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Morphological Characterisation and Documentation of Freshwater Macrophytes in Pontian, Johor's Water

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ABSTRACT

Freshwater macrophytes exist in varied life forms for example, emergent, submerged, floating-leaved and free-floating, and some of them have a heterophylly or multiple forms. The intraspecific variation in terms of morphology is common phenomena in macrophytes population; however, specific studies on morphology of freshwater macrophytes are still insufficient, especially in Pontian, Johor. Hence, this study aimed to characterise the freshwater macrophytes based on morphometrics and descriptive characteristics as well as to document their diversity and population. Thus, the morphometric measurements were done, the descriptive characteristics were documented in scientific photographs, the population of freshwater macrophytes was also estimated by using quadrat estimation technique. Additionally, the morphometrics of some collected samples were compared with the same sample in other studies to show the variation in the range of different measurements, the descriptive characteristics were also described as well as the population estimation assessed by area coverage was analysed in the form of statistical chart. The finding in this study showed variation in morphometric data and no intraspecific variation on phenotype of freshwater macrophytes in Pontian, Johor's water.

Keywords: Freshwater macrophyte, intraspecific variation, Malaysia, morphology

INTRODUCTION

Aquatic macrophytes are the most important element within an aquatic ecosystem (Jeppesen *et al.*, 1997), as it does not only undergo photosynthesis and act as producer, but also performed multiple ecosystem services such as carbon sequestration, habitat provision, nutrient uptake as well as cycling elements in an aquatic ecosystem (Wetzel, 1992; Kornijów *et al.*, 2004). In addition, freshwater macrophytes constitute a small ecological group with particular evolutionary processes, diverse reproductive patterns, excellent life cycles, unique physiologies, relatively high primary production rates and important structuring roles in shallow lakes, and potential for mass developments obstructing human use of water bodies (Li *et al.*, 2017). These features make aquatic macrophytes an important organism in the research of many fields as well as in environmental management.

The identification of freshwater macrophytes can be achieved by using the taxonomic keys based on vegetative characters and descriptive data, some researchers provided comprehensive descriptions and geographical distributions to aid in distinguishing between genera of aquatic macrophytes (Moody *et al.*, 2008). In order to identify freshwater macrophytes to species level, structural and morphological characterisation are necessary.

Freshwater macrophytes can be identified and classified according to their various zonation, habitats, or taxonomic groups (Oyedeji & Abowei, 2012). The key morphological characteristics to identify a freshwater macrophytes are observed in its flower, frond structure, stem, root structure, rhizome, or stolon (Bowden *et al.*, 2017). However, for most local people, and even researchers, identification of a species of freshwater macrophytes is rather problematic due to their morphological similarity. For example, *Lemna minuta* is an invasive species often mistaken as *L. minor* due to their small size and similar morphology (Ceschin *et al.*, 2016).

In Malaysia, freshwater macrophytes can be easily obtained from various kind of water bodies. Generally, they are beneficial to the aquatic environments, serving as primary producer, playing important roles in nutrient cycle, and acting as shelter for various fauna, such as the habitat for snakehead fish (*Channa striatus*) and walking perch (*Anabas testudineus*) found under the free-floating freshwater macrophytes *Salvinia molesta* in Sungai Perak (Ismail *et al.*, 2018). Unfortunately, some invasive alien species such as *Salvinia molesta* and *Alternanthera philoxeroides* are in some water bodies of Malaysia while forming dense population on the water surface that may reduce the habitat quality for waterfowls, reduce oxygen under the dense mat, and interfere navigation as well as recreational uses due to their high survival and reproductive rates (National Committee on Invasive Alien Species Malaysia, 2018).

The research, in particular, the morphological characterisation of freshwater macrophytes of different life-forms in Malaysia especially in Pontian, Johor needs to be addressed. Hence, our present study dealing with the morphological characterisation of freshwater macrophytes is important in order to provide data or documentary evidence of their occurrence in some specific location of Pontian, Johor. Therefore, the morphological characterisation based on morphometrics and descriptive characteristics as well as the documentation of species diversity and population estimation have been conducted to show the characteristic of each freshwater macrophytes including their intraspecific variation in Pontian, Johor's water. This report is the first report to characterise morphological characteristics and document the diversity of freshwater macrophytes in Pontian, Johor's water.

MATERIALS AND METHODS

Study Sites

The study sites were located in different water bodies along the oil palm plantation area of the Pontian District in Johor (Figure 1). A total of seven sampling stations were chosen based on accessibility after the ocular inspection was conducted in January of the year 2021. Only station 2 and station 5 (Figure 2(a)) were water ponds while the rest were river creeks (Figure 2(b)). The coordinates for each location were recorded by using smartphone's GPS application (Google Earth Android version 9.132.1.1). The field sampling activities were conducted during 18th of February, 3rd, 18th and 27th of March, respectively in the year of 2021. The sampling time for each sampling site varied from place to place with the accessibility to stations as the main consideration in deciding the sampling time, e.g., weather condition and the Movement Control Order (MCO) by local government during COVID-19 pandemic period. The surface area of each water bodies was estimated using Google Earth Pro (version 7.3.3.7786 64-bit) that can render 3-dimensional earth surface on satellite imageries to record and tabulate the result for further analysis.

Field Sample Collection

A total of 10 individuals for each species of freshwater macrophytes were collected in every sampling station and each individual was separated by 10 m to ensure that samples were not being taken from the same macrophyte population and to avoid repeated sampling the sample at the same point (Huang *et al.*, 2017). The freshwater macrophytes were removed or dug out carefully from the bottom substrate of the water body to preserve the whole structure. The collected samples were washed using an *in-situ* water to gently remove soil particles, invertebrates and excessive debris attached on the macrophytes. This step was carefully carried out to prevent the structure of macrophytes from being damaged or destroyed. The collected samples were stored inside plastic ice boxes before transferring to the laboratory.

Population Estimation

The population estimation of freshwater macrophytes at the particular sampling sites was expressed as macrophyte coverage over the water body surface at each sampling site. A simple modified PVC quadrat (1 m × 1 m) was used when conducting the field sampling to visually estimate the percentage of coverage of freshwater macrophytes. This technique was similar to the techniques proposed by Capers (2000) and Sharip *et al.* (2012) but without transect lines and diving activities due to the limitation of sampling devices and accessibility. The quadrat was placed randomly at each point (water surface) of sampling sites, a minimum number of two quadrats were placed on the smallest sampling site to give the

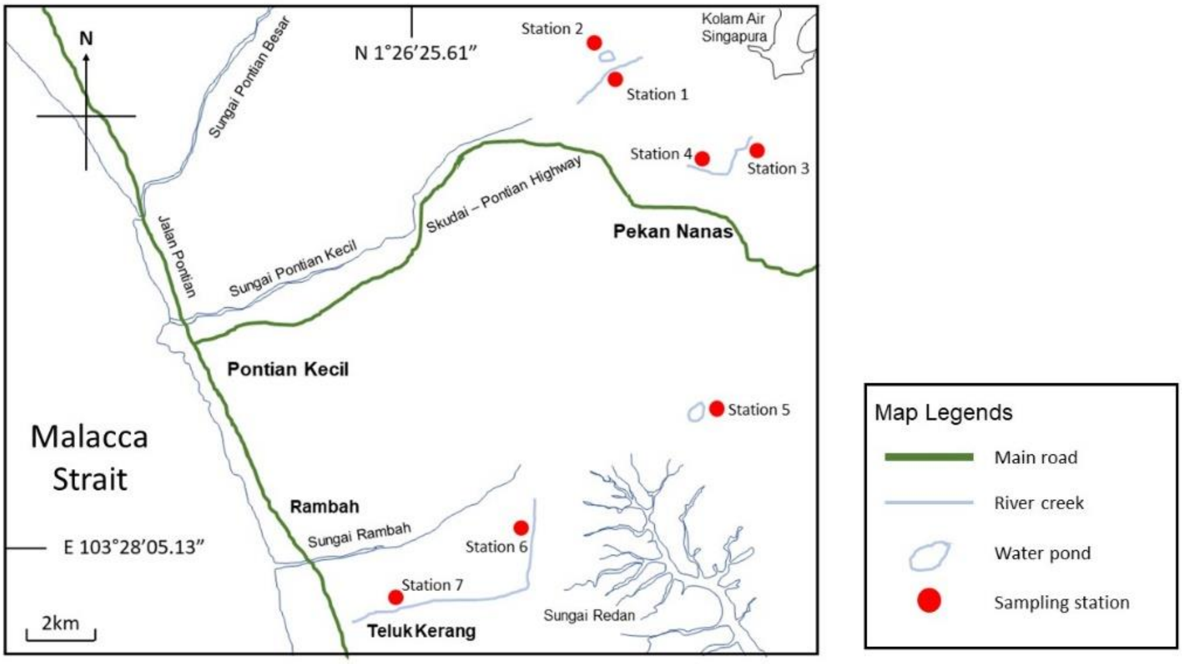

Figure 1. The map showing the locality of each labelled sampling station with different type of water bodies around the area of Pontian District in Johor (Google maps)

Figure 2. (a) The environment of water pond in Pontian, Johor. (b) The river creek with submerged species of freshwater macrophytes in Pontian, Johor

mean area of plant cover. For some submerged species of freshwater macrophytes like *Cabomba* sp., and *Ceratophyllum* sp., the quadrat was placed just above the macrophytes population at the water surface and observed the area coverage from the top view of the quadrat to give the estimation. The following formula was used to calculate the percentage of coverage estimated by using quadrat technique:

$$\frac{\text{Area of plant covered } (\text{m}^2)}{\text{Area of quadrat } (\text{m}^2)} \times 100\%$$

Vegetative Characters Documentation

For the morphological characteristics, vegetative characters that occur in most freshwater macrophytes were chosen according to Ceschin *et al.* (2016), with some informative characteristics added. The chosen key characteristics were further divided into descriptive characteristic and morphometric to specify the methods that were used to document the morphological characteristics of freshwater macrophytes. The selected characters are shown in Table 1.

Descriptive Characteristics

Smartphone camera (Xiaomi 10T pro 108 MP) was used to capture the details of the descriptive characteristics of freshwater macrophytes. A black background with ruler scale and the aid of fill light were used to take the photographs of the freshwater macrophytes, this method was similar to the method suggested by Dibble and Thomaz (2009) and Ferreiro *et al.* (2013) with slightly modification. Photographs were taken to document each of the descriptive characteristics based on the structure and morphology of macrophytes. For the descriptive characteristics, a total number of at least two photographs were taken. The resolution, brightness and contrast of the photographs were improved, and shades of the photographs were removed using image editing software (CorelDRAW® 2021 version 23.0.0.363 64-bit). All photographs were saved in Tag Image File format and arranged in publishable arrangement.

Morphometrics

For the morphometrics, the measurements were taken by using vernier caliper and ruler. The measurements of frond length and frond width were taken in the laboratory for every individual of every species. The number of contiguous frond and vein number were calculated directly by observing the structure and morphology of the particular freshwater macrophytes. All measurements were tabulated and recorded for analysis in this study.

RESULTS

Morphology

The measurements of morphometrics of the collected samples were recorded and tabulated in the form of data table (Table 2) while the descriptive characteristics were documented by arranging in scientific photographs (Figure 3-10).

Cabomba aquatica Aubl. measured 17.34 – 23.30 mm (20.32 ± 2.98) frond length, 0.47 – 0.57 mm (0.52 ± 0.05) frond width and 10.62 – 17.58 (14.10 ± 3.48) in the number of contiguous fronds (Table 2). The fronds of *C. aquatica* were heterophyllic occurring as submerged or floating fronds (Figure 3(a)). The floating fronds were orbicular while the submerged fronds were fine and needle-like arranged in a fan-shape (Figure 3(a)-3(d)). The frond color was bright green to darker green along the frond to the petiole (Figure 3(a)). Rhizomatous root structure and true root were absent, the flowers and flower buds of *C. aquatica* were bright yellow colour (Figure 3(e) & Figure 3(f)).

Cabomba sp. Aubl. measured 17.42 – 23.42 mm (20.42 ± 3.00) frond length, 0.43 – 0.55 mm (0.49 ± 0.06) frond width and 10.33 – 17.47 (13.90 ± 3.57) in the number of contiguous fronds (Table 2). The fronds of *Cabomba* sp. existed heterophylly which were the submerged fronds and floating fronds (Figure 4(a) – 4(c)). The shape of floating fronds of *Cabomba* sp. was elliptic (Figure 4(c)) while the submerged frond was feather-like and finely segmented (Figure 4(b)). The fronds were light yet bright green in colour (Figure 4(a)). Flower not seen.

Ceratophyllum sp. L. measured 17.60 – 23.64 mm (20.62 ± 3.02) frond length, 0.45 – 0.59 mm (0.52 ± 0.07) frond width and 14.10 – 19.50 (16.80 ± 2.70) in the number of contiguous fronds (Table 2). The frond shape of *Ceratophyllum* sp. was feather-like, with finely linear segments occurring in pairs along a central axis (Figure 4(e) & Figure 4(f)). The frond colour was lighter green at the fine needle-like segments while more to pale yellow at the central axis of the fronds (Figure 4a). Flower was not seen.

Table 1. The summary of the selected key characteristic to document the freshwater macrophytes (Ceschin *et al.*, 2016)

Descriptive Characteristics	Morphometrics
Frond shape	Frond length (mm)
Frond symmetry	Frond width (mm)
Frond colour	Number of contiguous fronds (n)
Root shape/ rhizome	Vein number (n)

Cyperus haspan L. measured 296.42 – 411.58 mm (354.00 ± 57.58) frond length, 1.86 – 2.54 mm (2.20 ± 0.34) frond width and 4.70 – 8.90 (22.00 ± 0.13) in the number of contiguous fronds (Table 2). The fronds shape of *C. haspan* were leaf blade reduced to sheath and symmetrical while the frond colour was light green slight darker at the tip of leaf blade (Figure 5(a – d)). The root structure was fibrous, short and elongated rhizome slender (Figure 5(e)).

Eichhornia crassipes (Mart.) Solms measured 20.14 – 26.52 mm (23.33 ± 3.19) frond length, 30.85 – 47.59 mm (39.22 ± 8.37) frond width and 5.58 – 8.82 (7.20 ± 1.62) in the number of contiguous fronds (Table 2). The frond shape was orbicular almost round at tip while ovate at the base of the frond, symmetrical and rosette arrangement (Figure 6(a)). The frond adaxial surfaces were darker green while abaxial side were lighter green (Figure 6(b)). The collected *E. crassipes* was rhizomatous and stoloniferous, roots were feather-like and white colour while root hairs were purple to black colour (Figure 6(c) & Figure 6(d)).

Eleocharis dulcis (Burm.f.) Trin. ex Hensch. measured 296.42 – 411.58 mm (354.00 ± 57.58) frond length, 1.34 – 2.02 mm (1.68 ± 0.34) frond width and 5.58 – 8.82 (10.60 ± 2.32) in the number of contiguous fronds (Table 2). The frond colour of *E. dulcis* was dark green while getting pale to yellow towards frond base (Figure 7(a)). The frond blades were symmetrical, root structure was rhizomatous and stoloniferous, the roots were reddish-brown colour while root hairs were pale brown until white colour (Figure 7(b) & Figure 7(c)).

Lemna minor L. measured 3.22 – 3.90 mm (3.56 ± 0.34) frond length, 1.97 – 2.37 mm (2.17 ± 0.20) frond width and 2.33 – 3.67 (3.00 ± 0.67) in the number of contiguous frond (Table 2). *L. minor*'s fronds had light green colour while the frond shape was obovate and symmetrical (Figure 7(d)). The root structure was simple root structure yet stoloniferous (Figure 7(e) & Figure 7(f)).

Hydrocotyle vulgaris L. measured 16.37 – 23.07 mm (19.72 ± 3.35) frond length, 13.99 – 21.47 mm (17.73 ± 3.74) frond width, 0.99 – 1.01 in the number of contiguous fronds and 11.82 – 12.78 (12.30 ± 0.48) in the number of vein (Table 2). The frond shape of *H. vulgaris* was asymmetrical shield-like or umbrella-like (Figure 8(a)). The frond colour was light green with obvious veinous structure on the adaxial surface of the frond (Figure 8(a)). The petiole and the root parts were generally same pale colour pigmentation, root structure was simple root structure yet stoloniferous (Figure 8(b) & Figure 8(c)).

Nuphar sp. Smith measured 128.52 – 147.46 mm (137.99 ± 9.47) frond length, 108.39 – 118.35 mm (113.37 ± 4.98) frond width, 6.85 – 10.15 (8.50 ± 1.65) in the number of contiguous fronds and 12.63 – 13.97 (13.30 ± 0.67) in the number of vein (Table 2). The frond shape for *Nuphar* sp. was reniform sagittate, symmetrical and dark green in colour (Figure 8(d)). The root structure was highly fibrous branched while white in colour (Figure 8(e)).

Limnobium laevigatum (Humb. & Bonpl. ex Willd.) Heine measured 28.00 – 43.68 mm (35.84 ± 7.84) frond length, 29.70 – 33.14 mm (31.42 ± 1.72) frond width, 3.33 – 4.67 (4.00 ± 0.67) in the number of contiguous fronds and 6.08 – 7.12 (6.60 ± 0.52) in the number of vein (Table 2). Heterophylly existed in fronds of collected *L. laevigatum* which were the free-floating form and emergent form or mixed form, which have different frond structure, respectively (Figure 9(a) & Figure 9(d)). For the free-floating form of *L. laevigatum*, the petioles were absent, frond shape was orbicular, rosette arrangement while frond width was higher than frond length (Figure 9(b)). For the emergent form, long petioles were present, the shape retained orbicular, but frond length was greater than frond width (Figure 9(d)). Both forms of fronds were symmetrical, in light green colour. Both forms of *L. laevigatum* had no different on root structure which were rhizomatous and stoloniferous, pale-green in colour, feather-like and slightly transparent root hairs (Figure 9(c) & Figure 9(e)). *Pistia stratiotes* L. measured 39.02 – 46.40 mm (42.71 ± 3.69) frond length, 30.41 – 34.83 mm (32.62 ± 2.21) frond width, 5.75 – 9.05 (7.40 ± 1.65) in the number of contiguous fronds and 5.82 – 6.78 (6.30 ± 0.48) in the number of vein (Table 2). The frond shape of *P. stratiotiotes* was spatulate in dense rosette arrangement, spongy-waxy adaxial surface and asymmetrical (Figure 10(a) & Figure 10(b)). The frond colour was brighter green at the tip of the fronds until yellow at the basal of the fronds (Figure 10(a) & Figure 10(b)). The root structure was rhizomatous and stoloniferous, light green in colour with many thicker root hairs along the root of *P. stratiotes* (Figure 10(c)).

Lindernia rotundifolia (L.) Alston measured 9.47 – 11.25 mm (10.36 ± 0.89) frond length, 9.65 – 11.61 mm (10.63 ± 0.98) frond width, 18.25 – 21.35 (19.80 ± 1.55) in the number of contiguous fronds 4.99 – 5.01 (5.00 ± 0.01) in the and number of vein (Table 2). The frond shape of *L. rotundifolia* was ovate-orbicular, sessile, and asymmetrical (Figure 10(d) & Figure 10(e)). The frond colour was bright light green and highly segmented often in pairs (Figure 10(d) & Figure 10(e)). The root structure was simple and less compact (Figure 10(f)).

Table 2. The morphometric data in comparison with same species or with close species

Species	Frond Length (mm)	Frond Width (mm)	Number of contiguous frond(s)	Number of Vein	Sources
Cabomba aquatica	17.34 – 23.30 (20.32 ± 2.98)	0.47 – 0.57 (0.52 ± 0.05)	10.62 – 17.58 (14.10 ± 3.48)	-	This study
Cabomba sp.	17.42 – 23.42 (20.42 ± 3.00)	0.43 – 0.55 (0.49 ± 0.06)	10.33 – 17.47 (13.90 ± 3.57)	-	This study
Ceratophyllum sp.	17.60 – 23.64 (20.62 ± 3.02)	0.45 – 0.59 (0.52 ± 0.07)	14.10 – 19.50 (16.80 ± 2.70)	-	This study
Ceratophyllum tanaiticum	10.00 – 20.00	-	-	-	Csiky *et al.* (2010)
Cyperus haspan	296.42 – 411.58 (354.00 ± 57.58)	1.86 – 2.54 (2.20 ± 0.34)	4.70 – 8.90 (6.80 ± 2.10)	-	This study
Cyperus haspan	94.10 – 95.90 (95.00 ± 0.90)	-	21.87 – 22.13 (22.00 ± 0.13)	-	Ayeni *et al.* (2015)
Eichhornia crassipes	20.14 – 26.52 (23.33 ± 3.19)	30.85 – 47.59 (39.22 ± 8.37)	5.58 – 8.82 (7.20 ± 1.62)	-	This study
Eleocharis dulcis	296.42 – 411.58 (354.00 ± 57.58)	1.34 – 2.02 (1.68 ± 0.34)	8.28 – 12.62 (10.60 ± 2.32)	-	This study
Hydrocotyle vulgaris	16.37 – 23.07 (19.72 ± 3.35)	13.99 – 21.47 (17.73 ± 3.74)	0.99 – 1.01 (1.00 ± 0.01)	11.82 – 12.78 (12.30 ± 0.48)	This study
Lemna minor	3.22 – 3.90 (3.56 ± 0.34)	1.97 – 2.37 (2.17 ± 0.20)	2.33 – 3.67 (3.00 ± 0.67)	-	This study
Lemna minor	1.90 – 4.50	1.30 – 3.30	2.00 – 4.00	1 – 5	Ceshin *et al.* (2016)
Limnobium laevigatum	28.00 – 43.68 (35.84 ± 7.84)	29.70 – 33.14 (31.42 ± 1.72)	3.33 – 4.67 (4.00 ± 0.67)	6.08 – 7.12 (6.60 ± 0.52)	This study
Lindernia rotundifolia	9.47 – 11.25 (10.36 ± 0.89)	9.65 – 11.61 (10.63 ± 0.98)	18.25 – 21.35 (19.80 ± 1.55)	4.99 – 5.01 (5.00 ± 0.01)	This study
Lindernia rotundifolia	4.50 – 12.00	3.00 – 8.00	-	3.00 – 5.00	Wannan (2019)
Nuphar sp.	128.52 – 47.46 (137.99 ± 9.47)	108.39 – 118.35 (113.37 ± 4.98)	6.85 – 10.15 (8.50 ± 1.65)	12.63 – 13.97 (13.30 ± 0.67)	This study
Pistia stratiotes	39.02 – 46.40 (42.71 ± 3.69)	30.41 – 34.83 (32.62 ± 2.21)	5.75 – 9.05 (7.40 ± 1.65)	5.82 – 6.78 (6.30 ± 0.48)	This study
Pistia stratiotes	36.21 – 38.39 (37.30 ± 1.09)	25.33 – 26.87 (26.10 ± 0.77)	5.29 – 9.55 (7.42 ± 2.13)	-	Galal & Falahat (2015)

Figure 3. (a) The whole structure of collected *Cabomba aquatica*. (b) Closer look into the frond structure of collected *C. aquatica.* (c) Detailed structure of the frond of *C. aquatica.* (d) The collected *C. aquatica* with flower bud and aerial leave attached on it. (e) The flower *of C. aquatica.* (f) Closer look into the flower structure of *C. aquatica*

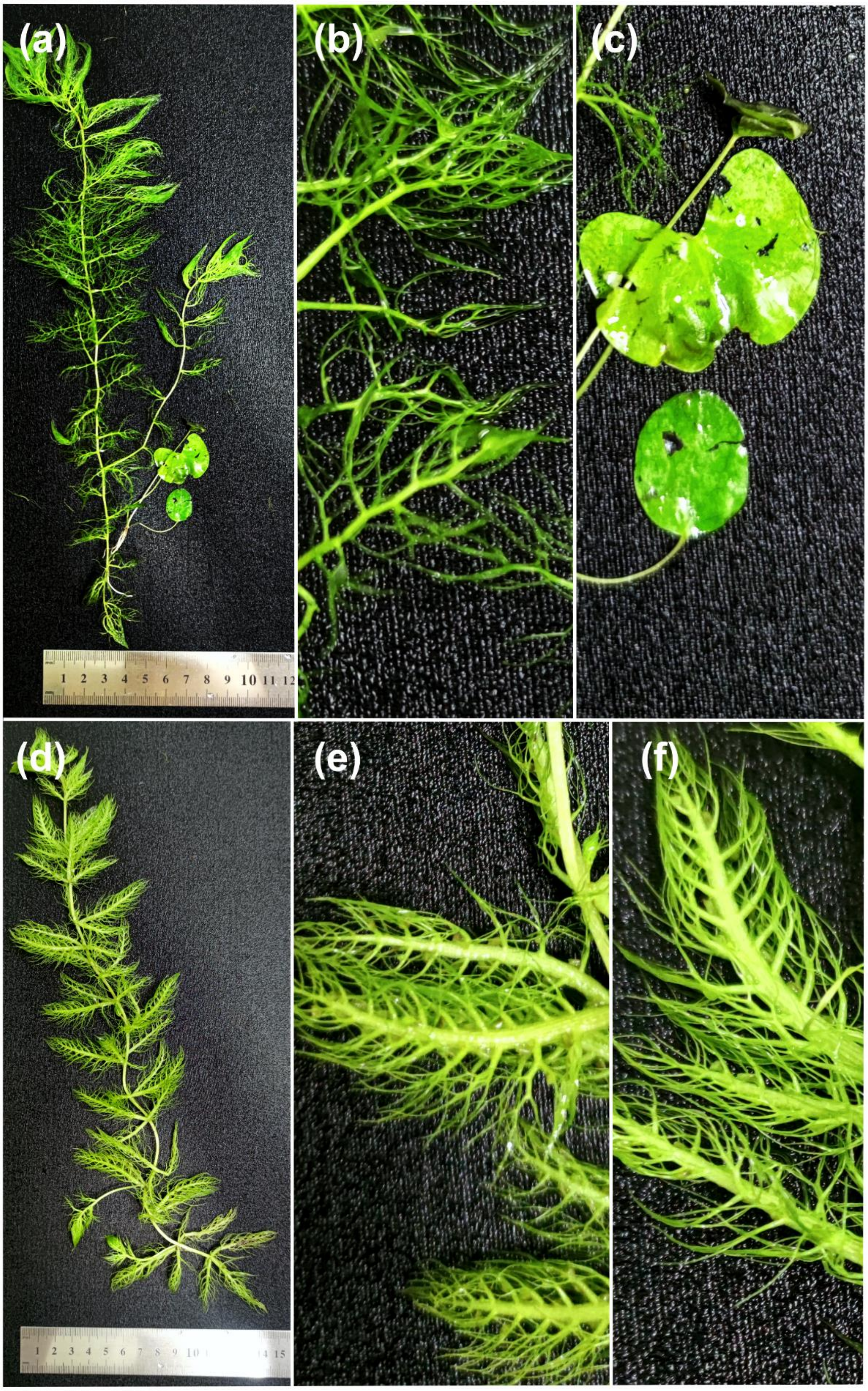

Figure 4. (a) The whole structure of collected *Cabomba* sp. (b) The detailed structure of the finely segmented frond of *Cabomba* sp. (c) Aerial leave of *Cabomba* sp. (d) The whole structure of collected *Ceratophyllum* sp. (e) The detailed structure of feather-like, with finely linear segments occurring in pairs along a central axis of the frond of *Ceratophyllum* sp. (f) Other segments of the fronds of *Ceratophyllum* sp.

Figure 5. (a) The whole structure of collected *Cyperus haspan*. (b) The flower of *C. haspan*. (c) The developing flower buds of *C. haspan.* (d) The closer look into the fronds of *C. haspan.* (e) The detailed root structure of *C. haspan*

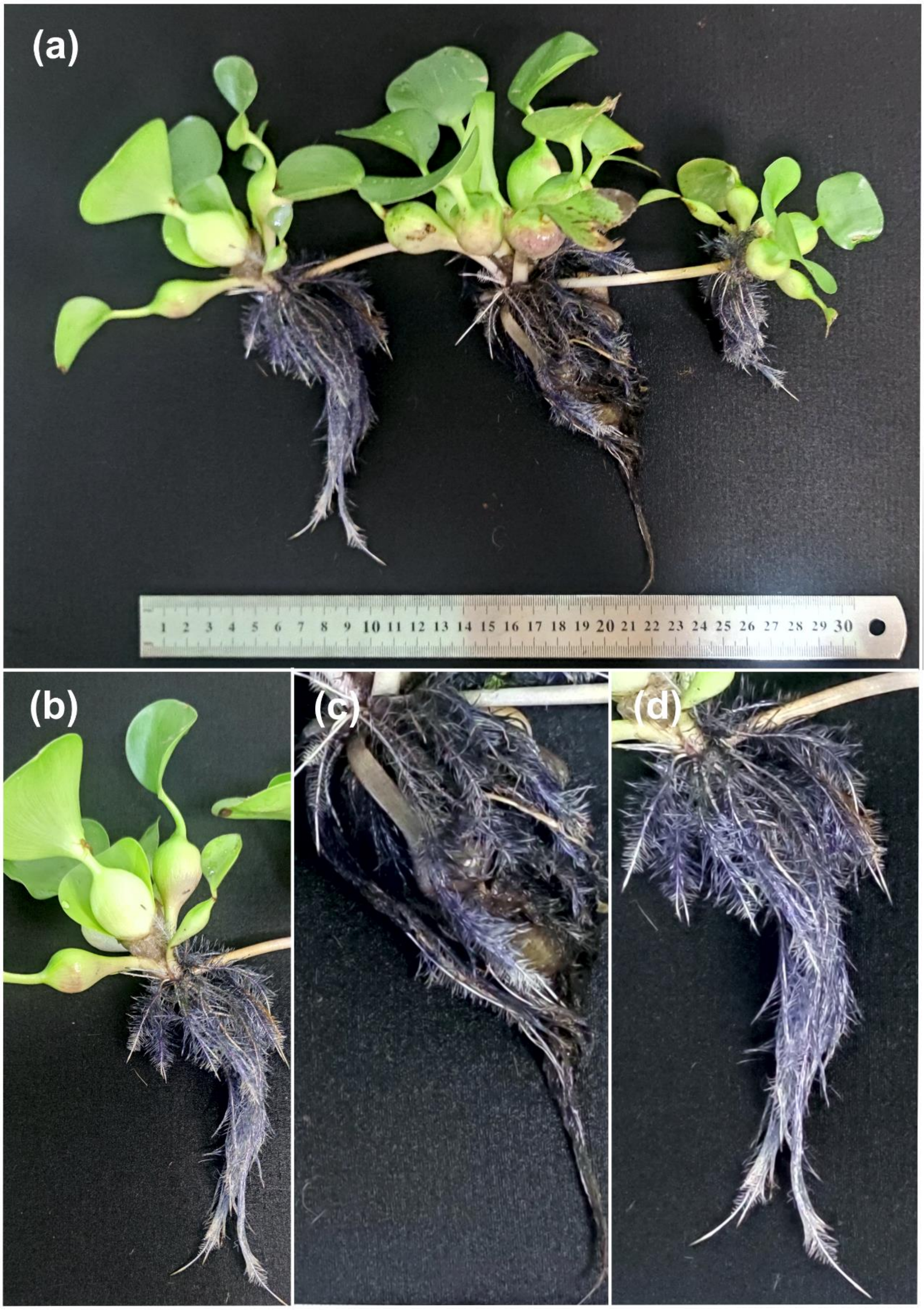

Figure 6. (a) The whole structure of three individuals of collected *Eichhornia crassipes* connected by stolon(s). (b) Closer look into one individual of collected *E. crassipes.* (c) The detailed root structure of *E. crassipes*. (d) The detailed root structure of other individual of *E. crassipes*

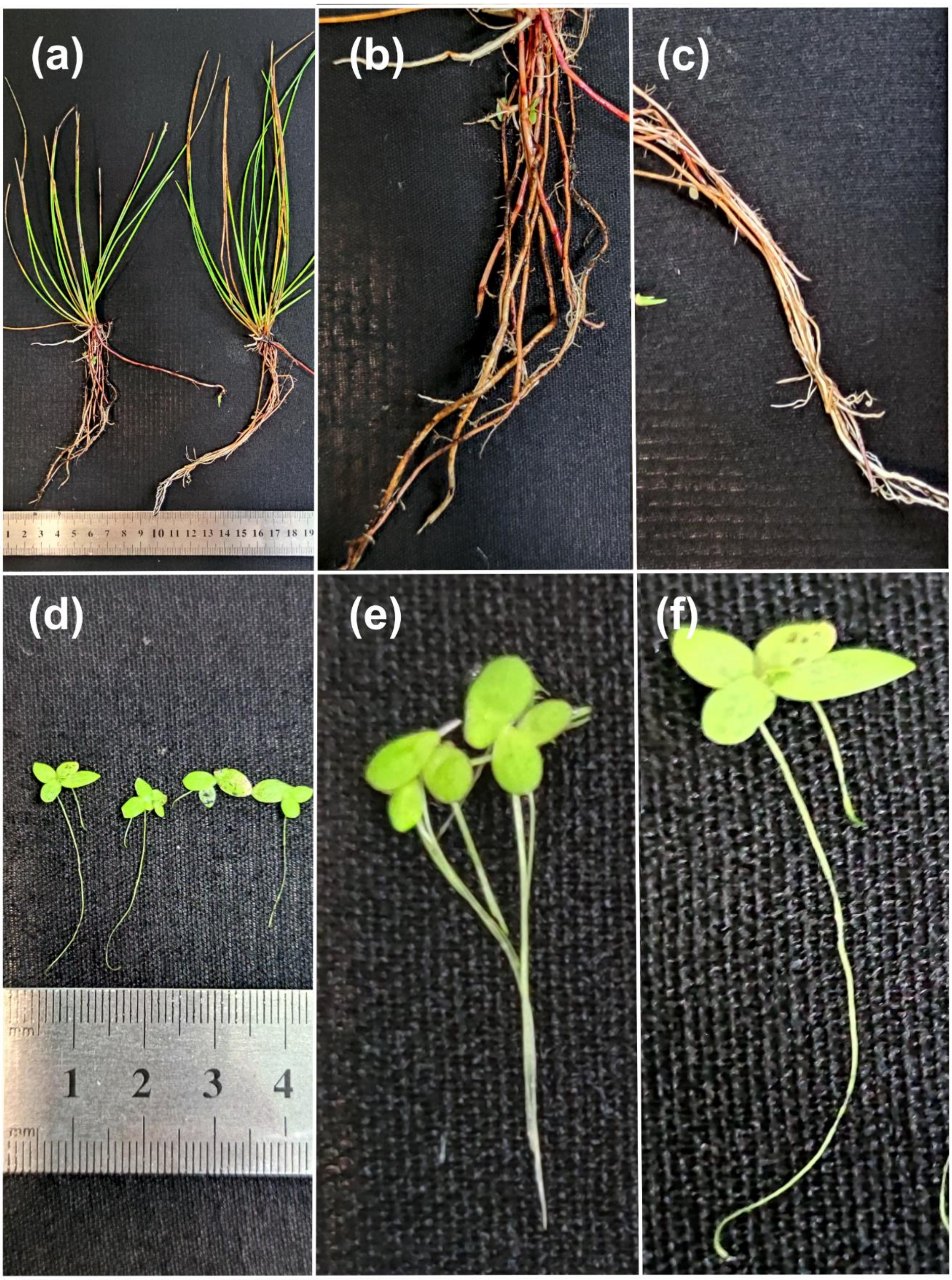

Figure 7. (a) The whole structure of two individuals of collected *Eleocharis dulcis*. (b) The detailed root structure of *E. dulcis*. (c) The detailed root structure of another individual of *E. dulcis*. (d) The whole structure of four individuals with different number of contiguous fronds of *Lemna minor*. (e) Two individuals of *L. minor* connected by stolon. (f) Closer look into the frond and root structure of collected *L. minor*

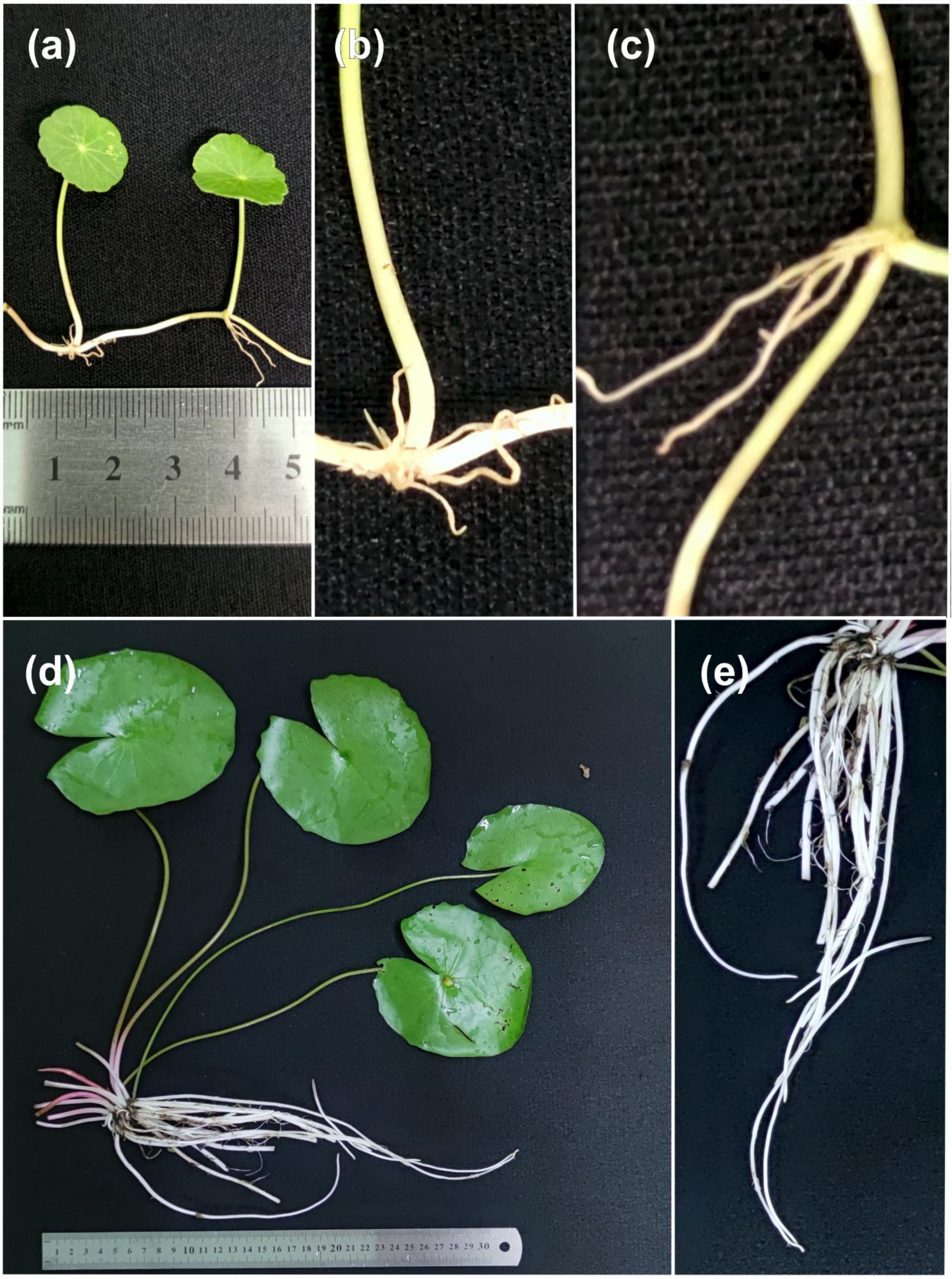

Figure 8. (a) The whole structure of two individual of *Hydrocotyle vulgaris* connected by stolon. (b) The detailed root structure of *H. vulgaris*. (c) The root structure of another individual of *H. vulgaris,* (d) The whole structure of collected *Nuphar* sp. (e) The root structure of *Nupahr* sp.

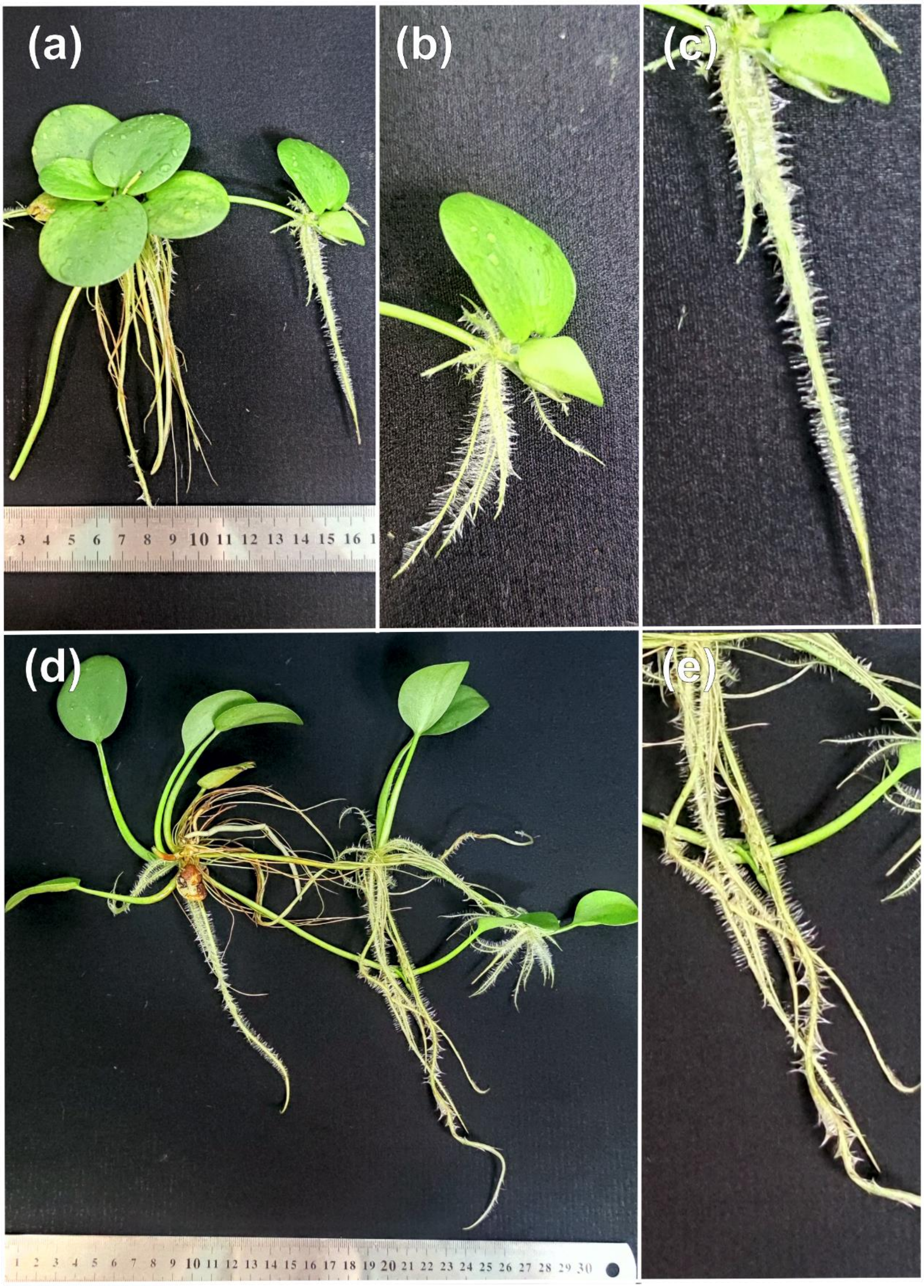

Figure 9. (a) The whole structure of two individuals of collected *Limnobium laevigatum* in free-floating form connected by stolon. (b) Closer view into one individual of *L. laevigatum* in free-floating form. (c) The detailed root structure of *L. laevigatum* in free-floating form. (d)The whole structure of three individuals of collected *L. laevigatum* in emergent form. (e) The detailed root structure of *L. laevigatum* in emergent form

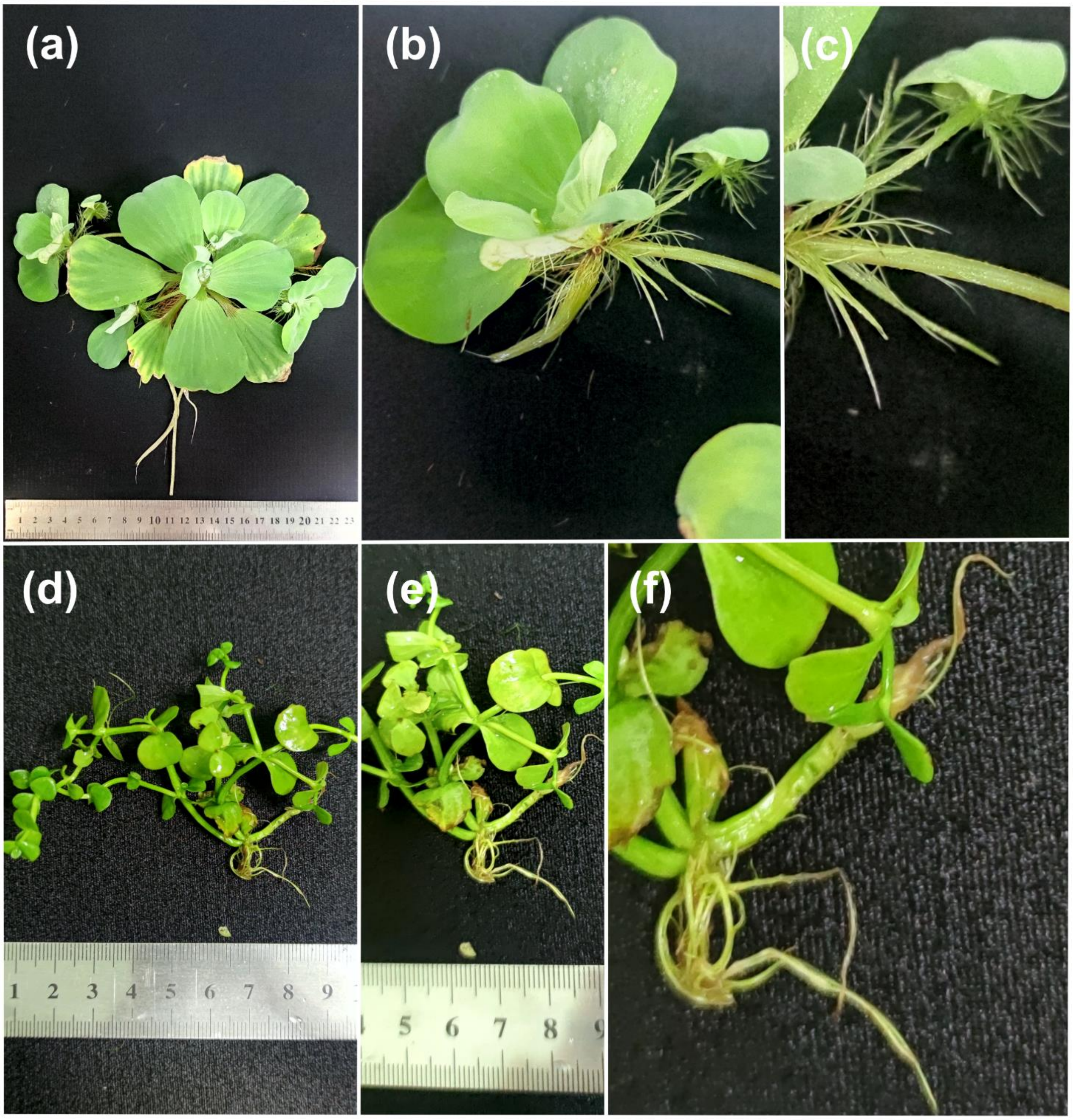

Figure 10. (a) The whole structure of three individuals of collected *Pistia stratiotes* connected by stolon. (b) The whole structure of another collected *P. stratiotes* connected by stolon. (c) The detailed root structure of *P. stratiotes.* (d) The whole structure of collected *Lindernia rotundifolia.* (e) Closer view of the structure of *L. rotundifolia*. (f) The detailed root structure of *L. rotundifolia*

Population Estimation

Table 3 summarised a total number of 12 species of freshwater macrophytes, 10 individuals per species were collected from the sampling sites in different river creeks and water ponds of Pontian, Johor. The estimated percentage of area covered by macrophytes in station 1 with the water body area of 571 m² was 18.68% dominated by *Limnobium laevigatum* (9.34%) and *P. stratiotes* (9.34%). The samples collected in station 2 with the water body area of 375 m² had only one species of macrophytes, which was *L. minor*, estimated percentage of coverage was 88.67%, followed by station 3 with the water body area of 104 m² were covered by *H. vulgaris* (8.00%) as well as station 4 with the water body area of 145 m² were covered by *E. crassipes* (44.00%). The estimated percentage of area covered by macrophytes in station 5 with the water body area of 1906 m² was 57.17% dominated by *C. aquatica* (42.67%) and *E. dulcis* (14.50%). The estimated percentage of area covered by macrophytes in station 6 with the water body area of 1372 m² was 56.68% dominated by *Cyperus haspan (31.34%)* and *Nuphar* sp. (25.34%). The estimated percentage of area covered by macrophytes in station 7 with the water body area of 1293 m² was 20.88%, recorded three collected species which were *Cabomba* sp. (5.33%), *Ceratophyllum* sp. (6.22%) and *L. rotundifolia* (9.33%).

Figure 11 summarised the estimation assessed by the percentage of area covered by macrophytes by using the quadrat population estimation technique summarised in 100% stacked bar chart. Coverage for station 1 was estimated 9.34% covered while 90.66% uncovered; Station 2 estimated 88.67% of area covered by freshwater macrophytes while only 11.33% was uncovered. Station 3 estimated the covered area was 8.00% while 92.00% was uncovered. Station 4 was 44.00% of the area covered by macrophytes while 56.00% uncovered. Station 5 was 57.17% of area covered by macrophytes while 42.83% was uncovered; station 6 was 56.68% of area covered by macrophytes while 43.32% uncovered. Station 7 estimated 20.88% of area covered by freshwater macrophytes while 79.12% was free water surface.

Table 3. The type for freshwater macrophytes collected from each station of the sampling site in Pontian, Johor with the area of water bodies within each location and the estimated percentage of area covered by macrophytes

Stations	Specimens	Locality	Area of water body (m²)	Estimated percentage of area covered by macrophytes (%)
1	*Limnobium laevigatum*	1°32’39.69” N, 103°29’09.34” E	571	9.34
	Pistia stratiotes	1°32’39.69” N, 103°29’09.34” E		9.34
2	*Lemna minor*	1°32’33.21” N, 103°30'56.28"E	375	88.67
3	*Hydrocotyle vulgaris*	1°31'17.43"N, 103°30’59.90” E	104	8.00
4	*Eichhornia crassipes*	1°31’05.85” N, 103°30’30.69” E	145	44.00
5	*Cabomba aquatica*	1°27’44.13” N, 103°30’21.15” E	1906	42.67
	Eleocharis dulcis	1°27’44.13” N, 103°30’21.15” E		14.50
6	*Cyperus haspan*	1°25’06.34” N, 103°26’08.12” E	1372	31.34
	Nuphar sp.	1°25’06.34” N, 103°26’08.12” E		25.34
7	*Cabomba* sp.	1°26'18.64"N, 103°28'9.03"E	1293	5.33
	Ceratophyllum sp.	1°26'18.64"N, 103°28'9.03"E		6.22
	Lindernia rotundifolia	1°26'18.64"N, 103°28'9.03"E		9.33

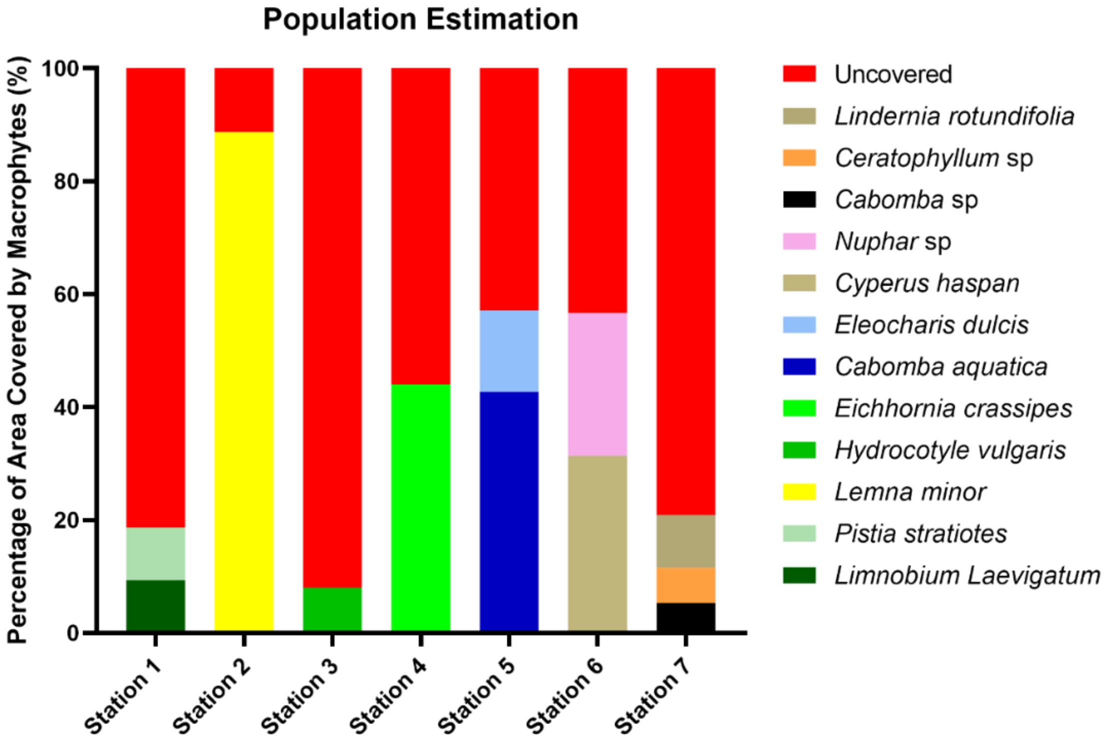

Figure 11. Population estimation assessed by percentage of area covered by macrophytes by using the quadrat population estimation techniques summarized in 100% stacked bar chart

DISCUSSION

Morphology

The morphometrics were obtained from 120 individuals of freshly collected macrophytes sample from different location around the oil palm plantation area of Pontian, Johor. A total of 12 species were collected and 10 individuals for each species were chosen to measure the selected vegetative characteristics occurring in freshwater macrophytes suggested by Ceschin *et al.* (2016). Most of the collected macrophytes lacked the number of veins from our study due to the absence of microscopy apparatus; however, the macrophytes with obvious vein structure were recorded in term of the range of vein number. The morphometric data of the five out of 12 species were compared with similar or closely related species from recent studies. The rest of the data were documented to characterise the morphometrics of macrophytes in Pontian, Johor. Some of the samples did not have the morphometric comparison due to the lack of specific studies or the related studies were outdated. In some related studies, no corresponding parameter was obtained to be included for morphometric comparison in our study.

Cabomba aquatica, *Cabomba* sp. and *Ceratophyllum* sp. were the submerge macrophytes collected in this study. *Cabomba aquatica* can be found in the freshwater environment in Kota Tinggi, Johor, as recorded by Siti-Munirah and Chew (2010), and the key characters to identify *C. aquatica* was the finely needle-like fronds arranged in fan-shape arrangement with yellow colour flower (Siti-Munirah & Chew, 2010). The *Cabomba* sp. was unable to be identified into species level because vital character which was the flower was absent from the sample collection in station 7. The *Cabomba* sp. can be misidentified into other species, for example *C. caroliniana* with white colour flower that was morphological similar to *C. aquatica.* Same situation for the identification of *Ceratophyllum* sp., the flower was absent in the sample collection; however, the morphological characteristics of the collected *Ceratophyllum* sp. such as the simple linear laminas or the laminas divided dichotomously into linear filiform segments with a central axis, following the description by Les (1993). This study only collected one floating leaved macrophyte, which was the *Nuphar* sp. Within the family of Nymphaeaceae, *Nuphar* and *Nymphaea* have higher similarities in term of morphology, the key characteristic to distinguish between these two genera was the colour of their flowers; yellow for *Nuphar* while white for *Nymphyaea*. Thus, *Nuphar* sp. can be identified following the dichotomous key and taxonomic description proposed by Schneider and Williamson (1993), which the frond of *Nuphar* was frond mostly floating, blades orbicular to lanceolate and rhizomatous. The identification was unable to reach to species level due to lack of flower, seed and fruit being collected in this study.

For the morphometric comparison, there were two species of *Cabomba* being collected in this study. *Cabomba aquatica* recorded an average of 20.31 ± 2.98 mm in frond length which was lower than that of *Cabomba* sp. However, the frond width and number of contiguous frond for *C. aquatica* measured an average 0.52 ± 0.05 mm and 14.10 ± 3.48, respectively which were higher than *Cabomba* sp. that was recorded 0.49 ± 0.06 mm for frond width and 13.90 ± 3.57 of the number of contiguous fronds.

The morphological feature for *Ceratophyllum* sp. recorded in this study was compared with the study conducted by Csiky *et al.* (2010) in term of frond length. The frond length of *Ceratophyllum* sp. measuring at 17.60 – 23.64 mm in this study was generally larger than the *Ceratophyllum tanaiticum* recorded by Csiky *et al.* (2010) ranging from 10.00 – 20.00 mm.

Hydrocotyle vulgaris, C. haspan, E. dulcis and *L. rotundifolia* were the emergent macrophytes collected this in study. *Hydrocotyle vulgaris* can be identified as it can be found in aquatic habitat (not all *Hydrocotyle* species were aquatic), and its veinous structure on the adaxial surface of the frond made *H. vulgaris* distinct from other species within same genus (Wang *et al.*, 2018). *Cyperus haspan* was identified by referring to the dichotomous key proposed by Tucker (1983) as *C. haspan* lack of leaf blades, sharp-pointed sheaths at the base of the culms and coarse granular surfaces. The distinct characteristics of *E. dulcis* compared with the other species within the genus of *Eleocharis* was the frond basal often purplish, sheaths glabrous, reduced and elongated terminal spikelet (Chavan *et al.*, 2014). *Lindernia rotundifolia* was identified using the dichotomous key and literature description proposed by Liang *et al.* (2012). The morphological characteristic to distinguish *L. rotundifolia* was the vein number was 3 - 5, the frond was minutely glandular-punctate on both surfaces, the branching from base of stem and the rooting at lower.

Next, there was a gap between the morphometric measurement of *C. haspan* in this study and the finding conducted by Ayeni *et al.* (2015). The frond length of *C. haspan* recorded in this study was average 354.00 ± 57.58 mm significantly higher than the frond length measured by Ayeni *et al.* (2015) average 95.00 ± 0.90 mm. However, the number of contiguous fronds was recorded average 6.8 ± 2.10 significantly less than the value obtained by Ayeni *et al.* (2015) which was average 22.00 ± 0.13. This observed the intraspecific variation in term of morphometrics between this study and the experiment conducted by Ayeni *et al.* (2015).

Generally, the morphometric data of *L. rotundifolia* recorded by Wannan (2019) were similar with the data obtained in this study. The frond length of *L. rotundifolia* in this study was measured 9.47 – 11.25 mm, this value was close to the value recorded by Wannan (2019) but wider in range which was 4.50 – 12.00 mm. The frond width of *L. rotundifolia* in this study were recorded 9.65 – 11.61 mm which was larger than the frond width recorded by Wannan (2019), ranging from 3.00 – 8.00 mm. The number of vein was narrower in range (4.99 – 5.01) for this study but the number of vein in Wannan (2019) study was wider in range (3.00 – 5.00).

Eichhornia crassipes, P. stratiotes, L. laevigatum and *L. minor* were the free-floating macrophytes collected in this study. *Eichhornia crassipes, P. stratiotes* and *L. laevigatum* were identified directly by using the morphological descriptions provided by Richards and Lee (1986), Bidarlord *et al.* (2019) and Howard *et al.* (2016), respectively. The species within the genera of these three species were not diverse and the morphological characteristics were distinct to other species within the same genera. However, the *L. minor* was morphological similar to *L. minuta* and often misidentified by researchers (Ceschin *et al.*, 2016). So, the identification of *L. minor* was done by referring the description in term of morphometrics and descriptive characteristics proposed by Ceschin *et al.* (2016). The best characters to distinguish *L. minor* was the round frond apex and obovate frond shape.

Thus, the frond length, frond width and the number of contiguous frond of *L. minor* in this study was generally similar with the features recorded by Ceschin *et al.* (2016). The frond length of *L. minor* recorded in this study was ranging 3.22 – 3.90 mm, frond width ranging from 1.97 – 2.37 mm, number of contiguous fronds ranging 2.33 – 3.67, the values were close to the measurements by Ceschin *et al.* (2016) where the frond length 1.90 – 4.5 mm, frond width 1.30 – 3.30 mm as well as number of contiguous fronds 2.00 – 4.00, respectively. The number of vein was recorded by Ceschin *et al.* (2016) ranging 1 – 5 veins but this feature was absent in this study.

In addition, the frond length and frond width of *P. stratiotes* measured in this study were slightly larger than the morphometric data from the experiment conducted by Galal and Farahat (2015). The frond length and frond width measured in this study were average 42.71 ± 3.69 mm and 32.62 ± 2.21 mm, respectively, slightly larger than the average value obtained by Galal and Farahat (2015) which were 37.30 ± 1.09 mm and 26.10 ± 0.77 mm, respectively. Nevertheless, the number of contiguous fronds recorded in Galal & Farahat (2015) was 7.42 ± 2.13 which more than the number of contiguous frond in this study that measured 7.40 ± 1.65 on average.

The descriptive characteristics of the freshwater macrophytes collected from Pontian Johor were documented by using scientific photograph techniques suggested by Dibble and Thomaz (2009) and Ferreiro *et al.* (2013) with slightly modification. The frond shape, frond symmetry, frond colour and root or rhizome structures or colour were described in the result of this study. For the descriptive characteristic comparison, the *E. crassipes* collected in this study were compared to the photograph taken by Das and Goswami (2015), as well as *L. laevigatum* compared to Howard *et al.* (2016), *P. stratiotes* compared to Chapman *et al.* (2017), *C. aquatica* compared to Silva and Leite (2011) and *L. rotundifolia* compared with the sample recorded by Wannan (2019). The rest of the sample collected in this study were not compared to other published literature because lack of specific photographs from their studies, but the related photographs can be compared easily with the open source from internet especially from the aquarium ornament plant dealers. Generally, little or no intraspecific variation on the phenotype of all freshwater macrophyte collected from the waters of Pontian, Johor.

Population Estimation

The population was assessed based on area covered by freshwater macrophytes in the river creeks and water ponds from the oil palm plantation area of Pontian, Johor. They were distributed differently, with no species population overlapping within the same environment. The quadrat estimation technique was partial count method suggested by Capers (2000) and Sharip *et al.* (2012) with slight modifications. There were several factors that could affect the macrophytes population distribution, such as the water quality, water transparency, water depth and substrate properties which may influence the occurance of species according to life forms that can be found within the water body (Harped *et al.*, 1995). The factors that were affecting the density of macrophytes within the same population could be the water nutrient contents, rate of water flow of the water body and the interspecific competition with terrestrial plant, phytoplankton, aquatic epiphyte, macroinvertebrate and bacteria (Gopal & Goel, 1993). Recent study also showed the degree of water health or pollution level has direct relationship with the growth population of aquatic macrophytes (Wang *et al.*, 2021).

Among the seven sampling stations, the highest estimated percentage of area covered by freshwater macrophytes was station 2, which was a 375 m² shallow water pond that was estimated covered by free-floating macrophytes, *L. minor* for about 88.67% within the water body. The water surface was dominated by *L. minor* only, for about 11.33% (42.49 m²) of the water body was free from *L. minor* population. The degree of invasion by *L. minor* may associate with the nutrient availability (Paolacci *et al.*, 2016). Station 2 was a lentic water body with less disturbance, the water body was estimated to have high nutrient availability as well as water hardness due to the runoff from oil palm plantation area that relied on chemical fertiliser. *Lemma minor* often occurred in nutrient-rich eutrophic water with habitat-dominating characteristics described by Ceschin *et al.* (2018).

The same situation occurred for sampling station 3 and station 4 which were the water bodies only occupied by one species of freshwater macrophyte. Station 3 was the smallest sampling site compared to other six sampling sites. It was a relatively smaller river creek (104 m²), found only one emergent species of macrophyte *Hydrocotyle* *vulgaris* at the shallowest part of the river creek that covered only 8% from its population and occupied the water surface area for about 8.32 m², 92% of the water body was free from any freshwater macrophyte population. Liu *et al.* (2016) claimed that *H. vulgaris* was considered as an invasive species that is able to form a population easily; however, the result shown that the least coverage by *H. vulgaris*, which may be due to the early invasion stage by *H. vulgaris* and relative lower nutrient input from station 3. Nevertheless, station 4 also considered as a relative smaller river creek (145 m²) compared to other sampling sites; the river creek colonised by one free-floating macrophytes species which was *Eichhornia crassipes*, covered the water surface for about 44% (63.8 m²) and left 56% (81.2 m²) uncovered. Station 4 was a lotic river creek, the condition maybe not favourable for the growth of *E. crassipes* proposed by Ho Thanh (2012) showed that the growth performance of *E. crassipes* in lentic water pond was significantly better than the growth performances in lotic river.

The most diverse population of freshwater macrophytes found in this study was station 7 which found three species of freshwater macrophytes including *Cabomba* sp., *Ceratophyllum* sp. and *L. rontundifolia*. The total percentage of area covered by macrophytes in this river creek (1293 m²) was 20.88%. Although the percentage of coverage was relatively lower than the other sampling station, the population was the most diverse. The obtained specimens were two submerged macrophytes species, which were the *Cabomba* sp. 5.33% (68.92 m²) and *Ceratophyllum* sp. 6.22% (80.42 m²) and one emergent species, *L. rotundifolia* 9.33% (120.64 m²). Among the three collected samples, *L. rotundifolia* had the highest percentage of coverage (9.33%) followed by *Ceratophyllum* sp. (6.22%) and *Cabomba* sp. (5.33%), respectively. About 79.12% (1023 m²) of the water surface in station 7 was free from macrophytes population. station 7 was estimated the largest area river creek (1293 m²); however, the macrophyte coverage was relatively lower compared to other sampling stations. The river creek located in station 7 was moving water, the disturbance maybe higher, the nutrient retainability in a same point within the river creek maybe lower compared to other river creek (Maine *et al.*, 1999), but the estimated area of the river creek was higher. This may provide a different habitat niche for different species of freshwater macrophyte (Oyedeji & Abowei, 2012). Two life forms of macrophytes were collected in Station 7 which were the emergent (*L. rotundifolia*) and submerged macrophytes (*Ceratophyllum* sp. and *Cabomba* sp.) These two life forms of macrophyte were occupying different niches in the same river creek, *L. rotundifolia* at the shallower part of the creek while *Cerataophyllum* sp. and *Cabomba* sp. occupying the deeper part of the river creek. It can be said that less interspecific competition will be occurred between the macrophyte community within the same habitat due to different habitat zonation (Roznere & Titus, 2017; de Assis Murillo *et al.*, 2019). So, station 7 was having a diverse population but less macrophytes coverage due to higher disturbance and lacking nutrient input.

Station 5 was a freshwater pond, located inside the oil palm plantation area, which was the largest estimated sampling area (1906 m²) in this study, found 57.17% of area covered by two life forms of freshwater macrophytes which were submerged and emergent macrophytes. The collected submerged species was *C. aquatica* estimated about 42.67% (813.29 m²) covering the water pond and the emergent species, *E. dulcis* covered about 14.50% (276.37 m²), leaving 42.83% (816.34 m²) free from macrophytes population. The situation occurred at station 1 and station 6, which were the water bodies found two species of freshwater macrophytes only. Station 1 was a river creek (571 m²) with estimated 18.68% covered by two types free-floating macrophytes, *P. stratiotes* and *L. laevigatum*. Both species of the macrophytes covered 9.34% (53.33 m²) of the water surface. About 81.32% (464.33 m²) of the water surface in station 1 was free from macrophytes population. Station 6 was a river creek (1372 m²) with 56.68% covered by one type of emergent macrophyte and one type of floating-leaved macrophyte, *C. haspan* and *Nuphar* sp.. *Cyperus haspan* covered 31.34% (429.98 m²) of the water surface while *Nuphar* sp. coovered 25.34% (347.66 m²) of the water surface. About 43.32% (594.35 m²) of the water surface was free from macrophytes.

CONCLUSION

Based on the population estimation in this study, the percentage of area covered by freshwater macrophytes in different water bodies around the area in Pontian, Johor was estimated. The morphometric data for five species of freshwater macrophytes including *Ceratophyllum* sp, *C. haspan*, *L. laevigatum*, *L. rotundifolia* and *P. stratiotes* were compared with recent studies as well as the morphometric data of other collected sample were recorded to characterise the morphometric measurement of freshwater macrophytes in Pontian, Johor. The descriptive characteristics as well as diversity were also documented in this study to support the characterisation of freshwater macrophytes in Pontian, Johor.

Future study on frond area by using digital determination software that can produce monochrome bitmap image yet produce mathematical data for statistical analysis and microscopic screening for detailed macrophytes structures examination is needed to be done to fill the gap of study in this field. In addition, more advanced sampling and quadrat estimation techniques such as SCUBA and underwater sampling as well as quadrat distribution with transect line can be done to give a more accurate population estimation for the freshwater macrophytes in Pontian, Johor water.

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