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Padina Spp.: Morphology Re-evaluation of the Israeli Brown Algae

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Research Article

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ABSTRACT

Padina spp. flourishes in the inter-tidal zone around the world, mainly in the Mediterranean Sea and the Atlantic Ocean, at ± 30 latitudes. In the Israeli shores, it grows primarily on abrasion platform of aeolianite ridges. In almost two year's observation, two *Padina spp*. growing in Tel-Baruch and other coastlines were monitored: *P. pavonica* which flourish most of the year, and *P. gymnospora* which flourish from May to December. This article described the phenotypic morphology pattern in which *Padina spp*. were expressed, with particular attention to *P. pavonica* and the differences between the two types of brown algae.

INTRODUCTION

Padina spp. (dichtyophyceae) grows widely around the globe and domain mainly the Mediterranean and the tropical shores [1-3]. Padina is one of the two brown algae known today that calcifies needle-shaped Aragonite [4,5]. These algae flourish up till sub-littoral zone, and its average growth rate, as measured in the island of Corsica, is 0.45 mm day^{-1} [6]. This littoral intertidal habitat provides some diurnal extreme environmental changes such as pH levels, salinity, and temperature, which can be changed radically at the ebb time [7]. It seems that *Padina spp*. are well adapted to this rapidly changing environment.

Another abiotic phenomenon in the Israeli coastline is sand movement. Sand is moving from the southern Nile Delta in Egypt, all the way up to the northern Haifa Bay [8], and while small grains remain in the deep water, big grains are carried nearshore [9] and sometimes covers the algal population.

In Tel-Baruch coastline, between the southern bathing area and the northern archeology site of Teller Rekkeit (N34°E32°), at approximately 100 meters of abrasion platforms, grow two types of *Padina*: *P. pavonica* and *P. gymnospora*.

At first sight, it is hard to distinguish between the two and after meticulous microscopic inspection, the morphological differences clarify. Besides the microscopic difference, there are another five - differences visible to the naked eye -, among which are thallus color, thallus shape and hair color that helps telling them apart.

Those visual differences are described in this paper.

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MATERIALS AND METHODS

Collecting Samples and Monitoring Algal Seasonal Growth

The algae were monthly collected from October 2014 to June 2016. Their amount and abandons, as well as environmental conditions and seasonal growth, were monitored during this period of time.

Microscopy Analysis

Fronds were collected from Tel-Baruch beach (N34°E32°) in approximately 100 meters of coastal length on a monthly basis from October 2014 to June 2016, a total sum of 24 collections. Mainly healthy fronds were collected and brought to the lab. In the lab, each frond was cleaned from epiphytes and sand and observed under LEICA binocular and AxioimagerZ1 microscope.

Healthy unbroken fronds were placed in 70% EtOH, fixed on aluminum plates, covered with carbon and analyzed under ESEM.

Histology Analysis

After collecting and cleaning the fronds from visible epiphytes, the samples were placed in 70% EtOH. Then, the fronds were transferred into an embedding machine using the following embedding protocol:

- 80% ethanol for 1 hour or until the start of the embedding
- 80% ethanol for 1 hour
- 96% ethanol for 1 hour
- 96% ethanol for 1 hour
- 96% ethanol for 1 hour
- 100% ethanol for 1 hour
- 100% ethanol for 1 hour
- 100% ethanol for 1 hour
- First clearing agent-chloroform (AR)-for 1 hour
- Second clearing agent-chloroform (AR)-for 3 hours
- First wax (Paraplast X-tra) at 60°C for 3 hours
- Second wax (Paraplast X-tra) at 60°C for 1 hour

After that, the embedded samples were placed into paraffin blocks (LEICA). The blocks were cut into 5 µm slices with a microtome and placed in a 37°C bath to straighten them up. The slices were gently placed onto a 'Superfrost+' slides, dried for one hour on a 40°C plate, and kept in 37°C incubator overnight. After fixation, the slides were observed under an AxioimagerZ1 microscope.

RESULTS

Evaluation of Algae Growth Seasons in Tel-Baruch Area

After almost two years follow up, repetitiveness in morphology and color was noticed. From October to December 2014, May to December 2015, and again from May until our last collection in June 2016, both *P. pavonica* and *P. gymnospora* were collected, whereas from January to April in 2015 and again in 2016, only *P. pavonica* was collected (**Figure 1**). Similar repetitiveness on the same algae and at the same location was described by Ramon and Friedmann early in the 60's [10].

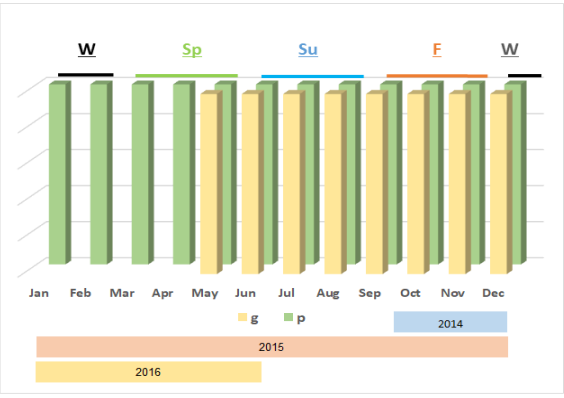

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Figure 1. Tel-Baruch's seasonal growth of *P. gymnospora* (g-yellow) and *P. pavonica* (p-green) during 2014-2016. While *P. pavonica* grows throughout the year, *P. gymnospora* appears on and off from May to December.

During the months which *P. gymnospora* was observed, in particularly May and October, most of the algae were covered by sand and half of the fronds were buried up (**Figure 2a**). In the months when *P. pavonica* grows, either side by side with *P. gymnospora* or alone, no sand movements were observed in Tel Baruch and in other Israeli shores (**Figure 2b**).

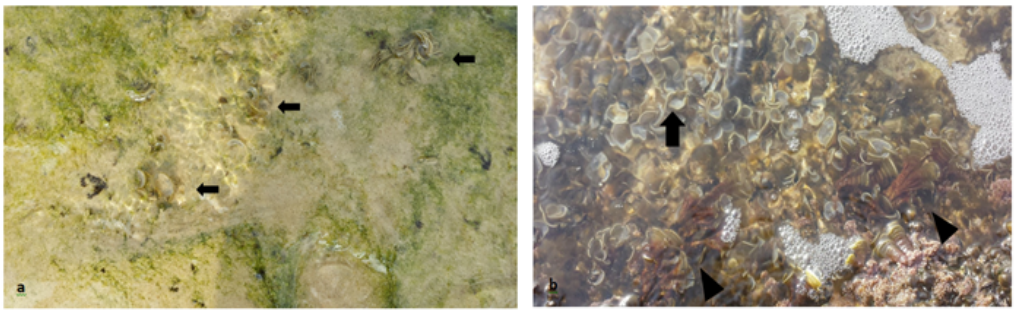

Figure 2. *P. gymnospora* and *P. pavonica* growth on an abrasion platform, Tel-Baruch a): *P. gymnospora* (arrow) grows on a kurkar covered by sand (October 2015, Tel-Baruch beach); b): *P. pavonica* (arrowheads) and *P. gymnospora* (arrow) grow side by side (October 2015) Palmachim beach.

Horizontal and Vertical Regions of the Thallus in *Padina Spp.*

In general, we divide the apical-basal axis of the thallus, into three:

- An apical region, which is the younger part of the frond
- A mid-thallus that narrows in the bottom
- Rhizoids, which are roots-like organelles that the algae use in order to hold onto the rocks they grow upon and are the older parts of the fronds

The thallus in a dorsoventrally-axis section shows a frontal ventral side and a back dorsal side.

Facing the ventral side, there are thick widely concaved stripes (**Figure 3a**), deposited with needles shaped aragonite, which are separated one from another by narrowly convex stripes (**Figure 3a and 3c**). The stripes are wider at the frond's middle apical-basal axis (**Figure 3b and 3c**, arrowhead) and get narrower at the sides of the fronds (**Figure 3b and 3c**). On the dorsal side of the thallus, parallel to and right behind the aragonite stripes, there were the reproduction stripes, and by looking at both sides, it seems that the aragonite stripes on the ventral side cover the reproduction stripes on the dorsal side (**Figure 3d**).

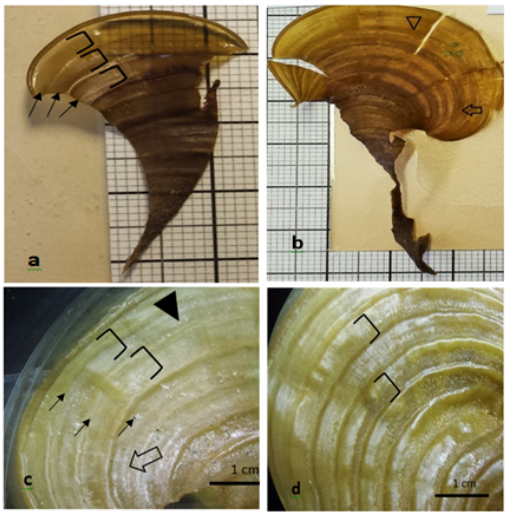

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Figure 3. General magnification of a piece of *P. pavonica* and *P. gymnospora* frond. a): *P. pavonica* narrow extra phaeoplasts convex (arrows) between a widely concaved $CaCO_3$ precipitation stripes (brackets); b): *P. pavonica* bigger frond demonstrating a wider middle stripe precipitation (arrowhead) getting narrower in the sides (arrow); c): *P. gymnospora* narrow convex (arrows) between a widely concaved $CaCO_3$ precipitation stripes (brackets); d): The dorsal side of *P. gymnospora* demonstrates that the reproduction stripes are parallel and located behind the aragonite ventral stripes.

There are hairbands on both ventral and dorsal sides every 2.5-3 mm [11]. On the dorsal side, the hairs delineate the reproduction cells and sometimes grows on top of them like a hairy cover, probably in order to protect the reproduction cells and to provide them with oxygen-rich micro-environment, but this idea needs more inspection. The hairs will be described in the next section.

Morphological differences between the two species: *P. pavonica* differs from *P. gymnospora* in several visible morphological differences. First, on a microscopic level, as implied by its name, *P. pavonica* has indusium, which is a thin membrane that covers both the hair and the reproduction cells, while *gymnospor*a does not [12,13].

Second, we observed five more practical and obvious characteristics:

- is more yellowish, and underwater, it can be almost transparent , while is more olive-greenish color
-
- While has only one stripe of dorsal reproduction cells located behind and towards the bottom of every ventrally Aragonite stripe , has two stripes of reproduction cells behind every aragonite stripe, and the reddish hairline are beneath the wider reproduction stripe . This observation support Ramon's and Friedmann's finding back in the 60's. However, they consider the reddish hairline to be a partition between the upper and the lower stripes, while we consider them to be a partition between reproduction segments, regardless the number of reproduction stripes in each segment
- while has a murky whitish reproduction cell, has colorful brownish haploidic gametes and greenish haploidic meiospores (sexual reproduction expression) co-expressed on the frond, and brownish diploid spores (a-sexual reproduction expression) (, respectively, left brackets)
- has unique two cell-lines with extra phaeoplasts, and those two cell-lines frames the Aragonite stripes

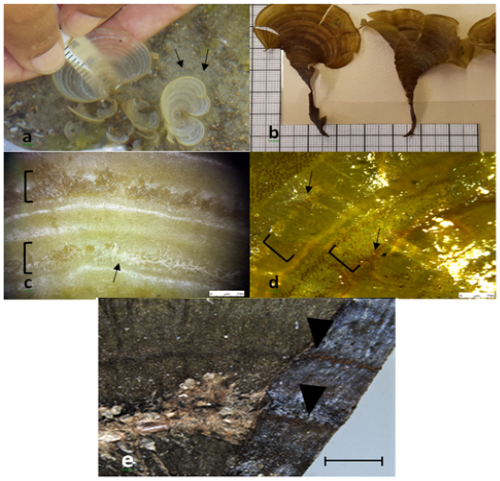

Figure 4. Tel-Bruch's *Padina spp*. morphology. The apparent differences between *P. gymnospora* (a, c) and *P. pavonica* (b, d). a): *P. gymnospora* is more yellowish and transparent under water; b): while *P. pavonica* is greenish and nontransparent; c): *P. gymnospora* has only one reproduction cell line at each segmentation, with a whitish hairline beneath it; d): while *P. pavonica* has two reproduction stripes: the upper one is consistently thicker and separated from the lower one (arrowhead) with reddish hairline. An AxioZ1 magnification of the red hairs in the smaller picture; e): *P. pavonica* has extra phaeoplasts (arrowheads) in between the calcification stripes (scale bar 1 cm).

Reproduction Organelles

Tel-Baruch's *P. pavonica* has several reproduction organelles and possesses two main reproduction cycles: a-sexual and sexual reproduction. In our observations, a-sexual reproduction occurred in the fall. During September and October 2015 and 2016, diploidic tetraspores were expressed **(Figure 5a)**.

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The sexual reproduction cycle transpires from winter to spring. During October and November 2015 and April 2016, haploidic organelles, i.e., dark-brown oogonia, light-brown antheridia and green meiospores, were expressed **(Figure 5b)**.

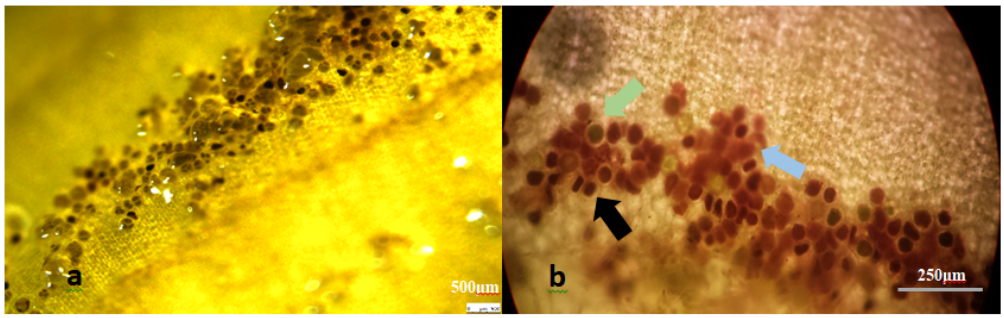

Figure 5. Reproduction organelles of *P. pavonica* a): A diploidic (2n) tetraspores representing a-sexual reproduction organelles; b): A haploidic (n) expression of sexual reproduction organelles. The darker female gametes (oogonia-black arrow), the light-brown male gametes (antheridia-pale blue arrow) and the meiospores (light green arrow).

This observation is not consistent throughout Israel's shores. On the same day of the harvest in Tel-Baruch, at October 7th, 2015, we have collected samples from Palmachim, ~25 Km (coastline) southern to Tel-Baruch, and unlike the algae in Tel-Baruch, the algae in Palmachim expressed a-sexual reproduction, i.e., tetraspores. These findings are not in accord with Brawley and Johnson's [14] study on brown algae in which they claim that gametes are expressed under low temperatures, whereas spores are expressed under high temperatures. However, since the Israeli climate is sunny most of the year, it is plausible that even the slightest change in temperature can affect the reproduction expression, making our observation a reasonable one [15].

Additional Observation

Throughout the two years of research, we saw a lot of epiphytes on the $CaCO_3$ stripes of *P. pavonica*, which looked not only attached but also merging with the needles **(Figure 6)**. It seems that the needles are growing onto the Foraminifera and the Diatoms that live on the algae. This merging was observed mainly in algae larger than 4.5 cm fronds (measured from the apical end to the rhizoids) rather than on the smaller 1 cm fronds. This observation makes us wonder if there is a symbiotic relationship between the epiphytes and *Padina spp*. regarding the light reflection, that is, whether the epiphytes add to the aragonite reflecting effect or whether they are parasitical leftovers. We also found microscopic worms on top of the frond along with other plankton which could be living along the hairs having some micro-habitat of their own. At the macroscopic levels, we've collected many time a large long marine centipede of some sort, which was 'resting' in-between the fronds, and at the beach, we observed small fish and crabs indicating that *Padina*'s mini-forest can be an ideal nursery environment for some species.

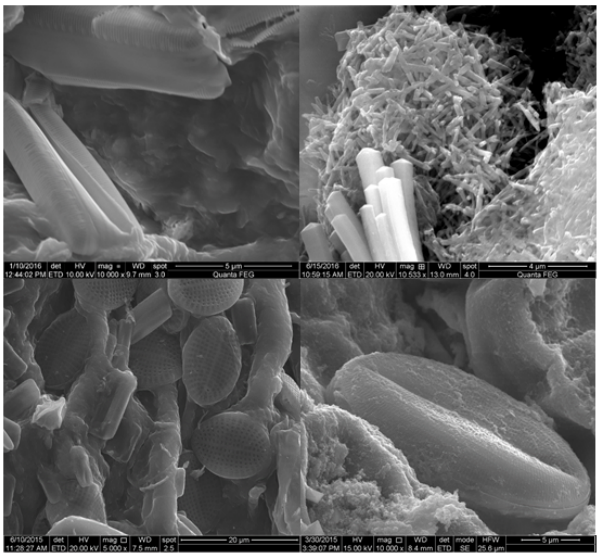

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Figure 6. Different types of epiphytes observed under ESEM. The epiphytes and some fragment of their skeleton are on top and sometimes merging into the $CaCO_3$ needles.

CONCLUSION

There is a lot to learn on the flora that inhabits the Israeli coastline. This observational research tries to add more information on the two *Padina spp*. inhabiting Tel-Baruch, Palmachim and other shores in Israel. These visual macroscopic and microscopic differences between *P. pavonica* and *P. gymnospora* needs more genetic proof and we still need to know whether the $CaCO_3$ precipitation is genetically encoded, phenotypically expressed or both. By understanding better this environment and its inhabitants, we can predict what can happen to them in consideration of global warming, plastic pollution, and more mankind's environmental effects.

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