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Research Article

# Morpho-anatomical Studies on the Genus *Padina* (Dictyotales, Phaeophycota) from the Coast of Karachi, Pakistan

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**Abstract:** Specimens of the genus *Padina* Adanson were collected from northern coast of the Arabian Sea at Karachi, Pakistan during March 2006–April 2009 and investigated for their morphology, anatomy and reproductive structures in details. The collection included five species which were studied for their size and shape of surface cells, presence and absence of intercellular spaces, cell-wall thickness, not studied by previous workers. Two recently described species, i.e., *P. afaqhusainii* and *P. nizamudinii*, were thoroughly investigated and compared with the other three species. The lack of hair bands in the former species and the presence of more layers in the middle part as compared to upper and lower parts in the latter species were observed as their characteristic features.

**Keywords:** Dictyotales, *Padina*, taxonomy, morphology, anatomy, reproductive structures

# 1. INTRODUCTION

The brown algal genus *Padina* Adanson is widely distributed in the warm temperate to tropical coastal regions, where it is found in the lower intertidal to deep subtidal zones [1]. Its thalli are flattened and generally fan shaped with an inrolled margin enclosing a marginal meristem by which growth is initiated [2, 3]. The thallus is calcified to a greater or lesser extent on both surfaces or only on the upper (superior) surface facing the inrolled margin. In some species prostrate rhizomes, called *Vaughaniella* stages, develop from a single apical cell, from which new erect thalli subsequently arise and become several cells thick [4].

Species of *Padina* are generally delineated based on the morphological features: (i) gross morphology, (ii) calcification of the thallus, (iii) presence or absence of *Vaughaniella* stage, (v) the position and arrangement of hair lines, (vi) the position and arrangement of tetrasporangial sori relative to the hair lines, and (vii) presence or absence of an

indusium, a hyaline cover over tetrasporangial sori. However, species—level taxonomy is still somewhat confused because of morphological differences between species that are difficult to describe, as well as considerable morphological variation *e. g.* thallus size and shape, the intensity of calcification, etc [6]. In addition, comparative studies of various *Padina* species have remained difficult owning to confusion in descriptive terminology and anatomy and insufficient information about morphological features of previously described species [7–9].

Occurrence of *Padina* at the coast of Karachi, Pakistan was initially reported by Anand in 1940 [10]. Later on, its various species were observed to grow at the Karachi coast [11-18], but only a few studies were made on their taxonomy [19]. Recently attention has been paid in this connection, and a large collection of its species growing in the coastal waters of Karachi was carried out. Five species were collected in this survey, which are being described in this article.

### 2. MATERIALS AND METHODS

The specimens were collected during March 2006-April 2009 from Manora, Buleji and Paradise Point, the coastal areas near Karachi, Pakistan. The material was brought to the laboratory, washed thoroughly and preserved in 4 % formalin-seawater solution for further investigation. Some material was preserved in the form of herbarium sheets and kept in the Herbarium, Department of Botany, Federal Urdu University, Karachi (FUU-SWH). Cross sections (C.S.) of the material were obtained by free hand cutting with the help of shaving blades, which were then stained with iodine, mounted in glycerine and sealed with the help of nail polish. Prepared slides were examined under Nikon PFX microscope, photographs were taken with F 601 camera and developed in a photolab with hp scanner. The photographic plates were prepared in Adop Photoshop 7.0 with the help of a computer.

### 3. RESULTS

The general observation and microscopic investigation of the collected specimens indicated the presence of five species of *Padina* which may be distinguished as follows:

Basal part of frond more than 5 cells layered 3

2. No hair band in the basal portion

1. Basal part of frond up to 5 cell layered ......2

- 3. Sporangial lines on both surfaces, hair lines absent...... gymnospora (4)

  Sporangial lines on one surface, hair lines not

Sporangial line one, abutting hair line

.....P. boergesenii (3)

These species differ from one another in a number of thallus characters (Table 1). Detailed studies indicated the following characters of the investigated species.

# 1. Padina afaqhusainii Aisha & Shameel: 2010: 320

**References:** Begum 2010: 219, Aisha & Shameel 2010: 320 [20, 21].

# Morphological Characters

Thalli greenish brown or olive green in colour; surface smooth, margin entire or slightly undulate; thalli 3 – 7 cm long, 3–5 mm thick at the base, 2.5 – 4.0 cm thick at the middle and 1.5 – 4.5 cm thick at the apex; base attenuate, basal portion reddish brown, attached with the help of a small, compact, rhizomatous holdfast, 3–6 mm broad and 4 – 7 mm long; basal region forms a long stipe, 0.6 – 1.7 cm long, 3–7 mm broad; in basal portion sporangial and hair lines not present; apical portion involute, apex slightly undulate; sporangial lines and hair lines alternate, sporangial lines 1–3 mm apart, hairs and sporangial lines alternate (Fig. 1).

#### **Anatomical Features**

In surface view: peripheral cells light brown, cubical or slightly elongated, with single phaeoplast, arranged in regular tires, thick-walled, 10.0-22.5  $\mu m$  in length and 12.5-35.5  $\mu m$  in breadth (Fig. 2); sporangial and hair lines alternate to each other; sporangia dark brown, rounded or oval in shape, arranged in rows (Fig. 3).

In the apical portion: thalli composed of 2-3 layers *i.e.* upper and lower peripheral layers and 1-2 cortical layers; peripheral cells small, quadratic or cubical, thin-walled, with a single large commashaped phaeoplast, 15-25 µm in length and 17.5-25.0 µm in breadth (Fig. 4); cortical cells slightly rectangular or quadratic, palisade like or horizontally elongated, thin-walled, poor in contents but in some cells few phaeoplasts present, 25.0-37.5 µm in length and 25.0-32.5 µm in breadth; thallus width 75 µm (Fig. 5).

In the middle part: thalli composed of 3-4 layers *i.e.* upper and lower peripheral layers and 1-2 cortical layers; peripheral cells small, quadratic or cubical, thin-walled, with dense phaeoplast,  $25.0-37.5 \mu m$  in length and  $25-37 \mu m$  in breadth; cortical cells large, thick-walled, inter-cellular spaces absent, poor in contents, in some cells phaeoplasts present, quadratic or slightly rectangular, 25-50

**Table 1** Comparison of characters about five species of *Padina*.

Thallus characters	P. afaq- husainii	P. antil- larum	P. boer- gesenii	P. gym- nospora	P. nizam- uddinii
Height (cm)	3-7	7-15	4-14	4-15	10-15
Breadth (cm)					
Upper	1.5-4.5	7-12	7-15	4.0-6.5	9-11
Middle	2.5-4.0	10-12	5-11	10-13	10-13
Basal	0.3-0.5	7.5-10.0	7-10	7-11	1-5
Colour	greenish brown	olive green	greenish brown	greenish brown	greenish brown
Surface	smooth	smooth	smooth	smooth	smooth
Margin	entire/ undulate	entire/ undulate	slightly undulate	slightly undulate	entire
Hair band	alternate	alternate	alternate	absent	not prominent
Sporangial lines	on both surfaces	on both surfaces	on one surface	on both surfaces	on one surface
Holdfast Length (mm) Breadth(mm)	4-7 3-6	7-20 5-15	7-10 5-7	8-12 5-8	5-9 3-5
Cell layers Upper Middle Basal	2-3 3-4 4	2-3 3-4 4 (-5)	2 2-3 3(-4)	3 (-4) 4-5 6-7 (-8)	3 (-4) 4 (-5) 6-7
Sporangia Length (μm) Breadth (μm)	75.0-82.5 50.0-62.5	20-60 15-35	75-125 50-75	20-45 17.5-32.5	50.0-62.5 50-55
Indusium	absent	absent	present	absent	absent

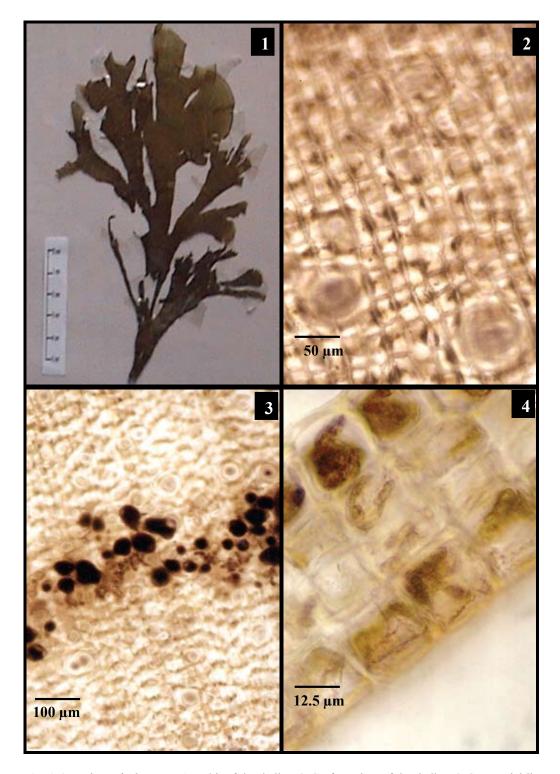
 $\mu m$  in length and 25 – 45  $\mu m$  in breadth; thallus width 125  $\mu m$  (Fig. 6).

In the basal portion: thalli composed of 4 layers *i.e.* upper and lower peripheral layers and 2 cortical layers; peripheral cells thick-walled, cell-wall thickness  $5.0 - 7.5 \mu m$ , cubical or slightly rounded, in some thalli thin cell-wall present with a single comma shaped or rod like phaeoplast,  $20 - 25 \mu m$  in length and  $20 - 25 \mu m$  in breadth (Fig. 7); cortical cells are more or less equal in size with peripheral cells, cubical or quadratic, poor in contents, thick-walled, cell-wall thickness 5.0 - 7.5

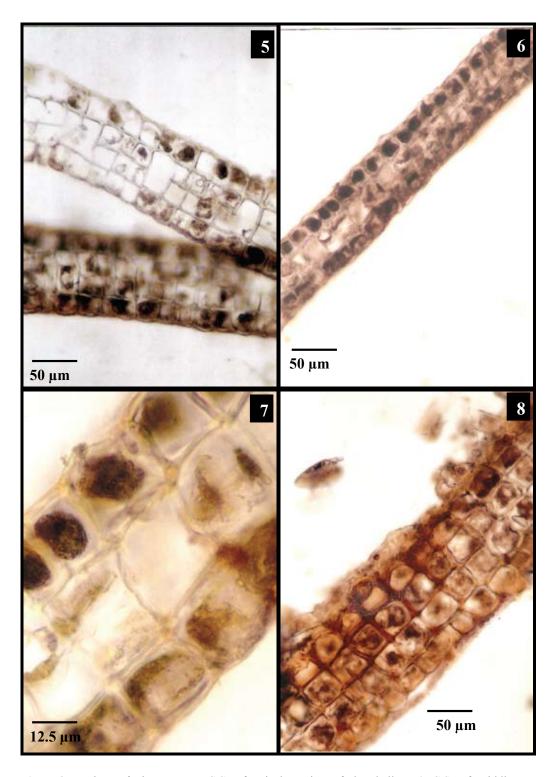
 $\mu$ m (Fig. 8), intercellular spaces absent, 20.0-27.5  $\mu$ m in length and 17.5-25.5  $\mu$ m in breadth; thallus width 150  $\mu$ m (Fig. 9).

# Reproductive Structures

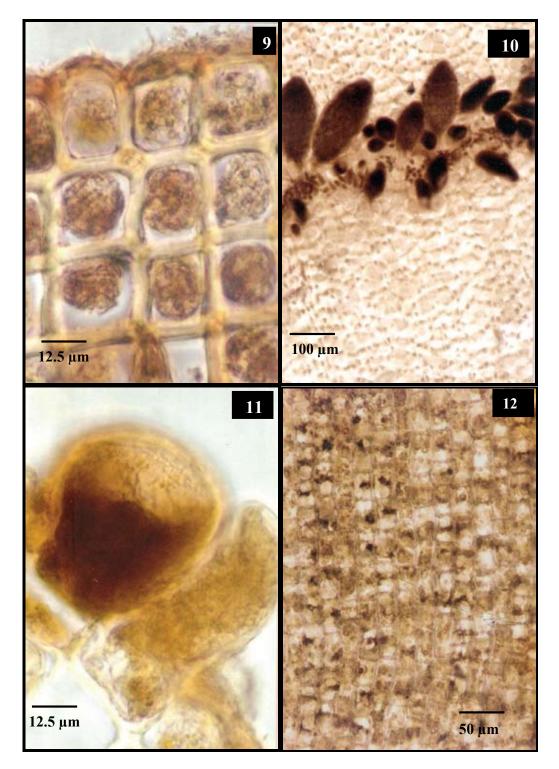
Sporangia found in sori on the surface of thallus (Fig. 10); dark brown in colour, oval or rounded, with a single basal cell, arise from peripheral cells, occur singly or in group of a few,  $75.0-82.5~\mu m$  in length and  $50.0-62.5~\mu m$  in breadth; basal cell small,  $25-30~\mu m$  in length and  $12.5-17.5~\mu m$  in breadth (Fig. 11).



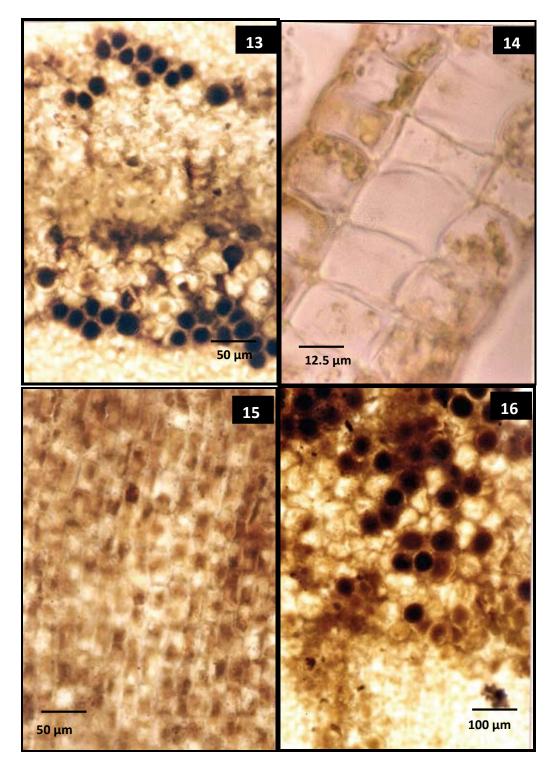
**Fig. 1-4.** *Padina afaqhusainii*: **1.** Habit of the thallus, **2.** Surface view of the thallus, **3.** Sporangial line in surface view, **4.** C.S. of thallus showing peripheral cells.



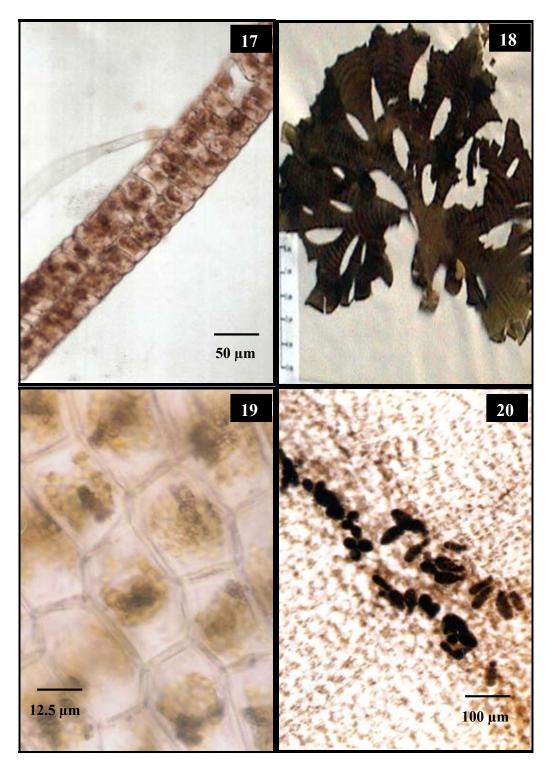
**Fig. 5-8.** *Padina afaqhusainii*: **5.** C.S. of apical portion of the thallus, **6.** C.S. of middle part, **7.** Enlarged view of middle portion, **8.** C.S. of basal part showing thick walls.



**Fig. 9-12.** *Padina afaqhusainii*: **9.** Enlarged view of basal part of the thallus, **10.** Arrangement of sporangia, **11.** Sporangium arising from a peripheral cell; **12.** *P. antillarum*: Surface view of thallus.



**Fig. 13-14.** *Padina antillarum*: **13.** Sporangial lines in surface view, **14.** C. S. of apical portion of thallus, **Fig. 15.** *P. boergesenii*: Surface view of the thallus, **Fig. 16.** Sporangia in surface view.



**Fig. 17.** *Padina boergesenii*: **17.** C.S. of apical portion showing unequal cells; **Fig. 18-20.** *P. gymnospora*: **18.** Habit of the thallus, **19.** Surface view of thallus, **20.** Sporangial line in surface view.

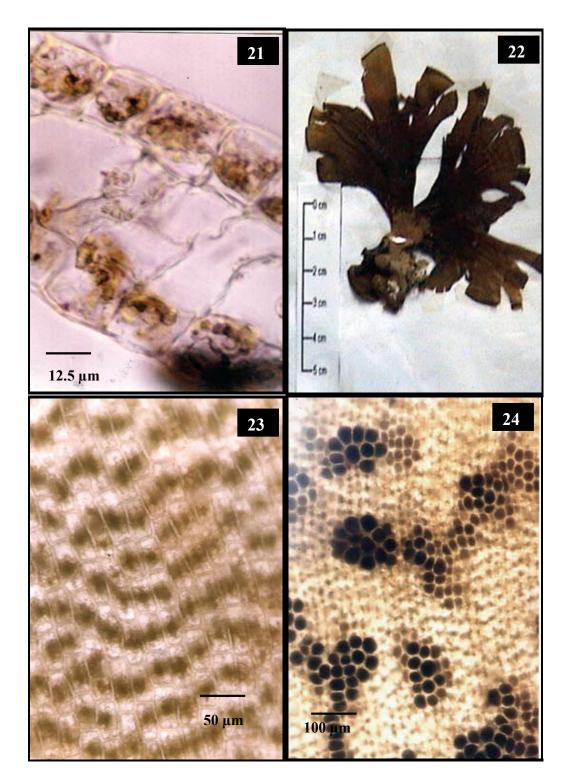


Fig. 21. *Padina gymnospora*: 21. C.S. of apical portion of the thallus; Fig. 22-24. *P. nizamuddinii*: 22. Habit of the thallus, 23. Surface view of thallus, 24. Sporangial sori in surface view.

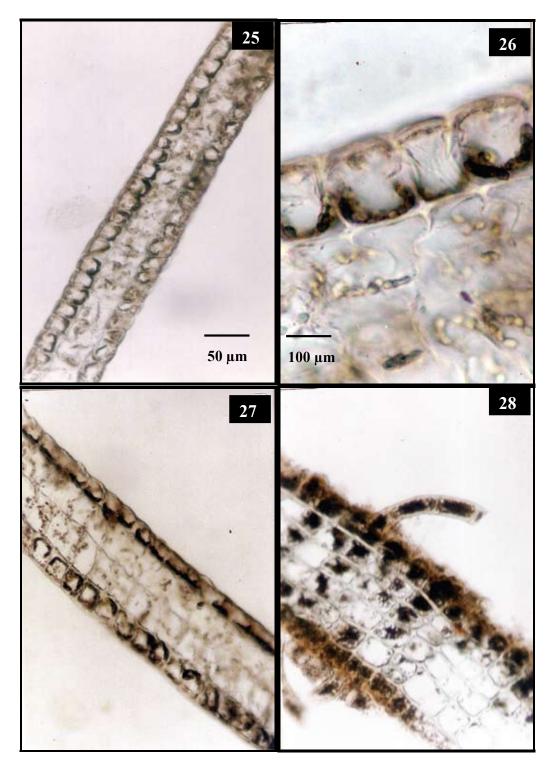
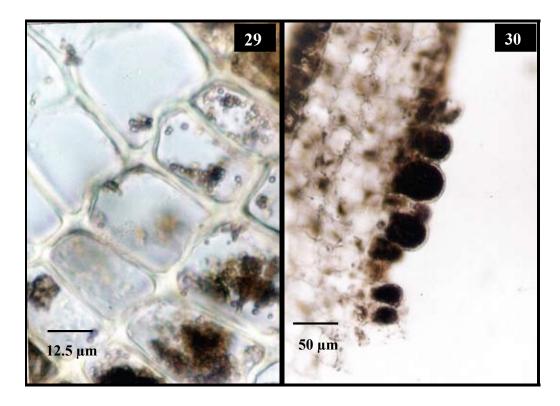


Fig. 25-28. *Padina nizamuddinii*: 25. C.S. of apical portion of the thallus, 26. Enlarged view of peripheral cells, 27. C.S. of middle part, 28. C. S. of basal portion.



**Fig. 29-30** *Padina nizamuddinii*: **29.** Cortical cells and intercellular spaces in the basal part, **30.** Sporangia arising from peripheral cells.



Fig. 31-32. Habit of the thallus: 31. Padina antillarum, 32. P. boergesenii.

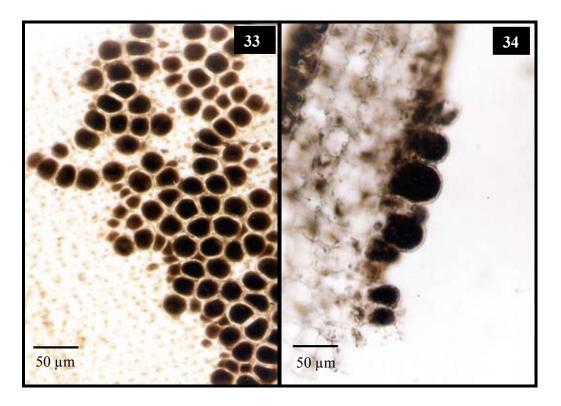


Fig. 33-34 Padina nizamuddinii: 33. Arrangement of sporangia, 34. Sporangia arising from peripheral cells.

**Specimens examined:** Collected as drift material or benthic on rocks of mid-littoral region, at Manora (*Leg.* Alia Abbas 6-4-2009); Goth Haji Ali, Buleji (*Leg.* Alia Abbas 31-3-2009).

Type locality: Manora, Karachi, Pakistan.

Local distribution: Karachi: Manora and Buleji.

Distribution in the Indian Ocean: Pakistan only.

### 2. Padina antillarum (Kützing) Piccone 1886: 36

**Basionym:** Zonaria antillarum Kützing 1859: 29. **Synonym:** Padina tetrastromatica Hauck 1887: 43 [22].

# Morphological Characters

Thalli olive green or dark green in colour, dichotomously or irregulary branched; margins smooth or slightly undulate, apex enrolled, surface smooth; sporangia present in double sporangial lines, sporangial lines and hair lines alternate to each other; attached with the help of a small, compact, holdfast, 0.5-1.5 cm broad and 0.7-2.0 cm long; thallus dived into many lobes, upto  $\frac{3}{4}$  part of the thallus, many clefts present on the thallus; thalli 7

-15 cm long, 7-12 cm thick at the apex, 10-12 cm thick at the middle and 7.5-10.0 cm thick at the base; some animal castings attached to the basal part on both surfaces (Fig. 31).

# **Anatomical Features**

In surface view: thalli dark brown, peripheral cells cubical or rectangular,  $7.5-20.0~\mu m$  in length and  $7.5-15.0~\mu m$  in breadth; double sporangial lines present at specific intervals (Figs. 12), sporangial lines alternate with the hair lines (Fig. 13). In the apical portion: thallus consists of 2-3 layers *i.e.* upper and lower peripheral layers and one layered cortex; peripheral cells cubical or squarish, thin walled, cell size more or less equal, with dense phaeoplasts,  $17.5-25.0~\mu m$  in length and  $20-25~\mu m$  in breadth (Fig. 14); cortical cells large, rectangular or slightly cubical, thin-walled, poor in contents, intercellular spaces absent,  $25.0-32.5~\mu m$  in length and  $12.5-25.0~\mu m$  in breadth. Other details have already been given previously [23].

**Specimens examined:** Manora (*Leg.* Alia Abbas 6-4-2009); Goth Haji Ali, Buleji (*Leg.* Alia Abbas 17-3-2007, 18-3-2008, 24-1-2009, 31-3-2009).

# 3. Padina boergesenii Allender et Kraft 1983: 87

*Synonyms: Padina fraseri* (Greville) Greville 1830: 15, *P. tristromatica* Levring 1942: 60 [22].

# Morphological Characters

Thalli greenish brown in colour, surface smooth; margins entire or slightly undulate, apex involute; attenuated base, attached with the help of a compact, small, rhizomatous holdfast, 0.7-1.0 cm long and 5-7 mm broad; sporangial lines and hair lines alternate, in between hair line a broad sterile portion present; sporangial lines broad, brown in colour, 0.5–1.0 cm apart; distance between hair lines 3–5 mm; thalli 4-14 cm long, 0.7-1.0 cm thick at the base, 5–11 cm thick at the middle and 7–15 cm thick at the apex, the apex and margins slightly undulate, at the upper portion thallus becomes fan-shaped; upper portion broad and gradually becomes narrow at the base; at the apical portion few concentric hair lines present and below this portion hairs and sporangial lines regularly present (Fig. 32).

#### Anatomical Features

In surface view, peripheral cells are elongated or slightly cubical, thin-walled arranged in regular manner, with a single phaeoplast;  $15-65~\mu m$  in length and  $12.5-35.0~\mu m$  in breadth (Fig. 15); sporangial lines arranged with hair lines (Fig. 16). In the apical portion: thalli composed of 2 layers, both layers unequal in size, upper layer consists of comparatively large cells, and lower layer contains small cells (Fig. 17), thin-walled, intercellular spaces absent, with dense phaeoplasts, large cells  $50-55~\mu m$  in length and  $20-30~\mu m$  in breadth, small cells  $32.5-35.0~\mu m$  in length and  $17.5-27.5~\mu m$  in breadth, cell-wall thickness  $2.5~\mu m$ ; thallus width  $75-100~\mu m$ . Other details have already been given previously [24].

**Specimens examined:** Manora (*Leg.* Alia Abbas 6-4-2009); Goth Haji Ali, Buleji (*Leg.* Alia Abbas 15-3-2006).

# 4. *Padina gymnospora* (Kützing) Sonder 1871: 47

**Basionym:** Zonaria gymnospora Kützing 1859: 29.

*Synonyms: Padina vickersiae* Hoyt *in* Howe 1920: 595, *P. crassa* Yamada 1931: 67 [22].

## Morphological Characters

Thalli fan-shaped, greenish brown in upper portion and brown in the basal portion, spliting into lobes; 4–15 cm long, 4.0–6.5 cm thick at the apex, 10–13 cm thick in the middle and 7–11 cm thick at the base; attached with the help of a small, compact, rhizomatous holdfast, 0.8–1.2 cm long and 5–8 mm broad; surface smooth, margins slightly undulate, apex enrolled, sporangial lines present on both surfaces of the thalli; sporangial lines 3–4 mm apart and concentric; hair lines absent, not prominent; on sporangial lines *in situ* syntagmatic germination presenting a velvety look (Fig. 18).

#### Anatomical Features

In surface view: horizontal rows of cells, peripheral cells variable in size, hexagonal, rectangular, a large dense phaeoplast present in the center of the cells, 23.5-80.0 µm in length and 25.0-32.5 µm in breadth (Fig. 19); sporangial lines present at specific intervals (Fig. 20). In the apical portion: thalli consists of 3 (-4) layers i.e. upper and lower peripheral layers enclosing 1 (-2) cortical layers, peripheral layers slightly unequal, dorsal layer consists of small, cubical cells and ventral layer composed of comparatively large cells, thinwalled with dense phaeoplast, 20-30 µm in length and 17.5-25.0 µm in breadth; cortical cells large, rectangular, palisade like, poor in contents, thinwalled, 25-50 µm in length and 20-30 µm in breadth (Fig. 21). Other details have already been given previously [25].

*Specimens examined:* Goth Haji Ali, Buleji (*Leg.* Alia Abbas 24-1-2009).

5. Padina nizamuddinii Aisha et Shameel 2010 References: Begum 2101: 230, Aisha & Shameel 2010: 326 [20 & 21].

# Morphological Characters

Thalli greenish brown, fan-shaped; surface smooth, margins entire, apex involute; 10 - 15 cm long, 9 - 11 cm thick at the apex, 10 - 13 cm thick at

the middle and 1–5 mm thick at the base; erect, attached with the help of a small, compact, solid holdfast, 3–5 mm broad and 5–9 mm long; hair lines not prominent, and sporangial lines seem to be concentric, dark brown; transverse, wavy bands present on the surface of the thallus (Fig. 22).

#### Anatomical Features

In surface view: peripheral cells rectangular, with single phaeoplasts,  $15-25 \mu m$  in length and  $7.5-15.9 \mu m$  in breadth (Fig. 23). All over the surface sporangial lines are present, hair lines are not prominent (Fig. 24).

In the apical portion: thalli composed of 3 (-4) layers *i.e.* upper and lower peripheral layers and 1 (-2) cortical layers; peripheral cells small, cubical, thin-walled, with a single & large phaeoplast, arrangement of phaeoplast in the cells is very unique and not found in other species, phaeoplasts arranged in the lower side of peripheral cells, and rod shaped, 25–30 μm in length and 12.5–25.0 μm in breadth (Fig. 25); cortical cells comparatively large, rectangular, thin-walled, with or without phaeoplast, intercellular spaces absent, arranged in regular tiers, 25–45 μm in length and 12.5–20.0 μm in breadth (Fig. 26).

In the middle part: thallus consists of 4 (-5) layers *i.e.* upper and lower peripheral layers enclosing 2 (-3) cortical layers; peripheral layers consist of small, cubical, thin-walled cells with dense phaeoplasts, 25.0–37.5 μm in length and 22.5–35.0 μm in breadth; cortical cells, cubical or slightly rectangular, thin-walled, poor in contents, intercellular spaces absent, 30.0–37.5 μm in length and 22.5–25.0 μm in breadth (Fig. 27).

In the basal portion: thallus composed of 6 (-7) layers (Fig. 28); upper and lower peripheral cells more or less equal in size, small, cubical or squarish, thin-walled with dense phaeoplasts, 25.0–30.0 µm in length and 25–30 µm in breadth; 4 (-5) layered cortex composed of large, thin-walled, cubical or squarish cells, with or without phaeoplast, intercellular spaces present, 20.0–32.5 µm in length and 25–30 µm in breadth (Fig. 29).

### Reproductive Structures

Tetrasporangia found in groups (Fig. 24); oval or

globular, stacked, dark brown or reddish brown in colour,  $50.0-62.5~\mu m$  in length and  $50-55~\mu m$  in breadth (Fig. 30).

**Specimens examined:** Benthic on shallow sandy pools at Goth Haji Ali, Buleji (*Leg.* Alia Abbas 31-3-2009).

Type locality: Hawksbay, Karachi, Pakistan.

*Local distribution*: Karachi: Manora, Hawksbay, Buleji and Nathiagali.

Distribution in the Indian Ocean: Pakistan only.

### 4. DISCUSSION

Padina is a commonly occurring genus of the family Dictyotaceae (order Dictyotales, class Dictyophyceae, phylum Phaeophycota; fide [26]) at the coast of Karachi. Its five investigated species were found to be distinguished from one another to some extent (Table 1). Padina afaqhusainii resembles P. glabra Gaillard in the absence of hairbands, non indusiate sporangial sori and lack of calcification but differ from it in having thallus organization of variable cell-layers from upper to lower parts. It is two cell-layered at marginal part and six-layered in the lower part, three layered near marginal portion of the basal part, but medullary or middle parts comprise of 4-6 layers of cells [21]. In P. glabra sporangia are borne mainly on the superior surface of the blade and has frequently on the inferior face but they do not occur in dense concentric sori [28], while club-shaped tetrasporangia are present in P. afaqhusainii on both surfaces of thallus and arranged in concentric layers.

Padina antillarum is traditionally known as *P. tetrastromatica* in Pakistan as well like other counties [27] which was recently designated as synonymous to this species [28]. It has 3-4 cell layers, and its most distinguishing characteristic is that tetrasporangia are girdling the hairline. It is often confused with *P. distromatica* Hauck but is different from it because the latter is always two-cell layered [29]. It can be distinguished from *P. gymnospora* by thicker blades of the latter species (6-8 layers in the basal region) and soral bands placed proximal to each successive hair zone [28].

Apparently P. nizamuddinii resembles P.

afaqhusainii, P. gymnospora and P. tenuis Bory. In P. afaqhusainii tetrasporangia are club shaped and arranged in concentric layers and phaeophycotean hairs are present in between two layers of reproductive organs in younger stage of sporangia but absent in growing or mature sporangia [21]. While in P. nizamuddinii the tetrasporangia are oval shaped alternating with single layer of phaeophycotean hairs. This species has anatomically quite distinct features such as middle part with more layers of cells (4-7) as compared to upper (3-4) and lower (2-5) parts of thallus [21]. This character is only found in old middle or mature part of thallus, whereas younger middle part has number of layers as in the lower part. On the basis of this peculiar arrangement of cells in middle part of the thallus this species differs from *P. tenuis*. This is a new species, recently reported from the coast of Karachi, [21]. The present study is the second report of its occurrence from this coast. Large thalli, as reported earlier, could not be collected. As initially reported, the hair bands are not present in the basal portion of the thallus. This appears to be a distinctive character of the species, as verified by Prof. Dr. Gerald Thompson Kraft (pers. comm.). This is the detailed investigation of the anatomical features of this species, which was not made previously. This is another new species, recently reported from the coast of Karachi Pakistan [21], and the present study is the second report of its occurrence from this coast. Thalli were found as large as initially reported, but internally the layers of cells were observed to increase from upper part to the basal portion. This is the first detailed investigation on the anatomy of this species. While examining its description, Prof. Dr. G. T. Kraft observed similarities of this species with the very widespread P. gymnospora (pers. comm.). But the new species differs from it in its peculiar arrangement of cells in the middle part of the thallus [20].

Inconsistent use of taxonomic terminology, uncertainty concerning diagnostic characters for species definition and the absence of DNA sequence data present difficulties in *Padina*. Taxonomy of a genus that is anatomically based can be extremely uncertain because of morphological plasticity. Several studies using chloroplast-uncoded *rbcL* and mitochondrial encoded *cox* 3 sequences as well

as meticulous morphological observations have documented species-level taxonomy of *Padina* from Japan, Hawaii, Malaysia, Indonesia, Palau and the Mediterranean Sea [30]. These have resulted in the proposal of several new diagnostic characters for species delineation. Recently molecular analysis using *rbc*L and *cox*3 genes as molecular markers confirmed the identification of several species of *Padina* from Japan [1, 5, 6]. Similar studies on Pakistani specimens, if made, may confirm their identity.

### 5. ACKNOWLEDGEMENTS

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