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Life history of two species of *Bryopsis* from the Straits of Messina (Italy)

Abstract

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Observations on cultured specimens of *Bryopsis* J.V. Lamouroux, a genus with many species of uncertain taxonomic status, carried out in order to obtain a better understanding of the life histories of *Bryopsidaceae*, are presented. In particular, the life histories of *B. hypnoides* J.V. Lamouroux and *B. corymbosa* J. Agardh showed by two populations collected in the Straits of Messina (the Mediterranean Sea) and grown in laboratory conditions, are here outlined. In both species the life history is diplobiontic and strongly heteromorphic. Gametophytes are either monoecious (in *B. hypnoides*) or dioecious (in *B. corymbosa*). The germling zygotes form microscopic sporophytes, which, after a period of dormancy from some months to a year or more, produce holocarpically stephanokontan zoospores. Both gametophytes and sporophytes are able to reproduce vegetatively. A life history of this kind is typical for the genus *Bryopsis*. This is the first report on the life history of *B. corymbosa*, a taxon described on Mediterranean specimens.

Introduction

The genus *Bryopsis* J. V. Lamouroux (1809) (Chlorophyta, Bryopsidales) comprises siphonous green algae, and is widely distributed from tropical to polar seas. The taxonomy of these algae is difficult since thalli are scarcely differentiated and reproductive structures are often not present in field material (Calderón-Sáenz & Schnetter 1989). Many species of *Bryopsis* were described, mainly from the Mediterranean Sea (Morabito 2000); however, morphological characters used to distinguish taxa are overlapping and the status of many species is often uncertain. Variations in branching pattern could be due either to ecological factors, or to genic flux between species (Womersley 1984). Observations of cultured specimens allowed a better understanding of the life histories of *Bryopsidaceae*, and led to a taxonomic reevaluation of this group. The genera *Pseudobryopsis* Berthold in Oltmanns (1904) and *Bryopsidella* J. Feldmann (1970) were established on species of *Bryopsis* and were separated on the basis of different reproductive structures or life histories.

Even though observations on the biology of the genus *Bryopsis* were carried out only on few species, different patterns of life cycle were reported. In the Mediterranean, data on life histories are particularly interesting because studies were carried out not later than the 70s.

They deal with two populations of *B. plumosa* (Hudson) C. Agardh from Naples (Schussnig 1930; Zinnecker 1935; Rietema 1970) and Banyuls (Rietema 1970; Kermarrec 1975) as well with populations of *B. muscosa* J. V. Lamouroux (Kermarrec 1975) and *B. hypnoides* J. V. Lamouroux (Neumann 1970; Rietema 1971a, 1971b) both from Banyuls. In all the above studies gametophytes, either monoecious or dioecious, are reported alternating to creeping microthalli (sporophytes), which, after a variable period of dormancy, can follow two pathways: some isolates produce holocarpically stephanokontan zoospores, which develop into gametophytes; in others the gametophytes arise directly from sporophytes. One population, as *B. plumosa* from Naples, can show both patterns (Rietema 1970).

Here, we outline the life histories of two populations of *B. corymbosa* J. Agardh and *B. hypnoides* J. V. Lamouroux collected in the Straits of Messina (Torre Faro and Reggio Calabria) and grown in laboratory conditions. Both species are Mediterranean taxa: the former species was established by J. Agardh (1842) on specimens collected in Livorno, Tyrrhenian Sea; the latter was established by J.V. Lamouroux (1809) from Sète, France. Unfortunately, the biology of *B. corymbosa* is still unknown in the Mediterranean Sea. Observations on biology and reproduction of these algae that could clarify their complex life histories mainly in the type area, are necessary for a correct identification.

Materials and methods

B. corymbosa J. Agardh was collected in Reggio Calabria, Italy; *B. hypnoides* J. V. Lamouroux was collected at Torre Faro (Messina), Italy. Cultures were initially obtained from fertile gametophytes: axes with gametangia were isolated, washed several times in sterile seawater and placed in sterile Petri dishes (10 cm Ø) with sterile seawater. In cultures of *B. corymbosa* (a dioecious species), each dish contained either axes with microgametangia only, or with macrogametangia only, or with both. In cultures of *B. hypnoides* (a monoecious species), each dish contained one fertile axis or more axes from several thalli. Cultures were incubated at 18 °C, in a 12:12 h light: dark cycle, under the light of a cool-white Triton Interpet 30 W fluorescent lamp, producing an irradiance of 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The release of gametes were observed as described by Burr & West (1970) and Speransky & al. (2000).

When zygotes were produced, 2 week old germlings were isolated and incubated under the same conditions, using modified Von Stosch medium (VS10; Gargiulo & al. 2001) in sterilised sea water, added with 5 mg l⁻¹ GeO₂ and 100000 U l⁻¹ Penicillin G, sodium salt. Culture medium was changed weekly and observations were carried out every two days; observations of released gametes were carried out daily in order to verify fertilisation and parthenogenesis. Vegetative isolation of thalli of both species was also carried out repeatedly to obtain unialgal stock cultures, maintained under the same conditions of sporophytes. Zoospores produced by sporophytes were isolated and cultured under the same conditions. Germling zygotes and sporophytes were fixed in 95% ethanol-acetic acid (3:1) and stained with 0,5 mg l⁻¹ DAPI (4,6 diamino-2 phenylindole) in seawater.

Results

Bryopsis corymbosa grows on rocks and concrete blocks outside the Port of Reggio Calabria, from the low tide water line up to 20-25 cm. Gametophytes (Fig. 1a) both in nature and in laboratory conditions produce biflagellate anisogametes (Fig. 1c) in proximal vegetative-like ramuli (Fig. 1b), microgametangia are orange and macrogametangia are dark green; thalli are dioecious. Gametophytes produce gametangia repetitively in culture; in the field thalli are reproductive from December to February.

In culture, gametes are differentiated on thalli not younger than 30 days; gametangia complete their differentiation in 48-72 hours and release gametes few minutes after the beginning of the light period of the next day by either a subapical papilla or sometimes two papillae in different positions. Macrogametes, provided with an eyespot, are $5,4 \pm 0,8 \mu\text{m}$ long and $3,6 \pm 0,6 \mu\text{m}$ wide; microgametes are $4,0 \pm 1,2 \mu\text{m}$ long, $1,9 \pm 0,5 \mu\text{m}$ wide and they lack eyespot. Microgametangia also bear anomalous gametes with different dimensions and with 2-4 flagella. Many zygotes (Fig. 1d) are present in dishes with gametangia of both sexes. Parthenogenesis occurs neither in dishes with macrogametangia nor in dishes with microgametangia. Zygotes enlarge and germinate unipolarly within 4 days after fertilisation (Fig. 1e): many settle on the bottom of dishes, while only few float. 10 day-old germlings form siphonous, irregularly branched creeping filaments (Fig. 1f). Within two months germlings reach their maximum size and stop growing (Fig. 1g). Siphon diameter is not uniform, even in the same thallus: average diameter is $146 \pm 30 \mu\text{m}$, average length is $6,6 \pm 1,8 \text{ mm}$. At maturity, sporophytes are able to reproduce vegetatively through fragmentation of thalli: a sporophyte produces a new branch connected by a thin siphon in which a septum develops (Fig. 1h). After 1-2 days this branched propagulum (Fig. 1i) is cut off and reaches appearance and dimensions of an old thallus within one month. Fragmentation of sporophytes is very common in culture. After a variable period of time, but not less than three months from fertilisation, few sporophytes produce holocarpically stephanokontan zoospores ($32,3 \pm 4,9 \mu\text{m}$ Ø) (Figs 1j-1k). They are released by breaking of cell wall, settle down and germinate within two days (Fig. 1l). Most of sporophytes have a period of dormancy of two years or more; zoospores production occasionally occurs in culture conditions. Zoospores germinate mainly bipolarly (Figs 1m-1n), but also in a different way with three or more germling siphons arising from the spore. Germlings produce plumose gametophytic thalli similar to those collected in nature, but more loosely branched.

Gametophytes reproduce vegetatively in several ways. In the field new axes frequently arise from creeping ones or by detachment of ramuli (Fig. 1o): proximal ramuli become plugged at their base and may develop a rhizoid before detachment; a scar is often visible on the parental thallus. Vegetative multiplication often occurs in nature. In laboratory conditions, any portion of the thallus, accidentally or voluntarily detached, is able to regenerate; in injured or punched siphons, cytoplasm rearranges in spherical masses that can regenerate an entire plant if the wall of the parental thallus is removed. Thalli with regenerated portions or with spherical masses of protoplasm inside damned siphons are also present in field material.

At the study site, *Bryopsis hypnoides* grows on concrete blocks from low tide water line down to 1 m. Its life history is quite similar to that of *Bryopsis corymbosa* with few dif-

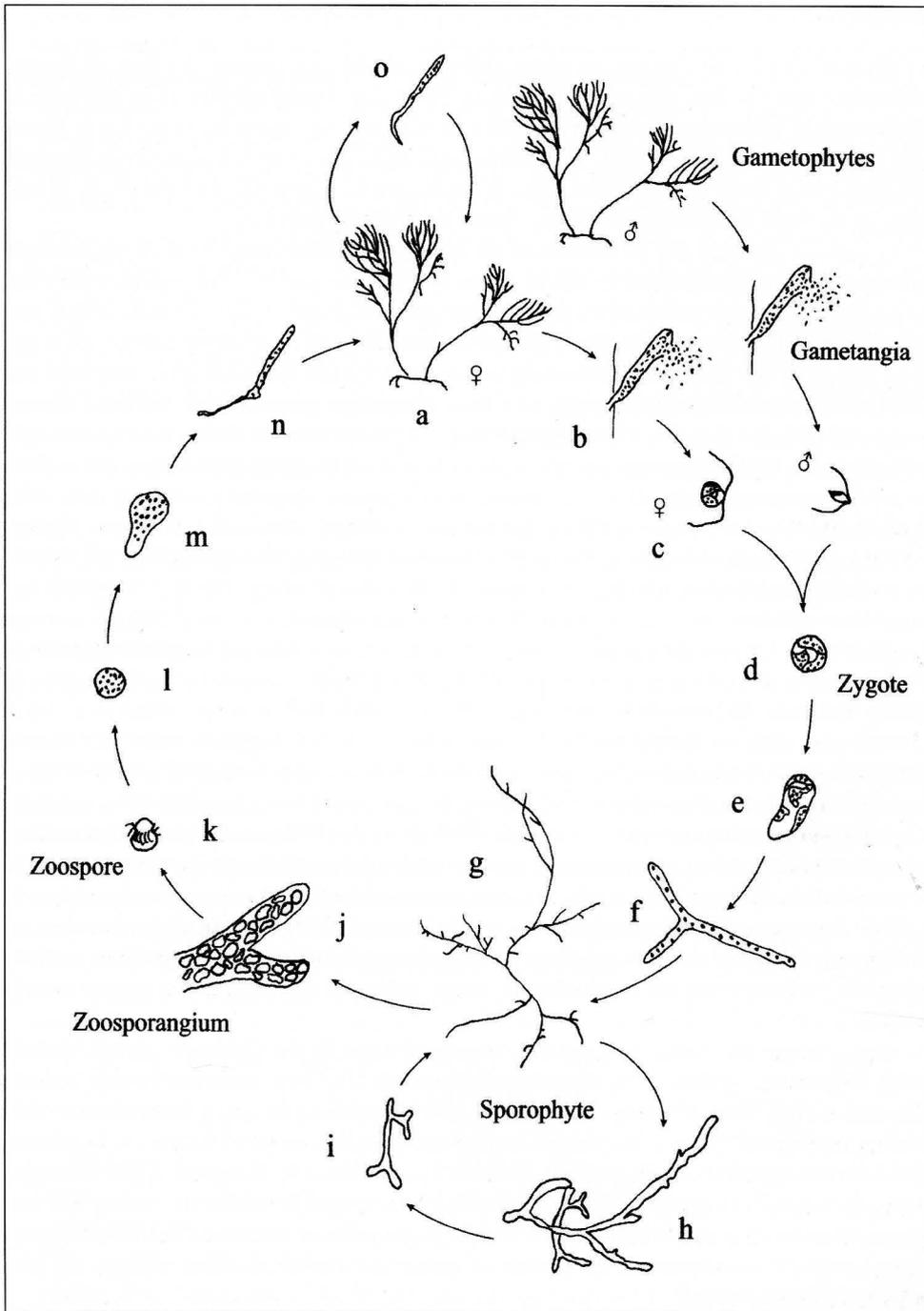


Fig. 1. Life history of *B. corymbosa* J. Agardh from the Straits of Messina (Reggio Calabria, Italy), as inferred in the present study.

ferences. Thalli are monoecious and each gametangium bears gametes of both sexes; gametangia are dark green. Macrogametes have an eyespot and they are $10,9 \pm 0,9 \mu\text{m}$ long and $4,4 \pm 0,6 \mu\text{m}$ wide. Microgametes are $7,3 \pm 1,2 \mu\text{m}$ long and $2,6 \pm 0,4 \mu\text{m}$ wide and have no eyespots. Gametes release is similar to that of *B. corymbosa*. Sexual reproduction occurs repetitively in culture: the species is homothallic since fertilisation occurs in both dishes with gametangia from different thalli and with gametangia from one thallus only. Development of germling zygotes is similar to that of *B. corymbosa*; sporophytes reach their maximum size after two months and then fragmentation begins. Average siphon diameter is $159 \pm 34 \mu\text{m}$, average length is $10,5 \pm 1,8 \text{ mm}$. Occasionally sporogenesis occurs in a few thalli not younger than three months. Sporophytes have a period of dormancy comparable to that of *B. corymbosa*. Gametophytes grown in culture are similar to those collected in the field, but the former have a looser branching pattern. Vegetative reproduction of gametophytes often occurs in nature and in culture as in *B. corymbosa*.

Discussion

Life histories of both *B. corymbosa* J. Agardh and *B. hypnoides* J. V. Lamouroux from the Straits of Messina, as showed in the present study, are diplobiontic and strongly heteromorphic (Fig. 1). Macroscopic gametophytes, monoecious in *B. hypnoides* and dioecious in *B. corymbosa*, produce biflagellate anisogametes. Microscopic sporophytes, after a period of dormancy ranging from some months to a year or more, produce holocarpically stephanokontan zoospores. Both gametophytes and sporophytes are able to reproduce vegetatively. A life history of this kind is typical for the genus *Bryopsis*.

This is the first report of the life history of *B. corymbosa*, a taxon described on Mediterranean specimens. Okuda (1994) investigated Japanese isolates of *B. corymbosa*, whose life history is similar to the Mediterranean one.

Considering his studies on some populations of *Bryopsis*, Rietema (1971b) pointed out a correlation between life histories and latitudinal distribution in European coasts. Mediterranean isolates of *B. plumosa* and of *B. hypnoides* (Rietema 1970, 1971a, 1971b) can follow two patterns in their life history, with either microthalli releasing stephanokontan zoospores (diplobiontic cycle) or with gametophytes arising directly from sporophytic thalli (monobiontic cycle); anyway they have a hard-breaking period of dormancy. Isolates from higher latitudes (from Biarritz, Roscoff and Rade de Brest, in Atlantic coasts) have only diplobiontic life histories, and dormancy easier to break (Rietema 1969, 1970, 1971b). Otherwise, isolates from more Northern latitudes (from some locations in the Baltic Sea and in the North Sea) have only a monobiontic cycle and a brief dormancy (Rietema 1969, 1975). Reports of other researchers on populations of *B. hypnoides* and *B. plumosa* from Europe (Neumann 1969, 1970; Kermarrec 1972) agree with those of Rietema, except for period of dormancy sometimes longer, and for the detection of parthenogenesis occurring occasionally in female gametes of *B. plumosa* from Britain (Kermarrec 1972). Nevertheless, observations carried out by other authors disagree with this pattern. Particularly, *B. plumosa* from Iceland has a very long period of dormancy, over 18 months, and has a monobiontic life history (Jónsson 1980). A population of *B. hypnoides* from Wales (Diaz-Piferrer & Burrows 1974) has a Mediterranean-like life history.

Variations on these patterns of development are known. Both macrothalli and microthalli of an isolate of *B. hypnoides* from Newfoundland are able to produce gametes (Bartlett & South 1973). In both cases zygotes grow into creeping filaments, while plumose thalli arise directly on microthalli, without any productions of zoospores. Such a life history doesn't fit at all with Rietema's interpretation. *B. hypnoides* from Newfoundland could be genetically separate from the European one. Our observations on *B. hypnoides* differ from those of Neumann (1970) on a population of this species from Banyuls, which does not produce zoospores and have gametophytes arising directly from sporophytic plants. However, they correspond to those of Rietema (1971a) on a population of the same species (as *B. monoica*) from Banyuls too. In the present study, both *B. hypnoides* and *B. corymbosa* have a life history that matches partially with the Mediterranean cycle of *Bryopsis* species as inferred by Rietema, since microthalli release only stephanokontan zoospores (diplobiontic cycle) and a monobiontic cycle has never occurred in tested conditions; they also have a hard-breaking period of dormancy. The break of the period of dormancy is an unsolved aspect of life history of *Bryopsis*, since it is related neither to photoperiod, nor to irradiance, nor to temperature (Díaz-Piferrer & Burrows 1974; Jónsson 1980), and sporophytes seem to need a period of maturation after which they develop under various conditions. This period of dormancy could be an adaptation to survive while conditions become favourable to trigger the production of erect axes of gametophytes (Richardson 1982). However further studies are required to clarify this feature.

Vegetative reproduction of gametophytes has a very important role in the life history of *Bryopsis* species. Several alternative ways can occur, as fragmentation of thallus, cutting off ramuli or cytoplasts formation, sometimes referred as aplanospores. They are frequent in culture conditions, as they are in the field (present study, Díaz-Piferrer & Burrows 1974; Richardson 1982). Brück & Schnetter (1993) described for the first time this way of multiplication on a population of *B. plumosa* from the Canary Islands. However, our observations on *B. corymbosa* and *B. hypnoides* from the Straits of Messina don't fit exactly with the ones on *B. plumosa*. Particularly, Brück & Schnetter (1993) reported for the latter species that sporophyte has a region specialized for vegetative multiplication, a thin branch that produce an adventitious thallus similar to the parental one. Later, by necrosis of the connecting axis, this "duplex structure" separates into independent plants. In species we have investigated, multiplication is due to adventitious branched propagula, that are cut off in a defined abscission zone and then grow to dimensions of mature thalli. Production of adventitious thalli by uninucleate sporophytes is very important in order to understand the life history of *Bryopsis*. Uninucleate microthalli of *Bryopsis* should be considered as sporophytes (see also Brück & Schnetter 1997), rather than a specialized zygote, as suggested by van den Hoek & al. (1995), because they are lasting and independent thalli, with their own development cycle, independent from sporogenesis and capable of vegetative multiplication. According to Brück & Schnetter (1993), only few sporophytes undergo sporogenesis in the field.

Further observations should be useful to highlight the biology of reproduction of this genus, particularly of microthalli development, both in the field and in controlled conditions, in order to test which physical or physiological factors control the induction of sexual *versus* asexual reproduction and seasonality of the two phases.

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