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ON THE LIFE HISTORY OF *ACETABULARIA ACETABULUM (= MEDITERRANEA)* IN THE LABORATORY AND IN NATURE

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Summary. - The authors compare the life cycle of *Acetabularia acetabulum (Dasycladaceae)* grown under articicial or under natural conditions. New evidence is presented, supporting the view that in nature *Acetabularia* behaves as a «pluriannual» plant.

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Résumé. - Les auteurs comparent le cycle de vie d'*Acetabularia acetabulum (Dasycladaceae)* dans des conditions artificielles ou naturelles de croissance. Les observations rapportées supportent l'hypothèse selon laquelle, dans la nature, *Acetabularia* se comporte comme une plante «pluriannuelle».

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Samenvatting. - De auteurs vergelijken de levenscyclus van *Acetabularia acetabulum (Dacycladaceae)* die groeien onder kunstmatige of natuurlijke omstandigheden. De resultaten ondersteunen de hypothese dat, in de natuur, *Acetabularia* een «meerjarige» plant schijnt te zijn.

ON THE LIFE HISTORY OF *ACETABULARIA ACETABULUM* (=*MEDITERRANEA*) IN THE LABORATORY AND IN NATURE

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1. INTRODUCTION

The green marine alga *Acetabularia acetabulum* (L.) Silva (1952)(= *A. mediterranea*, LAMOUROUX) is a giant unicellular (NäGELI, 1847; WORONINE, 1861) uninucleate (HäMMERLING, 1931) organism. It is used for studies not only on cellular and molecular biology (HäMMERLING and ZETSCHE, 1966; BRACHET, 1968; SCHWEIGER, 1969; PUISEUX-DAO, 1970) but also on radiobiolgy, radioecology and chemical and radioactive pollution of the environment (BONOTTO et al., 1976).

The morphogenesis of *Acetabularia* is very complex and not yet completely understood. At maturity, the cell forms a reproductive cap, which is constituted by a mean of 74 ± 0.45 rays, normally distributed (LONI and BONOTTO, 1971). According to present knowledge, cap formation in *Acetabularia* is controlled only *indirectly* by the nucleus, by means of "morphogenetic substances" (HäMMERLING, 1934a), which are identified with long-lived molecules of nuclear messenger RNA (m-RNA) (BRACHET, 1962, 1965; HäMMERLING and ZETSCHE, 1966; ALEXEEV et al., 1974, 1975). The development of the reproductive cap is the most investigated morphogenetic process, because it is easy to observe. Other important morphogenetic processes are cyst formation in the cap's rays and zoid differenciation in the cysts (BONOTTO and KIRCHMANN, 1970).
Moreover, of particular interest is the formation of the whorls and the rhizoids, which grow respectively at the apical and at the basal end of the stalk. Their development has been scarcely investigated, though they may have a significant physiological role (BONOTTO, 1969; GIBOR, 1973a,b; BONOTTO et al., 1976).

The life history of *Acetabularia acetabulum* and related species of the *Dasycladaceae* family was studied by a number of authors (WORONINE, 1861; HäMMERLING, 1931, 1932; SCHULZE, 1939; ARASAKI, 1942; CRAWLEY, 1966; PUISEUX-DAO, 1970; BONOTTO, 1975; KOOP, 1975, 1979a,b; see other references in BONOTTO and LüTTKE, 1980).

In spite of this effort, some aspects of the reproductive cycle of *Acetabularia* still remain obscure. The scope of this paper is to compare the life cycle of *Acetabularia* grown under artificial and under natural conditions and to discuss new evidence supporting an old view (WORONINE, 1861; de BARY and STRASBURGER, 1877), according to which *Acetabularia* may behave in nature as a "pluriannual" plant.

2. MATERIAL AND METHODS

The observations were made on algae *(Acetabularia acetabulum)* collected near Calvi (Corsica) and kept alive in filtered sea water and on cultured algae (LATEUR and BONOTTO, 1973), originating from the collections of Professor J. Brachet (University of Brussels) and of Professor S. Puiseux-Dao (University of Paris VII).

The algae were observed with a Spencer stereomicroscope equipped with a Polaroid camera. The basal part of the stalk and the rhizoids of *Acetabularia* cells grown in their natural habitat were freed from contaminant debris or from calcareous material with fine tweezers. The utilization of acids for dissolving encrusting calcareous materials (WORONINE, 1861; de BARY and STRASBURGER, 1877) is not advisable because the eventual cytoplasm present in the rhizoids would be damaged and the typical green colour of chlorophyll, contained in the chloroplasts of the basal stalk, would disappear.

3. OBSERVATIONS

3.1. THE LIFE CYCLE OF *ACETABULARIA* IN THE LABORATORY

Acetabularia acetabulum, although unicellular, may present several types of reproduction (Fig. 1). The plant may reproduce in the following 5 ways :

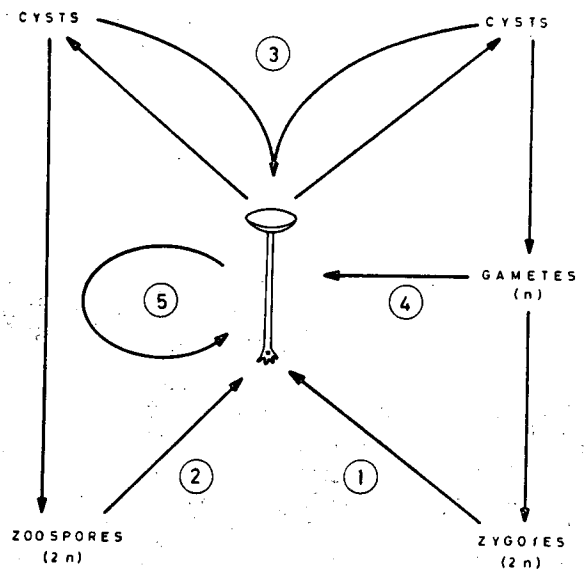

Fig. 1. Scheme showing the different possible types of reproduction of *Acetabularia acetabulum* (explanation in the text). (From BONOTTO, 1975).

1. By gamete fusion

Two isogamous (HÄMMERLING, 1931, 1932) or anisogamous (PUISEUX-DAO, 1962) gametes (having a positive phototropism) form by fusion (CRAWLEY, 1966, 1970) a zygote (having a negative phototropism), which gradually transform into a young plant (Fig. 2).

This is the type of reproduction most frequently occurring under artificial laboratory conditions (BONOTTO and LÜTTKE, 1982), and it is believed also to be the predominant one in nature.

2. By zoospore formation

The terms "spores" and "zoospores" were already utilized in Woronine's paper (WORONINE, 1861). The cysts would produce zoospores, which transform directly into a new plant. In this case gametogenesis is short-circuited. This type of reproduction has been reported for *Acetabularia acetabulum (= A. mediterranea)* (PUISEUX-DAO, 1962, 1970), *Acetabularia clavata* (VALET, 1968, 1969) and *Acetabularia parvula (= A. wettsteinii = A. moebii;* VALET, 1969) (PUISEUX-DAO, 1965). This mode of reproduction has been questioned by HÄMMERLING (1964).

3. By cyst germination

The cysts develop directly into a new plant (PUISEUX-DAO, 1970; BONOTTO, 1975). This type of reproduction was observed in *Acetabularia acetabulum (= A. mediterranea)* as well as in *Acetabularia dentata* (VALET, 1968, 1969) and in *Batophora oerstedii* (PUISEUX-DAO, 1970). It concerns not only the cysts formed in the cap but also those which sometimes develop in the stalk. In the latter case, the germlings perforate the cell wall. It is not known, however, if perforation occurs by mechanical breaking, provoked by germling growth, or by cell wall lysis, due to enzymatic action.

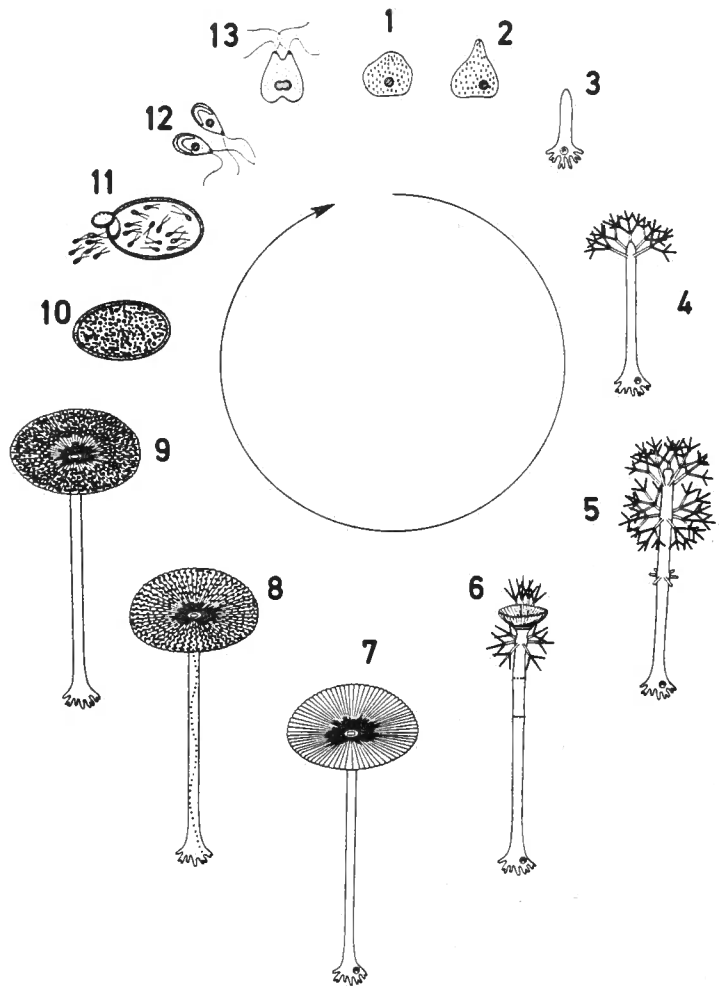

Fig. 2. Reproductive cycle of *Acetabularia acetabulum* most frequently occuring under artificial laboratory conditions.
1. zygote; 2. zygote showing polar growth; 3. stalk and rhizoids development; 4. whorl formation; 5. cap initiation : the form of the apical region changes (see : WERZ, 1965); 6. cap growth; 7. maximal cap and cell size (see : LONI & BONOTTO, 1971); 8. secondary nuclei formation and their migration into the cap's rays; 9. cyst formation; 10. cyst with nuclei and cytoplasmic organelles; 11. release of gametes; 12. two gametes; 13. gamete fusion. The secondary nuclei, the cysts and the gametes are haploid (see GREEN, 1973 and KOOP, 1975, 1979a,b). (From BONOTTO, 1975, modified).

4. By gamete parthenogenesis

In this case gametes develop parthenogenetically. Since gametes of opposite sign exist, theoretically haploid plants of different sign would be produced if both types of gamete germ. The early report by HÄMMERLING (1934b) on this possible form of reproduction has been discussed by PUISEUX-DAO (1970). Parthenogenesis in *Acetabularia* was observed more recently by GREEN (1976) and by PRIMKE et al. (1978). Haploid plants showed reduced growth and were unable to develop normally and to form a reproductive cap.

5. By germination of basal cytoplasm

We have repeatedly observed that adult plants at stage 9 or 10 (BONOTTO and KIRCHMANN, 1970) may produce a new cell by germination of basal cytoplasm remained in the rhizoids. This phenomenon was observed in cells bearing a single cap as well as in branched cells having 2 or 3 mature caps (see Fig. 4 in BONOTTO, 1975). Under our culture conditions, however, this type of reproduction may concern at most about 7 % of the algal population (BONOTTO, 1975). Since cultured *Acetabularia* have a transparent cell wall, due to poor calcification (BERGER et al., 1974), cytoplasmic residual bodies remained in the rhizoids (or in the stalk) may be easily seen under the stereomicroscope.

3.2. THE LIFE CYCLE OF *ACETABULARIA* IN NATURE

Most species of the *Dasycladaceae* growing in nature possess highly calcified cell walls (WORONINE, 1861; de BARY and STRASBURGER, 1877; LEITGEB, 1887). Both the superior (Fig. 3A) and inferior (Fig. 3B) side of the cap are calcified, as well as the stalk. If regeneration occurs (Fig. 3C and D : arrows), the newly formed stalk and cap become progressively calcified.

Because of the strong calcification of stalk and cap, it is difficult to observe cytoplasm migration into the cap rays and cyst formation.

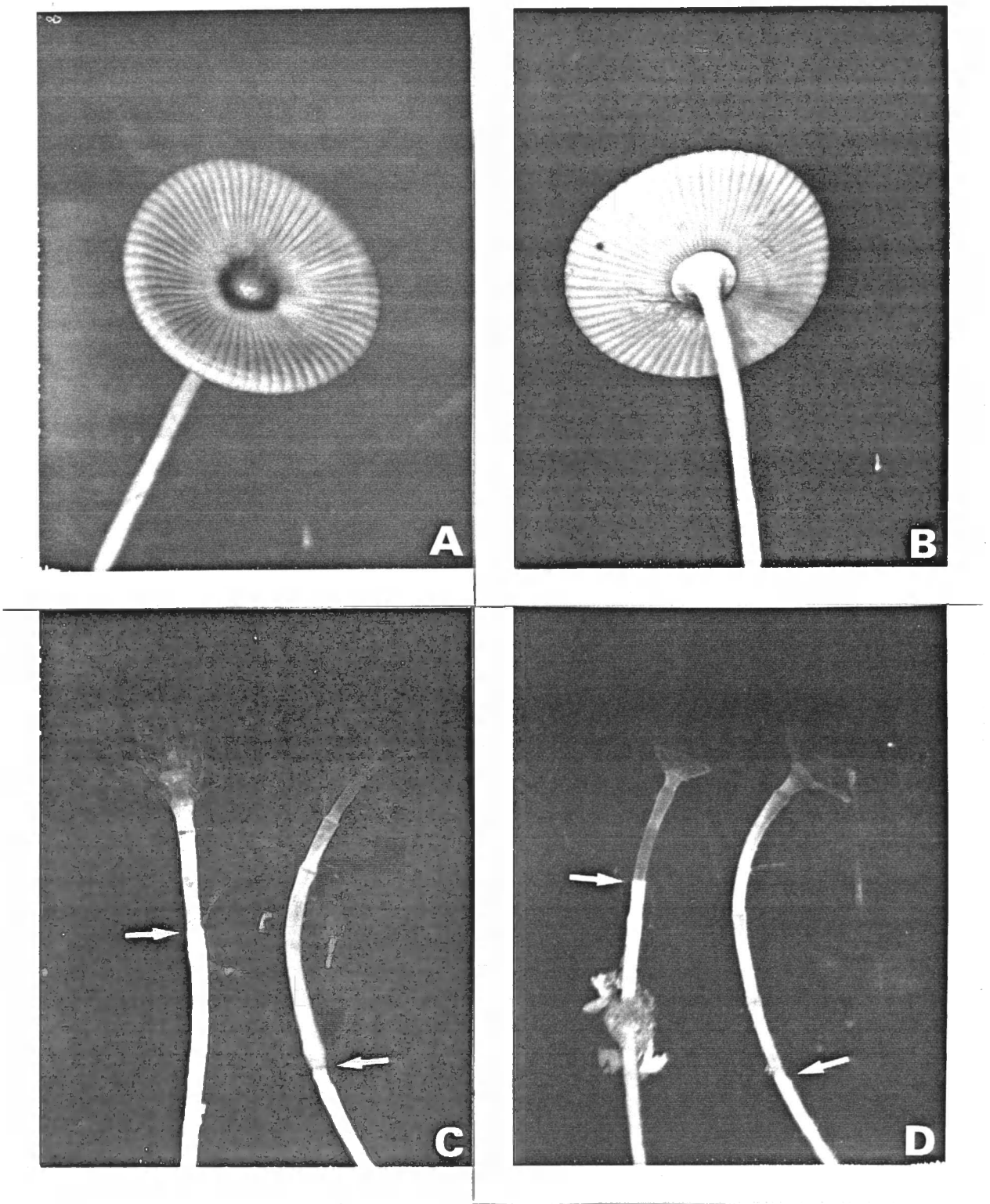

Fig.3. *Acetabularia acetabulum*. Cells collected near Calvi (Corsica), at depths of respectively 3m(A,B), 6m(C) and 15m(D). A : Superior side of a mature reproductive cap; B : Inferior side of the same cap; C : Stalk regeneration (arrows). The new stalk becomes progressively calcified as growth goes on. D : Stalk regeneration (arrows) and cap formation. The young caps are little calcified.

Early authors have used acids to dissolve the carbonates of the cell wall and the calcareous material around the rhizoids (WORONINE, 1861; de BARY and STRASBURGER, 1877). Acids may, however, damage the cytoplasm and destroy chloroplasts pigments. We have succeeded to make free the rhizoids of few plants simply by removing calcareous materials with fine tweezers. We have observed that rhizoids were located inside the limestone (Fig. 4A and B) and not at its surface. Moreover, the presence of small holes (Fig. 4A : arrow), having the size of a stalk suggest that some plants might have been eradicated. Three findings were of particular interest :

1) the basal part of the stalk was enlarged to form a calcified ring, just above the limestone surface (Fig. 4C and D : arrows);
2) the branched rhizoids were not calcified (Fig. 4C), so that some flexibility of the stalk was possible;
3) the rhizoids of algae having formed cysts still contained cytoplasm having chloroplasts, as inferred from its typical green colour. Fig. 4C and D shows that the rhizoids are penetrated inside the calcareous material, a phenomenon which has been reported by ARASAKI and SHIHIRA-ISHIKAWA (1979) for other *Acetabularia* species.

4. DISCUSSION

Acetabularia acetabulum may present 5 types of reproduction. This wonderful reproduction capability, however, is not an exception in the world of marine algae (DAWSON, 1966; LEVRING et al., 1969; BONOTTO, 1976). The mode of reproduction of *Acetabularia* seems to depend from the particular environmental conditions (PUISEUX-DAO, 1970). One may, thus, suppose that the plant might behave differently under artificial or under natural conditions. In nature, reproduction by cyst formation and zoid development seems quantitatively very important, since a single cell may produce several thousand cysts. It remains, however, unknown if under natural conditions :

1) both gametes and zoospores are formed or only one swarmer type;

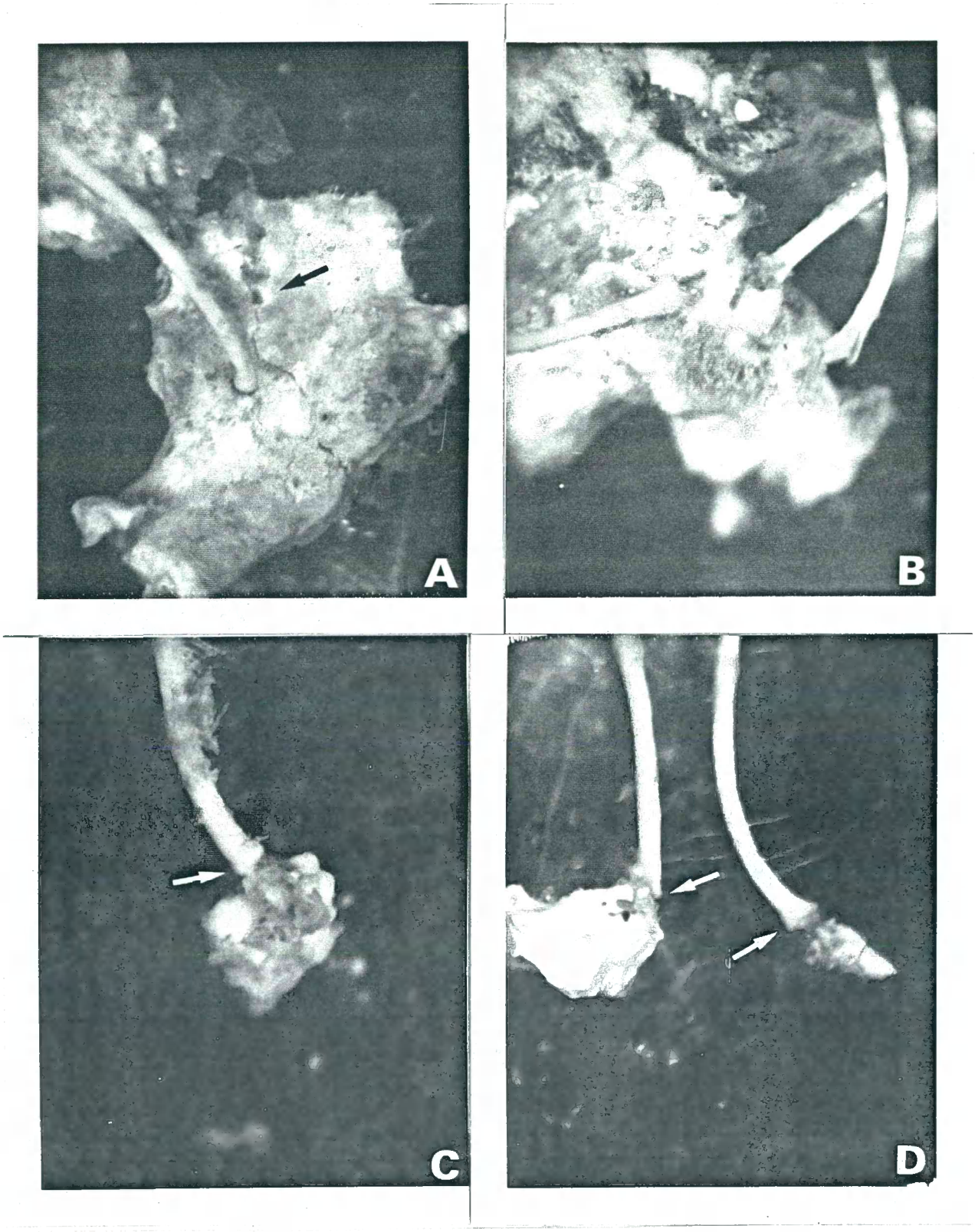

Fig. 4. *Acetabularia acetabulum*. Cells collected near Calvi (Corsica), at a depth of 15 m.

A : Basal part of one cell fixed on limestone. A small hole (arrow) show the probable emplacement of a cell, which may have been eradicated; B : Basal parts of three cells fixed on limestone; C : Basal part of a cell showing a ring-shaped calcification (arrow) and uncalcified rhizoids, containing cytoplasm with green chloroplasts; D : Basal part of two cells with the same ring-shaped calcification (arrows) and uncalcified rhizoids containing cytoplasm. Note the penetration of the rhizoids into the limestone.

2) parthenogenesis occurs;

3) direct cyst germination takes place.

Regeneration of a new cell from cytoplasm remained in the rhizoids, after cyst formation and loss of the mature cap, is probably much more frequent in nature than in the laboratory. Our observations on the rhizoids containing living cytoplasm confirm and extend those of WORONINE (1861) and of de BARY and STRASBURGER (1877).
Woronine writes :

> "...lorsque les spores sont entièrement formées, le chapeau tombe, et la tige de l'*Acetabularia* reste sous la forme d'un tube blanc calcaire, dans lequel toutefois se trouve une jeune cellule. Au printemps suivant ressort, ainsi que je l'ai dit plus haut, le bout de cette cellule (fig. 2, pl. 7), qui, vers la fin de l'été, se développe en chapeau; ce nouveau chapeau tombe à son tour, il ne reste alors que le tube, duquel se dévelopera l'année suivante un autre chapeau, et ainsi de suite".

On the other hand, de Bary and Strasburger confirm :

> "Wie Woronine beschrieben hat, ist *Acetabularia* eine Pflanze von mehrjähriger, jeder schirmtragende Stiel, oder kurz ausgedrückt, Schirmspross, von einjähriger Dauer. Am Ende der Vegetationsperiode sterben, unter den normalen Lebensbedingungen, die oberen Theile der Sprosse ab und zerfallen; nur die kalkigen Membranen der unteren Stielstücke bleiben wie Stoppeln auf dem Substrat stehen; der unterste, gewöhnlich aus Fuss und Basalstück bestehende Theil der Pflanze bleibt lebendig".

In nature, *Acetabularia* may thus survive during the winter, with the cytoplasm retracted into the rhizoids, which corresponds to the "Basalblase" of de BARY and STRASBURGER (1877). A new plant probably

develop from a residual body (the "Restkörper" of SCHULZE, 1939) remained in the rhizoids after the migration of the secondary nuclei. It would be of interest to known if this residual body contain a normal diploid nucleus. We known from the investigations of KOOP (1975, 1979a,b) that secondary nuclei are haploid and that "residual nuclei" may remain in the stalk and produce germlings. Theoretically a haploid secondary nucleus may remain in the rhizoids, but it would be difficult to explain how it could assure a normal cell regeneration, since experimentally obtained haploid cells (GREEN, 1976; PRIMKE et al., 1978) are abnormal, show poor growth and are incapable of generating a stalk with a cap.
In nature, the regeneration process would thus be continued by a presumably diploid nucleus.

In the *Dasycladaceae* family, other species were found to possess different modes of reproduction. *Cymopolia barbata*, for example, "has three different mechanisms by which it can adaptively maintain populations in a stressful grazing and tidal surf environment" (LIDDLE, 1979) :

1) gametes and zygotes formation;
2) spore germination;
3) cell regeneration from basal fragments.

Acetabularia , like *Cymopolia* may use different mechanisms to maintain its population in a competitive environment.

It is of interest to note that the rhizoids of *Acetabularia* were found inside the limestone and were not (or only poorly) calcified. According to the recent work of ARASAKI and SHIHIRA-ISHIKAWA (1979), who studied 7 species of *Acetabularia* , the rhizoid "perforates and penetrates into shell-pieces" and "most *Acetabularia* can survive during the seasons when the thalli disappear". The rhizoids are thus capable of penetrating into calcareous material, probably by a lytic process, like the shell-boring *Conchocelis*-phase of the red marine alga *Porphyra* (see literature in BONOTTO, 1976). If the substrate is very hard (e.g. glass)

the rhizoids would develop at its surface, as observed in laboratory culture (BONOTTO and SIRONVAL, 1977). New investigations would be necessary to know whether *Acetabularia* grows preferentially on calcareous substrates.

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