

## SCANNING ELECTRON MICROSCOPY OF *CORALLINA OFFICINALIS* AND *BOSSIELLA ORBIGNIANA* SSP. *ORBIGNIANA* (RHODOPHYTA, CORALLINALES) FROM ARGENTINA

By RICARDO SCROSATI\*

**Summary** The surface of intergenicula of *Corallina officinalis* Linnaeus and *Bossiella orbigniana* ssp. *orbigniana* Johansen from Argentina was analyzed with S.E.M. Mean diameter of epithallial concavities of *C. officinalis* is  $9.1 \pm 1.2 \mu\text{m}$ , while that of *B. orbigniana* ssp. *orbigniana* is  $7.1 \pm 0.8 \mu\text{m}$ , a statistically significant difference. Based on comparisons with previous reports, it appears that this parameter is taxonomically useful to distinguish among species of geniculate Corallinales, but more extensive studies including collections of several species from different localities are needed to fully accept its diagnostic use.

**Resumen** Se analizó la superficie de las intergenículas de *Corallina officinalis* Linnaeus y *Bossiella orbigniana* ssp. *orbigniana* Johansen de la Argentina a través de M.E.B. El diámetro promedio de las concavidades epitaliales de *C. officinalis* es  $9,1 \pm 1,2 \mu\text{m}$ , mientras que el de *B. orbigniana* ssp. *orbigniana* es de  $7,1 \pm 0,8 \mu\text{m}$ , una diferencia estadísticamente significativa. Basándose en comparaciones con reportes previos, este parámetro parece ser taxonómicamente útil para distinguir especies de Corallinales geniculadas entre sí, pero se necesitan estudios más extensos que incluyan colecciones de varias especies de distintas localidades para aceptar totalmente su uso diagnóstico.

### INTRODUCTION

Red algae of order Corallinales are distributed worldwide in marine waters. Based on external morphology of thalli, two groups are distinguished: the geniculate (or articulated) species and the non-geniculate species (Johansen 1981, Woelkerling 1988). Among the geniculate species, *Corallina officinalis* Linnaeus and *Bossiella orbigniana* (Decaisne) Silva are found on the Argentine coast (Pujals 1963, Johansen 1971, Kühnemann 1970, 1972a, b, Mendoza 1974, 1976).

Scanning electron microscopy is useful for the taxonomy of geniculate species, through the study of the surface of the calcified regions of thalli (Garbary 1978, Garbary *et al.* 1981, Garbary and Johansen 1982, Choi and Lee 1988, Economou-Amilli *et al.* 1990). After preparation of thallus material for S.E.M., the "cuticle" and the external wall of the epithallial cells, which lack calcium carbonate (Matty and Johansen 1981), change from their origi-

nal form. Thus, structures known as epithallial concavities are observed on the thallus surface, as a result of the projection of the lateral calcified walls of epithallial cells over their collapsed protoplasts (Garbary and Johansen 1982). Garbary (1978) concluded that the diameter of epithallial concavities and the distance between epithallial concavities are useful to distinguish among certain species of Corallinales. However, he pointed out that a considerable amount of intraspecific variation had been overlooked. In addition, he commented that future measurements of additional species whose values were intermediate to those of previously defined species could complicate the establishment of precise limits among species. The objective of this study was to make the above mentioned measurements on *Corallina officinalis* and *Bossiella orbigniana* ssp. *orbigniana* Johansen from Argentina, in order to increase the current data on this matter and to analyze the taxonomical usefulness of these parameters.

### MATERIAL AND METHODS

#### Material studied

*Corallina officinalis*. ARGENTINA. Buenos Aires province: Partido General Pueyrredón, Mar del Plata, Punta

\* Las Bases 681, 1706 Haedo, Buenos Aires, Argentina. Present address: The University of British Columbia, Department of Botany, Vancouver, B.C. V6T 1Z4, Canada.

Mogotes; 18-IV-1992; Scrosati 1501 (BAFC), "tetrasporic thalli, from the rocky intertidal level".

*Bossella orbigniana* ssp. *orbigniana*. ARGENTINA. Buenos Aires province: Partido General Pueyrredón, Mar del Plata, Punta Mogotes; 18-IV-1992; Scrosati 1502 (BAFC), "from the rocky intertidal level".

The thalli were fixed in 4% formalin in seawater shortly after collection. Thallus fragments were air-dried and processed following the standard procedures for S.E.M. analysis (D'Ambrogio de Argüeso 1986). The diameter of epithallial concavities and the distance between concavities

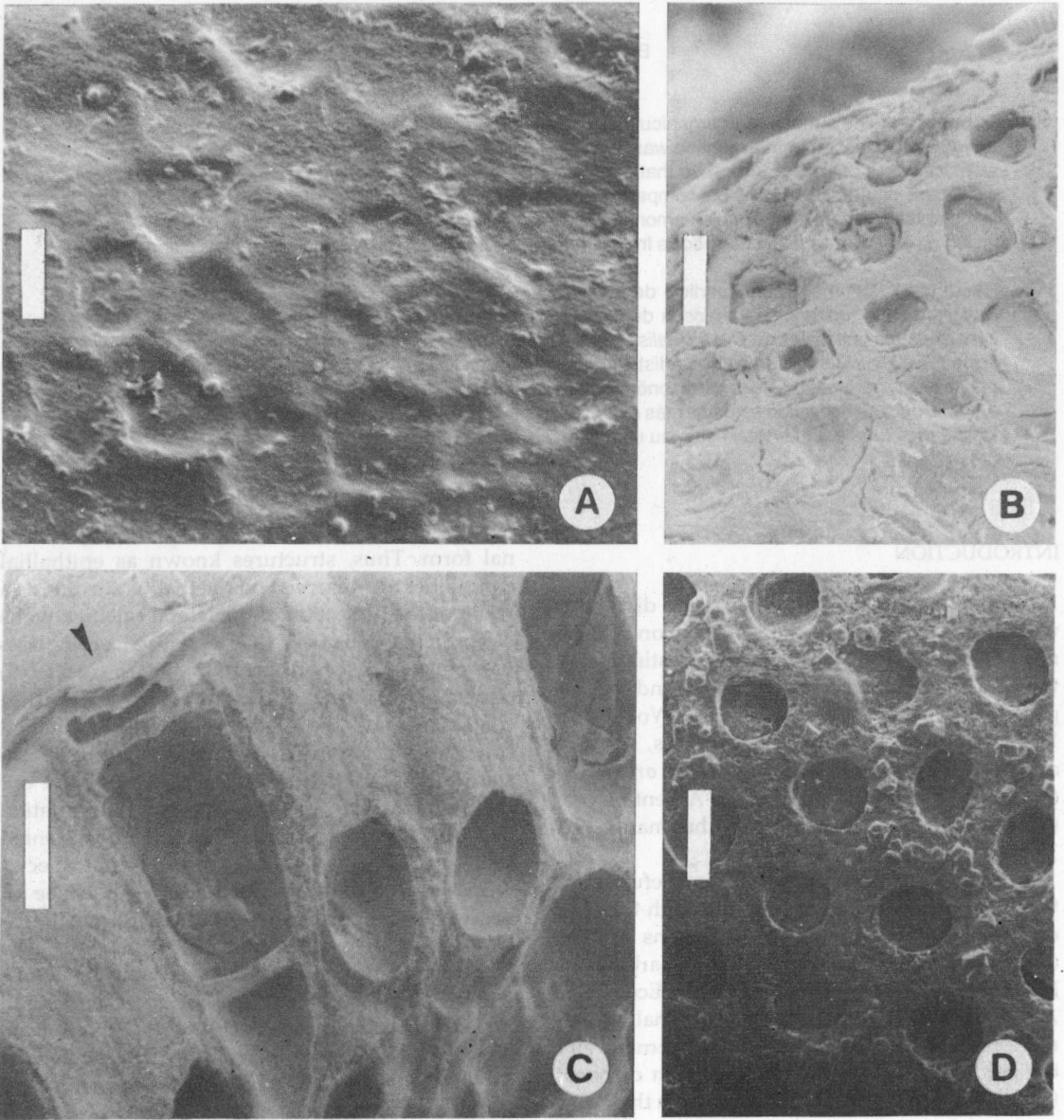


Fig. 1.— A: Surface view of the apical intergeniculum of a branch of *C. officinalis*. B: Surface view of an intergeniculum of *C. officinalis*, where the "cuticle" and the external layer of the cell walls have been lost. C: Section of an intergeniculum of *C. officinalis*, showing the remains of an epithallial cell (arrow) and cortical cells. D: Surface view of an intergeniculum of *Bossella orbigniana* ssp. *orbigniana*. Scale bars: 10  $\mu$ m.

were measured using photographs of S.E.M. Statistical comparisons were done using the *t*-test (Zar 1984), employing SYSTAT 5.0 (Wilkinson 1989).

## RESULTS AND DISCUSSION

*Corallina* and *Bossiella* belong to the tribe Corallineae (Corallinaceae, Corallinoideae), where intergenicular surfaces are referred to as "Corallina-type" (Garbary and Johansen 1982), *i.e.*, having round to irregular epithallial concavities. The surface of intergenicula of *C. officinalis* has already been observed using S.E.M. by Garbary (1978), Garbary and Johansen (1982), and Economou-Amilli *et al.* (1990). Surface views of intergenicula are presented in Fig. 1A and 1B, while fig. 1C shows a section of an intergeniculum of *C. officinalis* by first time in S.E.M. There, part of the structure of epithallial cells and the superficial cortical cells are showed. Epithallial cells have been analyzed under T.E.M. by Bailey and Bisalputra (1970), Giraud and Cabioch (1976, 1977), and Borowitzka and Veski (1978).

The mean diameter of epithallial concavities and the mean distance between concavities of *C. officinalis* from Mar del Plata (Fig. 1A) are shown in Table 1. The mean diameter of epithallial concavities is similar to that reported by Garbary (1978) in *C. officinalis* from the British Isles and that of Economou-Amilli *et al.* (1990) in thalli collected in Greece (Table 1). However, the mean distance between concavities differs from what Garbary (1978) found (Table 1). Economou-Amilli *et al.* (1990) mentioned that the distance between concavities was less than 2.5  $\mu\text{m}$ , but did not give mean values. It is important here to notice the high homogeneity

of the mean diameter of epithallial concavities found in specimens of this species collected from such distant localities. This suggests that this parameter may be species-specific, thus being useful to distinguish among different species. However, in order to accept this hypothesis it is necessary to expand the specific and geographical range of the observations. On the other hand, care has to be taken when measuring these parameters for further comparisons. For example, in certain regions of the intergenicula of *C. officinalis*, a thin superficial layer (probably composed of the "cuticle" and part of the external cell wall) may disappear during preparation for S.E.M. (Fig. 1B). Thus, epithallial concavities look different when compared to those regions where all layers are present. In that case, the mean diameter of epithallial concavities is  $8.1 \pm 2.1 \mu\text{m}$  ( $n = 32$ ), while the mean distance between concavities is  $3.5 \pm 1.2 \mu\text{m}$  ( $n = 30$ ). These values are significantly different ( $p < 0.05$ ) from those found before in intact regions of the surface.

Trichocytes were not found in *C. officinalis* from Mar del Plata. Trichocytes may or may not be present in thalli even from the same geographical locality (Garbary and Johansen 1982). The surface of conceptacles was similar to that of vegetative parts of the intergenicula, a characteristic found only in some species of Corallinales (Garbary 1978).

The mean diameter of epithallial concavities and the mean distance between concavities of *Bossiella orbigniana ssp. orbigniana* from Mar del Plata (Table 1, Fig. 1D) are the only values published for any species of *Bossiella*, and they are significantly different ( $p < 0.05$ ) from those measured in the intact regions of the surface of *C. officinalis*. The distinct appearance of the surface of both species when observed with S.E.M. may reflect differences between the species in cell wall structure.

Table 1.— Diameter of epithallial concavities (Dm) and distance between concavities (Dt) of intergenicula of different species of geniculate Corallinales. Values are expressed in  $\mu\text{m}$ , as mean  $\pm$  S.D.

Species	Dm	Dt	Source
<i>Corallina officinalis</i>	$9.1 \pm 1.2$ ( $n = 33$ )	$2.4 \pm 0.5$ ( $n = 30$ )	This study
<i>Corallina officinalis</i>	$9.8 \pm 0.4$	$1.7 \pm 0.2$	Garbary (1978)
<i>Corallina officinalis</i>	6-13	< 2.5	Economou-Amilli <i>et al.</i> (1990)
<i>Bossiella orbigniana</i> <i>ssp. orbigniana</i>	$7.1 \pm 0.8$ ( $n = 46$ )	$6.1 \pm 1.2$ ( $n = 45$ )	This study
<i>Yamadaea melobesioides</i>	$6.0 \pm 0.9$	$1.8 \pm 0.7$	Garbary <i>et al.</i> (1981)
<i>Yamadaea americana</i>	$12.1 \pm 1.3$	$1.4 \pm 0.4$	Garbary <i>et al.</i> (1981)

Within the tribe Corallineae, mean diameter of epithallial concavities of the intergenicula has only been reported in two other species: *Yamadaea melobesioides* Segawa, from Japan, and *Y. americana* Dawson *et* Steele, from Pacific North America (Garbary *et al.* 1981). These values (Table 1) appear to be different from those of *C. officinalis* and *B. orbigniana* ssp. *orbigniana*. However, the mean distance between concavities of the two species of *Yamadaea* is similar to that of *C. officinalis* (Table 1).

#### CONCLUSIONS

The results presented here and discussed in relation to previous studies indicate that S.E.M. is useful in delimiting geniculate species of Corallinales through measurement of surface features of the intergenicula. Mean diameter of epithallial concavities appears to be an important diagnostic character. This contribution also points out the need for a more extensive investigation of more geniculate species, collected in different parts of the world, in order to fully accept the taxonomic utility of this technique.

#### ACKNOWLEDGEMENTS

To Dr. María Laura Mendoza (CADIC, Argentina), Dr. David Garbary (St. Francis Xavier University, Canada), Lic. Carmen Pujals (MACN, Argentina), Ken Marr, M. Sc. (UBC, Canada), and an anonymous reviewer of the manuscript, for their appreciated help in many ways, and to the S.E.M. service of the Faculty of Dentistry of the University of Buenos Aires, for the processing of the material.

#### REFERENCES

- BAILEY, A. & T. BISALPUTRA. 1970. A preliminary account of the application of thin-sectioning, freeze-etching, and scanning electron microscopy to the study of coralline algae. *Phycologia* 9 (1): 83-101, fig. 1-31.
- CHOI, D. S. & I. K. LEE. 1988. On surface structures of *Amphiroa* (Corallinaceae, Rhodophyta). *The Korean J. Phycol.* 3 (2): 111-117, fig. 1-9.
- D'AMBROGIO DE ARGÜESO, A. 1986. *Manual de técnicas en histología vegetal*. Hemisferio Sur, Buenos Aires, 83 p.
- ECONOMOU-AMILLÍ, A., I. BITIS & M. PASCHOU. 1990. Morphological variability in *Amphiroa*, *Corallina* and *Jania* (Rhodophyta-Corallinaceae) from Greece. *Bot. Mar.* 33: 261-271, fig. 1-33.
- GARBARY, D. J. 1978. *An introduction to the scanning electron microscopy of red algae*. In: IRVINE, D.E.G. and J. H. PRICE (Eds.). *Modern approaches to the taxonomy of red and brown algae*, pp. 205-222, Academic Press, London.
- H. W. JOHANSEN & R. F. SCAGEL. 1981. Aspects of the morphology, ultrastructure and distribution of the two species of *Yamadaea* Segawa (Rhodophyta, Corallinaceae). *Jap. J. Phycol. (Sôru)* 29: 7-13, fig. 1-12.
- & H. W. JOHANSEN. 1982. Scanning electron microscopy of *Corallina* and *Haliptilon* (Corallinaceae, Rhodophyta): surface features and their taxonomic implications. *J. Phycol.* 18: 211-219, fig. 1-13, t. 1-2.
- GIRAUD, G. & J. CABIOCH. 1976. Etude ultrastructurale de l'activité des cellules superficielles du thalle des Corallinacées (Rhodophycées). *Phycologia* 15 (3/4): 405-414, fig. 1-19.
- 1977. Caracteres généraux de l'ultrastructure des Corallinacées (Rhodophycées). *Rev. Algol.* 12 (1-2): 45-60, fig. 1, pl. 1-6.
- JOHANSEN, H. W. 1971. *Bossiaella*, a genus of articulated corallines (Rhodophyceae, Cryptonemiales) in the eastern Pacific. *Phycologia* 10 (4): 381-396, fig. 1-32, t. 1.
- 1981. *The coralline algae: a first synthesis*. CRC Press, Boca Raton, 239 p.
- KÜHNEMANN, O. 1970. Vegetación marina de la ría de Puerto Deseado. *Op. lill.* 17: 1-123, fig. 1-141, pl. 1-11.
- 1972a. Bosquejo fitogeográfico de la vegetación marina del litoral argentino. *Physis (Buenos Aires), Secc. A*, 31 (82): 117-142, fig. 1-2.
- 1972b. Bosquejo fitogeográfico de la vegetación marina del litoral argentino (Continuación). *Physis (Buenos Aires), Secc. A*, 31 (83): 295-325.
- MATTY, P. J. & H. W. JOHANSEN. 1981. A histochemical study of *Corallina officinalis* (Rhodophyta, Corallinaceae). *Phycologia* 20 (1): 46-55, fig. 1-15, t. 1.
- MENDOZA, M. L. 1974. Distribution de quelques espèces de Corallinacées articulées sur les côtes d'Argentine. *Bull. Soc. Phycol. France* 19: 67-73, pl. 1.
- 1976. Estudio de las variaciones morfológicas externas, internas y citológicas de las *Corallineae* (Rhodophyta) de la Argentina. *Physis (Buenos Aires), Secc. A*, 35 (90): 15-25, pl. 1-6.
- PUJALS, C. 1963. Catálogo de Rhodophyta citadas para la Argentina. *Rev. Mus. Argent. Cs. Nat. "Bernardino Rivadavia"*, Bot. 3 (1): 1-139.
- WILKINSON, L. 1989. *SYSTAT: The system for statistics*. SYSTAT, Inc., Evanston, 638 p.
- WOELKERLING, W. J. 1988. *The coralline red algae: an analysis of the genera and subfamilies of nongeniculate Corallinaceae*. British Museum (Natural History), London, and Oxford University Press, Oxford, 268 p.
- ZAR, J. H. 1984. *Biostatistical analysis*. Prentice Hall, Englewood Cliffs, 718 p.