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ORIGINAL PAPER

Reproduction in the externally brooding sea anemone *Epiactis georgiana* in the Antarctic Peninsula and the Weddell Sea

E. Rodríguez · C. Orejas · P. J. López-González · J. M. Gili

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Abstract External parental care is uncommon among actiniarians but common in *Epiactis* species. Here, several aspects of reproduction are analyzed for of one of them, *Epiactis georgiana*. Samples were collected in December, January, February, March, and April in the Antarctic Peninsula and the eastern Weddell Sea, during 1998, 2000, 2002, and 2003. Most sexually mature individuals of *E. georgiana* are male or female, but some are hermaphrodites. This is the first report of hermaphroditism in *E. georgiana,* which is the third species of the genus with this sexual pattern. The results suggest that oogenesis starts in December and that at least two generations of oocytes overlap; a third generation is often brooded externally. Putative fertilization is likely internal, and larvae and/or embryos are externally brooded on the distal part of the adult column until an advanced developmental stage. Apparently *E. georgiana* reproduces seasonally, probably releasing the embryos/larvae in the last months of the austral spring (December). Inter-individual variability was observed in gametogenesis. In addition, specimens from the Antarctic Peninsula were larger than those from the Weddell Sea. This study represents the first step in understanding the reproductive mode of *E. georgiana*.

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E. Rodríguez and C. Orejas have equally contributed to this work.

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E. Rodríguez (✉)
Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA
e-mail: erodriguez@amnh.org

C. Orejas
Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Muelle de Poniente, s/n, 07015 Palma de Mallorca, Spain

P. J. López-González
Biodiversidad y Ecología de Invertebrados Marinos, Departmento de Fisiología y Zoología, Facultad de Biología, Universidad de Sevilla, Reina Mercedes 6, 41012 Sevilla, Spain

J. M. Gili
Department of Biología Marina y Oceanografía, Institut de Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta, 37–49, 08003 Barcelona, Spain

Introduction

The diversity and number of clades of marine benthic invertebrates employing parental care in the Southern Ocean is unusually high, leading to a wide array of hypotheses to explain the phenomenon (reviewed by Pearse et al. 2009). Although the reproduction of many Antarctic invertebrates has been studied in the last two decades (e.g., Arntz et al. 1992; Barthel and Gutt 1992; Gutt et al. 1992; Peck and Robinson 1994; Poulin and Féral 1996; Barnes and Clarke 1998; Chantiore et al. 2002; Strathmann et al. 2006; Kang et al. 2009 among others), anthozoans have received little attention. Only five studies have focused on the group (Brito et al. 1997; Orejas et al. 2001, 2002, 2007; Waller et al. 2008), despite their abundance (Arntz et al. 1994). In the deep sea, a habitat whose benthic invertebrate biology is often compared with that of Antarctica, some studies on anthozoan reproduction are available, most of them on octocorals (reviewed by Watling et al. 2011) and scleractinian corals (e.g., Waller 2005; Waller et al. 2008; Waller and Tyler 2011). The paucity of studies on

reproductive biology of sea anemones (Actiniaria) is notable in these habitats: only one study reports details of the reproduction of a sub-Antarctic species (Riemann-Zürneck 1975) and a few studies address deep-sea species (e.g., Van Praët 1990; Bronsdon et al. 1993; Mercier and Hamel 2009).

The highly variable reproductive strategies documented for Cnidaria (Fautin 2002) are especially pronounced in Actiniaria, in which a combination of sexual and asexual mechanisms is favored by their anatomical and physiological simplicity (Stephenson 1928; Uchida and Yamada 1968; Fautin 1991). High inter- and intra-specific variability, and geographical and interannual variation (Edmands 1996) occur, and patterns can be influenced by factors such as light or food availability (Hand and Uhlinger 1992; Lin et al. 2001; Chen et al. 2008).

Both external and internal parental care (brooding) have been described for Actiniaria (e.g., Chia 1976); brooding is especially common in Actiniidae (e.g., *Actinia*, *Epiactis, Aulactinia*) and Actinostolidae (e.g., *Stomphia*, *Actinostola*) and has been reported for several Antarctic actiniarians (Carlgren 1927; Riemann-Zürneck 1975, 1978; Dunn 1983; Fautin 1984). The externally and internally brooded offspring in actiniarians were assumed to have a sexual origin until genetic studies revealed that at least some brooded juveniles were asexually produced (see *Actinia equina:* Carter and Funnell 1980; Orr et al. 1982; *Actinia tenebrosa*: Black and Johnson 1979; Sherman et al. 2007; Sherman and Ayre 2008). However, in *Epiactis* species, juveniles can be genetically different from the progenitor (Bucklin et al. 1984; Edmands 1995; Edmands and Potts 1997), indicating a sexual origin for brooded offspring. External brooding is an uncommon strategy among actiniarians, being mainly recorded in subtidal austral and boreal species (Stephenson 1928; Fautin et al. 1989). However, in the genus *Epiactis*, external brooding is the most common reproductive pattern (Edmands 1995, 1996; Edmands and Potts 1997).

Here, we study the reproductive biology of the Antarctic externally brooding sea anemone *Epiactis georgiana*. This is a medium-size actiniarian (to 56 mm in diameter and 76 mm height), whitish in color, usually with a distinct marginal collar in which offspring are brooded (Fig. 1a). *Epiactis georgiana* is circumpolar in the Antarctic and sub-Antarctic (Rodríguez et al. 2007) and inhabits soft and hard substrates in a wide bathymetric range (118–1,227 m depth), although it is especially abundant at 400–500 m depth (Dunn 1983). The number of specimens collected during several Antarctic cruises in different seasons over 4 years provided an exceptional opportunity to study the reproductive mode of an Antarctic sea anemone in an ecological context. We identify and describe the pattern of

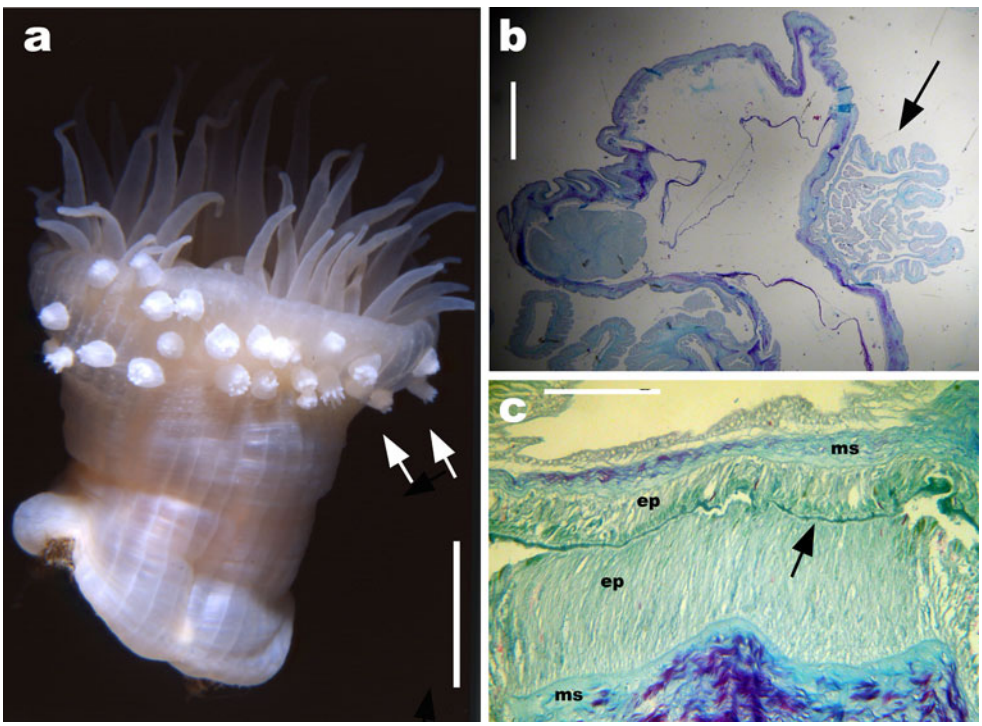

Fig. 1 *Epiactis georgiana;* **a** living female specimen brooding juveniles (*arrows*); these have open mouths and developed tentacles, **b** longitudinal histological section of column margin of a female and attached juvenile (*arrow*), **c** detail of defined layer (*arrow*) between epidermis of parental column and pedal disk of attached juveniles; it probably corresponds to mucus with which larvae and embryos come out. Abbreviations: *ep* epidermis, *ms* mesoglea. *Scale bars* **a** 40 mm; **b** 3 mm; **c** 0.2 mm

sexuality and the reproductive trends of *E. georgiana* through the months sampled and explore possible differences between the Antarctic Peninsula and Weddell Sea.

Materials and methods

Study areas and sampling

This study was conducted in the Antarctic Peninsula (AP) and the eastern Weddell Sea (WS) (Fig. 2). Sampling was carried out on board RV *Polarstern* during the EASIZ (Ecology of the Antarctic Sea Ice Zone) cruises II and III (in austral summer 1998 and 2000, respectively), ANDEEP-1 (Antarctic Benthic Deep-sea Biodiversity) cruise (in austral summer 2002), LAMPOS (Latin American "Polarstern" Study) cruise (in austral autumn 2002), and BENDEX (Benthic Disturbance Experiment) cruise (in late spring-summer 2003). For cruise details, see Arntz and Gutt (1999), Arntz and Brey (2001, 2003, 2005), and Fütterer et al. (2003). Specimens of *Epiactis georgiana* Carlgren, 1927 were sampled using Agassiz and bottom trawls. A total of 105 specimens collected from 36 stations have been analyzed (Table 1). Specimens were relaxed on board using menthol crystals, photographed alive, and subsequently preserved in 10 % sea-water-buffered formalin for histological analysis. The studied material has been deposited in the American Museum of Natural History (AMNH) in New York.

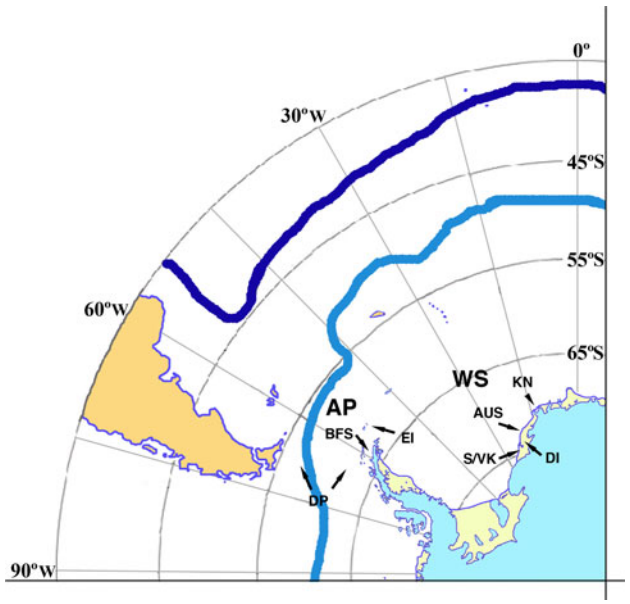

Fig. 2 Study area with detail of locations pooled into main areas of study. *Dark gray line*, subtropical front; *light gray line*, polar front. Abbreviations: *AP* Antarctic Peninsula, *AUS* Austasen, *BFS* Bransfield Strait, *DI* Drescher Inlet, *DP* Drake Passage, *KN* Kapp Norvegia, *EI* Elephant Island, *S/VK* South Vestkapp, *WS* Weddell Sea

Although in some cases, the distances between sampling stations within the AP and the WS were relatively great, due to the low number of specimens captured at some stations, we pooled specimens from sampling stations into two geographical zones for some of the data analyses: (1) "Antarctic Peninsula area" (AP) includes Drake Passage, Bransfield Strait, and Elephant Island; and (2) "Eastern Weddell Sea area" (WS) includes Kapp Norvergia, South Vestkapp, Drescher Inlet, and Austasen (Fig. 2).

Reproductive study

All specimens were examined to determine sex ratio and maturity classes. Sex ratio was calculated separately for each pooled area (65 specimens from AP, 40 from WS). Pedal disk diameter and column height were measured in preserved specimens; the number of pairs of mesenteries in a specimen was used to determine its maturity. The number of mesenteries is the most reliable way to determine maturity because although it is related to the size of the individual, it is not as variable as body size and might also relate to the sexual state (Dunn 1975a). In sea anemones, the number of pairs and cycles of mesenteries is characteristic at the genus or species level; the development of gametogenic tissue (sexual maturity) is usually associated with the development of a certain number of cycles (or pairs) of mesenteries (Stephenson 1928).

The development of gametogenic tissue was studied in histological sections of 51 specimens (29 from AP and 22 from WS, see Online Resource 1) sampled in December to April in different years. In subsequent discussion, we refer to the large, yolky structures inside females as "oocytes", but it is possible that these are not meiotically produced eggs, being instead asexual buds, or parthenogenic eggs rather than participants in mictic reproduction. Furthermore, we call those products of the same size and structure as these oocytes produced by hermaphrodites oocytes, and those products similar in size and structure to spermatic cysts produced by hermaphrodites spermatic cysts. Fragments of specimens were dehydrated in butanol (Johansen 1940) and embedded in paraffin. Histological sections of 7–8 µm thick were stained with Ramón y Cajal's Triple Stain (Gabe 1968). Individual sections were spaced at intervals of at least 200 µm (mean nucleus diameter from test sections), and intervening sections were discarded to avoid measuring the same cell repeatedly. Fifty gametes (when possible) were haphazardly selected from 30 female (16 from AP, 14 from WS) and 18 male (11 from AP, 7 from WS) specimens; however, in the case of oocytes, only those sectioned through the nucleus were measured; when the shape of the oocytes was irregular, the major axis was measured. Because oocytes and spermatic cysts should be equally affected by the decrease in size produced by the

Table 1 Sampling stations for studied specimens of *Epiactis georgiana*

Program[a]	Coordinates	Area[b]	Depth (m)	Date	*N*
EASIZ II	70°51.60′S 10°24.30′W	N/KN (WS)	282–283	30/01/1998	1
	70°53.60′S 10°28.10′W	N/KN (WS)	235–241	31/01/1998	3
	70°49.30′S 10°28.60′W	N/KN (WS)	281–301	01/02/1998	1
	71°09.70′S 12°28.70′W	N/KN (WS)	360–341	02/02/1998	1
	72°51.00′S 19°15.80′W	DI (WS)	391–395	03/02/1998	1
	73°33.50′S 22°15.30′W	S/VK (WS)	920–866	05/02/1998	2
	71°40.20′S 12°43.60′W	KN (WS)	244–248	15/02/1998	2
	71°17.00′S12°36.30′W	KN (WS)	415–416	16/02/1998	2
	70°50.60′S 10°35.50′W	KN (WS)	234–267	19/02/1998	2
	61°26.80′S 58°06.20′W	DP (AP)	1,047–1,227	19/03/1998	18
	61°33.90′S 58°11.00′W	DP (AP)	417–416	19/03/1998	1
EASIZ III	71°17.60′S 13°48.00′W	KN (WS)	615–648	31/03/2000	2
	71°11.30′S 12°15.40′W	KN (WS)	309–318	02/04/2000	3
	71°11.90′S 12°21.70′W	KN (WS)	323–312	03/04/2000	3
	70°50.40′S 10°35.20′W	AUS (WS)	226–266	07/02/2000	1
ANDEEP I	61°20.76′S 55°13.80′W	EI (AP)	270–264	31/01/2002	1
	61°21.34′S 56°02.65′W	EI (AP)	355–352	02/02/2002	1
	61°17.38′S 56°12.98′W	EI (AP)	327–317	02/02/2002	2
	61°17.87′S 57°02.29′W	EI (AP)	304–317	02/02/2002	3
	60°51.39′S 55°31.35′W	DP (AP)	279–292	03/02/2002	1
	60°49.42′S 55°39.56′W	DP (AP)	454	03/02/2002	30
	60°52.51′S 55°29.65′W	DP (AP)	242–250	03/02/2002	1
	61°01.41′S 55°58.98′W	EI (AP)	272–338	04/02/2002	1
	60°57.76′S 55°54.43′W	DP (AP)	202–212	05/02/2002	1
	61°00.97′S 55°46.16′W	EI (AP)	153–184	09/02/2002	1
LAMPOS	61°23.40′S 55°26.99′W	EI (AP)	282–276	25/04/2002	4
BENDEX	70°50,08′S 10°35.75′W	AUS (WS)	269–268	11/12/2003	1
	70°56.42′S 10°31.61′W	AUS (WS)	284–244	12/12/2003	1
	70°56.57′S 10°31.86′W	AUS (WS)	296–253	16/12/2003	2
	70°56.74′S 10°42.60′W	AUS (WS)	318–337	22/12/2003	1
	71°05.51′S 11°30.46′W	N/KN (WS)	286–287	23/12/2003	2
	70°52.74′S 10°52.72′W	N/KN (WS)	286–295	27/12/2003	2
	70°52.16′S 10°43.69′W	AUS (WS)	288–291	28/12/2003	1
	71°06.44′S 11°27.76′W	N/KN (WS)	268–277	28/12/2003	4
	71°07.20′S 11°26.47′W	N/KN (WS)	191–228	29/12/2003	1
	72°51.43′S 19°48.62′W	DI (WS)	598–576	31/12/2003	1
Total					105

Abbreviations: [a] research programs (see "Materials and methods" section). [b] Areas: *AUS* Austasen, *BFS* Bransfield Strait, *DI* Drescher inlet, *DP* drake passage, *EI* elephant Island, *KN* Kapp Norvegia, *N/NK* North Kapp Norvegia, *S/VK* South VestKapp, *AP* Antarctic Peninsula, *WS* Weddell Sea. *N*: number of specimens. Cruises, station numbers, and collecting gear information available from the authors

histological process (60–75 %, see Dunn 1975a), this shrinkage is factored out in comparisons. Oocytes inside the coelenteron were classified into three maturity classes: previtellogenic oocytes (<300 μm), early vitellogenic oocytes (300–600 μm), and late vitellogenic oocytes (600–1,000 μm). The development of the spermatic cysts was classified according to Wedi and Fautin Dunn (1983) as follows: stage 1 (E1), spermatic cysts only with spermatogonia; stage 2 (E2), spermatic cysts with spermatogonia, spermatocytes, and the first appreciable tailed sperm; stage 3 (E3), spermatic cysts completely mature with sperm dominance (see Online Resource 2). Putative gametes or zygotes free in the gastrovascular cavity of the female and those found in the brooding area were studied with scanning electron microscopy (SEM) to discriminate between large late vitellogenic oocytes and embryos/larvae

and to infer the type of fertilization (external/internal). The number of brooded embryos/larvae or juveniles and the number of mesenteries in each of these were counted and studied in histological sections. Pedal disk diameter and column height of brooded juveniles were also measured.

Data analysis

A chi-square test (χ_2^2) was used to test for significant differences in sex ratio between AP and WS. Measurements of pedal disk diameter, column height, and number of mesenteries were compared between sexes (pooled areas) and also between geographical zones (pooled sexes). Maximum relative frequencies of oocyte sizes and spermatic cysts stages (E1, E2, E3) were calculated for the sampled months, years, and geographical zones. Due to the lack of normality or variance homogeneity, the non-parametric tests U-Mann–Whitney (Mann and Whitney 1947) and Kruskal–Wallis (Kruskal and Wish 1978) with a Dunn post hoc test were applied in all cases. All results are presented as means ($\bar{X}$) ± standard deviation (SD).

Results

Sexual pattern

The studied populations of *Epiactis georgiana* consisted of 55 % females and 41.7 % males in AP and 47.5 % females and 50 % males in WS. A small proportion of hermaphrodite individuals (3.3 % in AP, 2.5 % in WS) and some sterile individuals (5 specimens in AP) were also detected (Table 2, Fig. 3a). No significant differences in sex ratio were detected for AP ($\chi_2^2 = 1.103$, $P = 0.29$) or WS ($\chi_2^2 = 0.256$, $P = 0.87$), and thus between zones. The sex ratio did not deviate significantly from expected equal frequencies.

Pedal disk diameter and column height ranged from 13.6 to 57.2 mm and 15.0–75.7 mm in females and males, respectively. We did not find a correlation between gamete and parent size (results not shown). The number of pairs of mesenteries ranged from 24 to 54. We found no significant differences in pedal disk diameter ($H_2 = 0.862$, $P = 0.6498$), column height ($H_2 = 0.370$, $P = 0.8311$), or number of pairs of mesenteries ($H_2 = 0.736$, $P = 0.6921$) between females and males. However, the comparison between AP and WS revealed a significantly higher pedal disk diameter ($U = 184$, $N_1 = 32.4$, $N_2 = 20$, $P < 0.005$), column height ($U = 43.5$, $N_1 = 37.1$, $N_2 = 13.9$, $P < 0.0001$), and number of mesenteries ($U = 3.500$, $N_1 = 38.9$, $N_2 = 12.2$, $P < 0.0001$) (Online Resource 3) in AP specimens.

The examined specimens of *Epiactis georgiana* had up to five cycles of mesenteries (Table 2); only the first two cycles (and some pairs of the third cycle in several specimens) were perfect (reaching the actinopharynx, see Fig. 3b). All mesenteries (except those in the youngest cycles, present only proximally) were fertile (including the directives) in most individuals. Specimens (except juveniles) were fertile once the third cycle of mesenteries (24 pairs) developed (Fig. 3b). The regularity in the number of pairs of mesenteries and directives indicates that *E. georgiana* does not reproduce asexually by fission (irregularities in arrangement of mesenteries are often associated with species with asexual reproduction, see Stephenson 1928). In hermaphrodite specimens, gametes of both sexes occurred in the same mesentery without vertical separation (Fig. 3a).

Gametogenesis

Oogenesis

Oogonia (8.0–35 μm in diameter) with a relatively large nucleus (15–17.5 μm in diameter) grew and multiplied in

Table 2 *Epiactis georgiana*

Maturity classes	Pairs of mesenteries (*N*)	Specimens (*N*)	Sterile specimens (*N*)	Females (*N*)	Males (*N*)	Hermaphrodites (*N*)
1	Up to 24	1	0	0	1	0
2	25–27	0	0	0	0	0
3	28–30	3	0	3	0	0
4	31–33	11	0	5	5	1
5	34–36	16	0	6	10	0
6	37–39	3	0	1	2	0
7	40–42	14	3	7	4	0
8	43–45	10	0	5	4	1
9	46–48	42	2	23	15	0
10	49 and more	5	0	2	2	1
Total		105	5	52	45	3

Number of individuals of each sex (females, males, and hermaphrodites) related to different maturity classes. *N* = number of individuals

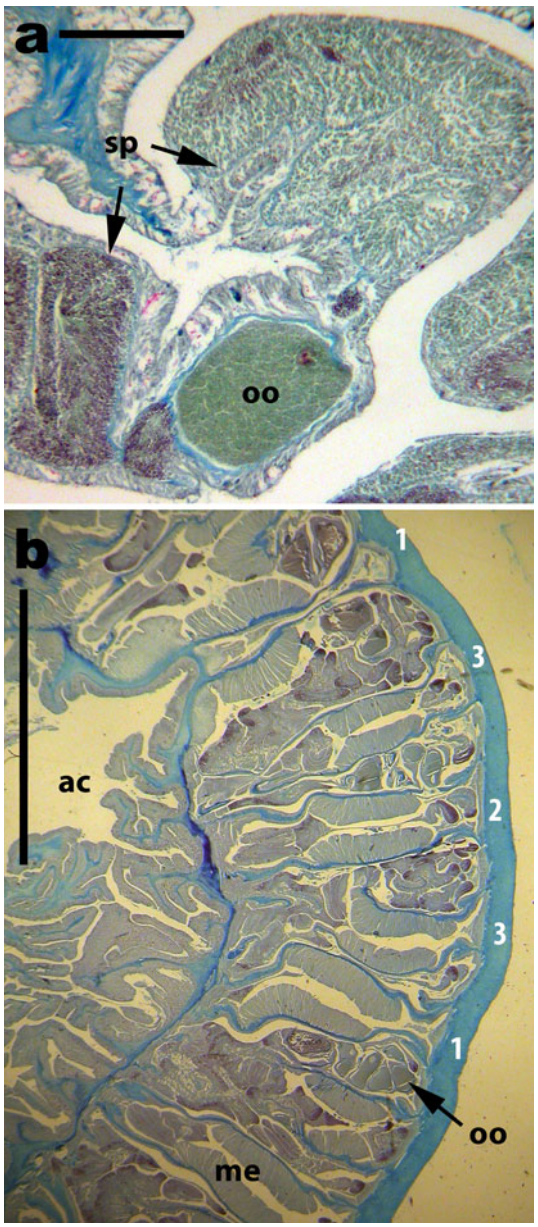

Fig. 3 *Epiactis georgiana*; **a** detail of gametes in a mesentery of hermaphrodite specimen, **b** cross section at actinopharynx level showing cycles of mesenteries; numbers indicate pairs of mesenteries of different cycles. Abbreviations: *ac* actinopharynx, *me* mesenteries, *oo* oocyte, *sp* spermatic cyst. *Scale bars* **a** 0.3 mm; **b** 5 mm

the gastrodermis; afterward, they migrated into the mesoglea, increasing their size (while decreasing the nucleus size) and concentrating yolk grains (small oval bodies) around the germinal vesicle (Fig. 4). Three maturity classes were distinguished: previtellogenic oocytes (<300 μm), each with a large nucleus; early vitellogenic oocytes (300–600 μm), each with large yolk granules; late vitellogenic oocytes (600–1,000 μm), with a prominent nucleolus in the central nucleus (Fig. 4c).

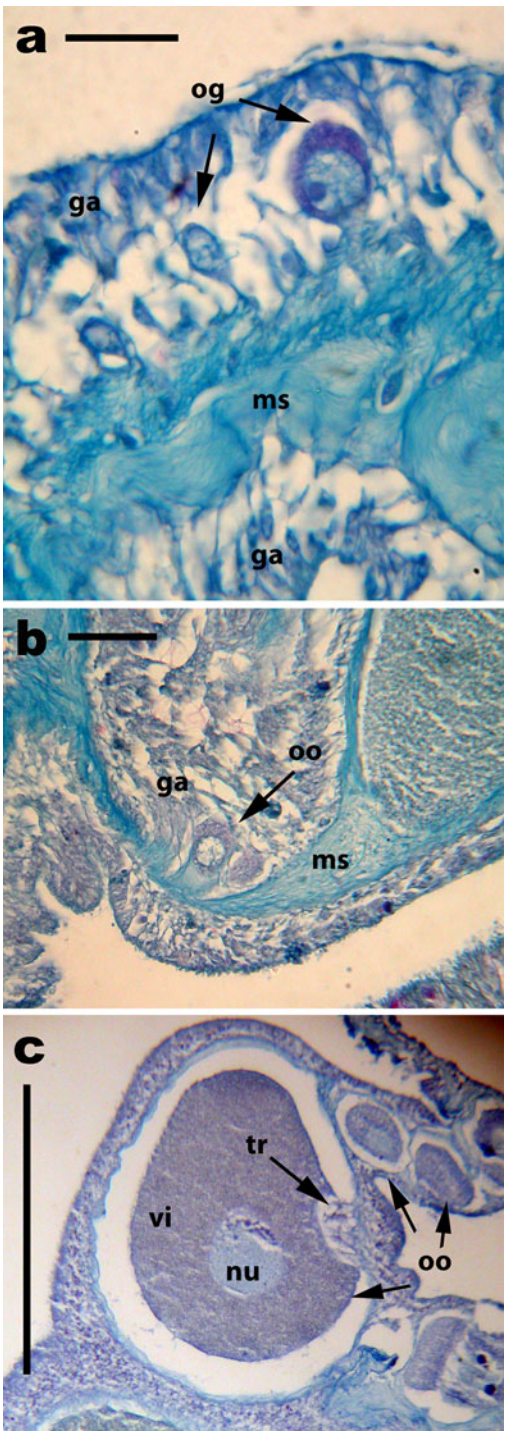

Fig. 4 *Epiactis georgiana*, histological sections of oogenesis; **a** first stage of development of female gametes: primary and secondary oogonia (8–10 μm and 30–35 μm in diameter, respectively) in gastrodermis with relatively large nucleus, **b** female cells (oocyte stage) migrated into mesoglea after reaching 30–45 μm diameter, **c** detail of two different sizes classes of oocyte (pre- and early-vitellogenic oocytes); note trophonema. Abbreviations: *ga* gastrodermis, *ms* mesoglea, *nu* nucleus, *og* oogonia, *oo* oocyte, *tr* trophonema, *vi* vitelo. *Scale bars* **a**, **b** 30 μm; **c** 0.25 mm

Spermatogenesis

Male gametes were recognizable when they formed spermatic cysts, each covered by mesoglea. The first developmental stage (E1) was a cyst with 24–28 spermatogonia. Visible spermatocytes (from 5 μm in diameter) in the center of the spermatogonia defined the next developmental stage (E2); at the end of this stage (spermatid), cells (to 2 μm in diameter) occupied the center of the spermatic cyst. In the final stage (E3), spermatozoids (to 1 μm in diameter) were visible in the mature follicles filling the center of the cyst with tails converging at the edge of the mesentery. The different stages of the process are summarized in Online Resource 2.

Although no oocytes or spermatic cysts were observed free in the gastrovascular cavity of *Epiactis georgiana,* we documented free embryos and/or larvae in the coelenteron of several specimens collected in December (see following section).

Reproductive trends

Based on measurements of 1,420 oocytes from 30 females, oocyte diameter ranged from 30 to 2,100 μm and did not differ significantly (pooling all specimens, sampling months and years, Online Resource 4) between AP and WS ($U = 242{,}415$, $N_1 = 723.8$, $N_2 = 696.8$, $P > 0.05$). However, results are presented separately to display the variability within and among months, years, and study areas (Fig. 5, Online Resources 4, 5, 6).

The frequency distributions of oocyte size differed among months (Fig. 5, Online Resources 4, 5). The diameter of oocytes from specimens collected in December (late spring) in WS showed the widest size range, followed by those from February and March in AP and WS. The diameter of oocytes from specimens collected in January (summer) in WS and April (autumn) in both AP and WS had the narrowest range. Samples for December and January were only available for WS. The most common stage in December (late spring) was the small previtellogenic oocyte (100–200 μm in diameter); we also found a small proportion of putative embryos/larvae (>1,000 μm in diameter) in this month (Fig. 5). The most prevalent oocyte size in the only specimen collected in January was ~600 μm, and there were no putative embryos/larvae. In WS, in February and March (summer), the most common oocytes were slightly larger than in December or January; this slight shift to a larger oocyte size continued in April (autumn) (Fig. 5). Although a similar trend was observed at AP in March and April, the distribution of oocyte sizes in February at AP mirrored that in December from WS. We observed high inter-individual variability within and among months and study areas (Online Resource 6); despite this variability, all studied females had previtellogenic and late vitellogenic oocytes, indicating at least two different oocyte cohorts (Figs. 4c, 5, Online Resource 5). However, caution in the interpretation of these results is advisable due to the low number of individuals analyzed for some months.

Based on measurements of 907 spermatic cysts from 18 individuals, spermatic cyst size was 35–380 μm and did not differ significantly in terms of the relative proportions of cyst stages (E1, E2, and E3) in a given month between AP and WS ($H_2 = 3.964$, $P = 0.1378$). However, results are presented separately to show the variability within and among months, years, and study areas (Fig. 6, Online Resources 4, 6). The frequency distribution of spermatic cysts in each month was related to the three defined maturity stages. Stage E1 was always the least common, followed by E2, and E3, except in March and April in WS, in which stages E2 and E3 were less common (Fig. 6).

SEM studies allowed the characterization of embryos and/or larvae in different developmental stages inside the coelenterons of two of the 52 females (both collected in December: Fig. 7, Online Resource 7). Furthermore, we found six additional females (not included in the study) externally brooding larvae (3–48 per polyp) in an early developmental stage in December (late spring) in WS; these females were collected in the same collection as seven recruits (free individuals completely developed but sexually immature).

Thirteen females (of the total 52 studied) were brooding offspring externally in the distal part of the column (Fig. 1, Online Resource 7). Only mature females brooded. In the brooding females, the epidermis was slightly thickened and more compact in the brooding area than in the rest of the column. We found a total of 19 (13 plus 6 additional ones, see above) female specimens externally brooding. Two of the brooding females were incubating embryos in the column collar in February and March (summer months) (Fig. 7b). One specimen collected in January was brooding juveniles in an early stage of development. The rest of the specimens brooding juveniles in a more developed stage (from 2 to 44 juveniles) were collected in February (Online Resource 7). Attached juveniles had a pedal disk diameter 3.5–13.0 mm and 12–30 pairs of mesenteries (Fig. 1b, Online Resource 7). Although some juveniles had developed the third cycle of mesenteries, none were fertile. Brooded juveniles had cnidae, and glandular cells completely developed in tentacles by the time six pairs of mesenteries had developed.

The number of brooded juveniles differed greatly with time and zone; we found 200 well-developed juveniles in February and three in April (Online Resource 5); no juveniles were detected in December. Juveniles were

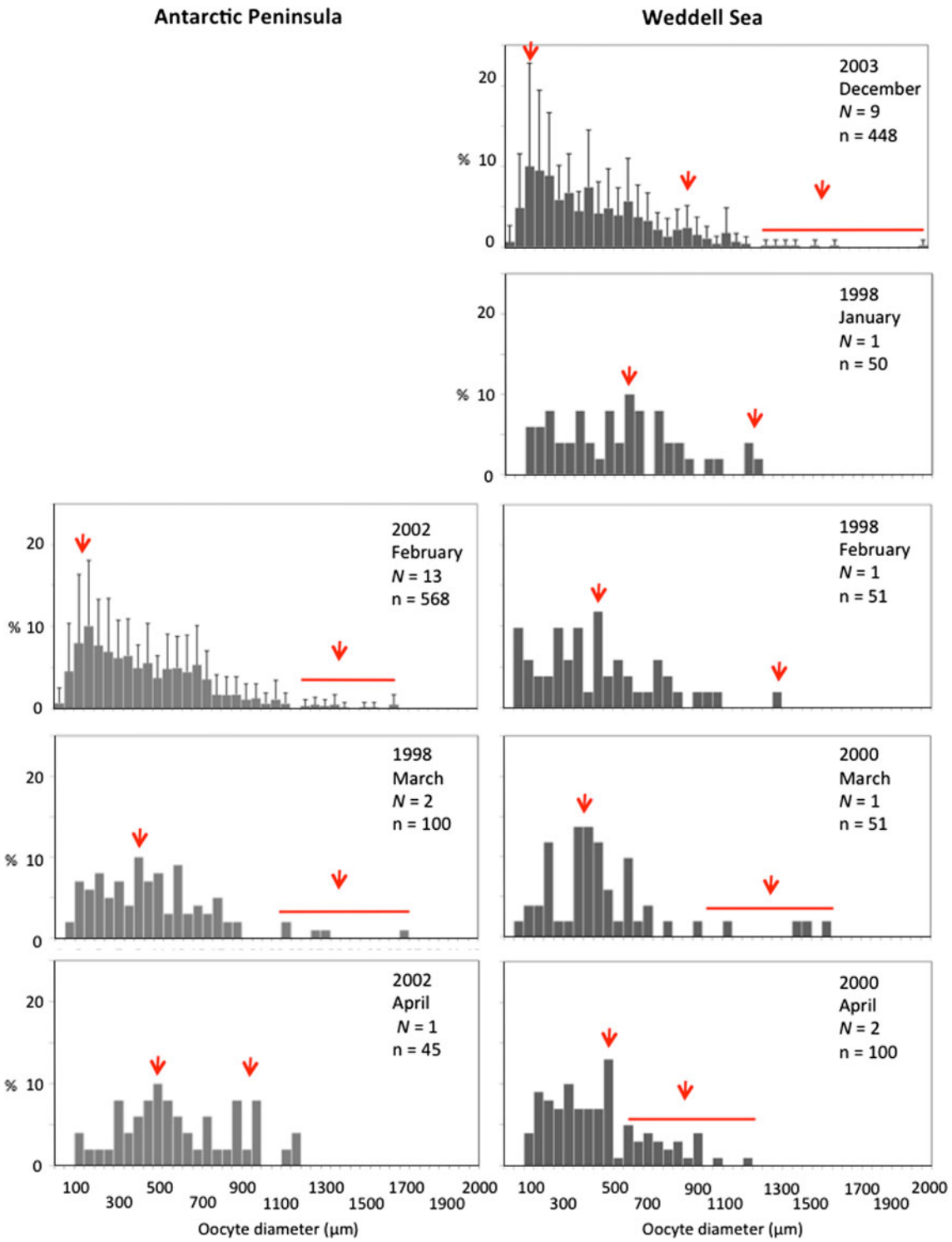

Fig. 5 Size frequency distribution (%) of oocytes separated by studied months, years, and zones (AP and WS). *Arrows* indicate suggested different oocyte maturity classes. *N* is number of females measured; *n* is number of oocytes measured

significantly ($U = 248$, $N_1 = 31$, $N_2 = 22.8$, $P = 0.05$) more abundant at AP than WS. We found the highest number of brooded juveniles in females with 48 pairs of mesenteries. We did not find any correlation (results not shown) between maternal size and number of juveniles.

Discussion

This is the first documentation of hermaphroditism for *Epiactis georgiana,* and the third time, this pattern of sexuality has been reported for the genus. Our data suggest

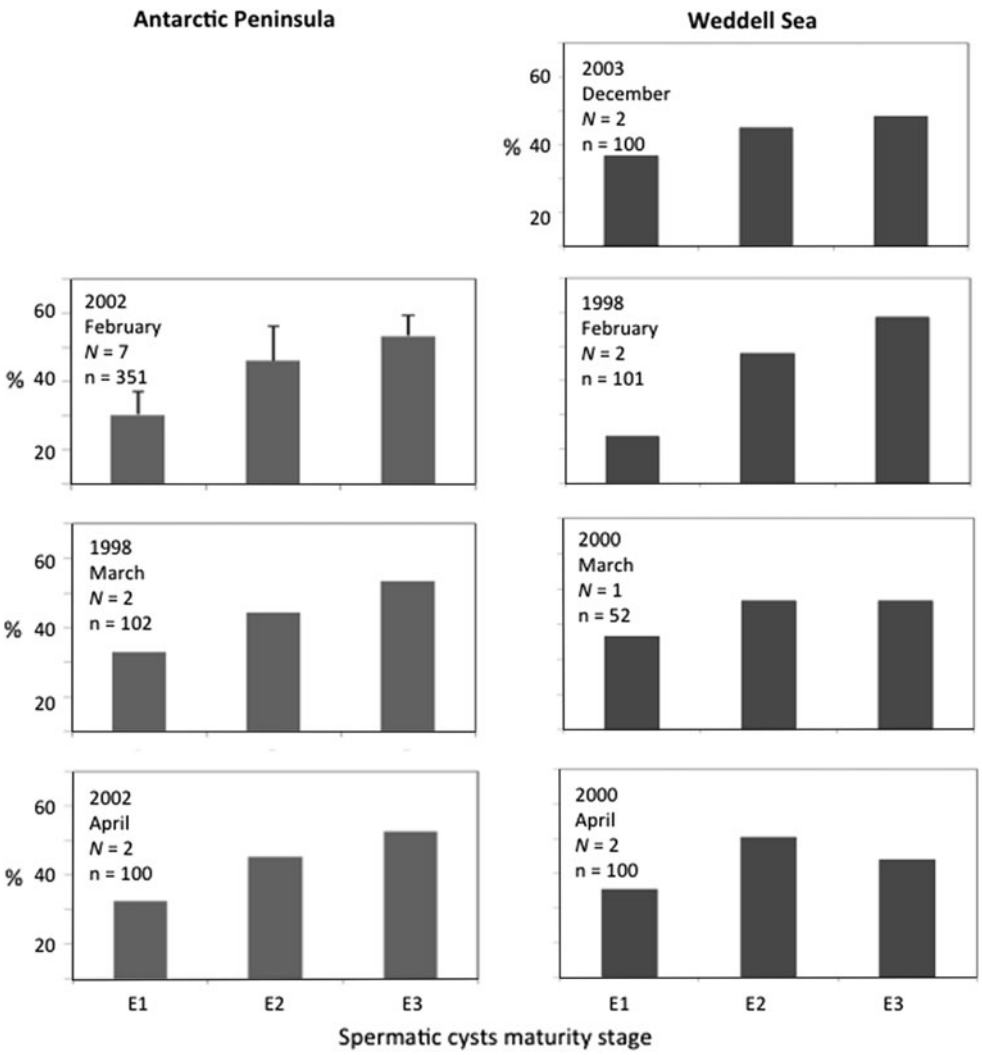

Fig. 6 Size frequency distribution (%) of spermatic cysts separated by studied months, years, and zones (AP and WS). Abbreviations: E1, E2, and E3 correspond to different developmental stages of spermatic cysts; *N* is number of males measured; *n* is number of spermatic cysts measured

that *E. georgiana* reproduces seasonally; oogenesis probably begins in December (late spring); and presumably fertilization takes place in the gastrovascular cavity of the female. The relatively slow development of oocytes results in the overlap of at least two, and often three, different generations in female specimens (Fig. 8).

Epiactis georgiana has a mixed sexuality: populations consist of females, males, and a low proportion of hermaphroditic individuals. Hermaphroditic individuals were previously not encountered (Carlgren 1927; Dunn 1983), probably due to their rarity and the difficulty of distinguishing small oocytes and spermatic cysts except in histological sections. A similar pattern with females, males, and a very low proportion of hermaphroditic individuals (but only one hermaphrodite among hundreds of individuals) has been reported for only two actiniarians: *Condylactis gigantea* (Jennison 1981) and *Paracalliactis stephensoni* (Van Praët 1990). Like *E. fernaldi*, *E. georgiana* is a simultaneous hermaphrodite; however, *E. fernaldi* broods its offspring internally (Dunn 1975a; Fautin and Chia 1986). The other externally brooding species of the genus, *E. prolifera*, is gynodioecious (only females and hermaphroditic individuals) (Dunn 1975a, b). Thus, *E. georgiana* shows a combination of sexual states previously unknown for the genus. Expression of hermaphroditism was not related to individual size in *E. georgiana* in contrast to *E. prolifera*, where females become hermaphrodites after reaching a particular size (Dunn 1975a).

Hermaphroditism increases fertilization probability in extreme and variable environments (Ghiselin 1969, 1974; Clark 1978; Bucklin et al. 1984). The extreme and locally variable environmental conditions of AP and WS (e.g., topography, deep-sea currents, iceberg scouring impact, etc.) might explain the advantages of hermaphroditism for *Epiactis georgiana*. Furthermore, the patchy distribution of Antarctic actiniarians (Rodríguez, unpubl. data) can reduce the probability of cross-fertilization (Levitan 1991; Coma and Lasker 1997). Hermaphroditism is usually, but not always, associated with self-fertilization in sea anemones (Edmands 1995); however, it is always (only one exception,

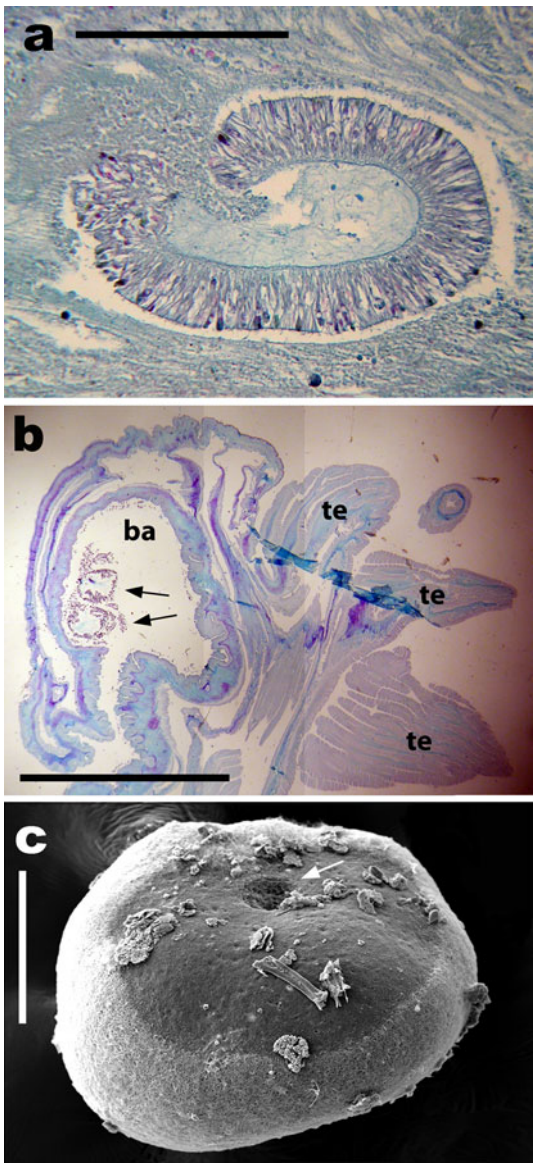

Fig. 7 *Epiactis georgiana*; **a** histological cross section of planula larva in gastrovascular cavity of female specimen, **b** embryos in gastrula phase (*arrows*) externally brooded in distal part of column (longitudinal section), **c** embryos in advanced developmental stage with cilia forming toward blastopore area (*white arrow*). Abbreviations: *ba* brooding area, *bl* blastopore, *te* tentacle; *Scale bars* **a** 0.15 mm; **b** 20 mm; **c** 0.2 mm

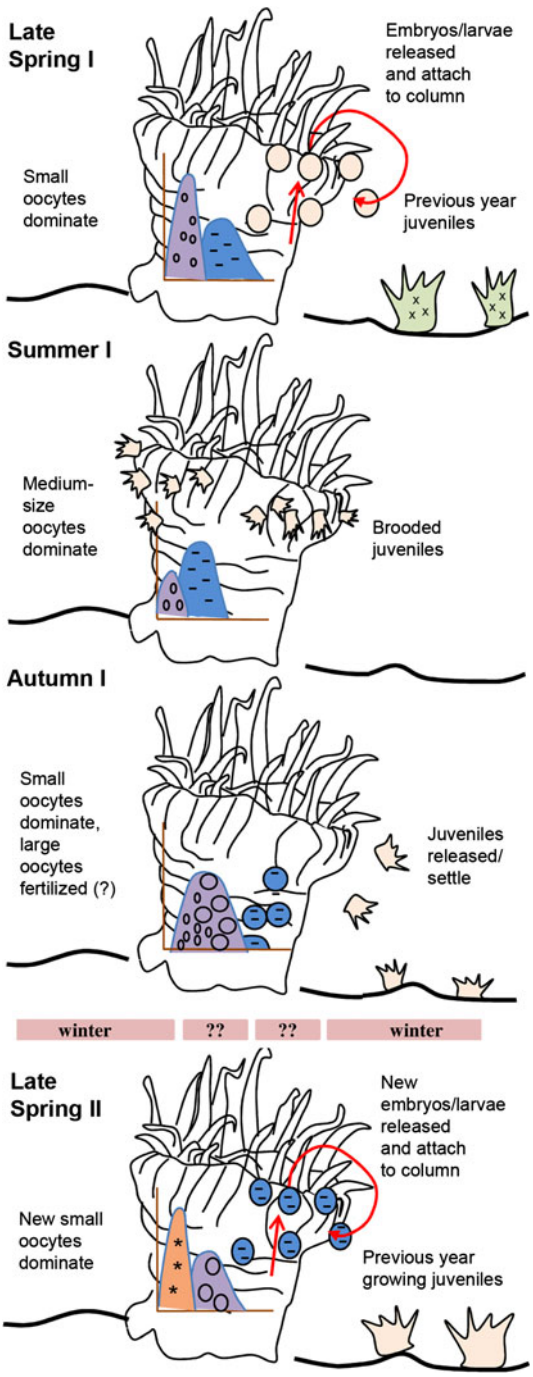

Fig. 8 Schematic representation of hypothetic reproductive cycle of *Epiactis georgiana*. A brooding female is represented through seasons of two consecutive years; each pattern (depicted by *asterisks*, *circles*, *dashes*, etc.) represents a different generation. Broken timeline and question marks represent missing data from winter; late spring (December); summer (January, February, and March); autumn (April)

see Bronsdon et al. 1993) associated with vivipary or some kind of brooding in this group (Shick 1991). Self-fertilization allows reproduction in widely distributed individuals (e.g., Ghiselin 1969, 1974; Clark 1978) and favors faster colonization of new areas from a small number of specimens (e.g., zones affected by a recent iceberg scouring event). Although this could be the case in *E. georgiana*, molecular studies are necessary to confirm the occurrence of self-fertilization and to make inferences about the advantages this trait could provide to this species.

The observed relationship between hermaphroditism and relatively small size for marine invertebrates (Ghiselin 1969; Charnov 1982) has been pointed out for the genus *Epiactis* (see Shick 1991). However, *E. georgiana* does not follow this trend: its body size (pedal disk 57.2 mm in

diameter) is more similar to the gonochoric species (e.g., in *E. lisbethae* and *E. ritteri*, pedal disk diameter is usually 50 mm or larger), rather than to the small bodied, hermaphroditic species (*E. prolifera* and *E. fernaldi*, pedal disk diameter usually 20 mm). Despite some studies that relate size and brooding strategy in marine invertebrates (e.g., Charnov 1982; Strathmann and Strathmann 1982; Strathmann et al. 1984), this relationship is still unclear (Poulin and Féral 1996). Nonetheless, the unusual and intermediate nature of external brooding could be linked with the allometry hypothesis, which seeks to explain the inverse relationship between brooding and small sizes in marine invertebrates (Strathmann and Strathmann 1982). We consider external brooding an intermediate reproductive strategy with respect to broadcast spawning and internal brooding: offspring develop in two different environments when externally brooded because part of the development occurs outside the parent, although with some parental protection. Furthermore, brooding offspring outside the adult alleviates space limitation for gamete production while still protecting the offspring; this might diminish the risk of the shift from the internal to external environments, probably the most critical time for offspring survival. The relatively large size of *E. georgiana* might represent an instance of the proposed trend toward "gigantism" in some marine invertebrates in the Antarctic (e.g., Chapelle and Peck 1999, 2004); however, this is only valid for some taxa (Woods et al. 2009) and requires detailed comparisons of sibling taxa. Although most Antarctic sea anemones tend to be relatively large (Rodríguez pers. obs.), a trend toward gigantism for actiniarians in Antarctica still has to be confirmed.

The differences in size between *Epiactis georgiana* from AP and WS (larger in the former) do not have a clear explanation based on previous comparative studies between the two zones. No differences in biomass and abundance for macro- and mega-benthos have been found between these two regions; depth, food supply, and seabed features seem to be more influential factors than latitude (Dayton 1990; Piepenburg et al. 2002). Linse et al. (2006) did not find any clear relationship between body size and latitude in gastropods, echinoderms, or bryozoans; nevertheless, they found a correlation with food availability and fecundity in one of the studied gastropods. As Brey and Haine (1992) found for Antarctic bivalves, statistically significant differences in size between specimens of *E. georgiana* from the two zones could be related to depth: more specimens from AP (particularly in the Drake Passage) were from greater depths (18 individuals came from 1,047 to 1,227 m and 30 from 454 m) than those from WS (maximum sampling depth 920 m). However, studies of gastropods in the North Atlantic showed an inverse relationship between size and depth (Olabarria and Thurston 2003). Observed differences could also just be due to samples coming from different years in each zone.

Epiactis georgiana starts developing oogonia and spermatic cysts once the third cycle of mesenteries (24 pairs of mesenteries) is completely developed, a pattern common for actiniarians (Carlgren 1949). However, maturity does not seem to be related to the number of developed cycles of mesenteries as immature (non-reproductive) brooded juveniles as well as mature (reproductive) adult individuals both showed 24–30 pairs of mesenteries; this suggests a relationship between maturity and size of the organisms. *Epiactis prolifera* also develops oocytes only once the fourth cycle of mesenteries is completed, with no relationship to season (Dunn 1975a). According to Dunn (1975a), the presence of fertile individuals of *E. prolifera* year round is due to the continuous presence of different size classes of individuals in the population (there is always some individual completing the development of the fourth cycle of mesenteries and starting oocyte production). Similarly, fertile individuals of *E. georgiana* were found in all collection months. However, in the case of *E. georgiana*, this seems due to the overlap of different generations of sexual products—as observed in other Antarctic cnidarians (e.g., gorgonians, Orejas et al. 2002, 2007)—rather than continuous reproduction.

The gradual increase in frequencies of larger oocytes from December (late spring) to April (autumn) suggests that oogenesis starts in the early austral spring (or end of the winter) and that reproduction in *Epiactis georgiana* occurs seasonally. Several studies of deep-sea actiniarians support this pattern: six of seven studied species reproduce seasonally (Van Praët 1990; Van Praët et al. 1990; Bronsdon et al. 1993; Fautin 1997; Mercier and Hamel 2009). This suggests that a seasonal reproductive cycle may be correlated with maximum phytodetritus abundance, as has already been suggested for deep-sea organisms in the North East Atlantic (Gage and Tyler 1991) and hypothesized for Antarctic ecosystems (food bank theory; Smith et al. 2006; Galley et al. 2008). However, for logistical reasons, samples in this study came from different years and sampling localities (sometimes far apart) with likely local environmental differences among them; in addition, sample size in some months and areas (e.g., January, April, December in AP, etc.) was small. These methodological limitations might affect the interpretation of the data, and reproductive asynchrony among years may occur. Indeed, these limitations could explain the observed difference between the oocyte size frequency distribution in February between AP and WS: the slight delay in the increase in larger oocytes in AP (mirroring data from December in WS) might be due to samples from February coming from different years in each zone.

Our data suggest that *Epiactis georgiana* probably releases embryos from the previous year in the last months of the austral winter or beginning of spring (December). The frequency distributions of oocyte diameter in the different months suggest that at least two generations of developing sexual products overlap in an adult female because two size classes can be distinguished from December to April despite the high observed inter-individual variability. Thus, up to three different generations (two phases of gametogenesis and brooded embryos on the exterior of the column) may exist in one female; each generation takes about 2 years to develop and be released from the parent (Fig. 8). The two-year reproductive cycle suggested here for *E. georgiana* is shared with several deep-sea and other actiniarian species with parental care (Dunn 1975a; Van Praët 1990; Van Praët et al. 1990), as well as with other polar invertebrates such as isopods (Luxmoore 1982), caridid decapods (Gorny et al. 1993), and gorgonians (Orejas et al. 2002, 2007).

The slight decrease in the relative frequency of spermatic cysts in stage E3 in March and April in WS suggests their liberation at the end of the austral summer. However, males from AP did not follow this trend. These differences between AP and WS could be due to environmental conditions but also to methodological limitations: only two male samples were available for those months and they came from different years in each zone. The early embryos/larvae inside the coelenterons suggest internal fertilization; however, molecular analyses are necessary to test whether they are of sexual or asexual origin (e.g., parthenogenesis, self-fertilization).

The developmental stage of externally brooded offspring in *Epiactis georgiana* suggests that embryos and/or larvae are released synchronously in all individuals around December (late austral spring) or a little earlier. This means that offspring are usually in an advanced developmental stage by summer. The approximately simultaneous development of the brooded offspring in the two study areas suggests similar size classes among the offspring. The release of embryos and/or larvae in austral spring is supported by (1) the presence of free embryos and larvae inside the female coelenterons in December, and (2) the intermediate level of development of the externally brooded offspring collected in January.

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