

Rapid Communication**New records of alien and cryptogenic marine bryozoan, mollusc, and tunicate species in Libya**Jamila Rizgalla^{1,*}, Andrew P. Shinn^{2,3} and Fabio Crocetta⁴¹Department of Aquaculture, Faculty of Agriculture, University of Tripoli, Tripoli, Libya²Institute of Aquaculture, University of Stirling, UK³Fish Vet Group Asia Limited, Saensook, Chonburi, 20130, Thailand⁴Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale, I-80121 Napoli, ItalyAuthor e-mails: jamarizgalla@gmail.com (JR), andy.shinn@fishvetgroup.com (APS), fabio.crocetta@szn.it (FC)

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Citation: Rizgalla J, Shinn AP, Crocetta F (2019) New records of alien and cryptogenic marine bryozoan, mollusc, and tunicate species in Libya. *BioInvasions Records* 8(3): 590–597, <https://doi.org/10.3391/bir.2019.8.3.15>

Received: 8 March 2019**Accepted:** 5 June 2019**Published:** 4 July 2019**Handling editor:** Mary Carman**Thematic editor:** April Blakeslee**Copyright:** © Rizgalla et al.This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International - CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).**OPEN ACCESS****Abstract**

New records of alien and cryptogenic invertebrate marine species in Libya are reported here based on field surveys conducted from June to November 2018, in a shallow bay next to the city of Tripoli. Those identified here include the cerithiid gastropod *Cerithium scabridum* Philippi, 1848, the goniadoridid sea slug *Okenia longiductis* Pola, Paz-Sedano, Macali, Minchin, Marchini, Vitale, Licchelli and Crocetta, 2019, the “spaghetti bryozoan” *Amathia verticillata* (delle Chiaje, 1822), and the ascidian *Symplegma brakenhielmi* (Michaelsen, 1904).

Key words: ascidian, bioinvasions, faunistic, gastropod**Introduction**

About one thousand alien species have been recorded in the Mediterranean Sea. Of the various pathways through which they may have been introduced, the movement through the Suez Canal (Galil et al. 2017), by both natural dispersal (Shefer et al. 2004) and assisted commercial shipping traffic, is one of the principal routes for possible introductions (Katsanevakis et al. 2014; Zenetos et al. 2017). The translocation of species through shipping activity may result from the discharge of ballast water or, in the case of biofouling organisms such as ascidians, bryozoans, and molluscs, they may be carried on the hulls of vessels (Gewing and Shenkar 2017) or attached to man-made debris such as ropes and plastic bottles (Barnes 2002; Miralles et al. 2018). This study was conducted in a shallow water bay close to the commercial port of Libya’s capital, Tripoli, in the south-eastern part of the Mediterranean Sea, and provides the first report of the presence of two alien and two cryptogenic species in Libya. The species include the molluscs *Cerithium scabridum* Philippi, 1848 and *Okenia longiductis* Pola, Paz-Sedano, Macali, Minchin, Marchini, Vitale, Licchelli and Crocetta, 2019, the bryozoan *Amathia verticillata* (delle Chiaje, 1822), and the tunicate *Symplegma brakenhielmi* (Michaelsen, 1904).

Historically, *C. scabridum* was among the first alien species to be recorded in the Mediterranean Sea; the first record of its occurrence was from Port Said, Egypt in 1883 (Zenetos et al. 2017). This species is commonly considered to be a Lessepsian migrant, and now has a wide distribution throughout the Indo-Pacific and the Red Sea (Zenetos et al. 2004). Following its first report from Egypt, *C. scabridum* has since colonised nearly the entire eastern reaches of the Mediterranean Sea, extending into the central Mediterranean to Italy and Tunisia (Gofas and Zenetos 2003; Antit et al. 2011; Zenetos et al. 2017; Servello et al. 2019).

Okenia longiductis is a goniadoridid sea slug that has been frequently misidentified as *O. zoobotryon* (Smallwood, 1910) in the Mediterranean Sea, and is commonly associated with the bryozoan *A. verticillata*. This sea slug has been recently described from Spain, Italy, and Slovenia (Trainito and Doneddu 2014; Ballesteros et al. 2016; Lipej et al. 2017; Pola et al. 2019).

Amathia verticillata, a bryozoan initially described from the Gulf of Naples (Italy) as *Hydra verticillata* delle Chiaje (1822), has a worldwide distribution with reports from the Mediterranean, Caribbean, Atlantic, and Indo-Pacific (Minchin et al. 2016). The putative alien status assigned to this species in the Mediterranean Sea has been debated in the recent literature (Galil and Gevili 2014; Marchini et al. 2015). In the absence of molecular data to support its status, the species is ranked here as cryptogenic.

Symplegma brakenhielmi, an alien tunicate that originated from the Indian Ocean (Monniot and Monniot 1997), has a worldwide distribution (Lambert and Lambert 1998). While it was originally limited to the eastern Mediterranean Sea, with reports from Lebanon, Turkey, Cyprus (Bitar and Kouli-Bitar 2001; Çinar et al. 2006; Ulman et al. 2017; Gerovasileiou et al. 2017), and the latest records from Italy, it has also expanded into the western Mediterranean Sea (Mastrototaro et al. 2019).

The goal of our field surveys is to document the presence of any new, previously undocumented, marine invertebrate species in the northwest Libyan coastal region of the Mediterranean Sea. The findings reported here may contribute to the body of information on alien species in Libyan waters resulting from ongoing surveys.

Materials and methods

Field surveys were conducted from 14th June to 7th November 2018, in a touristic region in western Tripoli, in an area known as “Regatta” or “Magribi Arabic Touristic Village”, which covers an area of approximately 90 hectares with a 2.2 km long coastline. Along this coastline, there is a shallow water, natural bay (32.853970; 13.054376) with two islands that are subject to tidal movements (Figure 1A); the bay is close to the commercial port of Libya’s capital, Tripoli. The natural bay consists of sandy and rocky substrates covered by algae; sea temperatures for the area range from 20–25 °C (World sea water

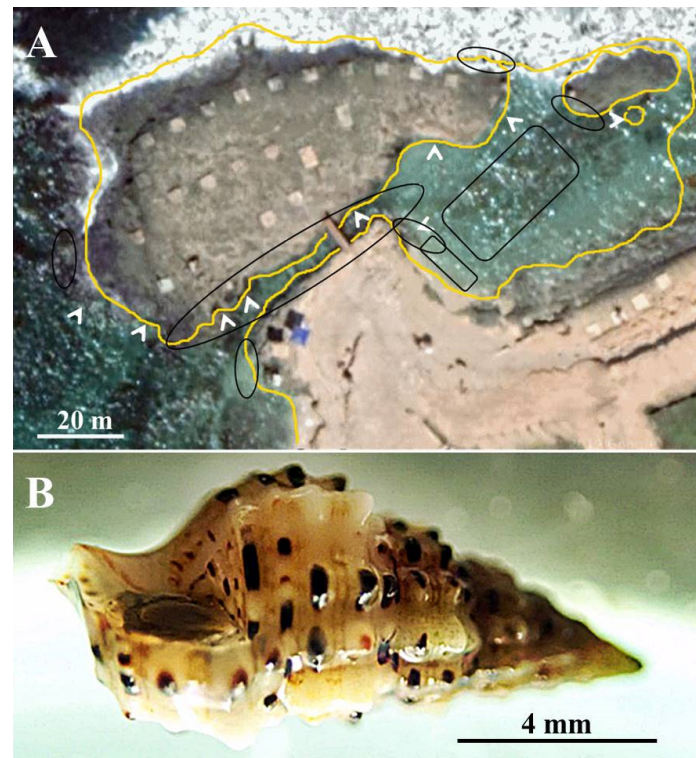


Figure 1. (A) Survey area (outlined in yellow) detailing where each of the alien and cryptogenic species from “Regatta”, Libya were found. The zones where specimens of *Cerithium scabridum* Philippi, 1848 were found are marked as black rectangles, while the white arrowheads indicate where specimens of *Okenia longiductis* Pola, Paz-Sedano, Macali, Minchin, Marchini, Vitale, Licchelli and Crocetta, 2019 and *Amathia verticillata* (delle Chiaje, 1822) were found, while the black ovals highlight where specimens of *Symplegma brackenhielmi* (Michaelsen, 1904) were found. (B) A live specimen of *C. scabridum*. Picture credit: J. Rizgalla.

temperatures 2019), and salinities range from 35.5 to 36.4 ppt (El Ezabi et al. 2016). Weather and the political situation permitting, field surveys consisted of a consecutive series of 2–6 hour-long, daily, shallow water, snorkel dives from 0 to 2 m depth at multiple locations within the bay.

During the surveys, marine specimens were photographed *in situ* using an Olympus Tough camera and then collected by hand and transferred into plastic containers. In the laboratory, specimens were observed under a dissecting (Hamilton) and compound microscope (Carl Zeiss Axiostar) and photographed. Subsequently, specimens were fixed in either 10% neutral buffered formalin (10% NBF) or 85% ethanol, and then stored in a private archive maintained by the first author. Egg-masses and five specimens of *O. longiductis* attached to *A. verticillata* were maintained for 10 days in an aerated sea water tank; these specimens were identified using descriptions provided by Pola et al. (2019).

Results

Four species were discovered during the dive surveys at the “Regatta” site and identified as *Cerithium scabridum*, *Okenia longiductis*, *Amathia verticillata*, and *Symplegma brackenhielmi*. Empty shells and living specimens of *C. scabridum* (Figure 1B) were usually found in large aggregations (i.e. > 30

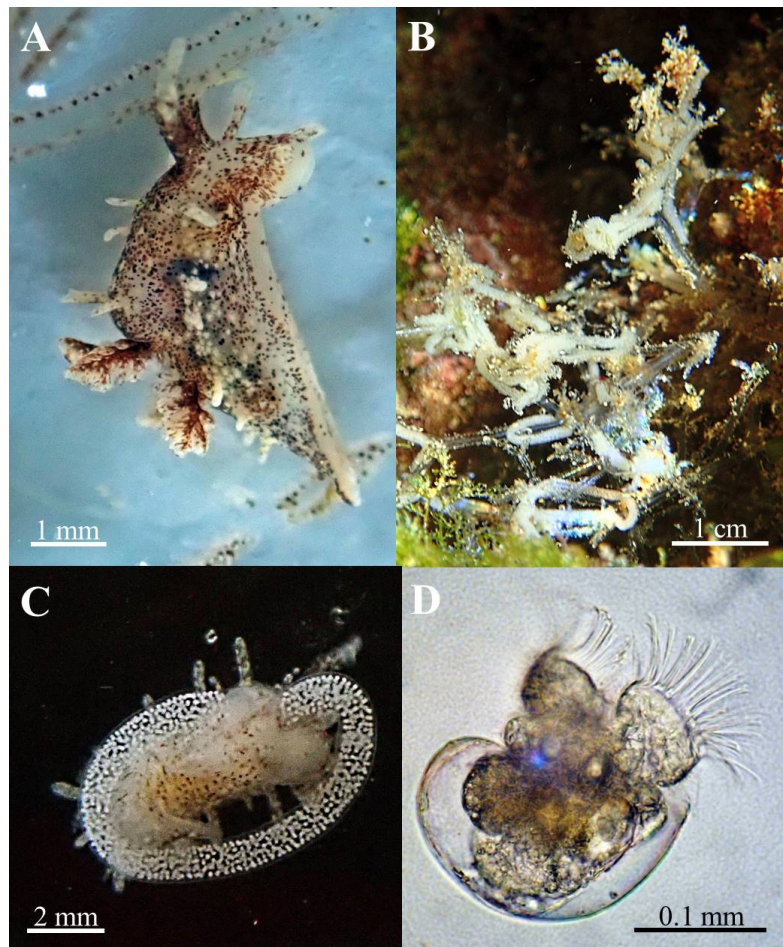


Figure 2. Alien and cryptogenic species documented from the study site at “Regatta”, Libya. (A) *Okenia longiductis* found on the bryozoan *Amathia verticillata*; (B) *Amathia verticillata* attached to a rocky substrate with egg-masses of *O. longiductis* attached to it; (C) an adult specimen of *O. longiductis* in the process of depositing an egg-mass on the surface of a plastic aquarium; and, (D) a free-swimming veliger of *O. longiductis*. Picture credits: J. Rizgalla.

individuals), commonly on sandy-muddy substrates or on rocks, at depths ranging from 20 cm to 1 m. A total of 10 empty shells and five living specimens were collected – two specimens were fixed in 10% NBF, and three specimens were preserved in 85% ethanol.

Okenia longiductis and its egg masses were found in association with the bryozoan *A. verticillata* (see Figure 2A, B), from late July until the end of the study period in November. These sea slugs were usually found moving over the bryozoan and egg-masses and were seen attached to the branched stolons (Figure 2B). Five specimens of *O. longiductis*, in association with the bryozoan, were transferred to a static, 1 litre, plastic, aerated seawater aquarium. These molluscs subsequently laid several egg-masses, with no discernible preference between the surface of the aerated tank (Figure 2C) and the branched stolons of *A. verticillata*. At least four egg-masses were laid by two individuals on the plastic container surface. The number of eggs per egg-mass was not determined. After approximately five days, veligers were observed in the tank (Figure 2D); a total of 15 specimens were preserved, with eleven being fixed in 10% NBF and four in 85% ethanol.

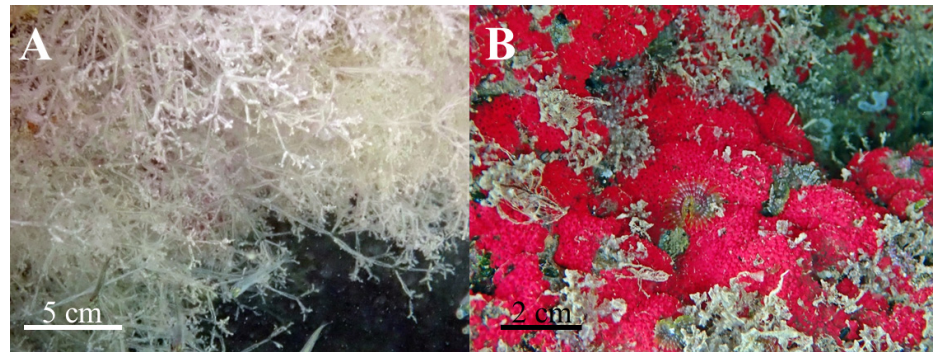


Figure 3. Cryptogenic and alien species from recorded at the study site at Regatta, Libya. (A) Colonies of the bryozoan *Amathia verticillata* and, (B) the tunicate *Symplegma brakenhielmi* attached to a rocky substrate. Picture credits: J. Rizgalla.

Amathia verticillata were frequently encountered in high densities (Figures 3A) throughout the entire study period, attached to rocky substrates that were also colonised by various unidentified sponges. While the species was commonly seen throughout June, July, and August, increasing in numbers from mid-July onwards, it was noticeably absent at the beginning of September, but then re-appeared mid to late September, although in reduced densities and in smaller-sized colonies. A total of two specimens were collected and fixed in 10% NBF and subsequently identified using the descriptions provided in Galil and Gevili (2014).

Specimens of the ascidian *Symplegma brakenhielmi* were found attached to the surface of exposed rocky substrates on both islands, in the channel between the two islands, and at depths ranging from 20 cm to 1 m (Figure 3B). A total of two specimens were collected, fixed in 10% NBF, and subsequently identified using the descriptions provided in Mastrototaro et al. (2019).

Discussion

The shallow water “Regatta” study site in the environs of a major commercial port suggests this may be a local hotspot and port of arrival for alien species (Rizgalla et al. 2018, 2019a, b). To date, a total of three alien (*C. scabridum*, *Haminoea cyanomarginata* Heller and Thompson, 1983, and *S. brakenhielmi*) and three cryptogenic species (*O. longiductis*, *Aplysia dactylomela* Rang, 1828 and *A. verticillata*) have been reported from the “Regatta” natural bay site, and *Fulvia fragilis* (Forsskål in Niebuhr, 1775) from the nearby Tripoli Harbour site (Rizgalla et al. 2018, 2019a, b; present paper). Among these, the ascidian *S. brakenhielmi* appears to be the most widespread across the study site. This taxon is known to be facilitated by its ability to live in most environments and on hard substrates (Lambert 2002), being a hull-fouling species with low nutrient requirements and having the capacity to establish in a wide range of environments (Lambert 2002; Gewing and Shenkar 2017; Ulman et al. 2017). *Cerithium scabridum* was also found in large aggregations, with colonies ranging in 3–10 mm in total height. This taxon had almost entirely replaced native small congeneric species, occupying

hard substrates along the eastern Mediterranean coastline (Mienis 2003), and is able to colonise a wide range of environments in all kinds of natural and man-made substrates (Ben Souissi et al. 2005; Albano and Trono 2008; Crocetta et al. 2009). Further and longer-term investigations of these species are required to better understand their occurrence, local distribution, density, and capacity to establish breeding populations. For example, while *A. verticillata* is known to cause adverse effects to fishing gears, aquaculture cages, and harbour structures, the prevalence of the species also appears subject to wide-seasonal fluctuations (Marchini et al. 2015; McCann et al. 2015; Jebakumar et al. 2017), and a similar situation may hold for the three sea slugs mentioned above.

Although the precise routes of introduction of these taxa into Libyan waters remains to be determined, they are already widespread in parts of the Mediterranean Sea, suggesting that their local spreading may have been through secondary natural dispersal from nearby populations. Two species recorded from the current study, however, are commonly cited as species associated with biofouling and shipping traffic; therefore, activity within Tripoli Harbour, situated close (i.e. 12 km) to the study site, may have also played a role in their local arrival.

Acknowledgements

The authors wish to thank Dr. Mary Carman and two anonymous reviewers for their comments on the manuscript.

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