A TAXONOMIC AND MORPHOLOGICAL STUDY OF CYPERACEAE

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ABSTRACT

This study aimed to investigate the taxonomic characteristics based on micromorphology and macromorphology of Cyperaceae species and to build the taxonomic key based on these characteristics. The research method involved the use of light and electron microscopy. Sampling was carried out in four areas of Tanjong Malim, Ipoh, Kuala Kangsar and Gerik covering different ecology of open areas, wetlands and limestone. The findings showed that four out of the thirteen studied species; *Cyperus compactus*, *C. compressus*, *C. iria*, and *C. kyllingia* are common species found in all studied areas. *Cyperus sphacelatus* is limited to Gunung Lang, Ipoh while *C. pulcherrimus* often found in Pulau Banding, Gerik. This study showed the diagnostic characteristics of flower and spikelet is in agreement with the classification of Koyama, Eiten, Dahlgren, Tucker, Bruhl and Goetghebeur. Its diagnostic macromorphological characters have successfully been applied for developing the taxonomic key of Cyperaceae. The findings also revealed the micromorphological characters of exine and cuticular ornamentation of spikelet can be used as supportive diagnostic evidence in identification of the species. Multivariate and phylogenetic analyses using 30 of both macromorphological and micromorphological characters showed that there is a close evolutionary relationship between species of *Cyperus*, *Bulbostylis* and *Fimbristylis*. The phylogenetic tree demonstrated a close evolutionary relationship with high bootstrap values for *C. compressus* and *C. sphacelatus*, moderate for *C. kyllingia* and *C. rotundus*, while the rest are weak. As a conclusion, this study has successfully manipulated both macromorphological and micromorphological characters in identification of Cyperaceae and a taxonomic key based on macromorphology has successfully constructed. This key can benefit the researchers to systematically identify the species of Cyperaceae especially in Malaysia.

PENELITIAN MORFOLOGI DAN PEMBINAAN KEKUNCI TAKSONOMI CYPERACEAE

ABSTRAK

Kajian ini adalah untuk meneliti ciri taksonomi makromorfologi dan mikromorfologi spesies Cyperaceae dan untuk membina kekunci taksonomi berdasarkan ciri tersebut. Kaedah penelitian melibatkan penggunaan mikroskop cahaya dan elektron. Persampelan dilakukan di empat kawasan iaitu Tanjong Malim, Ipoh, Kuala Kangsar dan Gerik yang meliputi ekologi berbeza iaitu kawasan terbuka, kawasan tanah bencah dan batu kapur. Dapatan kajian menunjukkan di antara tiga belas spesies yang dikaji, *Cyperus compactus*, *C. compressus*, *C. iria*, dan *C. kyllingia* merupakan spesies lazim yang dijumpai di semua kawasan kajian. *Cyperus sphacelatus* dijumpai terhad di Gunung Lang, Ipoh manakala *C. pulcherrimus* banyak dijumpai di Pulau Banding, Gerik. Kajian ini menunjukkan ciri-ciri diagnosis pada spikelet dan bunga adalah selari dengan pengelasan oleh Koyama, Eiten, Dahlgren, Tucker, Bruhl dan Goetghebeur. Ciri diagnosis makromorfologi telah berjaya dijadikan kekunci taksonomi. Dapatan juga telah menemui ciri mikromorfologi eksin dan hiasan kutikel pada spikelet boleh digunakan sebagai ciri diagnosis sokongan dalam pengecaman spesies. Analisis multivariat dan filogenetik dengan menggunakan 30 ciri makromorfologi dan mikromorfologi menunjukkan bahawa terdapat hubungan evolusi yang rapat antara spesies *Cyperus*, *Bulbostylis* dan *Fimbristylis*. Pohon genetik menunjukkan hubungan evolusi yang rapat dengan nilai *bootstrap* yang kuat di antara *C. compressus* dan *C. sphacelatus*, manakala *C. kyllingia* dan *C. rotundus* mempunyai hubungan yang sederhana. Spesies lain menunjukkan hubungan evolusi yang lemah. Kesimpulannya, kajian ini telah berjaya menggandingkan ciri makromorfologi dan mikromorfologi dalam pengecaman Cyperaceae dan kekunci taksonomi berdasarkan makromorfologi telah berjaya dibina. Kekunci ini dapat dimanfaatkan oleh para pengkaji untuk mengecam spesies Cyperaceae dengan lebih sistematik terutamanya di Malaysia.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Cyperaceae Juss., the third largest monocotyledonous family comprises 109 genera and approximately 5,500 species, has a cosmopolitan distribution (Muasya et al., 1998; Govaerts et al., 2007) and has been resolved as monophyletic and sister to Juncaceae (Bruhl, 1995; Goetghebeur, 1998). Ridley (1925) was the first to do extensive study on Malay Peninsula and reported 3000 species in the world under 24 genera. While, there are 154 species and about 24 genera recorded from Malaya (Henderson, 1974). Turner (1995) had recorded 176 species within 29 genera (Appendix A). Species of Cyperaceae as contained within the plant list (2010) belong to 113 genera. The plant list includes 18,977 scientific plant names of species rank for the Cyperaceae (Appendix E). Of these 5,732 are accepted species names. The plant list includes a further 8,375 scientific plant names of infraspecific rank. These are primarily included because names of species rank are synonyms of accepted infraspecific names.

Cyperaceae are well represented in temperate, sub-arctic, and especially tropical regions worldwide from sea-level to over 5000 m (absent from Antartica). The family has several very large cosmopolitan genera including *Carex* L. (ca. 2000 spp.) and *Cyperus* L. (ca. 600 spp.; Goetgthebeur, 1998). Genus *Fimbristylis* (L.) Vahl is the fourth largest genus within the Cyperaceae, having 306 species including several homogenous subunits (Bruhl, 1995; Wilson, 2007) distributed worldwide in tropical and temperate zones. While Bailey (1963) stated that most species occur in mesic to hydric habitats, though the family is represented in almost all terrestrial environments and abundant in swampy regions. These species are widely distributed, with the centers of diversity for the group occurring in tropical Asia and tropical South America (Reznicek, 2012). A large family found in all parts of the world, usually in damp, wet places, and very common weeds of rice-field in Malaysia (Henderson, 1974).

Goetghebeur (1998) organized the family into four subfamilies and 14 tribes based on a combination of characters from flower, inflorescence and embryo morphology. Goetghebeur's (1998) scheme is as follows with subfamilies followed by their respective tribes parenthetically: 1. Mapanioideae (Hypolytreae, Chrysitricheae); 2. Cyperoideae (Scirpeae, Fuireneae, Eleocharideae, Abildgaardieae, Cypereae, Dulichieae, Schoeneae); 3. Scleriodeae (Cryptangieae, Trilepideae, Sclerieae, Bisboeckelereae, two monotypic genera *Incertasedis* including *Exochogyne* and *Koyamaea*; and 4. *Caricoideae* (*Cariceae*).

However, Kern (1974) organized the family into two subfamilies and five tribes 1. Subfamily Cyperoideae: i. Tribe Hypolytreae (*Scirpodendron, Capitularina,*

Lepironia, Thoracostachyum, Mapania, Paramapania, Hypolytrum; ii. Tribe Cypereae: (*Scirpus, Fuirena, Lipocarpha, Eleocharis*, Bulbostylis, *Fimbristylis*, *Cyperus*; iii. Tribe Rhynchosporeae: (*Tetraria*, *Costularia*, *Carpha*, *Lepidosperma*, *Tricostularia, Schoenus, Oreobolus, Cladium, Machaerina, Gahnia, Rhynchospora*: 2. Subfamily Caricoideae: iv. Tribe Sclerieae: (*Scleria*) v. Tribe Cariceae: *Kobresia, Carex, Uncinia.*

The family is closely related to the Gramineae or Poaceae, from which it differs in the often 3-ranked leaves, solid stems, the absence of pallets and of regular empty glumes, and the presence, in most cases, of a perianth and 3 carpels (Bailey, 1963). However, they differ in many characteristics, particularly in the structure of the inflorescence. Along with the similarities to grasses there are many features that distinguish sedges (such as usually leaves arranged in threes, and usually a solid stem that is triangular in cross section, and usually a conspicuously bracteate inflorescence), but they are not all observable with the naked eye (Archer, 2000).

The basic inflorescence unit in Cyperaceae is a spikelet. Eiten (1976) described the cyperaceous spikelet as a racemosely branched structure consisting of an axis (rachilla) of potentially indefinite growth bearing lateral true flowers, each subtended by a floral scale. Indeed, the branching pattern or ultimate branching orders of the inflorescence are important characters used to divide the family into subfamilies, tribes, and sub-tribes (Holttum, 1948; Eiten, 1976). Spikelet and inflorescence structure, together with other evidence, forms the basis for classification within the family (Archer, 2000). Because the spikelet is very small and the inflorescence structure very complex, interpretation is difficult and there is still

controversy over recognition of subfamilies, tribes and genera (Archer, 2000). Most novice cyperologists must first familiarize themselves with myriad inflorescence and spikelet arrangement of the family in their flora before attempting the use of diagnostic key, and before accurate identifications can be made. This task is complicated by the extremely contracted inflorescences and reduced floral parts. The family, though well defined, comprises many taxonomically challenging taxa.

Over the past 15 years, suprageneric relationships in Cyperaceae have been evaluated using both morphological and molecular data. Using phylogenies inferred from morphological data, Goetghebeur (1998) classified the family into four subfamilies and 15 or 17 tribes whereas Bruhl (1995) classified the family into two subfamilies and 12 tribes. Both workers recognized the same number of genera in the family but differed in their interpretation of homologies in some morphological characters, especially of homologies in some morphological characters, especially in the flower and spikelets. A phylogeny based on DNA sequence data of 40 genera in Cyperaceae (Muasya et al., 1998) showed some incongruence with both classifications. The placement of the juncaceous genus *Oxychloe* has been questioned.

The family has considerable economic importance; many members are serious agricultural weeds, whereas others provide food, fuel and medicines together with construction, weaving, and perfumery materials (Simpson & Inglis, 2001). They also have importance in conservation as dominant components of many wetland ecosystems and are reliable indicators of habitat deterioration in such systems. The rhizomes of several species of *Carex* were formerly used as a remedy in syphilis (Bailey, 1963). The chief importance of sedges lies in their forming a major natural

constituent of wetlands and riverside vegetation, where their densely tangled rhizomes contribute to erosion control and water purification (Archer, 1998).

Nowadays, the number of large cities is increasing. Although small communities can find the necessary water locally water demands of large cities are drawn from extensive drainage areas or aquifers. Therefore, in order to prevent water quality degradation wastewater should be treated properly prior to disposal. Archer (1998) reported that a modern usage for sedges is in artificially constructed water purification beds, because the rhizomes of several species are able to grow anaerobically, at least for a period of time.

We should also not forget that two of the world's worst weeds, *Cyperus rotundus* and *C. esculentus* hail from the sedge family (Archer, 2000). He also said that in irrigated lands, ploughing spreads their tubers and corms to such an extent that the crop plant is totally smothered, necessitating the use of herbicides to combat these weeds (Archer, 2000). The efficient ways to destroy the species is needed, so the study of morphological characters of the species is very important. Furthermore the morphological studies of this family can help to destroy the weed that compete with others crops more effectiveness. One species, *Cyperus rotundus*, has the dubious reputation of being termed "the world's worst weed" (Holm et al., 1977). Nowadays the cost of pesticides to destroy this species is very high. The new ways must be discovered to destroy this weed in more effectives way with a minimize cost. The infestation pattern of weed species in Muda area changed between their surveys due to continuous adoption of a single weed control method. Repeated use of a particular herbicide greatly influenced weed species composition and dominance. Ismail et al.

(1998) observed that widespread use of molinate herbicide which selectively suppressed *Echinochloa crus-galli*has resulted in the gradual increase of *Leptochloa chinensis*. The plots repeatedly applied with 2-4 D amine resulted in drastic increased of *Echinochloa crus-galli* (Azmi, 2002) and *Fimbristylis miliacea* (Watanabe, 2000), whereas effective in suppressing *Scirpus grossus* and *Monochoria vaginalis* (Azmi, 2002). Therefore, Moody (1989) stated that herbicide use moves the agroecosystem towards low species diversity with new problem weeds appearing, stressing the need for an ecological approach to weed control. Moreover, annual weeds react very quickly to alternation of their environment.

1.2 Background of the Study

The morphology of the Cyperaceae has received attention from taxonomists for many years but the data gathered and used by them are largely from dissection and superficial examination. Since modern morphology has shown the importance of microscopic study, the more basic structure in this specialized family should be of value in tracing ancestry and specialization. Three genera used in these studies are *Bulbostylis*, *Cyperus* and *Fimbristylis*.

Furthermore, this research is very important to develop a resource base for Cyperaceae which will be available and of interest to a wide range of stakeholders, especially in conservation and sustainable use and for other researcher in further study. We hope to construct a key to species of the sedges which larger research involving families of high conservation value could be based. It will help to develop conservation assessments for Cyperaceae species in Malaysia and will allow

determination of priorities or provision of advice for both habitat and species conservation to be carried out more effectively and with greater accuracy.

In this study the focus is on the Cyperaceae in Perak which is the second largest state in Peninsular Malaysia with 21,006 km² land area, comprise of 6.4% the whole Malaysia by Emap (2009) and many types of forest and developed areas. Perak is also unique because it has great influence factors: Perak subprovince at the north-west part, Titiwangsa main range, and seasonal asiatic intrusion from the Asian mainland (Wong, 1998). In addition, Perak has a huge limestone hills complex, which many studies show that limestone hills are rich with endemic plant species (Wong 1998; Fatimah 2009).

In this study, the focus is on the wetland, open areas, which are known to be dominated by many Cyperaceae. The information is on the current, composition, abundance, importance and sedge status is needed for further study. Currently, a total of 31 species of *Cyperus* was recorded in FRIM for Perak. The selected sampling areas are Tanjong Malim, Gunung Lang, Ipoh, Pulau Banding, Gerik, and Chenderoh Lake, Lenggong. The four areas represent disturbed and less disturbed areas. Temengor is selected it is an 'Environmentally sensitive area' to disallow development, agriculture and logging. Tanjong Malim has abundant of plantations, waste land and quarries. Ipoh is selected because this area has limestone hills and maintained with regular cuttings and weedicide sprayings. While, Gerik is selected because it borders with Kelantan and close to Titiwangsa range which is less disturbed and near to the wetland, Pulau Banding. Chenderoh Lake is selected because it is a big

wetland interconnected with several small lakes and rivers, and this area is less developed.

In this study, the phylogeny of the Cyperaceae was evaluated using the macromorphological and micromorphological data sets. These analyses represent the first step in developing a comprehensive phylogeny analyses of the family.

1.3 Problem Statement

There is no comprehensive study on Cyperaceae in Malaysia for 89 years since Ridley (1925). Turner (1995) had listed the genera and species but the comprehensive studies on taxonomy and morphology of Cyperaceae was not done. Kern and Nooteboom (1983) covered the flora of Malesia. Henderson (1954) had described the brief of description of Cyperaceae. Yet, there are not much scientific research carried out to explore the family of Cyperaceae in Malaysia. The current revision is very important to record the status of sedges in Peninsular Malaysia as many of the species shows economic and biological importance. There is currently insufficient taxonomic capacity to keep abreast of the rate of new discoveries as our herbaria already contain numerous unnamed species (e.g. FRIM Herbarium (KEP), Universiti Putra Malaysia Herbarium, Universiti Pendidikan Sultan Idris Herbarium and others in Malaysia).

The taxonomy of the sedges genera is complex, and under review by botanists. Recent studies have resulted in the creation of several new genera, including the genera *Schoenoplectus* and *Bolboschoenus*; others including *Blysmus*, *Isolepis*, *Nomochloa*, and *Scirpoides* have also been used (Wilson, 2012).

Thus, this research aims to map the diversity of Cyperaceae in Perak to represent Peninsular Malaysia. The information of sedges species and its diversity and distribution will be important for future references.

There is conflict in identification of Cyperaceae species. Many of previous taxonomists used different diagnostic keys to identify the species and very much rely on reproductive morphology such as inflorescence, spikelets, glumes and nut. Those characters are known as macromorphology. In addition to macromorphological characters, Sminitand (1993), also use plant habit as part of his identification key. This research focuses not only focus on comprehensive macromorphological but also complemented by micromorphological characteristics. Simpson (2003) in pollen research on another species successfully discovered based on the specialization of some taxa in terms of their pollination biology and taxanomical significant. The key of genera and species using spikelets need to review for diagnose the best morphological characteristics used to identify Cyperaceae.

Many phylogenetic research that focus on certain DNA, but the placement of Cyperaceae does not resolved. In this study we evaluated the phylogeny of Cyperaceae using gross macromorphological and micromorphology data sets, analysed both individually and in combination. These analyses represent the first step in developing a comprehensive phylogeny of the family based on macromorphological and micromorphological data.

1.4 Research Objectives

The primary objectives of this research were to review and diagnose the best morphological characteristics used by the previous researchers. Species studied during the course of this research included *Cyperus compactus, C. compressus, C. digitatus, C. distans, C. iria, C. kyllingia, C. pulcherrimus, C. rotundus, C. sphacelatus, C. pilosus,* while the outgroup species are *Bulbostylis barbata*, *Fimbristylis dichotoma* and *F. miliacea*. Results was derived from a macromorphological and micromorphological study based on the examination of specimens from herbaria, supplemented with field studies in Perak. An enumeration of the specific problems investigated are as follows:

i) To review and diagnose the best macromorphological and micromorphology characteristic used to identify Cyperaceae.

ii) To construct key to species of Cyperaceae.

iii) To survey the diversity of Cyperaceae.

iv) To map the distribution of Cyperaceae.

1.5 Research Questions

i) What are the best macromorphological and micromorphology characters of taxonomic value of each species of Cyperaceae?

ii) What are the practical key characteristics that will characterise the species?

iii) How diverse is the species of Cyperaceae?

iv) What is the distribution of Cyperaceae?

1.6 Significance of the Study

i) We can review their viability and identify diagnostic characters used by the previous researchers.

ii) The practical key to species can be constructed in this research that will be used by other researchers.

iii) The knowledge of number, type, and economic important of species in Cyperaceae can be used by other researchers and contribute to conservation and weed control.

iv) The map of the Cyperaceae diversity is available for other references.

1.7 Research Frameworks

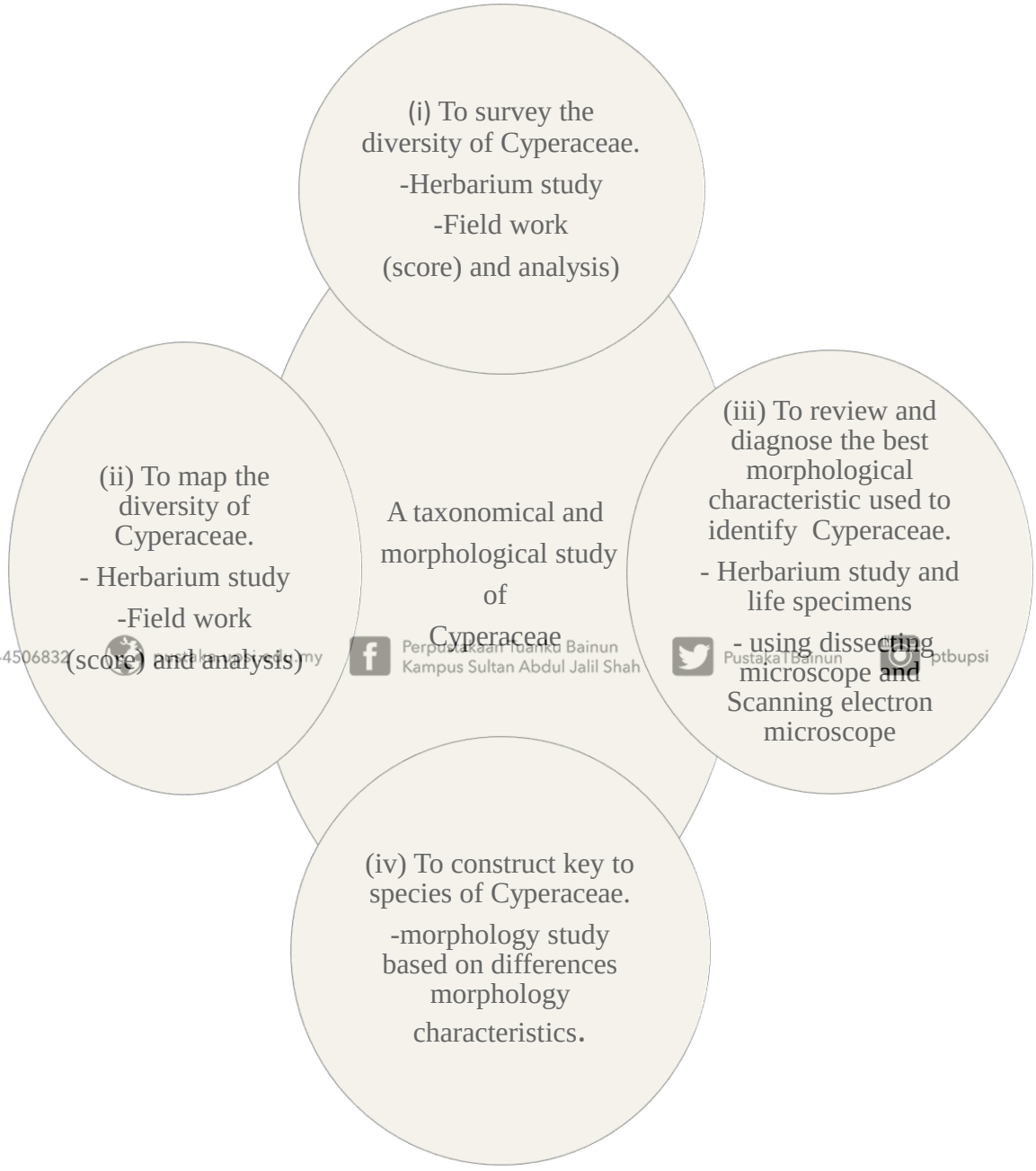

Figure 1.1. Research Frameworks

Taxonomy has had various meanings over the past one hundred and fifty years, and particular confusion has prevailed with systematic. The early documented use of the term systematic can be traced (as systematic botany) at least as far back as Linnaeus (1753, 1754), and it has persisted to the present day although in modified form. Linnaeus (1753) states that "we reject all the names assigned to plants by anyone, unless they have been either invented by the systematist or confirmed by them".

The research framework was divided into four objectives (Figure 1.1). This studies mostly use the macromorphological and a few micromorphological features (using SEM) and accordingly grouped based on similarities and/or differences was (and still is) called classification. Data are gathered from surveys and their morphology characteristics used to answer question about classification, phylogeny, and the process of evolution.

The surveys done in herbarium at FRIM, Kepong and field work was done in four areas. From the surveys, it was used to map the diversity and distribution of Cyperaceae. To diagnose the best morphological characteristic used to identify the species.

The key to species of *Cyperus* used spikelets to constructed using the morphological characteristics. The key is very important to recognize the species. In this study, we are leveraging of fieldwork and undertake revision of the sedge genus *Bulbostylis*, *Cyperus*, and *Fimbristylis* and the embedded genera of tribe Cypereae (in Family Cyperaceae) under Kern (1974) classification. *Cyperus* is among the third largest flowering plant genera worldwide.