

## **UNIVERSITI PUTRA MALAYSIA**

### ASSESSMENT OF MORPHOLOGICAL VARIATION OF MALAYSIAN HALODULE SPECIES COMPLEX;

# NORHAPIZAH AHMAD NAZRI

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MASTER OF SCIENCE UNIVERSITI PUTRA MALAYSIA

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#### ASSESSMENT OF MORPHOLOGICAL VARIATION OF MALAYSIAN HALODULE SPECIES COMPLEX

Ву

NORHAPIZAH AHMAD NAZRI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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This thesis is dedicated to my beloved one...kanda



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

#### ASSESSMENT OF MORPHOLOGICAL VARIATION OF MALAYSIAN HALODULE SPECIES COMPLEX

By

#### NORHAPIZAH AHMAD NAZRI

February 2007

#### Chairman: Associate Professor Japar Sidik Bujang, PhD

Faculty: Science

*Halodule uninervis* and *H. pinifolia* are widely distributed along the southwest, south and east coast of Peninsular Malaysia, Sabah and Sarawak. Eleven locations were selected for this study and each site has different habitat characteristics. Samplings and collections of *Halodule* plants were conducted from August 2002 until May 2004. From the study *Halodule* species occurred on various habitats and a variety of substrates; sand, coralline sand, coral degraded sand, loamy sand, calcareous sandy-mud, sandymud, mud and soft mud. They also grow in wide range of salinity (18-34‰) and depths (-1.5 to -7.0 m MSL). These species were found growing as a single pure population or exist in association with other seagrasses (e.g. *H. pinifolia*, *H. ovalis*, *H. minor*, *H. spinulosa*, *Halophila* species, *C. rotundata*, *C. serrulata*, *T. hemprichii* and *E. acoroides*), seaweeds (e.g. *Ulva reticulata*, *Acanthophora spicifera* and *Gracillaris* sp.) and fauna (e.g. fishes, echinodermata, cnidaria, mollusca and crustacea).

Halodule uninervis and *H. pinifolia* have the capability to adapt in different environments through changes in morphology. Plants on exposed site have short leaves with short erect stems while plants that are under shade and always in submerge condition have long leaves with long erect stems. In addition plants from an area of high sedimentation



also have long erect stems. Branching erect stems with roots at the nodes were found where the substrate is loose coralline sand. Results also showed that plants growing on substrate that contain mud tend to have wider leaves compared to plants on sand substrate. Studies found the types of substrate and spaces availability for the growth of plants have an affect on the morphological variation of rhizome internodes for both species. Leaf sheath morphology, pattern of leaf surfaces, and anatomical structures were not affected by habitat characteristics for both *H. uninervis* and *H. pinifolia*.

Laboratory culture studies on *H. uninervis* in different conditions, showed changes in morphological characteristics. The plants showed similar decrement in length of leaves (almost 50%) for different conditions (with cultures kept under the same amount of ambient light) suggest that light influence the leaf length characteristics. The leaves tend to be narrow for plants in natural substrate (3%) compared to artificial substrate (50%) suggesting that substrate types influenced the leaf width characteristics. Leaf tip morphology did not change among cultures.

Culture observation on *H. pinifolia* in laboratory condition showed that leaves tend to be narrow throughout the period of the study. Observation found that the leaf tip morphology of *H. pinifolia* changes under laboratory conditions. *Halodule pinifolia* exhibited the leaf tip morphology that belongs to *H. uninervis*.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

#### PENGENALPASTIAN KE ATAS VARIASI MORFOLOGI SPESIES KOMPLEKS HALODULE DI MALAYSIA

Oleh

#### NORHAPIZAH AHMAD NAZRI

February 2007

#### Pengerusi: Profesor Madya Japar Sidik Bujang, PhD

Fakulti: Sains

*Halodule uninervis* dan *H. pinifolia* boleh didapati di sepanjang barat daya, selatan dan pantai timur Semenanjung Malaysia, Sabah dan Sarawak. Sebelas lokasi telah dipilih untuk kajian ini dan setiap kawasan mempunyai ciri habitat yang berbeza. Persampelan spesies *Halodule* dijalankan dari Ogos 2002 hingga Mei 2004. Kajian mendapati spesies *Halodule* terdapat dipelbagai jenis habitat dan substrat; pasir, pasir berkarang, pasir serpihan karang, pasir loam, pasir lumpur bercengkerang, pasir lumpur, lumpur dan lumpur halus. Ia juga tumbuh pada julat kemasinan (18-34‰) dan kedalaman (-1.5 to -7.0 m MSL). Spesies ini di dapati tumbuh sebagai populasi tunggal atau tumbuh bersama spesies rumput laut yang lain (*H. pinifolia*, *H. ovalis*, *H. minor*, *H. spinulosa*, *Halophila* species, *C. rotundata*, *C. serrulata*, *T. hemprichii* and *E. acoroides*), rumpai laut (*Ulva reticulata, Acanthophora spicifera* and *Gracillaris* sp.) dan fauna (contoh: ikan, echinodermata, cnidaria, moluska and krustasia).

Halodule uninervis dan *H. pinifolia* mempunyai kebolehan untuk beradaptasi dalam persekitaran yang berlainan melalui perubahan pada morfologinya. Tumbuhan di kawasan yang terdedah mempunyai daun yang pendek berserta batang menegak yang



pendek manakala tumbuhan yang tumbuh di kawasan terlindung dan ditenggelami air mempunyai daun yang panjang berserta batang menegak yang panjang. Tumbuhan dari kawasan yang bersedimen tebal juga mempunyai batang menegak yang panjang. Batang menegak yang beranting dan mempunyai akar pada nodul dijumpai apabila tumbuhan tumbuh di atas substrat seperti pasir berkarang yang tidak padat. Kajian mendapati tumbuhan yang tumbuh di atas substrat yang mengandungi lumpur condong untuk memiliki daun yang lebar berbanding tumbuhan yang tumbuh di atas substrat pasir. Kajian mendapati jenis substrat dan ruang untuk tumbuhan tumbuh menyebabkan tindak balas variasi morfologi ke atas internod rizom untuk kedua-dua spesies yang dikaji. Morfologi seludang daun, corak sel permukaan daun dan struktur anatomi tidak dipengaruhi oleh ciri-ciri habitat untuk kedua-dua *H. uninervis* dan *H. pinifolia.* 

Kajian kultur di makmal ke atas *H. uninervis* dalam keadaan yang terkawal, di dalam keadaan yang berbeza menunjukkan perubahan pada ciri morfologi. Pemendekkan daun adalah sama (hampir 50%) dalam keadaan yang berbeza (dimana kultur diletakkan di bawah pencahayaan yang sama) menunjukkan cahaya mempengaruhi pemanjangan daun. Helaian daun menjadi tirus apabila tumbuh di dalam keadaan bersubstrat semulajadi (3%) berbanding didalam substrat tiruan (50%) menunjukkan jenis substrat mempengaruhi pelebaran daun. Morfologi hujung daun tidak berubah di antara kultur.

Pemerhatian kultur *H. pinifolia* di dalam keadaan makmal yang terkawal menunjukkan daun menjadi semakin tirus sepanjang tempoh kajian. Pemerhatian mendapati morfologi hujung daun *H. pinifolia* berubah apabila dikultur secara terkawal di makmal.



*Halodule pinifolia* mempamerkan morfologi hujung daun yang menyerupai hujung daun *H. uninervis*.



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I certify that an Examination Committee has met on to conduct the final examination of Norhapizah Binti Ahmad Nazri on her degree thesis entitled "Malaysian *Halodule* Species Complex; Assessment on the Morphological Variations" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

#### Tan Siew Goon, PhD

Professor Department of Biology Faculty of Science Universiti Putra Malaysia (Chairman)

#### Faridah Qamaruzzaman, PhD Dr

Department of Biology Faculty of Science Universiti Putra Malaysia (Internal Examiner)

#### Misri Kusnan, PhD

Dr Department of Biology Faculty of Science Universiti Putra Malaysia (Internal Examiner)

#### Misni Surif, PhD

Associate Professor Faculty of Science Universiti Sains Malaysia (External Examiner)

#### **GULAM RUSUL RAHMAT ALI, PhD**

Professor/ Deputy Dean School of Graduate Studies Universiti Putra Malaysia

Date: February 2007



This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

#### Japar Sidik Bujang, PhD

Associate Professor Faculty of Science Universiti Putra Malaysia (Chairman)

Aziz Arshad, PhD Associate Professor Faculty of Science

Universiti Putra Malaysia (Member)

#### Muta Harah Zakaria, PhD

Lecturer Faculty of Agriculture and Food Sciences Universiti Putra Malaysia Bintulu Campus (Member)

AINI IDERIS, PhD

Professor/ Dean School of Graduate Studies Universiti Putra Malaysia

Date: 9 August 2007



#### DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

#### NORHAPIZAH AHMAD NAZRI

Date: 25 June 2007



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- 4.18 Leaf tips of *H. uninervis* from Merambong; associated with 4.38 *Halophila* species on sandy mud; exposed; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.19 Leaf tips of *H. uninervis* from Tg. Adang Darat on mud; 4.39 exposed; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.20 Leaf tips of *H. uninervis* from Tg. Adang Laut; associated with 4.39 *E. acoroides* on calcareous sandy mud; under the effect of sedimentation; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.21 Leaf tips of *H. uninervis* from Pulau Tinggi; associated with *H.* 4.40 *ovalis* and *C. rotundata* on soft mud; under continuous submergence; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.22 Leaf tips of *H. uninervis* from Kuari, Pulau Gaya; mixed 4.40 meadows population on loamy sand; (a) on the same rhizome;
  (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf



- 4.23 Leaf tips of *H. uninervis* from Pulau Layang-Layang; pure 4.41 dense population in the lagoon of reef atoll on unstable loose coralline sand; under continuous submergence; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.24 Details of leaf surface morphology; (a) interveinal spaces 4.41 (40x); (b) detail of lateral-vein region (10x0.1); (c) detail of midvein region (10x0.1); TC- tannin cell; LV- lateral vein; MV- mid vein
- 4.25 Anatomical structure of *H. uninervis*; (a) Transverse section of 4.42 leaf blade (10x0.22); CVB- central vascular bundles; E-epidermal cells; (b) Transverse section of leaf sheath (20x0.4); CVB- central vascular bundle; (c) Transverse section of rhizome (4x10); PV- peripheral vascular bundle; (d) Detail of rhizome vascular bundle region (10x0.22); (e) Detail of rhizome components (10x0.22); E- epidermal cells; M-mesophyll cells; (f) Transverse section of root (10x0.25); M-mesophyll cells; VB- vascular bundles; (g) Detail of root vascular bundle region (20x0.4); (h) Detail of root cortex region (20x 0.4); E- epidermal cells; M-mesophyll cells;
- 4.26 The vegetative morphology of *Halodule pinifolia;* (a) Pure 4.49 dense population in the shallow area on sand in Merchang; (b) Dense growing with *Halophila* species in deeper area on sandy mud in Merchang; (c) Sparse pure population on coral rubble and degraded sand in Teluk Kemang; (d) Growing with *E. acoroides* on calcareous sandy mud and under the effect of sedimentation in Tg. Adang Laut; (e) Mixed meadows population on sandy mud in Lawas; Lb=leaf blade; Ls-leaf sheath; Lt-leaf tip; Es-erect stem; Rh-rhizome; N-node; Rt-root;∂-male flower; Scale bar = 1 cm
- 4.27 The vegetative morphology of *Halodule pinifolia*; (a) Patch 4.50 mixed population on fine sand in Bakau Pulau Gaya; (b) Mixed meadow population on loamy sand in Kuari Pulau gaya; (c) Dominant mixed meadow population on fine sand in Base Camp Pulau Gaya; (d) Mixed population on coralline sand in Pulau Selingan; Lb=leaf blade; Ls-leaf sheath; Lt-leaf tip; Eserect stem; Rh-rhizome; N-node; Rt-root; Scale bar = 1 cm
- 4.28 Leaf tips of *H. pinifolia* from Merchang; pure dense population 4.53 in the shallow area on sand; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.29 Leaf tips of *H. pinifolia* from Merchang; dense associated with 4.53 *Halophila* species in deeper area on sandy mud; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf



- 4.30 Leaf tips of *H. pinifolia* from Teluk Kemang; sparse pure 4.54 population on coral rubble and degraded sand; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.31 Leaf tips of *H. pinifolia* from Tg. Adang Laut; associated with *E.* 4.54 *acoroides* on calcareous sandy mud; under the effect of sedimentation; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.32 Leaf tips of *H. pinifolia* from Lawas; mixed meadows 4.55 population on sandy mud; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.33 Leaf tips of *H. pinifolia* from Bakau Pulau Gaya; patch mixed 4.55 population on fine sand; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.34 Leaf tips of *H. pinifolia* from Kuari Pulau Gaya; mixed meadow 4.56 population on loamy sand; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.35 Leaf tips of *H. pinifolia* from Base Camp Pulau Gaya; dominant 4.56 mixed meadow population on fine sand; (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.36 Leaf tips of *H. pinifolia* from Pulau Selingan on coralline sand; 4.57 (a) on the same rhizome; (b) on different rhizome; scale bar = 1 mm; m refer to mature leaf, y refer to young leaf
- 4.37 Details of leaf surface morphology; (a) detail of lateral-vein 4.58 region (20x0.4); (b) interveinal spaces (4x0.1); (c) detail of mid-vein region (10x0.22); TC- tannin cell; LV- lateral vein; MV- mid vein
- 4.38 Male flowers of *H. pinifolia* from Merchang; dense associated 4.60 with *Halophila* species in the deeper area on sandy mud; (a) Bud stage: male flower enclosed in leaf sheath; magnification 10x 63x 1.6; (b) Bud stage with open male flower: side view; SA- superior anther; IA- inferior anther; S- stalk; LS- leaf sheath; LB- leaf blade; magnification 10x 63x 2.0; (c) Bud stage with open male flower: back side view; magnification 10x 63x 2.5; (d) Bud stage with open male flower: male flower: pollen released from the anther; magnification 10x 63x 2.0



- 4.39 Male flowers of *H. pinifolia* from Lawas; mixed meadow 4.61 population on sandy mud; (a) Bud stage of open male flower: SA-superior anther; IA- inferior anther; S- stalk; LS- leaf sheath; LB- leaf blade; magnification 10 x 63 x 2.0; (b) Mature stage of male flower: elongation of stalk; magnification 10 x 63 x 0.71; (c) Mature stage of male flower: pollen released from the anther; magnification 10x 63x 2.0
- 4.40 Male flowers of *H. pinifolia* from Bakau, Pulau Gaya; patch 4.62 mixed population on fine sand; (a) Bud stage: male flower enclosed in leaf sheath; magnification 10x63x3.2; (b) Bud stage of open male flower: side view; magnification 10x63x3.2; SA-superior anther; IA- inferior anther; S- stalk; LS- leaf sheath; TC- tannin cell; (c) Bud stage of open male flower: front view; magnification 10x63x3.2
- 4.41 Flowers of *H. pinifolia* from Teluk Kemang; sparse pure 4.63 population on coral rubble and degraded sand; (a) Male flower: SA-superior anther; IA- inferior anther; S- stalk; LS- leaf sheath; scale bar 1mm; (b) Female flower: O- ovary; Sy-style; LS- Leaf sheath; scale bar 1mm
- 4.42 (a) Female flowers of *H. pinifolia* from Merchang; dense 4.64 associated with *Halophila* species in the deeper area on sandy mud; (b) Ovary of female flower; magnification 10x 63x 2.5; Lb-leaf blade, Ls- leaf sheath, Rt- root, Sy- style, O- ovary
- 4.43 Fruits of *H. pinifolia;* (a) Associated with *Halophila* species in 4.65 the deeper area on sandy mud in Merchang; (b) Mixed meadow population on sandy mud in Lawas; F- fruit; LB- leaf blade; LS- leaf sheath; RT- root; RH- rhizome; scale bar represent 1mm
- 4.44 Anatomical structure of *H. pinifolia*; (a) Transverse section of 4.70 leaf blade (10x0.22); CVB- central vascular bundles; A- air lacunae; (b) Transverse section of leaf sheath (10x0.22); CVB- central vascular bundles; A- air lacunae; E- epidermal cells; (c) Transverse section of rhizome (4x 0.1); (d) Detail of rhizome vascular bundle region (20x0.4); S- stele; En- endodermis; (e) Detail of rhizome components (10x0.22); TC- tannin cell; E-epidermal cells; A- air lacunae; M- mesophyll cells; (f) Transverse section of root (10x4); (g) Detail of root cortex region (10x0.25); E- epidermal cells; M- mesophyll cells
- 4.45 Plants collected from Merambong for culture in artificial 4.73 substrate; (a) Plant 1; (b) Plant 2; (c) Plant 3
- 4.46 Observation on new vegetative part of the plants in artificial 4.73 substrate were made by taking the plants out of the pot

