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Functional Anatomy of Air Conducting Network on the Pneumatophores of a Mangrove Plant, *Avicennia marina* (Forsk.) Vierh.

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Abstract: Pneumatophore of *Avicennia marina* (Forsk.) Vierh. were studied by Light Microscopy (LM) and Scanning Electron Microscopy (SEM) in order to relate their development and structure of their function as air conduits. Specifically, the developmental pattern is based on cell separation and expansion (schizogeny) rather than cell lyses and most appearance revealed that these roots have honeycomb aerenchyma type, with air spaces which run longitudinally down the root axis. During gas space formation, the cortex cells wider parallel to the root axis, elongate parallel to the root radius and shrank in the plane perpendicular to the radius leaving long and thin rows of cortex cells. The aerenchyma has a pronounced tubular structure, run parallel to the root axis, relatively straight and the walls of the tubes being made up of single layers of cells in regular rows. There appeared to be interconnections between tubes and no perforated membrane or transverse septa were observed on the tip of the each tube. As a longitudinal appearance, a network of aerenchyma revealed as many long tubes with tapering tip and connected each other create as a continuum. There are radial pores at the corner of the cortical cells. These pores seem to function as interconnections between the tubes and radial internal gas pathway. From transversal view, the aerenchyma is composed of cells in the form of a squat X, Y, I, or T united by the ends of their arms into a network. The path of air from the atmosphere through the lenticels into the aerenchyma and then to the feeding roots and cable root is described as well.

Key words: Aerenchyma, *Avicennia marina*, cortex cells, radial pores, schizogeny, lenticel

INTRODUCTION

Avicennia marina is a common mangrove species on tropical and subtropical sea shores, swamps and stream banks[1-4]. In mature tree, the root system of *A. marina* is complicated and quite similar to *Sonneratia alba*. It has four root types, i.e. cable roots, pneumatophores, feeding roots and anchor roots (Fig. 1). Cable roots run horizontally and radially for several meters from the tree. Pneumatophores grow vertically upward and expose their tips in air. Anchor roots grow vertically downward about 1 m depth. Feeding roots originated from pneumatophores just under the ground and grow horizontally. The pneumatophores are covered by an impermeable periderm. At low tide, lenticels on the surface of the pneumatophores allow gas exchange between the atmosphere and the internal structure of root. Therefore, the pneumatophores has important function as a highly specialized ventilation mechanism, enabling the plant to survive in anaerobic soil.

Roots of mangrove plants are typically posses an aerenchymatous cortex[5,6]. The aerenchyma considered to be an important adaptation to flooded soils because it provides a mechanisms for root aeration in environment characterized by low oxygen concentrations[7,8] and act as a pathway for oxygen to supply respiring roots[9].

Despite the importance of aerenchyma for the survival of mangrove species very little is known about the development and organization of aerenchyma tissue, in mangrove root system. The structure of pneumatophores of *A. marina* has been studied previously by Gordon and Dubinsky[10], however there is still no clear how this air conducting network develop in this species.

Aerenchyma formation process clearly varies among plant species, with one striking difference being that aerenchyma can be produced in either a constitutive or induced manner[5,11,12]. Root aerenchyma is apparently formed under all environmental conditions or in a constitutive manner in most wetland species[5,13-15]. For

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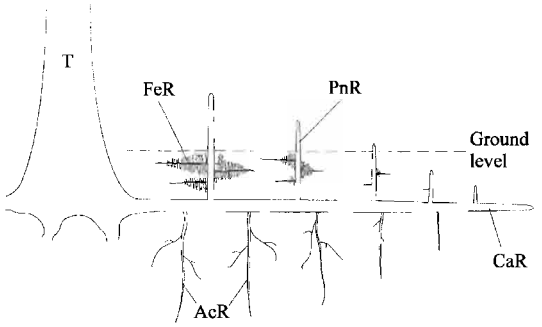

Fig. 1: Simplified diagram of the mature root system of *A. marina* that showing the different types of roots observed. CaR (cable roots), primary roots with lateral horizontal growth. PnR (pneumatophores), aerial roots with upward vertical growth from CaR. FeR (feeding roots), lateral horizontal root grown from PnR. AcR (anchor roots), vertical root grown downward from CaR, T, trunk

many wetland species, the formation of extensive aerenchyma is an integral part of normal root development and is considered constitutive and pre-adaptive[16,17]. In other species, root aerenchyma is not formed when roots are subjected to environmental stress, most notably low oxygen concentrations[8,18].

The present study examines the tissue differentiation process of the pneumatophores of *A. marina* from their apical meristems and three dimensional structure of matured aerenchyma system, to assess the relationships between tissue structure and habitat adaptation.

MATERIALS AND METHODS

Pneumatophore and other root types samples were taken from adult trees (10-15 cm in trunk diameter at base, 0.5-1 m tall) of *Avicennia marina* (Forsk.) Vierh., which grow naturally in Urauchi (24°23'N, 123°46'E) and Komi estuary (24°19'N, 123°54'E), Iriomote Island, Okinawa Prefecture, Japan, at March 2002, December 2002 and July 2003 and September 2004. The sampled trees are sparsely distributed in seaward outer fringes of mangrove forest where the plants are flooded by all high tides and easily influenced by strong winds and tidal forces. Around the sampled trees, we excavated root system (Fig. 1) of the trees during low tide and collected pneumatophore. Five adult trees were used as sample and for each root type; we collected from more than five cable roots. The longitudinal distances of cells or tissues are measured from the root tips in the meeting point of root cap initials and vascular cylinder initials.

Light microscopy observation: The samples were prepared for paraffin sectioning method. They were fixed and preserved in FAA (70% ethanol, 10% formalin, 5% acetic acid -90:5:5). The air in the tissue was evacuated by oil rotary vacuum pump. Dehydrated in an ethanol series and embedded in paraplast plus (Oxford Labs, USA) in 59°C. Sections were cut at 10-12 μm by rotary microtome HM 350 Microm, Heidelberg Germany and stained in Safranin-Fast Green[19-21] and permanently mount by Bioleit (Oken Shoji, Tokyo). Finally all of the observation work was done on a light microscope (BX 50 Olympus Co. Japan). Microscopic images were taken by Microscopy camera (Olympus PM-C35, Japan) and recorded on Fuji Film Neopan F ISO 32/16° films for black and white prints observed.

Maceration study was also carried out for cortex cell and sclereid observation. The samples were trimmed into slivers thinner than a toothpick and then keep in a mixture (1:1) of glacial acetic acid and 6% hydrogen peroxide at 60°C for 36 h. After this treatment, the macerated materials were washed in distilled water, stained in Safranin O. Macerated materials were mounted for observations.

The measurements and counts carried out on the cortex cells of the different root types were performed on 5 roots for each root type using computer assisted image analysis (IPLab Ver. 3.5-Scientific Imaging Software) by digitizing the images with a digital camera attached to a compound microscope.

SEM observation: Pneumatophores were cut into cubic of 5-8 mm length pieces using razor blade for SEM preparation using Resin Casting Method[22]. The tissue was fixed in FAA and dehydrated in a graded ethanol-*t* butyl alcohol and freeze dried at -10°C (HITACHI ES-2030 Freeze Dryer). The dried samples were embedded in styrene monomer-polyester resin with 1% benzoyl peroxide in the gelatin capsule and vacuum for several minutes. The samples were put at the oven 60°C overnight for polymerization. After removing gelatin, samples were trimmed using a chisel or sand paper. The trimmed sample was immersed into a mixture of hydrogen peroxide-acetic acid (1:1) to remove lignin for one day or more at the oven 60°C, after that it was washed in water and put on the sulfuric acid 64% to remove cell wall polysaccharides for one day. The resin cast was rinsed with water and cleaned by agitation in the water using mini supersonic cleaner. The sample was dehydrated again in a graded ethanol-*t* butyl alcohol and freeze dried. The dried sample was glued to the specimen stub that coated with conductive carbon tape. The sample was coated with platinum-palladium in vacuum evaporator (HITACHI E-1030 Ion

Sputter), observed and photographed on Scanning Electron Microscope (HITACHI S-4100).

RESULTS

A. marina produces characteristics pneumatophores that are uncovered at low tide and in contact with the atmosphere. Surfaces of pneumatophores had many little spot or mere pimples of lenticels (Fig. 2). The clorenchyma of the pneumatophores cortex beneath may be responsible for overall green color in the surface of pneumatophores. As relation to lenticels, there appear to be two kinds of pneumatophores which may be described briefly as the smooth and the rough types. The rough type has numerous lenticels, which project markedly from the surface and the smooth type (young pneumatophores) posses fewer or no lenticels, which still underground.

The root apical meristems of pneumatophores of *A. marina* are fundamentally same in structure with other root types, except for the root size (Table 1). The meristem apex is usually with three tiers of initials: root cap, cortical-epidermal and vascular cylinder initials (Fig. 3). The root apical meristem organization is a closed system, usually with clearly apparent boundary of cells between cortex and root cap. The most striking feature of the root is the distinct and extensive root cap with quite long files of cells. The tissue that present in all roots outside the curving boundary of the outermost later of

Table 1 Root diameters and cell numbers at different distances behind the root tip in four root types of *Avicennia marina*

Root types and distances behind the tip (mm)	Diameter (mm)	No cells in epidermal layers (cross section)	No of radial files of cells in cortex (longitudinal section)
Feeding roots			
2	1 51±0 01	272±36 6	18±0 5
6	2 01±0 01	326±34 8	16±0 4
10	2 32±0 01	392±46 5	14±0 8
20	3 02±0 01	402±59 8	13±0 5
Anchor roots			
2	1 89±0 01	344±25 5	30±1 2
6	2 52±0 01	385±37 1	29±0 8
10	3 13±0 01	404±48 5	28±0 8
20	4 86±0 01	433±49 6	26±0 5
Cable roots			
2	3 63±0 01	697±45 3	45±0 8
6	4 52±0 01	809±55 8	43±0 6
10	6 85±0 02	919±63 6	40±0 6
20	8 01±0 01	925±66 6	39±0 4
Pneumatophores			
2	2 06±0 02	381±42 7	36±0 5
6	3 13±0 02	409±40 5	34±0 8
10	3 88±0 02	519±51 2	30±0 5
20	6 35±0 02	530±49 7	29±0 5

-Number of cells in epidermal layer was measured by counting all the cells in epidermal layer as seen on Fig 4A-C
-Number of radial files of cells in cortex was measured by counting the column of cell files as seen on Fig 4D-F

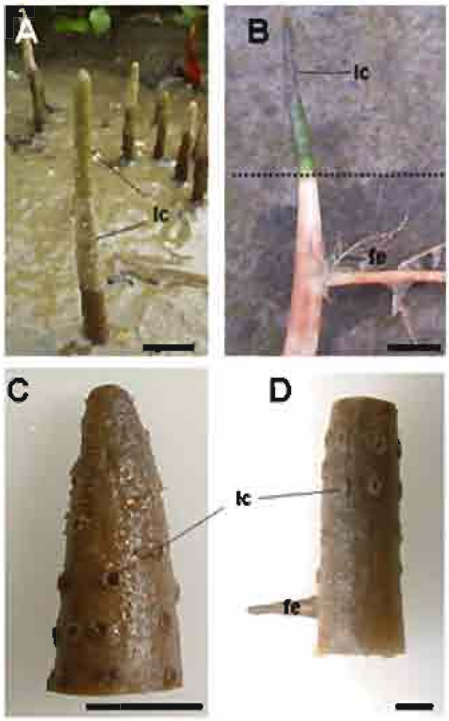

Fig. 2: Structure of pneumatophores as gas exchange place in *A. marina*. The lenticels mere pimples or spot on the surface of pneumatophores (A-B), Bar = 2 cm. The clorenchyma of the pneumatophore cortex beneath may be responsible for overall green colour and the dot line reveals the ground level. C) Root tip of pneumatophores with many lenticels , appearance as crater-like. Bar = 8 mm. D) More distance for root tip with young lateral root (feeding root) growth. Bar = 4 mm fe. feeding roots; lc. lenticel

ground meristem of cortex are the lateral root cap-epidermal initials, a later of cells encircling the columellar initials and outer cortical initials like an obliquely flattened band (Fig. 3). In pneumatophore this layer contains a distinctive wedge-shaped cell which can be observed near the juncture of the cortical and columellar initials.

Aerenchyma development: All the cells in the cortex are appeared intact in cross sections from close to the root apex (0-100 μm distance from the root apex). The cortex cells lacked intercellular spaces and were arranged in columns of cell files that extended from an endodermal layer to epidermal layers in each of root types (Fig. 4).

Cortex cells were relatively rounded or polygonal in cross section near the root apex, they are transversely enlarged, become thin and form arms cell during to their development. The initial stage of intercellular space

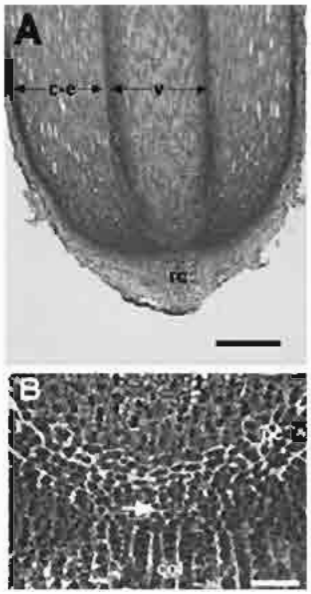

Fig. 3: Median longitudinal sections of pneumatophore of *Avicennia marina*. A) Pneumatophore tip (Bar = 400 μm). B) Higher magnification taken from meristem apex region of A. There are more than one tier of cortical-epidermis initials (arrows). The root cap initials are the layer below and the vascular initials are above the cortical-epidermis initials. c-e. cortex-epidermis; col. columella; pc. procambium; rc. root cap; v. vascular bundle

formation is revealed within 200 μm distant from the root tip. Behind the distance, the cell separation is more prominent and followed by longitudinal and then radial cell expansion leading to enlargement of the intercellular space to form aerenchyma, involving radial and longitudinal expansion of cells with arm formation (Fig. 4). Collapse nor dissolution of cells in the cortex of pneumatophores are not observed and it seems that in all root types the intercellular spaces were arisen schizogenously.

The number of cortex cells in radial files of pneumatophores was quite different with other root types (Table 1), pneumatophores have 36 files in average, whereas the cable roots have the most numerous files (45 files in average), while, the feeding roots have the fewest (18 files in average). However, the arrangement of cortex cells was generally same among the root types.

Root diameters increased as a distance from the root apex (Table 1). Within a given root of all root types, the number of cells in epidermal layers showed quite increased and cortical layers remained relatively constant from close to the root apex where there were little

Table 2 Cortex cell size from longitudinal sections taken at 0-200 μm (no intercellular spaces) and 1-2 cm (well developed intercellular spaces) distance the root apex

	Distances behind the apex meristem					
	0-200 μm		1-2 cm		% Increase	
Root types	Vertical	Radial	Vertical	Radial	Vertical	Radial
Feeding roots						
Mean±SD	9 3± 0 4	17 9± 2 1	36 0± 6 7	39 6± 5 7	387 1	221 2
Anchor roots						
Mean±SD	13 7± 1 9	21 3± 2 1	37 5± 3 1	47 0± 6 1	273 7	220 7
Cable roots						
±Mean±SD	20 5± 3 3	18 7± 2 2	36 7± 3 6	54 0± 3 6	179 0	288 7
Pneumatophores						
±Mean±SD	17 4± 2 4	18 7± 3 5	44 5± 8 3	44 8± 3 8	255 7	239 6

Table 3 Total average of aerenchyma diameter in matured roots

Root types	Measured point distant from the root apex (mm)	Radial (μm)	Tangential (μm)
Feeding roots	20	143 5±34 7	56 1±16 2
Anchor roots	20	134 5±25 4	49 3±8 6
Cable roots	50	252 5±45 2	115 2±16 7
Pneumatophores	50	235 1±42 2	103 5±14 1
Average		188 9±58 8	80 95±23 3

intercellular spaces to much further behind it (Table 1). It show that the root diameter growth by extension of cells and intercellular space.

In cross sections at 5-10 mm distant from the root apex, the radial files of cells became extent and separated with each other in some areas of the cortex. The intercellular spaces between radial files of cells become larger and larger as the distance increased (Fig. 4A-C). The longitudinal dimensions of cortex cells increased more than 200% in all root types (Table 2). The intercellular space formation process was observed also in longitudinal sections. The aerenchyma was developed by the separation of cortical cells along their longitudinal walls which parallel to the root axis. The part between 0-200 μm distance from the root apex, there were few intercellular spaces and cortical cells were tightly packed (Fig. 4D). The part over 6 mm distance from the root apex, cortical cells began to separate and some intercellular spaces appeared (Fig. 4E and F).

Aerenchyma structure: Most characteristic appearance in fully differentiated pneumatophores of *A. marina* on cross section is broad lacunose cortex. Lacunae are distributed between hypoderm and endodermis and arranged in a honeycomb-like structure in all root types (Fig. 4C). The lacunae are designated as aerenchyma because no liquid content was found in it when we cut sample pieces from the excavated roots.

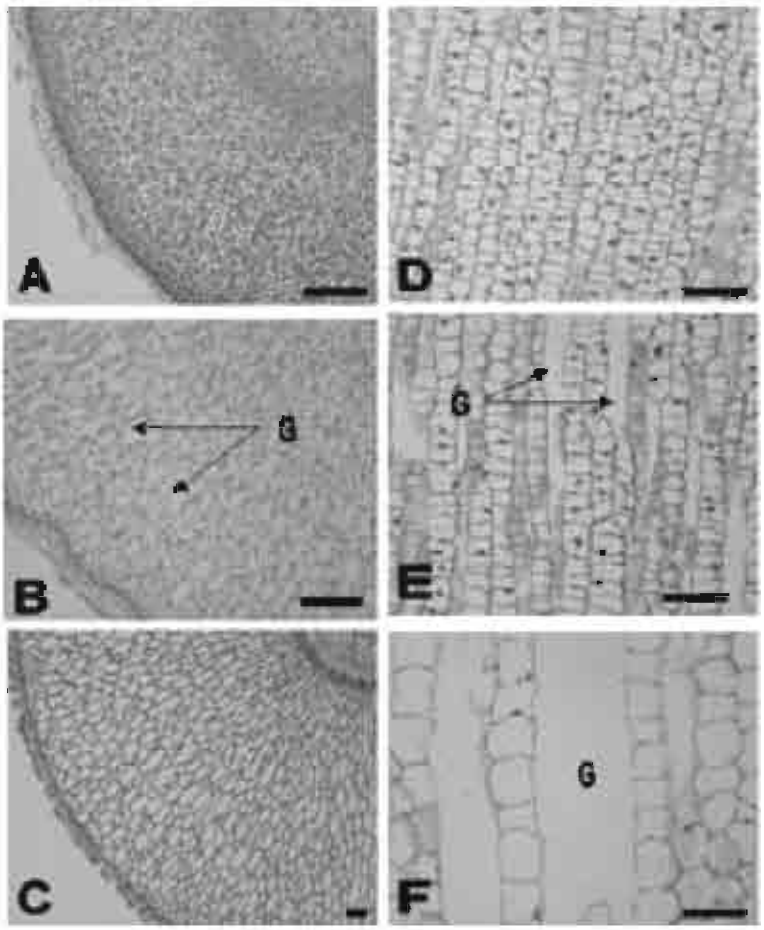

Fig. 4: Serial cross sections (A-C) and longitudinal sections (D-F) of pneumatophores of *A. marina* at different distance from the root apex. A) 3 mm (Bar=200 μm); B) 9 mm (Bar=200 μm); C) 2 cm (Bar=50 μm); D) 200 μm (Bar=50 μm); E) 6 mm (Bar=50 μm) and F) 12 mm (Bar=50 μm). G) gas space

It is tubular structure longitudinally to the root axis in indeterminate length and connect each others on their overlapping tips with very fine canals (Fig. 5B and D) and no perforated plate or transverse septa on the tip of the each tube (Fig. 5C and D). The aerenchyma is delineated by a layer of cortical parenchyma cells (aerenchyma wall cells). The aerenchyma wall cells are thin walled, transversely long rectangular or polygonal in lateral view (Fig. 5A).

The aerenchyma is radially elongated elliptical or oval in cross section, 134.5-252.5 and 49.2-115.2 μm in radial and tangential diameters, respectively (Fig. 6D and Table 3). An aerenchyma is surrounded by four to six wall cells with I, X, Y or T shape in cross section (Fig. 6D and 7). Its lateral surface is sculptured by transversely elongated rectangular or polygonal aerenchyma wall cells (Fig. 5A and 6B).

The interconnection between the tubular aerenchyma is performed by very fine horizontal intercellular spaces (pores), about 7-8 μm in diameter (Fig. 6C), between two adjoining wall cells (Fig. 6B). It is quite difficult to get full shape of these pores by resin casting method, because this fine structure is easily broken (Fig. 5C). Aerenchyma tubes connected longitudinally with overlapping area of their tapering ends just look-like as vessel elements, although the aerenchyma has no perforation plates.

Lenticels and root-root junction structure: Lenticels spread in the surfaces of pneumatophores and only clearly showed at the region of aerial part of these roots. These varied in size and range in morphology from classical crater-like (Fig. 2C and D) with a mass of fluffy tissue in the centre to unopened pustules. The lenticel of this species is composed of complementary (filling) tissue,

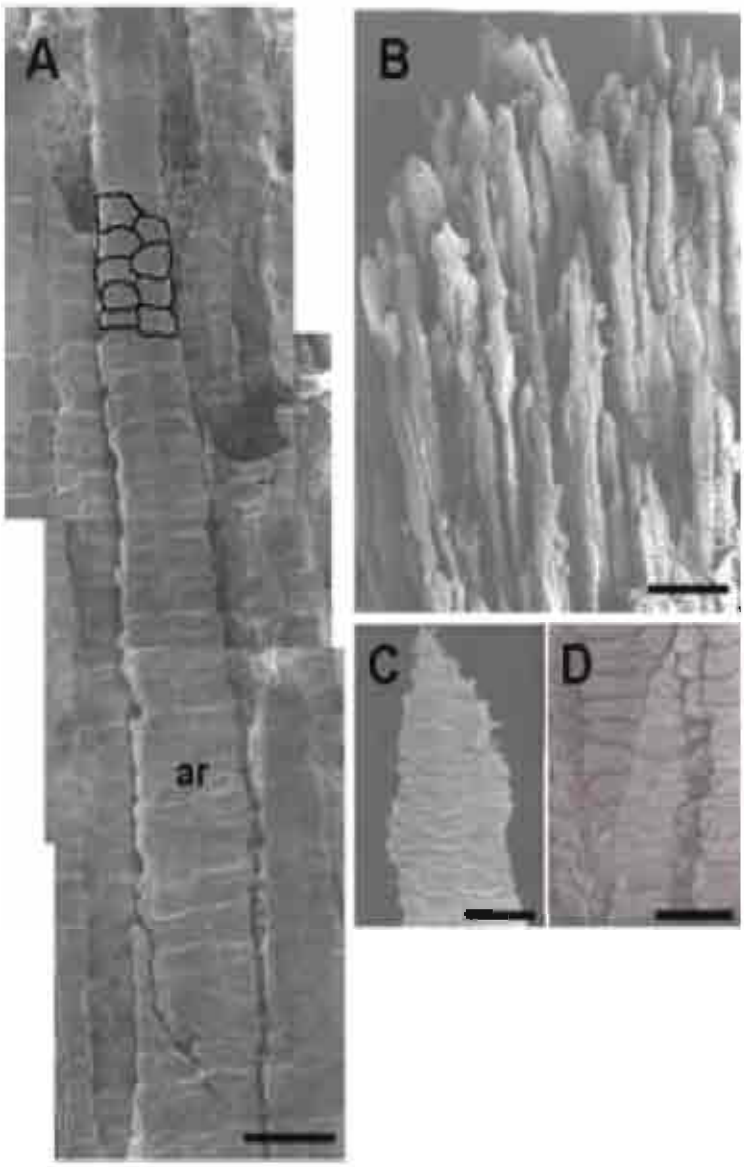

Fig. 5: Longitudinal view of aerenchyma resin cast of cable root of *A. marina* by SEM observation. A) Inner surface view of tubular structure of aerenchyma. There are no transverse septa that interrupt the longitudinal tubes. Lines draw in to emphasize the parenchyma cell pattern of elongated rectangular or polygonal that composed the walls of aerenchyma (Bar=200 μm). B) Tips of aerenchyma tubes that run parallel to the root axis (Bar = 420 μm). C) A tip of aerenchyma tube without perforation. Fine prickle-like protrusions on surface of the tip are residue of the fine pores (Bar = 75 μm). D) Overlapping tips between two tubes (Bar = 75 μm). ar. aerenchyma

which consists of thin walled spheroidal cells by intercellular spaces (Fig. 8 and 9). They concave disc, stacked with no intercellular matrix (Fig. 9 C and D). The cells may be suberized by positive stained of safranin.

Lenticels appear up to 0.5 cm below the pneumatophore tip and the uppermost lenticels are mere pimples on the surface of the pneumatophores in *A. marina*. As it grows, the complementary tissue ruptures the periderm and the lenticel become functional (Fig. 8D). After that, it supposed that a suberized layer is formed by the meristem beneath the complementary tissue of the lenticel. Eventually the meristem begins to form complementary tissue inside this suberized layer which eventually ruptures and is cast off along with all of the old

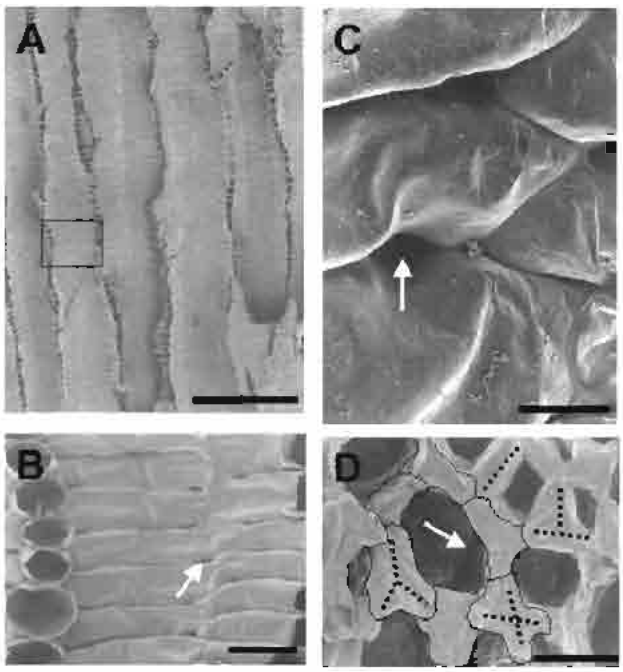

Fig. 6: SEM observation of cable root aerenchyma in *Avicennia marina* (not resin cast) showing pores in the walls of aerenchyma tubes. A) Longitudinal section of cable roots at 4 cm behind the tip (Bar = 375 μm); B) Magnification in the square part of figure A (Bar = 33 μm); C) More magnification of figure A in the square part that show the radial pores (arrow) (Bar = 15 μm); D) Cross section of cable root at 4 cm behind the tip and show the position of radial pores (arrow) (Bar = 60 μm); The dash line show the cells in the form of a squat I, X, Y or T united by the ends of their arms into a network

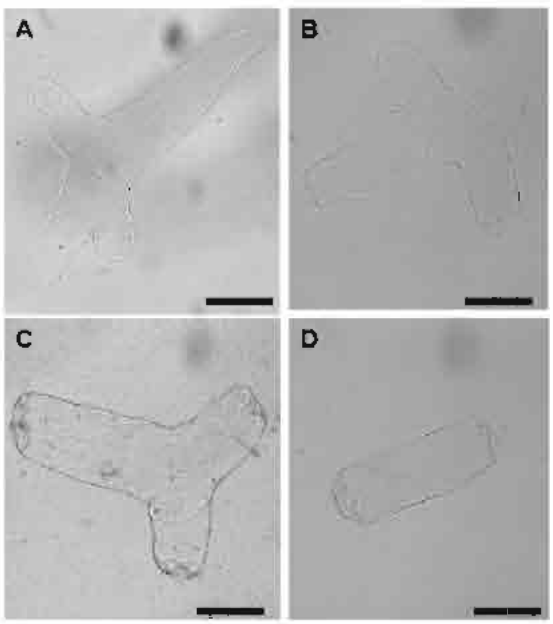

Fig. 7: Various types of arm cells on pneumatophores of *A. marina* observed from maceration method. A) X-shape, B) Y-shape, C) T-shape and D) I-shape. Bar= 50 μm

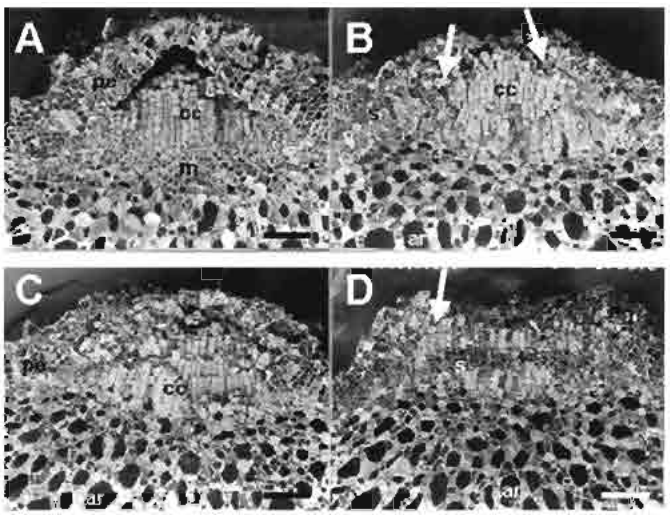

Fig. 8: Cross section of pneumatophore of *A. marina* at a lenticel which has undergone several growth cycles. A. closed lenticels (Bar= 100 μm). B. some periderm ruptured and lenticels start to open (Bar= 150 μm). C-D. open lenticels (Bar= 150 μm). ar. aerenchyma; cc.complementary tissue; m. meristem; pe. periderm; s. suberized tissue. Arrows indicate ruptured suberized layers with old complementary tissue

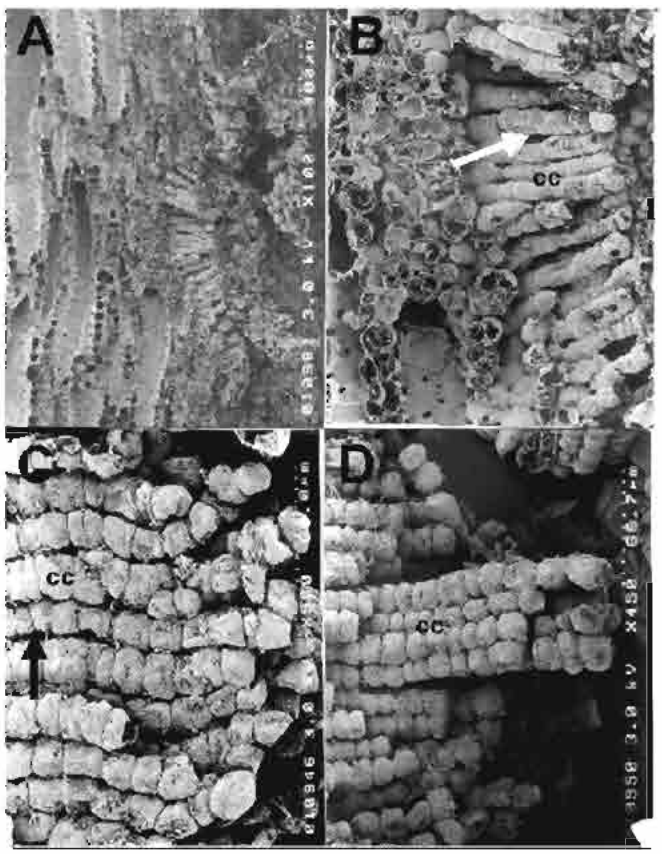

Fig. 9: Longitudinal section of pneumatophore at a lenticel (A,B) and complentary cells (C,D). Mass of complementary cells (cc) make regularly alignment and there is a intercellular space (arrows) between them and there is no intercellular matrix on this space

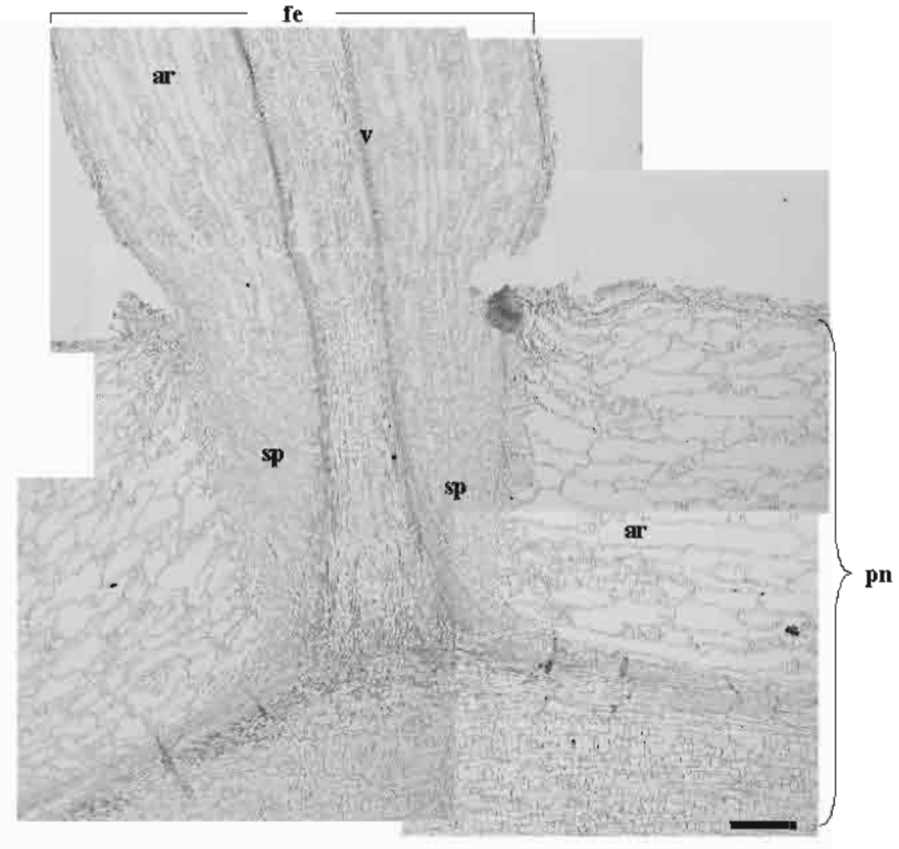

Fig. 10: Longitudinal section through the junction of pneumatophore (pn)-feeding roots (fe), showing the continuity of aerenchyma from the pneumaphore via mass of spongy tissue (sp) of parenchyma. ar. aerenchyma; v. vascular bundle. Bar=200 μm

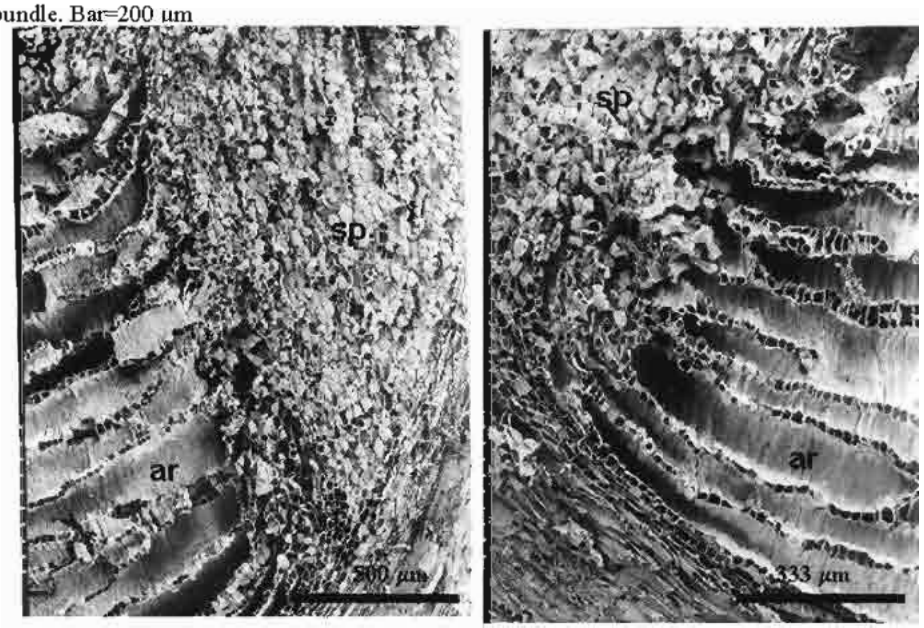

Fig. 11: Longitudinal section of pneumatophore of *A. marina* through the junction of cable root-pneumatophore. It showing that the end of tubular structure of aerenchyma (ar) of the cable roots make a little bend at the meeting point to mass of spongy tissue (sp) of parenchyma at base of pneumatophore

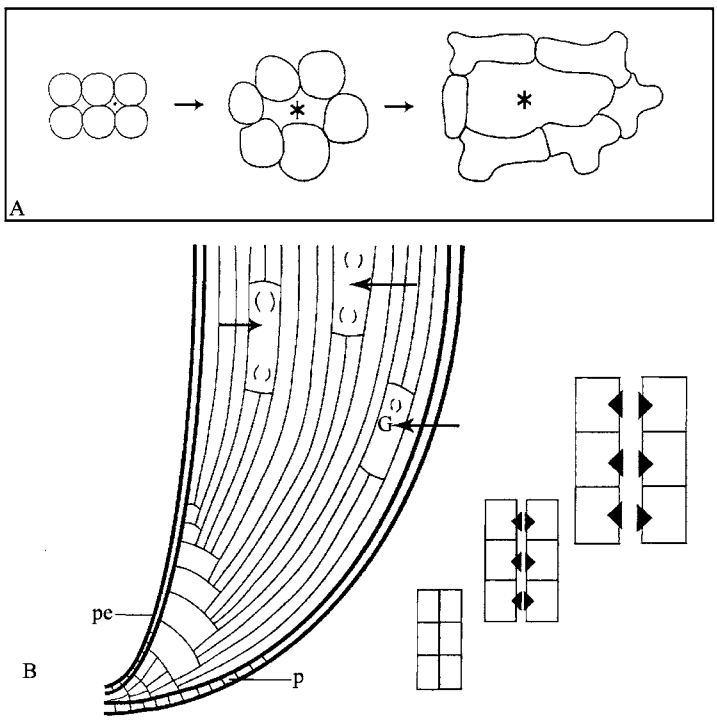

Fig. 12: Schematic of the cortex development from the apical meristem of *Avicennia marina*. A. Transverse view of growth of some radial files of parenchyma large cells producing gas spaces (aerenchyma lacunae) (asterisk) from intercellular spaces. The cortex cells become extended and folded making an arm cell (ar). B. The longitudinal view presents the cell file of the ground meristem from the several tiers at the tip. The innermost layer is proendodermis (pe) and the outermost layer is protoderm (p). The positions of separating cells of the developing gas spaces (G) are indicated by arrow

complementary tissue. A scar remains at the edges of the lenticel (Fig. 8D) shows a short section of the suberized layer and some complementary tissues. From the presence of several sets of scar tissue in some lenticels it is obvious that this may be happen many times in the life of one lenticel.

The lenticels development of these roots briefly describe as follows. The parenchymatous cells adjoining the aerenchyma tubular structure in the cortex increase in size and divide. The resulting daughter-cells give rise to complementary tissue which fills up the 'air-chamber' in regular form of files formation. A curved cell-layer, convex towards the inside, undergoes tangential divisions and becomes the 'lenticellar meristem' (Fig. 8A), which continually cuts off additional complementary cells on its outer side. The pressure of the steadily expanding regular files of complementary tissue causes the epidermis layer to bulge outwards and finally to burst and make the epidermis layer become rupture; the complementary tissue than protrudes in places, hence to rough surface of the lenticels. On older pneumatophores, which are already covered with periderm, the lenticels arise from the phellogen, which, at certain points, produces complementary tissue with abundant intercellular spaces in place of the normal uninterrupted layers of cork (Fig. 8 and 9B) and thus becomes locally converted into lenticelllar meristem. The layer of cork above a developing lenticel suffers the same fate as the epidermis in the previous explanation, being distended and finally ruptures by the growing complementary tissue.

As its growth, the complementary tissue ruptures the periderm and the lenticel becomes functional. After a while, a suberized layer was formed by the meristem beneath the complementary tissue (Fig. 8A) of the lenticel. Eventually the meristem begins to form complementary tissue inside this suberized layer which ruptures and its cast off along with all of the old complementary tissue (Fig. 8D).

In the longitudinal sections of pneumatophores of *A. marina*, it showed the quite similar condition as in cross section. However it showed clearly that the mass of complementary cells make regularly alignment and there are intercellular spaces or gaps between the files of complementary cells. These spaces or gaps may provide

access to the aerenchyma tubular structure in the cortex area (Fig. 9A and B). It was observed also there is no intercellular matrix on these spaces.

At the junction of branch and main roots there is an abrupt constriction of the branch and a diaphragm of small rounded cells passes obliquely across the cortex at this point (Fig. 10). This meeting point was composed as mass of spongy tissue with many intercellular spaces between the cells. The cortical lacunae of two roots intercommunicate only via the many small intercellular spaces of this layer.

There is a transition area of aerenchyma in the region between cortical tissue with longitudinal oriented cylindrical channels and a little loose spongy tissue with no apparent direction of orientation (Fig. 11). The continuity of gas space in this area appeared interrupted by the layer of mass cell that growth from the pericycle of previous root. However, this area is still support the continuum of gas space between the cylindrical channels of one root type to the others by intercellular space on the tissue of this mass tissue. The gas spaces in this spongy aerenchyma formed a continuum of gas spaces between the aerenchyma tubular structure of one root and the other root.

DISCUSSION

Aerenchyma development: Cortex development followed the same general pattern of schizogenous in pneumatophores examined here. The development of cortical cells produced aerenchyma gas space as summarized in Fig. 12. Air spaces developed was due in part to greater extension of the arms of cortical cells and in part to actual separate of numerous cells (Fig. 12A and B), their walls folding together so completely that was originally a cell, elongated and branched in the dimension perpendicular to root radius in cross section (Fig. 12A). Especially, the intercellular spaces appeared to be result of increased distance between cortex cells because the number of files of cells changed little as the distance from the root tip (Table 1). The mechanism that would produce these results is unknown, but appears complex. There must be rather tight adherence between cells of different files[23].

Aerenchyma formation in pneumatophores of this species is morphologically same with other mangrove species, *Sonneratia alba*[24]. The result also confirmed to the brief report of Curran[9] that revealed consistent schizogenous origin for the intercellular spaces in the same species. The longitudinal and radial expansions of growing aerenchyma cells appear same with other plants reported in literature. There is schizogeny occurring at these stages of root growth that involves intercellular space expansion. Initially, the expansion of the gas space is by growth of the cell walls between point attachments. A situation found in roots of other wetland species like *Filipendula ulmaria* and *Caltha palustris*[15], where aerenchyma in the form of broad lacunose cortex, giving the spongy appearance, develop by cell separations and enlargement of intercellular spaces following earlier schizogenous intercellular space formation.

Lawton *et al.*[25] reported that air spaces in *A. marina* are more random and lysigenous in origin. While our results did not confirm to this report, we observed that air space in *A. marina* was formed by schizogeny. The well-developed aerenchyma observed here was not the result of cells dying and fragmenting. Furthermore, while there was separation between cortex cells in different files, the deformation of cortex cells would significantly expand the definition of schizogeny[26].

Aerenchyma structure: Generally, the anatomy of aerenchyma in this species agrees closely with that of described by Baylis[27]. In particular, the intercellular spaces, although very long and slightly extended radially in pneumatophores[9], are apparently not in radial files as reported for *A. germinans*[28].

However in this study, the longitudinal tubular structure found in the aerenchyma is not like the network of lacunae of limited extend described by Baylis[28] for the same species in New Zealand, but confirmed to the study reported by Curran[9]. This is may be due to difference in age and growth-conditions of the plants, or to be difficulty of reconstructing in three dimensions using the light microscope. We are success to make appearance of singular aerenchyma tubular structure by resin casting method in this study that never reported by previously study on aerenchyma. The 'sheet of cells' illustrated by Baylis[27] can be reinterpreted as the walls of the aerenchyma tubes.

Baylis[27] described the pores which are visible in the longitudinal section of the pneumatophores aerenchyma, but in our results show that it found in cortex cells of all root types. These pores seem to function as interconnections between the tubes and support radially movement of gas on tubes. Since the aerenchyma tubes are continuous over long distances, it may be important that these interconnecting pores are small, serving to prevent lateral spread of water following damage to root without unduly hindering movement of gas[9,29].

The tubular structure of aerenchyma helps to explain the close agreement found in cable roots between measured diffusion rates and rates predicted from simple theoretical models that proposed by Curran[9], which do

not include allowance for tortuousity. Cortical cells are usually elongated in the plane of the root axis and joined together in files several or many cells in length. Consequently the longitudinal intercellular spaces are essentially tubular and non-tortuous in character.

The highly elongated tubular structure is extensively connected to one another, with the result that the intercellular space forms a continuum. The continuum of aerenchyma creates internal long-distance apoplastic gas transport pathways[30]. This condition will minimized the risk of asphyxiation (lack of oxygen) on the submerged organs when interfaces eliminated by inundation[17,31]. Also the structure of the aerenchyma is ideal for fulfilling dual functions: that is a transport system and storage tissue of the oxygen for high-tide respiration[32].

Structures of lenticels and root-root junction in relation gas pathway of mangrove roots: Lenticels have an important role on the gas pathway of root of *A. marina* as first gate that related to atmosphere directly. The SEM observations on the present study revealed different stages of the lenticels (Fig. 8). We suppose that these are different stages in the development of the lenticels; the partially-opened one (Fig. 8A), a partially-mature lenticels (Fig. 8B) and the fully-opened one (Fig. 8C and D). From the structural analysis we suggest that a developmental process taking place in the lenticels. The young immature lenticels are closed, with a relatively low number of complementary cells. Later, more and more complementary cells are formed, creating an increasing pressure inside the lenticels. When the pressure is high enough, the cork breaks open, forming the partially-opened state of the partially-mature lenticels. As the complementary cells continue to be formed, the additional pressure enlarges the opening, creating the fully-opened mature lenticels. Although gas exchange can occur in the partially-mature lenticels, the fully-opened mature lenticels is probably more effective in the aeration process, as its larger opening and the larger amount of complementary cells inside the lenticels, allow more air to diffuse into the lenticels and to move rapidly. Chapman[33,28], using light microscopy, describes lenticels of *Avicennia nitida* with and without an opening, but associated them with different kinds of pneumatophores. Thus, the suggested development stages for lenticels of *A. marina* may be similar in some or all the species in the genus.

The presence of the of complementary cells with no intercellular matrix will encourages free diffusion of air between the cells, whose concave shape also helps to fulfill this function. Since the reaction to Safranin was positive, it is possible that, because of their suberin and or cutin cover, the accumulation of complementary cells may act as a hydrophobic layer through which air may diffuse while water penetration is prevented.

In the pneumatophore describe in the present study, lenticels arise further down the pneumatophore than most subrisules and are a more permanent site of conductance. Lenticels seem to be rejuvenated several times throughout their functional life. Some lenticels are probably non-functional, especially an older pneumatophores on which the growth of algae is profuse. Pneumatophores which are covered in algae have lenticels which grow out in a fashion which is termed hypertrophy. Hypertrophic lenticels are functional and evident on regions of pneumatophores which are inundated most of the time. Hypertrophic lenticels were not studied here since the interest was mostly with aerenchyma development.

The periderm of pneumatophores is both water-and air-tight resulting in gases being kept in the root system and water out. The periderm covers all of the pneumatophore except at the sites of conductance, lenticels. Since lenticels are large and complex organs of gas exchange, they take time to produce. Therefore lenticels are not found in the tip most section of pneumatophores. If the pneumatophore is actively growing, then the dividing cells at the tip have a high oxygen demand. This demand could be a significant sink of the oxygen which the pneumatophore is supplying to the rest of the isolated root system. It is likely that this demand is met by the other structure like subrisules or horizontal structures in pneumatophores[34,35]. The observation that functional pneumatophores which are not actively growing do not posses subrisules supports this hypothesis.

The suberized layers which are produced by the phellogen along with the complementary tissue in lenticels have been termed closing layers[36]. The production of a closing layer in lenticels has been described previously[36]. Most authors postulate that the function of the closing layer is to hold together the rather loose aggregations of complementary cells. This may be the case in plant species which produce closing layers regularly alternating with layers of complementary cells. The production of closing layer in lenticels of *A. marina* is infrequent and may be a seasonal phenomenon. A closing layer is absent in many lenticels and I have never observed more that one intact closing layer in a lenticel such as was described for some species by Haberlandt[37] although some lenticels have several rings of scar tissue which probably represent ruptured closing layer. The closing layer of *A. marina* is heavily suberized and it is likely that a lenticel with an intact closing layer is non-functional. In this species, the production of closing layer may function as an anti-foulling mechanism whereby the

lenticels clears all settling algae and exposes a fresh conducting surface. Chapman[28] referred to a 'closing layer' at the point where the pneumatophore emerges from the cable root. This is a very different structure and the name should not be used for it. Three stages in the development of lenticels have been identified by Gordon and Dubinsky[10], viz., immature, partially-mature and mature. We suggest an extension of this developmental series to include the production of a closing layer and its eventual rupturing. These two steps may occur many times during the life of lenticels.

Continuity of the aerenchyma gas space system from the main axis into lateral roots can shown in the same way, despite the constriction apparent at their junction which is consequence of the method of cortical development of the newly emerged lateral root. This network fully supports the air conducting on the root system of *A. marina* to adapt on their habitat.

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