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Morphology of *Halophila ovalis* (R.Br.) Hook. *f.* from Peninsular and East Malaysia

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ABSTRAK

Halophila ovalis *telah disampelkan dari enam lokasi yang mempunyai ciri habitat berbeza; Merambong, Johor dari Semenanjung Malaysia (beting sub-tidal), Teluk Sepinong, Sabah (muara sub-tidal), Pulau Gaya, Sabah (kawasan terumbu karang yang musnah) dan Merchang, Terengganu (inter-tidal danau air masin), Teluk Kemang, Negeri Sembilan (inter-tidal terumbu karang) dan Punang, Sarawak (pantai pasang surut). Berdasarkan pada dimensi daun (panjang dan lebar) dan bilangan pasangan urat daun, tiga jenis variasi* H. ovalis *dapat dibezakan iaitu* H. ovalis *daun besar, sederhana dan kecil.* Halophila ovalis *daun besar dijumpai di kawasan sub-tidal contohnya Merambong, Teluk Sepinong manakala* H. ovalis *daun sederhana dan kecil didapati di kawasan pasang surut yang terdedah pada udara selama 3 hingga 4 jam semasa air surut contohnya di Punang, Merchang, Teluk Kemang dan Pulau Gaya. Saliniti dan kedalaman air yang berbeza untuk setiap habitat yang menyumbang kepada perbezaan bentuk dan dimensi saiz daun. Daun* H. ovalis *menunjukkan berbagai jenis bentuk dari ovate, obovate, oblong ke spatulate. Di kawasan yang lebih dalam, air yang keruh dan tanah berlumpur, daun* H. ovalis *berbentuk lebih memanjang dan petiolnya juga panjang.* Halophila ovalis *di Malaysia mempamerkan kepelbagaian morfologi terutamanya pada daun sebagai gerak balas pada faktor persekitaran yang berbeza di pelbagai habitat.*

ABSTRACT

Halophila ovalis *plants were collected from six locations with different habitat characteristics; Merambong, Johore of Peninsular Malaysia (sub-tidal shoal), Teluk Sepinong of Sabah (sub-tidal estuary), Pulau Gaya, Sabah (sub-tidal degraded coral), Merchang, Terengganu (inter-tidal lagoon), Teluk Kemang, Negeri Sembilan (inter-tidal degraded coral platform) and Punang, Sarawak (inter-tidal beach front). Based on the leaf dimensions (length and width) and number of paired cross-veins, three* Halophila ovalis *variants can be distinguished; big, intermediate and small-leaved. The big-leaved* H. ovalis *are found at sub-tidal areas e.g. Merambong, Teluk Sepinong, while intermediate- and small-leaved variants are from inter-tidal areas that are exposed to air for 3 to 4 hours during low tides e.g. Punang, Merchang, Teluk Kemang and Pulau Gaya. The salinity and depth in a particular habitat contribute to the variability in shapes and dimension of leaf sizes.* Halophila ovalis *leaves showed diverse shapes from ovate, obovate, oblong to spathulate. At deeper depths, turbid water and muddy substrate, leaves of* H. ovalis *are elongated in shape and with longer petiole length.* Halophila ovalis *in Malaysia exhibit morphological variability particularly in the leaves in response to the different environmental factors in the various habitats.*

INTRODUCTION

Malaysia has a total of 14 species seagrasses belonging to 8 genera: *Enhalus acoroides, Halophila beccarii, H. decipiens, H. ovalis, H. minor, H. spinulosa, Thalassia hemprichii, Cymodocea rotundata, C. serrulata, Halodule uninervis, H. pinifolia, Syringodium isoetifolium, Thalassodendron ciliatum* and *Ruppia maritima* (Japar Sidik *et al.* 2001; Japar Sidik and Muta Harah 2003). Amongst these species, *H. ovalis* exhibits morphology

variability in response to different environmental factors at various habitats (den Hartog 1970; McMillan *et al.* 1983; Japar Sidik *et al.* 2001). According to den Hartog (1970), *H. ovalis* is a 'collective species' consisting of a large array of uncertain taxa. Examples of this are subspecies *australis* (Doty and Stone 1966) den Hartog of South Australia, subspecies *bullosa* (Setchell) den Hartog of Samoa, Tonga and Fiji, subspecies *hawaiiana* (Doty and Stone 1966) den Hartog of Hawaii and subspecies *linearis* (den Hartog) den Hartog of Mozambique. Den Hartog (1970) categorised *H. ovalis* into subspecies based on the leaf sizes and pairs of cross veins. *Halophila ovalis* has 12-25 pairs of cross veins, while *H. minor* has 4-12 pairs of cross veins. Observations on *H. ovalis* indicate that they have a wide range of leaf shapes and sizes, pairs of cross veins and pigmentation (Japar Sidik *et al.* 2001; Muta Harah *et al.* 2003). In addition, it was also reported that *H. ovalis* has long petioles due to heavy sedimentation and large shifts of sand by water movements to obtain maximum sunlight and big leaf blade due to less surface irradiance reaching the bottom of deeper depth, shading or water turbidity reported at Merambong, Johore (Japar Sidik *et al.* 1999; Japar Sidik *et al.* 2001). These are some responses of *H. ovalis* towards the ever changing and unstable environment. Other morphological variabilities related to environmental factors are also reported by various researchers. *Halophila ovalis* with big leaf blades are found under shaded environment (Duarte 1991), in brackish water (low salinity compared to high salinity in marine waters) (Benjamin *et al.* 1999), low light due to depth (Beer and Waisel 1982; Longstaff and Dennison 1999; Short *et al.* 2001; Ralph 1999) and muddy substrate (Young and Kirkman 1975). *Halophila ovalis* with small leaves and short petioles are found in habitats with hard carbonate substrate (coralline sand less in free nutrient, Short 1987). Changes in plant morphology and natural habits in *H. ovalis* that is locally common certainly warrant investigation. This paper reports on the distribution, habitat characteristics and morphology of *H. ovalis.*

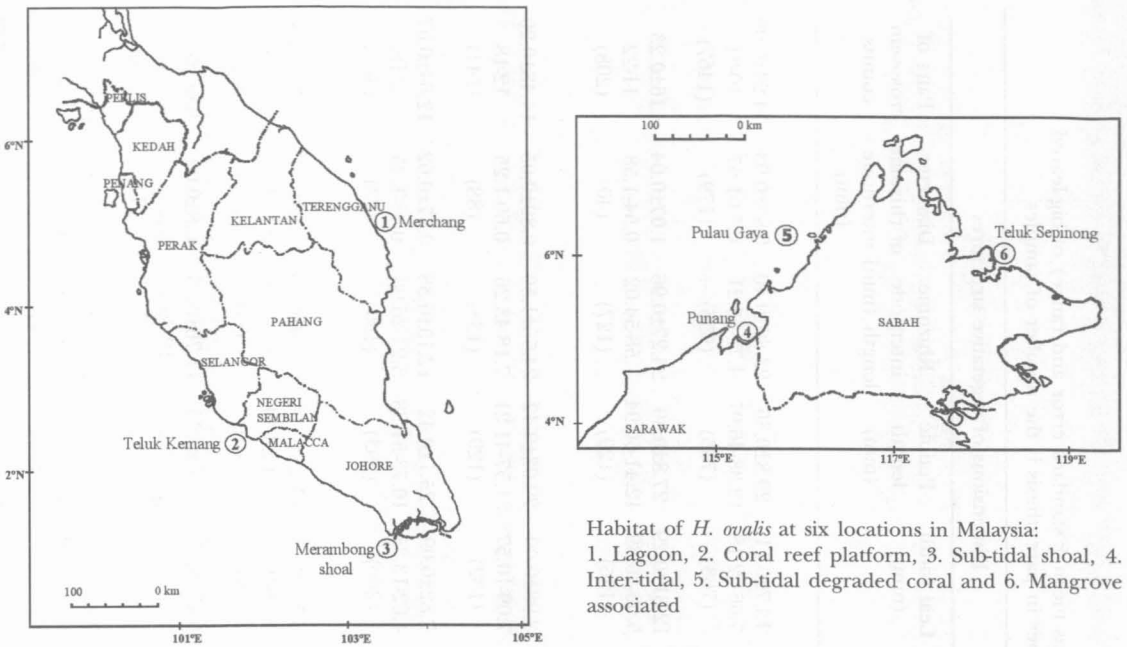
MATERIALS AND METHODS

Halophila ovalis were collected from six locations, comprising Merambong, Johore (01° 19' 00" N, 103° 36' 45" E), Merchang, Terengganu (05° 02' 15.0" N, 103° 17' 53.0" E), Teluk Kemang, Negeri Sembilan (02° 31' 00" N, 101° 47' 00" E), Teluk Sepinong, Sabah (05° 49' 3.42" N, 118° 09' 14.28" E), Punang, Sarawak (104° 54' 23.6" N, 115° 22' 36.3" E) and Pulau Gaya, Sabah (06° 00' 11.0" N, 116° 02' 30.4" E) (*Fig. 1*). At each location, the environmental parameters were recorded, e.g. substrate type, salinity, water temperature and depth. In the laboratory, the collected plants were cleaned of debris and sorted, e.g. vegetative structures comprising the length and diameter of rhizome internode, length and width of leaf blades and petiole length were measured using Mitotoyo Digital Vernier Caliper. Reproductive structures were recorded digitally and measured: male flower-pedicel length, tepal length and width, and anther length and width; female flower-style length, ovary length and width and hypanthium length and length and width of fruit were measured using UTHSCSA Image Tool for Windows software (Wilcox *et al.* 1996). The whole strand of the plants was recorded as digital images by using Nikon CoolPix. The mature leaves were recorded as digital images and pairs of cross veins were counted from the recorded images. The existing plant materials were processed for herbarium specimens as described by Menez *et al.* (1983) and some were preserved as wet specimens in 4% formalin in saline water. Leaf dimension data were analysed for various habitats and compared for differences or similarities by using Cluster Analysis using SPSS Program version 12.0.

RESULTS AND DISCUSSION

Distributions and Habitat Characteristics of H. ovalis

The distribution of *H. ovalis* is shown in *Fig. 1.* Details of the habitat characteristics of *H. ovalis* are illustrated in *Fig. 2* a - g showing; (i) inter-tidal degraded coral reef platform, e.g. Teluk Kemang (Japar Sidik and Muta Harah 2003; (ii) sub-tidal shoal e.g. Merambong; (iii) inter-tidal lagoon, e.g. Merchang; (iv) inter-tidal beach front, e.g. Punang; (v) sub-tidal degraded coral reef flat, e.g. Pulau Gaya; and (vi) sub-tidal estuary, e.g. Teluk Sepinong. In the above habitats the waters depth varied from -1.0 to -5.0 m MSL, temperatures between 25-34 ºC and salinity ranging between 29-31 psu (practical salinity unit). However, at the inter-tidal lagoon of Merchang, salinity fluctuated widely between 9.41-34.41 psu. In addition, *H. ovalis* plants in Merchang can tolerate a wide fluctuation in



Habitat of *H. ovalis* at six locations in Malaysia:
1. Lagoon, 2. Coral reef platform, 3. Sub-tidal shoal, 4. Inter-tidal, 5. Sub-tidal degraded coral and 6. Mangrove associated

Fig.1: Distribution and six study locations: Peninsular Malaysia – Merchang, Terengganu; Teluk Kemang, Negeri Sembilan; Merambong shoal, Johore; Sarawak-Punang and Sabah – Pulau Gaya; Teluk Sepinong

salinity resulting from freshwater input during monsoon and dry season respectively (Japar Sidik *et al.* 1999). *Halophila ovalis* were found to grow and adapt themselves in various substrates from coralline sand, e.g. Pulau Gaya, sand covered coral, e.g. Teluk Kemang; calcareous sandy mud, e.g. Merambong; muddy loam, e.g. Teluk Sepinong to muddy sand, e.g. Merchang and Punang (Table 1). It was also noted that *H. ovalis* in certain locations e.g. at Merchang, Punang and Teluk Kemang could withstand 3-4 hours exposure to air (den Hartog 1970). *Halophila ovalis* is a eurybiontic species that is capable of surviving in very turbid and polluted waters. It was found in different places with different ecological parameters. In clear water, *H. ovalis* distribution exceeded mid-tidal level to nearly 60 m in depth on substrates ranging from soft mud to coarse rubble (den Hartog 1970; Hillman *et al.* 1995).

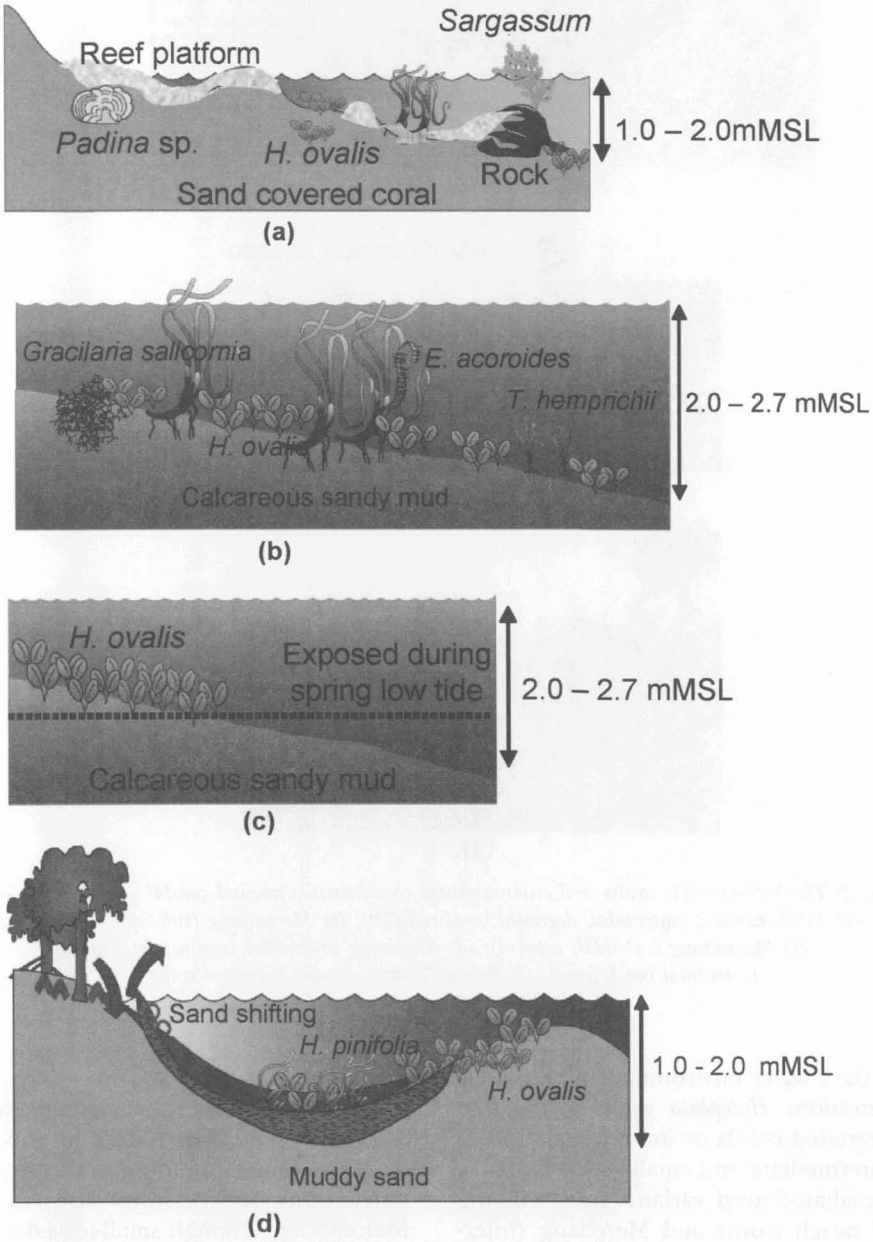
Fig. 2: The habitat of *H. ovalis* and environmental characteristics around coastal waters of Malaysia; (a) Teluk Kemang (inter-tidal, degraded coral reef flat), (b) Merambong (sub-tidal, unexposed), (c) Merambong (sub-tidal, exposed), (d) Merchang (inter-tidal lagoon), (e) Punang (inter-tidal beach front), (f) Pulau Gaya (sub-tidal, degraded coral reef) and (g) Teluk Sepinong (sub-tidal estuary).

Halophila ovalis *Variants*

Cluster analysis interpretation (*Fig. 3*) based on the leaf size dimension (leaf length and width) and pairs of cross veins distinguished *H. ovalis* into three categories: big-leaved, intermediate-leaved and small-leaved variants (Plate 1). Teluk Kemang (exposed), Pulau Gaya and Merambong (exposed) exhibit the small-leaved variants while the intermediate-leaved variants are from Merchang and Punang. The big-leaved variants are from Merambong (sub-tidal shoal, unexposed) and Teluk Sepinong (sub-tidal estuary) respectively. The details of vegetative structures such as leaf length and width, petiole length, rhizome internode length and diameter, pairs of cross veins are given in Table 1. Big-leaved *H. ovalis* plants were found mostly in a shaded environment, e.g. Merambong or in areas with light limitations, e.g. Teluk Sepinong. The big-leaved variants of Merambong were found growing under the shade of tall *Enhalus acoroides* canopy and found fully submerged even during the lowest tide level (*Fig. 2b*). The big-leaved variants from Teluk Sepinong are adapted to

TABLE 1
Habitat characteristics and vegetative structure characteristics (measurement given as mean ± standard error and range) of big-leaved, intermediate-leaved and small-leaved *H. ovalis* from the six locations. Number in parenthesis is the number of samples

	Habitat characteristics					Dimensions of vegetative structures					
	Associated habitat	Substrate type	Salinity psu	Water temperature (°C)	Depth (m MSL)	Leaf length (mm)	Leaf width (mm)	Petiole length (mm)	Rhizome internode length (mm)	Diameter of rhizome internode (mm)	Pairs of cross-vein counts
Big-leaved											
Merambong, Johore	Sub-tidal shoal, unexposed	Calcareous muddy sand	29.6-30.7	29.0-31.0	2.0-2.7	23.7±0.18 10.29-36.46 (761)	13.7±0.11 5.66-32.18 (763)	20.8±0.30 12.38-46.91 (753)	23.86±0.40 4.72-55.21 (656)	0.98±0.03 0.54-1.67 (179)	14.24±0.06 10-21 (1467)
Teluk Sepinong, Sabah	Sub-tidal estuary	Muddy loam	28.0-31.0	28.0-29.0	2.0-2.7	20.8±0.56 5.52-35.83 (129)	12.81±0.29 5.09-20.89 (126)	27.8±0.80 12.41-66.94 (129)	24.27±0.96 1.58-54.02 (127)	1.07±0.04 0.54-1.58 (40)	16±0.23 11-22 (208)
Intermediate-leaved											
Punang, Sarawak	Inter-tidal-beach front	Muddy sand	20.0-31.0	25.0-34.0	1.5-2.7	19.7±0.18 16.29-23.10 (76)	8.98±0.09 7.09-10.57 (132)	29.03±0.42 21.57-41.21 (129)	24.56±1.50 7.18-43.26 (138)	0.89±0.05 0.60-1.25 (88)	14.48±0.20 12-18 (144)
Merchang, Terengganu	Inter-tidal lagoon	Muddy sand	9.41-34.41	29.0-30.1	1.0-2.0	17.24±0.18 10.2-24.70 (255)	7.82±0.09 4.62-13.35 (260)	25.1±0.42 10.73-51.98 (293)	12.15±0.38 3.24-26.96 (199)	0.87±0.02 0.43-1.25 (73)	12.64±0.07 8-18 (460)
Small-leaved											
Merambong, Johore	Sub-tidal shoal, exposed	Calcareous sandy mud	29.6-30.7	29.0-31.0	2.0	11.36±0.09 6.68-15.89 (553)	6.54±0.05 3.56-9.65 (581)	11.28±0.14 2.31-23.44 (616)	13.79±0.23 2.6-27.00 (554)	0.60±0.019 0.16-1.47 (117)	9.78±0.06 7-15 (1150)
Pulau Gaya, Sabah	Sub-tidal degraded coral	Coralline sand	29.0-31.0	30.0-32.0	1.5-2.5	8.19±0.12 2.08-14.03 (474)	5.47±0.07 2.21-9.31 (475)	10.6±0.23 8.41-26.95 (505)	11.76±0.26 1.12-36.11 (803)	0.8±0.02 0.33-1.44 (113)	9.66±0.07 6-16 (1033)
Teluk Kemang, Negeri Sembilan	Inter-tidal; degraded reef flat	Sand covered coral	29.0-31.0	28.0-31.2	1.0-2.0	13.1±0.17 5.11-21.01 (473)	7.14±0.07 3.10-11.89 (473)	11.03±0.25 5.01-27.31 (425)	16.2±0.31 3.18-39.92 (541)	0.85±0.01 0.48-1.39 (136)	10.91±0.06 8-15 (806)


Sargassum
Reef platform
Padina sp.
H. ovalis
Rock
Sand covered coral
1.0 – 2.0mMSL
(a)
Gracilaria salicornia
E. acoroides
T. hemprichii
H. ovalis
Calcareous sandy mud
2.0 – 2.7 mMSL
(b)
H. ovalis
Exposed during spring low tide
Calcareous sandy mud
2.0 – 2.7 mMSL
(c)
Sand shifting
H. pinifolia
H. ovalis
Muddy sand
1.0 - 2.0 mMSL
(d)

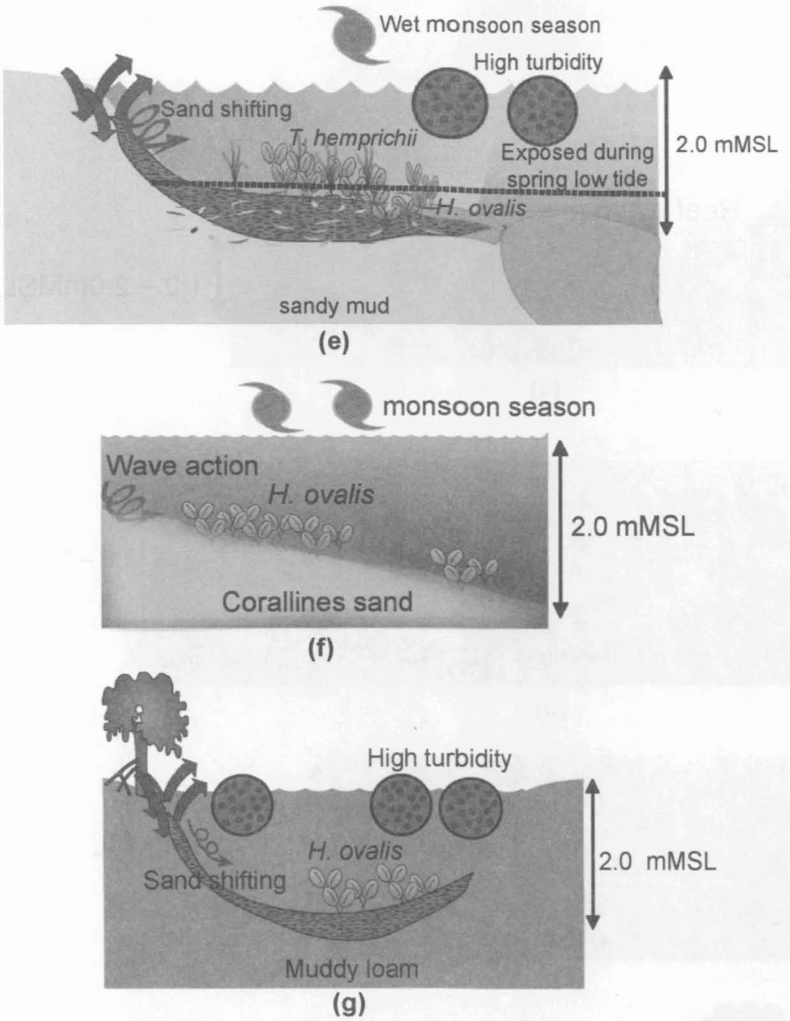


Fig. 2: The habitat of H. ovalis *and environmental characteristics around coastal waters of Malaysia: (a) Teluk Kemang (inter-tidal, degraded coral reef flat); (b) Merambong (sub-tidal, unexposed); (c) Merambong (sub-tidal, exposed); (d) Merchang (inter-tidal lagoon); (e) Punang (inter-tidal beach front); (f) Pulau Gaya (sub-tidal, degraded coral reef); and (g) Teluk Sepinong (sub-tidal estuary)*

living in a dark water environment which limits light penetration. *Halophila ovalis* plants that grow in degraded corals or in areas exposed to air have intermediate and small-leaved variants. The intermediate-leaved variants from Punang (inter-tidal beach front) and Merchang (inter-tidal lagoon) were observed to be under a wide range of salinity fluctuations. The small-leaved variants are found in degraded coral substrate, e.g. Pulau Gaya, in exposed areas e.g. Merambong or exposed degraded coral substrate, e.g. Teluk Kemang. McMillan (1983) reported that in *Halophila,* substrate types also correlated with leaf size dimension; small-leaved plants came from firm silty substrate while the large-leaved plants are from muddy calcareous sand which is always covered with water. In this study, big-leaved variants were found to be growing on the calcareous sandy mud substrate, e.g. at Merambong. Though small-leaved variants grow on the same substrate type, these plants have small leaves in response to the unfavorable environmental condition, i.e. they are exposed to air for a period of 3-4 hours during the low tide (Japar Sidik *et al.* 2001). The intermediate-leaved variants from Merchang and Punang exhibit purplish blotches or dots on the leaves (Plate 2d) and the Teluk Kemang small-leaved

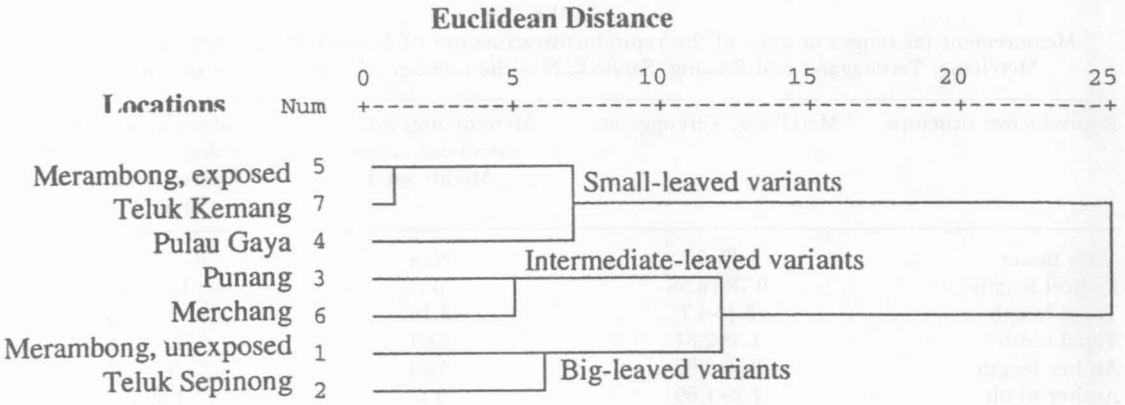


Fig. 3: Hierarchical Cluster Analysis using leaf size dimensions (leaf length, leaf width) and cross veins of H. ovalis *from six locations. The similar size leaf blades and cross veins were grouped by Euclidean Distance*

variants have pinkish pigment on the petiole near the base of the leaf (Plate 1e). These are responses to the high light conditions during exposure to air (Japar Sidik *et al.* 2001) and are believed to be UV-blocking pigments (Hemminga and Duarte 2000).

Leaves of *H. ovalis* at each habitat showed diverse shapes from ovate, obovate, oblong to spathulate. At deeper depths, turbid water and muddy substrates, e.g. Merambong (sub-tidal shoal, unexposed), Teluk Sepinong (sub-tidal estuary) the leaves of *H. ovalis* were elongated in shape and with longer petiole length (Plate 1a & 1b, Plate 2a & 2b). At shallower depths, clear water and coral sand, e.g. Pulau Gaya, Teluk Kemang, and Merambong (exposed) the *Halophila* leaves were more ovate to obovate in shape and with a short petiole length (Plate 1e, 1f & 1g, Plate 2e, 2f & 2g). However, in exposed areas and muddy sand, the leaves of *H. ovalis* were oblong and spathulated with pointed apex and with long petiole length in response to large shifts of sand from the rivers (Plate 1b & 1c, Plate 2c & 2d).

The least number of pairs of cross veins (6-16) were in small-leaved variants for Teluk Kemang, Merambong and Pulau Gaya, 8-18 for Punang and Merchang, while the Merambong big-leaved plants had 10-22 pairs of cross veins. In the previous studies, the number of pairs of cross veins of *H. ovalis* big-leaved was in the range of 10-25 as reported by den Hartog (1970); 12-25, (Sachet and Fosberg 1973) and 10-25 (Kuo 2000). The range in number of paired cross veins counts for the small-leaved *Halophila* populations were found to overlap with those of *H. ovalis* and *H. minor*. Based on Kuo (2000), *H. minor* posses 7-12 pairs of cross veins and *H. ovata* 4-12 pairs of cross veins, while according to den Hartog (1970) *H. minor* (Zoll) has 5-9 pairs of cross veins. In the present study, the small-leaved *Halophila* could be placed in the category of either *H. ovalis* or *H. minor*. To confirm the taxonomic uncertainty, a study on both variants should be conducted in culture conditions.

Reproductive Structures

Reproductive structures, male and female flowers and fruits were observed at a coastal lagoon area, Merchang; in exposed sub-tidal area, Merambong; and in an inter-tidal area, Punang. However, at Punang only opened male flowers were observed. Comparatively, the male flower of Punang had longer pedicel and anther length compared to those from Merchang and Merambong (Table 2). Den Hartog (1970) reported that female flowers had three styles, 10-20 mm long, ovary ovoid, 1-1.5(-2.5) mm long, and a hypantium of 3-5 mm length. Male flowers had tepals 3 which were mm long and 2 mm wide and a 10-20 mm long pedicel. For *H. minor* (Kuo 2000), the male and female flowers were smaller than *Halophila ovalis* in Merchang, Merambong and Punang, while the styles and hypantium were long, 4.99-21.79 mm and 1.17-6.61 mm.

CONCLUSION

1. *Halophila ovalis* in Malaysia exhibits morphological variability in response to the

TABLE 2
Measurement (as ranges in mm) of the reproductive structures of *H. ovalis* from Merambong, Johore, Merchang, Terengganu and Punang, Sarawak. N is the number of samples, nd = not observed

Reproductive structure	Merchang, Terengganu.	Merambong, Johore. Inter-tidal, lagoon Muddy sand	Punang, Sarawak. Sub-tidal Inter-tidal, Calcareous sandy mud Muddy sand
Male flower	N=6	N=4	N=5
Pedicel length	0.78-14.58	0.7	1.11-14.98
Tepal length	3.46-4.7	3.16	3.7-3.91
Tepal width	1.38-2.31	1.84	1.79-2.22
Anther length	2.67-3.82	3.03	3.4-4.45
Anther width	1.29-1.69	1.6	1.38-1.66
Female flower	N=6	N=6	nd
Style length	5.23-21.79	4.99-18.51	nd
Ovary length	0.83-1.42	0.99-1.77	nd
Ovary width	1.65-5.97	0.67-1.22	nd
Hypanthium length	1.07-5.97	1.17-6.61	nd
Fruit	N=3	N=4	nd
Fruit length	1.25-3.34	(1.93-3.82)	nd
Fruit width	0.82-2.58	(1.28-2.5)	nd

different environmental factors in the various habitats. Such responses are reflected in the leaf sizes and shapes. *Halophila ovalis* can be distinguished into three categories; big-leaved, intermediate-leaved and small-leaved variants. The big-leaved variants, e.g. at Merambong (unexposed sub-tidal) and Teluk Sepinong (sub-tidal estuary) in response to shaded conditions and light limitations have longer leaf length, petiole length, higher number of paired cross veins and are elongated in shape. The intermediate-leaved *H. ovalis*, e.g. from Merchang (inter-tidal lagoon) and Punang (inter-tidal beach front) were under exposure to air during low tide and in an environment of wide salinity fluctuation. Small-leaved *H. ovalis* from Pulau Gaya (sub-tidal degraded coral), Merambong (exposed sub-tidal) and Teluk Kemang (inter-tidal degraded coral reef flat) have relatively small leaves compared to the intermediate-leaved and big-leaved variants. They are observed either from shallow areas or exposed to air for a period of 3-4 hours during low tide.

2. The paired cross veins counts for the small-leaved *Halophila* populations show ranges that overlap with those of *H. ovalis* and *H. minor*. The small-leaved *Halophila* plants can be placed in either the category of *H. ovalis* or *H. minor*. To confirm the taxonomic uncertainty, a study on both variants should be conducted in culture conditions.

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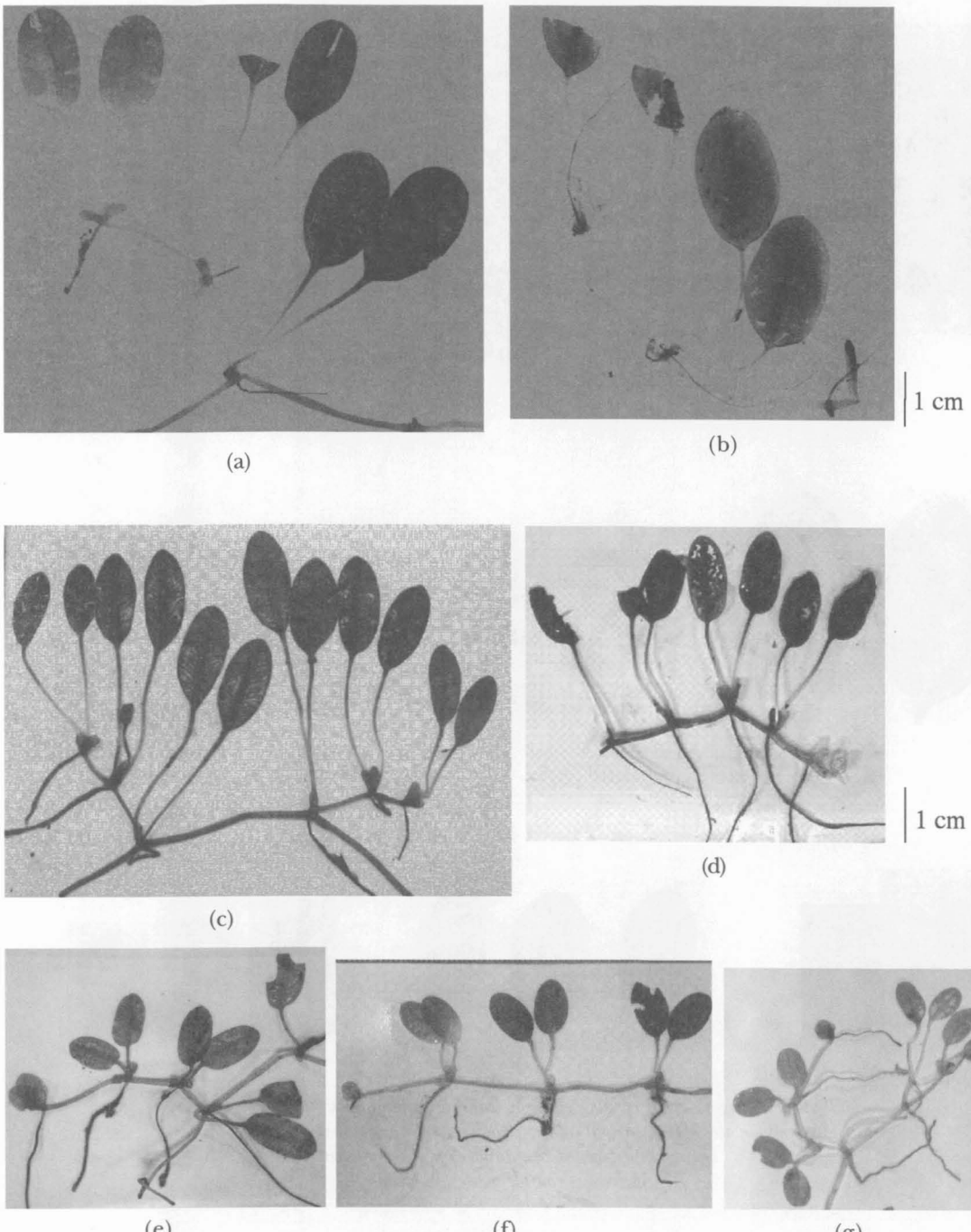

Plate 1: Cluster Analysis; a- big-leaved H. ovalis *from Merambong, unexposed sub-tidal shoal; b- big-leaved* H. ovalis *from Teluk Sepinong sub-tidal estuary; c- Intermediate-leaved* H. ovalis *from Punang, inter-tidal beach front; d- Intermediate-leaved* H. ovalis *from Merchang, inter-tidal lagoon; e, f, g- small-leaved* H. ovalis *from Teluk Kemang, inter-tidal coral reef flat, Merambong, sub-tidal exposed and Pulau Gaya, sub-tidal degraded coral reef respectively*

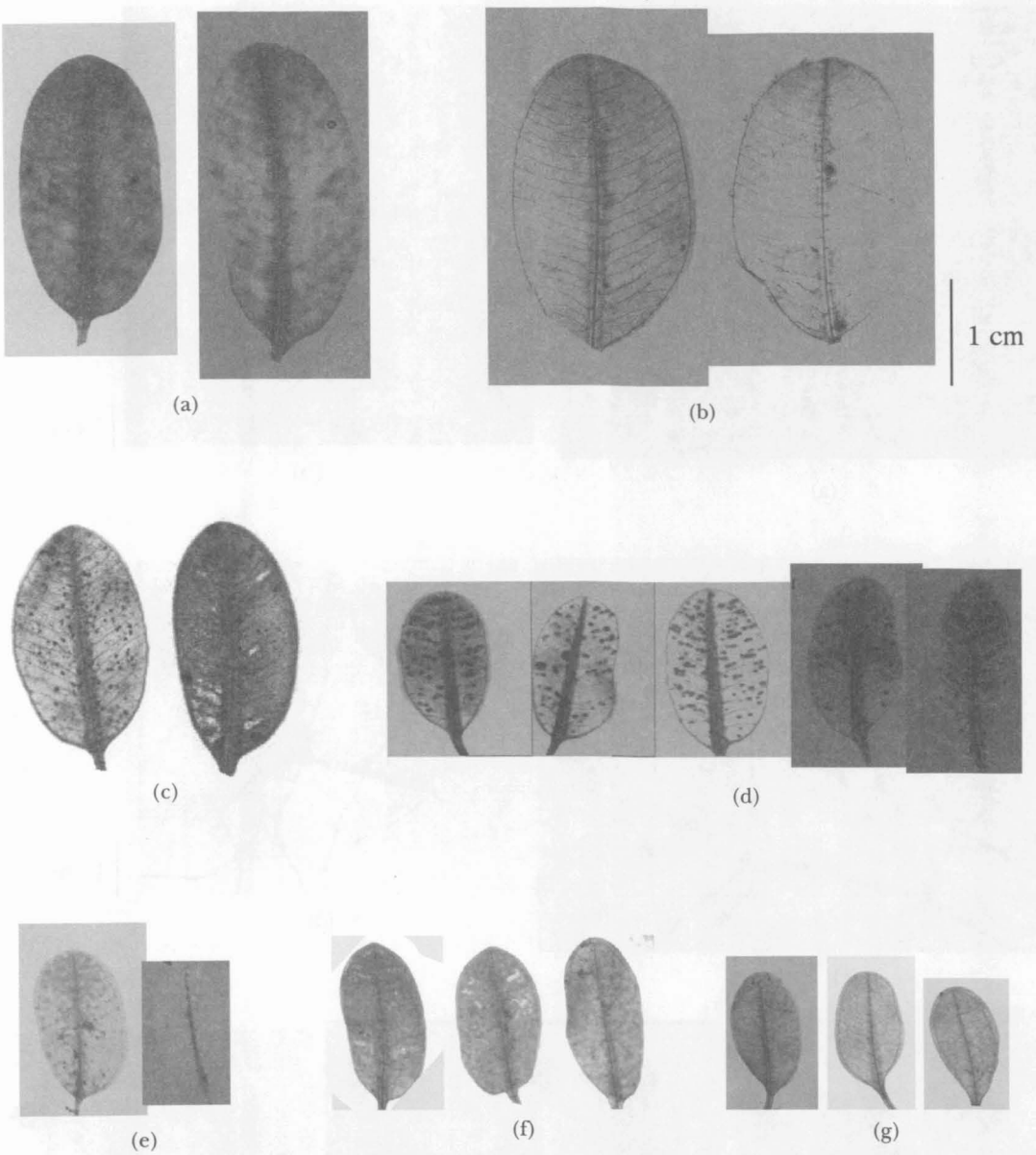

Plate 2: Category of H. ovalis *based on leaf sizes and shapes; a, b - big-leaved* H. ovalis-*ovate, oblong to spathulate; c, d - intermediate-leaved* H. ovalis-*ovate, oblong with pointed apex and e, f, g - small-leaved* H. ovalis-*round, ovate to obovate*

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