UNIVERSITI PUTRA MALAYSIA

MORPHOLOGY AND PROPAGATION OF BLYXA AUBERTII RICH

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MORPHOLOGY AND PROPAGATION OF *Blyxa aubertii* RICH.

BY

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ABSTRACT

This study is an attempt to furnish information with respect to the morphology and propagation of *Blyxa aubertii* Rich. from four locations; UPMKB, Baloi, Bintulu; Maradong, Sarikei and 19km golf field, Sibu. *Blyxa aubertii* is an annual plant can be found submersed in natural and man-made water bodies with 0.10-1.20 m water level. Live on silted clay substrate, water pH 5-6.6, temperature 26.4-27.9°C and dissolved oxygen 0.5-3.57 mg/L. *Blyxa aubertii* grows associated with other aquatic macrophytes such as *Eleocharis* sp., *Hydrilla verticillata, Nymphaea nouchali, Utricularia aurea* and *Utricularia bifida*. The vegetative morphology of *Blyxa aubertii* from UPMKB, Baloi, Maradong and Sibu are generally similar but the dimension analysis shows some variable. The number of leaves per shoot from UPMKB (7-800 leaves) is significantly higher when compared with Sibu (6-32 leaves), Maradong (8-27 leaves) and Baloi (10-23 leaves). The longest leaves were obtained from Baloi 26.90±8.43 cm followed by Maradong 15.18±3.73 cm, UPMKB 12.38 ± 3.19 cm and the smallest from Sibu 10.67±1.73 cm. The leaf widths of *B. aubertii* from UPMKB were similar to Maradong with 0.48 ± 0.09 cm and 0.48±0.12 cm respectively. However, these leaves width significantly different when compared with Baloi 0.68±0.12 cm and Sibu 0.35±0.04 cm. *Blyxa aubertii* leaves consist a lot of air spaces without stomata were observed. Flowers are hermaphrodite, covered by spathe in bud stages, borne on the hypanthium. *Blyxa aubertii* have three sepals, three petals, three stamens and three stigmas. Ovary as long as the spathe. The fruits of are elongated capsule 17.81-66.88 mm x 1.95-3.64 mm with peduncle 2.66-27.35 cm. Seeds count varies from 22-132 seed per fruit. *Blyxa aubertii* have ellipsoidal seed with 5-12 longitudinal ribs. The first sign of germination is the swollen of the seed follow by emergence of radicle, splitting of seed coat, emergence of cotyledon, first leaf, second leaves and third leaf within two months. *Blyxa aubertii* germinates from seed, grows into seedlings, juvenile plants, flowering, fruiting plants and finally disappeared all occurring within a period of approximately four and half months.

ABSTRAK

Kajian ini berusaha untuk memberi maklumat yang lebih mendalam mengenai tumbuhan akuatik *Blyxa aubertii* Rich. dari segi struktur morphologi dan propagasi dari empat kawasan iaitu UPMKB, Baloi, Bintulu; Maradong, Sarikei dan 19km padang golf, Sibu. *Blyxa aubertii* adalah tumbuhan setahun (annual) dan boleh ditemui hidup tenggelam di badan air semulajadi dan buatan manusia pada kedalaman 0.10-1.20 m. Tumbuh di atas substrat lumpur berpasir, pH air 5-6.6, suhu 26.4-27.9°C dan oksigen terlarut 0.5-3.57 mg/L. *Blyxa aubertii* tumbuh berasosiasi dengan makrofit akuatik yang lain seperti *Eleocharis sp., Hydrilla verticillata, Nymphaea nouchali, Utricularia aurea* dan *Utricularia bifida.* Morphologi vegetatif *Blyxa aubertii* dari UPMKB, Baloi, Maradong dan Sibu adalah sama tetapi analisis dimensi menunjukkan perbezaan. Bilangan daun per rumpun dari UPMKB (7-800 daun) adalah tinggi dibandingkan dengan Sibu (6-32 daun), Maradong (8-27 daun) dan Baloi (10-23 daun). Daun paling panjang didapati dari Baloi 26.90±8.43 cm diikuti oleh Maradong 15.18±3.73 cm, UPMKB 12.38±3.19 cm dan yang paling kecil dari Sibu 10.67±1.73 cm. Lebar daun *B. aubertii* dari UPMKB sama dengan Maradong masing-masing 0.48±0.09 cm dan 0.48±0.12 cm setiap satu. Walaubagaimanapun, lebar daun tersebut adalah berbeza berbanding dengan Baloi 0.68±0.12 cm dan Sibu 0.35±0.04 cm. Daun *Blyxa aubertii* mempunyai banyak ruang udara tanpa stomata diperhatikan. Bunga adalah hermafrodit, dilitupi kelopak bunga semasa peringkat kudup, terletak di atas 'hypanthium'. *Blyxa aubertii* mempunyai tiga sepal, tiga petal, tiga stamen dan tiga stigma. Ovari adalah sama panjang dengan kelopak bunga. Buah berbentuk kapsul memanjang 17.81-66.88 mm x 1.95-3.64 mm dengan pedunkel sepanjang 2.66-27.35 cm. Bilangan biji dalam setiap kapsul buah berjulat 22-132 biji. Biji benih berbentuk ellipsoidal dan membentuk 5-12 'longitudinal ribs'. Percambahan bermula dengan pembengkakan biji benih diikuti oleh kemunculan radikel, kulit biji merekah, kotiledon, daun pertama, daun kedua dan daun ketiga dalam jangkamasa dua bulan. *Blyxa aubertii* mengambil masa selama empat bulan setengah untuk bercambah dari biji benih, menjadi anak benih, membesar menjadi tumbuhan muda, berbunga, berbuah dan mati.

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Lastly, my appreciation is extended to those that I did not mention here and had offered their help during my project. This project will not complete without them. Thank you very much!

APPROVAL

I certify that this research project report entitled "Morphology and Propagation of *Blyxa aubertii* Rich." has been examined and approved as partial fulfilment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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LIST OF ABBREVIATIONS

1.	° C	: Degree Celsius
2.	%	: Percent
3.	cm	: Centimetre
4.	DO	: Dissolved Oxygen
5.	DPX	: DePeX mounting medium
6.	FAA	: Formalin acetic alcohol
7.	km	: Kilometre
8.	m	: Metre
9.	max	: Maximum
10.	mg/L	: Milligram per Litre
11.	min	: Minimum
12.	mm	: Millimetre
13.	N	: Sample Size
14.	NaOH	: Sodium hydroxide
15.	s.e	: Standard Error
16.	Temp.	: Temperature

CHAPTER 1

INTRODUCTION

Aquatic macrophyte is one of the aquatic resources, which include freshwater plants and marine plants. Aquatic macrophytes are the macroscopic (large enough to be observed by the naked eye) forms of aquatic and wetland plants found in or near water bodies (Soepadmo, 1986; Kubitzki, 1998; Jiang and Kadono, 2001; Muta Harah *et al.*, 2005a) or in nature growing association with free standing water level is at or above the surface of the soil (Kubitzki, 1998; Jiang and Kadono, 2001; Muta Harah *et al.*, 2005b). The term aquatic macrophytes also refers to a diverse group of aquatic plants and encompasses flowering vascular plants, mosses, ferns and macroalgae (Cook and Urmi-König, 1983) and includes plants whose photosynthetically active parts are submerged in water or floating on the water surface permanently or at least for several months each year (Cook *et al.*, 1974).

The aquatic macrophytes were classified into four groups base on growth form; (a) submersed: plant with roots in the soil and the upper part are always in the water (b) emergent: plants with roots in the soil and the upper part such as leaves are always in or above the water (c) floating: plants either have or devoid of roots are always float on the water surface (d) semi-aquatic: plants are growing near the water areas as mentioned by Pancho and Soerjani (1978), Seopadmo (1986), Mashhor (1988) and Muta Harah *et al.* (2005a; 2005b).

Blyxa Thouars ex Rich is one of the flowering submersed aquatic macrophytes (Randall, 1998), monoecious or dioecius aquatic herbs belong to family

Hydrocharitaceae. The genus *Blyxa* is classified under entomophilies (flowers bloom above the water surface and are pollinated by insects) as reported by Cook (1982) and Tanaka *et al.* (2004). In Malaysia, about 216 species from 30 families of aquatic flowering plants were found (Cheksum, 1998) while in Sarawak, Baki (1993) found only 150 species of the aquatic flowering plants. These include both monocotyledonous plants and dicotyledonous plants. The aquatic flowerings plants also known as angiospermous hydrophytes, are able thrive and complete their life cycle in habitats where water quality exerts the greatest influence on their growth and development.

Despite the noxious nature of this macrophyte, a number of studies (Pancho and Soerjani, 1978; Baki and Md Khir, 1983; Cook and Lüönd, 1983; Azmi, 1991) have noticed that some species of *Blyxa* are persistent weeds in those rice fields' areas. Several studies on the growth, morphology, reproduction and ecology of *Blyxa* have been done by Cook and Lüönd (1983) as well as Jiang and Kadono (2001). There are rarely studies in detail focus on biology and propagation of *Blyxa aubertii*. With regard to the above review, the objectives of this study are:

(i) To study the vegetative and reproductive structures of *Blyxa aubertii.*

(ii) To study the propagation of *Blyxa aubertii.*

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy

Kingdom : Plantae

Division : Magnoliophyta (flowering plants)

Subdivision : Magnoliophytina (Angiosperms)

Class : Liliopsida (Monocots)

Subclass : Alismatidae

Order : Hydrocharitales

Family : Hydrocharitaceae (tape-grass family)

Genus : *Blyxa* Noronha ex Thouars.

Species : *Blyxa* spp.

(Cook and Lüönd, 1983)

According to Jacobsen (1979) ten species of *Blyxa* have been recorded worldwide; *Blyxa aubertii* Rich., *B. echinosperma* Clarke, *B. hexandra* Cook and Lüönd, *B. japonica* Miquel, *B. novoguineensis* den Hartog, *B. octandra* Planch. ex Thw, *B. quadricostata* den Hartog, *B. radicans* Ridley, *B. senegalensis* Dandy and *B. vietii* Cook and Lüönd. Of the ten species, only two species were found in Malaysia, which are *B. aubertii* (Pancho and Soerjani, 1978; Seopadmo, 1986; Mashhor, 1988; Azmi, 1991; Mohd Zaini and Japar Sidik, 1994; Muta Harah *et al.*, 2005b) and *B. echinosperma* (Pancho and Soerjani, 1978; Soepadmo, 1986). *Blyxa aubertii* are synonyms to eight other species, which are *Blyxa oryzetorum, B. ceylanica, B. griffithii, B. malayana, B. ecaudata, B. muricata, B. graminea* and *B. coreana*. In

Malaysia *B. aubertii* locally known as 'Rumput Lumut' (Pancho and Soerjani, 1978) and 'Santawa bai kao' in Thailand (Naples, 2005).

2.2 General Description of *Blyxa*

Jiang and Kadono (2001) noted that *Blyxa* are glabrous, annual or both annual and perennial (Cook *et al.*, 1974; Cook and Lüönd, 1983) aquatic herbs. Leaves submersed, spirally arranged, radical or cauline or along a 15-60 cm, elongated with no clear differentiation into petiole and blade, up to 10 distinctly midrib with up to 28 tertiary parallel veins, faint cross-veins often present, unicellular spines margin (particularly towards apex). Two squamulae intravaginales at branches or more, triangular to narrowly triangular, white with entire margins. Two spathe unite, conduplicate bracts, tubular, narrowly transversely rhombic in transverse section, two or six nerved, two lobed at the apex, sessile or pedunculate. Elongated stems erect and floating or horizontal and stoloniferous or contracted to a forked or simple rootstock or a simple corm. Roots adventitious, endogenous and unbranched. Flowers unisexual or bisexual, female and hermaphrodite flower sessile, male pedicelled. Sepal three, linear or linear lanceolate, green, persistent. Petals three, linear, longer than sepals, white, flaccid, fringed. Stamens 3-9, one or more rudimentary, filaments capillary. Anther linear or lanceolate, erect, bilocular, latrorsely dehiscent. Pistil three, slender; ovary inferior, linear, slender beaked, three parietal placentae. Styles three, linear, connate at the base. Stigmas three, filiform. Fruit linear or linear lanceolate, wall membranous in the spathe. Seeds 10 or more, elliptic or fusiform, 1-2 mm, testa glabrous, membranous, smooth or with 3-8 longitudinal rows of tubercles or spines (Subramanyam, 1962; Pancho and Soerjani, 1978; Cook and Lüönd, 1983; Kubitzki, 1998). *Blyxa* is known to be quite varied

morphologically (Cook and Lüönd, 1983; Kadono, 1994). Plant size shows enormous variation according to environmental conditions such as water depth and nutrient conditions. Variation within the same population seems to cause by difference in germination time and competition afterwards.

2.2.1 Vegetative Description of *Blyxa aubertii*

Blyxa aubertii (Figure 1) is a submerged rosette plant with sharply pointed narrow lanceolate submerged leaves (Subramanyam, 1962; Keng, 1969; Cook and Lüönd, 1983; Keng *et al.,* 1998). The leaves are bright cellophane green (Randall, 1998), broaden at the middle, sheathing at the base, radical elongated leaves have no clear differentiation into petiole and blade (Subramanyam, 1962; Keng, 1969; Cook *et al.,* 1974; Cook and Lüönd, 1983; Keng *et al.,* 1998; Jiang and Kadono, 2001). Pancho and Soerjani (1978) reported that the plant can reach up to 10-130 cm in height. The leaves length are 2.5-60.0 cm or more and leaves width are 0.2-1.2 cm wide. The leaves connected by thin cross veins that variable in size and shape; total numbers of veins are 5-9, midrib vein usually distinct with 0-10 secondary and up to 28 tertiary parallels veins (Pancho and Soerjani, 1978; Cook and Lüönd, 1983). The leaves margin (Figure 2) are usually finely or minutely or entire serrate with unicellular spines particularly towards apex (Thieret *et al.,* 1969; Cook *et al.,* 1974; Jiang and Kadono, 2001). Blade of uniformed colour from margin to margin, with continuous intercellular space, linear, base grading into sheath, apex acute or bifid or attenuate, midvein without rows of lacunae along sides, abaxial surfacely without prickles or aerenchyma (Thieret *et al.,* 1969). The roots are adventitious, endogenus and unbranched (Cook and Lüönd, 1983).

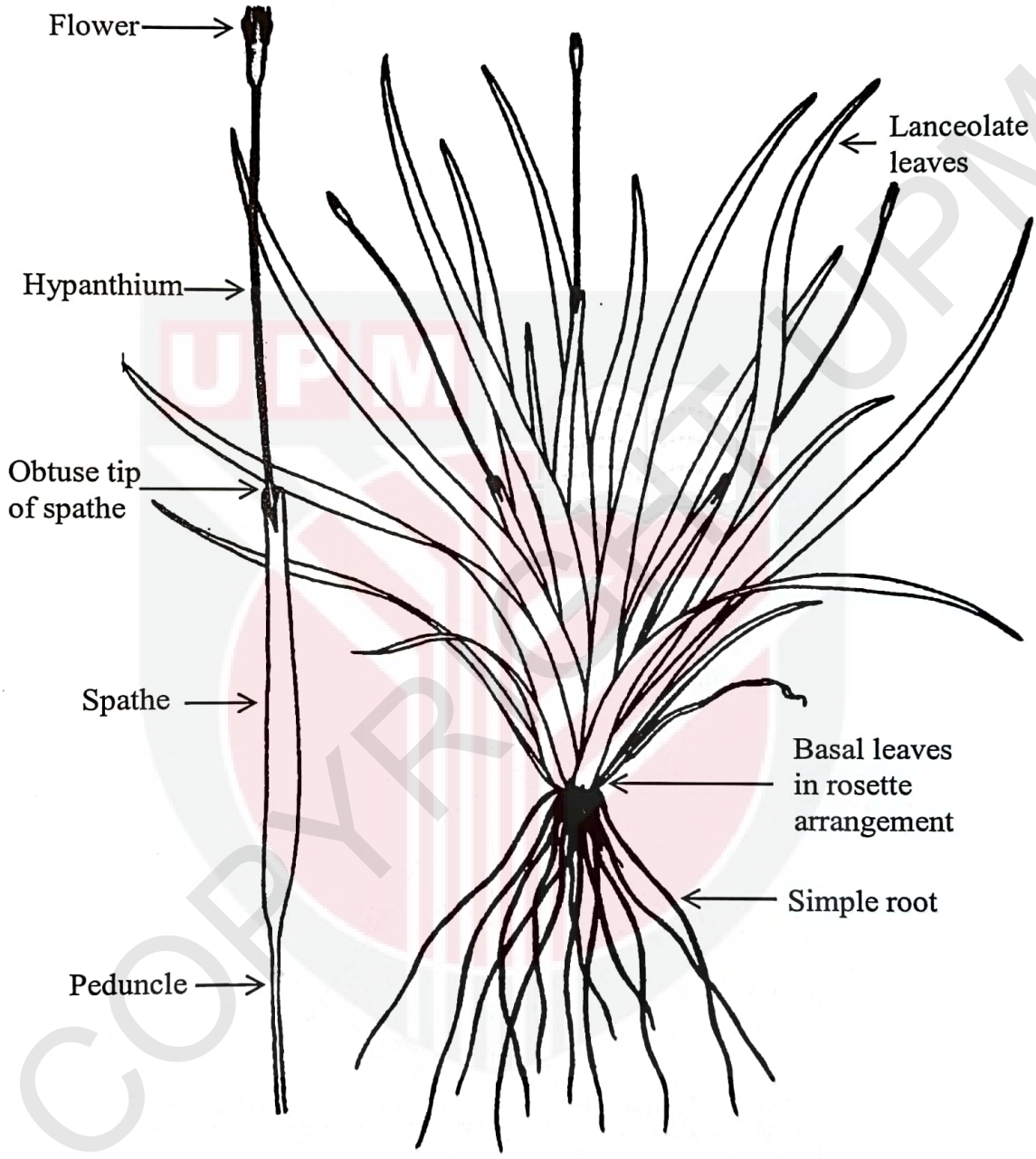

Figure 1: *Blyxa aubertii* habit (modified from Cook *et al.*, 1974)

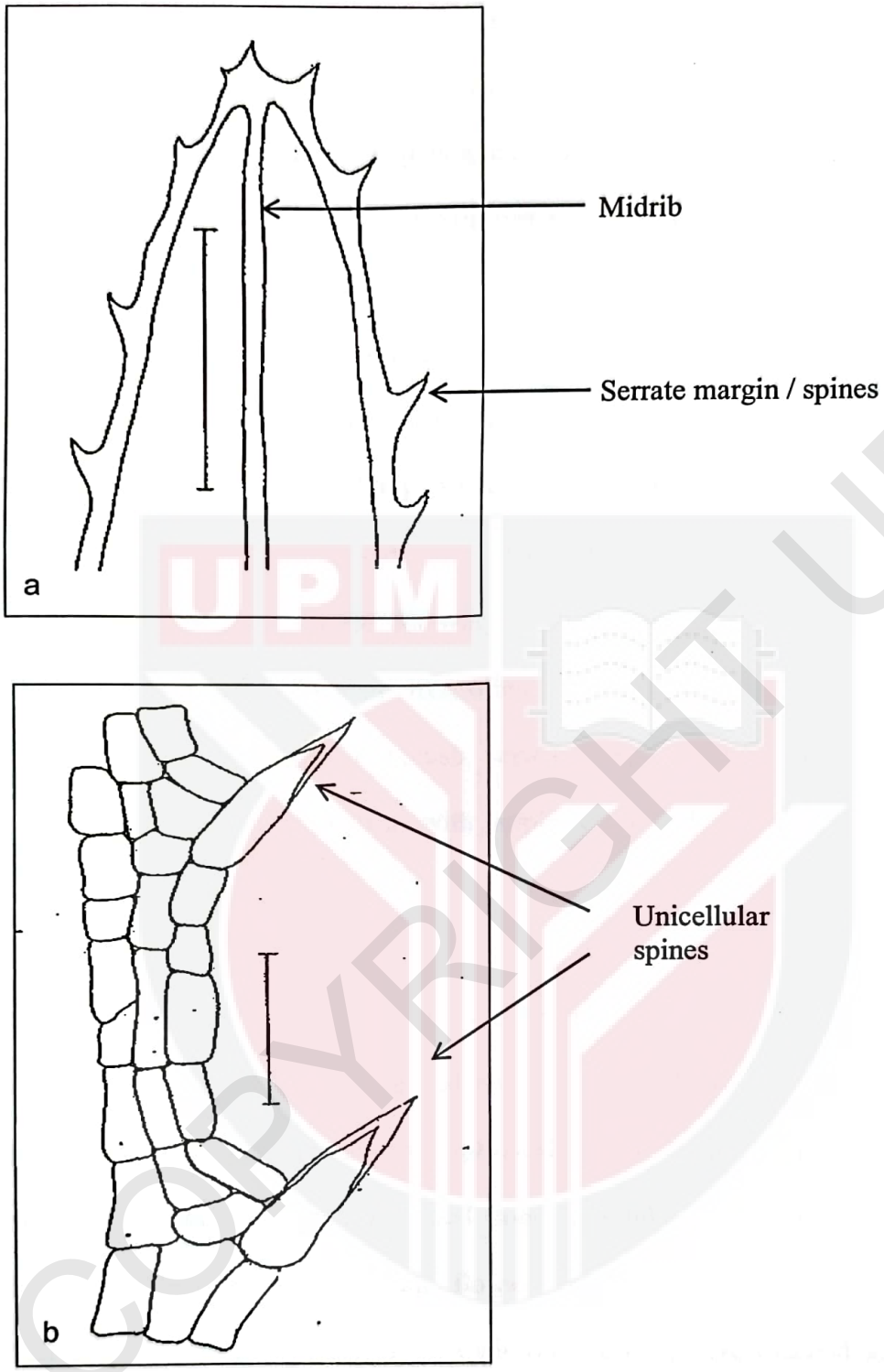

Figure 2: Leaf morphology. a-Leaves margin and midrib of *B. aubertii*, b-Unicellular spines of *Blyxa aubertii* (modified from Cook and Lüönd, 1983)

2.2.2 Reproductive Description of *Blyxa aubertii*

Blyxa aubertii is monoecious (Subramanyam, 1962; Pancho and Soerjani, 1978) or both monoecious and dioecious (Cook *et al.,* 1974) plants produced up to 22 flowers per shoot. The flowers are very small, bisexual, sessile, solitary or rarely paired in the flattened spathe, usually emergent or submerged. The flowers are highly autogamous with the occasional occurrence of submerged cleistogamous and emerged chasmogamous flowers (Pancho and Soerjani, 1978; Cook and Lüönd, 1983; Jiang and Kadono, 2001). According to Kubitzki (1998) the flowers of *B. aubertii* are lack of scent and nectar therefore the plants show extreme sexual dimorphism. The spathes are not winged and have an obtuse tip (Subramanyam, 1962). Petals three, linear, band like, often twisted within the calyx, flaccid, fringed or reduced or absent white to reddish. Sepals three, linear or linear-lanceolate, long, green, persistent and often stripe with purple (Cook and Lüönd, 1983; Cook *et. al.,* 1974).

The male flowers are borne on elongated pedicels, small, regular three numerous, partially acyclic. Stamens three and antesepalous. Filaments are distinct. Anthers latrorse or introrse, bisporangiate, spherical, echinate, in monads, long, fusiform, dehiscing before the stigmas emerged from calyx tube, connective elongation beak-like. The female and bisexual flowers are borne on elongated hypanthia. Superficially resemble those of the male but the petals are reduced to filamentous threads and the stigmas resemble the petals of the male flower in form, colour and position. Pistil is one celled with three placentae, inferior. Stigmas linear, 10-15 mm long, depressed ovate in transverse section, white or reddish. Ovary one locular or as

long as the spathes (or sessile within the spathe) and located inferiorly (Cook and Lüönd, 1983) and distally elongated into the hypanthium. Style one and simple.

The fruits are linear or linear-lanceolate about 3-6 cm long (Subramanyam, 1962) or elongated capsule disintegrating at maturity (Cook and Lüönd, 1983), membranous with 10 to numerous seeds. The seed can be in the elliptic or fusiform with 1-2 mm long and either smooth or spiny (Cook *et. al.,* 1974). The testa has eight and can be up to 12 irregular ribs longitudinal rows of distinctly tubercled ribs, connected by scarious membrane, long spines and apical and or basal tails absent (Subramanyam, 1962; Cook and Lüönd, 1983). Cook and Lüönd (1983) has detailed on variety of *Blyxa aubertii* which each of the variety can be differentiate by their seed morphology as shown in Figure 3.

2.3 Distribution and Habitat

Blyxa aubertii is widely distributed in Korea, Japan, New Guinea, Australia, Madagascar, India through South-East Asia (Indonesia and Malaysia), Africa, America, Taiwan, Queensland (Subramanyam, 1962; Thieret *et al.,* 1969; Cook *et al.,* 1974; Pancho and Soerjani, 1978; Cook and Lüönd, 1983; Jiang and Kadono, 2001) and Singapore (Peter *et al.,* 1991; Keng *et al.,* 1998) (Figure 4). In Malaysia, this plant were recorded in Kemubu, Kelantan, Barat Laut Selangor (Azmi, 1991) and Baloi, Kg. Iran, Taman Tumbina and Nyabau River, Sarawak, East Malaysia (Muta Harah *et al.*, 2005a).

Blyxa aubertii usually gregarious and can be found grow relatively in shallow area that was 20-50 cm depths in paddy fields (Cook *et al.,* 1974; Pancho and Soerjani,

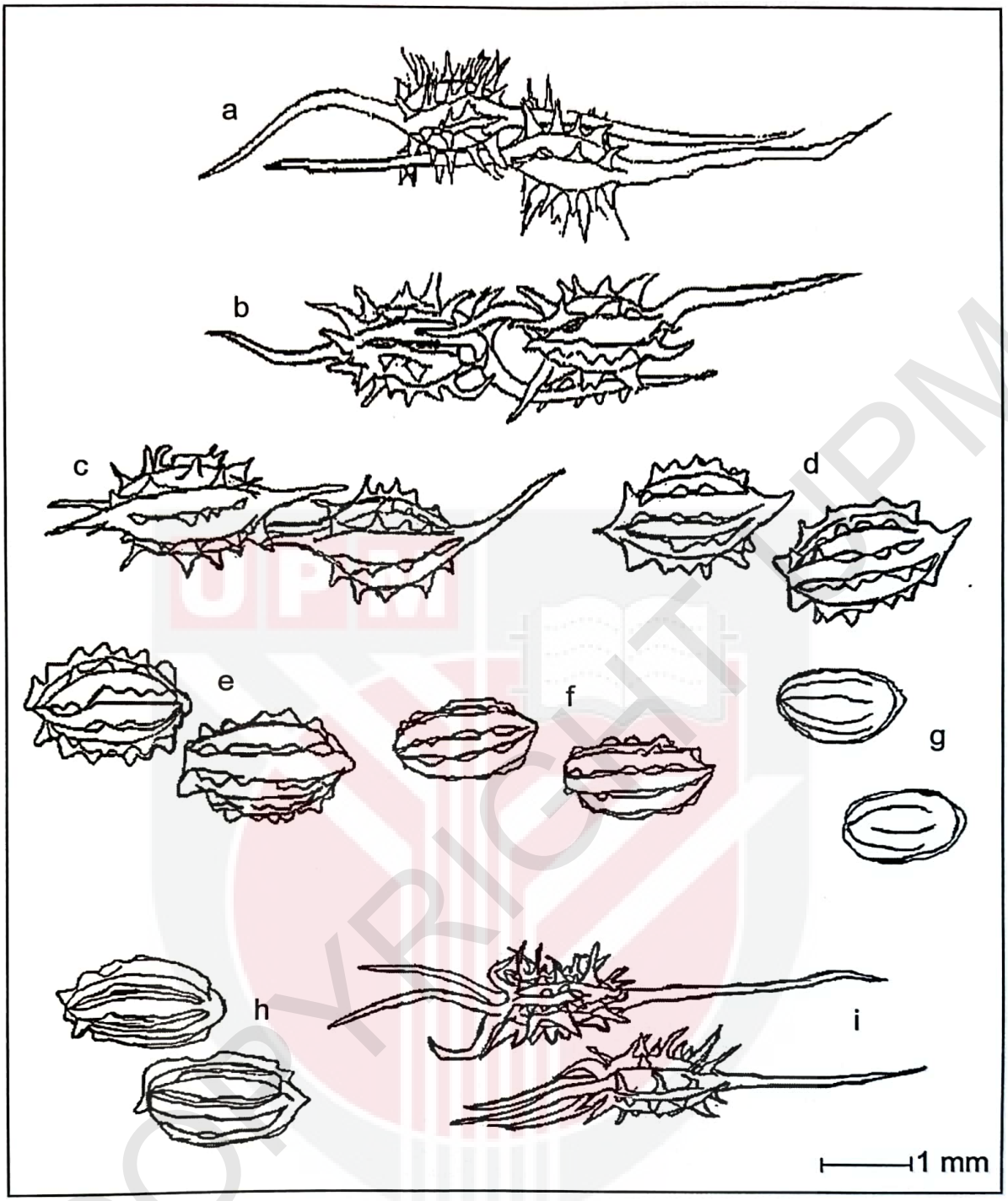

Figure 3: Seeds of *Blyxa aubertii*; a-f collected from India, Kerala, Ernakulam Distr. By Cook, Rix & Schneller, specimens in Z. a-var. *echinosperma*-no. 271 sheet 1; b-var. *echinosperma*-no. 293; c-var. *echinosperma*-, 'short-tailed'-no.189; d-intermediate-no.277; e-var. *aubertii*-no.240; f-var. *aubertii*-no.278A; g-var. *aubertii*, 'almost smooth'- Papua, New Guinea, Derbyshire 988 (L); h-var *aubertii*, 'ridged', Indonesia, Java, Backer 26449 (L); i-var. *echinosperma* 'multi-tailed' Philippines, Merrill 1244 (P); scale 1 mm. (source modified from Cook and Lüönd, 1983)

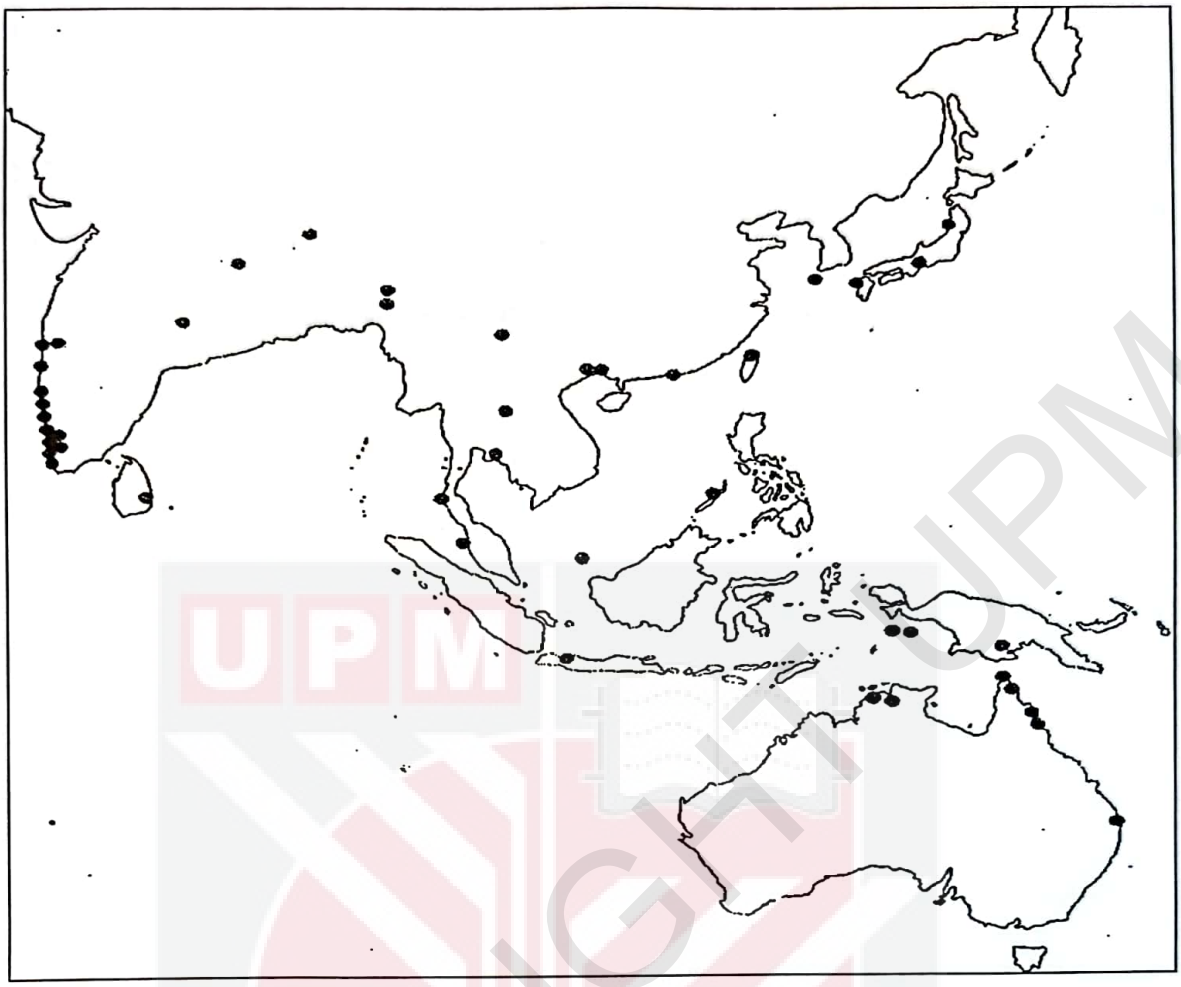

Figure 4: *Blyxa aubertii* distribution (Source from Cook and Lüönd, 1983)

1978; Cook and Lüönd, 1983; Azmi, 1991; Jiang and Kadono, 2001), irrigation ponds, irrigation ditches (Cook *et al.*, 1974; Pancho and Soerjani, 1978; Jacobsen, 1979; Soerjani *et al.*, 1987; Jiang and Kadono, 2001), pools, lakes (Subramanyam, 1962; Thieret *et al.,* 1969; Pancho and Soerjani, 1978; Cook and Lüönd, 1983; Jiang and Kadono, 2001), ponds, ditches (Keng *et al.,* 1998; Muta Harah e*t al.*, 2005a), marshes, streams, rivers, creeks and billabongs (Pancho and Soerjani, 1978; Yang, 1978; Mashhor, 1988; Azmi, 1991; Mohd Zaini and Japar Sidik, 1994; Keng *et al.,* 1998; Cowie *et al.*, 2000; Muta Harah *et al.*, 2005a). It grows well in soft acidic water with a nutrient rich substrate and under strong light (Randall, 1998).

2.4 Propagation

Virtually all the major flowering plant breeding systems are represented among aquatic plants, including the various forms of dicliny self-incompatibility autogamy and agamospermy. These are often combined with a wide range of methods for clonal propagation including rhizomes, runners, stolons, tubers and turions. Most of the pollination systems observed in terrestrial plants involving both winds (Cook, 1988) and insect vectors (Philbrick and Crow, 1992) are found in aquatic plants. In addition, many submerged aquatic plants exhibit hydrophily, which is a unique pollination system to aquatic plants (Cox, 1988; Philbrick, 1991). Hydrophily is subdivided into epihydrophily (on the water surface) and hypohydrophily (below the water surface) mentioned by Pettit (1984), Cox (1988), Les (1988) and Philbrick (1991).

Clonal diversity within and among populations of aquatic macrophytes are often low because recruitment occurs predominantly through vegetative reproduction (Les,

1988; Aspinwall and Christian, 1992; Laushman, 1993; Barrett *et al.,* 1993; Lokker *et al.*, 1994; Philbrick and Les, 1996; Wang *et al.*, 2005). Several structures and mechanisms of vegetative propagation in plants including (a) shoot fragments, (b) modified buds: turions, pseudoviviparous buds and gemmiparous buds, (c) modified stems: layers, runners, stolons, rhizomes and tubers, (d) modified shoot bases: bulbs and corms, (e) modified roots: creeping roots and taproots.

There are five mechanisms of reproductive propagation including (a) insect pollination or entomophily, (b) wind pollination or anemophily, (c) water pollination or hydrophily, (d) adaptations of hydrophillous pollen, (e) adaptations of hydrophilous stigmas (Rebeccae and Cyndie, 2003).

Tanaka (2000) and Cook (1982) have stated that *Blyxa* were in entomophily group which mean that the flowers bloom above the water surface and are pollinated by insects. However, Subramanyam (1962) and Jacobsen (1979) reported that *Blyxa aubertii* often do self-pollination, division of rootstock and seed production.

2.5 Importance of Aquatic Macrophytes

Many aquatic vertebrates and invertebrates utilize aquatic macrophytes for food and habitat protection. In fact, the metabolism of many flowing and limnetic ecosystems is dominated by photosynthesis and respiration of aquatic macrophytes, which are at the base of the detritus food web. Exchange of nutrients among the compartments of the sediment-plant-water system and calcium carbonate precipitation in plant beds can have striking effects on the water chemistry of aquatic ecosystems (Graham and Mark, 1980).

Aquatic and terrestrial macrophytes along with algae are essential components of ecosystems. Macrophytes are becoming more important for the monitoring and assessment of herbicides, effluents and industrial chemicals (Wang and Freemark, 1995). They produced oxygen and organic substances on which most other life forms depends (Benenati, 1990). Submerged macrophytes in the littoral zones in the Lake Pontchartrain estuary serve important nursery functions and contrast sharply with the simple soft bottoms that dominate much of the littoral and deeper portions of the system. The structural complexity of submerged aquatic macrophytes offers protection from predation, especially for recruiting fishes (Carr, 1994), and in some cases provides an increased availability of food resources, especially for smaller organisms (Boesch and Turner, 1984; Rozas and Odum, 1988). Macrophytes are beneficial to lake because they provide food and settler for fishes and aquatic invertebrates. They are considered as important components of the aquatic ecosystem not only as food source for aquatic invertebrates (Muta Harah *et al.*, 2005a, 2005b), but also as an efficient accumulator of heavy metals (Bianchini *et al.*, 2006). In Malaysia, more than 15 species are edible, 30 species from 16 families are claimed to have medicinal value. The aquatic macrophytes also considered as fertilizer, ornamentals and as raw materials for various types of handicrafts (Cheksum, 1998).

In Peninsular Malaysia *B. aubertii* are recognized as weed in the paddy field (Mashhor, 1988; Azmi, 1991). However, in Sarawak this species commonly consumed as vegetables or cooked (Muta Harah *et al.*, 2005a). These plants are also cultured as ornamental plants in aquarium and recreation area.

CHAPTER 3

METHODOLOGY

3.1 Sampling Sites

Fresh samples of *Blyxa aubertii* were collected from four locations; UPMKB ditches (N 3° 12' 23.8-34.1" E 113° 05' 0.4-5.7"), Maradong ditches (N 2° 07' 15.0" E 111° 39' 7.4), Baloi marsh (N 3° 07' 43.6" E 113° 02' 47.8") and 19km golf field, Sibu (N 2° 17' 29.5" E 111° 56' 6.2") (Figure 5, Plate 1).

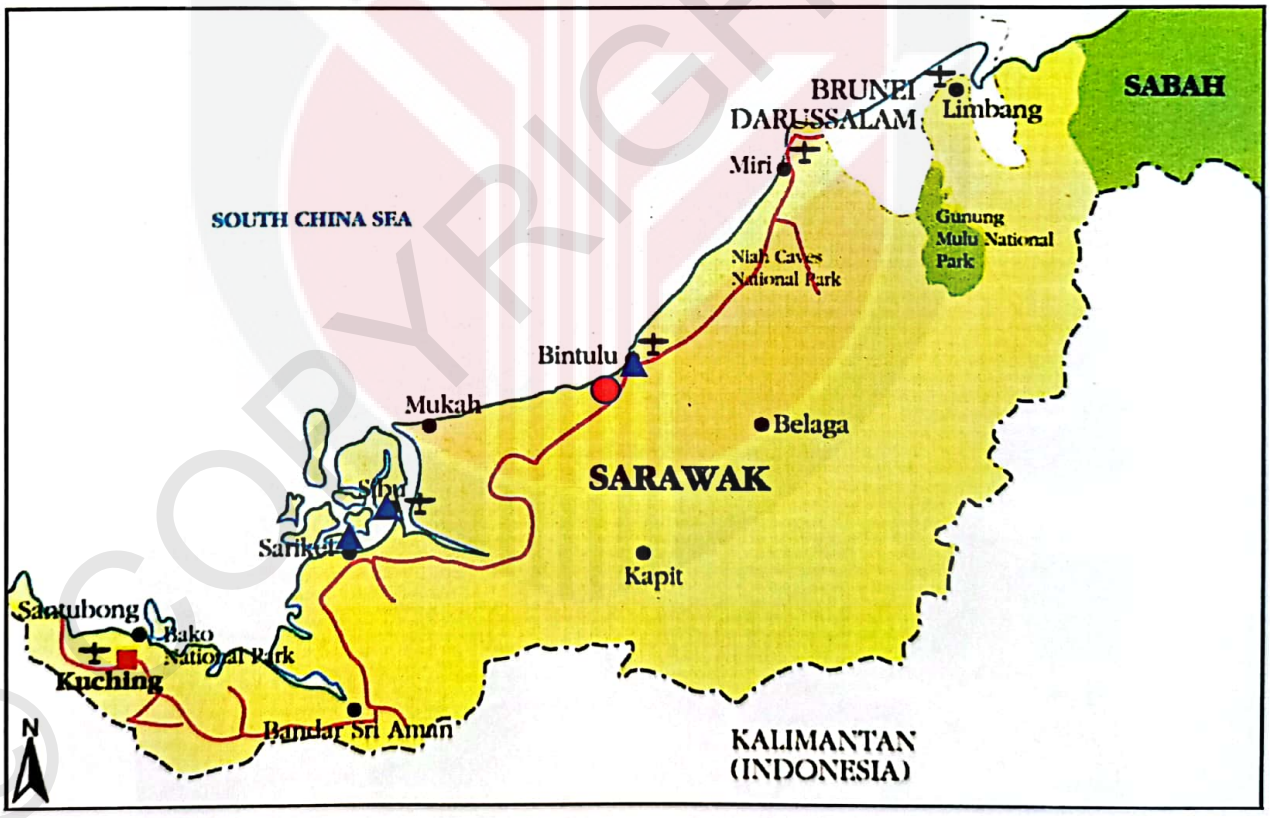

Figure 5: ▲ Sampling site ● Herbarium specimen source

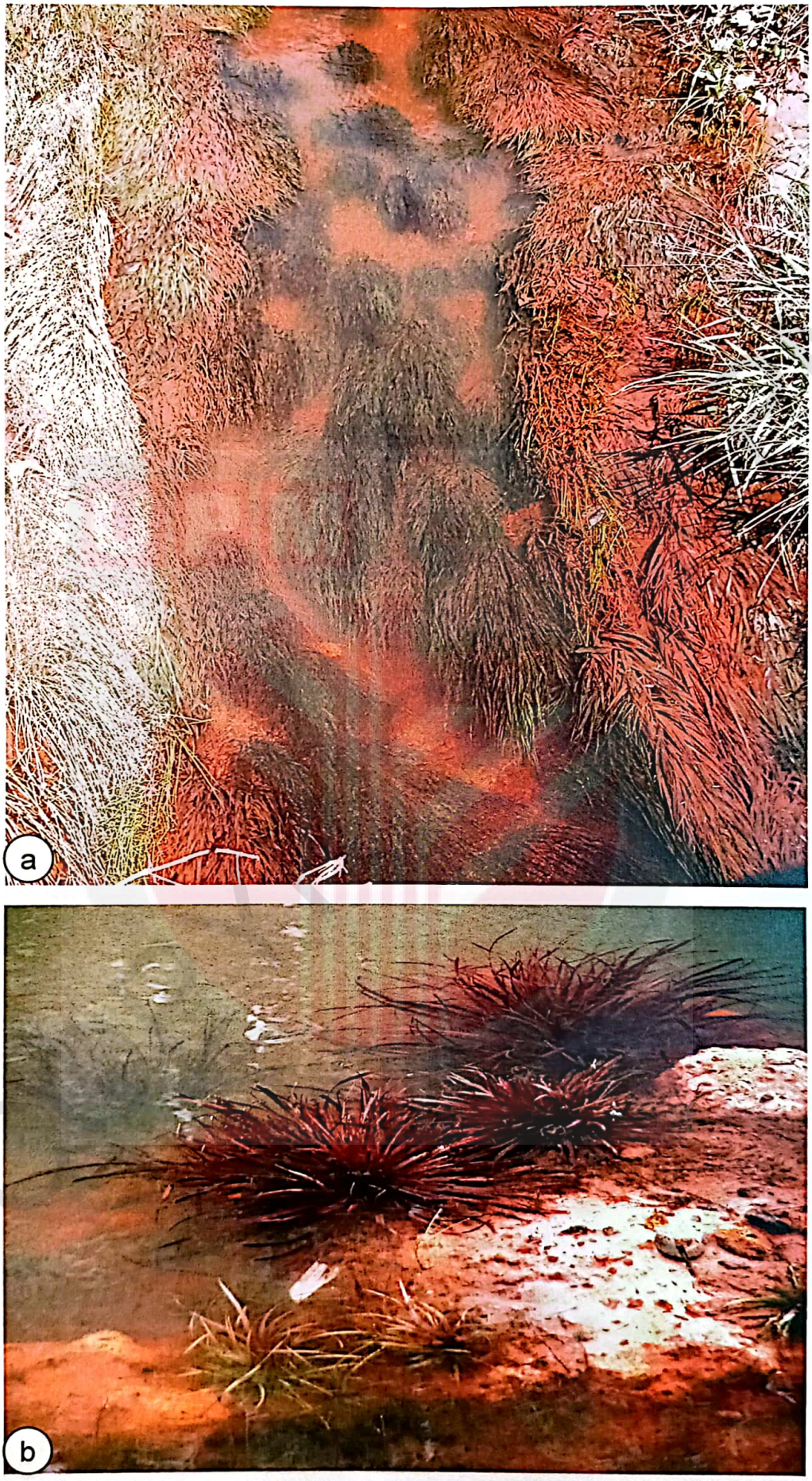

Plate 1: *Blyxa aubertii* was found growing submerged in natural and man-made water bodies. a-dense of *B. aubertii* at UPMKB ditches, b-patches of *B. aubertii* at Baloi marsh

3.2 Field Collections

Fresh samples of *Blyxa aubertii* were collected from the sites early in the morning or in the evening to reduced stress. Specimens with vegetative and reproductive parts were carefully gathered complete with the substrate culturing in the laboratory. Samples were washed before placed in the plastic bags to further observation in the laboratory. Fruits were collected from matured plants for seed germination study. Habitat types and environment conditions particularly water pH, water depth, dissolved oxygen (DO) and water temperature were measured using HANNA pH meter and YSI 200 DO meter for each collection site. Photographs were taken for each site for records.

3.3 Preservation and Herbarium

In the laboratory, samples were washed to removed debris and mud using running water. The specimens was arranged neatly on the paper and sprayed with 5% formaline solution until wet. The specimens then covered with a piece of newspaper layer by layer. A cardboard were put on top of the layers and all specimens were then press and tightened with string. Specimens were dried in the air circulation oven (35-37°C) for about seven to fourteen days. After seven days, specimens were take out and left overnight at room temperature. The samples then mounted on the herbarium paper. The samples then were carried out for morphological analysis. The herbariums were kept in the dark to prevent damages and discoloration modified method from Menez *et al.* (1983).

3.4 Morphological Study

For vegetative characteristics; leaf length and leaf width were measured using Mitutoyo Vernier Caliper, number of leaves per shoot were count and number of vein were count under compound microscope. Reproductive characteristic; female flower (ovary, fruit and stigma), spathe and male flower (sepals, petals, anthers and filaments) were measured and observed under compound microscope and Zeiss Dissecting Micro Stemi SVII, peduncle length, hypanthium length and roots were measured using Mitutoyo Vernier Calliper. Seed length and width were measured under Zeiss Disecting Micro Stemi SVII microscope.

3.4.1 Clearing Technique

Leaf cell surface morphology was conducted by using clearing technique for different parts of the leaves (leaf tip, leaf vein and leaf margin). The leaves (1 cm^2) were cut carefully and dip in 5-8% formaline solution. After that, samples were put in 15% Sodium hydroxide (NaOH) solution for 24 hours before washed with distil water for two minutes. Then samples were put in 15% Clorox for ten minutes to remove the green colour. Then samples were dip in safranin solution for one minutes and washed with distil water (until the red colour removed). The samples undergo series of alcohol (50%, 70%, 80%, 90%, and 95%) in two minutes each, then in 100% alcohol solution for 10 minutes and clove oil for 10 minutes. Samples were put on the slide, added with DePeX (DPX) mountant, cover with slide cover and then dried up overnight (Johnstone and Hudson, 1981).

3.4.2 Nail Polish

The leaves (1 cm^2) were cut carefully. The samples were polished using red and transparent colorant. Then, the samples were dried up for one minute at room temperature before stripe out the layers using cellophane tape. The layers then were put on a slide and observed under compound and dissecting microscope.

3.4.3 Anatomical Study

The leaf parts (leaf tip and middle leaf) of *Blyxa aubertii* were processed for anatomical studies. The methods for preparing the slides followed the procedures given by O'Brien and McCully (1981) and Cutler (1978). Sequences of the procedures were:

1. Fixation
2. Dehydration
3. Waxing
4. Embedding
5. Sectioning
6. Staining
7. Mounting
8. Observed under light microscope

The time element has been modified in dehydration and staining step based on the sample studies.

3.4.3.1 Fixation

The sample was cut horizontally (cross-section) using a sharp razor blade with minimum bruising or compression in formal acetic alcohol (FAA) solution. The cut tissues were then soaked promptly into separated vials (well labelling) containing 70% FAA solution at room temperature for overnight.

3.4.3.2 Dehydration

The cut tissues were transfer into pure tert-butyl alcohol (TBA) and then placed them in the room temperature for overnight.

3.4.3.3 Waxing

The paraffin wax solution was added into the vials that contained TBA solution in portion 1:1. The vials were placed in an oven at 60-62°C for 24 hours.

3.4.3.4 Embedding

Tissues were embedded in wax that served to hold and support the tissues against the impact of blade when sectioning. The tissues were embedded in mould, aluminium foil manually. A heated forceps was soaked into the aluminium mould containing wax and gently stirred to ensure that air bubbles do not remain in the wax-block. Tissues were removed into the wax in the aluminium mould using a heated forceps and placed in proper position. Aluminium mould was then labelled. It was then left to freeze overnight.

3.4.3.5 Sectioning

The embedded tissues were sectioned manually using sharp razor blade. The section formed a continuous ribbon. The ribbons were cut and placed on the slides that had two drops of 8% formalin. Then the slides were put on the heater at 60°C to flatten out the wrinkles in the wax and were taken off from the heater after the ribbons have stretches out and set aside for few minutes until the solutions cool and surplus formalin solutions was drained off. After dried, the slides were kept away from dust in oven 60°C for two weeks.

3.4.3.6 Staining

Stains used in the staining process were safranin and fast green. The slides were taken out slowly and transferred into Petri dish using forcep. A series of solutions and timing are shown below:

1. Xylene-3 minutes (to remove wax)
2. Xylene: Alcohol-3 minutes
3. 95% alcohol-2 minutes
4. 70% alcohol-2 minutes
5. 50% alcohol-2 minutes
6. Distilled water-dip
7. Safranin-dip
8. Tap water-until become clear
9. 50% alcohol-dip
10. 70% alcohol-dip
11. 95% alcohol-dip

12. fast green-dip

13. Xylene: Alcohol-dip

14. Xylene-1 minute

3.4.3.7 Mounting

A drop of DPX was put onto the slide and covered with cover slip before left for 24 hours to dry. Finally, the slides were then labelled.

3.5 Propagation

3.5.1 Seed Germination

Matured seeds collected from UPMKB ditches were spread in the tray contained natural substrate (Plate 2a) and tissue medium (Plate 2b) in the laboratory. The seed germination and stages of progress developments of *B. aubertii* seedlings were recorded and photographs were taken.

3.5.2 Vegetative Propagation

The culturing of plants samples from UPMKB ditches were conducted in the laboratory. Six aquarium tanks were filled with artificial substrate, as shown in the Plate 3. The artificial substrates are made from; (1) Stones 6-7 cm height (2) Gravel 2-3 cm height. Four *Blyxa aubertii* were planted in aquarium tank with 10-15 cm height of water from natural habitat and aeration were given using bubble tube. Any loss of water due to evaporation was maintained by addition of natural sources of water. All aquarium tanks were place near the window for full access to the light. Observations on leaf production, flowering and fruiting of the plants were recorded. The algae growths were removed once it appears. The images were taken using

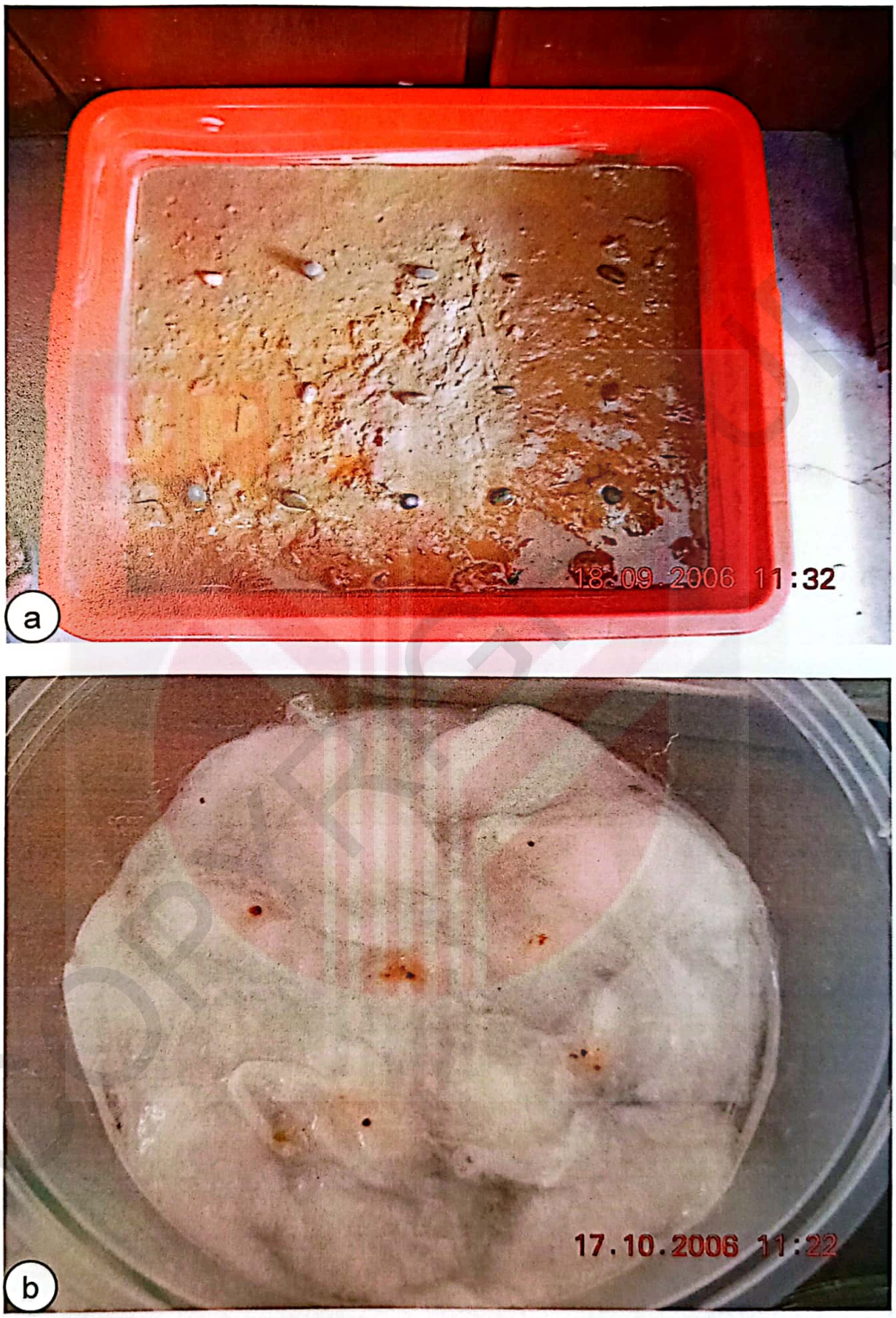

Plate 2: Seed germination medium. a-soil from natural habitat, b-tissue medium

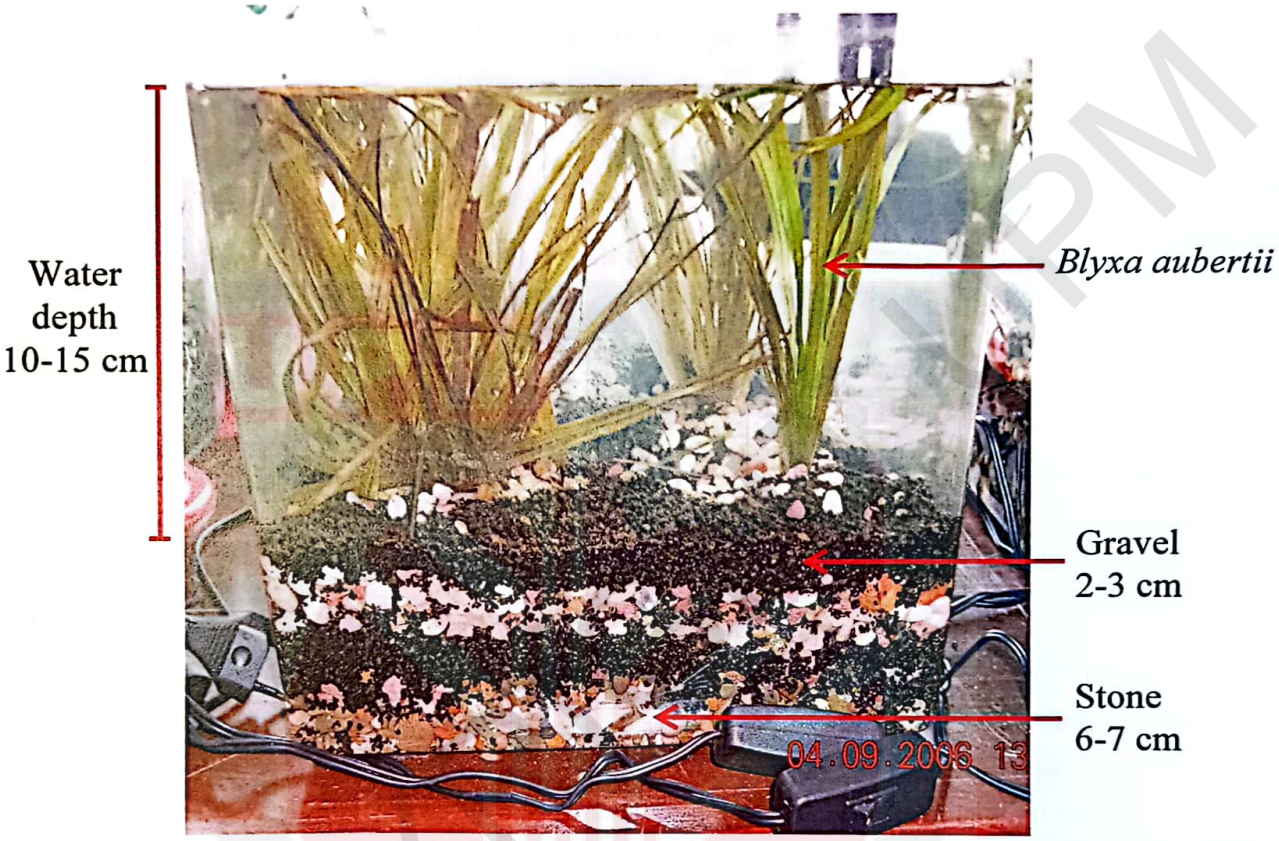

Plate 3: *Blyxa aubertii* culture in aquarium tank with artificial substrate in the laboratory

digital camera to access their morphologies.

3.6 Statistical Analysis

The vegetative measurement data: leaf length, leaf width, number of leaf per shoot, number of veins, root length for the specimens from four locations were compared by ANOVA test using SPSS 14.0 to evaluate hypothesis.

Ho = the means of the plant are all the same for different location

When the Ho is rejected, the Post Hoc Tests was conducted to find out the difference between specimens from different location (UPMKB, Baloi, Maradong and 19 km golf field, Sibu). The reproductive measurement data for specimens from UPMKB were analyzed to test the significant variance (t-test, $p<0.05$).

CHAPTER 4

RESULTS

4.1 Ecology of *Blyxa aubertii*

Blyxa aubertii was found growing abundantly in moving and stagnant water either in the natural or man made water bodies. The plant grew in ditches at UPMKB, Bintulu and Maradong, Sarikei; marshes at Baloi, Bintulu and 19 km golf field, Sibu. Plants were growing on various habitats submerged at depths of 0.1-1.20 m, water pH 5-6.6, temperature 26.4-27.9°C and dissolved oxygen 0.5-3.57 mg/L. The details of the habitat characteristics of *B. aubertii* are summarized in Table 1.

Blyxa aubertii was found associated with other aquatic macrophytes such as *Eleocharis* sp., *Limnocharis flava* (Plate 4), *Ludwigia hyssopifolia* and *Commelina diffusa* at UPMKB ditches. Those found in clear water UPMKB ditches form a higher number of leaves 7-800 per shoot and flowers (Plate 5a) while plants grown at marsh 19 km golf field, Sibu and Maradong ditches habitat were comparatively lower 8-32 leaves per shoot (Plate 5b).

In Maradong ditches this species was found associated with *Eleocharis* sp., *Fimbristylis* sp. and freshwater algae. In Baloi marsh, *B. aubertii* growing submerged at depths of 0.45-1.20 m associated with *Hydrilla verticilata, Nymphaea nouchali* and *Utricularia aurea* while at 19 km golf field, Sibu marsh *B. aubertii* is growing at shallow area at depths of 0.1-0.50 m mixed with *Utricularia bifida, Eriocaulon truncatum, Nymphea nouchali* and *Eleocharis* sp.

Table 1: Locations and habitat characteristics of *Blyxa aubertii*

No.	Location	Latitude N and Longitude E	Form and Association	Depth range (m)	Water pH	Water Temp. (°C)	Dissolved Oxygen (mg/L)
Ditches							
1.	UPMKB, Bintulu	3° 12' 34.1" N 113° 05' 0.4" E	Mixed with *Limnocharis flava, Eleocharis* sp., *Ludwigia hyssopifolia* and *Commelina diffusa*	0.35-1.05	5.0-6.6	22.9-27.9	0.55
2.	Maradong, Sarikei	2° 07' 16.0" N 111° 39' 10.4" E	Mixed with freshwater algae, *Fimbristylis* sp. and *Eleocharis* sp.	0.20-0.30	7.0	32.13	2.77
Marsh							
3.	Baloi, Bintulu	3° 07' 43.6" N 113° 02' 47.8" E	Mixed with *Hydrilla verticilata, Nymphaea nouchali* and *Utricularia aurea*	0.45-1.20	6.6	26.4	3.15
4.	19 km golf field, Sibu	2° 17' 29.5" N 111° 56' 36.2" E	*Nymphea nouchali, Utricularia bifida, Eleocharis* sp. and *Eriocaulon truncatum*	0.10 - 0.50	5.17	30.1	3.73

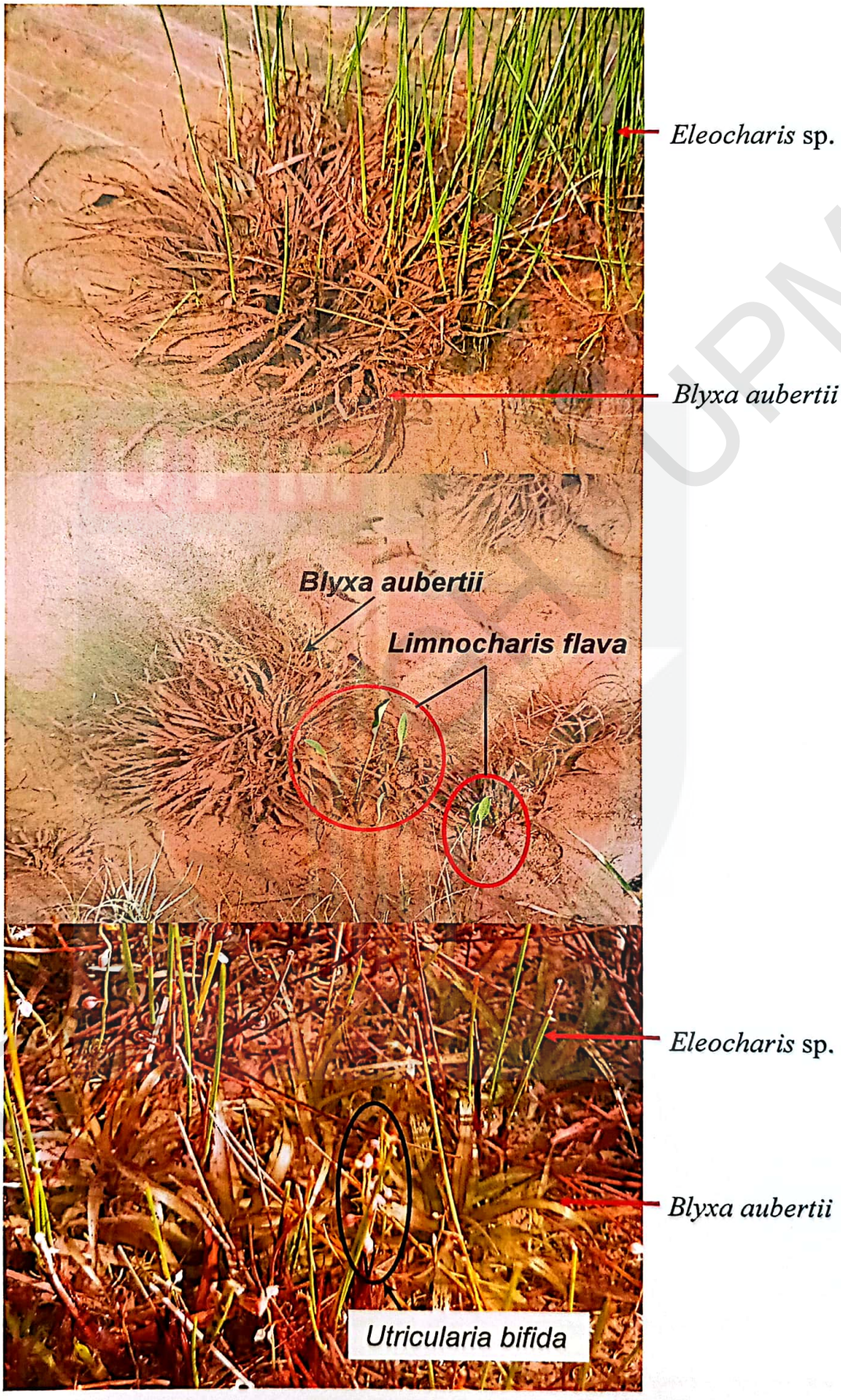

Plate 4: *Blyxa aubertii* was found submerged associated with other aquatic macrophytes such as *Eleocharis* sp., *Limnocharis flava* and *Utricularia bifida*

Plate 5: *Blyxa aubertii* was found grows up to 800 leaves per shoot in slow moving water of UPMKB ditches (a) compare with 8-32 leaves per shoot at (b) Baloi, Meradong and 19 km golf field, Sibu

4.2 Description on Vegetative Structure of *Blyxa aubertii*

The vegetative morphology of *Blyxa aubertii* from UPMKB ditches and Baloi marsh, Bintulu, Maradong ditches, Sarikei and 19 km golf field marsh, Sibu were generally similar. Illustration on the vegetative and reproductive morphology of *Blyxa aubertii* is presented in Plate 6. Dimension analysis (Table 2, ANOVA, DMRT, $p<0.05$) indicated that noticeable differences in vegetative structures are observed between the different locations.

4.2.1 Leaves

The leaves are green lanceolate arranged in a rosette (Plate 7) and rise from the rootstock or a simple corm. The leaves have no clear differentiation into petiole and blade (Plate 8), obligately submerged. *Blyxa aubertii* leaves may vary from few to numerous depends on locations. *Blyxa aubertii* from UPMKB ditches have significantly different with the highest 7-800 leaves per shoot when compared to 19 km golf field, Sibu marsh 6-32 leaves, Maradong ditches 8-27 leaves and Baloi marsh 10-23 leaves (Table 2).

The leaves lengths were varied per shoot and per location. The longest leaves were obtained from Baloi with 26.90±8.43 cm (4.76-59.72 cm) followed by Maradong with 15.18±3.73 cm (0.65-26.43 cm), UPMKB with 12.38±3.19 cm (3.42-29.90 cm) and the lowest from Sibu with 10.67±1.73 cm (2.45-23.25 cm). The leaf width of *B. aubertii* UPMKB were similar to Maradong with mean 0.48±0.09 cm (0.15–0.82 cm) and 0.48±0.12 cm (0.16–1.05 cm) respectively. However, these leaves width significantly different when compared with Baloi 0.68±0.12 cm (0.10–1.09 cm) and 19 km golf field, Sibu 0.35±0.04 cm (0.40–0.63 cm).

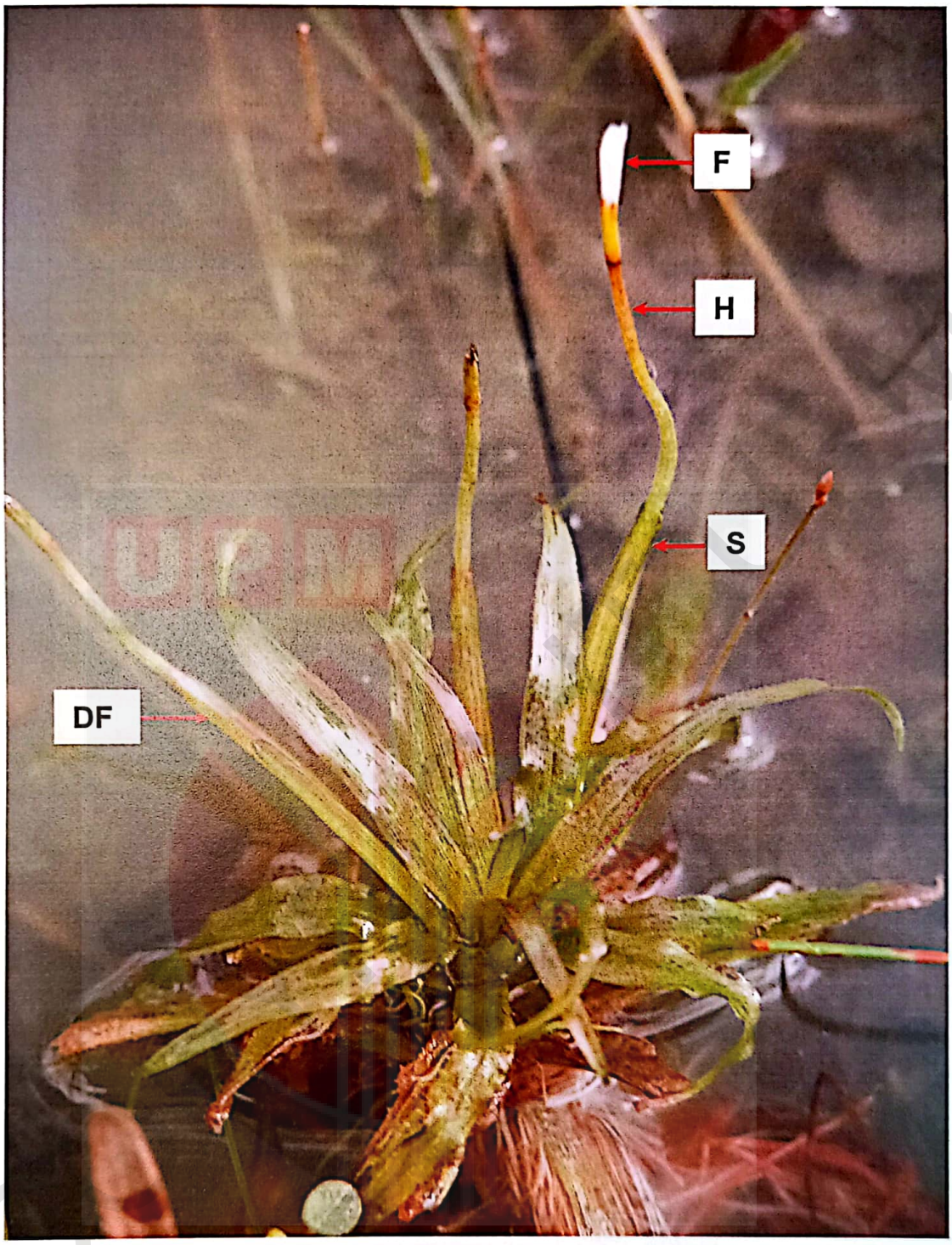

Plate 6: *Blyxa aubertii* submerged rosette plant, green in colour with hermaphrodite flower (F-flower, H-hypanthium, S-spathe, DF-developing fruit)

Table 2: Summary of dimension analysis (mean ± s.e) of vegetative structure of *Blyxa aubertii* at four sites, UPMKB, Baloi, Maradong and Sibu

	Parameter	N	UPMKB	N	Baloi	N	Maradong	N	Sibu
	Vegetative structure								
1.	number of leaf per shoot	31	115.16 ± 39.39^{a} (7–800)	10	15.30 ± 1.17^{b} (10–23)	14	17.14 ± 1.79^{b} (8–27)	42	15.17 ± 0.92^{b} (6–32)
2.	leaf length (cm)	251	12.38 ± 3.19^{c} (3.42–29.90)	153	26.90 ± 8.43^{a} (4.76–59.72)	240	15.18 ± 3.73^{b} (0.65–26.43)	637	10.67 ± 1.73^{d} (2.45–23.25)
3.	leaf width (cm)	251	0.48 ± 0.09^{b} (0.15–0.82)	153	0.68 ± 0.12^{a} (0.10–1.09)	240	0.48 ± 0.12^{b} (0.16–1.05)	637	0.35 ± 0.04^{c} (0.40–0.63)
4.	no of leaf vein	56	24.39 ± 0.88^{a} (12–48)	50	21.78 ± 1.01^{b} (2–31)	56	23.41 ± 0.75^{ab} (12–38)	80	22.09 ± 0.57^{b} (12–38)
5.	root length (cm)	19	6.41 ± 7.00^{b} (2.04–14.81)	10	12.31 ± 21.73^{a} (4.00–24.72)	11	13.15 ± 17.74^{a} (1.48–20.31)	42	7.66 ± 5.6^{b} (2.94–20.02)

Note: Different letters indicate significant differences at $p<0.05$ (ANOVA following Post-Hoc Tests, Duncan Multiple Range Test). i.e, a>b>c>d, ns-not significant. Values in the parentheses are the range

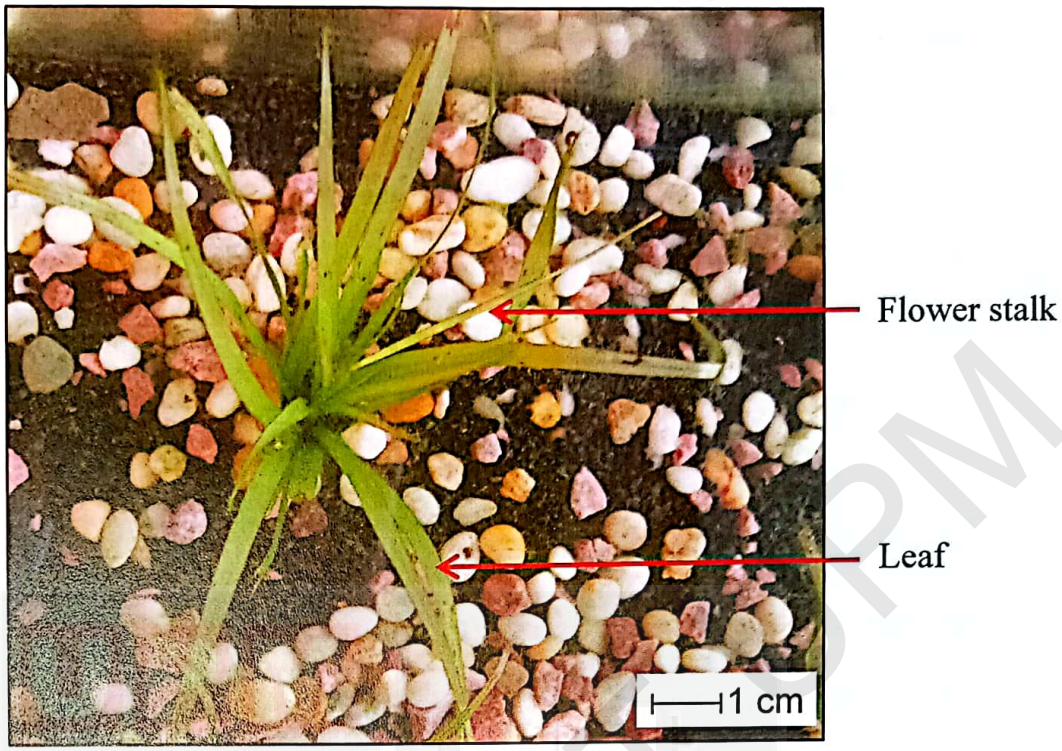

Plate 7: *Blyxa aubertii* leaves arranged in a rosette

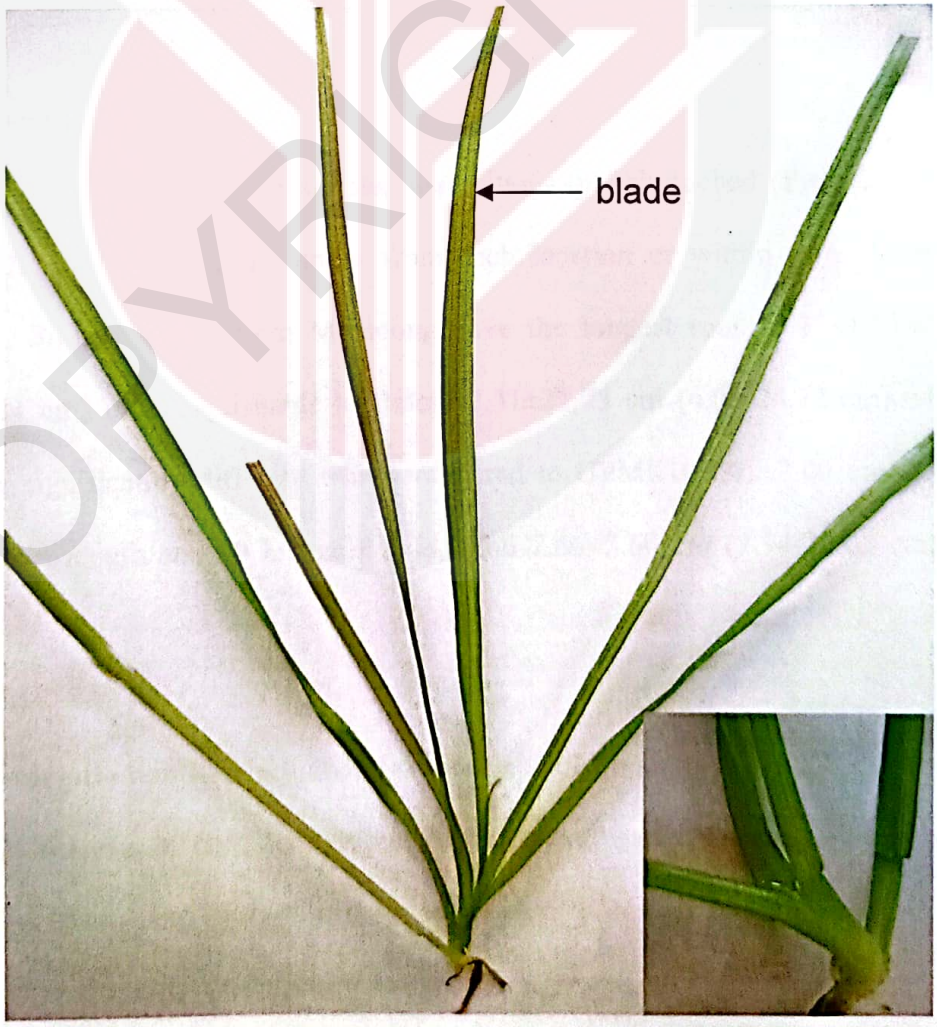

Plate 8 : *Blyxa aubertii* leaves are undifferentiated into petiole and blade

The leaves of *B. aubertii* show the numerous numbers of leaf veins and interconnecting nerves (Plate 9a). *Blyxa aubertii* from UPMKB have 12–48 leaf veins similar to Maradong 12–38 leaf veins while significantly different from Baloi and Sibu with 2–31 leaf veins and 12–38 leaf veins correspondingly. The leaves margins were minutely or serrated with unicellular spines (Plate 9b). The leaf cells are rectangular, without stomata, full with chlorophyll pigments and air vacuole (Plates 9c, 9d).

The anatomy of the leaf is uniform (Plate 10). A single layer of epidermal cell lies above parenchyma cell without stomata. Many large air spaces are present in the arenchyma and the air spaces are segmented by frequent septa. The vascular bundle is surrounded with 4-5 vascular tissues.

4.2.2 Roots

Blyxa aubertii roots are brown simple, adventitious or unbranched (Plate 11). The root length varied among specimens from each location or within same location (Table 2). *Blyxa aubertii* from Maradong have the longest root 13.15±17.74 cm (1.48-20.31 cm) indistinguishable to Baloi 12.31±21.73 cm (4.00-24.72 cm) while both were significantly different when compared to UPMKB 6.41±7.00 cm (2.04-14.81 cm) in length and 19 km golf field, Sibu 7.66±5.66 cm (2.94-20.02 cm) in length.

4.3 Description on Reproductive Structure of *Blyxa aubertii*

Dimension analysis of reproductive structure of *Blyxa aubertii* from UPMKB was measured in this present study. The matured plants of *Blyxa aubertii* produced

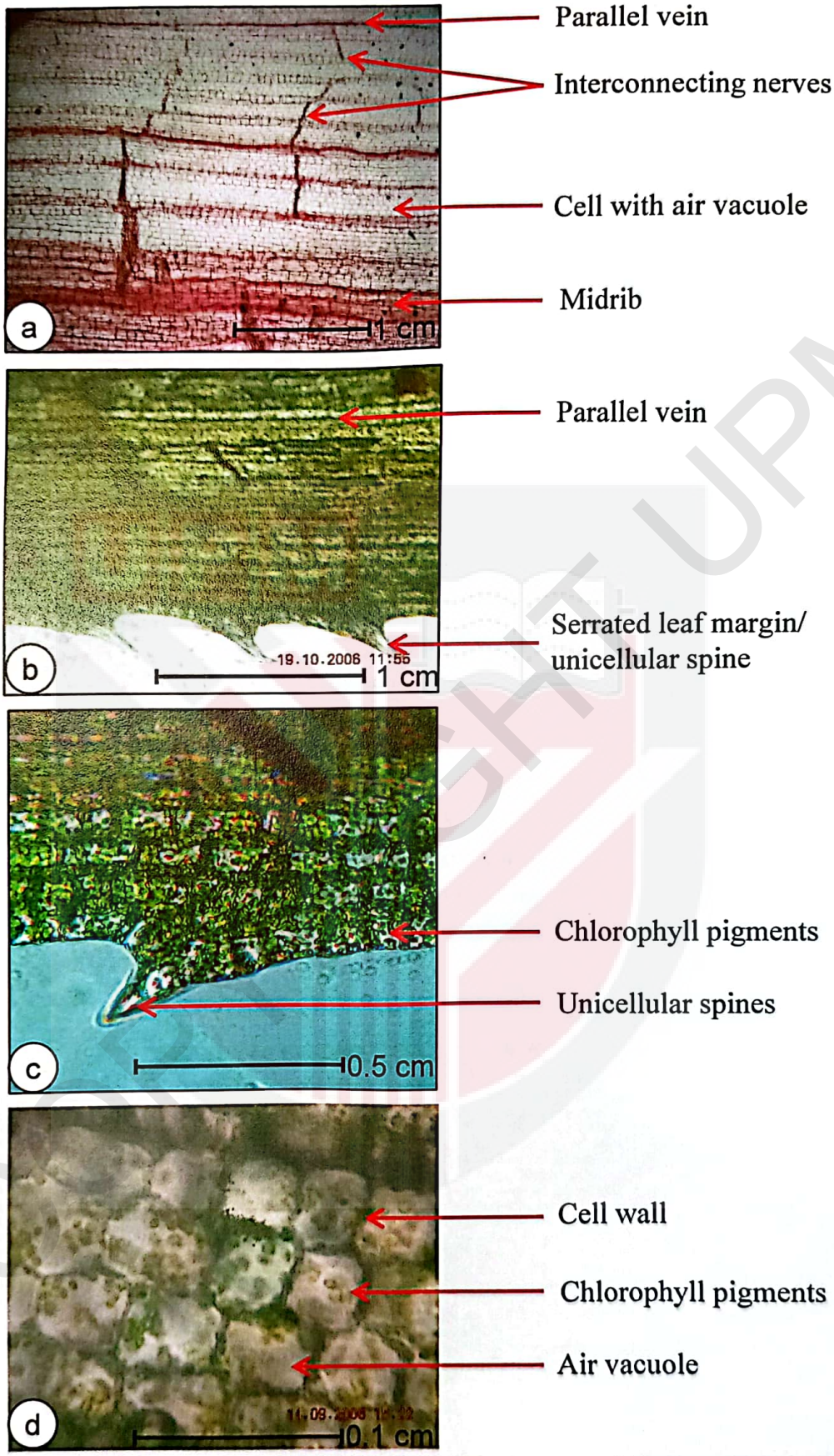

Plate 9: *Blyxa aubertii* leaf morphology. a and b-serrated leaf margin with unicellular spine (magnification 10x10 and 20x10), c-parallel veins with interconnecting nerve (magnification 15x10), d-rectangular shape cell with chlorophyll pigments and air vacuoles (magnification 40x10)

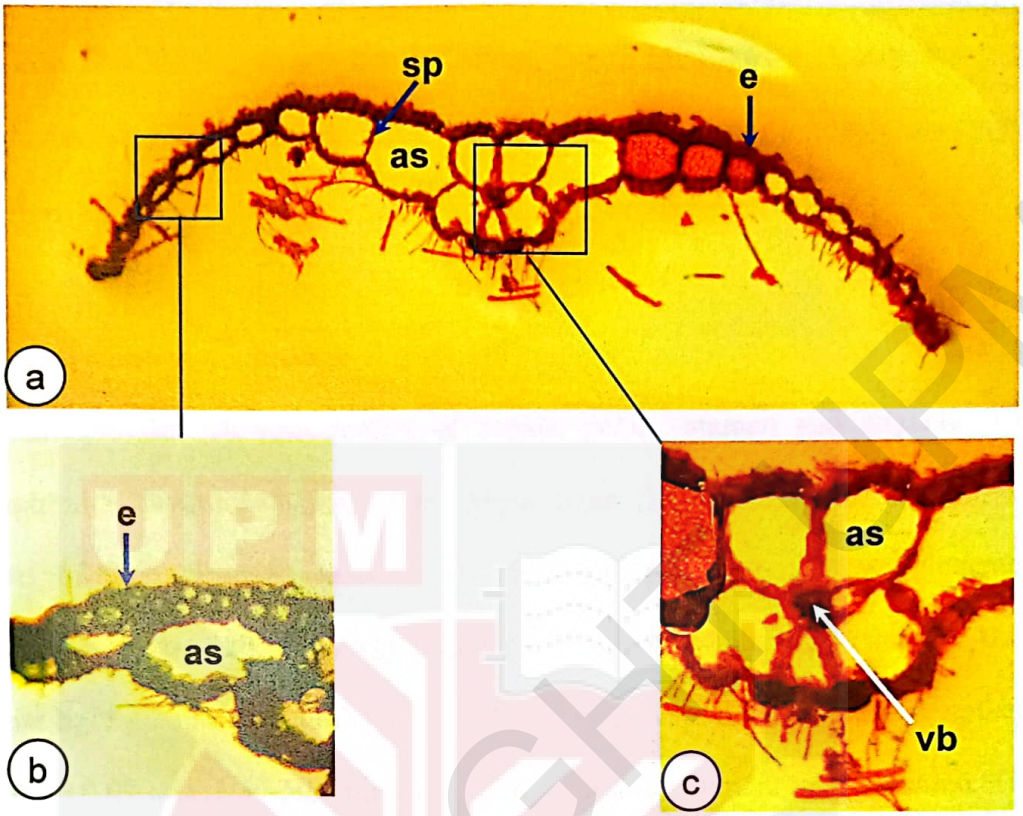

Plate 10: a-Cross section of *Blyxa aubertii* leaf, b-single layer of epidermal cell, c-vascular bundle with air space. e-epidermis layer, as-air space, sp-segmented septa, vb-vascular bundle

Plate 11: *Blyxa aubertii* roots. Brown simple, adventitious or unbranched roots

hermaphrodite flower bear male and female flowers on the same flower. The flowers are small, whitish, emerged above water surface, often solitary and rarely paired. Each flower has three greenish sepals varying length from 2.61-8.11 mm, linear to linear lanceolate. The three petals are white to pinkish and papillose with the length range from 4.89-10.80 mm.

4.3.1 Hermaphrodite Flower

The hermaphrodite flowers consist of sepals, petals, stamen and stigmas. The hermaphrodite flower is borne on the hypanthium (Plate 12a). The male flower consists of three stamens, three sepals and three petals borne from elongated hypanthium (Plates 12b, 12c). The hypanthium length is 3.86±1.72 cm (1.93-6.70 cm) and light green in colour (Table 3). The stamens were three in a whorl, anthers with 4.40±0.09 mm (3.30-5.56 mm) in length, pollen is spherical and echinated. The ovary is inferior sessile within the spathe, tubular and elongated into the hypanthium. The spathes consist of two overlapping green layers, elongated with obtuse tip. There are three stigmas per flower with varying length range from 5.01±0.19 mm.

4.3.2 Fruit and Seed

The fruits of *Blyxa aubertii* are elongated capsule disintegrating at maturity hold by the peduncle 15.01±8.20 cm (2.66-27.35 cm) in length and hypanthium 3.86±1.72 cm (1.93-6.705 cm) in length (Plate 13a, Table 3). The fruits are 48.20±2.27 mm x 2.72±0.10 mm. The seeds are numerous ranges from 22-132 seeds per fruit arrange

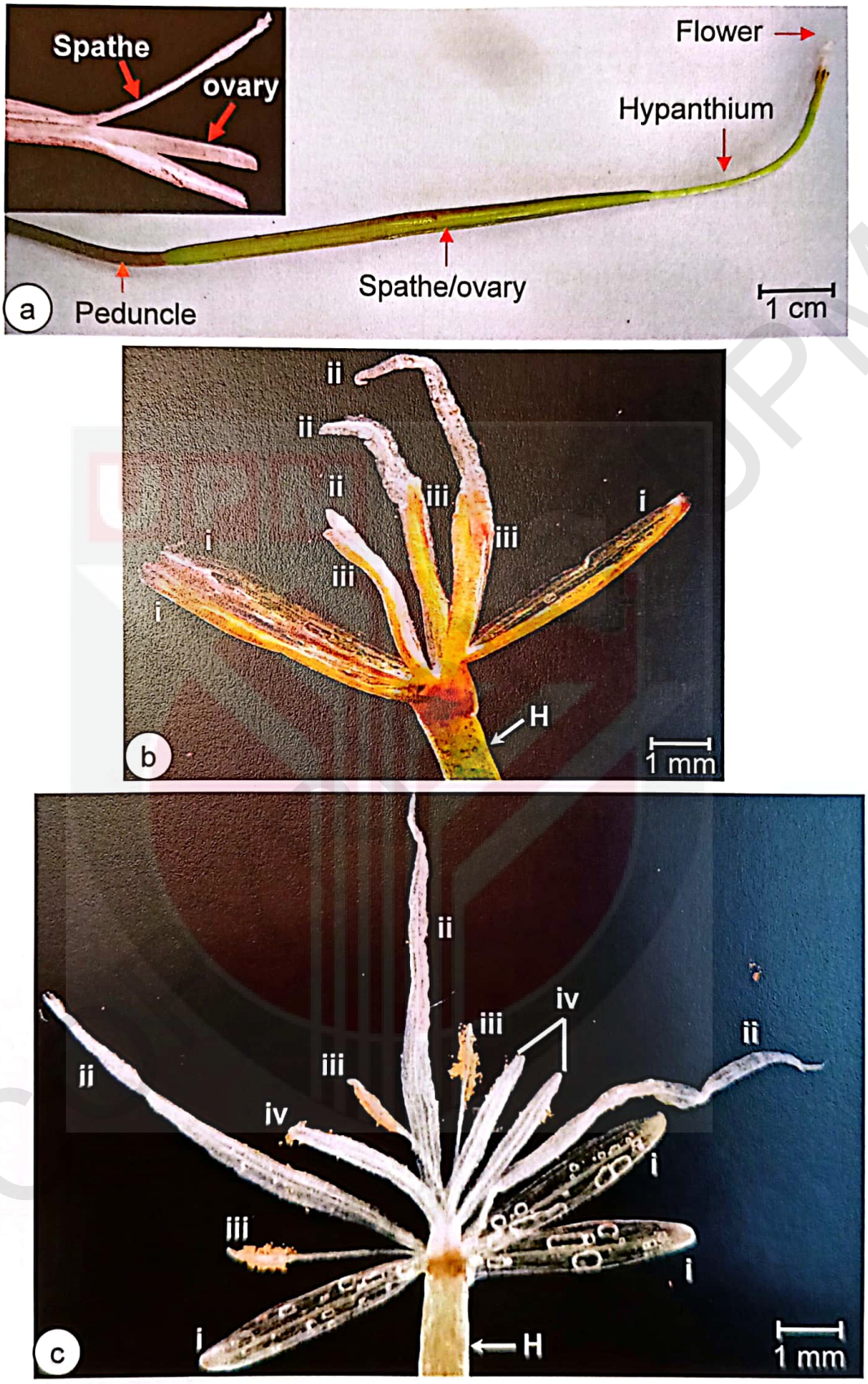

Plate 12: *Blyxa aubertii* hermaphrodite flower. a-flower borne on the elongated hypanthium, b-developing flower (magnification 0.8x10), c-matured flower consists of 3 sepals, 3 petals, 3 stamens, 3 stigmas (magnification 0.6x10). i-sepal, ii-petal, iii-stamen, iv-stigma, F-flower, H-hypanthium, S-spathe, P-peduncle

Table 3: Summary of dimension analysis (mean ± s.e given in mm) of reproductive structure of *Blyxa aubertii* from UPMKB

Parameter	N	Mean ± s.e (min-max)
HERMAPHRODITE FLOWER		
Male flower		
1. stamen length	60	4.40 ± 0.09 (3.30-5.56)
2. sepal length	90	5.14 ± 0.10 (2.61-8.11)
3. petal length	51	7.35 ± 0.24 (4.89-10.80)
4. peduncle length	46	150.05 ± 8.20 (26.64-273.50)
Female flower		
5. ovary length	60	55.68 ± 1.30 (30.10-70.80)
6. ovary width	60	2.11 ± 0.07 (1.00-3.10)
7. stigma length	51	5.01 ± 0.19 (3.29 -9.33)
Fruit and Seed		
8. hypanthium length	60	38.57 ± 1.72 (19.33-66.98)
9. fruit length	30	48.20 ± 2.27 (17.81-66.88)
10. fruit width	30	2.72 ± 0.10 (1.95-3.64)
11. number of seed per fruit	20	63.55 ± 6.50 (22-132)
12. seed length	52	1.36 ± 0.01 (1.14-1.54)
13. seed width	52	0.80 ± 0.01 (0.69-1.05)
14. no of longitudinal ribs per seed	52	8.87 ± 0.25 (5.00-12.00)

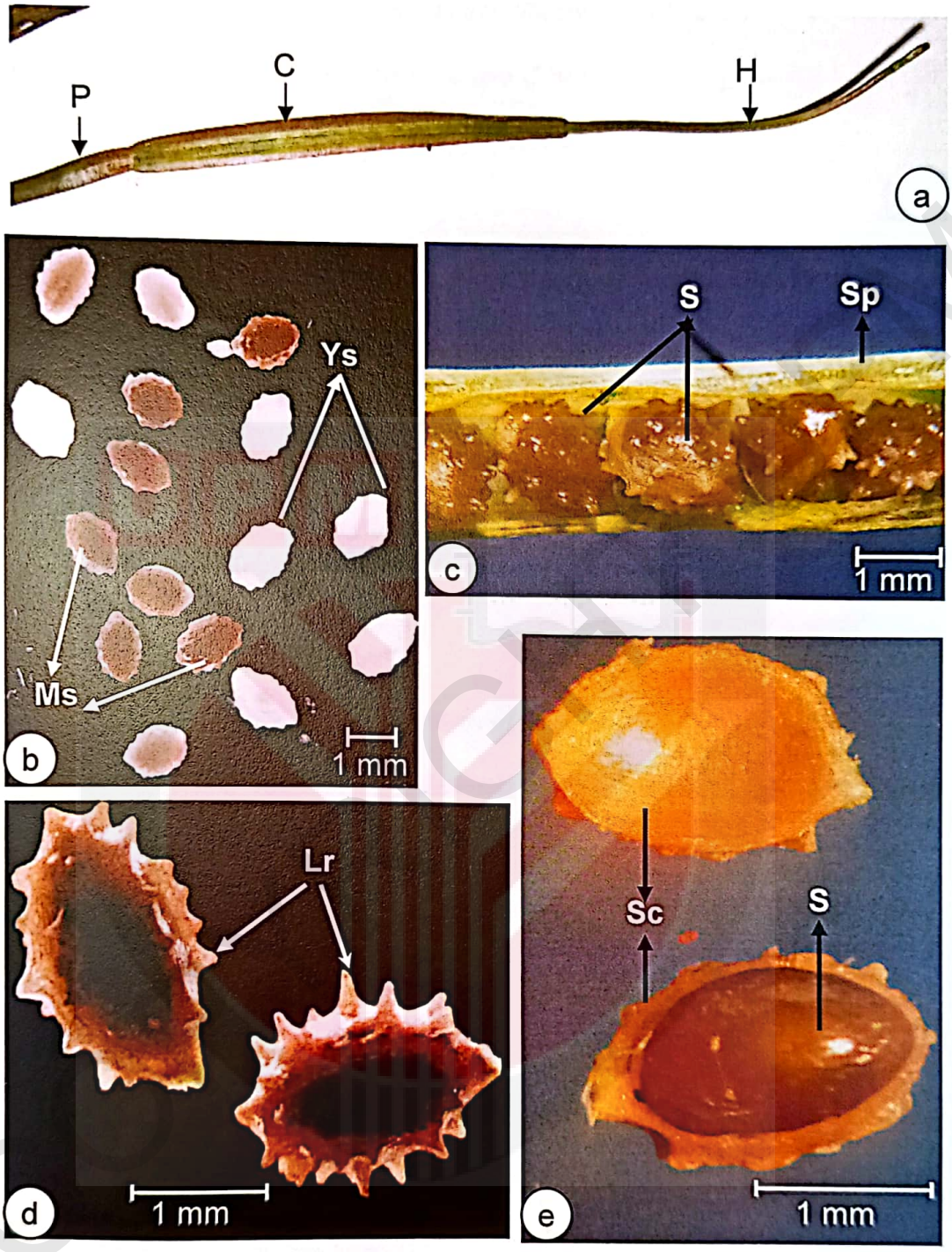

Plate 13: a-Fruit of *Blyxa aubertii* with elongated capsule, peduncle and hypanthium, b-young and mature seed (magnification 0.6x10), c-seed arrangement in the spathe (magnification 25x10), d-seed with longitudinal ribs (magnification 1.2x10), e-seed cover by seed coat (magnification 40x10). P-peduncle, C-capsule, H-hypanthium, Ms-matured seed, Ys-young seed, S-seed, Sp-spathe, Lr-longitudinal ribs, Sc-seed coat

in spathe (Plates 13b, 13c). The seeds are whitish to light green during young stage and turn into brown when matured. Seeds are ellipsoidal or fusiform with ridges, 5-12 longitudinal ribs (Plate 13d) at the seed coat (Plate 13e). The seeds are 1.36±0.01 mm x 0.80±0.01 mm.

4.4 Propagation

4.4.1 Seed Germination

Seeds of *Blyxa aubertii* showed low rates of germination over extended of time. No consistent germination trends were noted during the study. By 152 days, about 21% (out of the 100 seeds) had germinated and were at various stages of seedling development.

The germination and stages of development of *Blyxa aubertii* seedling in laboratory cultures are described. The first sign of germination in *B. aubertii* is the swelling of the seeds (Plate 14a) within 4-20 days culturing followed by the emergence of radicle (Plate 14b) from the base of the hypocotyls after 4-43 days (Table 4). Following the emergence of radicle, green cylindrical cotyledon elongated from the hypocotyls. The extension of the cotyledon pushed and released the seed coat (3-18 days). The cotyledon (Plate 14c) emerged in 5-24 days. The cotyledons persist up to two leaves stage. The first lateral root with root hairs was formed opposite to base of the cotyledon at two or three-leaf stage (Plate 14d). The second lateral root also formed within the same period from the base of the cotyledon (Plate 14e). After varying period of 16-25 days, the cotyledonary pocket produced the first true leaf and the succeeding second leaf (17-28 days), third leaf (21-57 days) and the other leaf

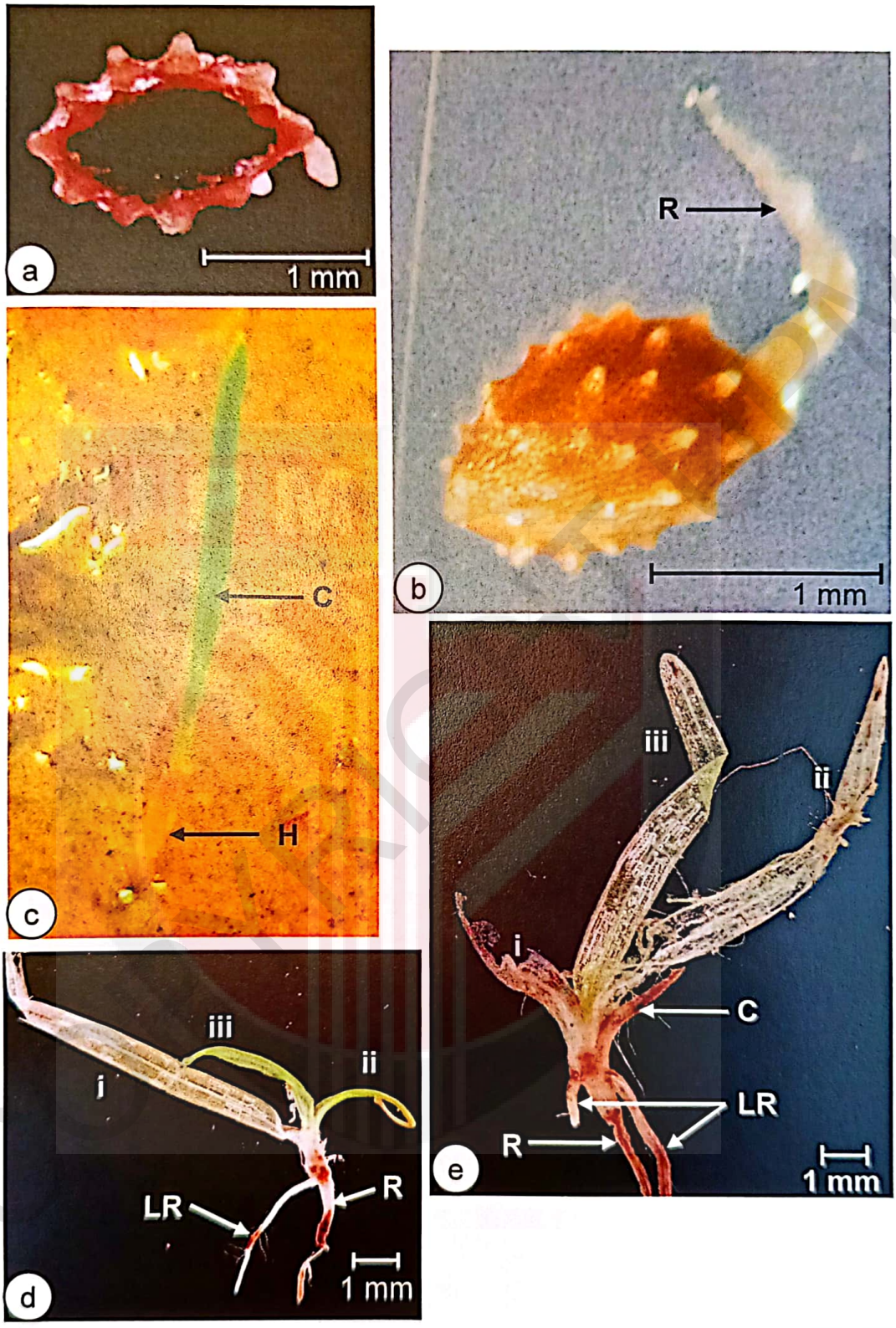

Plate 14: Germination and stages development of *Blyxa aubertii* seedling. a-matured seed, b-emergence of radicle, c-emergence of cotyledon and expansion of hypocotyl from the natural substrate, d-3-leaves seedling with lateral root, e-seedling with 3 leaves (one rotten) and dying cotyledon,. C-cotyledon, R-radicle, LR-lateral root, H-hypocotyl, i-first leaf, ii-second leaf, iii-third leaf

Table 4:Seed germination of *Blyxa aubertii* in natural substrate and the different development stages

Percent of seed germinated (%)		Days From The Beginning of Germination						
		Swollen seed	Emergence of radicle	Splitting of seed coat	Emergence of cotyledon	1-leaf	2-leaf	3-leaf
	4	4	16-21	3-7	7-10			
	1	5	33	18	-			
	4	6	16-43	3-10	5-7			
	2	7	4-5	7	-			
	3	8	15-17	7-18	24	16-25	17-28	21-57
	1	9	32	-	-			
	2	10	20-21	7-17	-			
	1	12	17	10	-			
	1	16	20	-	-			
	2	20	22	-	-			
TOTAL	**21%**	**4-20**	**4-43**	**3-18**	**5-24**	**16-25**	**17-28**	**21-57**

appeared relentlessly within 7 days gap. The cotyledon tends to drops within third to fourth leaves stage appeared (Plate 14 e).

4.4.2 Vegetative Propagation

From the results showed that only plants in the Tank 2 were successfully cultured. Total number of leaf per shoot (Figure 6) were increasing starting from the 1st week culturing until 6th week with the highest total number of leaves per shoot (52 leaves) were gain. The results showed that the plants start to decrease the total number of leaves per shoot in the 7th week and 8th after culturing before increased again. Culture plants in the other five tanks however show a fluctuation for every week. There were two plants flowering in Tank 2. The first plant was flowering in the 6th week, the ovary developed into fruit with seed (after fertilization) in 7th week and bursting after a week. The second plant was flowering in 7th week, fruiting in 9th week and the fruit burst in 10th week. These flowers wilted within one or two days (Plate 15). After 11th week culturing all the plant were starting to die. *Blyxa aubertii* from UPMKB germinates from seed, grows into seedlings, juvenile plants, flowering and finally fruiting plants all occurring within a period of approximately 12 weeks.

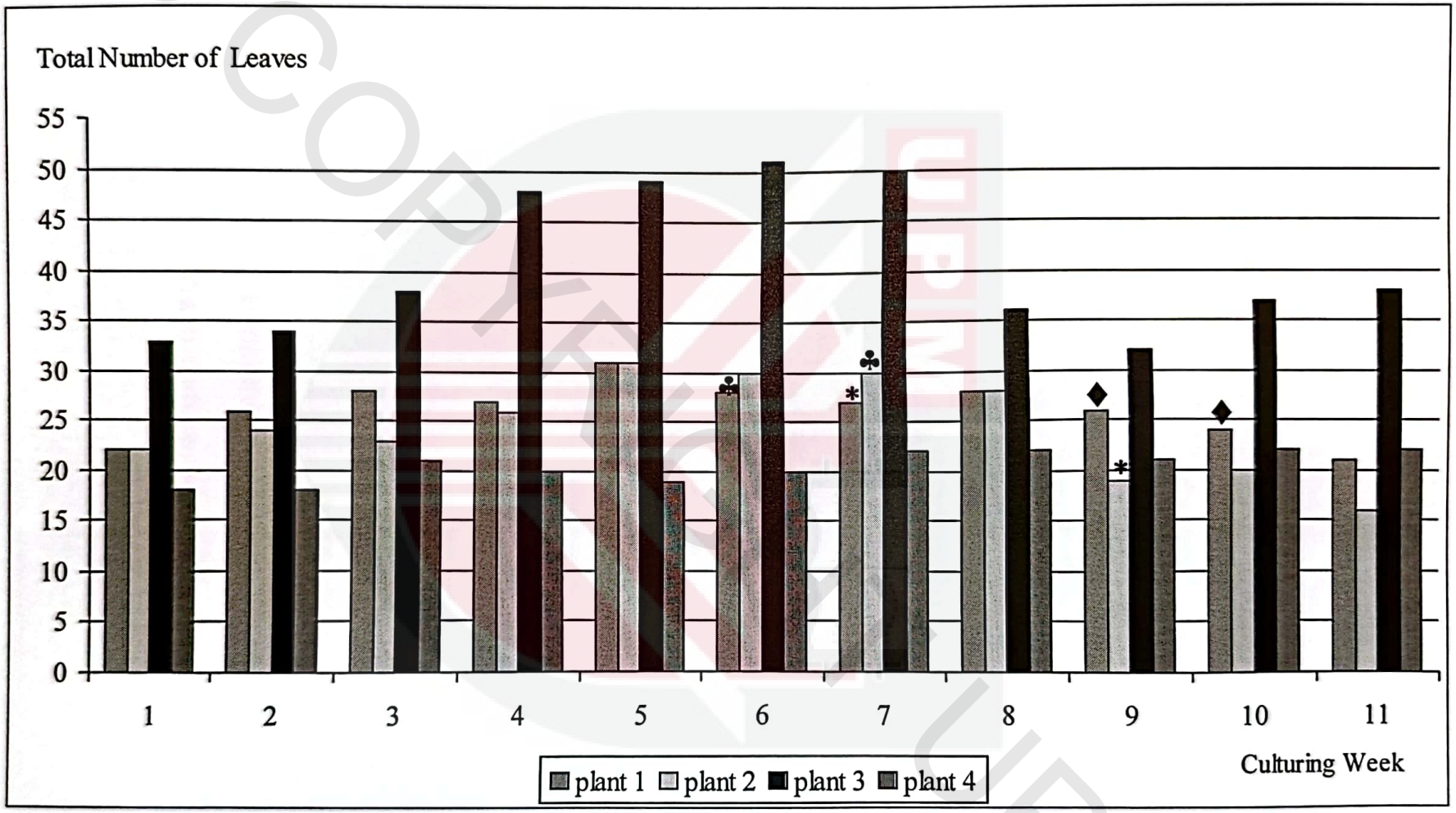

Figure 6: The changes of the total number of leaves per shoot of *Blyxa aubertii* cultured in aquarium tank 2 in the laboratory. ♣ - Flowering, * - Fruiting and ♦ - Fruit bursting

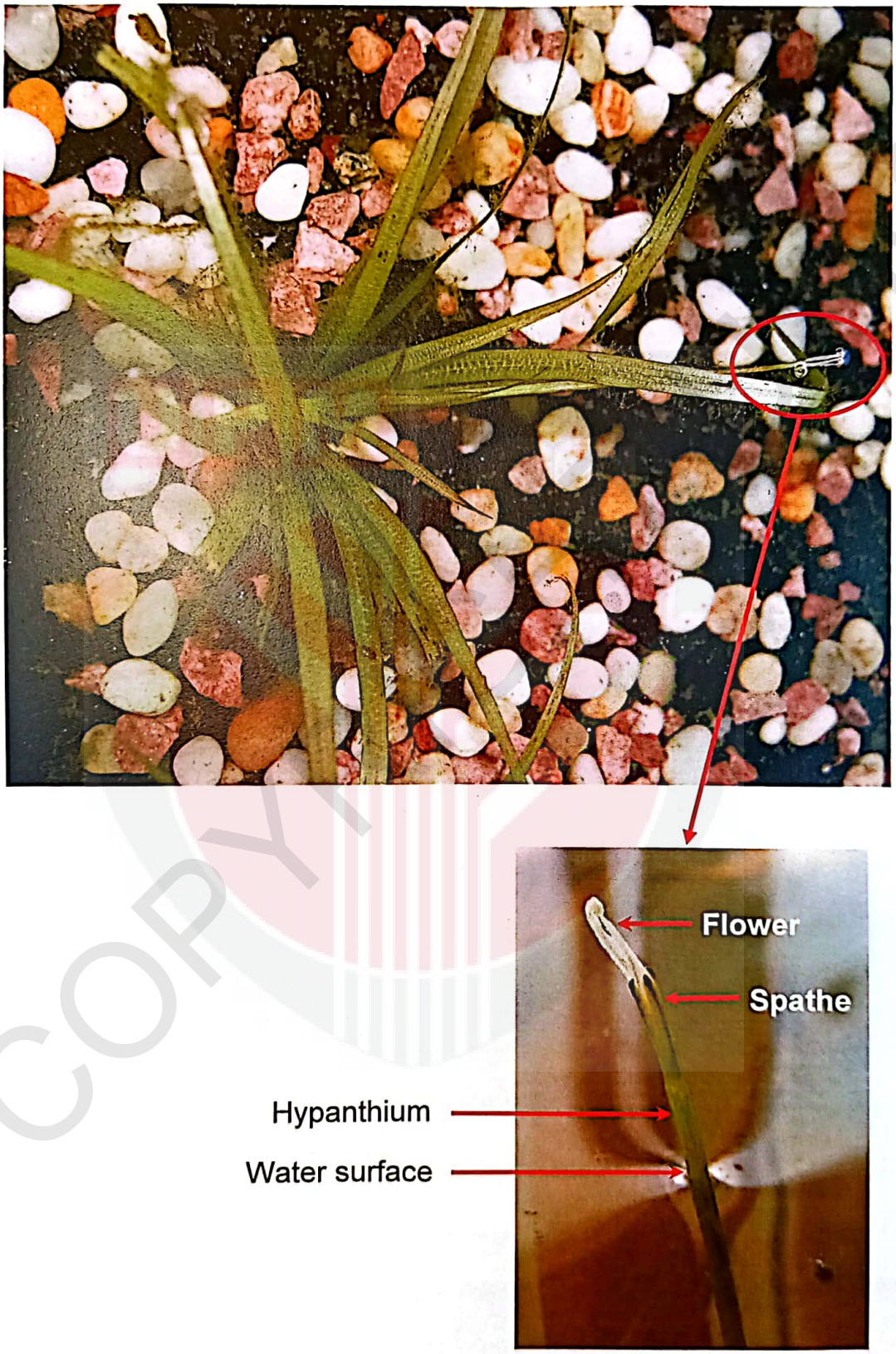

Plate 15: Flower was emerged to the water surface lasting one to two days after 90-109 days cultured in the laboratory

CHAPTER 5

DISCUSSION

5.1 Ecology of *Blyxa aubertii*

Blyxa aubertii are found submersed in natural marshes at Baloi and Sibu; man-made ditches at UPMKB and Maradong, Sarikei, Sarawak. In Peninsular Malaysia *Blyxa aubertii* were found in Kemubu, Kelantan and Barat Laut Selangor (Mashhor, 1988; Azmi, 1991). This species were found in association with slow-flowing or standing water level about 0.35-1.20 m. In Malaysia studies by Soepadmo (1986), Mashhor (1988), Azmi (1991), Mohd Zaini and Japar Sidik (1994), and Muta Harah *et al.* (2005a, 2005b) reported that *Blyxa aubertii* thrives well in rice field waterways, ditches, marshes, pools, ponds and other similar habitats. In other country this species also were found in association with shallow water bodies either natural pools, lakes, marshes, streams, rivers, creeks and billabongs (Subramanyam, 1962; Thieret *et al.,* 1969; Pancho and Soerjani, 1978; Keng *et al.,* 1998; Cowie and Osterkamp, 2000; Jiang and Kadono, 2001) or man-made: paddy fields, ponds, ditches, irrigation ponds, irrigation ditches (Henderson, 1954; Cook *et al.,* 1974; Pancho and Soerjani, 1978; Soerjani *et al.*, 1987; Keng *et al.,* 1998; Jiang and Kadono, 2001) water bodies.

In this present study *B. aubertii* was found associated with other aquatic macrophytes *Eleocharis* sp., *Limnocharis flava, Ludwigia hyssopifolia, Commelina diffusa, Fimbristylis* sp., *Hydrilla verticilata, Nymphaea nouchali, Utricularia bifida, U. aurea, Eriocaulon truncatum* and freshwater algae similar as reported by Muta Harah *et al.* (2005a). Earlier in 1993, Baki has recorded that *Blyxa aubertii* also found

growing associated with *Rhynhospora corymbosa* in rice fields of Samarahan, Sarawak.

5.2 Vegetative Structure of *Blyxa aubertii*

In this present study, the vegetative morphology of *Blyxa aubertii* from UPMKB, Baloi Bintulu, Maradong, Sarikei and 19 km golf field, Sibu are generally similar. *Blyxa aubertii* have green, lanceolate leaves similar to Cook and Lüönd (1983). However, Keng *et al.* (1998) have described the leaves as narrow while Soepadmo (1986) has described it linear or strap shape. The leaves have no differentiation into blade and petiole, leaves radically arranged or rosette and crowded at the base as stated by Henderson (1954), Subramanyam (1962), Pancho and Soerjani (1978) and Keng *et al.* (1998) while Soepadmo (1986) as well as Cook and Lüönd (1983) noted the leaves are spirally arranged.

According to Cook and Lüönd (1983) other species of *Blyxa* are varied in leaf shape. *Blyxa echinosperma, B. japonica, B. vietii* and *B. senegalensis* are linear-lanceolate leaf while *Blyxa hexandra, B. quadricostata, B. octandra, B. novoguineensis* and *B. radicans* have linear leaf. All of the species have radically or spirally leaf arrangement except for *Blyxa vietii* (leaves are cauline and clustered at the stem), *Blyxa novoguineensis* (the leaf are cauline) and *Blyxa radicans* (leaves are solitary and space along the stolon or clustered into rosette).

The leaf margins are serrated with unicellular spine as describe by Cook *et al.* (1974) as well as Cook and Lüönd (1983). All species in the genus *Blyxa* are identified with the presence of unicellular spine similar to the other six species (*Blyxa hexandra, B.*

echinosperma, B. japonica, B. octandra and *B. novoguinensis*) while two other species (*B. senegalensis* and *B. vietii.*) are different which both of it have multicellular projection of the unicellular spine as described by Cook and Lüönd (1983).

Leaf length of *B. aubertii* from UPMKB, Baloi, Maradong and Sibu are 0.65-59.72 cm. Cook and Lüönd (1983) stated the leaves are shorter with 2.5-6.0 cm followed by 12.70-45.70 cm (Henderson, 1954), 10-50 cm (Keng *et al.*, 1998) and 2.5-60 cm or more in length (Subramanyam, 1962). The leaf length also can reach up to 130 cm as described by Pancho and Soerjani (1978). *Blyxa echinosperma* has longest leaf (10-130 cm) followed by *B. quadricostata* (40 cm or more), *B. octandra* (6-40 cm), *B. radicans* (15-30 cm), *B.* hexandra (10-20 cm), *B. novoguineensis* (4-8 cm), *B. senegalensis* (6 cm), *B. aubertii* (2.5-6.0 cm) and *B. japonica* (2-5 cm). *Blyxa vietii* has the shortest leaf length about 4 cm only (Henderson, 1954; Pancho and Soerjani, 1978; Cook and Lüönd, 1983; Soepadmo, 1986; Keng *et al.,* 1998).

The leaves widths from Maradong 4.83±0.12 mm (0.09-10.91 mm) are similar to specimens from Baloi were 6.80±0.12 mm (0.09-10.91 mm) while significantly different from UPMKB 4.77±0.09 mm (1.54-8.23 mm) and Sibu were 3.45±0.36 mm (0.41-6.32 mm). Overall leaf width is quite similar to Subramanyam (1962) 0.2-1.2 cm, Pancho and Soerjani (1978) 0.5-1.0 cm as well as Cook and Lüönd (1983) 0.2-1.2 cm. The leaf width did not show wide range when compared with the other species of *Blyxa,* range from 0.1-0.8 cm (Henderson, 1954; Pancho and Soerjani, 1978; Cook and Lüönd, 1983; Soepadmo, 1986; Keng *et al.*, 1998).

In this present study, the parallel leaf veins range from 2-48 per leaf comparatively higher than reported by Subramanyam (1962) 0-38 parallel veins, Pancho and Soerjani (1978) 5-9 parallel veins and, Cook and Lüönd (1983) 38 veins. The result of this present study also shows the number of leaf veins of *B. aubertii* was relatively higher than *Blyxa octandra* and *B. novoguineensis* (20 veins each), *B. quadricostata* (18 or more), *B. radicans* (18 veins), *B. japonica* (6-12 veins), *B. echinosperma* (5-9 veins) and *B. vietii* (6 veins). *Blyxa senegalensis* and *B. hexandra* has the smallest number of leaf veins that were about two veins per leaf (Subramanyam, 1962; Pancho and Soerjani, 1978; Cook and Lüönd, 1983).

Blyxa aubertii leaves are lined with thin-walled epidermal cell and their inner tissues are filled with air spaces. These structural characteristics and growth habit have been interpreted as adaptation to low light intensity and oxygen concentration in water. In the natural habitat, the plant can grow up to 800 leaves per shoot and reddish in colour due to high light intensity.

Roots of *Blyxa aubertii* from four locations are unbranched, brownish in colour due to type of soil in the natural habitat, which are almost silted and clay. The colour only has been mentioned by Mohd Zaini and Japar Sidik (1994) as red or brownish due to laterite soil precipitation. The root length also varied among specimens from different location or within location. Root lengths of *Blyxa aubertii* from Baloi were the longest (4.00-24.72 cm), followed by Maradong (48-20.31 cm), 19km golf field, Sibu (2.94-20.02 cm) and the shortest from UPMKB (2.04-14.81 cm). The differences in the vegetative dimension may be due to the adaptation of the plant to survive in the different habitat conditions.

5.3 Reproductive Structure of *Blyxa aubertii*

Blyxa aubertii observed are monoecious and annual submersed plant, which is same as Subramanyam (1962), Pancho and Soerjani (1978) and Cook and Lüönd (1983). The flowers are hermaphrodite (bisexual flower), small and whitish in colour (Plate 2). This is similar to Henderson (1954), Pancho and Soerjani (1978), Cook and Lüönd (1983) and Keng *et al.* (1998) which have explained that *Blyxa aubertii* flower as bisexual. Among the 10 species of genus *Blyxa, o*nly *Blyxa aubertii, B. echinosperma, B. japonica* and *B. vietii* (maybe dioecious) are monoecious while the others are clearly dioecious.

Flowers have three green, linear to linear lanceolate sepals and three white to pinkish and papillose petals with length range 2.61-8.11 mm and 4.89-10.80 mm respectively. Henderson (1954) as well as Cook and Lüönd (1983) have accounted that the sepals are three, linear to linear lanceolate with dimension of 6-8 mm x 0.5 mm while the petals are three white to reddish, papillose with dimension of 17 mm x 0.5 mm. In the other review, Pancho and Soerjani (1978) only mentioned that the sepals are linear and the petals lengths are about 10-12 mm. The sepals of *Blyxa aubertii* are similar to sepal of *Blyxa echinosperma* (no dimension stated*)* and *Blyxa japonica* (only about 2.5-3.8 mm x 0.7-0.8 mm). For the other species, the sepals are then divided into two: female flower's sepal and male flower's sepal because the flowers are unisexual. The sepals are always green in colour except in *Blyxa octandra* (white), *Blyxa vietii* (pale violet) *and Blyxa novoguineensis* (opaque). The petals are also similar within the genus but *Blyxa japonica* is lanceolate instead of papillose with 8 mm x 0.2-0.8 mm. Most of the petals for the dioecious species are linear for the male sepals and filiform for the female sepals.

The stamens for *Blyxa aubertii* are three in a whorl standard as recorded by others references (Henderson, 1954; Thieret *et al.*, 1969; Cook *et al.*, 1974; Pancho and Soerjani, 1978; Cook and Lüönd, 1983). Base on Cook and Lüönd (1983), the stamens are wide-ranging among the genus. Some species can produced up to nine stamen per flower (*Blyxa octandra, B. vietii, B. senegalensis, B. quadricostata and B. novoguineensis)* while two species with six stamens (*Blyxa hexandra* and *B. radicans*). The rest of others species are similar to *Blyxa aubertii,* has three stamens. The stamens arrangements are similar in every species that is three in a whorl.

The fruits of *Blyxa aubertii* are elongated capsule (17.81-66.88 mm x 1.95-3.64 mm) disintegrating at maturity hold by the peduncle while Pancho and Soerjani (1978) reported that the fruit are linear (30-60 mm). The peduncle length from UPMKB (66.98 mm) are shorter than peduncle length stated by Pancho and Soerjani (1978) about 100-400 mm or more while Thieret *et al.* (1969) as well as Cook and Lüönd (1983) has stated that the peduncle length is 500 mm. When compare to the other species, the peduncle length can be up to 50 cm for every species except for *Blyxa vietii* (4 cm). The fruit for the other species in the genus *Blyxa* are uniformed. All the species has terete and cylindrical fruit except for *Blyxa echinosperma* that are linear more alike to *Blyxa aubertii*'s fruit (Cook and Lüönd, 1983).

In present study *Blyxa aubertii* have 22-132 seeds per fruit. Henderson (1954) Subramanyam (1962) had mentioned the numbers of seeds per fruits are numerous. There is no exact number of seed per fruit reported by any literature. *Blyxa aubertii* (Figure 7a) have ellipsoidal seed with 5-12 longitudinal ribs while *B. echinosperma* (Figure 7b) has spiny seed with tail-like spine. *B. japonica* (Figure 7c) and *Blyxa*

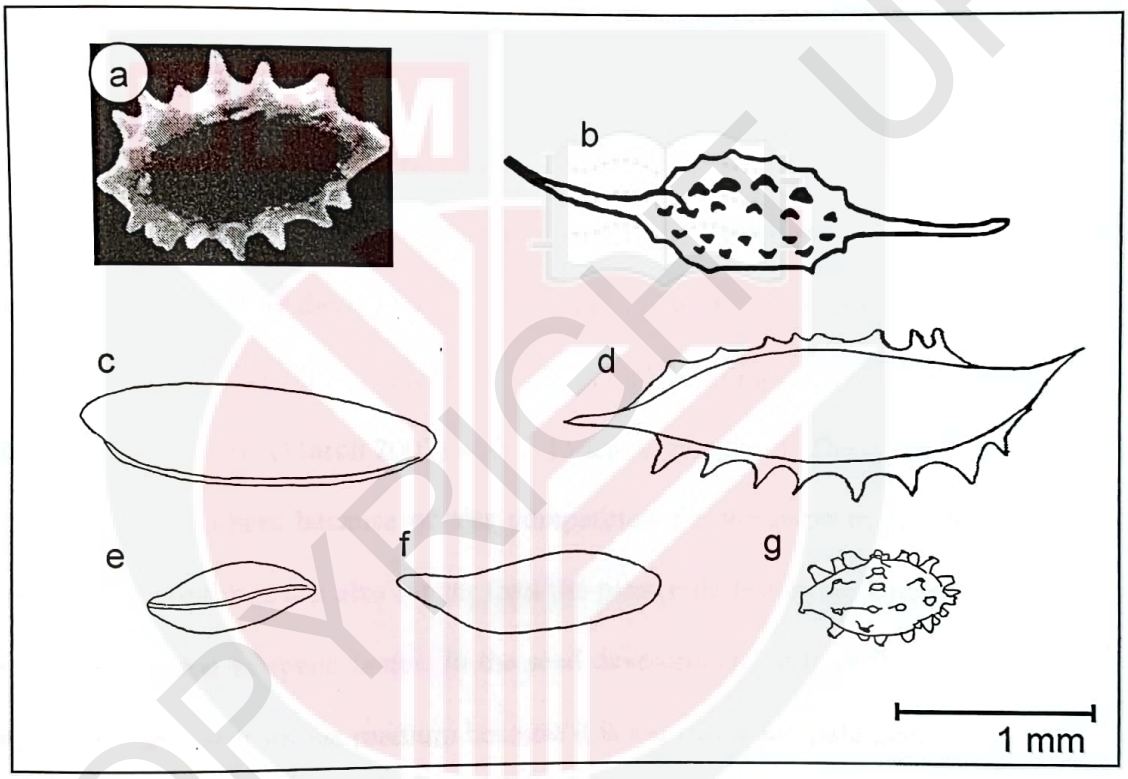

Figure 7: The different shape of seed in genus *Blyxa*. a-*Blyxa aubertii* (present study), b-*B. echinosperma* (Pancho and Soerjani, 1978), c-*B. japonica*, d-*B. senegalensis*, e-*B. vietii*, f-*B. hexandra*, g- *B. octandra* (Cook and Lüönd, 1983)

senegalensis (Figure 7d) *has* fusiform seed but to distinguish both species, the *Blyxa japonica*'s seed are smooth while *B. senegalensis* has slightly s-shape. The seed of *Blyxa hexandra* and *B. vietii* (Figure 7e) are also fusiform but the seed of *Blyxa hexandra* (Figure 7f) are unevenly fusiform with one end short, straight or curved beak. The seed of *Blyxa octandra* (Figure 7g) are oblong to elliptic while there is no information for other three species (*Blyxa novoguineensis, B. radicans* and *B. quadricostata*).

5.4 Propagation

Seeds germination of *Blyxa aubertii* in the laboratory showed low rates of germination over extended of time. No consistent germination trends were noted during the study. By 57 days, about 21% out of 100 seeds had germinated and were at various stages of seedling development. Over 21 seedlings, only three seedlings are survival until now (March 2007) with 5-15 leaves per shoot. These seedlings may live longer than others because of less competition for the nutrient and light. The leaves of the seedlings are also longer than the plant cultured in the aquarium tanks, which may be due to space factor. In the seed development, it is difficult to see the cotyledon emerged from the medium because it is too small and pale green in colour. The cotyledon however persist up to three leaves stages and die when fourth leaf appeared. No study has been done before on the seed germination of *B. aubertii*.

For the vegetative propagation, half of the plants cultured were dead after two month of culture and this may due to low aeration and frequent measurement activity. The most important factors for the culture to grow well in the aquarium tank are the light and aeration, because without this factors it might caused the plants stunted and died.

Within the same period, in-situ observation at the natural habitat have produced up to 55 leaves while in the tank culture the plants only produced up to 50 leaves. *Blyxa aubertii* from UPMKB germinates from seed, grows into seedlings, juvenile plants, flowering, fruiting and finally decaying all occurring within a period of approximately four months and half which is growing faster than reported by Jiang and Kadono (2001) that *Blyxa aubertii* were start to decaying within six months. This may be due to different type of experiment done by present study (in laboratory) and, Jiang and Kadono (2001) that studies by in-situ observation.

CHAPTER 6

CONCLUSION

In this present study *Blyxa aubertii* are fulfilled the characteristics and descriptions as reported by Subramanyam (1962), Pancho and Soerjani (1978), Cook and Urmi-König (1983), Cook and Lüönd (1983) and Kubitzki (1998). There are similarities morphology between specimens from all locations. The leaves are green, lanceolate and spirally arranged and rise from the rootstock. The roots are simple, adventitious and brownish in colour.

The reproductive parts are giving no doubt to clarify that the plants studied are *Blyxa aubertii.* The flowers are hermaphrodite flower bears female and male flowers on the same flower. The ovary are enclosed in the spathe and developed into fruit after fertilization happen. The ellipsoidal seed are the most important feature to determine *Blyxa aubertii.* The seed has 5-12 longitudinal ribs.

For the propagation, *Blyxa aubertii* are preferred reproductive propagation (which mean by seed germination) other than vegetative propagation (root splitting). Seeds of *Blyxa aubertii* show low rates of germination over extended of time. No consistent germination trends were noted over study period. Only 21% (out of the 100 seeds) germinated in the natural substrate. The length of survival for seedling varies with 10-200 days. From both observation on the seed germination and plant culture in aquarium tank shows that *Blyxa aubertii* germinates from seed, grows into seedlings, juvenile plants, flowering and fruiting plants and finally disappeared, all occurring within a period of approximately in or four and a half months.

The tank cultures for *Blyxa aubertii* need very gentle care because from observation, this plant is fragile and hardly to grow in the stress condition. As the conclusion, I would like to suggest for further research on nutrient content of this plants and its significance to the environment.

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Morphology and Propagation of *Blyxa aubertii* Rich." by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the Faculty.

Robiah Binti Awang

Date: 4/05/2007