

Morphology and vegetative reproduction of the introduced species *Hypnea cornuta* (Rhodophyta, Gigartinales) in the Mar Piccolo of Taranto (Italy), Mediterranean Sea

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Abstract

Hypnea cornuta, a species previously introduced into the eastern Mediterranean Sea, has been discovered recently in the Mar Piccolo of Taranto (southern Italy). The specimens are in good agreement with Indo-Pacific descriptions and reference material. It is highly probable that *H. cornuta* has been introduced either from eastern Mediterranean or Indo-Pacific populations via ship traffic or shellfish transfers (mussels). Since being introduced, *H. cornuta* has given rise to a stable both attached and free-floating population throughout the Mar Piccolo. Both attached and unattached plants are described. Field and culture observations have confirmed the reproductive role of the stellate branchlets as propagules. Vegetative reproduction both by propagules and fragmentation seems to be the only mode of reproduction in the Mar Piccolo.

Keywords: *Hypnea cornuta*; introduced species; Mediterranean Sea; morphology; propagules.

Introduction

Currently, the red algal genus *Hypnea* J.V. Lamouroux (1813) comprises 47 recognised species (Guiry and Nic Dhonncha 2004). In the Mediterranean Sea, eight species have been reported: *H. cornuta* (Kützinger) J. Agardh, *H. esperi* auctorum (as *H. esperi* Bory de Saint-Vincent), *H. furnariana* Cormaci, Alongi et Dinaro, *H. musciformis* (Wulfen) J.V. Lamouroux, *H. nidifica* J. Agardh, *H. spicifera* (Suhr) Harvey (as *H. harveyi* Kützinger), *H. spinella* (C. Agardh) Kützinger (as *H. cervicornis* J. Agardh) and *H. valentiae* (Turner) Montagne (Furnari 1984, Cormaci et al. 1993, Boudouresque and Verlaque 2002). *Hypnea musciformis*, the type species of the genus, which is widely distributed in the warm and temperate seas, was described from the Mediterranean Sea (Wulfen 1791, as

Fucus musciformis; type locality: Trieste), as well as *H. furnariana*, an entity known only from the type locality to date (Maddalena Peninsula, near Syracuse, Sicily) (Cormaci et al. 1993). All the others are considered to be introduced and migrants from the Red Sea to the Mediterranean Sea through the Suez Canal (Lessepsian migrants), with the exception of *H. spinella*, whose mode of introduction into the Mediterranean Sea is unknown (Verlaque 1994, Ribera and Boudouresque 1995). However, the Mediterranean reports of *Hypnea esperi* and *H. nidifica* are poorly documented (Forti 1928, Lipkin 1972). Moreover, *H. nidifica* is currently considered as a synonym of *H. spinella* (Abbott 1999, as *H. cervicornis*), and the identity of *H. esperi* Bory de Saint-Vincent (1828; type locality: Concepción, Chile, SE Pacific) – a superfluous, and thus illegitimate, substitute name for *Fucus nootkeanus* Esper (1802; type locality: Nootka Sound, Vancouver Island, NW Atlantic Ocean) – is uncertain (Silva et al. 1987, 1996). J. Agardh (1851) thought that it might be conspecific with *H. nidifica*.

Hypnea cornuta, described from Guinea (eastern Atlantic Ocean) (Kützinger 1849, as *Chondroclonium cornutum*, J. Agardh 1852, as *H. cornuta* var. *cornuta*), has been widely reported from various localities in the world (Mshigeni and Chapman 1994 and references therein). The first Mediterranean report of the species was from Rhodes Island, Greece [Reinbold 1898 as *H. valentiae* (Turner) Montagne], shortly after the opening of the Suez Canal in 1869. Successively, *Hypnea cornuta* has been reported from Egypt (Aleem 1948, 1993) and Israel (Nemlich and Danin 1964, Friedlander and Zelikovitch 1984, Lundberg 1991).

In the Mar Piccolo of Taranto (southern Italy), only *H. musciformis* and *H. spinella* (as *H. cervicornis*) have been reported to date (Cecere et al. 1991, 1992). In December 2000, some plants of a *Hypnea* species never found previously, were collected attached to scattered pebbles during routine sampling. The thalli were characterised by the presence of stellate processes, typical of *Hypnea cornuta*.

The aim of the present paper is to give a description of Mediterranean material of *Hypnea cornuta*, never described in detail to date, and to outline the phenology of the population thriving in the Mar Piccolo of Taranto.

Material and methods

Study site

The Mar Piccolo of Taranto, a coastal nearly-enclosed basin located to the north of the town of Taranto (40° 28' N, 17° 15' E), has a surface area of ca. 20 km² and is divided by two promontories of land into two smaller

basins, which are called the First and the Second Inlets. Seawater temperature ranges from 7.1°C to 33.6°C and salinity from 34.3 psu to 37.7 psu (Alabiso et al. 1997). The maximum depth is twelve and eight meters in the central part of the First and Second Inlet, respectively.

Most of the hard substrata are artificial. The soft substrata are sandy near the shore and muddy in the central zone of both the Inlets.

The Mar Piccolo of Taranto has the most important Italian naval base, which is located in the First Inlet, and the biggest mussel farm in Italy, the facilities of which are widely distributed in both Inlets.

Sampling and observations

In autumn 2003, a qualitative visual survey was carried out to verify if *Hypnea cornuta* had spread throughout the basin or remained confined to the site of first finding.

To monitor vegetative and reproductive phenology, qualitative monthly observations were carried out from December 2000 to November 2003. For this purpose, several attached and unattached plants were hand-collected from hard substrata at a depth of about 0.5 m and from free-floating communities, respectively. The collected material was brought to the laboratory, where it was preserved in 2.5% formaldehyde-seawater and then examined with both stereo and light microscopes (Leica Microsystems®, Wetzlar, Germany). For light microscopy, thalli were sectioned by hand or a Leitz Kryomat microtome (Ernst Leitz GmbH®, Wetzlar, Germany). Sections were observed either unstained or stained with Aniline Blue acidified by a drop of HCl 0.5%.

The following dried samples of *Hypnea cornuta* deposited in the University Herbarium, Berkeley (UC) were also examined:

- Tropical Atlantic Ocean: 1404865, Cuba: Camaguay Prov., Tarifa near Nuevitas, leg. E.Y. Dawson # 7619, det. W.R. Taylor, 19.v.1949; 1463438, Florida: Virginia Key, Bear Cut, leg. M. Diaz-Piferrer, det. M. Diaz-Piferrer, 26.v.1964; M 260948, Santo Domingo: Hotel Hamaca, Boca Chica, D.N., leg. L.R. Almodovar, M. Montero, M. Garcia, and F. Geraldès, det. L.R. Almodovar.
- Red Sea: 1461724, Dahlak Archipelago: Romia Islet, leg. G.F. Papenfuss, det. G.F. Papenfuss and Y.M. Chiang, 29.iii.1962; 1461725, Dahlak Archipelago: Entedebir Islet: Goliath Bay, leg. G.F. Papenfuss, det. G.F. Papenfuss and Y.M. Chiang, 29.iv.1962.
- Indian Ocean: M 058363, Australia: Northern Territory, Port Bradshaw (12° 27'S, 136° 42'E), leg. R.L. Specht # A56, det. H.B.S. Womersley, 25.vii.1948.
- Pacific Ocean: M 098859, Vietnam: Cua Be, 1 km South of Cua Be, leg. E.Y. Dawson # 11092, det. E.Y. Dawson, 25.i.1953.

Dried and liquid preserved samples from Taranto have been deposited in the Herbarium of the Istituto Sperimentale Talassografico of Taranto (TAR).

Culture observations

In order to confirm the hypothesis that stellate processes were real propagules able to give rise to new specimens,

qualitative culture observations were carried out. Several stellate processes, easily detached from the collected plants, were cultured for one month in small glass tanks stored in a culture room (BICASA®, Milan, Italy). Some of them were put onto an artificial hard substratum (“cal-pomice”, a very common building material comprising set-cement and “pumice-stone”) without medium agitation. Others were cultured free-floating in the vessels with a light medium agitation via aquarium aerators. Culture conditions were the following:

- culture medium: 3 l of plain filtered (45 µm glass filter) seawater;
- irradiance: 92 µmol m⁻² s⁻¹ (the highest achievable in the store by means of OSRAM L fluorescent tubes cool white 18W);
- photoperiod: 12L:12D;
- temperature: 21±1°C (recorded in the field during the collection of material to be cultured).

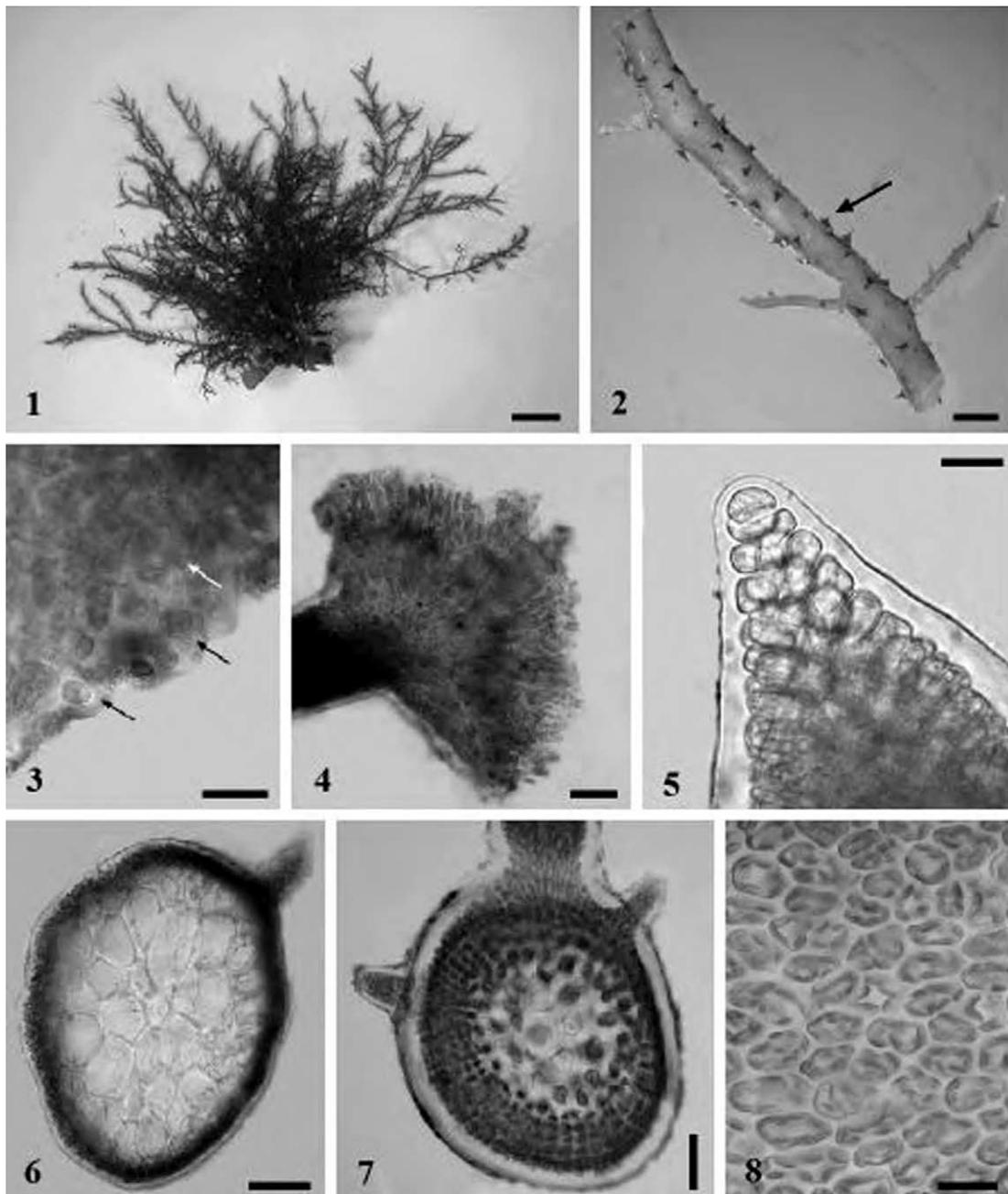
Results

Description

Attached and unattached plants, both collected in the Mar Piccolo, were morphologically different.

Attached plants

Plants caespitose or loosely intricate-caespitose, up to 30 cm in length, soft in texture and reddish-brown in colour; axes sub-terete, up to 1 mm in diameter at the base, pointed at the apex, spirally branched up to the fourth order (Figure 1); branching angle less than 90°; axes and branches loosely clothed with stellate processes (Figure 2); branches, up to 550 µm in diameter, and spinous ultimate branchlets, 60–70 µm in diameter, slightly constricted at their base; extensive basal system of prostrate axes attached by secondary discoid holdfasts produced by the cortical cells at any axis level; apical rhizoidal cells prominent, globular to oval, with a thick outer cell wall (Figure 3); rhizoids multicellular, unbranched and secondary-pit connected to form discoid holdfasts (Figure 4); axes growing from an obliquely dividing apical cell (Figure 5); in transverse sections of the upper portion of the axis, axial cell, 20 µm in diameter, surrounded by 5–6 pericentral cells (35–65 µm×30–45 µm), 2–5 layers of cells gradually smaller towards the periphery, one layer of pigmented cortical cells (6–12 µm×6–9 µm) and a cuticle of about 9 µm (Figure 6); in transverse sections of the basal portion of the axis, axial cell, ca. 60 µm in diameter, surrounded by 5–6 pericentral cells, with a thick cell wall, up to ten layers of starch filled cells and a thick cuticle, up to 30–40 µm thick (Figure 7); cell wall lenticular thickenings lacking; in surface view, outermost cortical cells polygonal rounded (Figure 8); stellate processes, endogenous in origin and deriving from the transformation of any order branch primordium, produced by pigmented axes; each consisting of a central body from which three to six rays issue, usually differing from each other in both length and diameter (Figure 9); stellate process primordia differing from branch primordia



Figures 1–8 *Hypnea cornuta*.

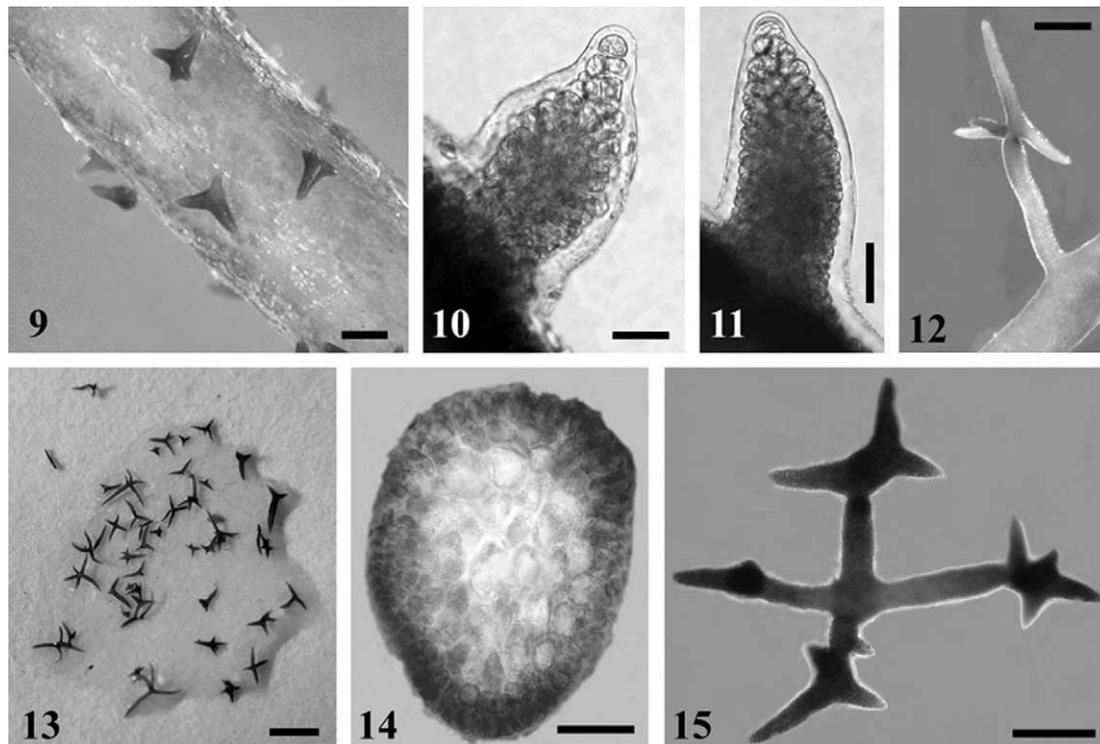
(1) Mature plant. Scale=5 mm. (2) Detail of axis bearing stellate processes (arrow). Scale=1 mm. (3) Early development of a discoid holdfast (white arrow: cortical cells; black arrows: thick outer wall of apical rhizoidal cells). Scale=40 μm . (4) Attachment disc. Scale=30 μm . (5) Apex. Scale=15 μm . (6) Transverse section of axis at 3 mm below the apex. Scale=40 μm . (7) Transverse section of axis just above the basal holdfast. Scale=200 μm . (8) Cortical cells in surface view. Scale=15 μm .

in having an inflated outline (Figures 10–11); stellate processes sometimes also deriving from the branch apical part (Figure 12); stellate processes easily detaching owing to a slight basal constriction that functions as an abscission zone (Figure 13); in transverse sections, axial cell of the stellate process, 12–21 μm in diameter, surrounded by five pericentral cells, 16–30 μm in diameter, 4–5 layers of cells, the outer cortical cells being strongly pigmented and bigger than those of the mother-plant (14–20 $\mu\text{m} \times 11$ –16 μm); cells filled of floridean starch granules (Figure 14); rays of stellate processes sometimes forming new stellate processes in their turn (Figure 15).

Unattached plants

Unattached plants characterised by the lack of a basal system, irregular branching up to the fourth order, a brownish to greenish colour, curved apical regions of both branches and branchlets and blunted apices with an inconspicuous apical cell (Figures 16–18). Like attached plants, unattached plants also bore stellate processes.

In autumn, several ball-like plants, up to 15 cm in diameter, were collected with branches of similar length radially arranged on the parent fragment and apices attached onto the axes by secondary rhizoids, giving the



Figures 9–15 *Hypnea cornuta*.

(9) Stellate processes on the axis. Scale=250 μm . (10) Stellate process primordium. Scale=25 μm . (11) Branch primordium. Scale=40 μm . (12) Stellate process formed at a branch apex. Scale=1 mm. (13) Different development stages of detached stellate processes. Scale=1 mm. (14) Transverse section of a stellate process. Scale=40 μm . (15) Stellate process forming other processes at the apex of each ray. Scale=300 μm .

plants sturdiness and a typically entangled look (Figure 17). These plants were often entangled with each other by means of coiled apices. Last order lateral branches of unattached plants were more pigmented and contained numerous floridean starch granules (Figure 16).

Field and culture observations

The visual examination carried out in autumn 2003 showed that *Hypnea cornuta* has spread throughout the Mar Piccolo, both along the coast and in the central zones of the basin, giving rise to both attached and unattached populations.

In the attached populations, erect fronds were present from July to December, when they became detached and decayed. Prostrate axes and the most basal parts of erect axes over-wintered and in summer produced new erect fronds. In late autumn-early winter, unattached thalli also fragmented and decayed, and only the small starch-filled fragments remained, either attached to various substrata (Figure 19) or free-floating. Plantlets growing out from stellate processes were frequently found attached to different substrata (e.g., stones, animals, seaweeds). Such plantlets were easily distinguishable by the presence of stellate processes at their base. In the free-floating multi-species seaweed community, many drift stellate processes were also observed which gave rise to unattached plants.

All the plants, either attached or unattached, always bore stellate processes. Neither fertile tetrasporophytes nor gametophytes were ever collected.

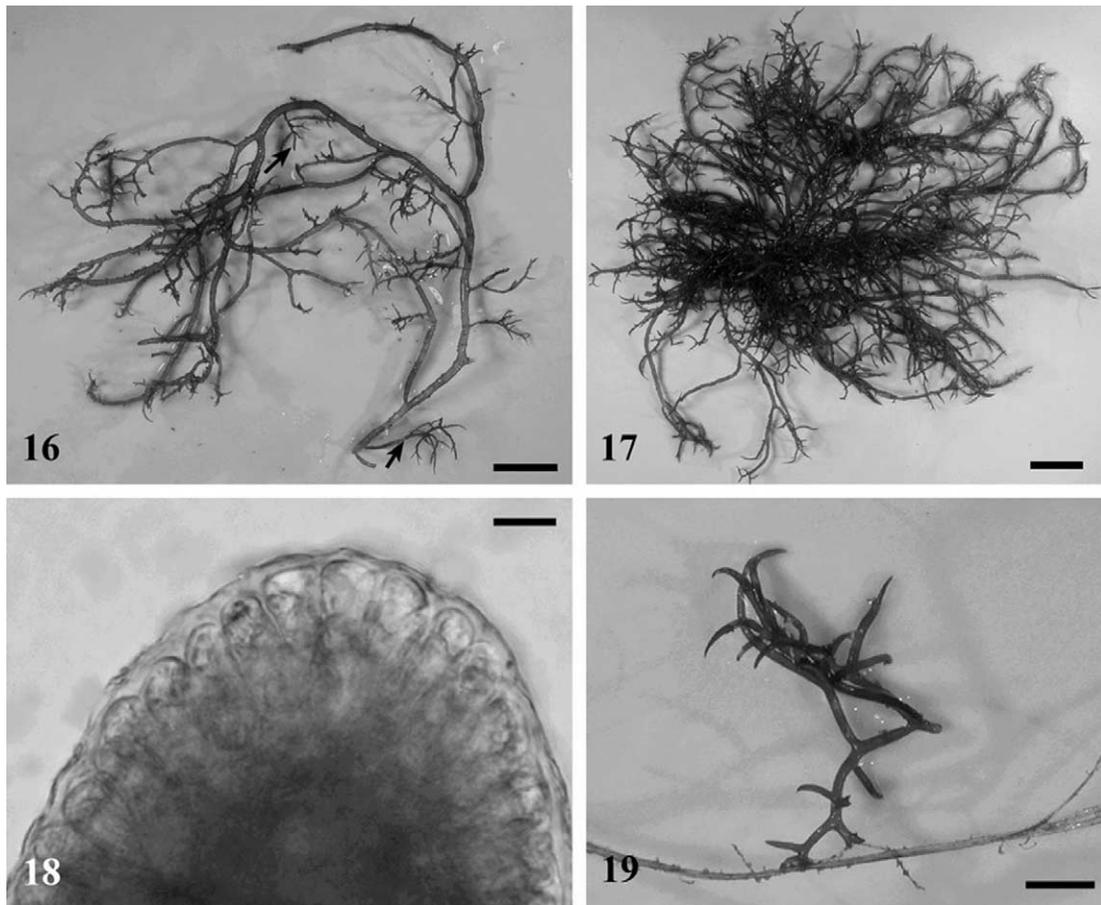
In culture without agitation, some detached stellate processes settled down onto the hard substratum. Their arms curved downward, attached by rhizoids and discoid holdfasts, then curved upward and gave rise to uprights and so to new attached plantlets (Figure 20). In culture with a slight agitation, free-floating stellate processes gave rise to unattached plantlets (Figure 21).

Discussion

The alga from the Mar Piccolo agrees with J. Agardh's (1852) characterisation of *Hypnea cornuta* var. *cornuta* (currently *H. cornuta*) from Guinea (see illustrations of Agardh's voucher specimens in Lewmanomont 1997, figs 1–4). The identity of the Italian material was recently confirmed by the analysis of the *rbcl* gene sequence (Yamagishi et al. 2003). Therefore, *Hypnea cornuta* is reported for the first time from the central region of the Mediterranean Sea.

In the Mar Piccolo, *Hypnea cornuta* can be considered as recently introduced, as it was not recorded in the area before, and was collected for the first time in the vicinity of potential introduction sources (e.g., mussel farms, naval harbours) (Ribera and Boudouresque 1995). Moreover, in this basin, at least six exotic seaweed species have been already found (Cecere 1989, Perrone and Cecere 1994, Cecere et al. 2000, Cecere and Petrocelli 2004).

The introduction of non-indigenous species in marine coastal environments is now world-wide. In the Mediter-

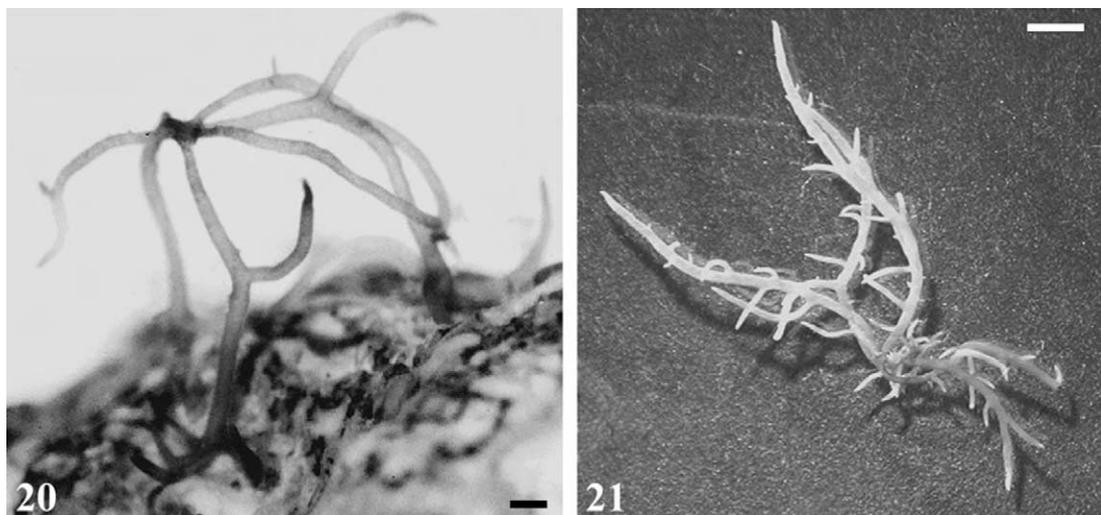


Figures 16–19 *Hypnea cornuta*.

(16) Greenish plant with more pigmented segments (arrows). Scale=10 mm. (17) Ball-like plant. Scale=1 cm. (18) Blunted apex. Scale bar=15 μ m. (19) Drift fragment re-attached onto a plastic line. Scale=3 mm.

ranean Sea, in the last twenty years the number of introduced species has doubled in comparison with the beginning of the twentieth century (Boudouresque 1998). The phenomenon is surely closely related to the increase of certain human activities, such as aquaculture and recreational and commercial navigation (Occhipinti Ambrogi 2001).

Concerning the origin of *Hypnea cornuta* in the Mar Piccolo, an eastern origin seems highly probable because the Mar Piccolo specimens are in better agreement with the Red Sea and Indo-Pacific populations (Weber van Bosse 1928, Tanaka 1941, Nasr 1947, Rayss 1959, Jaasund 1976, Mshigeni 1976a, Basson 1979, Khoja 1987, Wynne 1995, De Clerck and Coppejans



Figures 20–21 *Hypnea cornuta*.

(20) Plantlet attached onto the substratum. Scale=300 μ m. (21) Unattached plantlet developed from a drift stellate process. Scale=2 mm.

1996, Bangmei and Yongqiang 1997, Chiang 1997) than with Atlantic populations, which differ in having a thicker cuticle and larger cortical cells (Børgesen 1920, Chapman 1963, Afonso Carrillo et al. 1984). Two possible types of introduction, by ship traffic (fouling, ballast water) or shellfish transfer can be considered: either direct introduction from the Red Sea or the Indian Ocean or secondary introduction from the eastern Mediterranean basin (Egypt, Israel, Rhodes Island).

Since its introduction in the Mar Piccolo, *Hypnea cornuta* has developed stable attached and unattached populations. Free-floating plants, which exhibit all the features of unattached seaweeds (Norton and Mathieson 1983), undergo decay at the beginning of winter in parallel to the attached plants. The starch-filled fragments, derived from the decay process, probably over-winter and give rise to new free-floating and attached plants in early summer, as has been shown for the drift population of *Acanthophora nayadiformis* (Delile) Papenfuss (Cecere et al. 1994, Cecere et al. 1999).

Hauck (1887) was the first to consider stellate branchlets as putative propagative organs similar to the propagules of *Sphacelaria*. Various authors have since supported this hypothesis (Weber van Bosse 1928, Edwards 1970, Jaasund 1976, Mshigeni 1976a, Lipkin and Silva 2002), but vegetative reproduction of *Hypnea cornuta* by means of stellate processes has been observed so far only in culture, and briefly mentioned once in a congress abstract (Lipkin 1977). The present *in situ* and in culture observations confirm the propagative role of the stellate processes in the vegetative reproduction of *H. cornuta*. From a morphological point of view, endogenous *Hypnea cornuta* propagules differ from the exogenous pseudo-stellate processes of some other *Hypnea* species, which are produced by *in situ* germination of carpospores or tetraspores (Mshigeni 1976b). Like most of the asexual propagules of algae (Feldmann 1977), they are characterised by the presence of protuberances that facilitate the attachment to the substratum. Moreover, they contain floridean starch granules, as reported for other vegetative propagules (Okamura 1902, Gordon-Mills and Womersley 1987, Maggs and Hommersand 1993) and this suggests that they could also be over-wintering organs, as observed in *Acanthophora nayadiformis* (Cecere and Perrone 2002) and *Alsidium corallinum* C. Agardh (Cecere et al. 2002). Since in the genus *Hypnea* stellate propagules occur in only two species, *H. cornuta* and *H. stellulifera* (J. Agardh) Yamagishi et Masuda (Yamagishi et al. 2003), they are a useful taxonomic character at species level (Masuda et al. 1997), as has also been demonstrated for other algae (Cecere and Perrone 2002, Cecere et al. 2002).

In the Mar Piccolo, *Hypnea cornuta* lacks any fertile tetrasporophyte or gametophyte, as already observed in China and Taiwan (Bangmei and Yongqiang 1997, Chiang 1997).

In spite of the lack of sexual reproduction and its recent introduction, the species has already spread throughout the Mar Piccolo, unlike other introduced species, which remained confined to the sites of first sighting (Cecere et al. 2003, Petrocelli unpublished data). This is probably due to the vegetative reproduction described above.

Currently in the Mar Piccolo, *Hypnea cornuta* does not show a behaviour as invasive as that of *Caulerpa racemosa* (Cecere and Petrocelli 2004). However, the possibility that in the future it will cannot be excluded any more since vegetative reproduction has proven very effective for the spread of some introduced algae with demonstrated invasive behaviour (Boudouresque and Verlaque 2002, Renoncourt and Meinesz 2002, Squair et al. 2003).

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