Universidade de São Paulo

Desenvolvimento, anatomia e evolução de caracteres florais em Cyperaceae (Poales)

Mariana Maciel Monteiro

São Paulo
2020

Mariana Maciel Monteiro

DESENVOLVIMENTO, ANATOMIA E EVOLUÇÃO DE CARACTERES FLORAIS EM CYPERACEAE (POALES)

DEVELOPMENT, ANATOMY AND EVOLUTION OF FLORAL CHARACTERS IN CYPERACEAE (POALES)

São Paulo

2020

Universidade de São Paulo

Desenvolvimento, anatomia e evolução de caracteres florais em Cyperaceae (Poales)

Development, anatomy and evolution of floral characters in Cyperaceae (Poales)

Mariana Maciel Monteiro

Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para obtenção de Título de Doutor em Ciências, Programa: Ciências Biológicas (Botânica)

Orientador: Prof. Dr. Diego Demarco

São Paulo

2020

Ficha Catalográfica

Monteiro, Mariana Maciel.
Desenvolvimento, anatomia e evolução de caracteres florais em Cyperaceae (Poales) / Mariana Maciel Monteiro; orientador Diego Demarco -- São Paulo, 2020.

168 páginas.

Tese (Doutorado) – Instituto de Biociências da Universidade de São Paulo. Departamento de Botânica.

1. Cyperaceae. 2. Cyperoideae. 3. Mapanioideae. 4. Ontogenia. 5. Anatomia. I. Demarco, Diego. II. Título.

Bibliotecária responsável pela catalogação: Elisabete da Cruz Neves. CRB - 8/6228.

Comissão Julgadora:

_____	_____
Prof(a). Dr(a).	Prof(a). Dr(a).
_____	_____
Prof(a). Dr(a).	Prof. Dr. Diego Demarco Orientador

Dedico aos pós-graduandos desse país, que mesmo com pouco incentivo, respeito e motivação, dedicam sua vida e sua saúde (mental e física) e movem a ciência no Brasil.

“It is important that students bring a certain ragamuffin, barefoot irreverence to their studies; they are not here to worship what is known, but to question it.”

Jacob Bronowski

The ancient man

AGRADECIMENTOS

Apesar de levar meu nome como personagem principal, esse trabalho é fruto da colaboração, apoio e incentivo de pessoas que foram fundamentais nessa caminhada. Assim, agradeço imensamente:

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão de bolsa de estudos no país e no exterior (PDSE-PrInt EDITAL Nº 01/2019 - 88887.368005/2019-00). O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001. Ao edital PRPG 03/2017 de Mobilidade Internacional Santander pelo auxílio recebido.

À Universidade de São Paulo pela infraestrutura que permitiu a execução desse trabalho e pela participação na minha formação.

Ao meu orientador, Dr. Diego Demarco, por me receber e aceitar o desafio de me orientar com Cyperaceae, sem mesmo me conhecer. Agradeço a confiança que sempre teve no meu trabalho, pelo respeito as minhas escolhas e pelos conselhos que ajudaram a me construir profissionalmente.

À Dr. Aline Oriani, pelo apoio e tempo dedicado a esse trabalho, sempre colaborando e incentivando meu senso crítico. Sua colaboração e amizade são extremamente importantes para mim. Agradeço os seus conselhos, que vão além da vida acadêmica e os levarei eternamente comigo.

Many thanks to Dr Louis Ronse De Craene (The Royal Botanic Gardens, Edinburgh), Dr Gerhard Prenner and Msc Chrissie Prychid (The Royal Botanic Gardens, Kew) for receiving and supervising me in their respective Institutions during the execution of this Thesis. Unquestionably, working with each of you transcended the scientific relevance and taught me a fundamental point of view towards respect and collaboration.

Many thanks to Dr. Anna Heigh, curator of Monocots collection at The Royal Botanic Gardens, Kew, for your assistance and prompt help with the herbarium samples. To Frieda Christie, Microscopy technician, for the assistance with SEM during my time at The Royal Botanic Gardens, Edinburgh.

Many thanks to Maria Conejero, Imaging/EM Lab Technician at Jodrell Laboratory (The Royal Botanic Gardens, Kew) for your kind assistance, patience and prompt help with SEM, as well as for the friendship, for cheering my working days and for the enriching talks that I will always take with me.

À Dr. Vera Lucia Scatena, pelo incentivo e por me pressionar a ir além do que creio que posso ir. Obrigada por acreditar em mim, por me direcionar e me ensinar a me respeitar como pessoa e pesquisadora. A você minha eterna admiração.

À Dr. Nanuza Luiza de Menezes pela inspiração e conversas motivacionais que resgataram tantas vezes dentro de mim o sentido das minhas escolhas. Aprendi muitas coisas na nossa convivência, mas um de seus ensinamentos mais marcantes para mim é a sua preocupação genuína, aquela que transcende somente a boa educação e que nos fortalece de saber que alguém se importa. Obrigada.

Ao Dr. Paulo Takeo Sano, que me proporcionou a experiência de conhecer a Serra do Cipó, uma viagem que resgatou em mim o sentido das minhas escolhas. Sua lucidez e sensibilidade são virtudes que me inspiram.

Aos membros da banca examinadora por disporem seu precioso tempo neste trabalho.

Aos técnicos do Laboratório de Anatomia Vegetal, Gisele Costa, Tássia Santos e Irwandro Pires pelo apoio durante a execução deste trabalho. Agradeço igualmente aos colegas de trabalho e professores do Laboratório de Anatomia Vegetal, com os quais sem dúvida tive a oportunidade de dividir experiências e aprendizados.

Às minhas companheiras de sala, Ellenhise Ribeiro, Maria Camila Medina e Natalie Capelli pelo companheirismo, paciência e cumplicidade. É maravilhoso quando temos a oportunidade de conviver com pessoas que admiramos e que nos ensinam tanto, cada uma de vocês tem um papel essencial na pessoa que sou. Nossos papos filosóficos e a leveza da nossa amizade tornaram essa trajetória prazerosa e enriquecedora. Com o mesmo carinho, agradeço à Thália Gama e Karina Gagliardi, que apesar de não estarem perto fisicamente, os laços que criamos serão eternos e a amizade, carinho e ajuda de vocês no início da minha trajetória na USP foram essenciais e tornaram tudo mais leve. A vocês todo meu carinho e admiração.

Aos meus amigos do Laboratório de Sistemática Vegetal, em especial à Gisele Alves, Marcelo Kubo e Guilherme Antar. Agradeço por estarem sempre por perto e sempre

dispostos em dividir comigo os desafios e as alegrias. Conviver com vocês foi um imenso aprendizado e exemplo, levarei um pouco de cada um comigo por onde eu for.

Aos amigos que tornaram a distância de casa mais curta e me proporcionaram momentos inesquecíveis durante meu estágio no Kew Gardens, em especial ao Marcelo Kubo, Guilherme Antar, Karine Valdemarin, Arthur de Souza, Raquel Negrão, Maycon Jordan, Ethiéne Guerra, Luciana Silva, Tarciso Leão, Maria Conejero e Roberta Gargiulo. Dividir a experiência desse lugar fantástico com pessoas admiráveis como vocês, não tem preço.

Many thanks to my landlady Mary Stephens, for the incredible talks and that hilarious good humour that made me feel so good living at your house. Also, thanks to Katrina and Adam for showing me a lovely side of England.

Agradeço aos meus amigos de sempre, aqueles que cresceram comigo e fizeram parte da formação da minha personalidade, aqueles que encontrei durante a faculdade e que formamos laços que jamais se romperam, aqueles que passaram pela minha vida nesses longos anos de formação e que foram essenciais para que eu continuasse minha caminhada.

Agradeço à Deus e a minha família, de sangue e de alma, que sempre me deram força de seguir em frente, em especial a minha mãe, fonte da minha honestidade e acolhimento, e ao meu pai, fonte da minha coragem e disciplina. Ao meu companheiro Augusto Giaretta, que me ensinou que tristeza compartilhada se torna mais leve e alegria compartilhada se multiplica, você é minha inspiração.

RESUMO

Cyperaceae possui ca. 5500 espécies distribuídas em 87 gêneros e divididas em duas subfamílias: Cyperoideae (76 gêneros) e Mapanioideae (11 gêneros). Inflorescências em Cyperaceae apresentam uma ampla variação em número de eixos laterais, ordens e comprimentos que resultam em uma variedade de arquiteturas, levando a muitas interpretações controversas. A família apresenta inflorescências descritas como panículas compostas com vários eixos laterais; entretanto, diferente da descrição usual de panículas, os eixos laterais não terminam em flores e sim, em estruturas reprodutivas que diferem entre as subfamílias. Em Cyperoideae, os eixos laterais terminam em espiguetas contendo várias flores. Em Mapanioideae, os eixos laterais terminam em espigas, que ao invés de conterem flores em cada bráctea, apresentam várias unidades denominadas espicoides. Os espicoides já foram previamente interpretados tanto como flores únicas, assim como inflorescências reduzidas. Considerando tais particularidades, esta tese teve como objetivo investigar os caracteres morfológicos de espiguetas e espicoides através de uma abordagem da anatomia, desenvolvimento e aspectos evolutivos. Espiguetas e espicoides de várias espécies foram analisados através de técnicas de microscopias de luz e eletrônica de varredura, testes fisiológicos e reconstruções de caracteres ancestrais. Os resultados mostraram que mudanças no início do desenvolvimento, principalmente reguladas por pressões mecânicas, parecem influenciar a variação no número e posição dos órgãos florais nas subfamílias. Além disso, caracteres florais (i.e. estilopódio) parecem ter uma notável influência no sucesso da germinação e na diversidade de gêneros de Cyperoideae. Para Mapanioideae, os resultados mostraram que processos no desenvolvimento sugerem ser consistentes na mesma tribo e o desenvolvimento dos espicoides do gênero monotípico *Diplasia* favoreceu sua interpretação como uma inflorescência reduzida. Finalmente, enfatiza-se o valor em entender as variações morfológicas dos espicoides em Mapanioideae como uma fonte de caracteres informativos para uma subfamília com notáveis relações filogenéticas inconclusivas. Tais resultados reforça a importância de combinar estudos anatômicos, ontogenéticos e evolutivos com o intuito de elucidar aspectos sobre diversidade e relações em uma família excepcional como Cyperaceae.

Palavras chave: Cyperaceae, Cyperoideae, Mapanioideae, ontogenia, anatomia.

ABSTRACT

Cyperaceae comprise ca. 5500 species distributed in 87 genera divided in two subfamilies: Cyperoideae (76 genera) and Mapanioideae (11 genera). Inflorescences in Cyperaceae exhibits a wide range of variation in number of lateral axes, orders and length that results in a variety of architectures, leading to many controversy and misinterpretation. The family exhibits inflorescences described as compound panicles with several lateral axes; however, different from the usual description of panicles, the lateral axes do not end in flowers, instead they hold reproductive units that differ between subfamilies. In Cyperoideae the lateral axes end in spikelets which hold several flowers. In Mapanioideae the lateral axes end in spikes, but instead of holding flowers in each bract, they hold several units known as spicoids. The spicoids have been interpreted either as a single flower or as a reduced inflorescence. Considering such particularities, this thesis aims to investigate the morphological features of spikelets and spicoids through a survey of their anatomical, developmental and evolutionary aspects. Spikelets and spicoids of several species were analyzed through scanning electron and light microscopy, added to physiological tests and ancestral character reconstruction. The results showed that changes in the beginning of development, mostly regulated by mechanical constraints, appears to influence the variation in number and position of floral organs in both subfamilies. Moreover, floral traits (i.e. stylopodium) seems to have a notable influence in the germination success and diversity of Cyperoideae genera. For Mapanioideae, our results show that developmental processes appear to be consistent within tribes and the development of the spicoid in the monotypic genus *Diplasia* favours to interpret it as a reduced inflorescence. At last, we emphasized the value of understanding the morphological variation of spicoids in Mapanioideae as a source of informative features to a subfamily with notable inconclusive phylogenetic relations. Such results reinforce the importance of combining anatomical, developmental and evolutionary studies in order to elucidate the diversity and relationships in an outstanding family such as Cyperaceae.

Keywords: Cyperaceae, Cyperoideae, Mapanioideae, ontogeny, anatomy.

.

General Introduction

GENERAL INTRODUCTION

Cyperaceae comprise ca. 5500 species distributed in 87 genera occurring in a wide variety of environments (Govaerts *et al.*, 2018). The family is recovered in two subfamilies, Cyperoideae and Mapanioideae (Simpson *et al.*, 2007; Muasya *et al.*, 2009, Hinchliff & Roalson, 2013; Semmouri *et al*., 2019). Cyperoideae consist of 76 genera with a pantropical distribution among which *Carex* L. (1997 spp.), *Cyperus* L. (955 spp.) and *Rhynchospora* Vahl (361 spp.) exhibit the highest number of species (Goetghebeur, 1998; Simpson *et al.*, 2007; Muasya *et al.*, 2009; Govaerts *et al.*, 2018). Alternatively, Mapanioideae consist of 11 genera (five monotypic) with a more restricted distribution (Simpson *et al*., 2007; Muasya *et al.*, 2009) being *Mapania* Aubl. (100 species) and *Hypolytrum* Pers. (61 species) the most species-rich genera (Simpson, 1992; Alves *et al.*, 2001; Govaerts *et al.*, 2018).

The differences between the subfamilies are not only restricted to species richness, but also concerning their particular inflorescence and floral features. The general architecture of Cyperaceae inflorescence consist of a main axis from which the ramifications of successive orders originate, thus the whole structure is known as a synflorescence. Each ramification of the synflorescence comprises a group of reproductive units, known as coflorescence (Goetghebeur, 1998; Vegetti, 2003; Guarise & Vegetti, 2008).

Cyperoideae synflorescences are interpreted as compound panicles with several lateral axes that terminate in spikelets, the smallest unit in the whole inflorescence. The spikelet is described as an open spike composed of an axis holding several bracts that may or may not subtend a flower (Eiten, 1976; Bruhl, 1995; Goetghebeur, 1998; Vegetti, 2003). Flowers in Cyperoideae exhibit a typical Bauplan of two trimerous perianth whorls, one whorl of stamens and a trimerous gynoecium (Vrijdaghs *et al*., 2009). In Mapanioideae, a synflorescence with a main axis holding several lateral axes resembles Cyperoideae architecture. However, in the former the lateral axes terminate in spikes which instead of holding flowers, it holds several reproductive units named

spicoid (Kern, 1974; Simpson, 1992; Bruhl, 1995; Goetghebeur, 1998). The spicoids have been described as flowers, because of their structure composed of two bracts, two stamens and a central gynoecium (Kern, 1974; Eiten, 1976; Goetghebeur, 1998). However, for some species, the spicoids exhibit bracts between stamens and gynoecium, which raised questions whether it should be interpreted as a reduced inflorescence where each stamen is considered a unisexual male flower and the central gynoecium, a female flower (Dahlgren *et al*., 1985; Simpson, 1992; Bruhl, 1995; Richards *et al.*, 2006; Prychid & Bruhl, 2013; Monteiro *et al.*, 2016, submitted).

Considering that spikelets (Cyperoideae) and spicoids (Mapanioideae) are the functional units of the inflorescence (Snell, 1936; Holttum, 1948; Eiten, 1976; Dahlgren *et al.*, 1985; Simpson, 1992; Vegetti, 2003; Vrijdaghs *et al*., 2009), it is fundamental to investigate the morphological variation that undergoes in such structures in order to elucidate the possible factors influencing such changes and how consistent and informative they are for the relationships in each subfamily.

SPIKELET AND FLORAL VARIATION IN CYPEROIDEAE

Two main aspects are remarkable in Cyperoideae: 1) the wide diversity of floral morphology among and within genera and; 2) the high number of species concentrated in few genera (*Eleocharis* R.Br., *Fimbristylis* Vahl, *Rhynchospora* Vahl, *Bulbostylis* Kunth, *Carex* L., *Cyperus* L. and *Scleria* P.J.Bergius).

Variation on floral morphology in Cyperoideae

The morphological variation in the spikelets is mainly related to the arrangement of the bracts (glumes) along the axis (phyllotaxis), their fertility and floral sexuality (Dahlgren *et al.*, 1985). However, interpreting the morphology in mature flowers may hamper their understanding due to their reduced and complex nature. Therefore, it is fundamental to investigate processes occurring during the floral development, especially at the organ initiation, that may affect each floral whorl and consequently the final floral morphology (Ronse De Craene, 2016, 2018). It is

well known that genetic interaction regulates the identity of floral organs, however morphological factors such as mechanical constraints of surrounding organs (i.e. bracts) are also fundamental in modulate the final morphology of the flowers (Chandler, 2014; Ronse De Craene, 2018). Such approach led to the first chapter of this thesis:

1. The effect of spatial constraints on changes in floral development of Cyperoideae spikelets (Cyperaceae) – This chapter focus on elucidating the influence of spatial constraints, mainly applied by glumes, on the further development of flower primordia in Cyperoideae species. Floral development and anatomy were investigated in species varying in phyllotaxis and floral sexuality. This study was developed in The Royal Botanic Garden, Edinburgh, Scotland (Mobilidade Internacional Santander) and it is under review in *The Botanical Journal of the Linnean Society*.

Morphological traits on the most diverse genera of Cyperoideae

It is notable that despite the diversity of genera in Cyperoideae, the highest species number are concentrated in about seven genera: *Carex* (1997 spp.), *Cyperus* (955 spp), *Rhynchospora* (361 spp.), *Fimbristylis* (321 spp.), *Eleocharis* (295 spp.), *Scleria* (258 spp.) and *Bulbostylis* (220 spp.) comprising together ca. 80% of the total number of species in the family (Escudero & Hipp, 2013). The major radiation of these genera occurred when the climate was becoming drier (Escudero & Hipp, 2013) which may be associated with the acquisition of some morphological traits that in the right ecological or genetic condition, may have improved their reproductive success (Ronse de Craene *et al*., 2003; Endress, 2010a, 2011; Escudero & Hipp, 2013). Curiously, for these seven genera, except for *Carex* and *Cyperus*, a noticeable reproductive feature is shared: a thickening at the base of style right on top of the ovary apex, known as "stylopodium". Such structure occurs in non-phylogenetic related genera (Muasya *et al*., 2009; Hinchliff & Roalson, 2013; Semmouri *et al*., 2019). It becomes distinct in the mature flower and may be persistent (as a beak) in the mature nutlet (Cyperaceae fruit) or detach from the nutlet with the rest of the style (Vrijdaghs *et al*., 2004, 2009; Gonzalez & López, 2010; Reutemann *et al.*, 2012; Monteiro *et al.*, 2017). The origin of the

stylopodium is unclear and its function has been a matter of debate, mainly associated with a possible role in facilitating water entrance for seed germination, but never tested before. Such questions led to the second chapter of this thesis:

2. Development and function of the stylopodium in Cyperoideae (Cyperaceae): a potential adaptative innovation – This chapter aimed to analyze the stylopodium of five Cyperoideae species to assess the ontogenetic origin, identify the mechanism of detachment and any structure related to water supply. Moreover, we investigate the physiological influence of the stylopodium in seed germination. We intend to submit this study to *International Journal of Plant Sciences*.

SPICOID AND FLORAL VARIATION IN MAPANIOIDEAE

Mapanioideae exhibit a considerably lower number of genera and species compared to Cyperoideae (Goetghebeur, 1998; Govaerts *et al*., 2018) and the latter comprises the vast majority of studies in terms of development of flower, inflorescence and phylogenetic relationship (i.e. Vrijdaghs *et al*., 2004, 2005a, 2005b, 2007, 2009, 2010, 2011; Muasya *et al*., 2009; Reutemann *et al.*, 2012; Hinchliff & Roalson, 2013; Monteiro *et al.*, 2017; Semmouri *et al*., 2019). Therefore, many gaps remain towards the knowledge of Mapanioideae considering the nature and morphological variation of the spicoids. Some aspects in this matter are remarkable such as: 1) It is unclear if a pattern of development is shared among Mapaniodeae spicoids. 2) Little is known about the morphological variation of the spicoids among genera and their significance in an evolutionary perspective.

Development patterns on Mapanioideae spicoids

Considering the patterns on floral development, in Cyperoideae despite the wide diversity in floral morphology, the floral development was reported as following a general scirpoid ontogenetic pattern, in which the first organs to appear are the stamen primordia, followed by gynoecium primordium and later on, if present, perianth parts (Vrijdaghs *et al*., 2009). For Mapanioideae, developmental studies mainly focus on the debated nature of the spicoids as flowers

or inflorescences (Richards *et al.*, 2006; Prychid & Bruhl, 2013; Monteiro *et al.*, 2016), lacking a comparative approach among the development processes in such species. Taking that in account, it led to the third chapter of the thesis:

3. Spicoid ontogeny in *Diplasia* (Cyperaceae): an approach on the developmental processes operating in Mapanioideae spicoids – This chapter has a complete ontogenetic analysis of the spicoid of the monotypic genus *Diplasia* and compare these data with other developmental studies for Mapanioideae (Richards *et al.*, 2006; Prychid & Bruhl, 2013; Monteiro *et al.*, 2016) in order to elucidate the variability of developmental features and their influence in the final morphology of Mapanioideae spicoids. Furthermore, we discuss evidence that may help to interpret *Diplasia* spicoid as a flower or inflorescence. This is study was developed in The Royal Botanic Gardens, Kew (Doutorado sanduíche – PDSE/ CAPES) and is under review in *Plant Systematics and Evolution*.

Morphological variation on Mapanioideae spicoids: an evolutionary perspective

It is notable the importance of understanding the morphological features of spicoids for each Mapanioideae genera, for which most of the information are restrict to taxonomic descriptions (i.e. Holttum, 1948; Kern, 1974; Eiten, 1976; Bruhl, 1995; Simpson, 1992; Goetghebeur, 1998; Alves *et al*., 2001). The lack of an evolutionary perspective towards variation in the spicoid morphology also reflects in the high level of uncertainty in the phylogeny of Mapanioideae (Simpson *et al.*, 2007; Muasya *et al*., 2009; Hinchliff & Roalson, 2013; Spanlik *et al*., 2016; Semmouri *et al*., 2019). The value of reproductive traits to establish relationships led to the fourth chapter of this thesis:

4. Spicoid morphology of Mapanioideae (Cyperaceae): an evolutionary perspective – In this chapter we analyzed 49 species from the 11 genera of Mapanioideae, corresponding to ca. 27% of the total number of species from the subfamily. We focused on stable and variable features of the spicoids and mapped such features in the molecular phylogeny (Semmouri *et al*., 2019), through

a character reconstruction. Thus, we could infer about the ancestral spicoid morphology and their significance in an evolutionary perspective. This is study was developed at The Royal Botanic Gardens, Kew (Doutorado sanduíche – PDSE/ CAPES) and we intend to submit it to *American Journal of Botany*.

LITERATURE CITED

Alves M, Thomas WW, Wanderley MGL (2001) Typology of the inflorescence in species of *Hypolytrum* Rich. (Cyperaceae) from Brazil. *Beiträge zur Biologie der Pflanzen* 72: 59-73.

Bruhl JJ (1995) Sedge genera of the world: relationships and a new classification of the Cyperaceae. *Australian Systematic Botany* 8: 125-305.

Chandler JW (2014) Patterns and polarity in floral meristem and floral organ initiation. *Critical Reviews in Plant Sciences* 33:457-469.

Dahlgren R, Clifford H, Yeo P (1985) *The families of the monocotyledons: evolution and taxonomy*. Springer-Verlag: Berlin.

Eiten LT (1976) Inflorescence units in the Cyperaceae. *Annals of the Missouri Botanical Garden* 63: 81-112.

Endress P (2010a) Flower Structure and Trends of Evolution in Eudicots and Their Major Subclades1. *Annals of the Missouri Botanic Gardens* 97:541–583.

Endress P (2011) Evolutionary diversification of the flowers in angiosperms. *American Journal of Botany* 98: 370–396.

Escudero M, Hipp A (2013) Shifts in diversification rates and clade ages explain spcies richness in higher-level sedges taxa (Cyperaceae). *American Journal of Botany* 100(12):24003–2411.

Goetghebeur P (1998) Cyperaceae. In: Kubitzki K, ed. *The families and genera of vascular plants*. Springer: Berlin, Germany, 141–190.

Gonzalez AM, López MG (2010) Development and morphology of the gynoecium and nutlet in two South-American *Bulbostylis* (Cyperaceae) species. *Flora* 205:211–220.

Govaerts R, Simpson DA, Goetghebeur P, Wilson KL, Egorova T, Bruhl JJ (2018) *World checklist of Cyperaceae*. Kew: The Board of Trustees of the Royal Botanic Gardens, Kew. Available at: http://www.kew.org/wcsp/monocots/

Guarise NJ, Vegetti AC (2008) The inflorescence structure of *Cyperus* L. section *Luzuloidei* Kunth (Cyperaceae). *Plant Systematics and Evolution* 271: 41-63.

Hinchliff CE, Roalson EH (2013) Using supermatrices for phylogenetic inquiry: an example using the sedges. *Systematic Biology* 62(2): 205-219.

Holttum RE (1948) The spikelets in Cyperaceae. *Botanical Review* 14: 525-541.

Kern JH (1974) Cyperaceae. In: van Steenis CGGJ, ed. *Flora Malesiana*, 435-753. Noordhoff: Leyden, The Neth, 435-753.

Kukkonen I (1984) On the inflorescence structure in the family Cyperaceae. *Annales Botanici Fennici* 21: 257-264.

Monteiro MM, Scatena VL, Oriani A (2016) Anatomy and development of the reproductive units of *Mapania pycnostachya* and *Hypolytrum schraderianum* (Mapanioideae, Cyperaceae). *Australian Journal of Botany* 64: 389-400.

Monteiro MM, Scatena VL, Oriani A (2017) Comparative floral anatomy of *Rhynchospora consanguinea* and *Rhynchospora pubera* (Cyperoideae, Cyperaceae). *Plant Systematics and Evolution* 303: 283-297.

Muasya AM, Simpson DA, Verboom GA, Goetghebeur P, Naczi RFC, Chase MC, Smets E (2009) Phylogeny of Cyperaceae based on DNA sequence data: current progress and future prospects. *Botanical Review* 75: 2-21.

Prychid CJ, Bruhl JJ (2013) Floral ontogeny and gene protein location rules out euanthial interpretation of reproductive units in *Lepironia* (Cyperaceae, Mapanioideae, Chrysitricheae). *Annals of Botany* 112: 161–177.

Reutemann AG, Lucero L, Guarise N, Vegetti AC (2012) Structure of the Cyperaceae inflorescence. *Botanical Review* 78: 184–204.

Richards JH, Bruhl JJ, Wilson KL (2006) Flower or spikelet? Understanding the morphology and development of reproductive structures in *Exocarya* (Cyperaceae, Mapanioideae, Chrysitricheae). *American Journal of Botany* 93: 1241-1250.

Ronse De Craene LP (2016) Meristic changes in flowering plants: How flowers play with number. *Flora* 221: 22-37.

Ronse De Craene LP (2018) Understanding the role of floral development in the evolution of angiosperm flowers: a clarification from different perspectives. *Journal of Plant Research* 131: 367-393.

Ronse De Craene LP, Soltis PS, Soltis DE (2003) Evolution of floral structures in basal Angiosperms. *International Journal of Plant Sciences* 164: 329–363.

Semmouri I, Bauters K, Léveille-Bourret E, Starr JR, Goetghebeur P, Larridon I (2019) Phylogeny and systematics of Cyperaceae, the evolution and importance of embryo morphology. *Botanical Review* 85: 1-39.

Simpson DA (1992) *A revision of the genus Mapania. Royal Botanic Gardens*, Kew, Publishing: London.

Simpson DA, Muasya AM, Alves M, Bruhl JJ, Dhooge S, Chase MW, Furness A, Ghamkhar K, Goetghebeur P, Hodkinson TR, Marchant AD, Reznicek AA, Nieuwborg R, Roalson EH, Smets E, Starr JR, Thomas WW, Wilson KL, Zhang X (2007) Phylogeny of Cyperaceae based on DNA sequence data: a new *rbc*L analysis. *Aliso* 23: 72-83.

Snell RS (1936) Anatomy of the spikelet and flowers of *Carex, Kobresia* and *Uncinia*. *Bulletin of the Torrey Botanic Club* 63: 277-295.

Spalink D, Drew BT, Pace MC, Zaborsky JG, Starr JR, Cameron KM, Givnish TJ, Sytsma KJ (2016) Biogeography of the cosmopolitan sedges (Cyperaceae) and the area-richness correlation in plants. *Journal of Biogeography* 43(10): 1893–1904.

Vegetti AC (2003) Synflorescence typology in Cyperaceae. *Annales Botanici Fennici* 40: 35-46.

Vrijdaghs A, Goetghebeur P, Muasya AM, Smets E, Caris P (2004) The nature of the perianth in *Fuirena* (Cyperaceae). *South African Journal of Botany* 70: 587–594.

Vrijdaghs A, Caris P, Goetghebeur P, Smets E (2005a) Floral ontogeny in *Scirpus*, *Eriophorum* and *Dulichium* (Cyperaceae), with special reference to the perianth. *Annals of Botany* 95: 1199-1209.

Vrijdaghs A, Goetghebeur P, Muasya AM, Caris P, Smets E (2005b) Floral ontogeny in *Ficinia* and *Isolepis* (Cyperaceae), with focus on the nature and origin of the gynophore. *Annals of Botany* 96: 1247-1264.

Vrijdaghs A, Goetghebeur P, Smets E, Caris P (2007) The *Schoenus* spikelet: a rhipidium? A floral ontogenetic answer. *Aliso* 23: 204-209.

Vrijdaghs A, Muasya AM, Goetghebeur P, Caris P, Nagels A, Smets E (2009) A floral ontogenetic approach to homology questions within the Cyperoideae (Cyperaceae). *Botanical Review* 75: 30-51.

Vrijdaghs A, Reynders M, Larridon I, Muasya AM, Smets E, Goetghebeur P (2010) Spikelet structure and development in Cyperoideae, Cyperaceae: a monopodial general model based on ontogenetic evidence. *Annals of Botany* 105: 555-571.

Vrijdaghs A, Reynders M, Muasya AM, Larridon I, Goetghebeur P, Smets E (2011) Spikelet and floral ontogeny in *Cyperus* and *Pycreus* (Cyperaceae). *Plant Ecology and Evolution* 144: 44-63.

General Conclusions

GENERAL CONCLUSIONS

The results obtained in this thesis brings a new perspective towards the understanding of floral traits in Cyperaceae, as well as their evolutionary significance. Furthermore, it raised new questions to impulse the knowledge of such fascinating family. Changes at the beginning of floral development showed to be fundamental in regulating variations in each floral whorl and this remarkable matter was reinforced by our results where we showed a relation between the arrangement of the glumes as a fundamental constraint operating in the flower primordium geometry of Cyperoideae species. This seems to affect the available space for organs emergence, and together with genetic and biochemical interactions, influencing the variation towards the number and position of organs in each floral whorl.

Moreover, the influence of floral traits in the reproductive success and diversity of Cyperoideae genera was approached concerning the stylopodium, which was recovered as a homologous structure, evolving several times during Cyperoideae evolution. The results shed light in a notable relationship between the presence of a stylopodium as a structure possibly increasing the germination fitness of the species. The presence of stylopodium in some of the most diverse genera of Cyperaceae raise questions whether the acquisition of such structure should be investigated as a possible key innovation, possibly promoting the germination success of such species to a variety of environmental conditions and contributing to their wide diversification.

Developmental studies showed to be fundamental to understanding the processes regulating the floral variety in both subfamilies. For Mapanioideae, based on a comparative study on the development of the spicoids, we showed that developmental patterns appear to be consistent within tribe. Features such as the sequence of organ appearance, the size and shape of the spicoid primordium are the main developmental

variations among the species, influencing the spicoid's morphological variation in Mapanioideae. Furthermore, developmental features such as the sequence of bracts emerging alternate with the stamens and the presence of two lateral bracts arising as separate structures, allowed us to argue in favour of interpreting the spicoids of *Diplasia* as reduced inflorescences.

At last, we emphasized the value of understanding the morphological variation of spicoids in Mapanioideae as a source of informative features to a subfamily with notable inconclusive phylogenetic relations. The morphological features of the spicoids were considered informative at generic level since some genera with more than one species share a stable spicoid architecture. However, similarities in the spicoid morphology of different genera were observed, reinforcing the need of reinvestigate their phylogenetic boundaries combining molecular and morphological data. The ancestral character reconstruction allowed us to infer that the hypothetical ancestral morphology of the spicoid is composed of two lateral keeled bracts, two lateral stamens and a dimerous pistil, which resembles the morphology of *Hypolytrum* spicoids. Moreover, based on the most recurrent changes for each variable morphological character, we infer that evolutionary changes in the subfamily were mostly driven by the acquisition of structures.

Such results highlight the importance of morphological studies in elucidating gaps throughout the evolutionary story of Cyperaceae genera, especially concerning the wide range of morphological variation of the reproductive units found in the family. Therefore, further developmental, anatomical and evolutionary studies are crucial to push forward the knowledge towards such a rich and extraordinary family.