Rev. Mus. Argentino Cienc. Nat., n.s.
27(1): 87-93, 2025
ISSN 1514-5158 (impresa)
ISSN 1853-0400 (en línea)

External malformations in the green sea urchin *Arbacia dufresnii* (Blainville, 1825) in populations from Golfo Nuevo, Patagonia, Argentina

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Abstract: Malformations in sea urchins can manifest in various forms, often influenced by environmental and biological factors. Adult *Arbacia dufresnii* (Blainville, 1825) exhibit pentaradial symmetry, spines in the ambulacral areas, absence of spines in the interambulacral areas, and a rhomboidal periproct composed of four ossicles. This report aims to document the presence of external malformations in the green sea urchin *Arbacia dufresnii* in the population of Golfo Nuevo, Patagonia, Argentina (42°42′ S 64°30′ W). The specimens were collected at Golfo Nuevo. A total of 319 specimens were collected, out of which 5 showed malformations. The malformations include anomalous pentaradial symmetry, spines in the interambulacral areas, and non-rhomboidal periprocts covered by three supra-anal plates. These findings suggest the need for monitoring external malformations in populations of *A. dufresnii* and other Echinoidea, as they could provide crucial information about the health and status of these populations. Malformations may serve as biomarkers of environmental stress, pollution, or habitat condition changes, which are essential for effective conservation measures.

Key words: *Arbacia dufresnii*, test, malformations, Golfo Nuevo, Echinoidea

Resumen: Malformaciones externas del erizo de mar verde *Arbacia dufresnii* (Blainville, 1825) en poblaciones del Golfo Nuevo, Patagonia, Argentina. Las malformaciones en los erizos de mar pueden manifestarse de diversas formas, y suelen depender de factores ambientales y biológicos a los que estén expuestos. Los adultos de *Arbacia dufresnii* (Blainville, 1825) poseen simetría pentámera, espinas ubicadas en las áreas ambulacrales y la ausencia de estas en las áreas interambulacrales, así como un periprocto de forma romboidal compuesto por cuatro osículos. El objetivo de este reporte es documentar la presencia de malformaciones externas del erizo de mar verde *Arbacia dufresnii* en la población del Golfo Nuevo, Patagonia, Argentina (42°42′ S 64°30′ O). Los ejemplares fueron recolectados en el Golfo Nuevo. Se recolectaron 319 individuos, 5 de los cuales mostraron malformaciones. Las malformaciones reportadas son: simetría pentámera anormal, presencia de espinas en áreas interambulacrales y periproctos no romboidales cubiertos por tres placas supra-anales. Esperamos que los resultados promuevan el monitoreo de las malformaciones externas en las poblaciones de *A. dufresnii* y otros Echinoidea, lo que podría proporcionar información crucial sobre la salud y estado de las mismas. Las malformaciones podrían ser biomarcadores de estrés ambiental, contaminación o cambios en las condiciones del hábitat, lo cual es fundamental para implementar medidas de conservación efectivas.

Palabras clave: *Arbacia dufresnii*, testa, malformaciones, Golfo Nuevo, Echinoidea

INTRODUCTION

The Class Echinoidea (sea urchins), Holothuroidea (sea cucumbers), Ophiuroidea (brittle, serpent star or ophiuroids), Crinoidea (sea lilies and feathers stats) and Asteroidea (sea stars) constitute the Phylum Echinodermata (echinoderms) (Hickman *et al.*, 2008). This group of animals exhibits numerous distinctive features, including a complex internal transport system known as the water vascular system (WVS), composed of the madreporite plate, which connects the organism's interior to the external environment, and the ambulacral feet, appendages with an ampulla and a podium, with diverse functions (Hickman *et al.*, 2008). Echinoderms possess an endoskeleton composed of numerous calcareous ossicles with accessory structures like spines, spicules, and pedicellariae (Hickman *et al.*, 2008; Borrero Pérez *et al.*, 2012). Sea urchins are capable of regeneration the test and their external appendages (Carnevali, 2006; Emerson *et al.*, 2017; Maboloc & Fang, 2023). They exhibit an indirect life cycle, with embryos and larvae displaying bilateral symmetry (Hickman *et al.*, 2008). Through the process of metamorphosis, involving corporal changes, they acquire both internal and external secondary pentaradial symmetry, although some groups phenotypically display bilateral symmetry (Hickman *et al.*, 2008; Benavides Serrato *et al.*, 2011; Borrero Pérez *et al.*, 2012).

In Echinoidea ossicles are fused to form a test that can exhibit pentaradial symmetry ("regular" sea urchins) or bilateral ("irregular" sea urchins) symmetry (Larrain Prant, 1975; Hickman *et al.*, 2008; Swalla & Smith, 2008; Pérez, Gil & Rubilar, 2014). The test, consists of twenty columns of ossicles grouped in pairs, forming the five ambulacral areas (AA) and five interambulacral areas (IA), alternating with each other resulting in the juxtaposition of AA with IA (Fig. 1) (Borrero Pérez *et al.*, 2012; Pérez, Gil & Rubilar, 2014). The AA holds the tube feet of the WVS and spines (Hickman *et al.*, 2008). Each column of calcareous plates extends from the oral area to the apex of the test in the aboral area (Fig. 1) (Borrero Pérez *et al.*, 2012). The apical system is the region where the terminal ossicles of the AA (ocular plate or ambulacra) and IA (genital plate or interambulacral) converge (Fig. 1) (Borrero Pérez *et al.*, 2012; Pérez, Gil & Rubilar, 2014). Each genital plate has a perforation called genital pore or gonopore through which gametes are released (Fig. 1), each connected to a gonad (Hickman *et al.*, 2008). One of the genital plates correspond to the madreporite plate of the WVS (Fig. 1) (Hickman *et al.*, 2008; Pérez, Gil & Rubilar, 2014). The five genital plates are adjacent to the periproct, which is compose of supra-anal calcareous plates (Fig. 1) (Bernasconi, 1925; Hickman *et al.*, 2008). Additionally, sea urchins have a complex chewing called "Aristotle's lantern", composed of five teeth used for cutting and scraping food, which are visible in the oral area (Larrain Prant, 1975; Hickman *et al.*, 2008; Pérez, Gil & Rubilar, 2014).

Arbacia dufresnii is a sea urchin with a broad distribution range, from the Río de la Plata (Argentina - 35° S) to Puerto Montt (Chile - 42° S), including Antarctica, the Malvinas Islands (Bernasconi, 1925; 1942; Larrain Prant, 1975; Brogger *et al.*, 2013), and smaller islands from the Atlantic Ocean (Caselle *et al.*, 2018). As in other sea urchins, it has ecological significance by influencing productivity in its habitat (Newcombe *et al.*, 2012; Pérez, Gil & Rubilar, 2014). At the Golfo Nuevo, *A. dufresnii* is currently cultivated in Puerto Madryn by the company ERISEA S.A. for biotechnological purposes. The primary product derived from this species focuses on its female gametes as a source of antioxidants (Rubilar & Cardozo, 2021; Rubilar *et al.*, 2021). During its life cycle, this species typically exhibits primary bilateral symmetry in the embryo/larval development and secondary pentaradial symmetry after metamorphosis (Brogger, 2005; Fernández *et al.*, 2019; Gianguzza, 2020). The test of juveniles and adults are "regular" with well-defined ambulacral and interambulacral areas, and a rhomboidal periproct covered by four ossicles or supra-anal plates (Fig. 1) (Bernasconi, 1925; Larrain Prant, 1975; Brogger, 2005).

Malformations in sea urchins can be manifested in various ways, depending on the environmental and biological factors they are exposed to. These malformations may include alterations in symmetry, such as a reduced or increased number of symmetrical parts, absence or arrangement in spines, anomalies in test plates or in the periproct, and deformities during early development (Hylton Scott, 1939; Larrain Prant, 1975, Munar, 1984; Gambardella *et al.*, 2021). These malformations can be caused by a variety of factors, including chemical pollution, diseases, changes in water temperature and salinity, as well as genetic factors (Dupont *et al.*, 2010, Kroeker *et al.*, 2010; Clark, 2020; Gambardella *et al.*, 2021; Han *et al.*, 2022; González Pisani *et al.*, 2024). The presence of these malforma-

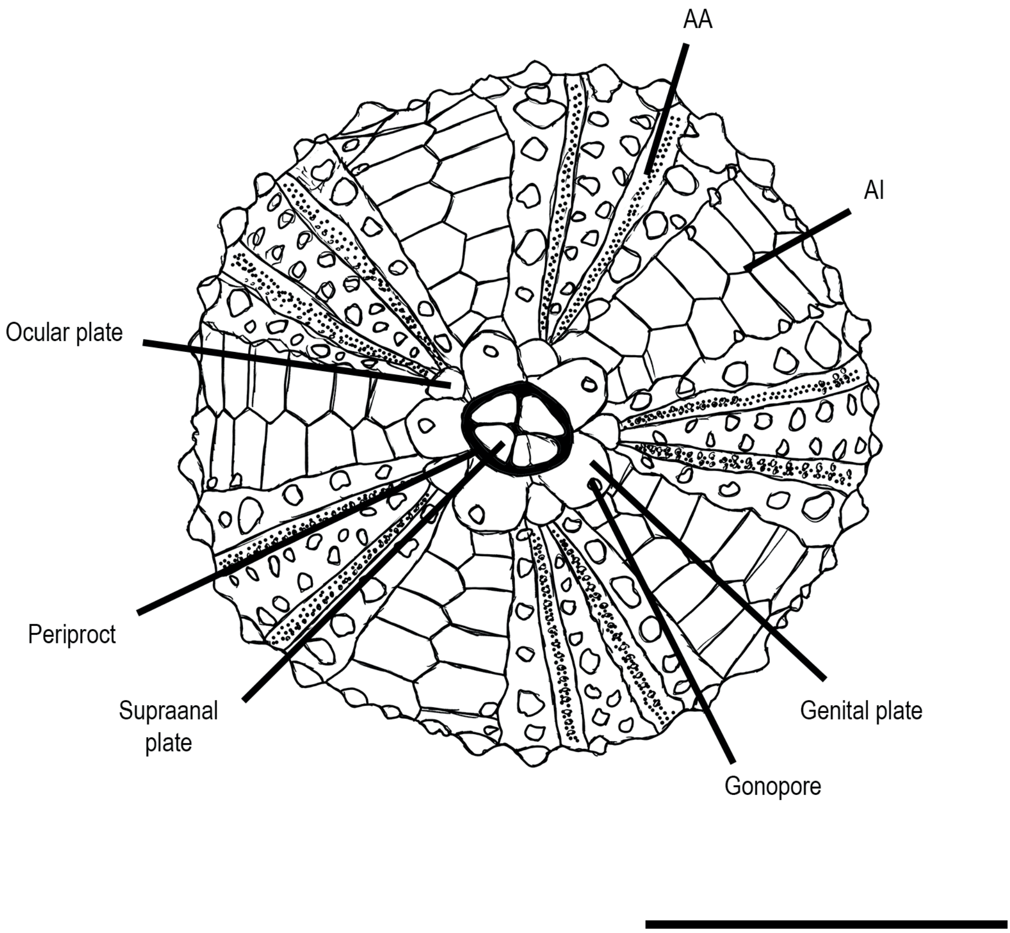

Fig. 1. Diagram of the aboral zone of the test of the sea urchin *Arbacia dufresnii*. AA: ambulacral area; IA: interambulacral area. Detail of the apical zone of the test: genital plates genital pore, ocular plates, and periproct composed of four supra-anal plates. Scale 10 mm.

tions can serve as an indicator of environmental health and habitat quality in marine ecosystems (Laitano *et al.*, 2013; Savriama *et al.*, 2015; Gouveia *et al.*, 2022).

MATERIAL AND METHODS

Sea urchins of *Arbacia dufresnii* were collected during November 2023 by SCUBA diving at Punta Cuevas, Golfo Nuevo, Chubut, Argentina (42°46'44'' S, 64°59'52'' W); under the regulatory authorization granted by the Ministry of Agriculture and Livestock of the province of Chubut (Disp. N°73/2023–DFyFS MAGIyC). Specimens were transported to the laboratory and acclimated for 15 days in aquaculture recirculating systems supplied with natural seawater in the Experimental Aquarium of the Center for the Study of Marine Systems (CESIMAR CONICET-ARGENTINA), with constant aeration, at 13 ± 0.5 °C and 12:12 light: dark cycle.

All individuals collected were characterized based on their coloration and spines arrangement (Hylton Scott, 1939; Larrain Prant, 1975), and measured for the test height and maximum diameter using a caliper (± 0.1 mm). To determine sex, spawning was induced by injecting 0.3 ml of a 0.5 M KCl saline solution into the peristomial membrane (Brogger, 2005; Fernández *et al.*, 2019). Malformations were identified by comparing the external morphology observed in the organisms test with descriptions from previous authors (Bernasconi, 1925; Larrain Prant, 1975; Brogger, 2005; Epherra, 2010). Specimens displaying any external malformation were drawn and photographed to capture their morphological features in detail, using an iPhone 14 Pro Max and stereoscopic microscope (Stemi

2000, Carl Zeiss) equipped with a digital camera (Microscopy Service, CCT CONICET-CENPAT).

RESULTS AND DISCUSSION

A total of 319 adults of *Arbacia dufresnii* were collected, 164 females and 150 males in a ratio of 1.09:1; and 5 indetermined specimens. Both test and spine colors characteristic of the species were recorded, according to Larrain Prant (1975). The dominant pigmentation on the test was green (91.11%) with brown spines (85.19%) (Table 1). The test average was 14.53 ± 2.14 mm of height and 28.95 ± 3.04 mm of maximum diameter, which is an intermediate diameter compared to Brogger (2005) at 30.7 ± 4.9 mm and Epherra (2010) at 25.9 mm.

A total of specimens were collected, out of which 5 individuals showed external anomalies in the test, consisting of:

Presence of spines in areas where they should be absent (Bernasconi,1925; Larrain Prant, 1975):

- Spines in the ossicles' sutures of the interambulacral area (IA) (Fig. 2A, B, C, D). This anomaly was present in 100% of the malformed specimens.
- Spines enclosing the genital plates in the apical area where the IA terminates (Fig. 2B). This was registered found in the 20% of the malformed specimens.

Periproct's malformation (Bernasconi, 1925; Larrain Prant, 1975):

- Periproct was covered by three ossicles (Fig. 2C) instead of four supra-anal plates (Bernasconi, 1925). Larrain Prant (1975) reported this anomaly and detailed that variation of the number of ossicles in the periproct are particular, generally present a higher number of these plates. This anomaly was found in 40% of the malformed specimens.

Symmetry anomaly in the test (Bernasconi,1925; Larrain Prant, 1975):

- Absence of an ambulacra area (AA) (Fig. 2D). This anomaly was present in 20% of the malformed specimens.

The specimen with anomalous pentaradial symmetry was assessed for maximum diameter using two approaches: AA-IA diameter was measured by placing one caliper point over an ambulacral area and the other over an interambulacral area; IA-IA diameter was measured by positioning the caliper between two IA, one positioned in front of the other. The specimen measured 15.22 mm height; the AA-IA diameter was 28.72 mm and the IA-IA diameter was 26.16 mm. On the oral area, the ambulacra and the Aristotle's lantern displayed the typical morphology. At the apex, there were five genital plates, each with a gonopore (Fig. 2D). Upon spawning induction, the organism released gametes through all its gonopores, demonstrating the functionality of its gonads. The released gametes exhibited a whitish pigmentation typical of males to *A. dufresnii* (Brogger, 2005). At the apex, the periproct consisted of four ossicles (Fig. 2D). Unusually, it displayed four AA and four IA (Fig. 2D). Due to the absence of one AA, the IA that would normally be on its sides fused into a single IA that was longer than the others (Fig. 2D). The aboral area showed sparse spines and a vestigial AA (Fig. 2D), suggesting that the AA formation did not complete. Sea urchins are capable of regeneration their external appendages (Carnevali, 2006; Maboloc & Fang, 2023) but the specimen had not sutures on test evidencing regeneration. The regeneration may not produce macroscopic abnormalities (Emerson *et al.*, 2017; Maboloc & Fang, 2023). Consequently, this anomaly positioned the atypical IA in front of two AA and one IA (Fig. 2D), contradicting the typical pattern observed in Echinoidea (Borrero Pérez *et al.*, 2012; Pérez, Gil & Rubilar, 2014).

This is not the first evidence of absence of an AA in *A. dufresnii* in Argentina. Hylton Scott (1939) reported a test with four AA and four IA, thus naming it tetramerous or tetraradial, in Punta Delgada, Patagonia (42°45′59″ S 63°38′13″ W). However, this specimen reported by Hylton Scott (1939) differs in some aspects from the one described in this study; the AA and IA had a symmetrical length and the test has a cross-like appearance because the AA faces the other AA, and the IA faces other IA. Also, its apical area has four genital plates, one of them with two pores, suggesting it had five gonads. On the other hand, since Hylton Scott (1939) only found the bare test, the sex, number and functionality of the gonads, as well as the symmetry of the oral area, could not be determined. Hylton Scott (1939) suggested that the specimen found at Punta Delgada exhibited internal pentaradial symmetry and external bilateral symmetry. The specimen sampled at Punta Cuevas, reported in this study, exhibits internal pentaradial symmetry. Externally, it showed five teeth in the Aristotle's

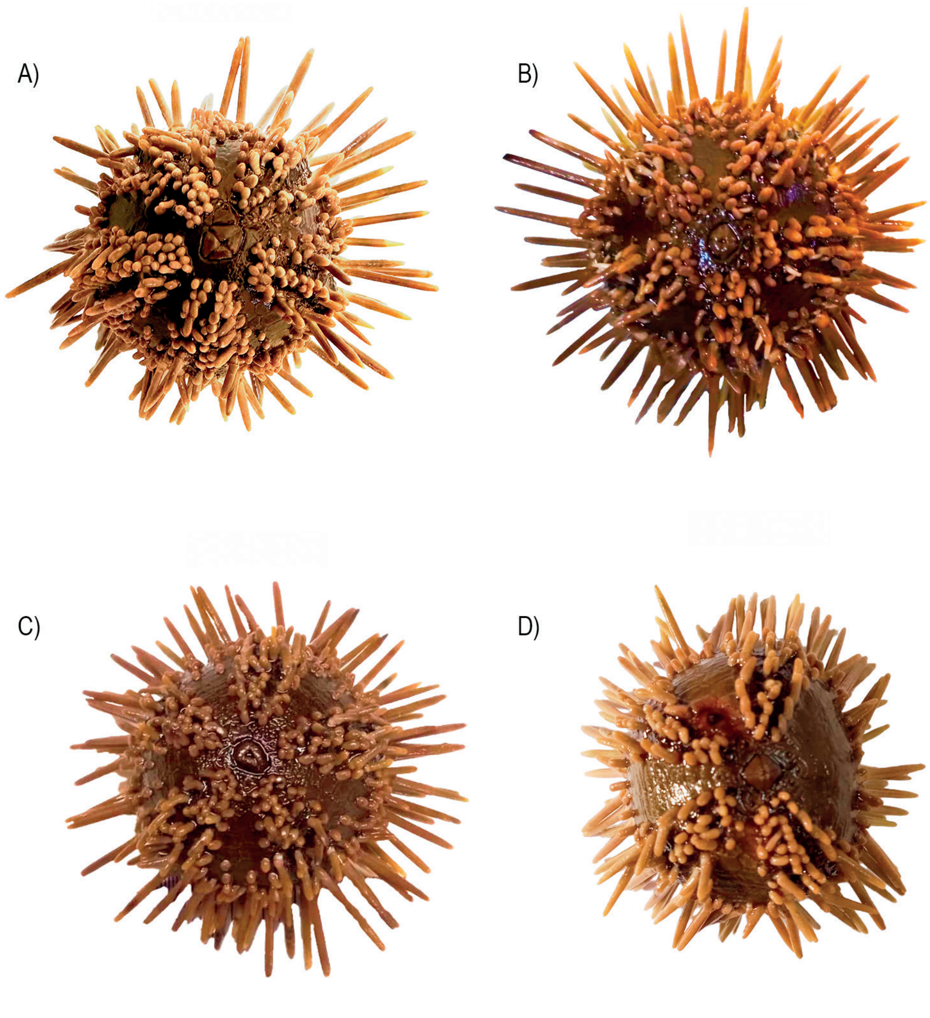

Fig. 2. External malformations found in natural populations of *Arbacia dufresnii* in Golfo Nuevo, Chubut, Argentina (42°46'44'' S, 64°59'52'' W). A) Specimen with spines in interambulacral areas (IA). B) Specimen with spines along and at the apex of the IA. C) Specimen with spines in IA and periproct covered by three supra-anal plates. D) Specimen with spines in IA and absence of an ambulacral area. Scale 10 mm.

lantern, five genital plates with their respective gonopores, and four AA and IA. Therefore, the external symmetry of this specimen cannot be defined as pentaradial or bilateral. These findings could suggest an adverse effect on the metamorphosis process of the specimens, during which pentaradial symmetry is established, although initially the anomaly does not seem to affect either its survival or gamete release.

The presence of spines in the IA has not been reported previously for this species, and there is no literature associating this feature with other Echinoidea. Larrain Prant (1975) described abnormal periprocts composed of three, five or more ossicles, emphasizing the rarity of this malformation. On the other hand, pentaradial symmetry predominates in Echinodermata, although there are evolutionary asymmetries, such as supernumerary arms in some Asteroidea, external radial symmetry in Holothuroidea or external bilateral symmetry in "irregular" Echinoidea (Hickman *et al.*, 2008; Benavides Serrato *et al.*, 2011; Borrero Pérez *et al.*, 2012). In other cases, external tetraradial symmetry is an atypical morphological feature, as seen in *A. dufresnii* (Echinoidea - Hylton Scott, 1939), *Ophiomyxa pentagona* (Ophiuroidea - Munar, 1984) *Echinaster sepositus*, *Astropecten jonstoni*, *A. aranciacus* and *Marthasterias gla-*

Table 1. Relative frequency (%) of test colorations in adult sea urchins *Arbacia dufresnii* in the population of Puerto Madryn, Golfo Nuevo, Chubut, Argentina (42°46’44’’ S, 64°59’52’’ W).

		Test	
	Pigmentation	Green (%)	Brown (%)
Spines	Brown (%)	76.67	8.52
	Green (%)	11.48	0.37
	Purple (%)	2.96	0.00

cialis (Asteroidea - Munar, 1984). The limited information on external malformations in Echinodermata complicates comparisons between cases (Hylton Scott, 1939; Munar, 1984; Savriama *et al.*, 2015).

The external malformations of the shell are caused by a variety of factors, such as pollution (Kroeker *et al.*, 2010; Clark, 2020; Han *et al.*, 2022). These malformations can serve as bioindicators of habitat quality and environmental health in marine ecosystems (Laitano *et al.*, 2013; Savriama *et al.*, 2015; Gouveia *et al.*, 2022). The Mar Argentino (Patagonia, Argentina), the habitat of *Arbacia dufresnii*, is subject to significant anthropic impact caused by tourism, fisheries, and pollution (Brogger *et al.*, 2013; Matteucci & Dadon, 2024). Reported chemical pollutants include heavy metals, microplastics, petrogenic hydrocarbons and others contaminants (Häder *et al.*, 2020; Ríos *et al.*, 2020; Gomez & Sturla Lompré *et al.*, 2024; González Pisani *et al.*, 2024). Despite the low frequency of occurrence of these external malformations (<2%), it is suggested to conduct monitoring to further study them, aiming to detect possible causal factors, analyze their biological effects on organisms and assess their potential as biomarkers of environmental stress.

ACKNOWLEDGEMENTS

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT 2018-01729) and Instituto de Conservación de la Ballena (Premio Australis - 2023). We extend special thanks to Téc. Mariano Moris, and Lic. Esteban Nicolás Sarrá for their technical support in the experimental aquarium (CCT CONICET-CENPAT), Téc. Norberto Andrés de Garín for his technical assistance in microscopy (CCT CONICET-CENPAT), to the Nautical Service at CENPAT-CONICET for their collaboration in sampling activities, and especially to “Kevin”, who made this work possible.

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Doi: 10.22179/REVMACN.27.874

Recibido: 13-VII-2024
Aceptado: 11-IV-2025