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Vegetative and reproductive anatomy of *Sargassum lapazeanum* (Fucales: Sargassaseae) in the south-western Gulf of California, Mexico

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An analysis of the vegetative and reproductive anatomy of *Sargassum lapazeanum* was performed based on extensive sampling in three areas within its geographical range: a) San Juan de la Costa, b) Punta Calera, c) Punta Machos. Material was collected from low intertidal to subtidal populations and evaluated based on variations present within and between populations. Our goal was to comprehensively analyze the features of the vegetative and reproductive anatomy of this species. Our observations showed that this species has cauline, which is anatomically composed of three tissues layers. This species is dioic, with male and female conceptacles in the same receptacle but with different maturation times. The present study confirmed the presence of cauline in this species; this structure was previously described for some species in the Gulf of California, such as *Sargassum horridum*, and for several species in Japan. This structure could be responsible for the growth of primary branches and may also generate a new plant. This trend might explain the perennial nature of this species at the population level. Field and laboratory experiments could help to delimit the ecophysiological conditions under which this structure starts to develop.

Key Words: antheridia; cauline; morphology; oogonium; *Sargassum lapazeanum*

INTRODUCTION

Most of the features used to classify species in the order Fucales at the generic level are morphological, and the diagnostic features are based on the number of eggs produced per oogonium (Ohno et al. 1995, Cho et al. 2006): four in *Ascophyllum*, two in *Pelvetia*, and one in *Cystoseira* and S*argassum* (Ohno et al. 1995, Cho et al. 2006). However, classification to the species level is also based on morphological features (Chapman and Chapman 1977, Abbott 2002), and few studies have considered classifying species based on differences between their vegetative and reproductive structures (Gardner 1910, Moreira and Suarez 2002). Moreover, some structures, such as the cauline, which is a protuberance that behaves like an adventive embryo (Yoshida et al. 1999), are present in many species that have not yet been investigated. *Sargassum* is a tropical genus with wide morphological plasticity (Abbott 2002) that causes confusion regarding the actual number of species in the genus. Most of the currently recognized species have not been studied in anatomical detail to determine whether or not their features could be used to delimit species belonging to this genus. Thus, a next logical step would be to gain a better understanding and further knowledge about the vegetative and reproductive structures of as many species within this ge-

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nus as possible.

Anatomical knowledge about *Sargassum* species living in the Gulf of California is not accurate. *Sargassum lapazeanum* Setchell & Gardner is a very common species in the southwestern gulf (Andrade-Sorcia et al. 2008), but no comprehensive investigation of its anatomical features has been undertaken (Andrade-Sorcia et al. 2008, Norris 2010). Most of the current knowledge of this species is related to a recent morphological analysis (Andrade-Sorcia et al. 2008) and population dynamics studies (Rivera and Scrosati 2006, 2008). In the latter studies, survivors of this species were linked to material that remained after an extreme summer, but there was no explanation of how the remaining thalli survived the summer or grew in October. Additionally, Andrade-Sorcia et al. (2008) highlighted the extreme morphological variations in species of this genus and the urgent need to understand the boundaries of these species in relation to their anatomical structure. Thus, the goals of this study were: 1) to analyze the anatomy of the caulines and 2) to describe the anatomy of the receptacles of *Sargassum lapazeanum*.

MATERIALS AND METHODS

Study site

Plants were collected in 2003, 2004, and 2005 from three sites in each of three localities along the southwestern Gulf of California: San Juan de la Costa (24°19'34" N and 114°34'10" W), Punta Calera (24°21.0' N and 114°16.0' W), Punta Machos (26°6´20˝ N and 111°18'30" W). At each site, 30 plants with several fronds each were randomly selected for morphological and anatomical analyses.

Samples were embedded in 4% formaldehyde and sea water. All material collected was used to analyze the vegetative (cauline) and reproductive (receptacles) structures and the variability in these structures within fronds. Fifteen receptacles were taken from each frond and processed by histological techniques.

Anatomical and morphological processing

Ninety samples were collected from the southwestern Gulf of California and preserved in 4% formaldehyde and sea water. A total of 45 thalli with receptacles were chosen, and twelve receptacles were taken from each thallus; all of the specimens were labeled with the date and location. We selected a group of specimens after dehydration in an alcohol series (30, 60, 90, and 100%) and butane for

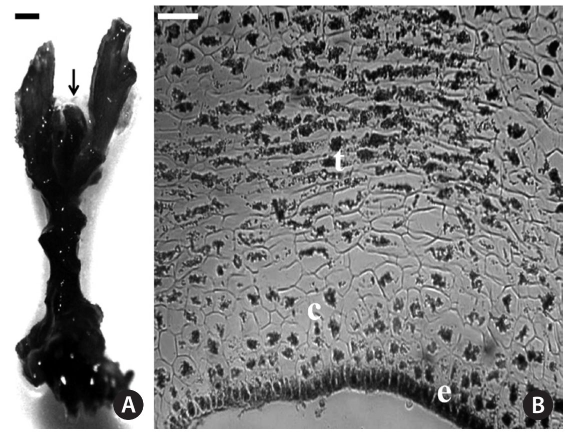

Fig. 1. (A) *Sargassum* cauline between branches (arrow). (B) Cross section: trumpet cells (t), cortical cells (c), epithelial cells (e). Scale bars represent: A, 1 cm; B, 200 µm.

30 minutes each. After we embedded the tissue in paraffin-butane and paraffin, we prepared 10 µm sections that were mounted permanently.

RESULTS

The anatomical analysis showed that the basal part of the thalli contained a real cauline with three tissue layers (Fig. 1). These layers were maintained over the stipe where a thicker cortical area and a wide medullar section were present in all samples. The upper part of the stipe showed a significant increase in width with cell layering, additionally, the holdfast showed the epithelial and cortical cells (Fig. 2). The blade cross section revealed epithelial cells, medullar cells, and trumpet cells (Fig. 3). The cryptostomata was composed of epithelial cells, cortical cells, medullar cells and paraphyses, and these structures were different than those of antheridial cells. Epithelial cells, cortical cells, and a blank space were found in the longitudinal bladder section (Fig. 4).

We found that the thallus receptacle structures looked like a *Sargassum* blade, with a thorny morphology; they were 5 mm in length and could have been male or female receptacles. They varied in number from the base to the top of the thallus, and, in some cases, many were observed on the upper part of the thallus. When receptacles are mature, they have an inner wall and the contents are discharged and remains just outside of the ostiole. We found a long mucilaginous stalk material on the receptacles, which was embedded in the receptacle wall to help the egg remain attached to the receptacle wall. At this moment in the process the receptacle remain open

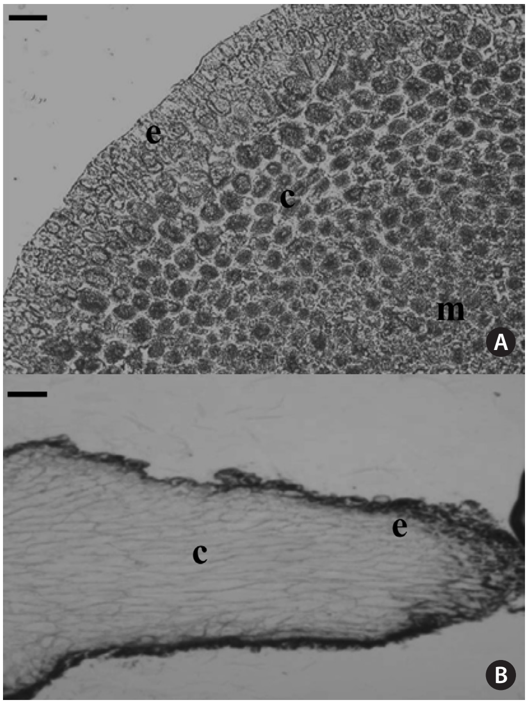

Fig. 2. (A) Stipe cross section: epithelial cells (e), cortical cells (c), medullar cell (m). (B) Holdfast cross section: epithelial cells (e), cortical cells (c). Scale bars represent: A & B, 200 µm.

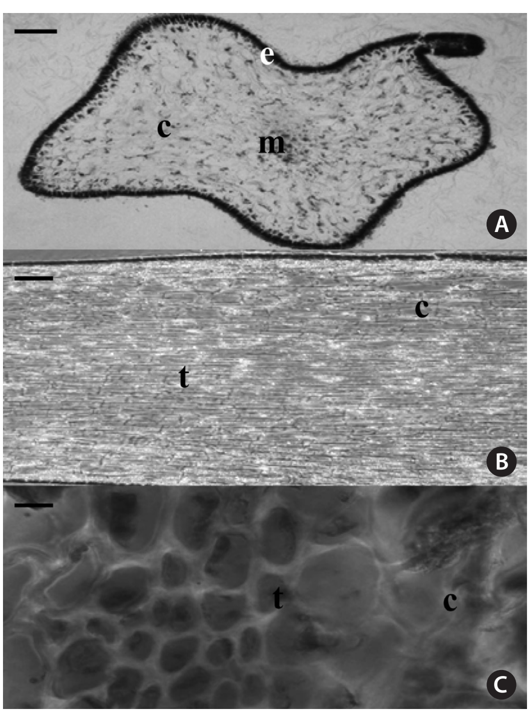

Fig. 3. (A) Blade cross section: epithelial cells (e), cortical cells (c), medullar cells (m). (B) Blade longitudinal cut: cortical cells (c), trumpet cells (t). (C) Close up of trumpet cells (t), cortical cells (c). Scale bars represent: A-C, 200 µm.

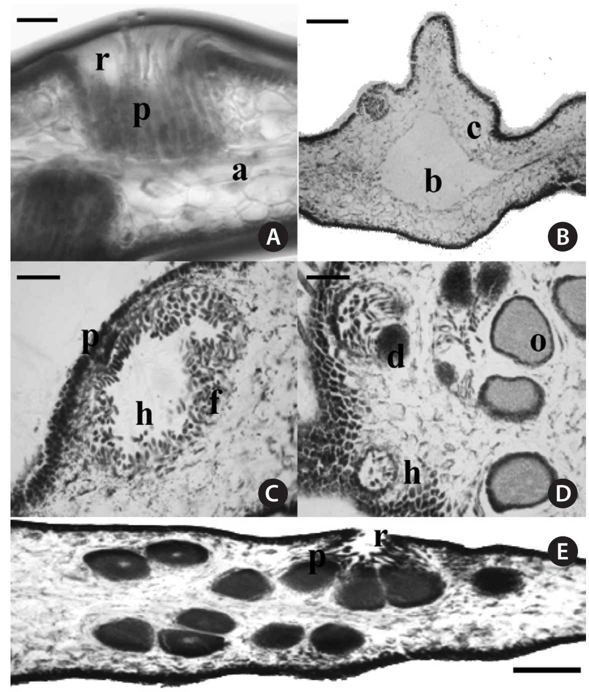

Fig. 4. (A) Cryptostomata anatomy: base of the capsule (a), paraphises (p), pore (r). (B) Bladder cross section: capsule (b), cortical cells (c). (C) Male conceptacle: paraphises (p), base of the conceptacle (f), antheridia (h). (D) Conceptacle with zygote (d), mature oogonium (o), antheridia (h). (E) Mature conceptacle: paraphises (p), pore (r). Scale bars represent: A-D, 200 µm.

but with a disc-shaped gelatinous plug (Fig. 5).

This species has a cylindrical receptacle in which many conceptacles were present. We found female and male caps in sections from the same receptacle and occasionally we found oogonium and antheridial cells within the same conceptacle; antheridia have a pedicel over the conceptacle wall. In contrast, the oogonium and antheridia were in the same conceptacle. Additionally, we could see the paraphises near the pore, and the nucleus of the oogonium.

DISCUSSION

Norris (2010) reviewed the taxonomy of the genus *Sargassum* in the north Gulf of California and reported that all of Dawson's (1944) sections should be raised to the species level. However, he did not clearly compare *Sargassum lapazeanum* in relation to other "species" recognized in the complex and in relation to other species in the genus. This species has been described as having oogonia that are thicker than the antheridial receptacle but,

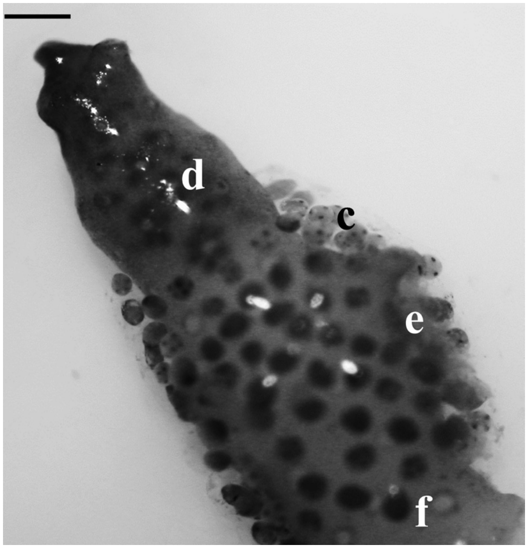

Fig. 5. Receptacle with open conceptacle pores, *Sargassum* zygote (c), receptacle wall (d), mucilaginous stalk material (e), open conceptacle pore (f). Scale bar represent: 1 mm.

in our study, we found them together, making it impossible to use this character to delimit the species. Gillespie and Critchley (1997) reported that the morphology of the receptacle should be different between species, and, moreover, that these differences are affected by time and the habitat in which the thallus was found. They reported that when thalli live in the surge zone, the receptacle has to be more branched than if the plant lives in quiet waters, and if this receptacle characteristic is combined with short blades then the level of fertilization would increase. Additionally, *S. lapazeanum* has short blades and complex structures that help with sexual reproduction. Although our studies have found this ontogenetic sequencing, we strongly suggest additional experiments in the laboratory to evaluate fertilization among species. This species is dioic, with the presence of male and female conceptacles in the same receptacle, but with different maturation times.

The present study confirmed the presence of caulines in this species, a structure that has previously been described in some species from the Gulf of California, such as *Sargassum horridum* (Foster et al. 2007) and several species in Japan (Yoshida et al. 1999, Ajisaka and Lewmanomont 2002). This structure could be responsible for the growth of the primary branches or it could generate a new individual plant. Our observations showed that the caulines were consistent in all samples, unlike in some Japanese species (Ajisaka and Lewmanomont 2002), which were spatially variable. At the population level, this trend might explain the perennial nature of this species (Rivera and Scrosati 2006, 2008). A new plant from *Sargassum* eggs forms a disc that eventually forms the holdfast. Then, the new thalli generates caulines and these caulines generate new thalli with a hapteroidal holdfast as found in *Sargassum macrocarpum* (Yoshida et al. 1999). Field and laboratory experiments might help to delimit the ecophysiological conditions under which this structure starts to develop. Furthermore, studies are needed to determine how widely distributed this species and genera of the Fucales group are.

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