

The risk of the nudibranch *Baeolidia rieae* (Mollusca: Gastropoda: Heterobranchia) in the international aquarium trade

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Abstract: *Baeolidia rieae* Carmona, Pola, Gosliner & Cervera, 2014 was described based on a single specimen from the subtropical Amami-Oshima Island (SW Japan). In this study, we recorded *B. rieae* feeding on a zoantharian, *Zoanthus* sp., in an aquarium store in Barcelona (Spain). The morphological and molecular match from the original description corroborated its identification. We discuss the possible origin of the *B. rieae* in aquarium stores, its resistance to ordinary disinfection processes, the harm it may cause to commercial coral species, and how it might develop into an invasive species if it continues to be found in aquarium stores around the world without treating the problem properly. Finally, we describe for the first time the spawn of *B. rieae* and its eggs features, and provide new morphological and biological data.

Résumé : *Risque associé au nudibranche Baeolidia rueae (Mollusca : Gastropoda : Heterobranchia) via le commerce international d'aquariophilie. Baeolidia rieae* Carmona, Pola, Gosliner & Cervera, 2014 a été décrite avec un seul spécimen de l'île subtropicale d'Amami-Oshima (sud-ouest du Japon). Dans cette étude, nous signalons *B. rieae* se nourrissant du zoanthaire *Zoanthus* sp. dans un magasin d'aquarium à Barcelone (Espagne). La correspondance morphologique et moléculaire de la description originale a corroboré son identification. Nous discutons de l'origine du *B. rieae* dans les aquariums, de sa résistance aux processus de désinfection ordinaires, des dommages qu'il cause aux espèces de corail commerciales et de la façon dont il pourrait se développer en une espèce envahissante s'il continue à être trouvé dans les magasins d'aquarium du monde entier sans traiter correctement le problème. Enfin, nous décrivons pour la première fois la ponte de *B. rieae* et les caractéristiques de ses œufs, et fournissons de nouvelles données morphologiques.

Keywords: Commercial interest • Aquarium hobby • Allochthonous species • Invasive species • Nudibranch

Introduction

Since the 1990s, saltwater aquarium keeping has increased in popularity as hobby among aquarists (Lecchini et al., 2006; Williams et al., 2012). The advances in breeding marine organisms in captivity under controlled conditions and the development of new and better technologies for aquariums have facilitated the fondness for home aquariums (Reynoso et al., 2012). Exotic marine plants and animals are prized for their beauty, making the ornamental trade lucrative, creating a scenario where people pay high amounts of money to get specimens of the most beautiful species, principally exotic fish species, crustaceans, mollusks and corals. Therefore, for some of them the international trade needs to be regulated to prevent their extinction. The commercial interest for certain species results in circumstances where the value of an ornamental marine fish species can overpass 80 times the value it would have in fisheries (Williams et al., 2012). International ornamental and aquarium coral fisheries involve the annual trade of hundreds of thousands of small coral pieces worth millions of dollars (Pratchett et al., 2020).

Apart of other products like aquariums, air pumps, food, medications and other supplies, the primary product of the aquarium industry is fish, although the world market is limited in the diversity of collected species (Lecchini et al., 2006). Aquariums containing living corals started to become popular since the late 1980s (Delbeek, 2001). In the last decades, the growing popularity of reef aquaria has increased the types and quantity of species in trade, in the period 2007 and 2016, the world coral trade totaled just over 19 million kg (Pavitt et al., 2021). Nowadays, hundreds of coral species and other invertebrates are exported for aquarium markets, an increasing portion of the coral sold comes from aquaculture, but the majority is still collected from the wild (Pratchett et al., 2020). Over 90% of coral trading occurred in Indonesia where there are about 2.500.000 hectares of coral reefs (Hadi et al., 2020) and the culture and trade of soft corals is done in a sustainable way (Rhyne et al., 2014).

The trade of live marine animals between countries promotes the movement of organisms around the world and has been considered as an important vector of non-indigenous species entry, with a serious potential of becoming invasive species (Morrysey et al., 2011; Maceda-Veiga et al., 2013). It is reported that freshwater ecosystems have been receptors for many commercial exotic species for both involuntary and deliberate introductions so, in general, the aquarium trade has been responsible for the

establishment of many fish, plant and mollusk species into areas where they are not native (Duggan, 2010). Every non-indigenous species can cause changes in ecosystems and as marine invasive they can become a major threat to marine coastal habitats (Mantelatto et al., 2018).

The nudibranchs that feed on zoantharians (zoa-eating nudibranchs) have been known to the aquarium community for several decades and are considered a pest to *Zoanthus* species in tropical marine aquariums. Several individuals of the West Pacific aeolid nudibranch *Baeolidia rieae* Carmona, Pola, Gosliner & Cervera, 2014 were found four times feeding on specimens of *Zoanthus* Lamarck, 1801, a commercial zoantharian genus, in the aquarium store Urban Natura (Naturalis Insignia SL) and once again in a private aquarium, both in Barcelona (Spain). In this study we provide new morphological data about this species and a first description of its spawn. We hypothesized the possibility of *B. rieae* eggs to travel with live zoantharians and survive to disinfestation processes to finally hatch once they reached maturity in the aquarium store under optimal conditions. We discuss the importance of the presence of this supposed Japanese species associated to commercial zoantharian species, how they could cause an important economic impact for consumers and stores, and how they could develop to an invasive species if the problem is not tackled correctly.

Materials and Methods

Morphology characters

A total of twenty-one specimens of *Baeolidia rieae* have been found feeding on the zoantharian *Zoanthus* sp. in tropical aquariums in the city of Barcelona in four independent findings from Urban Natural aquarium store and one specimen found in a private tropical aquarium (Supplementary Material, Table S1). For host identification, we used the works of Reimer et al. (2014 & 2015) covering the Indo-West Pacific, which is the most likely area of origin for the hosts and the snails. We collected and transferred them to the laboratory where they were kept alive by using small aquariums with air diffusers. All specimens were measured alive, photographed and recorded with an Olympus XZ1 compact camera and with a Bresser Mikrokular full HD camera. The specimens were observed *in vivo* with a binocular loupe and their morphological characteristics were recorded in detail (shape, coloration, cerata,

rhinophores, oral palps, digestive gland, and foot) before preserving in 96% alcohol.

To analyse the radula structure, three specimens of 8 mm were dissected. Buccal bulb and jaws were carefully removed and photographed with the binocular loupe. The buccal bulb was immersed in a 7% KOH solution for twelve hours, digesting the organic part of the bulb, leaving only the radula and the jaws. After carefully cleaning and drying the radula, it was mounted altogether with the jaws on a disk with double-sided tape and taken to the University of Barcelona Scientific-Technical Services (CCiTUB) where it was coated with a thin layer of gold to observe it in the scanning electron microscope (SEM).

Specimens collected on June 21, 2017 laid several spawns when kept in small aquariums in the laboratory. Spawns were observed, photographed and drawn under an optic microscope. The width of the spawn cord was measured and its total length was calculated. Additionally, the length of some eggs was measured. The total number of eggs in the spawn was calculated by counting the eggs from a spawn area of 500 μm^2 .

Molecular analyses

Briefly, a piece of 3 mm^2 of foot of one 8 mm specimen was selected for DNA extraction. Afterwards, the Cytochrome Oxidase Subunit I (COI), Histone 3 (H3) and ARN ribosomal 16S (16S) markers were amplified using the polymerase chain reaction (PCR). Once cleaned, samples were sent for sequencing to the CCiTUB (Supplementary Material).

DNA sequences were assembled, followed by edition and trimming. BLAST was carried out to perform a rapid assessment of the taxonomic genus to which the animals collected belonged to. To delimit putative molecular species of the BLASTed genus, the ASAP software was used taking the COI sequences from the *Baeolidia* species extracted from Genbank (Supplementary Material Table S2).

Finally, Bayesian Inference and Maximum Likelihood analyses were performed for a dataset concatenating the COI, 16S and H3 *Baeolidia* sequences extracted from Genbank (Supplementary Material).

Results

Baeolidia rieae Carmona, Pola, Gosliner & Cervera, 2014

Type locality and habitat

Japan, Amami-Ohoshima Island found in 7 m depth of water close to green zoantharians.

Type material

Holotype: CASIZ 184525, one specimen, dissected, 3 mm in length preserved, Japan, Amami-Ohoshima Island, collected by Rie Nakano, 06 March 2010. *Baeolidia* sp. B: Carmona et al., 2013.

Material examined

On June 21, 2017, five specimens found in Urban Natura aquarium store in Barcelona (Spain), with lengths of 5-9 mm; an 8-mm specimen was dissected for anatomy and for DNA extraction. On July 27, 2017, nine specimens found in Urban Natura aquarium store in Barcelona (Spain), with lengths of 2-10 mm; an 8-mm specimen was dissected for anatomy. On September 28, 2017, four specimens found in Urban Natura aquarium store in Barcelona (Spain), with lengths of 5-8 mm. On February 2, 2018 two specimens found in Urban Natura aquarium store in Barcelona (Spain) with lengths of 8 and 11 mm; the 8-mm specimen was dissected for anatomy. On March 29, 2019, one specimen from a private tropical aquarium in the city of Barcelona, length 8 mm. It has been impossible to determine the geographical origin of the zoantharian *Zoanthus* prey on which the nudibranchs fed in the aquariums.

External morphology (Fig. 1)

Relatively small specimens, between 3 and 11 mm. Short and wide body narrowing towards the tail. Translucent white-yellowish color, with brownish-green spots covering the whole body and some white spots above the notum. The foot, protruding little from the body, has rounded edges and a brownish-greenish reticule (Fig. 1A & B). The oral tentacles are short. The rhinophores have the same color as the body, are short, smooth on the front, with up to 8-10 tubercles on the back and side, all uneven and irregularly arranged (Fig. 1C & D). The specimens have large and remarkable cerata, of elongated foliaceous shape, narrow at the base and slightly curved inwards. The green colored cerata might be related to pigments found in the green zoantharian *Zoanthus* sp. in on which they were feeding (Fig. 1E & F). The cerata tip is mucron-shaped and translucent with pearly white pigmentation at the apex. The digestive gland within the cerata has short and irregular ramifications. There are between 4 and 5 ceratas groups along the body. In each group there are between 3 and 6 ceratas which decrease in size as they approach the tail. The first two groups are well separated from the following. Specimens kept alive in the aquarium without the *Zoanthus* specimens losing

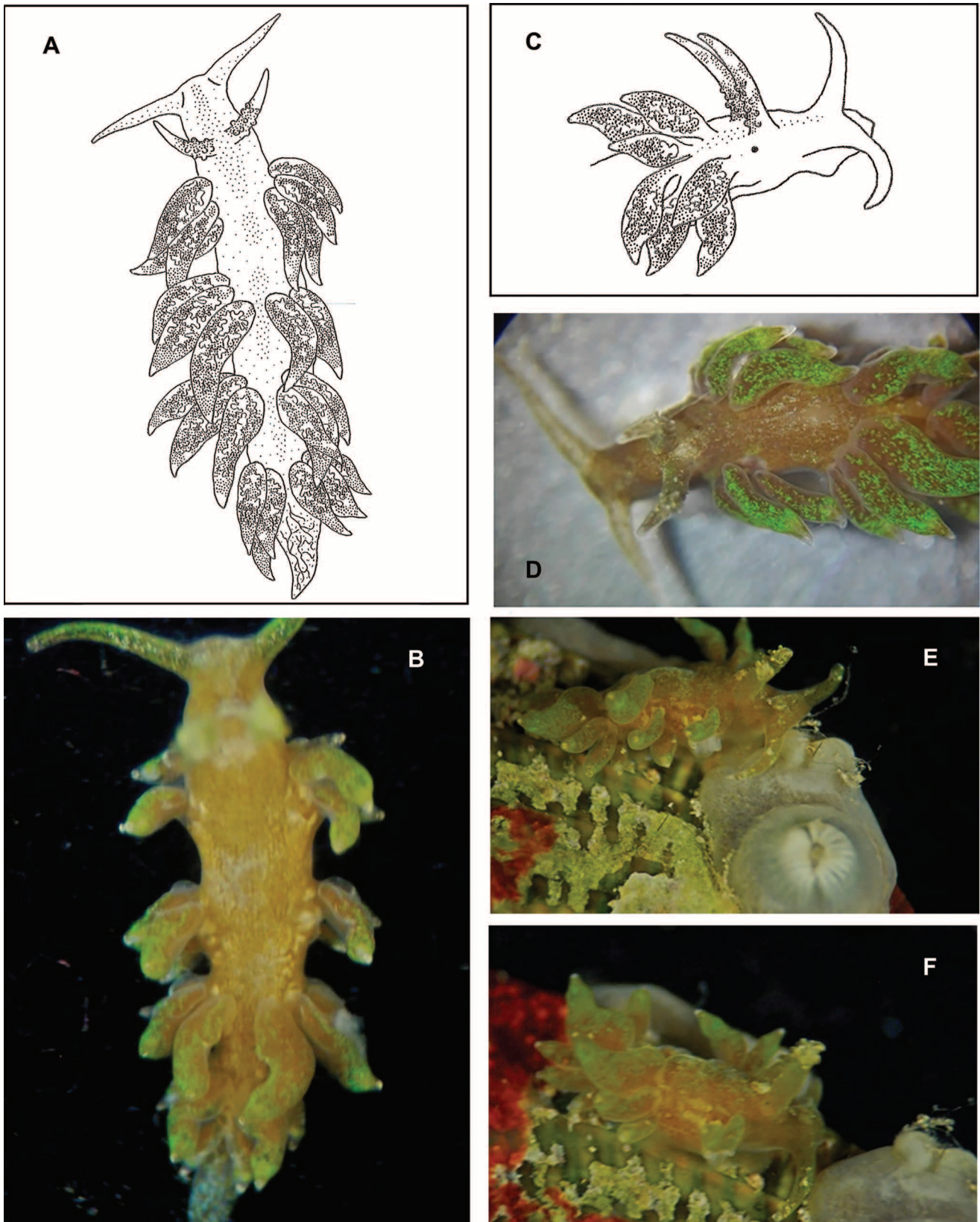


Figure 1. *Baeolidia rieae*. **A.** Drawing of a 9-mm specimen. **B.** Image of a live 5-mm specimen. **C.** Drawing of details of head, rinophores and ceratas. **D.** Image of the head and cerata. **E & F.** Photographs of two specimens feeding on a green zoantharian.

their green and brown tones and turning whitish in a couple of days. Once preserved, the cerata lose their green color but maintain a clearly foliaceous appearance with a narrow apex (Fig. 2A1 & A2).

Anatomy (Figs 2 & 3)

The buccal bulb extracted from an 8-mm specimen measured 1.3 x 1 mm and has the appearance of a small bivalve mollusk (Fig. 2B1 & B2). When the oral bulb was removed, the cerebral ganglia was observed just above it and the eyes above the cerebral ganglia. The jaws are slightly yellowish and almost completely cover the oral bulb (Fig. 2C1 & C2). The radula extracted from one of the 8 mm specimen measured about 700 µm and had a radular formula of 25 x 0.1.0. The radula of other two 8 mm specimens had a radular formula of 18 x 0.1.0 and 20 x 0.1.0 (Fig. 3A & B). The monoseriate radula has up to 25 pectinate teeth. The teeth have between 42 and 45 elongated and pointed denticles on each side of the central cusp (Fig. 3D). The denticles near the central cusp are shorter than the rest, growing on both sides. The teeth decrease in size and number of denticles as they approach the posterior end of the radula.

Egg-mass morphology (Fig. 4)

The spawn is a narrow cord wound around 5 rounds with a transparent matrix and abundant small eggs (Fig. 4A & B). Because it is coiled and could break, it could not be measured exactly, but it was seen that it stretched approximately 20 mm long and 0.5 mm wide. The eggs are whitish in color and are very tight together. Up to 36 eggs could be counted in an area of ~ 500 µm² (Fig. 4C), thus estimating a quantity of 1440 eggs in the entire cord. These eggs have a rather oval shape and, at their longest diameter, measure between 90 and 120 µm (Fig. 4D).

Geographical distribution

To date, only known from the (sub)tropical coasts of Japan. After the description it has only been recorded in the same Amami-Ooshima Islands, Japan and has been photographed by Jun Imamoto.

Molecular analyses

We obtained sequences of 710 pb for COI, 473 pb for 16S and 347 pb H3.

Our BLAST matched our COI, with values ranging from 78.55 to 95.74%, 16S (from 89.93 to 100%) and H3 (from 96.65 to 100%) to those of the genus *Baeolidia* being the highest values those regarding to

B. rieae Carmona et al., 2014 (COI = 95.74%, 16S = 100%, H3 = 100%).

The ASAP analysis performed with the COI gene distributed the genus *Baeolidia* in nine putative species, gathering the animals found in the aquariums to *B. rieae* (p-value = 0.152) (Supplementary Material, Table S3). Finally, the phylogenetic tree obtained with both Bayesian and Likelihood inferences also gathers the individual found in the Barcelona aquarium to *B. rieae*, with a node value of PP = 1, BS = 100, and separated from its sibling clade with a PP = 1 and BS = 98 (Fig. 5).

Discussion

Zoantharians (Cnidaria: Anthozoa: Hexacorallia) are a group of marine benthic cnidarians that play an important ecological role in many marine ecosystems. Some zoantharians are important rock-encrusting anthozoans which are commonly used in marine aquariums since they are easy to maintain, thus being highly recommended for beginner aquarists (Panné Huidobro, 2012). They also have a wide colour range that makes them interesting for marine aquarium keepers. These characteristics make Zoanthidea order one of the most appreciated and commonly commercialized taxa in marine aquariums.

Nudibranchs are more commonly known as predators that feed over species of sponges, ascidians, bryozoans and cnidarians with some preferences or specialization to their prey (McDonald & Nybakken, 1997). Most aeolid nudibranchs feed on hydrozoan or anthozoan cnidarians and store the stinging nematocysts from the cnidocytes in sacs at the tips of their cerata (Coll et al., 1985). However, there are many aeolid nudibranchs that feed on Anthozoa and have a symbiotic relationship with the zooxanthellae algae obtained from their prey (Marin & Ros, 1991).

As also occurs in other groups of Anthozoa, zoantharians such as *Zoanthus* spp. and *Palythoa* spp., establish symbiotic relationships with zooxanthellae (Kamezaki et al., 2013) of the *Cladocopium* genus (LaJeneusse et al., 2018; Wee et al., 2019). Aeolid nudibranchs can keep zooxanthellae coming from its diet inside their glandular digestive cells which confers the same color of the prey, providing a great camouflage advantage. Additionally, zooxanthellae may enhance sea slugs the ability to survive long periods of food shortage by taking profit from the endosymbiotic zooxanthellae (Burghardt et al., 2005). Here we report for the first time the symbiosis relationship between the nudibranch

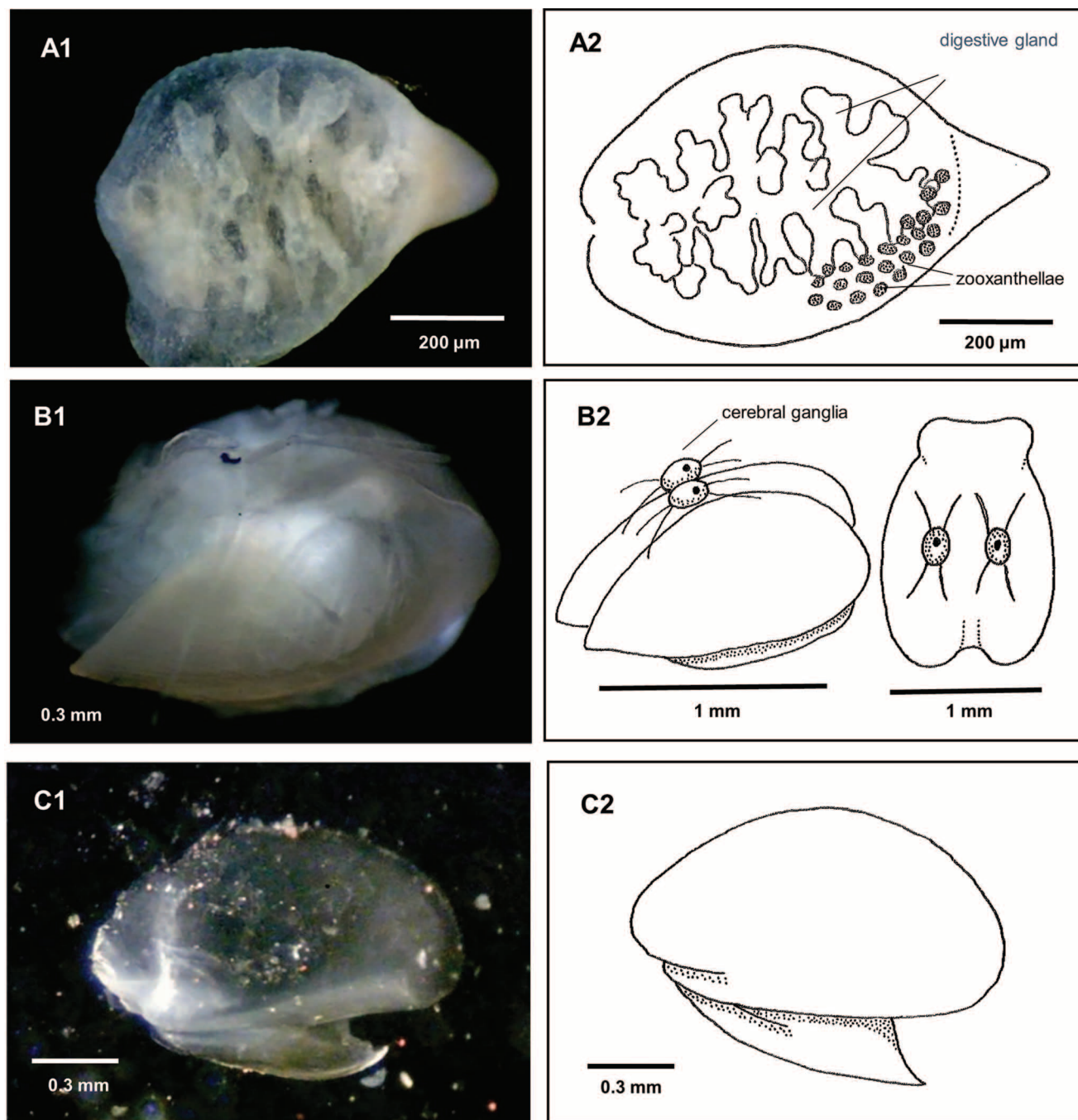


Figure 2. *Baeolidia rieae*. **A1 & A2.** Details of a conserved cerata observed with optical microscope; groups of zooxanthellae can be seen in the cerata of preserved specimens. **B1 & B2.** Bucal bulb observed with optical microscope. **C1 & C2.** Jaws dissected with optical microscope.

Baeolidia rieae and zooxanthellae algae coming from their prey, the green *Zoanthus* species, being this eating behavior a problem in aquaculture companies and aquarium stores. *Baeolidia rieae* can be particularly harmful in reef aquariums, damaging the polyps of *Zoanthus* colonies when feeding, taking

down partial or entire pieces of the *Zoanthus* prey in a few days. This fact produces the appearance of this nudibranch in saltwater aquariums to be considered as a pest due to its rapid reproduction rate, since it was observed how in a few days laid their spawns.

Corals are very sensitive organisms that have

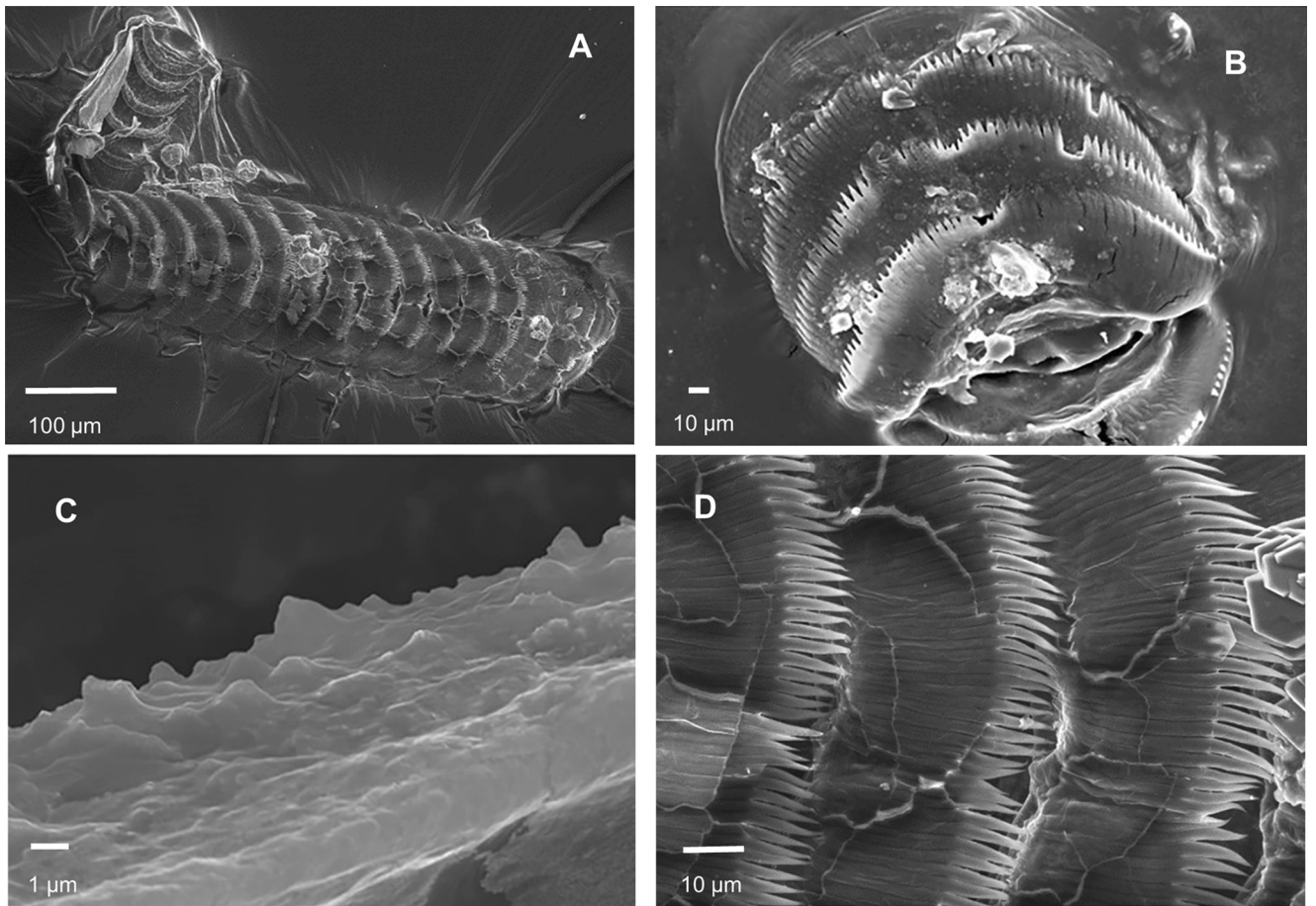


Figure 3. *Baeolidia rieae*. SEM images of the radulae and jaws. **A.** View of entire radula. **B.** View of the proximal part of the radula. **C.** Masticatory border of the jaw. **D.** Detail of tooth radulae and denticles.

become increasingly plagued with diseases in both wild and captive populations. The three most common coral afflictions observed in aquaria are color changes, rapid tissue necrosis and brown jelly infections (George, 2011). In addition of bacteria, fungi and protozoan, predators such as fish, gastropods or other invertebrates cause physical lesions. Treating coral before it enters to an aquarium is a good precaution to reduce or avoid the risk of bacterial infection and help to eliminate parasites and unwanted hitchhikers that may possibly devastate the entire stock (Bartlett, 2013). A product commonly used among aquarist is REEF DIP™ which is an iodine-based treatment. This product has been demonstrated to be effective against bacteria, fungi and protozoa and highly recommended to treat *Montipora*-eating nudibranch pests despite the fact that it has never been proved (Bartlett, 2013). Thus, the usage of this type of disinfectants in each new piece of coral incorporated into the aquarium store is not a guarantee to eliminate them, being possible to find several nudibranchs attached to the

base of the coral, feeding and damaging its polyps in the next two or three days after the coral implant. Due to the small size of *B. rieae* and its greenish color they have a great capacity to camouflage and can go unnoticed. It is well known that the major of mollusk egg masses are quite protected with chemical defenses as antimicrobial compounds, are resistant against low salinity and can persist in front of UVR light (Przeslawski et al., 2004; Benkendorff et al., 2011). Collected specimens laid their spawn when felt under stress in laboratory. The deposition of egg masses by nudibranchs is thought to be a stress response (Benkendorff et al., 2011) to ensure the survival of their progeny. This behavior hints that nudibranchs, when either taken away from the coral farm, transported or treated with disinfectants (under stress), lay their spawn in the pieces of coral, which then hatch when installed at the aquarium store under favorable conditions. It is not the first time that nudibranchs have been observed as parasites in the aquarium world even when corals have been treated with some iodine-

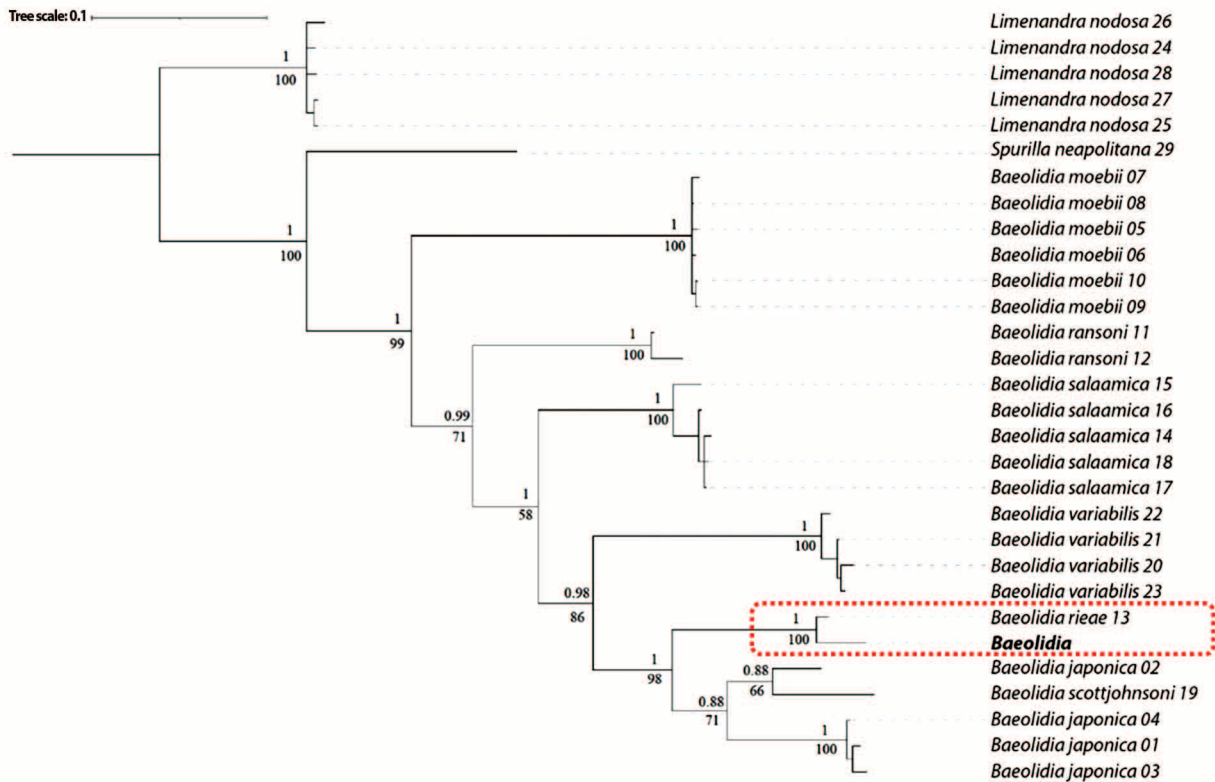


Figure 5. *Baeolidia rieae*. Phylogenetic tree obtained with COI + 16S + H3 markers. Represented on the upper side of the nodes is the PP values and on the lower side the BS values.

based product. The nudibranch *Phestilla subodiosa* Wang et al., 2020 was found feeding on a *Montipora* hard coral, which are also economically important in the aquarium industry (Mehrotra et al., 2020; Wang et al., 2020). Thus, some treatment must be sought to avoid nudibranch pests and keep corals alive avoiding large economical losses.

Although there are not many studies on alternative compounds to use as effective disinfectants, it has been proposed the use of molluscicides (Borneman, 2007). There are other compounds such as copper, aluminum, potassium permanganate or acetogenins derived from plants that are used against freshwater and terrestrial adult and egg masses snails (Singh & Singh, 2004) which may present similarities to marine organisms. Borneman (2007) found that with the application of potassium permanganate all pest aeolid nudibranchs and their egg masses were eliminated. So, further studies may be performed to postulate these elements as toxics when used in marine aquariums and corals farms.

An alternative to chemicals and freshwater baths known among aquarists is the biological control of pests by using cleaner and predator organisms

(Grutter et al., 2002). The biological function of labridae on the wild has been also used to control and eliminate nudibranch pests (Gochfeld & Aeby, 1997; Wang et al., 2020). To carry out this biological disinfestation process, Urban Natura store use *Halichoeres marginatus* Rüppell, 1835, one of the most known and commercial wrasse fish species. Although the effectiveness of these fishes has been tested it has not been possible to prove their effectiveness against egg masses (Gochfeld & Aeby, 1997) or to determine a critical stage when an organism cannot handle the whole pest by itself.

Conditions derived from climatic changes have resulted in altered population dynamics, geographic ranges, ecosystem structure, composition and function of native species. Climate change and biological invasions are key processes affecting global biodiversity, and their effects have to be considered altogether (Chang et al., 2009). In the last 20 years, peaks of 30°C of temperature, similar to tropical waters, have been registered in some Mediterranean Sea areas affecting the coralligenous community causing several massive mortality events (Coma et al., 2009). Due to the increasing

temperature derived from global warming and the constructions on the coastline made by the human species, new habitat conditions and ecological instability derived from niche-belonging species extinction, could provide an opportunity for alien species to be introduced in areas where they were not able to survive, establishing and prospering as a population (Walther et al., 2009). For this reason, the presence of tropical nudibranchs in aquariums are an actual threat. Public awareness of the dangers associated with the introduction of exotic species is very important. Aquarium trading represents one of the five major routes for introduction of allochthonous aquatic species (Chang et al., 2009; Duggan, 2010) and mollusks include the greatest number of invading taxa, with more than 200 species introduced in the Mediterranean Sea (Sabelli & Taviani, 2014). Recently, it has been found that many alien nudibranch species have settled in the Mediterranean Sea in the last decade (Camps-Castellà et al., 2020; Galià-Camps et al., 2020). Furthermore, some zoantharian species of the genera *Epizoanthus*, *Gerardia*, *Parazoanthus*, *Palythoa* and *Zoanthus* are present in the Mediterranean Sea (Aguilar, 2007), and this may enhance the probability of *B. rieae* to find a homologous food resource in the Mediterranean Sea. So, if the presence of undesired alien species in marine aquariums is not treated properly, it could cause them to accidentally reach the sea and find the ideal conditions to settle down and prosper, thus becoming an invasive species that could displace native ones and could become a threat to marine coast ecosystems.

Finally, it is necessary to investigate and report new methods and products to disinfect corals and to avoid this type of nudibranch or other coral predator blooms in commercial aquariums. In addition, we consider of great importance the scientific follow-ups to all procedures derived from dealing with commercial species, scientific dissemination and citizen science to report any unusual observations in the sea. Aquarists must be well informed and ensure the correct use of the products as well as full understanding of the species which they trade with.

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