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On the oviposition and egg masses of *Conus regius* (Neogastropoda: Conidae) from northeastern Brazil

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Abstract: Characteristics of the egg masses of *Conus regius* Gmelin, 1791 are described and figured for the first time for the coast of Brazil based on a female specimen found in the process of oviposition during the day in the state of Bahia, northeastern Brazil. Two clusters of egg masses were found in the subtidal zone of Itapuã beach attached to rocky substrate in a completely unprotected site. Oviposition likely began at least one day earlier, since the specimen had already affixed an entire egg mass and was ovipositing a second cluster at the time it was found. The egg masses were arranged in short, irregular rows of three to nine closely spaced capsules in parallel and facing the same direction. One egg mass cluster consisted of 34 capsules. *Conus regius* capsules are semi-transparent, vasiform in side view, higher than broad and have flattened to slightly convex sides with slight wrinkles constituted by transversal ridges. *Conus regius* is on the IUCN Red List of Threatened Species, but is still intensively collected in the study area and surrounding coastal environment by fishermen for the purposes of selling shells and as a food source.

Keywords: *Gastropoda*, *Conoidea*, reproduction, ootheca, Bahia.

Sobre a oviposição e massas de ovos de *Conus regius* (Neogastropoda: Conidae) no nordeste do Brasil

Resumo: Características de massas de ovos de *Conus regius* Gmelin, 1791 são descritas e figuradas pela primeira vez para a costa do Brasil com base em um espécime encontrado em processo de oviposição, durante o dia, no estado da Bahia, nordeste do Brasil. Dois aglomerados de massa de ovos foram achadas na zona subtidal da praia de Itapuã fixa a substrato rochoso em uma área completamente desprotegida. O processo de oviposição, provavelmente, começou um dia antes, uma vez que o espécime já tinha afixado uma massa de ovos e estava depositando um segundo aglomerado de ovos no momento em que foi encontrado. As massas de ovos apresentaram arranjos em fileiras curtas e irregulares contendo três a nove cápsulas estreitamente espaçadas, paralelas e posicionadas na mesma direção. A primeira massa de ovos observada conteve um total de 34 cápsulas. As cápsulas de *Conus regius* são semitransparentes, vasiformes em vista lateral (mais altas do que largas) e possuem parte lateral suavemente convexa a achatada com rugas suaves constituídas por elevações transversais. *Conus regius* está na Lista Vermelha de Espécies Ameaçadas da IUCN, mas ainda é intensamente coletada na área de estudo e ambientes costeiros circunvizinhos por pescadores por conta da venda das conchas e como fonte alimentar.

Palavras-chave: *Gastropoda*, *Conoidea*, reprodução, ooteca, Bahia.

Introduction

Conus Linnaeus, 1758 is a highly diversified genus of predatory marine neogastropods, with approximately 770 recent species worldwide (Bouchet & Gofas 2015). They live predominantly in shallow tropical habitats (Kohn 1966, Penchaszadeh 1984, Olivera et al. 1990, Kohn

1998, Duda et al. 2001, Terlau & Olivera 2004, Peters et al. 2013, Flores-Garza et al. 2014). Species of *Conus* are among the most well-known mollusks due to the beautiful color patterns of the typically heavy, obconical or biconical shells (Terlau & Olivera 2004, Kohn 1998). These species are also particularly dangerous due to a harpoon-like radular tooth that injects neurotoxins into prey (Olivera et al. 1990,

Terlau & Olivera 2004, Braga et al. 2005, 2013, Haddad et al. 2006, 2009, Peters et al. 2013, Flores-Garza et al. 2014) and that can also injury and even death to humans (Olivera et al. 1990, Kohn 1998, Terlau & Olivera 2004, Haddad et al. 2006, 2009, Flores-Garza et al. 2014). There is economic importance in the extraction of compounds of medical and pharmacological interest (Olivera et al. 1990, Terlau & Olivera 2004, Braga et al. 2005, 2013, Gowd et al. 2005, Haddad et al. 2006, 2009, Peters et al. 2013) and as a food source for fishing communities on some Pacific islands (Terlau & Olivera 2004) and in northeastern Brazil.

Species of *Conus* that inhabit the intertidal to subtidal zones usually live in a heterogeneous habitat (Kohn 1959, 1998, Peters et al. 2013, Flores-Garza et al. 2014). They are quiescent when exposed to sunlight and typically seek shelter in shaded areas under algae, rocks and the sediment (Kohn 1998). Most species forage actively at night (Kohn 1998, Terlau & Olivera 2004, Flores-Garza et al. 2014) preying on a diversity of worms (e.g., polychaetes), hemichordates, echinurans, crustaceans, fishes and other mollusks (e.g., bivalves and gastropods) (Olivera et al. 1990, Kohn 1966, Cruz et al. 1978, Zehra & Perveen 1991, Kohn 1959, 1998, Duda et al. 2001, Terlau & Olivera 2004, Gowd et al. 2005, Haddad et al. 2006, 2009, Flores-Garza et al. 2014).

Conus are dioecious gastropods (Kohn 1961a, Flores-Garza et al. 2014). Reproductive aspects related to spawning, egg masses and larvae have been studied for a number of species worldwide (Lebour 1945, Ostergaard 1950, Natarajan 1957, Kohn 1961a,b, D'Asaro 1970a,b, Nybakken 1970, Bandel 1976, Cruz et al. 1978, Perron 1981a,b,c, Penchaszadeh 1984, Zehra & Perveen 1991). Females deposit dozens of large, usually flask-shaped egg capsules on or within different substrates (Perron 1981c, Zehra & Perveen 1991, Kohn 1998). Each egg capsule may contain a few to hundreds of embryos (Kohn 1998).

Conus fauna on the coast of Brazil includes about 20 species, five of which are endemic to the country (Rios 2009). *Conus regius* Gmelin, 1791 is a common, potentially dangerous and predominantly nocturnal species (Braga et al. 2005, 2013, Haddad et al. 2006, 2009, Rios 2009). It mainly lives on and under rocky, coral reefs and calcareous reef environments from Florida to Brazilian waters and actively feeds on worms, especially polychaetes (Bandel 1976, Braga et al. 2005, 2013, Lee 2009, Tunnell et al. 2010, Haddad et al. 2006, 2009, Rios 2009). This species may also be found half buried in the sand (Bandel 1976). Egg capsules from *C. regius* were succinctly described and poorly illustrated only by D'Asaro (1970a) and Bandel (1976) from south Florida – Bahamas and the caribbean sea of Colombia, respectively. *Conus regius* was once recognized as a very abundant shallow-water species, especially in northeastern Brazil (Haddad et al. 2009), but is currently on the IUCN Red List of Threatened Species (IUCN 2017).

To the best of our knowledge, so little information has been published about the reproductive biology of any Atlantic *Conus* species. Thus, the purpose of the study is to provide additional information on the oviposition and mainly egg capsules of *Conus regius* based on a specimen found in the subtidal zone in northeastern Brazil.

Material and Methods

1. Study Area

This study was conducted in the shallow subtidal zone of Itapuã beach (12°57'27"S, 38°21'21"W), which is located approximately 20 km from the city of Salvador in the state of Bahia, northeastern Brazil (Silva et al. 2009). The study site has a coastline of approximately 600 m (Silva et al. 2009) and reef platforms that are under the direct influence of wave impacts from the Atlantic Ocean (personal observation) (Figure 1).

The study area and surrounding coastal environment is a high-energy region characterized by a slightly steep subtidal zone composed of sandy, carbonate and rocky substrates as well as reef outcrops that are nearly exposed at low tide (Alves & Cerqueira 2000). The hard substrates undergo a strong weathering process in the region originating sediments composed of sand, quartz and carbonate (Bittencourt 1975). The environmental complexity of Itapuã and other beaches of Salvador offers a variety of micro-habitats and macro-habitats that favor the establishment of diverse communities of invertebrates (Alves & Cerqueira, 2000), especially mollusks, which constitute the second most diversified phylum in the world (Lima et al. 2017).

The area is under the influence of urbanized areas that input nutrients and domestic sewage. Moreover, fishing pressure on Itapuã beach has drastically reduced the populations of fishes, crustaceans and mollusks.

2. Field observations and laboratory procedures

This paper is based on observations of the oviposition and egg capsules of a specimen of *Conus regius* on 12 December 2016 at low tide in the subtidal zone of Itapuã beach. The female in oviposition was observed for approximately 25 minutes, was not handled during the oviposition process and was not collected to be deposited in a scientific collection. This species is well known in the literature and is threatened with extinction, especially at the study site, where it is captured for the purpose of selling the shell and as a food source. The rocky substrate with the first attached egg mass was manipulated for approximately four minutes to photograph it out of the water and for the removal of a few egg capsules. The egg capsules removed were preserved in 70% ethanol. The capsules were measured in the laboratory using the three dimensions described by Kohn (1961a) as well as an additional measure: ea – length of escape aperture, hp – height

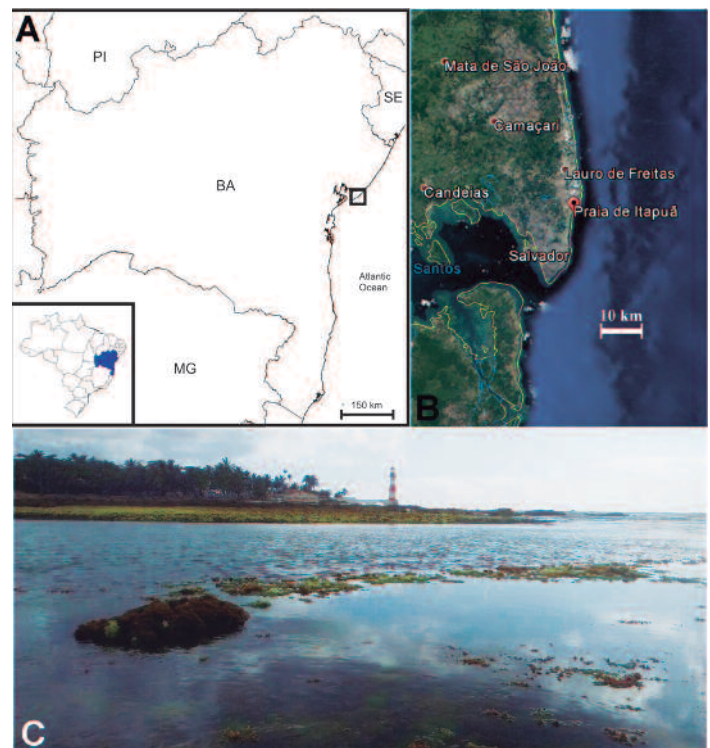


Figure 1. (A) Map of the Brazil (small square) highlighting the state of Bahia and the state of Bahia (large square) highlighting the location of the Itapuã beach (northeastern Brazil); (B) Map of stretch of the coast of Bahia showing the location of the Itapuã beach; (C) Photo of the area of observation and collection of spawning of *Conus regius* at Itapuã beach. Abbreviations: SE — Sergipe, BA — Bahia and MG — Minas Gerais.

of peduncle, mh – maximum height, and mw – maximum width. The total number of capsules in the egg mass was counted during manipulation of the substrate. Illustrations were also prepared from drawings.

The specimen of *Conus regius* shown here (Figure 2A) is deposited in the Coleção Zoológica do Laboratório de Zoologia, Universidade Federal de Campina Grande (UFCG_MOL 01), Centro de Formação de Professores, Cajazeiras, Paraíba, Brazil. The capsules are housed in the malacological collections of the Laboratório de Invertebrados Paulo Young, Universidade Federal da Paraíba (UFPB MOLL 3629: 03 capsules), João Pessoa, Paraíba;

Museu de Zoologia, Universidade de São Paulo (MZSP 132042: 04 capsules), São Paulo, Brazil and UFCG_MOL 02: 06 capsules.

Results

1. Oviposition

A specimen of *Conus regius* (shell measuring approximately 54 mm in total length) was found ovipositing during sunny daylight (approximately 8:50 a.m.) in the subtidal zone of Itapua beach (Figure 2). It was found

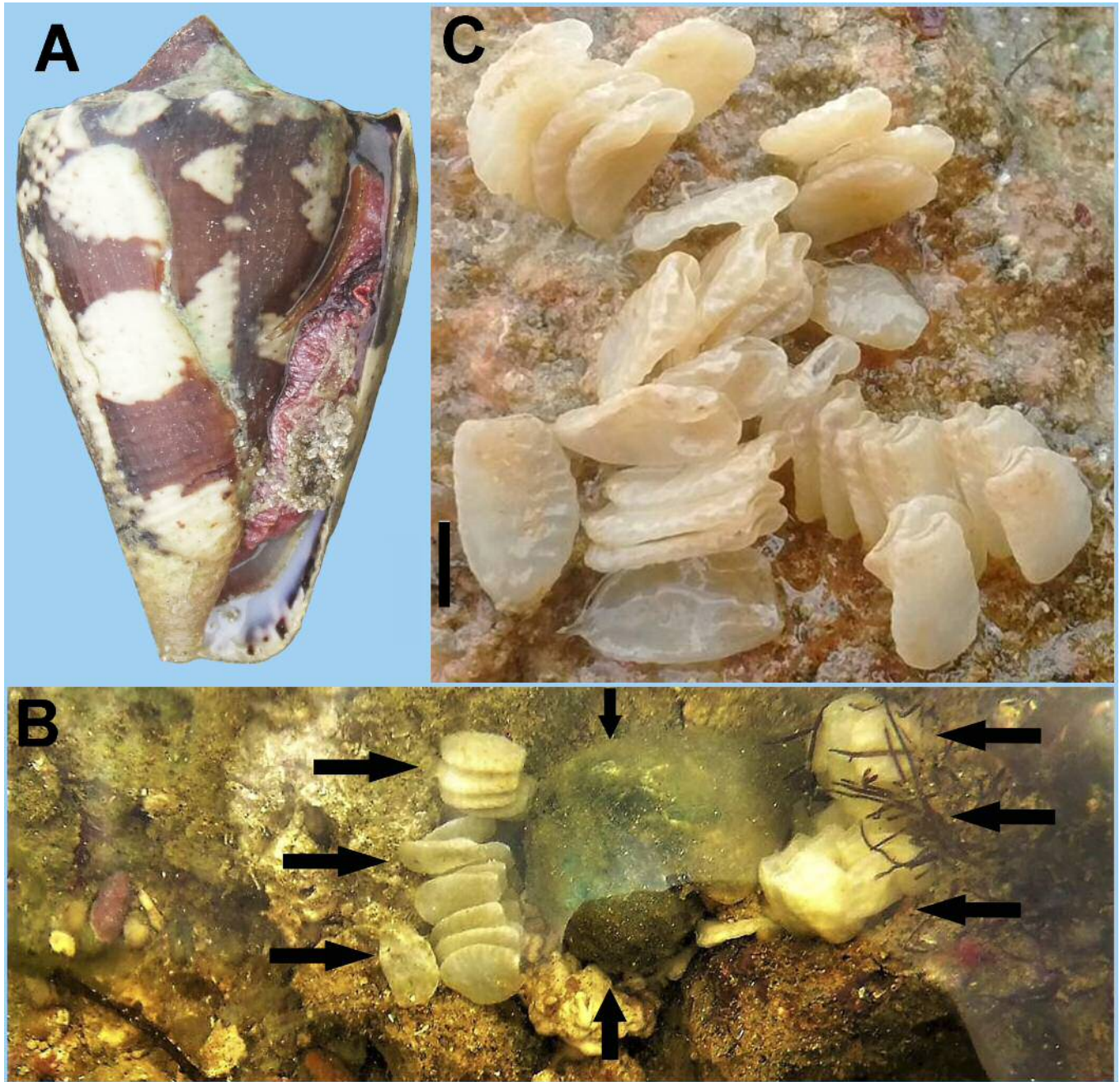


Figure 2. *Conus regius* from coast of Bahia: (A) Specimen collected from Morro de São (state of Bahia, northeastern Brazil – UFCG_MOL 01: 54 mm); (B) Female specimen in oviposition process on Itapua beach (vertical arrows pointing to specimen; left horizontal arrows pointing to first egg mass; right horizontal arrows pointing to second egg mass being deposited); (C) Clusters of egg masses photographed out of water (same as Figure B indicated by left horizontal arrows; scale bar of nearest ootheca: 5 mm).

in a completely unprotected site with a mixed substrate of gravel, sand and small to medium-sized pieces of rocks at depth of approximately 30 cm. The oviposition process had likely begun at least one day earlier, since the specimen had already affixed an entire egg mass cluster to the substrate (Figure 2B – left horizontal arrows). It was observed ovipositing the second egg mass cluster at the time it was found (Figure 2B – vertical and right horizontal arrows). The second cluster was being affixed to the rocky substrate, which was partially covered with macroalgae filaments. No other spawn was found on the surrounding substrates. The specimen remained relatively motionless during the oviposition process, which was observed for approximately 25 minutes. It was not possible to photograph in detail and observe the entire extrusion process of the second egg mass cluster due to the rising tide.

2. Egg masses and capsules

Two clusters of egg masses of *Conus regius* were found on the substrate (Figure 2B – left and right horizontal arrows). One cluster had likely been produced by the specimen (Figure 2B – left horizontal arrows) and another was being extruded and affixed to the substrate at the time it was found (Figure 2B – horizontal and right horizontal arrows). Both clusters were well separated from each other (Figure 2) and arranged in short, irregular rows of three to nine closely spaced egg capsules exhibiting the same pattern (one alongside the other) and oriented in parallel facing the same direction, all attached at the underside of the piece (Figure 2C). In each cluster, immediately adjacent groups of rows of egg capsules were situated at various angles (Figure 2C). The first egg mass cluster consisted of about 34 capsules (Figure 2B – left horizontal arrows) and was carefully removed from the water to be better studied and photographed (Figure 2C). Most of the capsules were empty (without embryos) in this cluster (Figure 2C). The second egg mass cluster in oviposition contained about 18 capsules (Figure 2B – vertical and right horizontal arrows). This cluster under construction was not handled or studied in detail so as not to interrupt the oviposition process. A minimum of 52 egg capsules in both egg masses were likely laid by the female. The clusters of egg masses were affixed to the substrate without any overlapping or confluence of the pieces of the capsules.

Conus regius has thin, semi-transparent, vasiform (side outline) capsules (Figure 2C). Such capsules are much higher (mh – about 12 to 14 mm) than broad (mw – about 7 to 8.5 mm) (Table 1) with flattened to slightly convex sides that are slightly and unevenly wrinkled by few, low and rather well-spaced transversal ridges running from apical region to peduncle (Figures 2C and 3). Capsules with typically convex lateral edges and ridges (Figures 2C and 3). Each capsule rises vertically from a short stalk with slight skew to one side (Figures 2C and 3). Capsules composed of two equal membranous halves along axis interrupted only by escape aperture (Figures 2C and 3). Capsular apex usually sigmoid shaped (Figures 2C and 3). Escape aperture very narrow and elongated (slit shaped: ea – about 3.9 to 5.5 mm), slightly concave extending along capsular apex (Figures 2C and 3). Peduncles short (hp – about 2 to 3 mm) (Table 1), narrow to enlarge, usually positioned in central part of capsular base (Figures 2C and 3). Basal membranes discrete and fused between peduncles (Figures 2C and 3). Empty capsules of opaque white (semi-transparent) color. Full capsules (with embryos) are light pinkish brown. Most of the capsules in the egg masses photographed were apparently empty (Figure 2C).

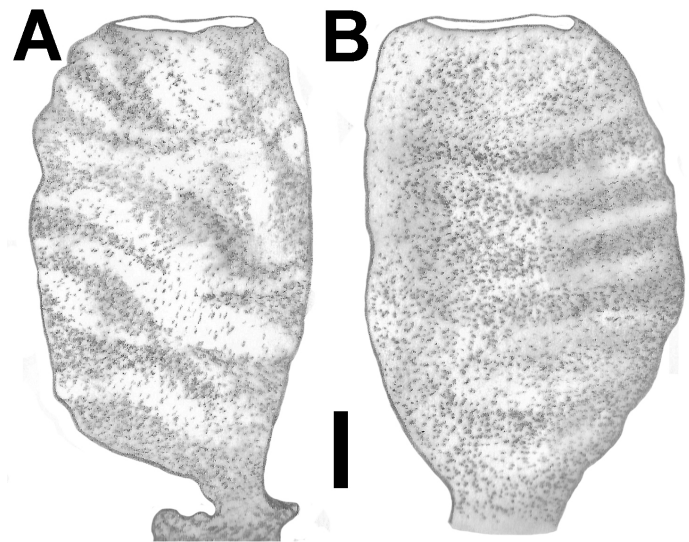


Figure 3. Two egg capsules of *Conus regius* from the coast of Bahia: **A.** Ootheca well wrinkled with narrow peduncle positioned in subcentral region of capsular base and part of basal membrane; **B.** Ootheca moderately wrinkled with wide peduncle positioned in central region of capsular base. Scale bar: 2 mm.

Table 1. Linear measurements (mm) of capsules of *Conus regius* found in the subtidal zone of Itapuã beach.

	<i>Conus regius</i>			
	N	R	M	SD
ea	11	3.90–5.50	4.62	0.56
hp	10	2.00–3.00	2.40	0.49
mh	11	12.0–14.0	12.7	0.75
mw	12	7.00–8.50	7.89	0.41

Discussion

Conus regius is one of the about 10 conid species widely distributed in the western Atlantic (Rosenberg 2009) and is found in a considerable variety of coastal habitats on rocks (Diaz & Puyana 1994), rocky reefs (Warmke & Abbott 1962) or similar reef structures (Abbott 1974), calcareous environments (Tunnell et al. 2010) and sediments as well as under rocks (Bandel 1976, Redfern 2013), gravel and calcareous algae bottoms (Rios 1994) associated with coral reefs (Bandel 1976), coral fragments (D'Asaro 1970a), dead corals and cavities under semispherical coral colonies, sea-grass bottoms or even buried in the sand (Bandel 1976). This species typically lives in these habitats on the coast of northeastern Brazil (Haddad et al. 2009), even on substrates exposed to the atmosphere at low tide. The coast of the state of Bahia, including the Abrolhos Archipelago is recognized as an area of important diversity of *Conus* due to the considerable environmental complexity of the coastline and islands, which results in a large variety of marine habitats (Coltro 2004). However, *C. regius* and congeners are currently rather rare in the habitats of Itapuã beach and adjacent areas of the coast due to environmental degradation and the intensive collection by fishermen for the sale of shells and as a food source.

Egg masses of *Conus regius* have been rarely studied in the western Atlantic (see D'Asaro 1970a: 434–435, fig. 9F–H, Bandel 1976: 184–185, fig. 9). In previous studies, egg capsules of the species was found only on coral fragments in Florida (D'Asaro 1970a) as well as under *Acropora* fragments and hollow hemispherical corals in Colombia and Curaçao (Bandel 1976). Unlike these studies, the specimen observed herein was

in an unprotected site with a mixed substratum of gravel, sand and rocks (Figure 2B). Geraldo Oliveira has studied mollusks on the coast of Bahia (especially Itapuã beach) for approximately 40 years and this is the first time that the researcher has found a female specimen in oviposition and egg masses in the region.

For *Conus regius*, D'Asaro (1970a: fig. 9F–H) and Bandel (1976: fig. 9) also reported the arrangement in closely spaced rows of egg capsules and figured them with essentially the identical shape to those studied herein (Figures 2C and 3). Bandel (1976) also found an arrangement in the number of capsules (four to ten in rows) similar to that of the present study. However, the capsules examined herein had a more conspicuous and frequent pattern of transversal wrinkles (Figures 2C and 3). Additionally, the egg capsules in this study (mh – about 12 to 14 mm) have intermediate dimensions to those analyzed by Bandel (1976: mh – 10 to 11 mm) and described by D'Asaro (1970: mh – 15 to 16.5 mm) for a specimen of *C. regius* from Florida.

Among the species of the western Atlantic, *Conus regius* has an capsule morphology somewhat similar to those of *C. ermineus* Bom, 1778 (see Bandel 1976: fig. 12A–B; Penchaszadeh 1985: fig. 4) and *C. mus* Hwass, 1792 (see Lebour 1945: fig. 31a), especially in the outline, conspicuous pattern of transversal wrinkles and elongated, narrow escape aperture. On the other hand, the egg capsules of *C. regius* differ in shape from those previously observed for *Conasprella puncticulata* (Hwass in Bruguière, 1792) (see Bandel 1976: fig. 10), *C. stearnsii* (Conrad, 1869) (see D'Asaro 1986: fig. 4D–F), *Conus anabathrum* Crosse, 1865 (see D'Asaro 1986: fig. 4A–C), *C. largillierii* Kiener, 1847 (see Bandel 1976: fig. 11) and *C. spurius* Gmelin, 1791 (see Penchaszadeh 1985: fig. 1A–D), especially in the rather regular outline and narrower escape aperture and peduncle.

The coastal ecosystems of Brazil exhibit an important biodiversity of Conidae (Coltro 2004, Rios 2009) that has been insufficiently studied, especially with regard to reproductive aspects. The conid fauna and the entire coastal invertebrate community have been drastically affected by multiple anthropogenic impacts (Migotto & Marques 2006, Peters et al. 2013). The destruction and pollution of habitats constitute the most serious global threat to marine biodiversity (Gomes et al. 2000, Amaral & Jablonski 2005, Migotto & Marques 2006, Peters et al. 2013). In particular, populations of Conidae have undergone considerable decline in recent times, with a number of species currently on the IUCN Red List of Threatened Species (IUCN 2017). In 2011, the IUCN assembled a committee to assess the threat levels of more than 600 species of *Conus*, with the results in the paper prepared by Peters et al. (2013) (Dr. Alan J. Kohn, personal communication, august 2017). The IUCN committee voted not to deem *Conus regius* as “threatened”, but rather with status “Least Concern” (IUCN 2017). Conids have undergone population reduction especially in the study site due to intensive collection by fishermen for the purposes of selling the shells and as a food source. To reverse the decline of such populations, local governments need to develop conservation strategies and act more effectively to impede the capture of threatened mollusks, especially conids that are recognized as having economic importance in terms of a food source and the extraction of compounds of medical and pharmacological interest.

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