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Note

First record of the invasive Rhodophyte *Hypnea cornuta* (Kützing) J. Agardh 1851, in the Northern Tunisian coast (Bizerte lagoon, West Mediterranean Sea)

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Abstract: The rhodophyte *Hypnea cornuta* is native to the Indo-Pacific Ocean. It has been introduced into the Mediterranean since the 19th century in Greece and has since widely spread in the eastern Mediterranean subregion. The first record of *H. cornuta* in Tunisia was in the southern coast, at Djerba. This paper reports for the first time, the detection of this species in the northern Tunisian coast (Bizerte lagoon) in May 2019. Thalli of *H. cornuta* were sparse, with a coverage area of less than 5 %. This finding of *H. cornuta* in Bizerte lagoon indicates its ongoing expansion in the western Mediterranean basin, and the main vector of this expansion is likely large commercial vessels and recreational boats.

Keywords: *Hypnea cornuta*; non-indigenous species; Invasive species; propagules; stellate branchlets; Tunisian coast.

1. Introduction

In the Mediterranean Sea, 986 alien species (more than 5% of the recorded species), including fishes, crustaceans, molluscs, worms, macrophytes, etc. were introduced from their native geographical ranges, the Atlantic Ocean via the strait of Gibraltar, the Indo-Pacific Ocean and the Red Sea via the Suez Canal, since its opening in 1869 (Zenetos et al., 2012).

Currently, the Mediterranean Sea hosts more than 1000 validated non-indigenous species (NIS) (Zenetos et al., 2022).

The number of introduced macrophytes in the Mediterranean reached 128 species, according to Zenetos et al. (2012), and this number is likely in a progressive increase.

In Tunisia, studies on marine macroflora (seagrasses, such as *Posidonia oceanica,* and seaweeds) were carried and continue to be carried out in different areas (Ben Brahim et al., 2007 a, b; Ben Maiz et al., 1987; Ben Maiz, 1995; Ktari & Langar, 2004).

Regarding the introduced macrophytes in Tunisia, a total of 27 alien species were reported by Sghaier et al. (2016). The native geographical range of the

rhodophyte *Hypnea cornuta (*Rhodophyta; Florideophyceae; Gigartinales) is the Indo-Pacific Ocean.

The alien invasive *Hypnea cornuta* has been introduced into the Mediterranean Sea by shipping, oyster cultivation and via Suez Canal, being first found at Rhodes Island (Greece), in 1894 (Verlaque et al., 2015). It is now widespread in the eastern Mediterranean (Egypt, Palestine, Syria, Lebabon, etc.) and western Mediterranean (Italy). In Tunisia, the invasive alien rhodophyte *Hypnea cornuta* was recorded for the first time in the southern coast of Tunisia, at Djerba in 2009 (Sghaier et al., 2016).

This paper aims to report the first observation of *Hypnea cornuta* in the North of Tunisia, in Bizerte lagoon.

2. Material and methods

2.1. Sampling site

A routine monitoring of seaweeds, mainly the genus *Gracilaria*, in the Bizerte lagoon (latitude: 37°8'-37°14'N, longitude 9°46'-9°56') was undertaken monthly, from January 2019 to December 2019. Sampling was conducted in front of the Lagoon Tunisian Society (LTS) shell-fish farm (**Figure 1**).

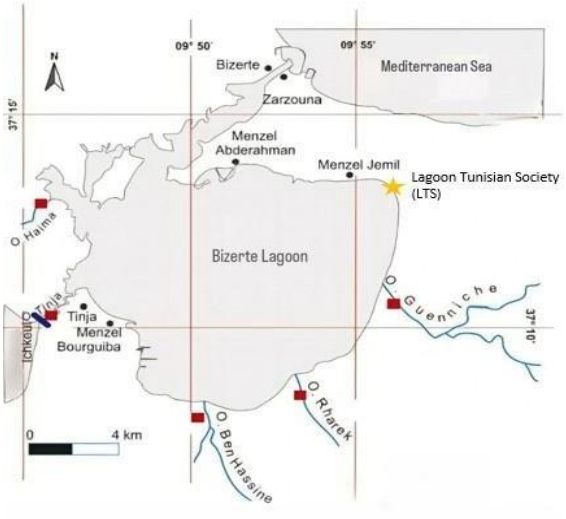

Figure 1. Sampling site of *Hypnea cornuta* in Bizerte Lagoon

During seaweed investigation, the rhodophyte *Hypnea cornuta* (**Figure 2 a)** was detected and collected for the first time in May 2019, at 0.5m depth.

For the assessment of coverage area, a 1 m x 1 m metallic quadrate divided into 25 small quadrates was used.

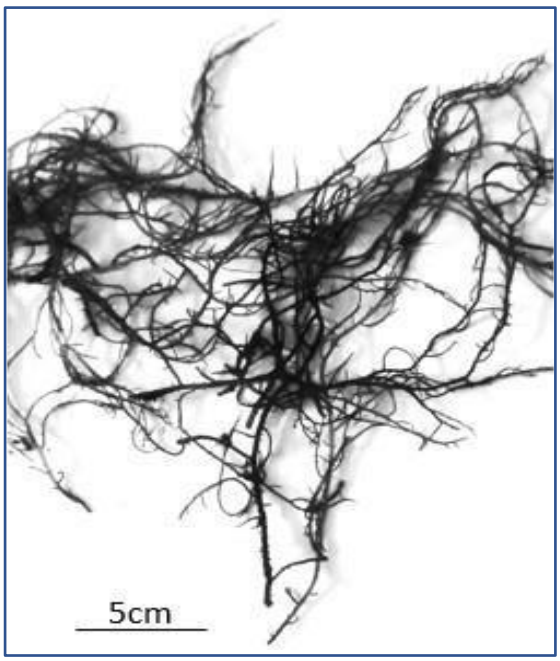

Figure 2 a. *Hypnea cornuta* (fresh specimen)

2.1. Microscopic observation

After seaweed collection, samples were brought to the laboratory of the INSTM in plastic bags. Then, fresh algae were placed in Petri dishes containing approximately 15 ml of fresh lagoon seawater.

The observation of the thalli was undertaken several times during the study period in the same sampling site, and the identification was carried out using a stereomicroscope (Wild Heerbrugg type) and a light microscope (Nikon PFX, type 104), with different magnitudes. Photos of stellate branchlets were taken using a Leica M165 FC stereomicroscope at a magnitude of 0.73. The observed samples were compared with the relevant literature

and online databases, such as AlgaeBase. Unfortunately, molecular tools for seaweed identification were not available; therefore, species identification was based only on microscopic observations.

3. Results

3.1. Morphological description, stereomicroscope and light microscope observation of Hypnea cornuta

The collected samples of *Hypnea cornuta* were attached to either sand substrate or rocky one. The thalli are reddish, sometimes brownish, green or translucent and rough when compared to its congener species *Hypnea musciformis*, which is soft and has particular flat hooks at the tips of many axes and branches. The collected thalli of *H. cornuta* were, in general 10 to 30 cm high, but in June and July 2019, they reached 50 cm tall.

At the sampling site, the *H. cornuta* population was very sparse, with a coverage area not exceeding 5 %, compared to other macrophytes, such as *Ulva rigida, Caulerpa prolifera*, *Gracilaria gracilis*, *Gracilaria bursa-pastoris*, and the Magnoliophyte *Cymodocea nodosa*.

Stereomicroscope and light microscope observations of the fresh collected thalli of *Hypnea cornuta* allowed us to identify the species. In addition to the spiny branches on the axes, the presence of stellate branchlets (**Figure 2 b**) allowed us to confirm the species, unequivocally. Additionally, many unattached stellate branchlets were observed at the bottom of the Petri dish. These branchlets are considered as propagules that enable sexual reproduction in *Hypnea cornuta,* as described by Cecere et al., (2004). During stereomicroscope and light microscope observations, reproductive organs were absent from the collected specimens.

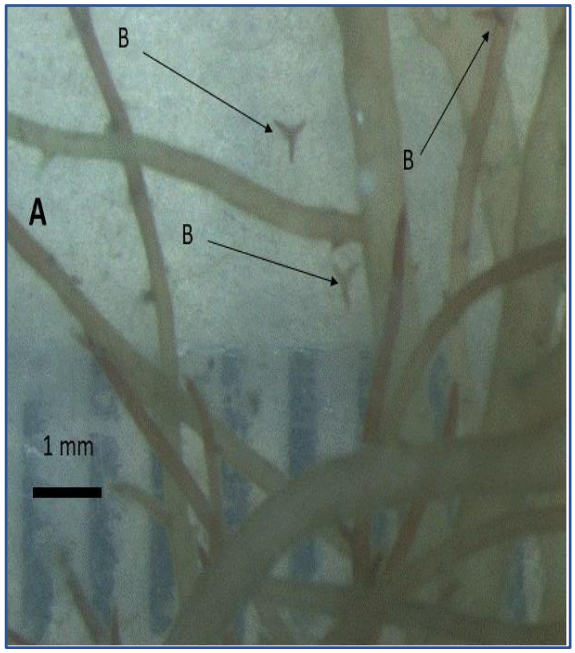

Figure 2 b. *Hypnea cornuta*, Bizerte lagoon, Tunisia, May 2019, A: General aspect of the thallus, B: axes bearing stellate branchlets and free stellate branchlet in the bottom of Petri dish (arrowed). Scale bar (ruler) (A & B): 1 mm

4. Discussion and conclusion

In the present study, *Hypnea cornuta* was detected for the first time in the Bizerte lagoon in May 2019, although it was likely introduced many years earlier. During the seaweed sampling, from January to December 2019, the species was observed at the sampling site until October but was absent in November and December. The coverage area of *H. cornuta* did not exceed 5 %. Observations of thalli, using the stereomicroscope and light microscope, combined with comparisons to the literature (Cecere et al., 2004; Sghair et al., 2016) and the AlgaeBase allowed us to accurately identify the species as an invasive non-indigenous species (NIS), which has been introduced in the north of Tunisia, as well as in the south of the country (Sghair et al., 2016).

It is now well known that NIS introduction hotspots are mainly ports, harbours, marinas, and aquaculture facilities.

Shipping was identified a likely vector for over half of NIS in European waters, through both biofouling of recreational vessels and ballast water discharges from large commercial boats (Zenetos et al., 2022). The substrates of ports, harbours and marinas provide suitable habitats for the secondary spread of alien species in the neighboring areas. This pattern has been observed at the global scale, particularly in the Mediterranean Sea (Occhipinti et al., 2011; Verlaque et al., 2015) and especially along the southern coast of Tunisia (Missaoui et al., 2006; Hattour & Ben Mustapha, 2015).

Along the northern coasts of Tunisia, especially in Bizerte region, many commercial ports and marinas receive large commercial vessels, as well as fishing and recreational boats. All these structures are considered the main introduction vectors of invasive alien species (IAS) - a subset of non-indigenous species (NIS) - in the Bizerte Lagoon, mainly through biofouling and ballast waters which transport specimens and propagules from one area to another.

On the other hand, Teixeira & Creed (2020) reported a status hierarchy for NIS as follows: contained, detected, established and invasive species. Regarding *Hypnea cornuta*, which was observed in the Bizerte lagoon, this NIS species can be considered well established in its new range. In fact, after the first record in May 2019, the species was detected in the subsequent years, at the same sampling site, during the same months (from May to October). Its coverage area remained below 5%, and no apparent ecological or socio-economic impacts have been observed to date. From a scientific perspective «invasive» refers to a species' ability to survive, reproduce and spread in the invaded region, whereas political frameworks often connect invasiveness to impact. In this context, invasive species interfere with the survival capacity of other species. Therefore, the introduction of NIS is considered as one of the main causes of biodiversity loss (Afonso et al., 2020; Mannino et al., 2023) and can cause measurable impacts on socio-economic activities or human health (Zenetos et al., 2022).

In the future, *H. cornuta* could expand its range in the Bizerte lagoon, and invade other areas through stellate branchlets, which serve as propagules in the vegetative reproduction. Consequently, its spread could threaten the biodiversity of other macrophytes, including both seaweeds and magnoliophytes. The geographical range of *Hypnea cornuta* could also expand in the future and reach other countries in the western Mediterranean Sea, beyond Tunisia and Italy. Therefore, monitoring this species is crucial to assess its impact on biodiversity, and various human activities.

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