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PHYCOERYTHRINES
SYMBIONTES
ASCIDIES
SPONGIAIRES
CYANOBACTERIES

RÉSUMÉ — Cette recherche a pour but d'analyser les caractéristiques pigmentaires de diverses Cyanophycées préalablement décrites en symbiose avec les Eponges et les Ascidies (Duclaux, 1977; Lafargue & Duclaux, 1979). Elle permet de tester l'hypothèse de l'existence d'une même espèce *Synechocystis trididemni* Lafargue & Duclaux associée à deux espèces d'Ascidies morphologiquement très proches : *Trididemnum cyanophorum* Lafargue & Duclaux et *T. tegulum* Kott. Les caractéristiques de la phycoerythrine suggèrent que *T. cyanophorum* contient un symbionte différent de celui de *T. tegulum.*

PHYCOERYTHRIN
SYMBIONT
ASCIDIAN
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ABSTRACT — This investigation contribute to our knowledge of the pigment content of certain previously described Cyanophycea found in symbiotic association with sponges and ascidians (Duclaux, 1977; Lafargue and Duclaux, 1979). It is opportunity to test the hypothesis of a single symbiont *Synechocystis trididemni* Lafargue and Duclaux associated both with *Trididemnum cyanophorum* Lafargue and Duclaux and *T. tegulum.* Phycoerythrin characteristics suggest that *T. cyanophorum* contains a cyanophyte symbiont which is different from the cyanophyte symbiont of *T. tegulum.*

INTRODUCTION

Lafargue and Duclaux (1979) reported an association between a didemnid ascidian *(Trididemnum cyanophorum)* and a cyanophyte on reefs off Guadeloupe. They named this cyanophyte *Synechocystis trididemni.* Cox *et al.* (1985) found that cyanophytes from *Trididemnum tegulum* and several sponges might be identical to *S. trididemni.* In the algae, pigment composition together with cytology, metabolites and reproductive mode are the principal criteria for identification and classification.

The intention of this investigation was to contribute to our knowledge of the pigment content of certain previously described cyanophyceae found in symbiotic associations with sponges and ascidians (Duclaux, 1977; Lafargue and Duclaux, 1979). It was the opportunity to test the hypothesis of a single symbiont *Synechocystis trididemni* associated both with *T. cyanophorun* and *T.tegulum.*

MATERIAL

The hosts were collected by SCUBA diving : all sponges are Mediterranean species from near Banyuls-sur-Mer (F). The Didemnid ascidians are from the west coast off Guadeloupe (West Indies).

For pigment analysis, small fragments of the host colony (sponge or ascidian) were finely ground as this was the only way to separate the symbiotic algae from their host. The material used was either :

lyophilized : A (host : *Trididemnum cyanophorum* Lafargue & Duclaux, 1979, symbiont : *Synechocystis trididemni* Lafargue & Duclaux);

formolized : B (host : *Trididemnum palmae* Monniot 1984, symbiont : closely related to or identical with *S.trididemni) ;*

fresh : C (host : *Ircinia fasciculata* Pallas, 1766 containing *Aphanocapsa feldmanni* Fremy Feld., 1933 and *A. raspaigellae* Fremy Feld., 1933 or host : *Petrosia ficiformis* Poiret, 1789 containing *A. feldmani* Fremy Feld., 1933.

METHOD

Extraction and chromatographic analysis of liposoluble pigments from A

Pigments were extracted by 100 % acetone followed by 90 % acetone. The pigments were then transferred to diethyl-ether (Uvasol Merck) by NaCl (10 %) partition. The pigment solution is dehydrated by anhydrous cristalline NaCl, then concentrated by evaporation under nitrogen atmosphere to a volume of 1 ml. 50 µl of this solution was used for a bidimensional thin-layer chromatrography (TLC) over 0,25 µm Merck cellulose (Jeffrey, 1968). Developing solvents are n-propanol/petroleum ether (60-80°C) (2 :98) for the first dimension and chloroform (Merck)/petroleum ether (60-80°C) (30 :70) for the second dimension. Major spots have been dissolved in their appropriate solvent for a study of their absorption spectrum and subsequent identification.

Extraction of liposoluble pigments of B and C

Blotting of the material between filter paper leaves, followed by a 90 % acetone extraction. Fluorescence excitation and emission spectra were done on the crude extract.

Extraction of hydrosolubles pigments from A and C

For extraction the material is ground with fine sand in a 0,2 M NaH2PO4 buffer at pH 6.8. After centrifugation the supernatant is treated with 0,5 S NH4 2SO4 in order to precipitate and partially purify the phycobilins. The pigments are re-suspended in a 0.2 M NaH2PO4 buffer at pH 6.8 and their fluorescence excitation and emission spectra are recorded on an Aminco-Bowman spectrophotofluorometer. In the spectra, apparatus-specific perturbations due to the Xenon lamp, the Hamamatsu R446 photomultiplier, etc... have not been corrected for.

Identification of species of the genus Trididemnum

The reliability of data from pigment analysis of symbionts depends on accurate identification of the hosts. *Trididemnum cyanophorum, T. solidum* and *T. palmae* are all very similar and have been confused. They can be distinguished by their spicules. *T. solidum* and *T. cyanophorum* have spicules with 12-14 oblong rays in the observable hemispherical sector (Fig. 1A), whereas the spicule rays of *T. palmae* are shorter and numerous (19-31 normally 22-27, Fig. 1B), displaying a quite uniform morphology. In *T. cyanophorum*, on the other hand, there are two spicule forms : the blunt rays may stand isolated (F. Monniot, 1984, Pl. 1D) or be fused (Fig. 1A). This spicule morphology was constant in all colonies studies (several thousands in the case of *T. cyanophorum*, approx. one hundred in the case of *T. palmae).* The material of Galeta, Panama that Olson was kind to send us was a mixture of *T. solidum* (Fig. 2A) and *T. palmae* (Fig. 2B). The same is true for the types of *T. solidum* at the American Museum of Natural History (AMNH). The Bermuda specimem AMNH 1301 may, by its spicules be conspecific with *T. palmae* (Fig. 3A and B), whereas the Puerto Rico specimens AMNH 288 and AMNH 283 seem to be *T. solidum* (Fig. 4A and B).

RESULTS

All specimens contain chlorophyll *a* and phycobilins as shown by TLC for A (Fig. 5) and by fluorescence excitation (Fig. 6) and emission spectra (Fig. 7) recorded at several wavelength pairs for the specimens A and C. The total absence of accessory chlorophylls should be noted. This pigmentation is characteristic of true cyanophyceae.

In the case of the formolized *T. palmae* specimen, degradation products of chlorophyll *a* (pheopigments) are present. No trace of degradation products of other chlorophylls could be detected suggesting that its symbiont is also a Cyanophyceae.

For all three species, phycoerythrin is the major phycobilin. For *Synechocystis trididemni*, symbiont of *T. cyanophorum*, the fluorescence excitation spectrum shows two conspicuous peaks at 498 and 546 nm respectively (Fig. 6). They indicate the presence of two types of chromophores in the protein : phycourobilin and phycoerythrobilin (or a related chromophore). There is only one emission peak at 569 nm following excitation fixed between 475 and 558 nm. One can observe a shift of the major excitation peak towards the longer wavelengths (554 nm) for emission beyond 640 nm, but in this case the excitation maximum at 498 nm subsists. Moreover, an excitation peak near 618 nm indicates the presence of C-phycocyanin and its chromophore (phycocyanobilin).

For the symbionts of *Ircinia fasciculata*, the fluorescence excitation of phycoerythrin (for emissions fixed between 570 and 600 nm) is quite similar to the former. For emission wavelengths between 640 and 700 nm, one can observe an excitation peak at 554 nm with a shouldering at 618 nm. Only a slight change of inflection subsists at 498 nm. The emission spectrum shows two distinct peaks : a larger one at 571 nm and a secondary one between 642 and 645 nm (Fig. 7). The ratio of the peaks at 571 and 645 nm varies clearly with excitation wavelength (3.97 at 500 nm; 1.91 at 558 nm). The above characteristics suggest the existence of two components in the

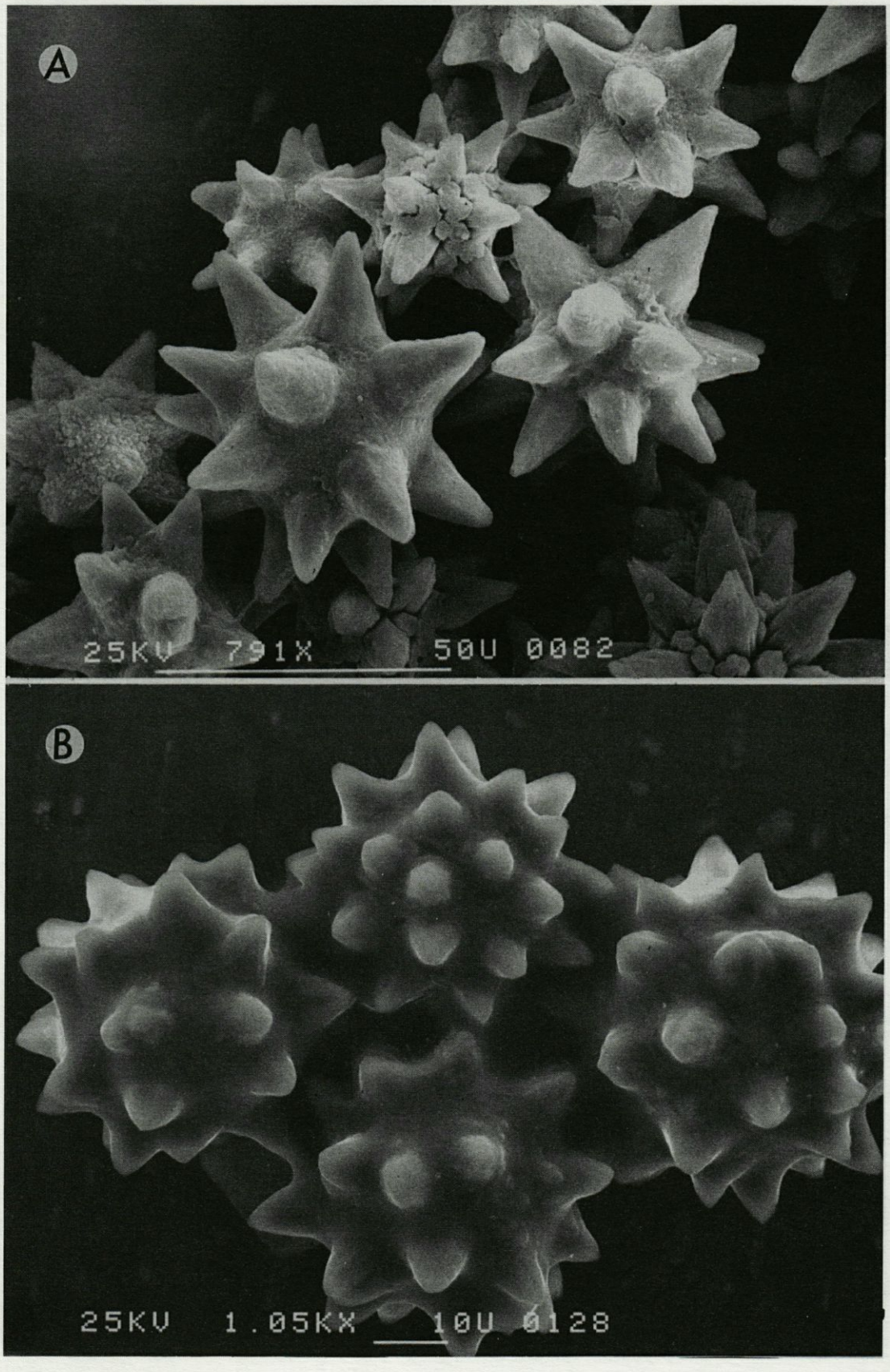


Fig. 1. — A, *Trididemnum cyanophorum* Lafargue and Duclaux, 1979, Guadeloupe. B, *Trididemnum palmae* Monniot, 1984, Guadeloupe.

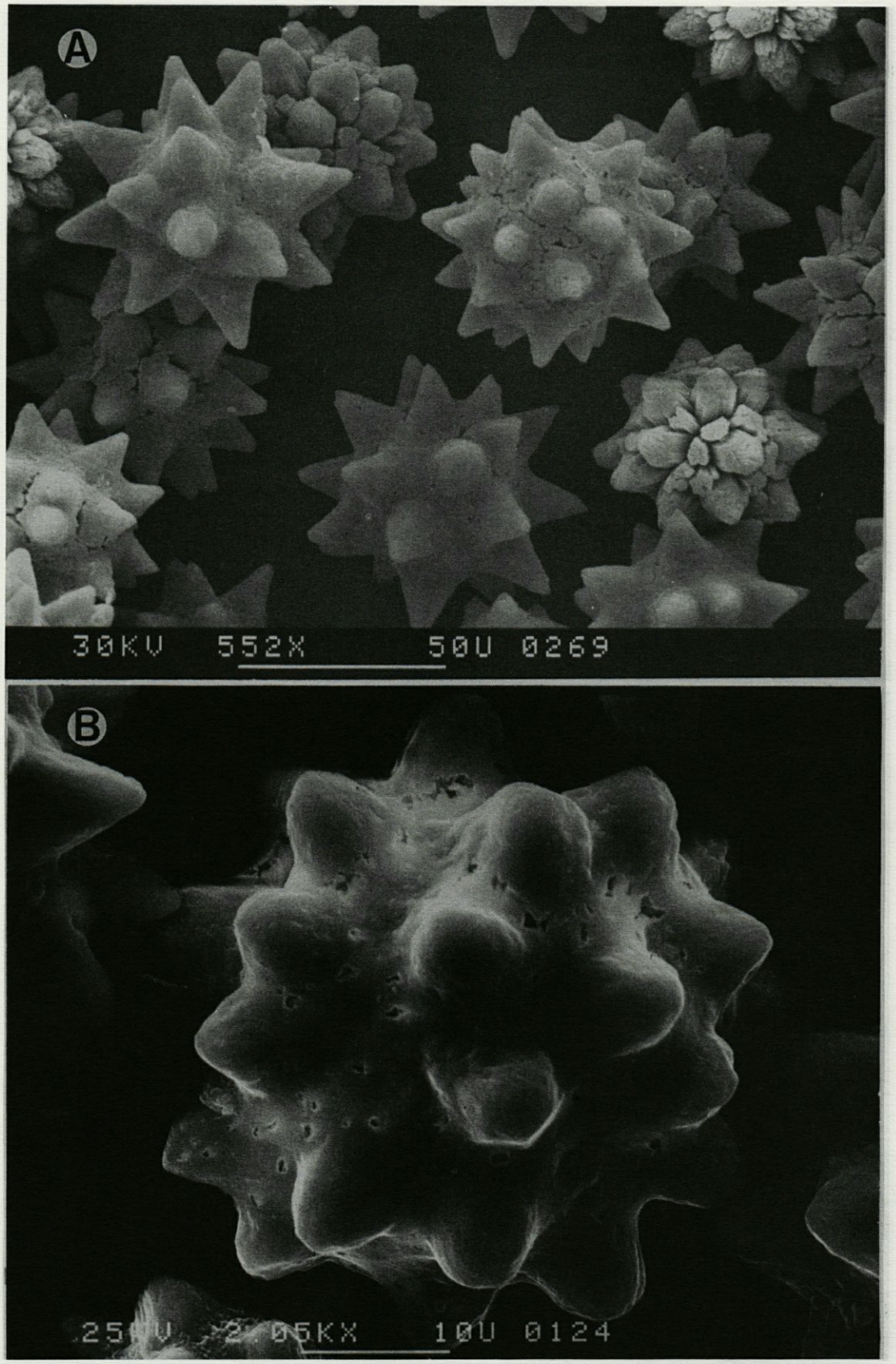


Fig. 2. — A, *Trididemnum cyanophorum* Lafargue et Duclaux, 1979 Panama. B, *Trididemnum palmae* Monniot, 1984, Panama.

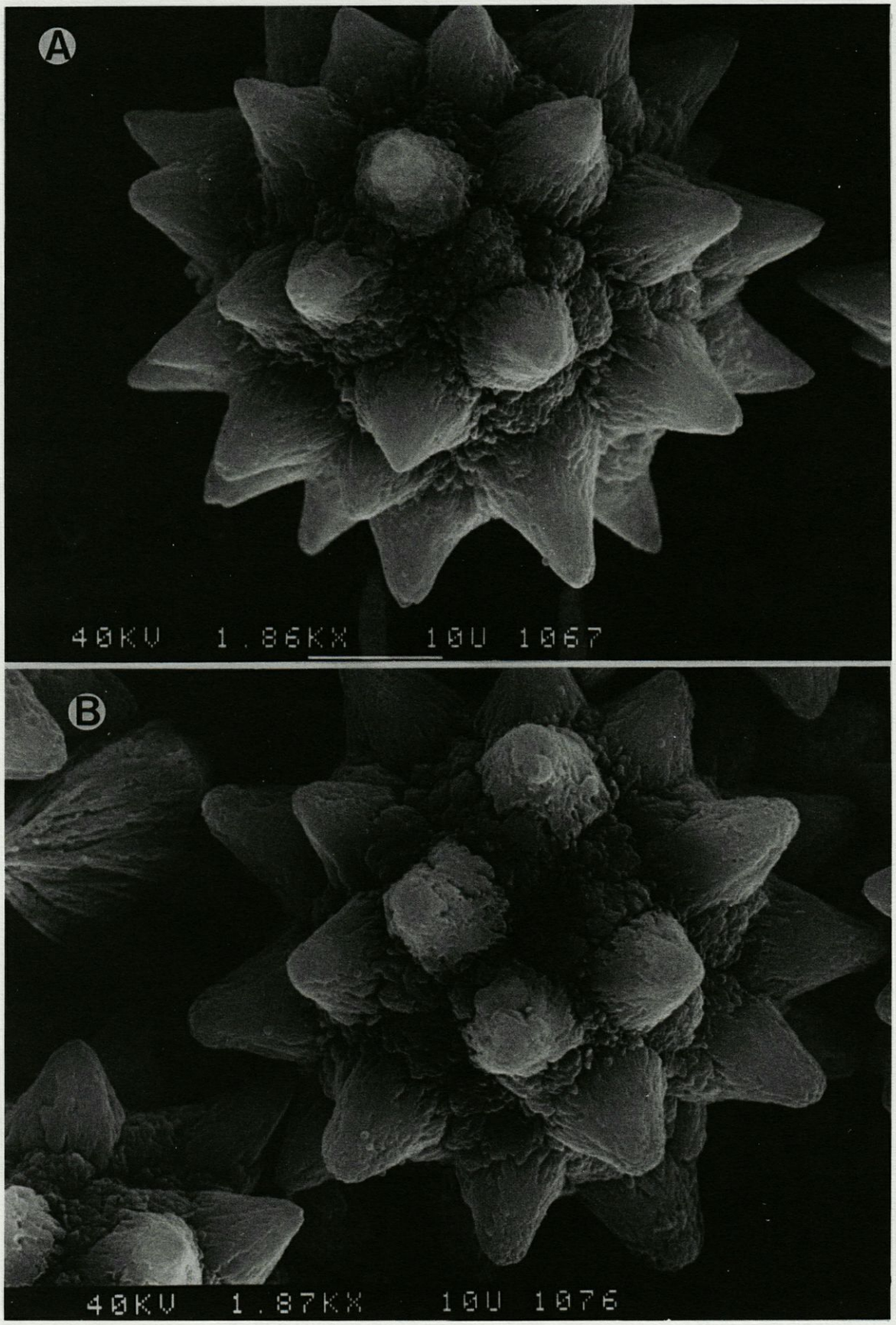


Fig. 3. — A and B, *Tridemnum solidum* Van Name 1902, Bermuda (AMNH). These spicules are similar to those of *T. palmae* Monniot, 1984.

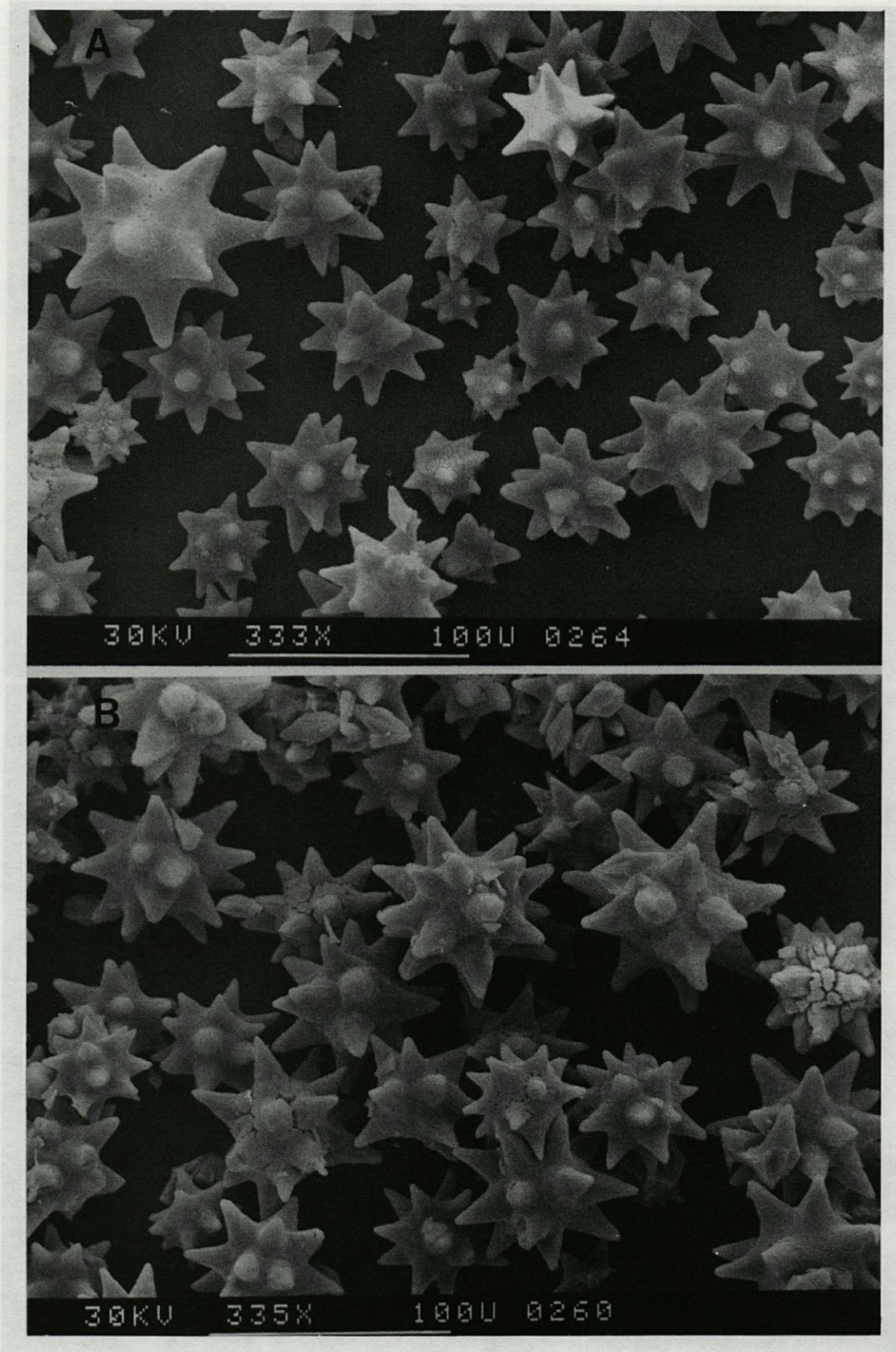


Fig. 4. — A and B, *Tridemnum solidum* Van Name 1902, Puerto Rico (AMNH). The spicule form is typical for this species.

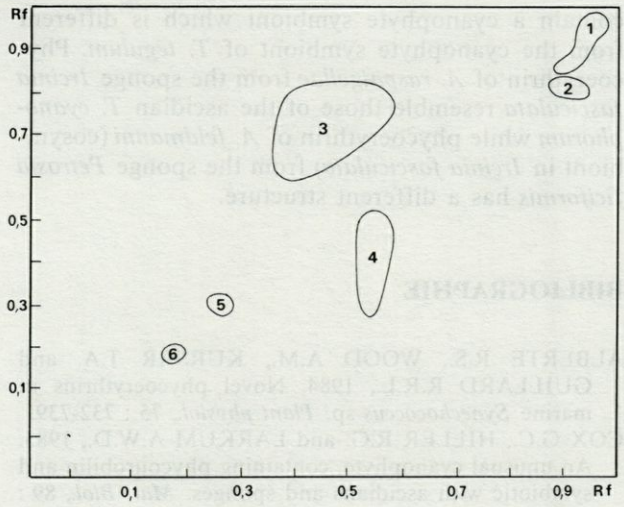
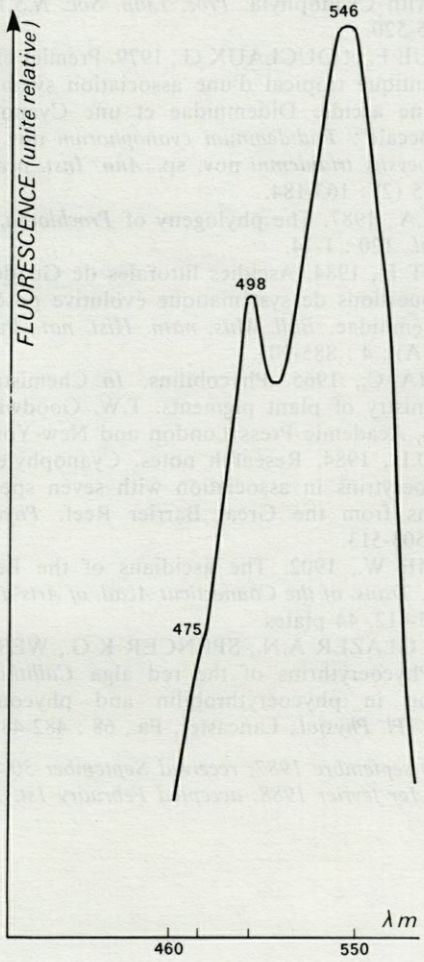


Fig. 5. — Two-dimensional thin layer chromatography (TLC) of the liposoluble photosynthetic pigments of *Synechocystis trididemni* Lafargue and Duclaux, 1979 from *Trididemnum cyanophorum* Lafargue and Duclaux, 1979. Solvants : 1st dimension (vertical) 2 % n-propanol in petroleum ether (60-80°C); 2nd dimension (horizontal) : 30 % chloroform in petroleum ether (60-80°C). 1 : carotenes; 2 : pheophytin; 3 : chlorophyll *a*; 4,5,6 : xanthophylls.



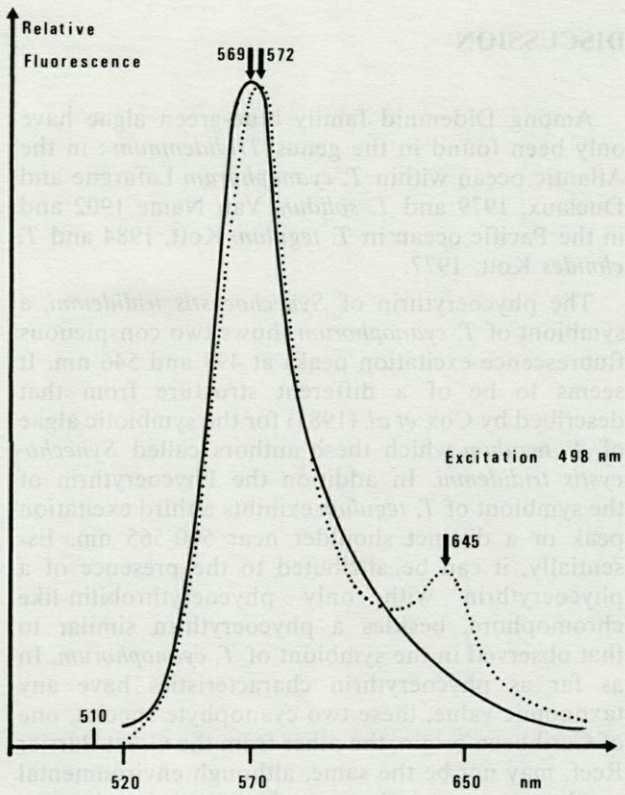


Fig. 7. — Fluorescence emission spectrum of 0,2 M phosphate buffer (pH 6,8) extracted phycobilins of *Synechocystis trididemni* Lafargue and Duclaux (———) from *Trididemnum cyanophorum* Lafargue and Duclaux and of *Aphanocapsa feldmanni* Fremy Feld. and *A. raspaigellae* Fremy Feld. (----) from *Ircinia fasciculata* (Pallas). Excitation wavelength, 498 nm.

phycoerythrin fraction, the first one belonging to *Aphanocapsa raspaigellae*, the second one to *A. feldmanni* (see below).

For *Aphanocapsa feldmanni*, symbiont of *Petrosia ficiformis*, only one conspicuous excitation peak (at 556 nm) is visible for the whole emission spectrum which presents a major peak at 575 nm and a small one at 636 nm. The fluorescence ratio 575/636 only shows little variations with excitation wavelength (4.7 at 470 nm; 4,0 at 558 nm). This species phycoerythrin is only formed by phycoerythrobilin-like chromophore. With emission fixed at 636 nm, a shoulder at 618 nm excitation reveals the presence of C-phycocyanin. However there could be an association between phycoerythrobilin and phycocyanobilin within the same protein (as R-phycocyanin in red algae : O'Heocha, 1965).

Fig. 6. — Fluorescence excitation spectrum of *Synechocystis trididemni* Lafargue and Duclaux, 1979, phycobilins extracted by 0,2 M phosphate buffer at pH 6,8. Emission wave-length : 590 nm.

DISCUSSION

Among Didemnid family blue-green algae have only been found in the genus *Trididemnum* : in the Atlantic ocean within *T. cyanophorum* Lafargue and Duclaux, 1979 and *T. solidum* Van Name 1902 and in the Pacific ocean in *T. tegulum* Kott, 1984 and *T. clinides* Kott, 1977.

The phycoerythrin of *Synechocystis trididemni,* a symbiont of *T. cyanophorum* shows two conspicuous fluorescence excitation peaks at 498 and 546 nm. It seems to be of a different structure from that described by Cox *et al.* (1985) for the symbiotic algae of *T. tegulum* which these authors called *Synechocystis trididemni.* In addition the Phycoerythrin of the symbiont of *T. tegulum* exihibts a third excitation peak or a distinct shoulder near 560-565 nm. Essentially, it can be attributed to the presence of a phycoerythrin with only phycoerythrobilin-like chromophore, besides a phycoerythrin similar to that observed in the symbiont of *T. cyanophorum.* In as far as phycoerythrin characteristics have any taxonomic value, these two cyanophyte species, one of Caribbean origin, the other from the Great Barrier Reef, may not be the same, although environmental conditions may influence phycoerythrin chromophore composition (Yu *et al.*) 1981. The spectral characteristics of phycoerythrin we analyzed are not rare in Cyanobacteria (Alberte *et al.*, 1984) but seems different from that generally found within the symbionts of ascidians (Parry, 1984; Cox *et al.*, 1985).

Comparison of the phycoerythrins of the symbionts of *Ircinia fasciculata* and of *Petrosia ficiformis* shows that *Aphanocapsa raspaigellae* contains a phycoerythrin similar to the pigment of *Synechocystis trididemni* whereas that of *Aphanocapsa feldmanni* have a different structure (one excitation peak at 556 nm).

Whatever their individual phycoerythrin characteristics, all these symbionts of didemnid ascidians display a pigment composition that is typical for Cyanobacteria. These are prokaryotes containing chlorophyll *a* only, plus different phycobilins.

CONCLUSION

Some researchers argue that coccoid endosymbiotic cyanobacteria found in ascidians and sponges would belong to the same species *Synechocystis trididemni* (Cox *et al.*, 1985; Lewin, 1987). Phycoerythrin characteristics suggest that *T. cyanophorum* contain a cyanophyte symbiont which is different from the cyanophyte symbiont of *T. tegulum.* Phycoerythrin of *A. raspaigellae* from the sponge *Ircinia fasciculata* resemble those of the ascidian *T. cyanophorum* while phycoerythrin of *A. feldmanni* (cosymbiont in *Ircinia fasciculata*) from the sponge *Petrosia ficiformis* has a different structure.

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