

How the unattached form of *Acanthophora nayadiformis* (Rhodophyta: Ceramiales) produces storage and perennating organs

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In the Mar Piccolo of Taranto (southern Italy, Mediterranean Sea), an enclosed basin with lagoonal features, *Acanthophora nayadiformis* is one of the major components of the free-living seaweed community to which the related attached population does not contribute consistently. Loose thalli of *A. nayadiformis* exhibit morphological features very different from those of the attached specimens and possess a peculiar vegetative cycle including an overwintering phase. Free-living morphs are characterized by monopodial stoloniferous growth; in contrast, both determinate and indeterminate branching as well as the typical ramisymphodial growth pattern of the species are lacking. In autumn, free-living thalli fragment giving rise to stolon pieces which undergo deep structural changes in their sub-apical parts including both storage of floridean starch and extensive cortical thickening. Soon afterwards the uncorticated parts decay, whilst the modified fragments are able to overwinter and give rise to new fronds during next spring.

INTRODUCTION

The typical *Acanthophora nayadiformis* thallus shows a ramisymphodial growth pattern, which is responsible for its distinct morphology compared to that of the other species in the genus (Perrone et al., 2006). It is a perennial species due to secondary modifications of the basal parts which make the thallus able to perennate, so that in late autumn, when senescent fronds detach just above the modified parts and decay, both stumps and prostate system overwinter and produce new uprights in the next spring (Cecere & Perrone, 2002). *Acanthophora nayadiformis* also produces specialized vegetative propagules, the anatomy and developmental pattern of which have been investigated in detail (Perrone et al., 2005). Once shed, such propagules promptly sink, attach onto the substratum and give rise to new plantlets, representing, therefore, an advanced means of vegetative propagation of the attached form. The propagules shed in late autumn remain in a dormant state until next spring, whereupon they resume growth (Cecere & Perrone, 2002; Perrone et al., 2005, 2006). In the Mar Piccolo of Taranto, *A. nayadiformis* is also found unattached, as one of the major components of the free-living seaweed community, which is the most important vegetation in the basin (Cecere et al., 1992).

Periodical surveys showed that both the presence and the persistence of *A. nayadiformis* in the unattached seaweed community were ensured by effective means of both vegetative propagation and self-perennation which were distinct from those of the attached form. This study was undertaken to describe the characteristic morphology of the *A. nayadiformis* unattached specimens as well as their unique perennating strategy.

MATERIALS AND METHODS

Study site

The Mar Piccolo of Taranto (Ionian Sea, Mediterranean Sea) is an inner, semi-enclosed basin, showing lagoonal characteristics, located to the north of the town of Taranto (40°28'N 17°15'E). Seawater temperature ranges from 7.1 °C to 33.6 °C and salinity from 34.3 psu to 37.7 psu. In the unattached seaweed community, irradiance ranges from 5 to 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Photon Flux Density was measured weekly during sunny days between 1000 and 1200 noon by a AQUAMAT RS-232 Probe bearing a Photosynthetic Photon Flux Rate quantum sensor AQPL-UV 912 (IDROMAR®, Genova, Italy).

Sampling and field observations

Qualitative monthly field collections were carried out from the unattached seaweed community by SCUBA diving from June 2002 to June 2004. The collected material was brought to the laboratory where it was preserved in 5% formaldehyde-seawater for subsequent examination with both stereo and compound light microscopy (Leica Microsystems®, Wetzlar, Germany). For light microscopy, thalli were sectioned either by hand or with a Leitz Kryomat microtome (Ernst Leitz GmbH®, Wetzlar, Germany). Sections were observed either unstained or stained with aniline blue acidified by a drop of 0.5% HCl. Microscopic observations were also performed on the cultured material. First order axis was taken as a measure of the total thallus length.

Observations in culture

Laboratory cultures were carried on thallus fragments collected in autumn with the aim of monitoring their morphological modifications step by step.

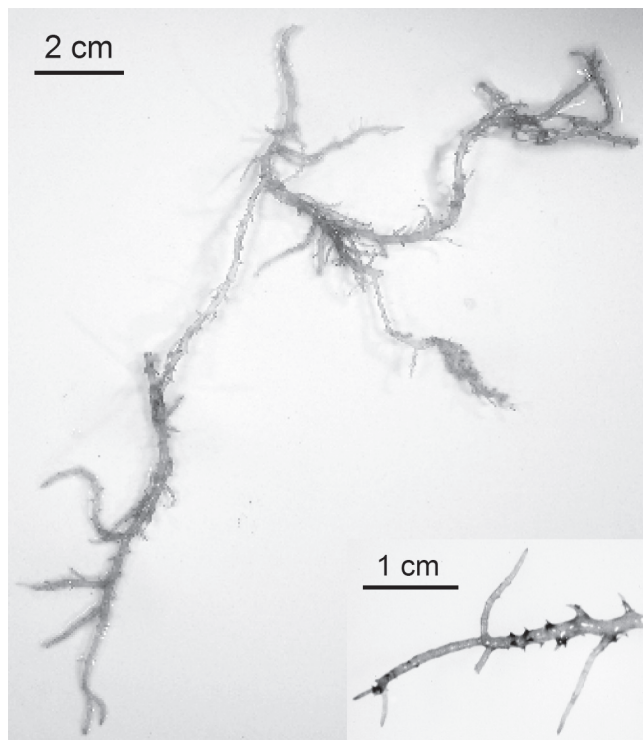


Figure 1. *Acanthophora nayadiformis*. Unattached summer thallus. Inset: apical part of a stolon-like axis.

Fragments were cultured in small aquaria (15-l) stored in growth chambers. The culture medium consisted of plain filtered (45 μm glass filter) seawater from the collection site, slightly agitated by bubbling air and was changed every four days. The irradiance was $60 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$ (OSRAM L cool white 18 W fluorescent tubes). Temperature and photoregime were adjusted monthly according to measurements made weekly in the field during the study period (11 °C, minimum temperature measured in February and 19 °C, maximum in November; L:D ranged from 8:16 in December to 14:10 in April). Cultures lasted from November to April.

RESULTS

Observations on freshly collected thalli

The morphology of the unattached specimens of *Acanthophora nayadiformis* was highly variable throughout the year and quite different from that of the attached form.

In summer, the unattached thalli reached a maximum length of 12 cm and exhibited a stoloniferous habit due to the transformation of the characteristic spinous upright apices into blunt apices that grew as terete stolons, sometimes bent and entangled with thalli of the same or other species present in the unattached community (Figure 1). Distal stolons typically grew according to a monopodial pattern only and were characterized by the absence or scarce production of determinate branches (trichoblasts and spines), scarce and irregular indeterminate branches to, at most, the second order, and sometimes bearing dwarf spines (up to 200 μm broad and 450 μm long) (Figure 1). At 5 mm from the apex, stolons had a diameter of 500 μm on average and in transverse section showed: the axial cell (30 μm in diameter on average), five periaxial cells (up to 140 μm in diameter), two layers of inner cortical cells (60–70 μm in

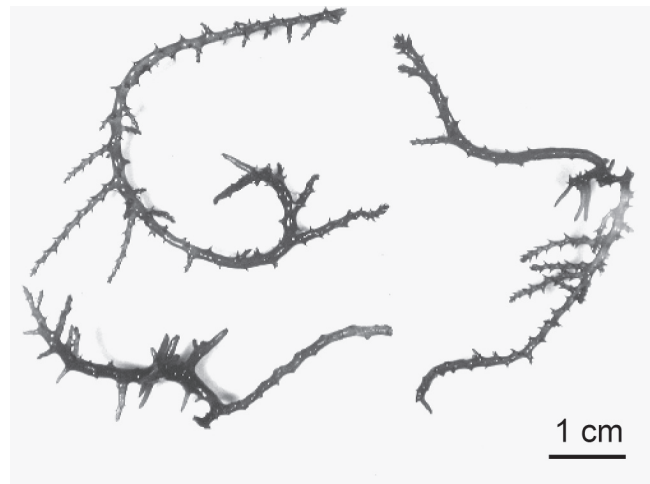


Figure 2. *Acanthophora nayadiformis*. Fragments of unattached autumn thalli.

diameter), few rhizoidal filaments (up to 15 μm in diameter) around the axial cell that intermingled with inner cortical cells, one layer of pigmented outer cortical cells (up to 15 μm in diameter).

Some anastomoses were observed among branches of the same thallus, thalli of the same species or other species with which they became entangled. The unattached thalli of *A. nayadiformis* brought neither reproductive organs nor the cone-shaped vegetative propagules typical of the attached specimens. Unattached thalli also completely lacked holdfasts, including both the simple rhizoidal discs of sporelings and the prostrate system typical of the mature thalli of all the species in the genus.

In late autumn, the unattached form of *A. nayadiformis* was only represented by irregular fragments, 4 cm long at most, entrapped in the dense seaweed assemblage. These were distal pieces of stolons, with their sub-apical parts greatly thickened and deeply pigmented (Figure 2). In winter, only these modified thallus fragments were present (Figure 3), some of which were occasionally found attached onto other entangled thalli by means of rhizoidal attachment discs. Winter fragments were dark red to brownish in colour, about 2 cm long and 1.5 mm in thickness. Their morphological features were insufficient to assign them to a red macroalgal taxon; only their anatomical characteristics allowed us to recognize that they belonged to *A. nayadiformis*. In transverse section such fragments showed: (1) an enlarged axial cell (about 80 μm in diameter); (2) five periaxial cells (105–135 $\mu\text{m} \times 90$ –180 μm); (3) up to four layers of roundish to polygonal inner cortical cells (up to 180 μm in diameter) with a thickened cell wall (up to 30 μm); (4) many large rhizoidal filaments (up to 24 μm in diameter) around the axial cell and intermingled with inner cortical cells; (5) up to five layers of deeply pigmented elongated to roundish outer cortical cells (15–20 $\mu\text{m} \times 20$ –30 μm), with a cell wall up to about 3 μm in thickness, not uniformly distributed around the external boundary. Periaxial cells, inner cortical cells and rhizoidal filaments were all filled with large floridean starch granules, up to 15 μm in diameter. In spring, thalli 1.5–2 cm tall with very short first order branches growing as stolons were present in the unattached seaweed community (Figure 4). Unattached plantlets derived from spores were never found.

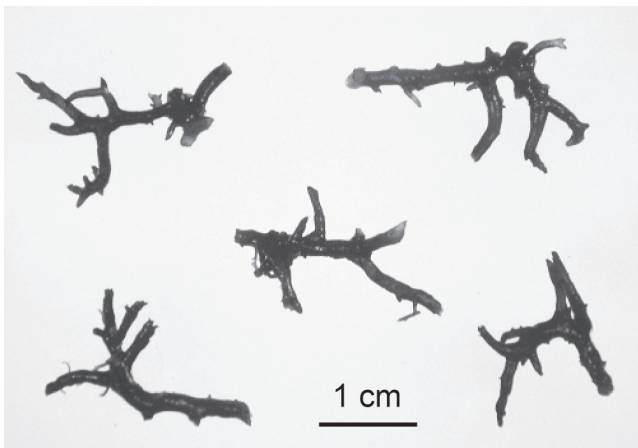


Figure 3. *Acanthophora nayadiformis*. Tuberized winter fragments.

Observations on cultured thalli

At the beginning of the culture, the thallus fragments collected in autumn stopped growing in length and the subapical part of the stolons began to grow in thickness for a length of at most 2 cm, producing many layers of pigmented cortical cells. The inner cortical and medullary cells enlarged and filled with massive amounts of floridean starch granules; their cell walls thickened. This modification was completed within 1–1.5 months. Subsequently, the unmodified parts of the thallus decayed and decomposed so that, in winter conditions in the growth chamber, only the above-stated fragments remained in culture, staying in a dormant state until late February. At the switch to spring conditions in the growth chamber, they give rise to new fronds that exhibited only monopodial growth and regularly formed determinate and indeterminate branches. Within 1–2 months, the consumed winter parent fragments decayed allowing each new axis to become independent.

DISCUSSION

Previous studies showed that *Acanthophora nayadiformis* was present throughout the year in the unattached algal bed of the Mar Piccolo, with very low biomass values in winter months (0.5–1 g fw 0.25 m⁻²), high values in summer, reaching the highest values in September and October (about 800 g fw 0.25 m⁻²) (Cecere et al., 1992). Spore contributions to the unattached community seem to be quite negligible, since plantlets derived from spore germination, which had the distinct morphology observed in the attached population (Cecere & Perrone, 2002), were never found; moreover, both carpospores and tetraspores showed low viability and slow germination potential in *in vitro* preliminary experiments (E. Cecere, unpublished data).

Typical unattached thalli of *A. nayadiformis* showed many of the features of other unattached seaweeds, as described by Norton & Mathieson (1983): they were morphologically very different from the attached specimens of the same species, lacked a holdfast, had bent thalli, remained vegetative, and did not even produce tetrasporangia. Mature unattached thalli were represented in summer by slender, little branched stoloniferous axes with no or scarce dwarf spines and bent apices intertwined around other unattached individuals.



Figure 4. *Acanthophora nayadiformis*. Unattached spring plantlet derived from a tuberized winter fragment.

Compared with the attached form of *A. nayadiformis*, which is characterized by a ramisymphodial organization, four distinct types of branch primordia and specialized vegetative propagules (Cecere & Perrone, 2002; Perrone et al., 2005), the unattached morph exhibited only monopodial development, did not form determinate laterals and produced only one type of indeterminate branch primordium, previously defined as ‘terete’, which typically develops into a stolon (cf. Cecere & Perrone, 2002). Finally, it never formed cone-shaped vegetative propagules. The anatomical structure of both attached and unattached morphs was the same, but in the unattached form every cell element was much smaller.

Distal stolon-like axes have been also observed in free-living thalli of another species of the genus, *A. spicifera* (Vahl) Børgesen, frequently reported as unattached in environments similar to the Mar Piccolo basin. In the unattached *A. spicifera* from the Virgin Islands, morphological features equivalent to those shown by the free-floating *A. nayadiformis* had been also pointed out by Børgesen (1918). Collado-Vides et al. (1994), describing the unattached seaweed community from a Mexican Caribbean coastal lagoon, drew an apex of *A. spicifera* quite different from that of the typical uprights of this species (cf. Perrone et al., 2006), probably because it was growing as a stolon apex. Our observation of some herbarium specimens [Herbarium abbreviations follow Holmgren et al. (1990)] of *A. spicifera* from lagoonal environments (13.XII. 1961, Puerto Rico Aguirre, no. 962221 681 L; 26.V.1964, Florida Virginia Key, unnumbered sheet, L; 20.I.1906, St Croix, no. 1216, C) confirmed that such plants also bear stolon-like apices (Cecere & Perrone, personal observations).

In the attached thalli of *A. nayadiformis*, stolons develop as terete, smooth, irregularly and scarcely branched axes without determinate laterals typical of the genus, such as trichoblasts and spines (Perrone et al., 2006), and have the tendency to basipetal growth and production of attachment rhizoids.

Stolons of cultured vegetative propagules of *A. nayadiformis* have proved to be able to change their development into upright axes under high irradiance and long-day experimental conditions (Perrone et al., 2005). In the unattached form, in

contrast, the opposite trend was observed in the field: both main and lateral apices, although initially produced from primordia of upright axes, early transformed into stolon apices and developed as typical stolons bearing rhizoids.

As in other macroalgal species, morphogenetic trends to a stoloniferous growth in the unattached *A. nayadiformis* might be caused by both exogenous and endogenous factors. Nutrient deficiency, low light intensity, and low water exchange, that frequently occur inside the algal mats, have also proved to be responsible for stoloniferous growth in cultured *Pterocladia capillacea* (S.G. Gmelin) Santelices & Hommersand (Felicini et al., 2002). Among the endogenous factors, a loss or a reversal of thallus polarity could be caused by the lack of a fixed orientation.

With regard to the lack of reproductive organs in the unattached *A. nayadiformis*, many algal species are infertile or at the most tetrasporic, when free-floating. Some interpretations have been suggested for this widespread phenomenon, such as lack of fixed orientation interfering with the receipt of the reproductive stimulus, abrasion damage and inadequate irradiance levels (Norton & Mathieson, 1983). In the unattached form of *A. nayadiformis*, in contrast, the lack of both reproductive organs and vegetative propagules could be merely caused by the stoloniferous growth pattern. Stolons, in fact, are greatly modified branches which lack attributes, such as trichoblasts and spines, that are necessary for the production of both reproductive organs and vegetative propagules (Cecere & Perrone, 2002).

In the Mar Piccolo, a massive fragmentation involving most of the unattached components of the seaweed beds occurs every year in late summer (Cecere & Petrocelli, unpublished data). Asexual reproduction via vegetative propagation by fragmentation is very common among macroalgae and the fragments show high survival and reattachment capacity (Norton & Mathieson, 1983). Since fragmentation occurs at an unfavourable season for growth of the unattached thalli of *A. nayadiformis* in the Mar Piccolo, natural decay of the fragments might be expected. On the contrary, deep structural modifications lead fragments to overwinter. Fragmentation could, therefore, represent an effective means of vegetative propagation of the unattached form of *A. nayadiformis*, and to verify and quantify this phenomenon could be an interesting challenge for future research.

The 'tuberized' overwintering fragments of the unattached form of *A. nayadiformis* are so strongly modified morphologically that they were not readily recognized as belonging to this species. Thallus modification involves the subapical parts of distal stolons, which undergo: (1) an extensive centrifugal growth of the outermost cortical cells; (2) the enlargement of every inner cortical and medullary cells; (3) the storage of massive amounts of floridean starch; and (4) the thickening of the cell walls. An analogous modification, involving both frond stumps and stolons, was already described in the attached morph as a means for preparing its prostrate system for perennation (Cecere & Perrone, 2002). In both attached and unattached thalli, this process occurs in late autumn when temperature and day length fall. Such thallus modification had not been previously observed among unattached seaweeds, and it is really

similar to the higher plant tuberization. It is also remarkable that short days and low temperatures seems to be triggers for the 'tuberization' process in a red macroalgal thallus, as happens in higher plants (Anjum & Villiers, 1997). Stolon 'tuberization', in fact, was also achieved in *in vitro* cultured stolon fragments of *A. nayadiformis* under autumn conditions (short days and low temperatures at our latitude) and in a medium without either nutrients or growth promoters.

In *A. nayadiformis*, in as much as the cone-shaped vegetative propagules, characteristic of the attached form, proved to be an effective mean for both population increase and intermediate distance spread (Perrone et al., 2005), so the overwintering 'tuberized' fragments characteristic of the unattached morph could play a crucial role in the maintenance of the free-living population, overcoming the unfavourable season. They both could be compared to turions and tubers of many hydrophytes, respectively (Perrone et al., 2005).

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