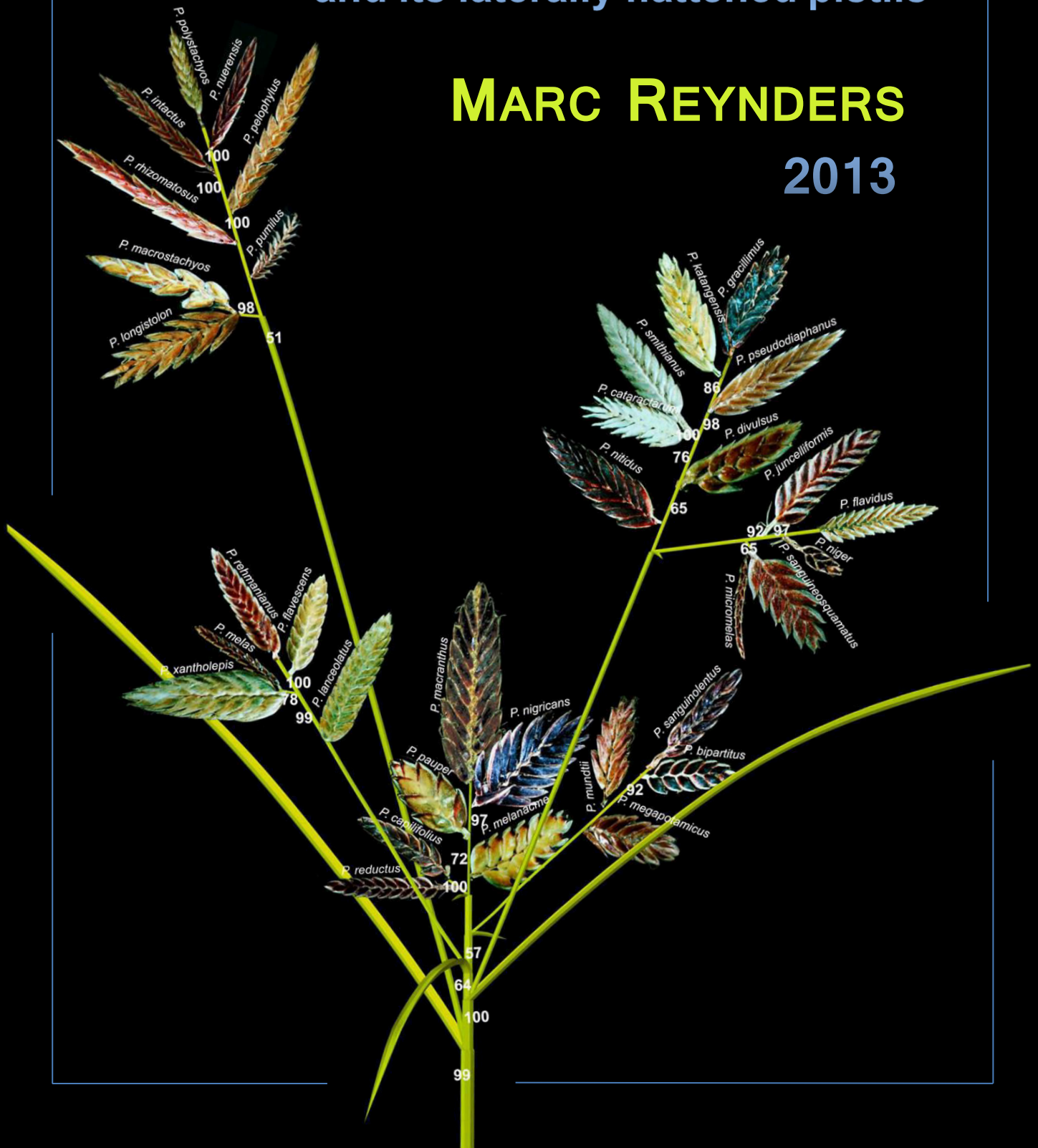The challenging taxonomy and evolution of C$_4$ *Cyperus* (Cyperaceae)

A focus on *Pycreus*

and its laterally flattened pistils

MARC REYNDERS

2013

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COVER ILLUSTRATION:

Front: Stylised cladogram of a ETS1f maximum parsimony analysis

Side: Spikelet of *Pycreus nigricans*

Back: Cypertoon

All pictures were made by M. Reynders, unless stated otherwise.

The challenging taxonomy and evolution of C$_4$ *Cyperus (Cyperaceae)*

A focus on

Pycreus

and its laterally flattened pistils

MARC REYNDERS

Thesis presented in order to obtain the degree of
Doctor in Sciences.

UNIVERSITEIT
GENT

Faculteit
Wetenschappen

Spermato-
phytes

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I Foreword

People frequently ask what I have been studying for so many years and my answer is: 'Cyper grasses' (in Dutch: Cypergrassen)! And they reply: 'Uh, Super grasses?' Well, 'super' they are indeed, what a great plant family to study and what an intriguing evolution they went through! I am very proud to be able to present this PhD study on sedges. However, this piece of work would not have been there in the current form, without the help of many colleagues, family and friends:

My foremost thanks go to Paul Goetghebeur, supervisor of this thesis, who at first gave me the opportunity to make my master thesis on the sedges of Rwanda and Burundi. Paul, thank you for your contagious enthusiasm for botany and Cyperaceae! I also want to thank you for the opportunity to start and continue for a second time this PhD project on *Pycreus* for many years, for your open mindedness and belief in the creativity of your research team, for your patience and support.

Special thanks go to the rather small, but very collaborative international cyperologists community. First of all to our "*Cyperus* team" in Ghent: Kenneth Bauters, Wim Huygh and Isabel Larridon. I'm really proud we managed to produce so many papers the past years. Isabel, thanks for your work on the nomenclatural and phylogeny papers and for the reading. Wim, thanks for your collegiality and cooperation on the molecular work. Kenneth, thanks for your help with the lab work, your input for the phylogeny paper and for continuing your research on sedges! Alex Vrijdaghs from K.U.Leuven was most helpful in sharing his experience and data for the ontogeny papers. Alex, I also want to thank you for the many hours we spend behind the SEM, our long discussions on sedge ontogeny, your very critical notes that lifted our papers to the next level! Muasya, many thanks for your input on the molecular phylogenetic part, for the many silica gel samples and for sharing your field work experience, especially in Madagascar. Dave Simpson, thank you for your positive comments on our papers and for warmly welcoming us in Kew.

Thank you also to all of my former colleagues at Gent University, especially: Pieter Asselman, Elke Bellefroid, Peter Chaerle, Leander Depypere, Yannick De Smet, Kristof de Vos, Bram D'hondt, Carolina Granados Mendoza, Rosette Heynderickx, Bieke Lybeer, Jorinde Nuytinck, Eduardo Cirez Rodriguez, Dirk Rosseel, Marie-Stéphanie Samain, Dirk Stubbe, Kobeke Van de Putte, Liesbeth Vanderschaeve, Mieke Verbeken, Ronald Viane, Marleen Vlaeminck, Adelin Van Heuverswyn and the many others. Thanks for your friendship, positive collaboration on both research and student labs, the conversations during the breaks (my apologies for being absent from most of those breaks), so many tips & tricks on the various research methods and equipment's, for the glimpses into the botanical world outside of sedges, …

II Abstract

Cypereae form one of the largest and most complex tribes of the sedge family (Cyperaceae). Recently, two clades have been revealed within the tribe, the largest of which includes the giant genus *Cyperus* and its closest allies. However, thirteen genera of the generally accepted classification of Goetghebeur (1998) appear to be nested within *Cyperus*. The taxonomic status of many of these taxa has been under discussion since they are based on different combinations of a limited set of derived characters. *Pycreus*, the largest of these segregate genera, is characterised by laterally compressed dimerous pistils of which the derivation from the general trimerous situation was not yet understood. It shares this pistil with *Kyllinga* and *Queenslandiella* that both are, as is *Pycreus*, embedded in the *Cyperus* clade which uses C_4 photosynthesis.

The recent insights from molecular phylogenetics make a reevaluation possible of the taxonomic status of the thirteen different segregate genera of *Cyperus* and of the taxonomic value of the characteristics that have been used to delimitate these taxa. This is currently tackled in a joint international research effort, using a combination of molecular phylogenetics, ontogeny, anatomy and morphology, to understand evolutionary patterns in Cyperaceae and to build a modern classification of sedges. This research strategy is situated on three taxonomic levels: family to tribal level (macro-scale), tribal to generic level (meso-scale) and infrageneric level (micro-scale). The current thesis is embedded in this international research context and focusses mainly on meso-scale objectives (C_4 *Cyperus* and the position and taxonomic state of its segregate genera, including *Pycreus*) and micro-scale objectives (the infrageneric taxonomy of *Pycreus*).

At first, a complete nomenclatural survey is presented of all generic and subdivisional names that have been published for the taxa now included in the *Cyperus* clade (around 350 names), along with an evaluation of their validity, legitimacy and priority. Types are indicated and where necessary lectotypes are designated. This nomenclatural survey serves as a base for the selection of representative taxa in the molecular, ontogenetic, anatomical and morphological studies. In addition it forms an essential tool when building a modern revision for the clade. In the current thesis only names for taxa in which *Pycreus* species have been placed are included.

Next, to be able to reevaluate the taxonomical value of derived pistils in the *Cyperus* clade, especially the laterally compressed dimerous pistils of *Pycreus*, an elaborate ontogenetic study of *Pycreus* and *Cyperus* species was performed. This study shows that both taxa follow the general ontogenetic patterns of spikelets and flowers found throughout Cyperoideae. In addition, the ontogeny and anatomy of the different types of pistils was reviewed with addition of new ontogenetic and anatomical data. These demonstrate that in Cyperoideae the pistil wall starts from an annular primordium (which evolved from congenitally fused carpels) on top of which the stigma

primordia develop. The development of the central ovule is decoupled from the ovary wall development. Vascular patterns follow the development of the primordia and vascular bundles are formed where necessary. The presence of an annular gynoecial wall primordium appears to have opened new possibilities for the development of the stigma primordia in new positions independent from the constraints of individual carpels.

An elaborate molecular phylogenetic study was performed on the C_4 *Cyperus* clade using ETS1f, *rpl32-trnL, trnH-psbA*. Although relationships within the C_4 *Cyperus* clade are still largely unresolved in a large polytomy, early emerging branches show better resolution than in previous studies. *Pycreus* appears to be para- or polyphyletic and in addition no relationships have been found between *Pycreus, Kyllinga* and *Queenslandiella*. Therefore, we have to admit, laterally compressed dimerous pistils have most likely originated multiple times in the clade. Subsequently, the most appropriate classification strategy for these taxa is sinking them into *Cyperus.* This also seems to be the most appropriate strategy for all other segregate genera based on a reevaluation of the taxonomical value of their key characters. Only for the C_4 *Cyperus* clade (accommodated in *C.* subgenus *Cyperus*), which is nested within a grade of species using C_3 photosynthesis (accommodated in *C.* subgenus *Anosporum*), an evolutionary classification strategy has been adopted. This is based on the evolutionary value of the origin of C_4 photosynthesis which had led to a major radiation of species.

On the micro-scale, it is not yet possible to present a modern classification for *Pycreus* since molecular phylogenetic relationships are largely unresolved. Therefore, results are presented as several case studies. First, in an elaborate SEM study, the taxonomical value of the nutlet epidermis was reevaluated. Next, the reestablishment of *P.* sect. *Tuberculati* is discussed. Finally, the new classification strategy for the *Cyperus* clade was applied on *Pycreus* and necessary combinations and nomina nova under *Cyperus* are listed along with some critical notes on synonymisations of several taxa.

III Samenvatting

De tribus *Cypereae* vormt één van de grootste en meest complexe takken van de Zeggenfamilie (Cyperaceae). Recent werden twee clades opgelost binnen de tribus, de grootste hiervan omvat *Cyperus* en aanverwante genera. Maar, dertien genera uit de algemeen aanvaarde classificatie van Goetghebeur (1998) blijken genest te zitten in het reuzengenus *Cyperus*. De taxonomische status van veel van deze genera wordt echter reeds lang betwist daar deze genera gebaseerd zijn op verschillende combinaties van een beperkte set aan afgeleide kenmerken. *Pycreus*, het grootste van deze satelliet genera, wordt gekenmerkt door lateraal samengedrukte dimere stampers waarvan het ontstaan vanuit de trimere toestand nog niet duidelijk was. *Pycreus* deelt dit stamper type met *Kyllinga* en *Queenslandiella*, twee andere genera die, net als *Pycreus,* genest zitten in de *Cyperus* subclade die gebruik maakt van C_4 fotosynthese.

De recente inzichten uit moleculair fylogenetisch onderzoek maken een herevaluatie mogelijk van de taxonomische status van de dertien satelliet genera van *Cyperus* en van de taxonomische waarde van de hiervoor gebruikte diagnostische kenmerken. Dit werd aangepakt tijdens een internationaal samenwerking. Door middel van moleculair fylogenetisch, ontogenetisch, anatomisch en morfologisch onderzoek werd gepoogd de evolutionaire patronen beter te begrijpen en een modern classificatiesysteem te ontwikkelen voor Cyperaceae. Deze onderzoeksstrategie speelde zich af op drie niveaus: familie tot tribus niveau (macro-niveau), tribus tot genus niveau (meso-schaal) en het infra generisch niveau (micro-niveau). De huidige thesis is genest binnen deze internationale onderzoek context en focust hoofdzakelijk op meso- niveau (C_4 *Cyperus* en de positie en taxonomische status van de satelliet genera, inclusief *Pycreus*) en micro-niveau (de infra generische taxonomie van *Pycreus*).

In eerste instantie werd een volledige nomenclatuur lijst voorgesteld van alle generische en infra generische namen van taxa in de *Cyperus* clade (350 namen) samen met een evaluatie van de geldigheid, legitimiteit en prioriteit. Types werden aangegeven en lectotypes werden aangeduid waar noodzakelijk. Dit nomenclaturaal overzicht diende als basis voor het selecteren van representatieve taxa voor het moleculair fylogenetisch, ontogenetisch, anatomisch en morfologisch onderzoek op *Pycreus* en *Cyperus*. Daarnaast vormt het een onmisbaar instrument voor het maken van een moderne revisie van de groep. In de huidige thesis worden enkele de namen die verband houden met *Pycreus* weergegeven.

Vervolgens, om de taxonomische waarde van afgeleide stamper types in de *Cyperus* clade te kunnen revalueren (meer bepaald de lateraal samengedrukte stampers van *Pycreus*), werd een uitgebreide ontogenetische studie uitgevoerd van aartjes en bloemetjes van *Pycreus* en *Cyperus* soorten. Deze studie toont dat de soorten van beide genera de algemene ontwikkelingspatronen

6

volgen die teruggevonden waren overheen de hele onderfamilie Cyperoideae. Daarnaast werd de ontogenie en anatomie van de verschillende stamper types gerevieuwed en met toevoeging van nieuwe data. Deze studie toonde aan dat de ontwikkeling van de wand van het gynoecium vertrekt vanuit een ring primordium (dat zelf evolueerde vanuit congenitaal versmolten carpellen). Op de top hiervan ontwikkelen zich vervolgens de stigma primordia. De ontwikkeling van de centrale ovule vanuit het bloem-apex primordium is ontkoppeld van het gynoecium wand primordium. De ontwikkeling van vaatbundels volgt de ontwikkeling van de primordia waardoor vaatbundels worden gevormd waar noodzakelijk. De aanwezigheid van een ring-primordium heeft nieuwe mogelijkheden geopend voor de ontwikkeling van stigma primordia in posities onafhankelijk van de vaste posities van individuele carpellen.

Een uitgebreide ontogenetische studie werd uitgevoerd van de C$_4$ *Cyperus* clade met gebruik van ETS1f, *rpl32-trnL, trnH-psbA* merkers. Hoewel verwantschappen binnen in de C$_4$ *Cyperus* clade nog grotendeels onopgelost bleven in een grote polytomy, vertonen de laagst geplaatste subclades een betere resolutie dan in voorgaande onderzoeken. *Pycreus* is para- tot polyfyletisch en daarnaast werden voorlopig nog geen verwantschappen gevonden tussen *Pycreus, Kyllinga* en *Queenslandiella*. Daarom moeten we toegeven dat lateraal samengedrukte stampers waarschijnlijk meerdere keren ontstaan zijn in de C$_4$ *Cyperus* subclade. Bijgevolg vormt de best geschikte classificatie strategie voor deze taxa een opname in *Cyperus*. Dit lijkt momenteel eveneens de meest geschikte strategie te zijn voor alle andere satelliet genera van de *Cyperus* clade, gebaseerd op een evaluatie van de taxonomische waarde van de kenmerken. Enkel voor de C$_4$ *Cyperus* clade (die overeenstemt met *C.* subgenus *Cyperus*), die genest zit binnen een groep van soorten die C$_3$ fotosynthese gebruiken (die overeenkomt met C. subg. Anosporum), werd een evolutionaire classificatie strategie toegepast. Dit is gestoeld op de evolutionaire waarde van het ontstaan van C$_4$ fotosynthese dat heeft geleid tot een grote radiatie van soorten.

Op micro niveau was het nog niet mogelijk om een modern classificatie voor te stellen voor Pycreus, daar de moleculair fylogenetisch relaties nog grotendeels onopgelost blijven. Er werden wel enkele case studies uitgevoerd over de taxonomie van *Pycreus*. Een eerste vormt een uitgebreide SEM studie waarin de taxonomische waarde van de vruchtwand epidermis werd geëvalueerd. Vervolgens werd het heroprichten van de kleine sectie *Tuberculati* besproken. Tenslotte werd de nieuwe classificatie strategie voor de *Cyperus* clade toegepast op *Pycreus* waardoor verschillende nieuwe combinaties en nomina nova werden gemaakt samen met enkele kritische bemerking over de synonymie van een aantal taxa.

IV Outline of this thesis

Chapter 1 first briefly addresses the research context of this PhD project and collaborative framework with other cyperologists. International cooperation on sedges plays on different scales: family scale, tribal and generic scale and subgeneric scale. The current research is mostly focused on the evolution of the *Cyperus* clade (meso-scale) and its segregate genus *Pycreus* (micro-scale). Next the taxonomical context of this research is presented, starting with Cyperaceae and their characters and phylogeny to pass through the tribe Cypereae to *Cyperus* and the challenging taxonomy of its segregate genera. Special attention is given to *Pycreus* and its laterally compressed pistils. Finally an outline is given of the research strategy followed on meso- and micro-scale together with the objectives of the research presented in the following chapters.

Chapter 2 gives a brief focus on the plant material used for botanical research and different types of samples required for modern botanical research.

Chapter 3 lists our results of the nomenclatural survey of generic and subdivisional names for *Pycreus*. Validity and legitimacy of names is discussed and lectotypes are designated where necessary.

Chapter 4 shows our results on the ontogeny of spikelets and flowers of *Cyperus* and *Pycreus*. The species of these taxa follow the main developmental patterns that have been found throughout cyperoid sedges.

Chapter 5 reviews the pistil development of Cyperoideae with addition of new data on the anatomy and development of the vasculature in the pistil of *Pycreus, Kyllinga, Queenslandiella* and *Cyperus*. A general developmental hypothesis is presented which allows understanding the origin of derived dimerous gynoecium types found in Cyperoideae.

Chapter 6 discusses the molecular phylogenetic analysis of the C_4 *Cyperus* clade with a reevaluation of the generic status of the different specialised genera nested within this clade (including *Pycreus*).

Chapter 7 includes the results of an elaborate scanning electron microscopy study of the nutlet epidermis of 50 *Pycreus* species together with a discussion the taxonomical value of nutlet characters and character states in *Pycreus.* These have been used as a key characters in previous subgeneric classifications of *Pycreus*.

Chapter 8 reviews the small section *Tuberculati*, which was erroneously synonimised by Kükenthal (1935-36) with his section *Muricati*.

Chaper 9 lists necessary combinations and nomina nova for *Pycreus* names that need to be sunken into *Cyperus* with application of our new classification strategy In addition, several cases on synonymimy are discussed.

Chapter 10 gives a general discussion and integration of the results from the different research lines. An important focus is laid on the selection of the most appropriate classification strategy for the different segregate lineages of the paraphyletic genus *Cyperus*. Finally, future prospects and possible research lines are discussed for *Cyperus* and *Pycreus.*

Chapter 11 lists the references that have been cited in this work.

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For Keegan

and Luna

'Duizend zonnen en één waaier' by Keegan Reynders, June 2013

1 Introduction

Botany, the science of the vegetable Kingdom, is one of the most attractive, most useful, and most extensive departments of human knowledge. It is, above every other, the science of beauty.

Joseph Paxton (1803-1865)

← Fig. 1.1 Swamp at Gen. McArthur, Samar Island, Philippines; "dream destination" for Cyperologists.

Spikelet theme: *Pycreus cataractarum*

1.1 In this chapter…

The introductory chapter sets out the framework and goals of the research presented in this thesis:

First, in 1.2, a brief introduction is given on the disciplinary context of this research as some confusion exists on the definitions of the different disciplines within modern botany. Next, 1.3 highlights the research context and collaboration.

The taxonomic framework is addressed in 1.4. Herein, key questions are highlighted in the systematics and taxonomy of the sedges that go flat: C_4 *Cyperus* and more specific of *Pycreus*. We will gradually zoom in on the research topic: first introducing the Cyperaceae family, then visiting the rich evolutionary history of the challenging Cypereae tribe, to end with *Pycreus* and its laterally flattened dimerous pistils.

Finally, A more detailed overview of the objectives of the *Pycreus* PhD project is given in 1.5. The goals are set out within two taxonomic scales that were introduced in 1.3: the meso-scale, focusing on the relationships and evolutionary processes in *Cyperus* s.l. and the micro-scale that focuses on the infrageneric relationships within *Pycreus*. This chapter interconnects research and taxonomic frameworks with the results that are set out in chapters 3 to 9.

1.2 The disciplinary context of this research

1.2.1 Modern botany and cyperology

Let us start with a quote of Johann Hermann Baas: "Botany, the eldest daughter of medicine" (Baas & Handerson, 1889: 843). Early descriptive botanical works were indeed mostly in function of medicinal uses (e.g. Dodoens, 1554). In contrast, modern botany has grown into a multidisciplinary study with many output areas. Various data types are combined to build solid hypotheses about plant life evolution in all of its aspects.

Within the diversity of plant life, various groups show very specific characteristics and evolutionary patterns each requiring an appropriate approach. For example, the research presented in this thesis can be situated within the branch of botany that studies Cyperaceae or sedges, named: **cyperology**. Consequently, scientists studying sedges are called cyperologists (see fig. 1.2).

Fig. 1.2 Cyperologists in the field in Madagascar & the Philippines.

1.2.2 Taxonomy

Taxonomy studies how individual organisms can be grouped at various ranks (e.g. Judd *et al.*, 2007; Harper, 2011). A taxon is a certain group of organisms based on mutual characters. Each taxon bears a name governed by nomenclatural rules (e.g. McNeill *et al.*, 2012). Besides classification, taxonomy also deals with identification of organisms.

While 18th and 19th century classifications were purely based on morphology (e.g. Manktelow, 2010), during the 20th century, the insights from plant systematics, and more precisely, from molecular phylogenetics (e.g. Angiosperm Phylogeny Group III, 2009), put taxonomy in an evolutionary context. Consequently, taxa are now generally based on common ancestry (reflected by shared characters or similarity).

To be able to handle the large amounts of information and names that are inherent to taxonomy, data basing has become an important aspect of modern plant taxonomy (ITIS, 2012).

1.2.3 Systematics

Systematics aims to interpret the data obtained from various kinds of investigations in the search for evolutionary relationships of different taxa. These hypotheses are then translated in the taxonomic treatments. The progress in plant systematics largely depends on the development of new technologies (Mayr, 1982).

In particular, from the start of the 20th century, the development of different types of light microscopy, anatomical techniques and later electron microscopy allowed for exploration of new types of data (e.g. anatomy and ontogeny), resulting in a more accurate interpretation of relationships. From the second half of the 20th century, statistical methods were developed to investigate relations in a more standardised and objective way (e.g. Hennig, 1950; Sokal & Sneath, 1963). Today, molecular phylogenetics forms the basis of our evolutionary hypotheses, which are further tested with data from morphology, ontogeny, anatomy, phytochemistry, etc. (APG III, 2009).

1.3 Research context and collaboration

1.3.1 International research strategy on Cyperaceae

Cyperaceae or sedges form a large and widespread family of grass-like plants, which are known for their complex morphological evolution and taxonomy. Within the family, the giant genera *Carex* and *Cyperus* and their relatives are some of the best examples of groups with complex morphological evolution (e.g. Starr & Ford, 2009).

With the availability of new research techniques, there is a joint international effort towards insight in the evolution of sedges, leading to a modern classification of the family and its genera. Two main strategies are followed: firstly, a molecular phylogenetic approach (e.g. Simpson *et al*., 2007; Muasya *et al.,* 2009a) and secondly, in parallel, studies (ontogeny, morphology, anatomy, etc.) are being conducted to resolve homology problems and to test the molecular phylogenetic hypotheses (e.g. Vrijdaghs, 2006; Vrijdaghs *et al*., 2009, 2010). Both strategies are conducted on three different scales: family to subfamily level (macro-scale); tribe level (meso-scale) and (sub-) generic level (micro-scale).

1.3.2 The current PhD study focusing on C$_4$ *Cyperus* and *Pycreus*

The research presented in this thesis is framed within this joint international research strategy on Cyperaceae and more specifically on Cypereae. It focuses primarily on the meso- and micro-scale. The main objectives of this study are: 1) meso-scale: to unravel the links between *Pycreus* and related taxa (C$_4$ *Cyperus* and its segregate genera), with a focus on the origin, nature and taxonomic value of the laterally compressed pistils that characterize *Pycreus*. This with special attention to the reevaluation of the generic status of *Pycreus* and other segregate genera of *Cyperus*. 2) micro-scale: to reveal the relationships within *Pycreus* towards a revision of the taxon. Fig. 1.3 shows how the current research (thick lines) is embedded within the relevant international research context.

Cyperaceae

Classification : Goetghebeur P., UGent
Nomenclatural database: Govaerts R., RBG Kew
Molecular phylogeny: Simpson D.A., RBG Kew
                 Muasya A.M., University of Cape Town
Ontogeny (Cyperoideae): Vrijdaghs A., KU Leuven

Cypereae (Muasya A.M., University of Cape Town &
Research Group Spermatophytes, UGent)

Cyperus **s.l.** (Research Group Spermatophytes, UGent)

C_3 *Cyperus* (Larridon I., UGent)

C_4 *Cyperus* (Research Group Spermatophytes, UGent)

Pycreus (Reynders M., UGent)

Kyllinga (Huygh W., UGent)

Ascolepis & Lipocarpha (Bauters K., UGent)

Fig. 1.3 Research context of the current study. The three different scales of collaboration corresponding to different taxonomic ranks are shown in three colors: the macro-scale or family/ subfamily level in red; the meso-scale corresponding to the tribal to generic ranks in blue. Also, subdivisional relationships within *Cyperus* s.l. are treated on this collaborative scale; and the micro-scale in green, which includes the specific research topics of the different cooperating researchers on *Cyperus* s.l. This micro-scale corresponds to groups or segregate genera within *Cyperus* s.l. For macro- and meso-scales, the international researchers are listed that are involved in the collaborative context of the current research together with their expertise. Only subfamilies, tribes and genera relevant for the research context are shown in the diagram.

1.3.3 Collaborative framework

At the time this research started in October 2004, there was already an established international collaboration network (involving persons from the Research Group Spermatophytes) on molecular phylogenetic research and ontogeny of the sedge family:

At Gent, Molecular phylogenetic research was started in 2000 in collaboration with David A. Simpson of the Royal Botanic Gardens, Kew, UK (e.g. Dhooghe *et al.*, 2003). Dr. Simpson specialises in mapanioid sedges and family level molecular phylogenetics (e.g. Simpson *et al.*, 2003, 2007). Later cooperation on molecular phylogenetics was extended with Dr. A.M. Muasya (University of Cape Town) who has expertise in family level molecular phylogenetics and a specific interest in Cypereae (e.g. Muasya *et al.*, 2009a, 2011). In 2005, the Research Group Spermatophytes became a partner in the Centre for Molecular Phylogeny and Evolution (CeMoFE, UGent), where the molecular lab work of the current and related studies was performed (e.g. Larridon, 2011).

In 2001, collaboration was started with Alex Vrijdaghs (KU Leuven, Belgium) who works on floral and inflorescence ontogeny to resolve homology problems within the sedge family. This resulted in numerous publications on the topic (Vrijdaghs *et al.*, 2004, 2005a, b, 2006, 2009, 2010, 2011; Larridon *et al.*, 2011b; Reynders & Vrijdaghs *et al.*, 2012). Within this cooperation, also a palynological study was performed (Nagels *et al.*, 2009).

With the current PhD project (which started in October 2004, a new cycle of Cyperaceae research was started at the Research Group Spermatophytes. In 2005, Wim Huygh joined the group with a PhD study on *Kyllinga*, a taxon showing similar derived pistils as *Pycreus.* Next, in 2007 Isabel Larridon started with a PhD study on C_3 *Cyperus* and its segregate taxa (*Courtoisina, Oxycaryum* and *Kyllingiella*). Finally in 2010, Kenneth Bauters made a phylogenetic study of *Ascolepis* and *Lipocarpha* for his MSc thesis (Bauters, 2011) and currently he has a PhD project on Sclerieae.

1.4 Taxonomic framework

1.4.1 The sedge family

1.4.1.1 Cyperaceae and their characters

      With its approximately 109 genera and over 5500 species (Govaerts *et al.*, 2007) the Cyperaceae is the third largest monocot family in the world after Orchids and Grasses. The family is characterised by its species diversity and it is also cosmopolitan, only being absent from Antarctica (Goetghebeur, 1998; Govaerts *et al.*, 2007). Sedges are adapted to many different habitats (figs. 1.1 & 1.5). Nevertheless, they are predominantly wetland dwellers, often forming the dominant elements in such environment (then called sedgelands, e.g. fig. 1.6). Cyperaceae also have considerable local economic importance as many members are notorious agricultural weeds, whereas others provide food, fuel, and medicines together with construction, weaving, and perfumery materials (Simpson and Inglis, 2001). More recently sedges, are gaining interest as bio-indicators for the environmental quality of wetlands (e.g. Shuping *et al.*, 2011) and in natural water purification facilities (e.g. Lu *et al.*, 2006). Several sedges are also useful in climate change monitoring (Simpson *et al.*, 2011).

      Cyperaceae combine a vast number of characters that, in most cases, allows them to be recognized as sedges, even only from vegetative parts (e.g. Goetghebeur, 1998). In their habit, most sedges resemble members of Grasses (Poaceae) and Rushes (Juncaceae) (Fig.1.4A). However, triangular culms with leaves in three ranks and closed leaf sheaths allow them to be distinguished from the other two families, although there are several exceptions. Important generative characters for Cyperaceae are the small, strongly reduced and mostly wind pollinated flowers, pistils with a single basal ovule (Goetghebeur, 1998) and pseudomonad pollen (Nagels *et al.*, 2009).
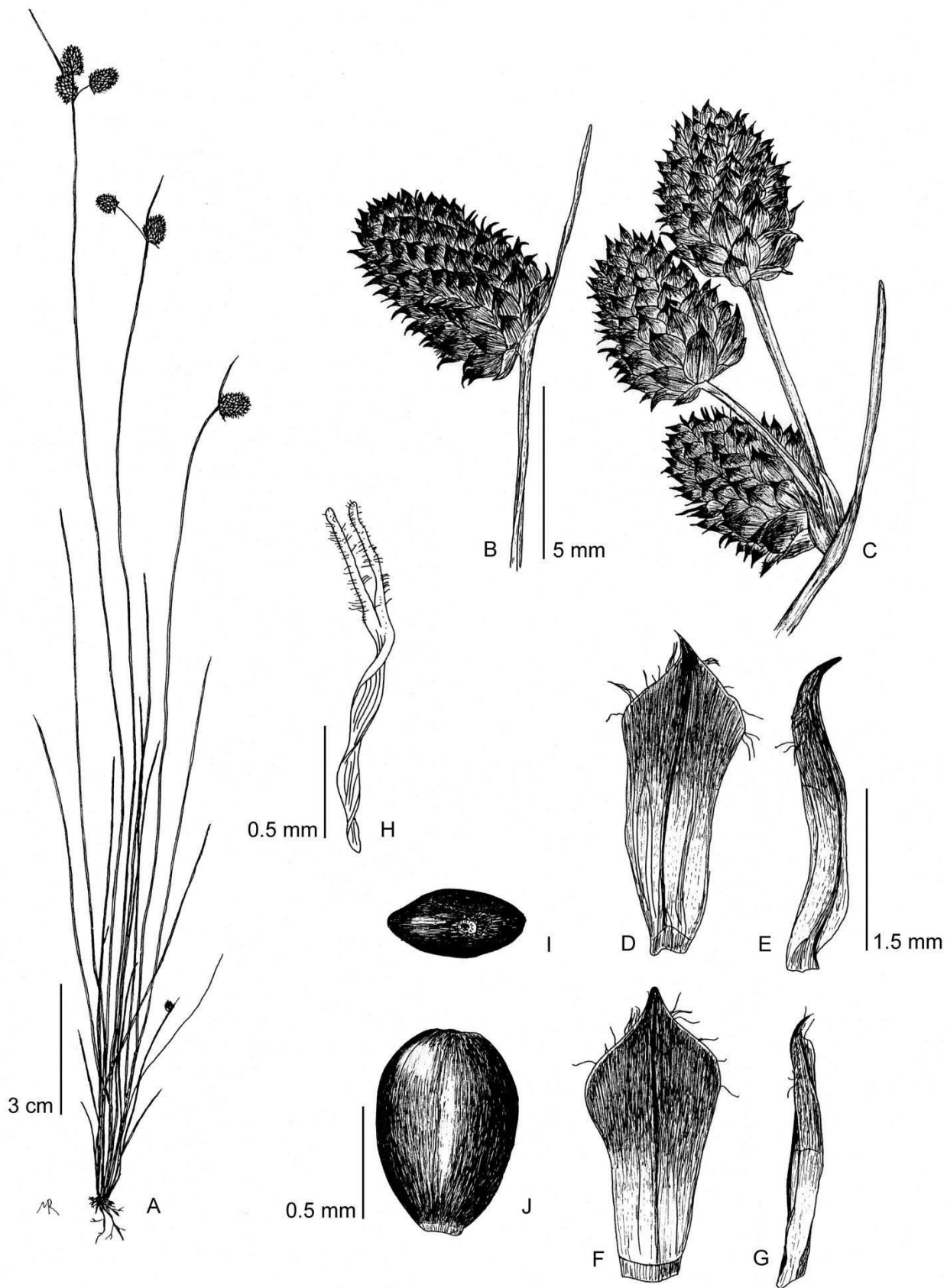
Fig. 1.4 *Nemum atracuminatum*. A. Habit. B & C. Inflorescence with spikelets. D-G. Glumes. H. Stigma branches. I & J. Nutlet. After Larridon *et al.* (2008a).

Fig 1.5 Examples of tropical sedge habitats. (1) Samar Island, Philippines: A. Coastal rainforest in Gen. McArthur, the habitat of *Paramapania parvibracteata.* B. Seashore with *Cyperus rotundus*. C. Rice fields. (2) Madagascar: D. Rocky pools on an inselberg. E. Rock vegetation at Andringitra National Park. F. Lakeshores with seepage zones, Lac Froid. G. Swamp dominated by *Costularia sp.*, Ranomafana National Park.

Flowers are arranged in spikelets (Fig.1.4B-C) with a central axis or rachilla on which the flower bearing bracts or glumes are arranged (see e.g. Goetghebeur, 1998; Vrijdaghs *et al.*, 2010) (Fig.1.4D-G). Due to reduction and contraction processes, many derived inflorescence types originated. The flowers of sedges are usually trimerous, small and bisexual to unisexual. When a perianth is present, it consists of two whorls of tree bristle hairs (e.g. fig. 1.15D) or scales. These have an air-capturing function, which helps the mature nutlets float on water for dispersal. There usually is a single whorl of three stamens. Gynoecia are tri- or dimerous, unilocular and have a single ovule with basal placentation. The fruit is a nutlet (Goetghebeur, 1998; Fig.1.4I-J).
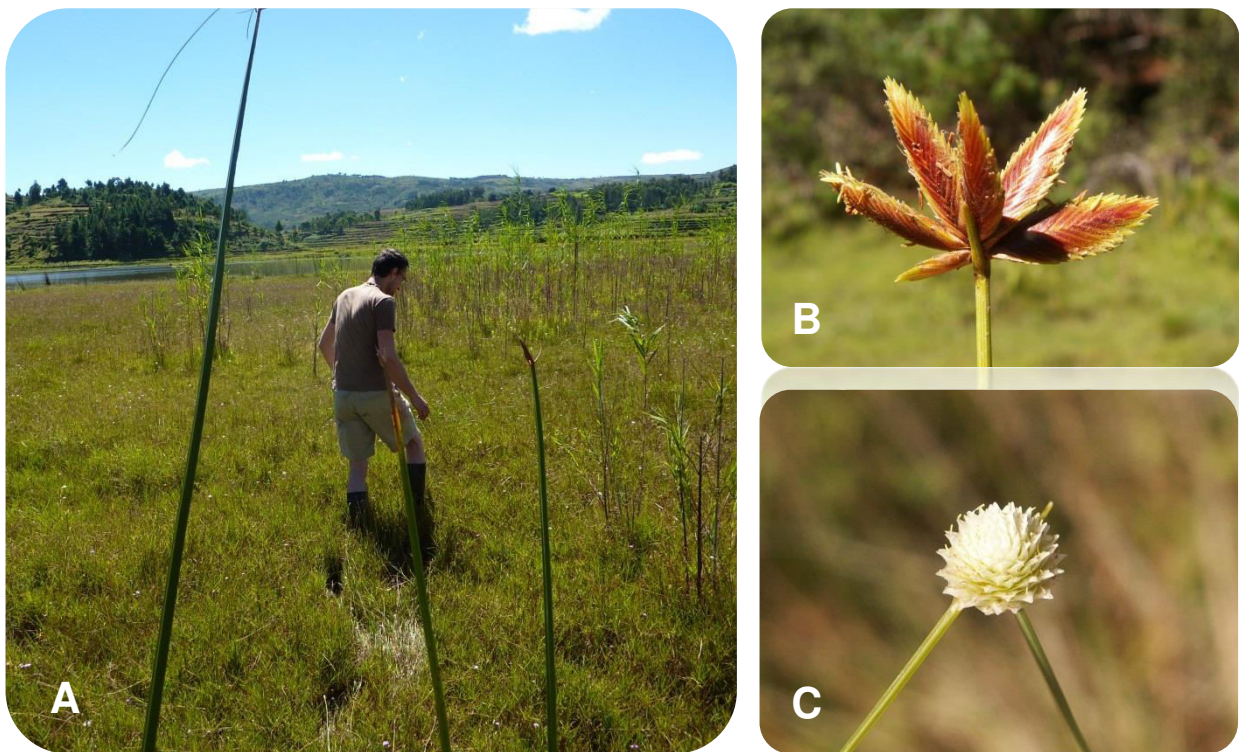
Fig. 1.6 Floating mats on a lake near Antsirabe, Madagascar. A. These mats are formed by the entwined stolons of *Pycreus mundtii* and according to Haines & Lye (1983) these are strong enough to hold crocodiles and most likely also cyperologists in their quest for the many sedges that inhabit these mats. B. *Cyperus pectinatus*. C. *Ascolepis brasiliensis*. Pictures taken by I. Larridon (A) & M. Reynders (B & C).

1.4.1.2 Cyperaceae in an evolutionary context

As "nothing in Biology makes sense except in the light of evolution" (Dobzhansky, 1973), the next part will focus on the larger evolutionary context of Cyperaceae and subsequently discuss the general phylogenetic relationships within the family.

Cyperaceae within the Poales

The latest classification of angiosperms based on molecular phylogenetic data (A.P.G. III, 2009), places Cyperaceae in the order Poales (Stevens, 2001 onwards; Fig.1.7A). This is a plant order containing 18 families of which four encompass over 1000 species. Poales are estimated to have originated ca. 113-106 mya (Janssen & Bremer, 2004; Leebens-Mack *et al.*, 2005), which coincides with the Gondwana break-up. As many early diverged members of the different Poales families seem to be linked to the South American Gondwanan shield, the origin of Poales could be assumed to be South American (Janssen & Bremer, 2004). Africa is also rich in Poales. However, it is not yet clear whether vicariance following continental drift, or long distance dispersal forms the basis for these distribution patterns. Most Poales families have gynoecia with 2-3 loci and numerous ovules, but fusion and reduction processes of the pistils are common (e.g. Ronse Decraene *et al.*, 2002; Rudall & Bateman, 2004). In sedges, the superior gynoecium consists of congenitally fused carpels, A single style and most often two or three stigmas (e.g. Vrijdaghs, 2006).

Within Poales, Cyperaceae are consistently sister to Juncaceae and Thurniaceae (Chase *et al.*, 1993; Plunkett *et al.*, 1995) in a clade also containing Mayacaceae, Eriocaulaceae and Xyridaceae (fig. 1.7A). The molecular confirmation of the relationships between Thurniaceae, Juncaceae and Cyperaceae (often referred to as 'cyperid clade'; Stevens, 2001 onwards) did not come as a surprise as several characters hold these families together (e.g. Takhtajan, 1997). The most important characters for the cyperoid clade are porate pollen in tetrads (becoming pseudomonads in Cyperaceae) and chromosomes with diffuse centromeres. The latter are often accompanied by considerable variation in chromosome numbers (see e.g. Escudero *et al.,* 2012). In addition, these families share an affinity for wetland habitats. Within the cyperoid clade, Cyperaceae originated around 88 mya and the crown group diversification started ca. 76 mya (Janssen & Bremer, 2004; Besnard *et al.*, 2009).
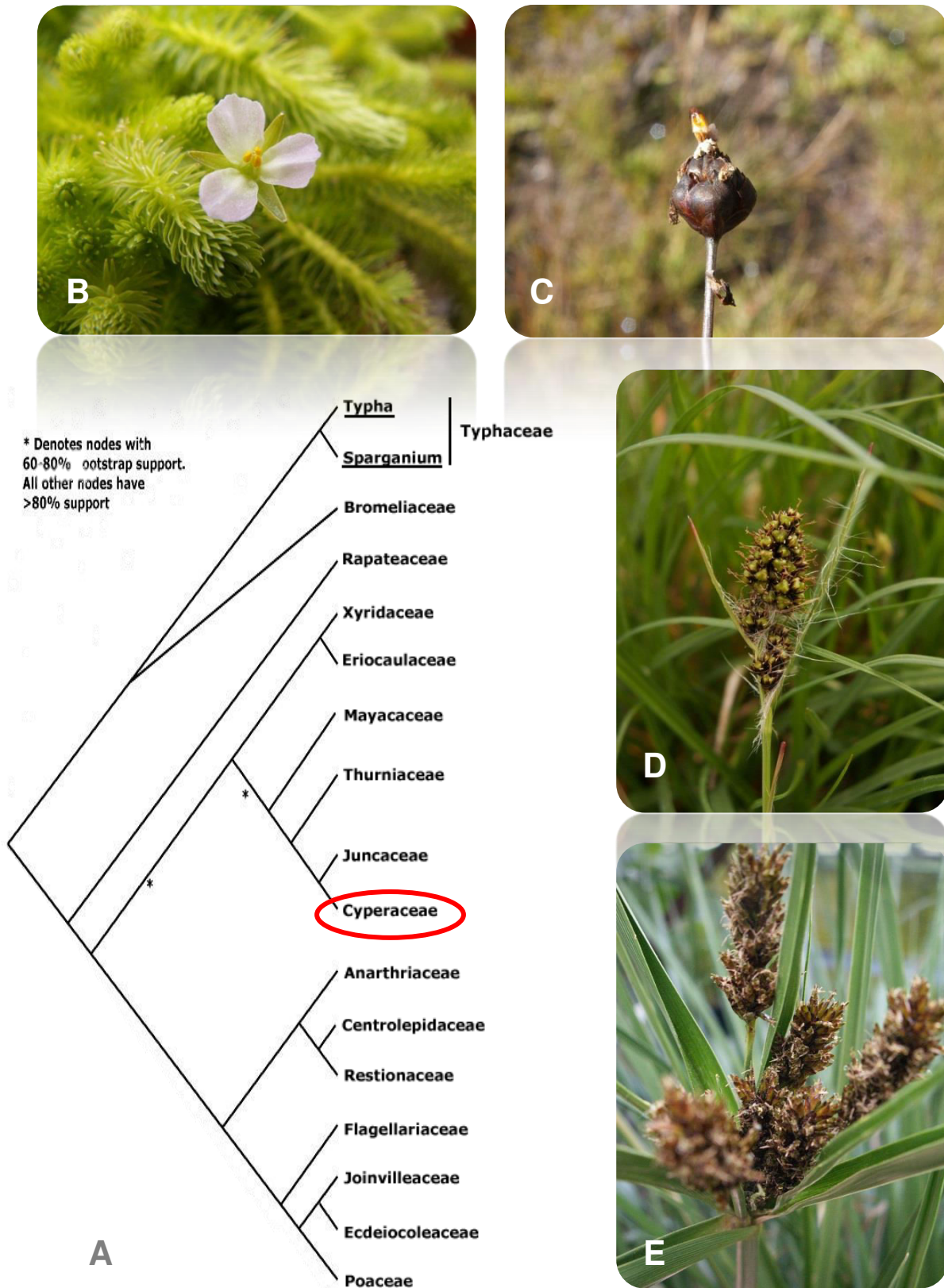
Fig. 1.7 A brief overview of Poales. A. Phylogenetic relationships of the families in the Poales sensu APG III (2009). Cyperaceae are encircled in red. After Stevens (2011 onwards). B. *Mayaca fluviatilis* (Mayacaceae), BG UGent. C. *Xyris* sp. (Xyridaceae), Madagascar. D. *Luzula abyssinica* (Juncaceae), BG UGent. C*. Cyperus ustulatus* (Cyperaceae), BG UGent.

Tribal relationships within Cyperaceae

With growing knowledge, the need for a classification of the large sedges family became apparent. From the 19th century on, genera had been grouped in tribes and these tribes into subfamilies. A major challenge in sedge classification is the strongly derived and reduced nature of many inflorescences and flowers. Homology assessments are therefore not straightforward and sometimes even impossible when using morphology alone (Goetghebeur, 1986). Next, the choice priority in diagnostic value that was granted to conflicting morphological characters proved to be challenging as well (e.g. Goetghebeur, 1986; see fig. 1.20). Consequently, during the 19th, 20th and 21th centuries, many adjustments were made to the subfamilial classification of sedges reflecting growing insights from morphology, anatomy, biochemistry, embryography, palynology and eventually also molecular phylogenetic analysis.

During the 19th century, classifications were primarily based on the presence of unisexual or bisexual flowers (e.g. Jussieu, 1789; Bentham, 1883). At the start of the 20th century, cyperologists started to use a broad range of flower and inflorescence characters (e.g. Clarke, 1908; Chermezon, 1937). From the middle of the 20th century, morphological, anatomical, embryographical, biochemical and physiological characters were added to produce the first modern classifications based on cladistic analysis (e.g. Bruhl, 1995; Goetghebeur, 1998). Embryography showed to be especially reliable for tribal delimitations in Cyperaceae (Van der Veken, 1965; Goetghebeur, 1986). Within Cyperaceae, Goetghebeur (1998) recognized four subfamilies and 16 tribes.

Finally, from the nineties to date, molecular phylogenetic studies, integrating other available data such as those from ontogenetic studies, allowed testing of evolutionary hypotheses and this will lead to a more stabilised classification of sedges. Simpson *et al.* (2007) and Muasya *et al.* (1998, 2002 & 2009a), produced a well resolved and supported backbone phylogenetic hypothesis for Cyperaceae. The resulting molecular phylogenetic classification (fig. 1.8) largely reflects the tribal classification of Goetghebeur (1998). Mapanioideae and Cyperoideae, each correspond to a well supported clade (Simpson *et al.*, 2007; Muasya *et al.*, 2009a) and have both distinct morphological characteristics (see next paragraphs).
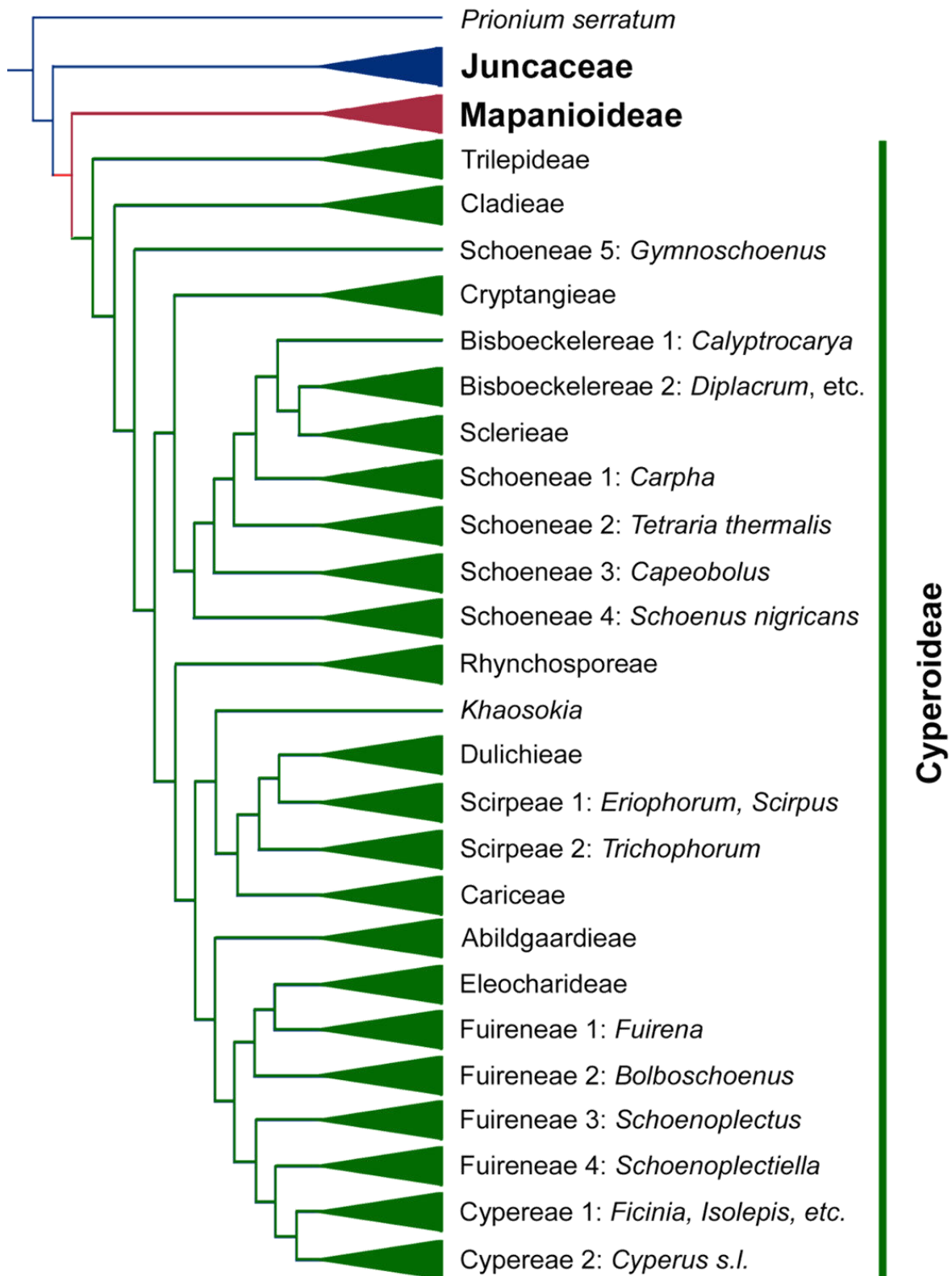
Fig. 1.8 Simplified cladogram of tribal relationships within Cyperaceae, adapted from a strict consensus tree from Muasya *et al.* (2009a).

On the other hand Sclerioideae and Caricoideae are merged into Cyperoideae. In addition, within Cyperoideae, several tribes from the classification of Goetghebeur (1998) appear to be paraphyletic (e.g. Schoeneae, Scirpeae and Fuireneae).

Mapanioideae, enigmatic forest dwellers

Mapanioideae seem to possess peculiar characters such as complex flowering units. These flowering units consist of two large opposite and usually dorsally scabrid scales enclosing a spadix-like axis with variable numbers of smaller scales and stamens with in the center one rather large gynoecium (e.g. Kern, 1974; fig. 1.9D). In many taxa, there are also three scales between the stamens and the gynoecium. It has been unclear whether these flowering units should be interpreted as flowers (the euanthial hypothesis; Goetghebeur, 1986, 1998) or compacted spikelets composed of unisexual flowers, also called 'spicoids' (the synanthial hypothesis; cf. Kukkonen, 1984; Simpson, 1992; Simpson et al. 2003). Recently a study combining floral ontogeny and gene protein localization in *Lepironia* supports the synanthial hypothesis as the evolutionary origin of the reproductive unit in Mapanioideae. The two lateral scales are interpreted as a split prophyll (Prychid & Bruhl, 2013). Ontogenetic studies in Mapanioideae are still limited due to the difficult access to plant material (Vrijdaghs *et al.*, 2006; Richards *et al.,* 2006).

Diversification within Mapanioideae began ca. 33 mya (Stevens, 2001 onwards). Fossil records represent members from the Eurasian Eocene ca. 48 mya (*Volkeria messelensis* & *Caricoidea*, Smith *et al.,* 2009). Mapanioid sedges such as *Scirpodendron* (fig. 1.9A-B) have for a long time been considered to be 'primitive' members of the family (e.g. Kern, 1974). This is based especially on their preferences for old biomes such as rainforest habitats, containing many basal lineages of Angiosperms (e.g. Couvreur *et al.*, 2011).

Nevertheless, the actual Mapanioid sedges have to be seen as highly derived since in the corresponding clade many derived characters accumulated in the ca. 40 million years between its origin and its diversification. Mapanioideae now consist of two tribes: Hypolytreae and Chrysitricheae, containing 13 genera (Simpson *et al.*, 2003). Hypolytreae developed a specific, uniporate pollen type (l.c.).

Fig. 1.9 Mapanioid sedges from Gen. McArthur, Eastern Samar, Philippines. A. *Scirpodendron ghaeri,* habit. B. *S. ghaeri,* mature inflorescence. C. *Paramapania parvibracteata*, mature inflorescence. D*.* SEM picture of one flowering unit in *P. parvibracteata* adapted from fig. 7D of Vrijdaghs *et al*. (2006). Abbreviations: f. filament; isc, inner scale; lsc, lateral scale; sg, stigma; st, style.

Cyperoideae, an extraordinary diversity

In contrast to Mapanioideae, floral and inflorescence morphology of Cyperoideae are better understood. Floral units in Cyperoideae were confirmed to be homologous to standard pentacyclic and trimerous monocot flowers (Vrijdaghs, 2006).

Cyperoideae show high diversification levels with several giant genera such as *Carex* (ca. 2000 species, mainly from temperate to subarctic regions) and *Cyperus* (ca. 950 species, mainly tropical) (Starr & Ford, 2008; fig. 1.11). This diversification started around ca. 77 mya, which falls close to the crown group age of the family. Trilepideae, corresponding to the earliest emerged clade within the cyperoid clade, is linked to the Gondwana shield in South America (*Trilepis*) and granite outcrops (inselbergs) on the African mainland and Madagascar

Fig. 1.10 Trilepideae, the basalmost clade in Cyperoideae, have been surviving on Inselbergs for millions of years. By inactivation of their chlorophyll, they can survive long periods of drought. In wetter conditions, these 'resurrection plants' are green within a few days. A. Inselbergs near Yaounde (Cameroon), covered with *Afrotrilepis pilosa*. B. *Microdracoides squamosus*. C. Creeping rhizomes of *Afrotrilepis pilosa*.

(*Afrotrilepis, Microdracoides* & *Coleochloa*; fig. 1.10). Resulting from their resilience in the landscape over 70 million years, these habitats are known to have accumulated many highly adapted palaeoendemics (e.g. Porembski & Barthlott, 2000).

Of the 14 tribes of Goetghebeur (1998) that are currently included in the Cyperoideae, four were found to be paraphyletic and need new circumscriptions (e.g. Muasya *et al.*, 2009a; fig. 1.8). While most smaller tribes were included in the family level phylogenetic analyses (Simpson *et al.*, 2007; Muasya *et al.,* 2009a), the larger tribes received additional interest in more detailed molecular phylogenetic studies. This was already the case for Abildgaardieae (Ghamkhar *et al.*, 2003), Cariceae (Starr & Ford, 2008), Cypereae (Muasya *et al.*, 2002; Larridon *et al.*, 2011a, 2013), Rhynchosporeae (Thomas *et al.*, 2009), Schoeneae (Verboom, 2006) and recently also Sclerieae (K. Bauters, UGent). Examples of species of most tribes are shown in fig. 1.11.

Noteworthy in the light of the current research is the multiple occurrence of C_4 photosynthesis within Cyperoideae. During the late Eocene, C_4 photosynthesis arose in at least five different Cyperaceae lineages opening possibilities for the colonization of open habitats (Besnard *et al.*, 2009). Lineages that developed C_4 photosynthesis are found in *Rhynchospora, Abildgaardieae* (*Bulbostylis, Nemum*), *Eleocharis* (2 origins) and *Cyperus* s.l. (Besnard *et al.*, 2009). Most of these lineages are rich in species indicating the adaptive importance of the C_4 innovation. C_4 photosynthesis is also linked to several types of Kranz anatomy of leaves and culms (Bruhl & Perry, 1995; Soros & Bruhl, 2000; Edwards & Voznensenskaya, 2011) and has been useful (besides embryography) to test the classification of several taxa with unclear relationships, especially in Cypereae (e.g. *Ascolepis* & *Lipocarpha*) (Goetghebeur, 1986).
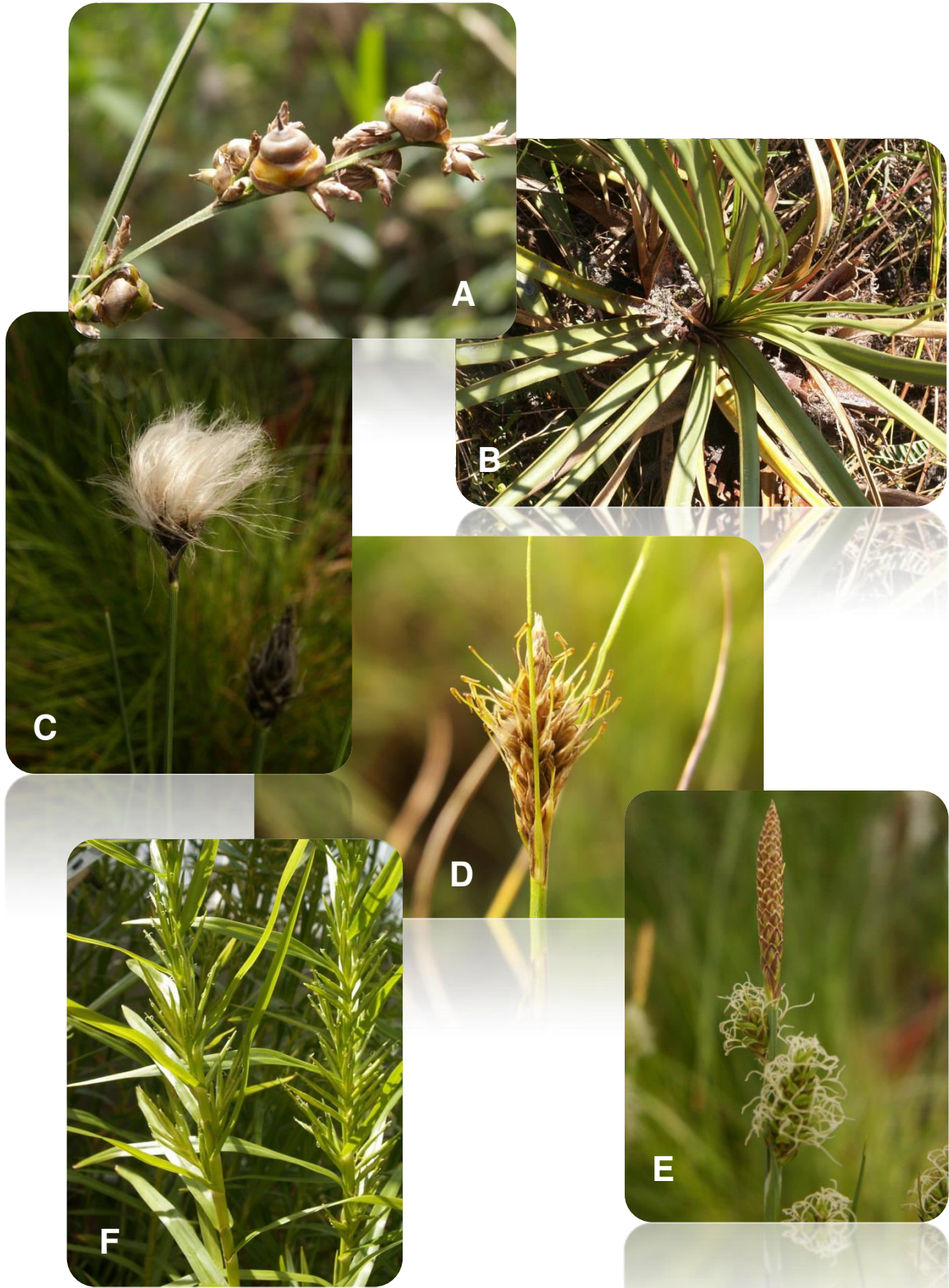
Fig. 1.11a Diversity of Cyperoideae part 1. A. *Scleria depressa* (Sclerieae), Cameroon. B. *Costularia* spec. (Schoeneae), Madagascar. C. *Eriophorum* spec. (Scirpeae), BG UGent. D. *Uncinia* spec. (Cariceae), BG UGent. E. *Carex* spec. (Cariceae), BG UGent. F. *Dulichium arundinaceum* (Dulichieae), BG UGent.
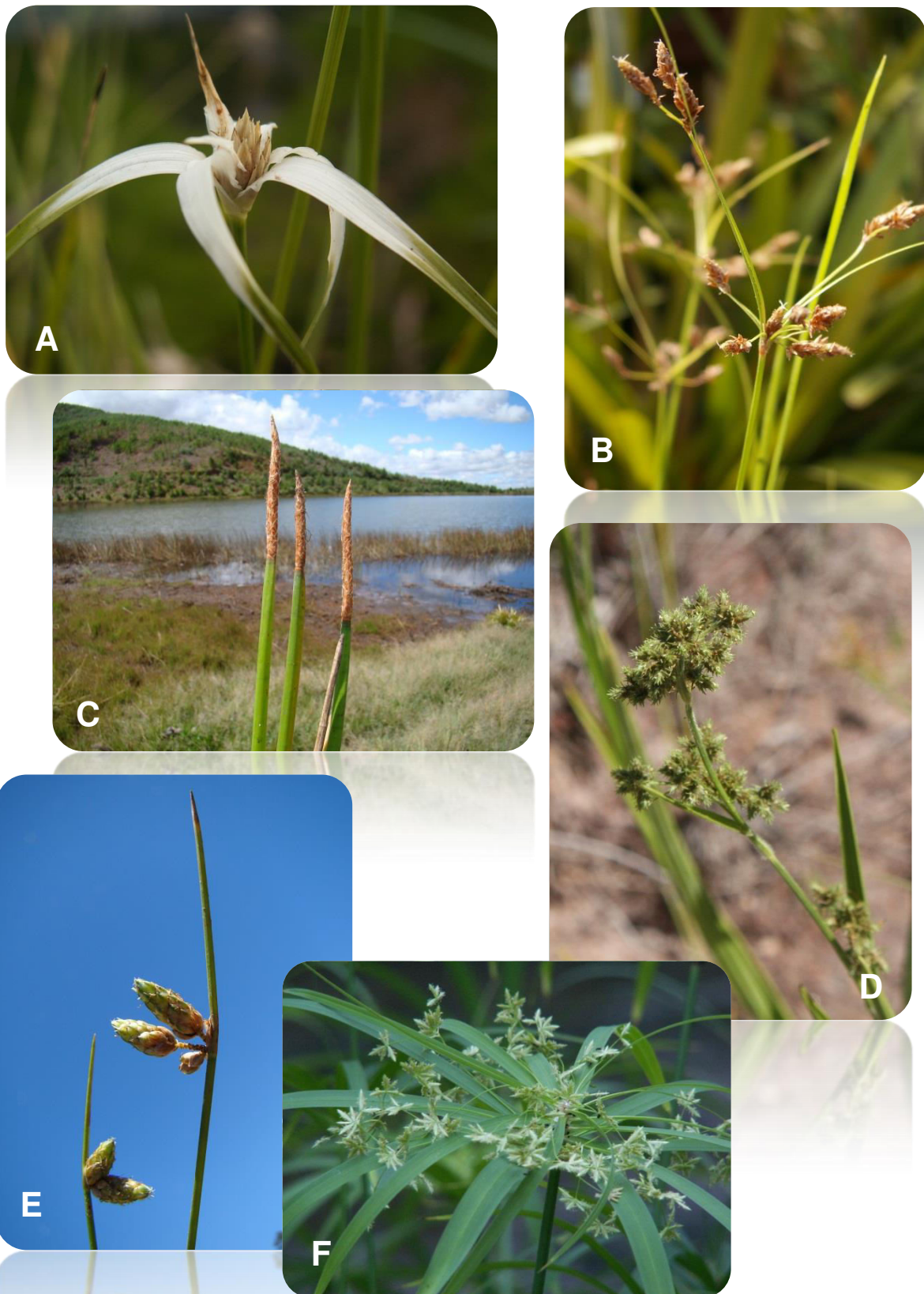
Fig. 1.11b Diversity of Cyperoideae part 2. A. *Rhynchospora colorata* (Rhynchosporeae), BG UGent. B. *Fimbristylis dichotoma* (Abildgaardieae), BG UGent; C. *Eleocharis acutangula* (Eleocharideae), Madagascar. D. *Fuirena ciliaris* (Fuireneae), BG UGent. E. *Schoenoplectus* spec. (Fuireneae), Madagascar. F. *Cyperus involucratus* (Cypereae), BG UGent. Pictures taken by M. Reynders (A, B, D & F), W. Huygh (D) & A.M. Muasya (C & E).

1.4.2 The Cypereae

Cypereae correspond to one of the crown clades of Cyperaceae and is the most speciose sedge tribe in the tropics. The clade arose within a grade representing the Fuireneae sensu Goetghebeur. *Schoenoplectiella* forms the clade sister to the Cypereae clade (Muasya *et al.*, 2009a; fig. 1.8). Morphological diversification within the tribe resulted in several types of highly specialized inflorescences. Generic circumscriptions and affinities within Cypereae are challenging as the amount of homology problems is among the highest in the sedge family (due to multiple origins of several characters as pistil dimerisations, deciduous spikelets, inflorescence condensations, etc.). In this chapter, a detailed overview of the history and the challenges for the taxonomy of this tribe are given.

1.4.2.1 Current delimitation of Cypereae

Cypereae originally included only genera characterised by spikelets with distichously placed glumes and reduced flowers without perianth: corresponding to the genus *Cyperus* and its most obvious relatives (e.g. Colla, 1836). More recently, when combining molecular, morphological, embryographical, anatomical, chemical and ontogenetic studies, Cypereae have grown to include also species with spirally arranged glumes (Haines & Lye, 1983; Goetghebeur, 1986, 1998) and perianth parts (Vrijdaghs *et al.*, 2006; Muasya *et al.*, 2009a, 2012). The only diagnostic criterion left is the *Cyperus-* type embryo (or the related *Ficinia-* type embryo) (Muasya *et al.*, 2009b).

Based on molecular phylogenetic data, two main clades were recognised within Cypereae (Muasya *et al.*, 2002, 2009a) as shown in fig. 1.12. The *Ficinia* clade consists of species with a generally southern (African) distribution, characterised by usually spirally arranged glumes and pseudolateral inflorescences. This clade currently includes six genera: *Dracoscirpoides* (fig. 1.15), *Erioscirpus*, *Ficinia* (fig. 1.13A), *Hellmuthia*, *Isolepis* (fig. 1.13B) and *Scirpoides*. *Dracoscirpoides, Erioscirpus* and *Hellmuthia* possess (remnants of) perianth parts (Vrijdaghs *et al.*, 2006; Muasya *et al.*, 2009a, 2012; Yano *et al.*, 2012). *Isolepis* is paraphyletic containing *Ficinia* (Muasya *et al.*, 2009a). The latter is characterised by a gynophore (Vrijdaghs *et al.*, 2005b). In *Isolepis levynsiana* (formerly *Cyperus tenellus*), glume placement is distichous (Muasya *et al.*, 2006, 2007).
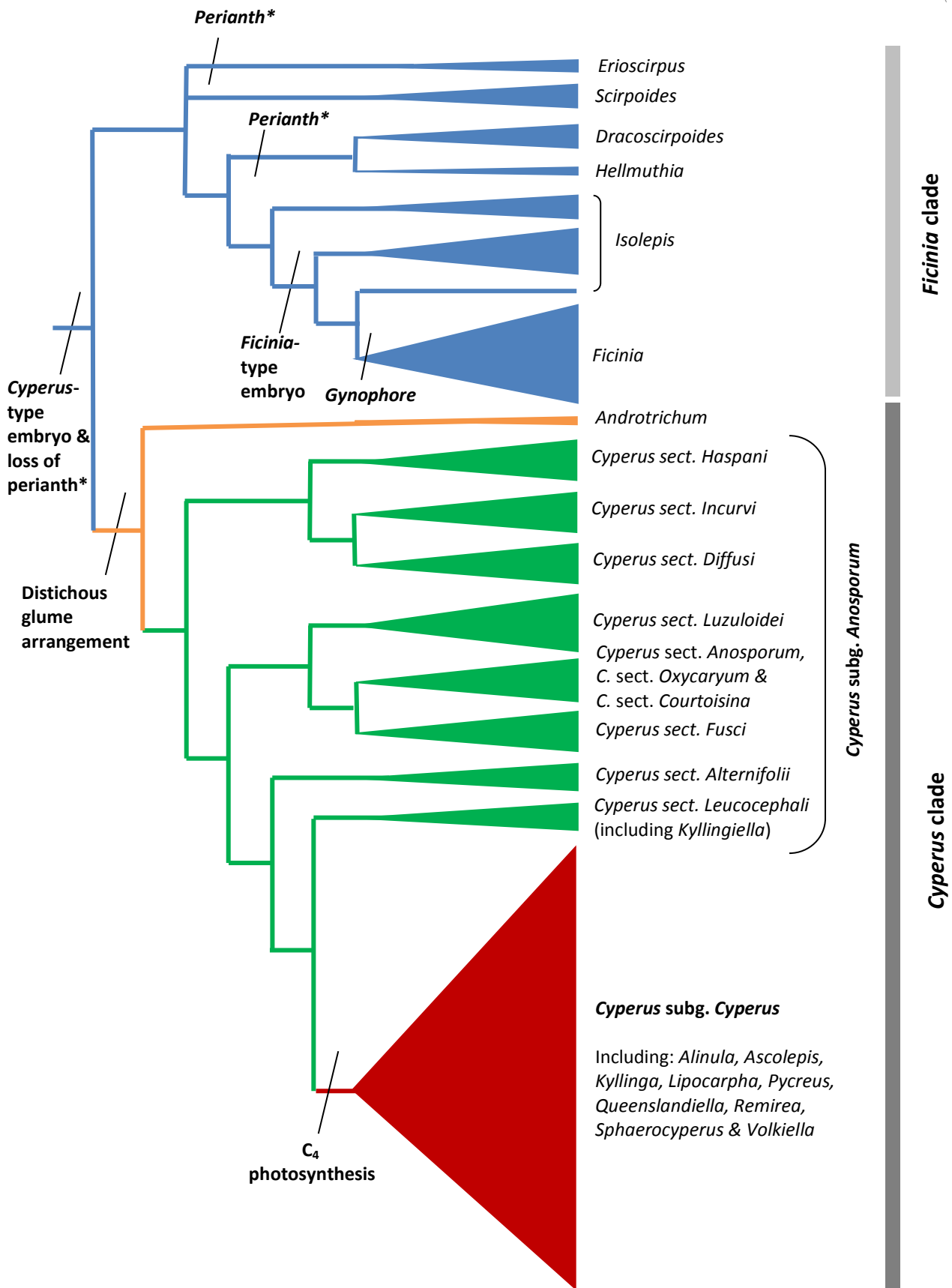
Fig. 1.12 Cladogram of Cypereae combining topologies from Muasya *et al.*, 2009a (*Ficinia* clade & *Androtrichum*), Yano *et al.*, 2012 (position *Erioscirpus*) & *Larridon et al.,* 2011a (*Cyperus* s.l.). Color codes: Blue= genera of the *Ficinia* clade. Orange= *Androtrichum*. Green= C_3 *Cyperus* grade. Red= C_4 *Cyperus* clade. * Either perianth got lost as the first node with 2 reversals or got lost at three different nodes.

The *Cyperus* clade is highly diversified, consisting of *Cyperus* s.s. and related genera (fig. 1.12). Most species in the clade bear spikelets with distichous glumes. *Androtrichum* is sister to the rest of the *Cyperus* clade, while 12 other currently recognized genera (following the classification of Goetghebeur, 1998) are nested within *Cyperus* s.s.: *Alinula*, *Ascolepis*, *Courtoisina*, *Kyllinga*, *Kyllingiella*, *Lipocarpha*, *Oxycaryum*, *Pycreus*, *Queenslandiella*, *Remirea*, *Sphaerocyperus* and *Volkiella*.
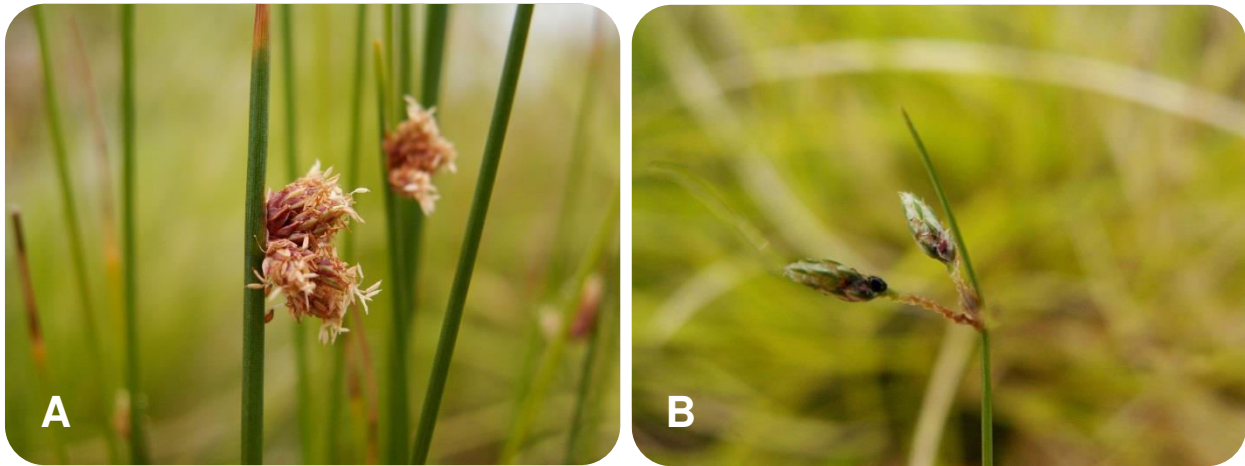
Fig. 1.13 Examples of species from the *Ficinia* clade, characterised by a pseudolateral inflorescence and spirally arranged glumes. A. *Ficinia nodosa*. B. *Isolepis cernua*. Pictures taken in the BG Ugent.

1.4.2.2 A history of misunderstanding and homoplasy

The taxonomy of Cypereae has seen many changes and differing, strongly opposing views during its history (e.g. Goetghebeur, 1986; Bruhl, 1995). Generally, two main reasons can be identified: at first, interpretations of the strongly reduced morphologies of certain taxa were not obvious, and secondly, there were different opinions on which diagnostic characteristics are the most reliable.

Before reaching the current delimitation, which largely corresponds to the tribal classification of Goetghebeur (1986, 1998), Cypereae have seen a long history of misinterpretations of the many derived morphologies present in the group. For example, *Lipocarpha* (fig. 1.14) was moved around within the family, first placed in Hypolytreae (e.g. Nees, 1834; Kunth, 1837; Boeckeler, 1871) subsequently in Scirpeae (e.g. Clarke, 1908; Kern, 1974), and even receiving its own tribe Lipocarpheae (e.g. Koyama, 1982). Eventually,

investigation of other characters (such as embryos and chlorocyperoid anatomy) combined with a more correct interpretation of the inflorescence lead to the current classification of the genus in Cypereae (Goetghebeur, 1986). With the latest treatment based on molecular phylogenetic data, *Lipocarpha* sensu Goetghebeur is found to be polyphyletic and will be sunken into *Cyperus* s.k. (Bauters, 2011; Bauters *et al.*, submitted).

Different choices in the priority of characters important for generic delimitations have led to disagreements between the cyperologists studying this group. This is especially the case for the status of the segregate genera within *Cyperus* s.l., which have largely been circumscribed by different combinations of a limited set of characters (fig. 1.20). Inevitably, some of these characters represent homoplasies (Goetghebeur, 1986).
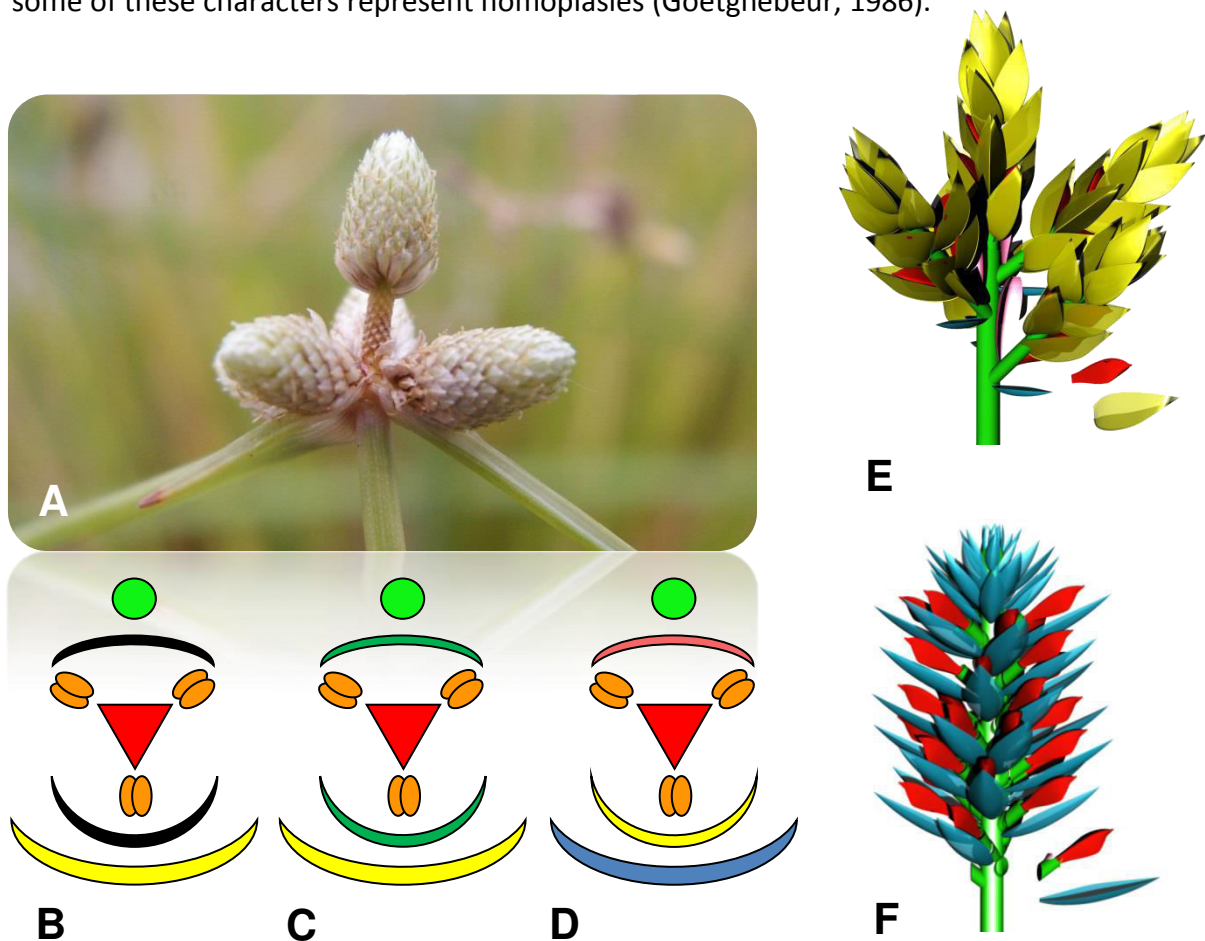
Fig. 1.14 The challenging morphology of the *Lipocarpha* inflorescence. A. Head-like inflorescence of *L. chinensis* (BG UGent). B, C & D. Models showing the different interpretations of the flowering units as mapanioid flowering units (B), scirpoid flowers with perianth (C) or reduced spikelets with a prophyll and first glume surrounding the flower (D). E & F. 3D models showing the difference between normal spikelets with spirally arranged glumes (E) and a pseudospikelet with reduced deciduous spikelets in the axis of the spikelet bracts (F). Color codes: Black= mapanioid scales. Blue= spikelet bract. Green (dark)= perianth. Green (pale)= spike axis & rachilla. Orange= stamens. Pink= Spikelet prophyll. Red= pistil. Yellow= glume.

Cypereae in the 18th & 19th centuries

Already in 1753, Linnaeus laid the foundation for distinguishing species in the larger genera such as *Cyperus* and *Scirpus*, *Cyperus* species being characterised by glumes with a distichous arrangement and *Scirpus* species having spirally arranged glumes.

During the following century, more and more species were added to the family and many new genera were described to house aberrant species with specific morphologies (e.g. Nees, 1834). With the growing amount of genera within the Cyperaceae, a more elaborate classification became necessary and the first tribal classifications were effected around large genera such as *Carex, Rhynchospora, Scleria, Cyperus* and *Scirpus* s.l.. Earlier circumscriptions of the tribe Cypereae were rather narrow, including only *Cyperus* and several taxa with an obvious relationship with *Cyperus* such as *Pycreus, Kyllinga* and *Mariscus* (e.g. Colla, 1836; Clarke, 1908; Kükenthal, 1935-36).

While several tribes had very reliable diagnostic characters such as utricles or large ornaments on the fruits, Scirpeae and Cypereae were primarily distinguished based on the position of the glumes, respectively spiral and distichous arrangements (Clarke, 1908). As spirally arranged glumes are a widespread character state in Cyperaceae, *Scirpus* and Scirpeae became a dump for all species not obviously linkable to one of the other tribes (e.g. Dhooghe *et al.*, 2003; Simpson *et al.*, 2007) and as a consequence an important amount of species now belonging to Cypereae was hidden in the heterogeneous *Scirpus* until beyond the mid of the 20th century. The application of modern techniques resulted in major shifts in our understanding of the evolutionary patterns in sedges and of the reliability of morphological characters used for taxonomic delimitations within the family (Goetghebeur, 1986).

Embryography: breaking of the Scirpeae bubble!

During the 1960s, Van der Veken (1965) studied the embryo morphology all over Cyperaceae. When focusing on the shapes and positions of the cotyledon, embryonic root, germination furrow and coleoptile, these characters appeared to be well conserved within certain tribes as Cariceae and Cypereae. Cypereae were found to be homogenous, all containing an embryo of the *Cyperus*-type. In contrast, in Scirpeae, the embryo types showed to be very heterogeneous, suggesting that Scirpeae, and more specifically *Scirpus* with its spirally arranged glumes, does not represent a natural entity. Actually Scirpus served as a

large taxonomical garbage bin to collect species that did not fit in any other, better circumscribed taxonomical entity.

With the acceptance of the heterogeneity of *Scirpus*, many, mostly small, genera were resurrected or newly described to accommodate aberrant species previously hidden in *Scirpus* such as *Alinula, Ascolepis, Kyllingiella, Lipocarpha, Oxycaryum, Remirea, Rikliella, Sphaerocyperus* and more recently also *Dracoscirpoides* and *Erioscirpus* (now all in Cypereae) (e.g. Raynal, 1973; Haines & Lye, 1983; Muasya *et al*., 2012; Yano *et al*., 2012).

The classification of Goetghebeur (1986)

While most embryographical studies up to then remained rather descriptive, Goetghebeur (1986) combined these findings with his observations on inflorescence typology and any other data available from literature on anatomy and morphology to evaluate the taxonomical value of all known sedge genera and their tribal classification. A general criterion was that each tribe should contain only a single embryo type.

Consequently, Cypereae were circumscribed to contain taxa with a *Cyperus*-type embryo (Goetghebeur, 1986). Based on this criterion, Goetghebeur (l.c.) identified six scirpoid genera (*Ficinia, Isolepis, Desmoschoenus, Scirpoides, Kyllingiella* and *Oxycaryum*) having more affinities with Cypereae than with Scirpeae. He initially placed *Isolepis, Ficinia* and *Desmoschoenus* in a separate tribe Ficinieae differing from Cypereae by a deeper furrow above the primary root in the embryo. Later, Goetghebeur (1998) combined both tribes under Cypereae s.l. as some species of *Isolepis* also seem to have an embryo of the *Cyperus*-type. The absence of perianth parts were seen as a strong common character of Cypereae to distinguish them with the scirpoid tribes.

In Cypereae, Goetghebeur (1998) ultimately listed 26 different genera. *Oxycaryum, Kyllingiella, Scirpoides* and *Androtrichum* were considered to be the most primitive taxa based on a weakly differentiated embryo. However, this type of embryo is also present in many C_3 *Cyperus* species, and even more differentiated lineages with C_4 photosynthesis, such as *Ascolepis* and *Lipocarpha*. Moreover, *Oxycaryum, Kyllingiella* and *Scirpoides* also share spikelets with a spiral glume arrangement which he assumed to be the primitive condition in Cypereae (Goetghebeur, 1986). Within the core Cypereae, Goetghebeur (l.c.) discussed several conflicts in generic characteristics.
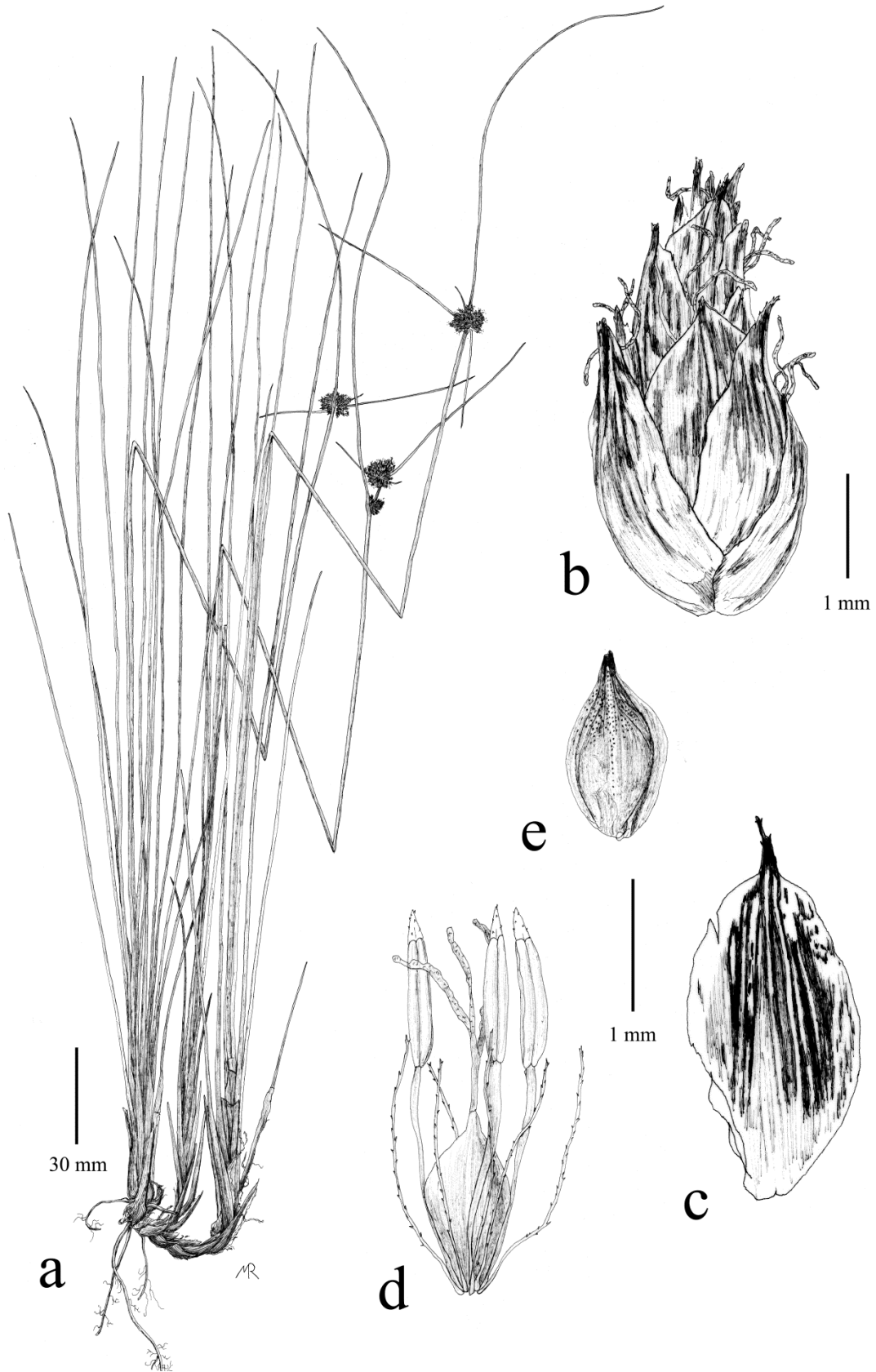
Fig. 1.15 *Dracoscirpoides surculosa,* a new genus and species of Cypereae. A. Habit. B. Spikelet. C. Glume. D. Flower (abaxial view). E. Fruit (abaxial view). All drawn from Browning 647 (GENT). After fig. 6 of Muasya *et al.*, 2012.

For example laterally compressed dimerous pistils can be found in species with deciduous glumes (*Pycreus*) and also in species with deciduous spikelets (*Kyllinga* and *Queenslandiella*). However, also deciduous spikelets and deciduous glumes can be found in species with trimerous pistils. Hence either or both laterally flattened dimerous pistils or deciduous spikelets must have originated at least twice within the group (convergent evolution). This is only one of the examples in the *Cyperus* clade where the phylogenetic homology question for the derived characters has been unresolved for a long time. This will be further addressed in 1.4.3.

The era of molecular phylogeny: even more homology questions in Cypereae!

Results from molecular phylogenetic analyses (Simpson *et al*., 2007; Muasya *et al.,* 2009a; Yano *et al*., 2012) support Cypereae sensu Goetghebeur (1998) as corresponding to a single clade (further called the Cypereae clade). However, some smaller adjustments needed to be made that have an important impact on the circumscription of the tribe and of its genera (Muasya *et al.,* 2009b).

First there was the recent addition of three small, perianth bearing, genera (*Hellmuthia, Dracoscirpoides* and *Erioscirpus*) to the *Ficinia* clade, one of the two subclades of the Cypereae clade. *Hellmuthia* had been classified in Hypolytreae as its two laterally placed perianth scales had previously been confused with the scales present around the flowering units in mapanioid sedges (Goetghebeur, 1998; Vrijdaghs *et al*., 2006). *Dracoscirpoides* Muasya is a newly described genus including two species that were not yet removed from *Scirpus* s.l.: *Scirpus falsus* and *S. ficinioides*. During the description of the new genus, a third and novel species was discovered and described (Muasya *et al*., 2012; fig. 1.15). *Erioscirpus* is characterised by cotton-like long perianth bristles and contains two species from the southern Himalaya (Yano *et al*., 2012).

Secondly, the molecular studies of Muasya *et al.* (2002, 2009a) showed that distichous glume arrangements had multiple origins in the Cypereae clade and multiple reversals of distichous to spiral glume arrangements also occurred. Concerning distichously arranged glumes a first origin seems to have occurred somewhere at the base of the *Cyperus* clade (depending whether *Androtrichum* would be mono- or paraphyletic, one of the two species has a spiral glume arrangement while the other has a distichous arrangement) and a second

origin of distichously arranged glumes within the main clade of *Isolepis* (*Isolepis levynsiana*; Muasya *et al*., 2006, 2007). In addition, in the *Cyperus* clade, spiral glume placement appears to be a multiple reversed condition from a distichous arrangement, as both *Kyllingiella* and *Oxycaryum* (both with spirally arranged glumes) seem to cluster among the C_3 species of *Cyperus* (characterised by distichous glumes). As a consequence, the character that Linnaeus (1753) once presented as the key character in the distinction between the two genera *Cyperus* and *Scirpus,* was later on extended to Scirpeae and Cypereae, is not valid anymore for the distinction between different genera and sometimes not even separate species in Cypereae (Muasya *et al.*, 2009b).

Thirdly, in *Cyperus* the species with C_4 photosynthesis form a well-supported clade (Muasya *et al*., 2002; Larridon *et al*., 2011a). However, this clade contains nine genera accepted in the classification of Goetghebeur (1998; fig 2.10). Unfortunately, the relationships between the different specialized lineages are not resolved since most of C_4 *Cyperus* forms one large polytomy. *Cyperus cuspidatus* and relatives consistently form the earliest emerging clade in the C_4 *Cyperus* phylogenies (Muasya *et al.*, 2009b; Larridon *et al*., 2011a).

1.4.3 *Cyperus* s.l.

1.4.3.1 A giant genus

When including the 12 segregate lineages, *Cyperus* s.l. encompasses ca. 950 species and forms the largest sedge genus in the tropics. Frodin (2004) lists *Cyperus* s.l. as the 23rd largest angiosperm genus, and it can be considered as a giant genus as it contains over 500 species. In human culture nutsedges or flatsedges have had a wide variety of uses, e.g. for paper making (*C. papyrus*), weaving & building materials (*C. iria, C. textilis, C. malaccensis,*…), essential oils (*Cyperus scariosus*), food source (*C. esculentus*) and ornamental plants (*C. haspan, C. involucratus*, …) (Simpson & Inglis, 2001).

Growth forms are adapted to various, especially wetland, habitats. Species are known from forest floors, swamps, savanna, sand dunes, seashores, rock outcrops, high altitude mountains, rice fields, etc. (e.g. Haines & Lye, 1983). Growth size varies from a few centimeters to several meters. The basic inflorescence in the *Cyperus* clade is an anthela of spikelets with a basal whorl of leaflike bracts (involucre). Two main types can be found, a first where the spikelets are grouped in digitate clusters and a second where spikelets are grouped

in spikes(see fig. 1.16). The anthela can be modified in various ways. Common modifications are contractions or elongations of axes and reductions in numbers of branches and spikelets. Spikelets are highly variable in shape, sizes and numbers; they have (with a few exceptions) a distichous glume arrangement, which makes them appear flat! In many species, the spikelets are deciduous as a whole and then often bearing a single maturing fruit. Empty glumes may also be present. The flowers are small and perianthless with one whorl of 3-1 stamens and a tri- to dimerous gynoecium. Nutlets are usually small and are dispersed either separately or enclosed within a deciduous spikelet/ partial spikelet.

Eucyperus
(C_3 photosynthesis)

Pycnostachys
(digitate spikelet clusters)

Chlorocyperus
(C_4 photosynthesis)

Choristachys
(spikes of spikelets)
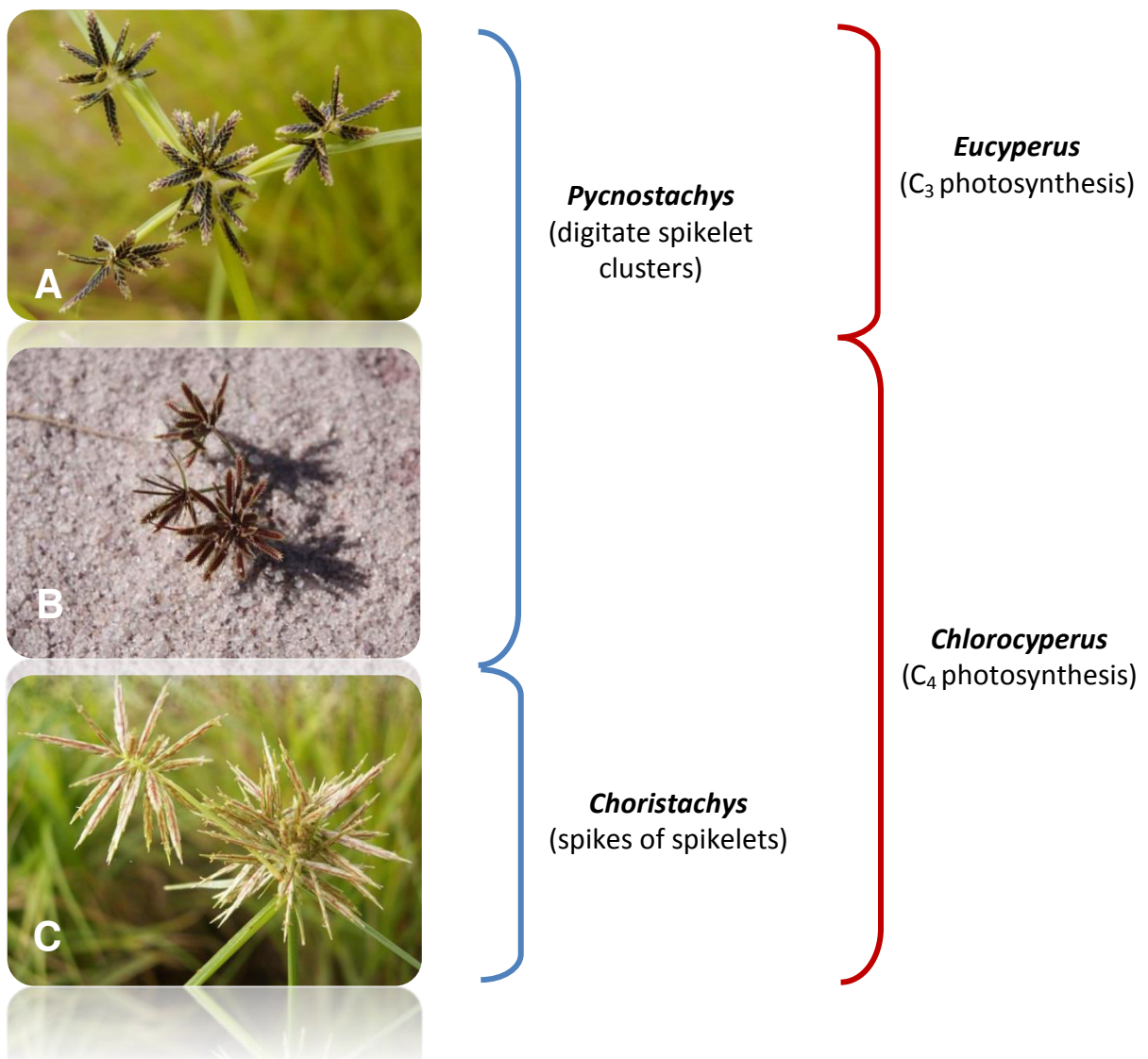
Fig. 1.16 Subdivisions described in *Cyperus* s.s. showing the inflorescences of: A. *Cyperus fuscus* (BG UGent) with digitate spikelet clusters and C_3 photosynthesis. B. *Cyperus waterlotii* (Madagascar) with digitate clusters and C_4 photosynthesis. C. *Cyperus congestus* (BG UGent) with spikes of spikelets and C_4 photosynthesis. Pictures taken by M. Reynders (A & C) and W. Huygh (B).

Within *Cyperus*, two subdivisions were distinguished, based on either photosynthetic type (C_3 or C_4 photosynthesis, linked with a specific anatomy type; e.g. Rikli, 1895) or the inflorescence typology (spikelets in digitate cluster or in spikes; e.g. Clarke, 1893; Kükenthal, 1935-36; fig. 1.16). Anthelate inflorescences with spikelets with digitate clusters and derived forms seemed to be primarily linked with C_3 photosynthesis and spikes of spikelets are exclusively found in species with C_4 photosynthesis. However, as demonstrated by Goetghebeur (1986; fig. 1.16) these two classification systems are not congruent since several C_4 *Cyperus* species (e.g. *C. cuspidatus*) possess digitately clustered spikelets. Isotope analyses confirmed the photosynthetic type of these species (Bruhl & Wilson, 2007; Larridon *et al.*, 2011a).

Several authors considered the C_4 pathway to have originated in a single event within the *Cyperus* clade (Raynal, 1973; Goetghebeur, 1986, 1998; Soros & Bruhl, 2000). This was confirmed by molecular phylogenetic studies which show a highly supported C_4 *Cyperus* clade which is nested within a grade of clades with C_3 photosynthesis (Muasya *et al.*, 2002; Besnard *et al.*, 2009; Larridon *et al.*, 2011a). Larridon *et al.* (2011b) suggested a classification of two subgenera within *Cyperus* s.l. Species with a C_4 photosynthetic pathway are accommodated in *Cyperus* subgenus *Cyperus* and species with C_3 photosynthetic pathway in a paraphyletically circumscribed *Cyperus* subg. *Anosporum*.

1.4.3.2 *Cyperus* subgenus *Anosporum*

Cyperus subg. *Anosporum* sensu Larridon *et al.* (2011b), includes the *Cyperus* s.l. species using C_3 photosynthesis (C_3 *Cyperus*) and forms a grade at the basis of the phylogenetic hypothesis of the genus (Larridon *et al.*, 2011a; fig. 1.12). This subgenus encompasses around 150 species (fig. 1.17). Spikelets in C_3 *Cyperus* are digitately clustered and different lineages show condensation of the inflorescence through prophyll branching, serial axillary budding or combinations of both (Guarise & Vegetti, 2008; Larridon *et al.*, 2011c). Most species of this group prefer shaded conditions with low seasonality such as forest edges and even forest floors in deep shade. Nevertheless, a few lineages are adapted to more open, permanently wet conditions. The majority of species are hemicryptophytes, geophytic and therophytic species are rare in this group.
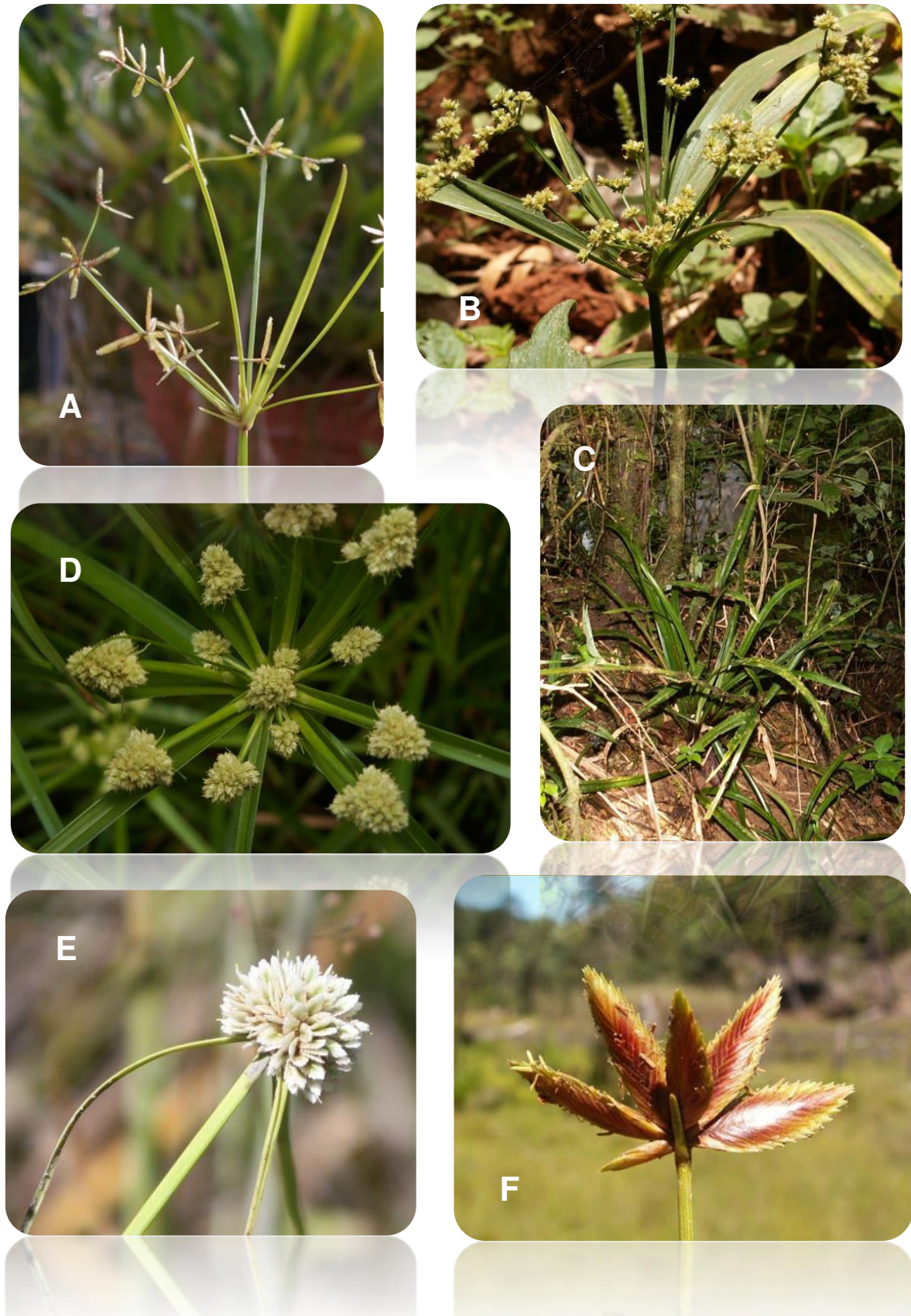
Fig. 1.17 Species in *Cyperus* subg. *Anosporum*. A. *C. haspan* (*Haspani*). B. *C. buchholzii* (*Diffusi*). C. *C. rufostriatus* (*Incurvi*). D. *C. luzulae* (*Luzuloidei*). E. *C. pulchellus* (*Leucocephali*). F. *C. pectinatus* (*Anosporum*). Pictures taken by M. Reynders, except pict. E by W. Huygh at BG UGent (A & D), Cameroon (B) & Madagascar (C, E & F).
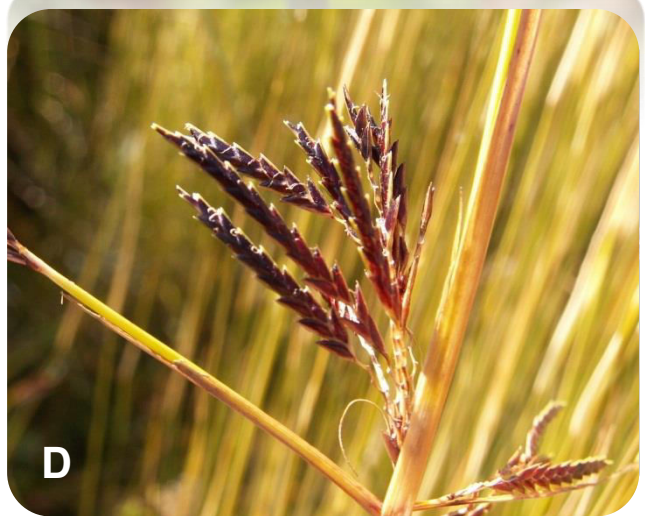
Fig. 1.18 Diversity within *Cyperus* subg. *Cyperus*. A. *Cyperus papyrus*. B. *C. capitatus*. C. *C. waterlotii*. D. *C. micrantherus*. E. *C. strigosus*. F. *C. vestitus*. Pictures taken at BG UGent (B & E) and Madagascar (A, C, D & F).

While the relationships among the species with C_3 photosynthesis were unresolved in the molecular phylogenetic study of Muasya *et al.* (2002), Larridon *et al.* (2011a) managed to obtain a well-supported phylogenetic hypothesis for this group and subsequently a new classification could be proposed for *Cyperus* subg. *Anosporum* (Larridon *et al.*, 2011c; fig. 1.12).

Courtoisina, Oxycaryum and *Kyllingiella* are nested within the C_3 *Cyperus* grade (Larridon *et al.*, 2011a). More profound morphological and ontogenetic research shows transitional states between these tree taxa and related C_3 *Cyperus* species. Therefore it is most appropriate to sink *Oxycaryum, Courtoisina* and *Kyllingiella* into *Cyperus* (Larridon *et al.*, 2011b). *Kyllingiella* and *Oxycaryum* both show spiral glume arrangements, a character that is now confirmed to have originated in multiple reversals from the standard distichous glume arrangement in the clade. *Kyllingiella* is now merged with *Cyperus* sect. *Leucocephali,* together forming the sister clade of C_4 *Cyperus*. Although species of this section use the C_3 photosynthetic pathway, they prefer more open and

drier growth conditions than most other members of C_3 *Cyperus* (Larridon *et al.*, 2011c)*.*

1.4.3.3 *Cyperus* subgenus *Cyperus*

The C_4 Cyperus clade

Cyperus subgenus *Cyperus* encompasses the *Cyperus* species with C_4 photosynthesis (C_4 *Cyperus*). The C_4 *Cyperus* clade contains around 800 of the 950 species of Cyperus s.l., reflecting high diversification rates for this group. Besnard *et al.* (2009) calculated that this clade arose around 10 mya, a period of aridifications. The species are successful as they are found in all tropical habitats with open and at least seasonally wet conditions (e.g. Haines & Lye, 1983; Li *et al.*, 1999; Stock *et al.*, 2004). Therophytic and geophytic species are common within the clade, enabling the species to survive periods of drought. In addition, several species show high resistance to disturbance (e.g. burning and grazing; *Pycreus fibrillosus*), while others are able to survive various limiting conditions such as salty (e.g. *Remirea maritima*) or oligotrophic soils. Last but not least, many species are highly competitive and form dominant elements (e.g. *C. papyrus*) or are notorious weeds (e.g. *C. rotundus*; Holm *et al.*, 1977, 1978).

Fig. 1.19 Representative species of the former genera *Mariscus* and *Juncellus,* now placed in *Cyperus*. A. *Cyperus cyperoides,* with deciduous spikelets set in pedicillate spike. B. *Cyperus laevigatus,* with dorsiventrally compressed pistils. Pictures taken at the BG UGent.

The segregate genera of C$_4$ *Cyperus*

Morphological diversification of C$_4$ *Cyperus* is reflected in the presence of 9 so-called segregate genera that were recognised (Goetghebeur, 1998) based on several specialised characters such as deciduous spikelets (*Mariscus, Queenslandiella*) combined with inflorescence compaction (*Kyllinga, Mariscus*), empty glumes (*Remirea, Sphaerocyperus*) or advanced spikelet reductions (pseudospikelets in *Alinula, Ascolepis, Lipocarpha* and *Volkiella*). Also, dimerisations of the gynoecium were used to delimitate several taxa (*Pycreus, Kyllinga, Queenslandiella and Juncellus*), see figs 18 & 19.

Unclear homology questions cast a large shadow on the stability of the classification and especially on the generic recognition of the segregate genera. Goetghebeur (1986) addressed the conflict in generic characteristics present in *Cyperus* and its closest relatives. Three morphological features, which were assumed to be reliable in generic delimitations, are present in all possible combinations in different groups: C$_3$ vs. C$_4$ photosynthesis, deciduous glumes vs. deciduous spikelets and trimerous vs. dimerous pistils (fig. 1.20). Inevitably the unique origin of one of these characters implies the multiple origins of the others. As a consequence, there were major disagreements on the generic statuses of the different

Fig. 1.21 Diversity of C$_4$ *Cyperus* segregate genera. A. *Pycreus flavescens*. B. *Kyllinga bulbosa*. C. *Remirea maritima*. D. *Queenslandiella hyalina*. E. *Lipocarpha nana*. F. *Lipocarpha chinensis* and *Ascolepis brasiliensis*. Pictures taken at BG UGent (A & B), GENT herbarium (C & D) and Andringitra National Park, Madagascar (E & F).

Fig. 1.20 Conflicts in generic characteristics in *Cyperus* s.l. Columns indicate different pistil types (from left to right: trimerous, dorsiventrally flattened dimerous, laterally flattened dimerous). Rows indicate deciduous glumes vs. deciduous spikelets (from top to bottom). Colors of names indicate photosynthesis type (C_3 = blue, C_4 = red). Underlined taxa have pseudospikelets, which can be seen as the extreme situation of deciduous spikelets. Taxa with transitional morphologies are placed in overlapping boxes.

taxa in the various taxonomic treatments (e.g. Chermezon, 1934; Kükenthal, 1935-36; Koyama, 1961; Haines & Lye, 1983; Tucker, 1987, 1994; Goetghebeur, 1998).

Goetghebeur (1986) assumed that C_4 photosynthesis originated in a single evolutionary event in Cypereae. While C_4 photosynthesis originated in several different sedge groups, in Cypereae, all C_4 taxa represent a single Kranz anatomy type (the chlorocyperoid anatomy; Rikli, (1895). Goetghebeur (1986) admitted a high possibility for a paraphyletic *Cyperus* from which the 9 mentioned segregate genera arose. However, he did not consider paraphyly to be an obstacle for recognizing these specialised lineages as separate genera as long as they themselves originated in a single evolutionary event.

On the other hand, deciduous spikelets are present in species that clearly belong to unrelated groups of species, both in C_3 and C_4 Cyperus, which was also discussed in detail by Lye (1992). Moreover, some species, such as *Cyperus distans*, possess populations showing intermediate morphologies with deciduous spikelets or deciduous fruits or both on the same plants. From the nineties on, deciduous spikelets are not considered as a valid generic character anymore unless combined with other specialised morphologies with a higher taxonomical value. As a consequence, *Mariscus* species were sunken into *Cyperus* while other taxa with additional characters such as laterally compressed pistils (*Kyllinga, Queenslandiella*), glume wings (*Courtoisina*), pseudospikelets (*Alinula, Ascolepis, Lipocarpha*) or empty glumes at the base of the spikelet *(Remirea* and *Sphaerocyperus)* are still upheld in the classification of Goetghebeur (1998).

Next, many Cypereae species possessing dimerous pistils were classified in separate genera. Dorsiventrally flattened pistils are common in many different sedge lineages. *Cyperus* species with dorsiventrally flattened dimerous pistils were previously placed in a separate genus *Juncellus* (e.g. Clarke, 1908). However, since some species within *Cyperus* s.l. (e.g. *C. alopecuroides*) possess both trimerous and dorsiventrally flattened dimerous pistils, *Juncellus* could no longer be maintained as a separate genus (Goetghebeur, 1986).

Also, several of the other Cypereae genera include species with dorsiventrally flattened pistils, such as *Lipocarpha* and *Oxycaryum*. Dorsiventrally flattened pistils seem to have originated many times in Cypereae and therefore are not reliable for generic delimitations. In contrast, laterally flattened pistils are restricted to three Cypereae lineages (with only few exceptions elsewhere in Cyperoideae: *Lagenocarpus amazonicus* and *Rhynchospora rubra* ssp. *rubra*).

Goetghebeur (1986) postulated a single origin for laterally flattened pistils in Cypereae (see further in chapter 1.5.2). The use of this character for generic delimitations implies the postulation of multiple origins of deciduous spikelets (Lye, 1992; fig. 1.20).

In previous studies, phylogenetic relationships in the C$_4$ *Cyperus* clade are poorly resolved (Muasya *et al*., 2002; Larridon *et al*., 2011a) as most species and segregates are nested within a major polytomy. Consequently, the question whether the segregate genera are monophyletic is still unresolved. A more elaborate phylogenetic investigation with focus on the segregate genera, along with a discussion of characteristics and classification strategies for C$_4$ *Cyperus* is presented in chapter 6. More detailed cladograms are also produced for *Kyllinga* (Huygh *et al*., unpublished data) and *Lipocarpha* s.l. (Bauters *et al*., submitted).

1.4.4 *Pycreus* and its laterally compressed pistils

1.4.4.1 *Pycreus*

With its ca. 120 species, *Pycreus* is the largest of currently recognised segregate genera of *Cyperus* s.l. and is easily recognised by the combination of laterally compressed dimerous pistils and deciduous glumes and nutlets (e.g. Clarke, 1908; Goetghebeur, 1986; fig. 1.22).

The first species belonging to this taxon was already described by Linnaeus in his Species Plantarum (1753), as *Cyperus flavescens* (fig. 1.22E). With the description as a separate genus, Palisot de Beauvois (1816) emphasized the close relationship with *Cyperus* in using the name *Pycreus*, which is an anagram of *Cyperus*. At that time the new genus contained only *Pycreus polystachyos* (fig. 1.22A), which therefore serves as the type species of *Pycreus*. As relationships with *Cyperus* are obvious, many authors were not convinced of the separate generic status for *Pycreus* (e.g. Kükenthal, 1935-36; Haines & Lye, 1983; Tucker, 1994). Nevertheless, whether considered as a separate entity or as a subdivision of *Cyperus*, *Pycreus* has been consistently treated as a well circumscribed taxon.

Africa forms the center of diversity for *Pycreus* where it is especially well represented in the Soudano-Zambesian phytochorion. Several lowland species also managed to spread around the Indian Ocean and/or to the New World where a few smaller radiations followed, for example in the southern US (e.g. Corcoran, 1941) and Madagascar (e.g. Muasya *et al*., 2012). A few therophytic species also spread to temperate regions (e.g. *P. flavescens*, fig.

Fig. 1.22 Diversity of *Pycreus* species. A. *P. polystachyos*. B. *P. holosericeus*. C. *P. divulsus*. D. *P. sanguinolentus*. E. *P. flavescens*. F. *P. nitidus*. Pictures taken by M. Reynders except pict. A by W. Huygh at BG UGent (B, D & E) and Madagascar (A, C & F).
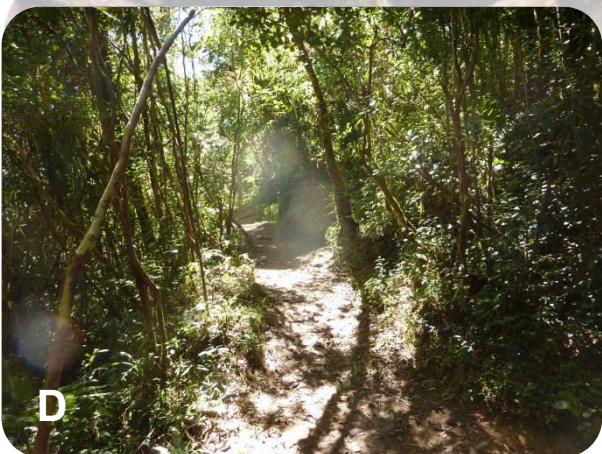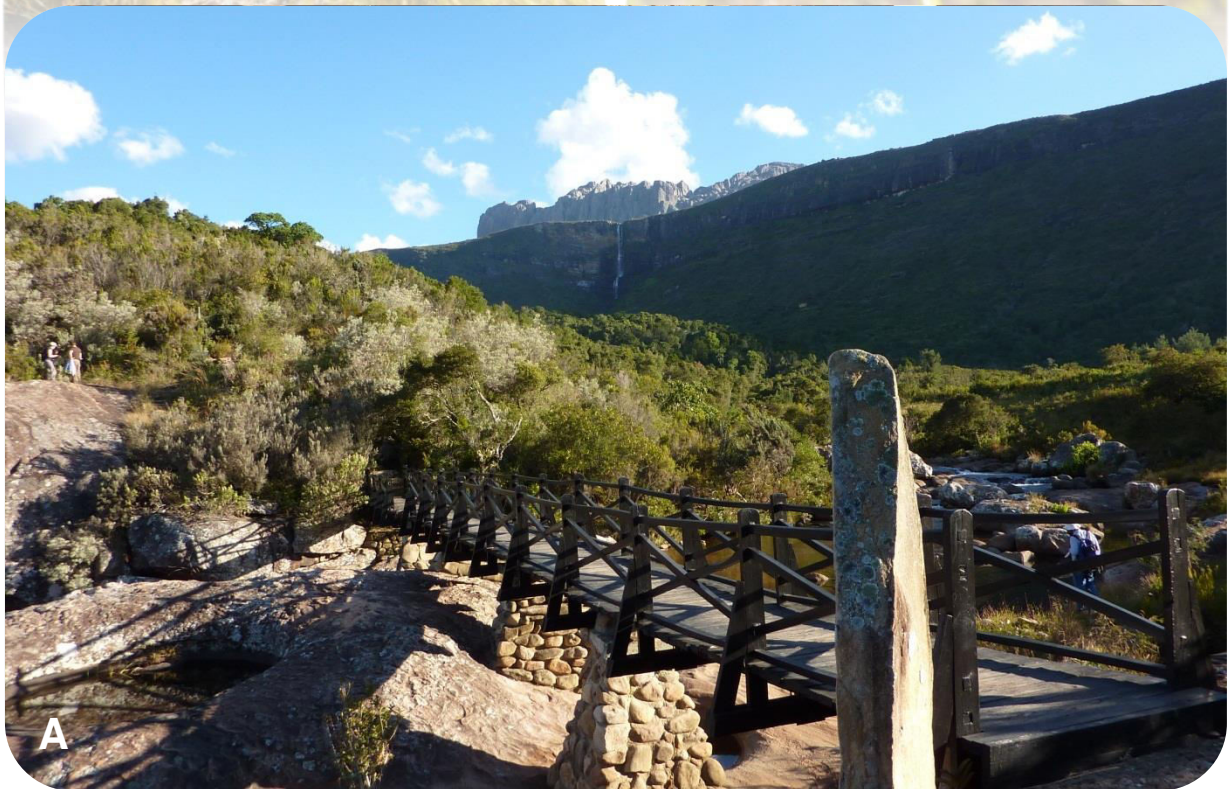
Fig. 1.23 Different niches of *Pycreus* species at Andringitra National Park, Madagascar. A. Bridge over river with the high plateau on the background, a top location for sedges (picture taken by I. Larridon). B. Moorlands on the high plateau with *P. nigricans* (C) as dominant species. D. Mountain forest with *P. ferrugineus* (E) along paths. F. Seepage zone on the rocks near the river, rich in sedges as *P. flavescens* var. *vicinus* (F). H. Peat like packages along seepage zones, formed by *P. atropurpureus* (I). J. The edges of the seepage zones are prone to fluctuations in humidity and inhabited by very small therophytes as *P. reductus* (K), which is here photographed on its type locality.

1.22E). Habitat preferences are diverse, from coastal sand dunes to salt marches and thermal springs, dambos, floating prairies and high altitude mountains. Typically, most species prefer exposed conditions, only a few species are tolerant for part-shade (e.g. *Pycreus ferrugineus;* Chermezon, 1937; fig. 1.23D-E). Short living, pioneering therophytes or hemicryptophytes are mostly found in temporal habitats in the lowlands (ditches, dambos, rice fields, edges of rock vegetation, etc.; e.g. *Pycreus polystachyos* (fig. 1.22A)*, P. macrostachyos, P. capillifolius, P. reductus* (fig. 1.23J-K), whereas taller and more competitive species dwell in or next to more permanent water bodies in different zones of the water banks and sometimes they even form floating mats (e.g. *Pycreus nitidus* (fig. 1.22F)*, P. mundtii*; e.g. Haines & Lye, 1983; fig. 1.6). One species is hydrophytic (*P. waillyi;* l.c.). Slow growing, tussocky and stress tolerant species can be found in high altitude mountain pastures and bogs (e.g. *P. nigricans* (fig. 1.23B-C)*, P. permutatus, P. atronervatus*). Many species have narrow niche preferences e.g. *Pycreus cataractarum* only grows along fast flowing rivers and *P. fontinalis* only near thermal springs (Kükenthal, 1936).

Pycreus species use C_4 photosynthesis linked with the chlorocyperoid anatomy type and possess spikes of spikelets, hence many authors considered *Pycreus* to be related to the C_4 species of *Cyperus* and other Cypereae genera with similar characteristics (e.g. Rikli, 1895; Palla, 1908). Goetghebeur (1986) was already aware of the probable paraphyletic nature of *Cyperus*. Nevertheless, *Pycreus* was maintained as a separate genus based on the presence of a distinct morphological character: its laterally compressed pistils.

The first molecular phylogenetic analysis of *Cyperus* s.l. (Muasya *et al*., 2002) confirmed *Pycreus* to be nested within the C_4 *Cyperus* clade. However, only a few species were included in this study and their position remained unresolved within a major polytomy. A more elaborate phylogenetic analysis representing the diversity present in *Pycreus* and other segregate lineages of C_4 *Cyperus* is presented in chapter 6.
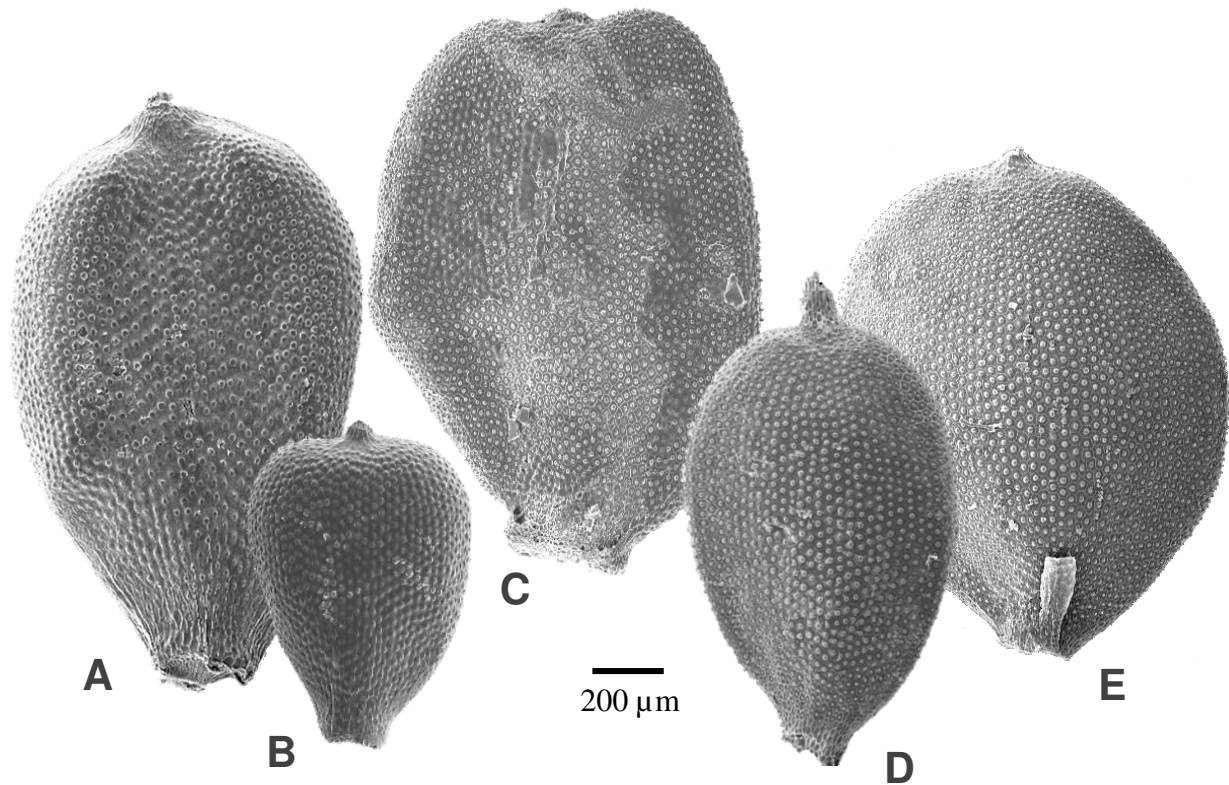
Fig. 1.24 Examples of laterally compressed nutlets in Cypereae. A. *Pycreus macrostachyos* (Berhaut 2643). B. *Pycreus capillifolius* (Reekmans 8811). C. *Queenslandiella hyalina* (Kilian & Lobin 2097). D. *Kyllinga polyphylla* (Reekmans 8399). E. *Kyllinga squamulata* (Reekmans 4304). SEM pictures taken by Marcel Verhaegen (BR).

1.4.4.2 Laterally compressed dimerous pistils

The laterally compressed dimerous pistils are the most important diagnostic characteristic of *Pycreus* which make the spikelets seem even flatter than in most other *Cyperus* s.l. species. Although rare in sedges, laterally flattened pistils are not restricted to *Pycreus* alone. Two other segregate genera of C_4 *Cyperus* also possess this pistil type, namely *Kyllinga* and *Queenslandiella* (fig. 1.24). Both of the latter taxa are characterised by deciduous spikelets, while in *Pycreus* mature nutlets and glumes are shed separately (fig. 1.20). As Goetghebeur (1986) explained, there is a conflict in the simultaneous presence of laterally compressed pistils and deciduous spikelets within *Cyperus* s.l.. Subsequently it was uncertain which of the two characteristics originated first.

As deciduous spikelets originated both in C_3 and C_4 lineages, these were not considered to be reliable for generic delimitations in Cypereae (Lye, 1992). On the other hand, many authors were convinced about the unique and derived nature of laterally compressed

dimerous pistils in Cypereae. Consequently, these pistils were considered as a reliable taxonomic character. Blaser (1941a) hypothesized a complex reorganization of the pistil vascular system in *Pycreus*, considering it to be a derivation from a scirpoid vasculature type and not from the *Cyperus*-type. However, Blaser's assumption is not congruent with the other morphological and anatomical data on the relationships between *Pycreus* and *Cyperus*. Since there was no clear hypothesis on the origin of laterally compressed pistils, this character was considered to have originated from complex processes, and therefore it was hypothesized to have arisen probably only once (Goetghebeur, 1986). Better understanding of the origin of laterally compressed pistils in the context of pistil evolutionary processes within Cyperoideae is needed for a correct evaluation of its taxonomical value. This topic is addressed in chapter 5 with combination of ontogenetic and anatomical data.

Finally, the question remains whether the laterally compressed pistils in *Pycreus, Kyllinga* and *Queenslandiella* originated from a single evolutionary event, or do we have to consider two or perhaps even tree separate origins? In chapter 6 this question will be further discussed.

1.4.4.3 Infrageneric classification of *Pycreus*

High morphological diversification within *Pycreus* led to the establishment of many names and subdivisions under this taxon as shown in chapter 3.

The latest revision for the complete genus with subdivisional treatment dates from Kükenthal (1936) who distinguished 9 sections divided among two unranked groups based on the shape of the nutlet epidermal cells (fig. 1.25). The *Isodiametrici* possess isodiametric cells (fig 2.23A), while the *Zonati* have elongated cells (fig 2.23B). These two unranked groups and their sections were based on the classifications of Clarke (e.g. 1908). Mariën (1969, unpublished thesis) and Van der Veken (unpublished data) suggested a third group '*Mixtae*' for the species with cells of intermediate length.
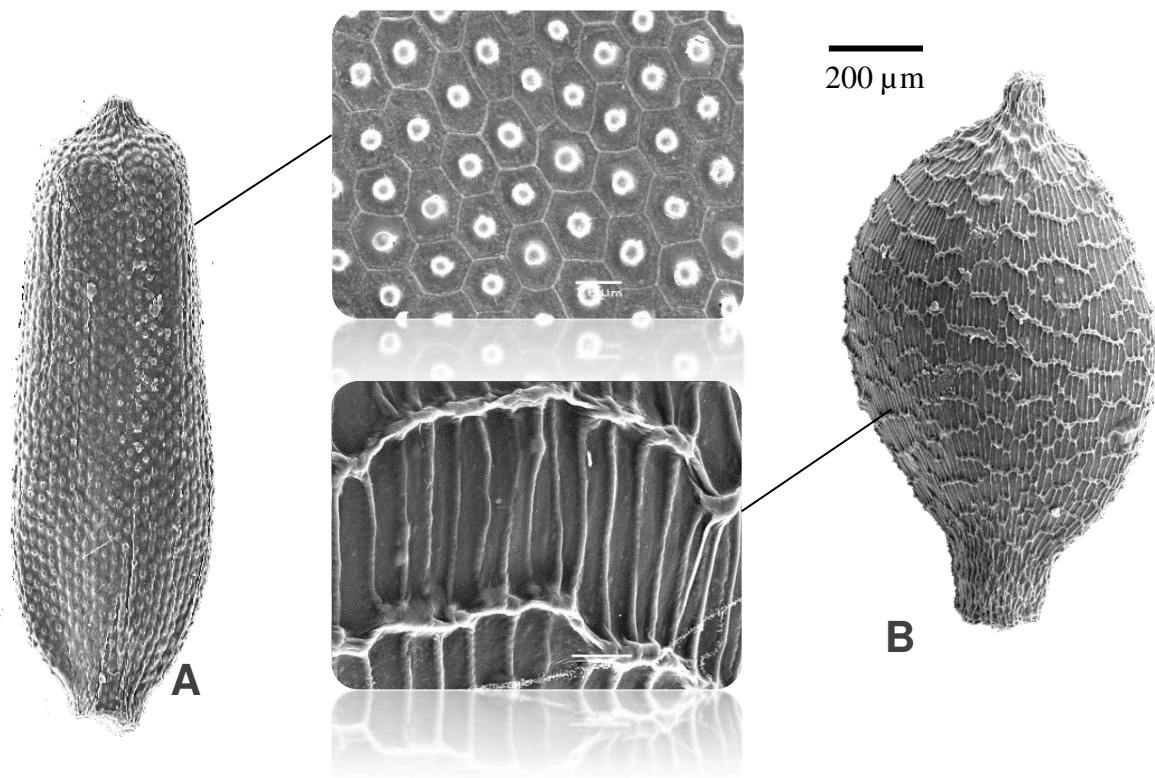
Fig. 1.25 Comparison of the two types of nutlet epidermal cells that were used in infrageneric classifications of *Pycreus*. A. Nutlet of *Pycreus intactus* with isodiametric cells containing one central silica body (Reid 559). B. Nutlet of *Pycreus flavescens* with elongated (or zonate) cells without silica bodies (Reid 1079). SEM pictures taken by Marcel Verhaegen (BR).

1.5 Research objectives

1.5.1 The *Pycreus* PhD research project

1.5.1.1 Main focus and justifications

The research presented in this thesis aims to contribute to a solution in understanding the evolutionary relationships within the diverse *Cyperus* s.l. (meso-scale), its homology, taxonomic and nomenclatural problems with a focus on *Pycreus* and its infrageneric taxonomy (micro-scale).

Understanding the evolutionary history of *Pycreus* and the production of a (preliminary) revision for this taxon were the original aims of this PhD research. However, to be able to address taxonomical questions in *Pycreus*, first a more profound understanding was necessary of evolutionary processes and relationships within the *Cyperus* clade, Cypereae and even the Cyperoideae subfamily. Only when morphologies and larger scale relationships are understood (meso-scale), more solid taxonomic treatments can be produced on the lower taxonomical levels (micro-scale) (e.g. Larridon *et al.*, 2011b), especially since the generic status of *Pycreus* needs to be reevaluated (this is further addressed below).

1.5.1.2 The grey zone between genus and subgenus

An important challenge in working with taxa such as *Pycreus* is their unstable generic status. In the standard binomial system that is used to name infrageneric taxa, the genus name always forms a part of the name for an infrageneric taxon (McNeill *et al.*, 2012). As a consequence, the choice to place a taxon on a generic or subgeneric level has an impact on perception of and communication about a certain species (and other infrageneric names) and also on the application of the nomenclatural rules. For example, it has impact on the nomenclature as names may have different priority when used under different genera (certainly concerning homonymy) (l.c.).

Therefore, segregate taxa such as *Pycreus* fall in a grey zone of taxonomic instability as they are balancing between a generic and subgeneric status. *Cyperus* and related genera have seen a long history of unstable classifications as different authors made different choices of priority in the taxonomical importance of the conflicting characters that were used for generic delimitations in Cypereae (Goetghebeur, 1986). Moreover, many authors changed their opinions about the generic status of *Pycreus* at least once (e.g. Govindarajalu, 1978 & 1979 vs.

1990; Koyama 1961 vs. 1991; Lye, 1981c vs. Haines & Lye, 1983). As a major consequence, this disagreement of generic status has led to a very complex nomenclature for *Pycreus* and other similar taxa such as *Kyllinga* (e.g. Reynders *et al*., 2011).

Deciding on the generic status of *Pycreus* and similar taxa, is the most important question in this PhD dissertation. To evaluate this status, a solid knowledge is needed on molecular phylogenetics and on the evolutionary and taxonomical value of the main diagnostic features of *Pycreus*. The decision on the generic status of *Pycreus* affects its taxonomical treatment as sorting out priority of names in nomenclature (1500 names for 120 species) is dependent on this decision.

For the above reason and within the limited time frame of this PhD study, the main focus of this PhD research was on the meso-scale. On the micro-scale, at least some research lines with possibilities towards a modern revision of *Pycreus* were executed with a focus on nomenclature and the evaluation of the traditional diagnostic features.

1.5.2 The choice of reference classifications

In the current era, molecular phylogenetic studies form the basis for the testing of our existing classification systems (Hillis *et al*., 1996). Novel insights in plant relationships and evolution obtained from such studies require many adjustments in our taxonomic treatments as in (molecular) systematics all valid taxa need to be monophyletic (l.c.). This has been especially of impact on the taxonomy of giant genera that were found to primarily represent paraphyletic groups containing specialized lineages (e.g. *Salvia,* Walker, 2004; *Croton,* Berry et al., 2005; *Euphorbia,* Horn *et al*., 2012). However, as molecular phylogenetics form a very solid and statistical method for the investigation of plant relationships the resulting classification systems are generally considered to bring more stability in plant taxonomy in the future (APG, 2009). Until such a modern molecular taxonomy has been produced, the taxonomic status of the taxa involved remains uncertain. As addressed above, also the taxonomic status of *Pycreus* was still unstable at the start of this research project. For practical reasons, a widely accepted reference classification is used, which forms the null hypothesis that will be reevaluated during further investigations.

1.5.2.1 Familial and tribal reference classification

We follow Simpson *et al*. (2007) and Muasya *et al*. (2009a) for the phylogenetic hypothesis based subfamilial and tribal classification of sedges, which largely reflects the classification of Goetghebeur (1998), see fig. 1.8. On tribal level, several taxonomic changes still need to be executed for the paraphyletically circumscribed tribes. However, as Cyperoideae and Cypereae both form monophyletic entities, their delimitations and circumscriptions are currently stabilizing (e.g. Yano *et al.*, 2012).

1.5.2.2 Generic reference classification

In the current research and also the joint research effort on *Cyperus* and related genera, the generic classification of Goetghebeur (1998) is folowed, which treats *Pycreus* on the generic level. Although Muasya *et al.* (2002) already confirmed that *Pycreus* is nested within *Cyperus* s.l., for practical reasons and until the production of more solid cladograms for the group, the classification of Goetghebeur (1998) remains widely used in the international research community (Govaerts *et al.*, 2007; Muasya *et al.*, 2009b).

1.5.2.3 Infrageneric reference classification

The most recent detailed infrageneric classification for *Pycreus*, is of Kükenthal (1935-36), who treats the taxon on the subgenus rank under *Cyperus*. The taxonomical value of the characters used by Kükenthal needs to be reevaluated as well as the legitimacy of his sectional names. A modern revision and infrageneric classification has not yet been produced.

1.5.3 Research strategy and goals

Research strategy and goals are shown in fig. 1.26, 1.27 & 1.28. The goals are divided among two research scales. (1) At first, research goals are formulated which are important on genus level or higher with *Pycreus,* if considered on generic level, as reference point (meso-scale). Within this chapter the macro-scale objectives are included in the meso-scale, as within the context of this PhD thesis, the main purpose of the study on derived pistils is understanding pistil evolution in *Cyperus* s.l. (2) Below genus level (micro-scale), research lines are treated that are important for the subdivisional classification of *Pycreus* and its species.

1.5.3.1 Meso-scale: *Cyperus* s.l. and its segregate taxa

As highlighted in 1.2, the meso-scale objectives were investigated in a collaboration within the Research Group Spermatophytes, UGent. Research strategy and objectives of this joint study on meso-scale are shown in fig. 1.26. In fig. 1.27, the meso-scale strategy diagram is repeated with specific objectives concerning the relationships of *Pycreus* with the remainder of the *Cyperus* clade, which is the part that is presented in this thesis.

The most important objective on the meso-scale is to reevaluate the generic status of the segregate genera of the *Cyperus* clade and subsequently to set out a strategy for an integrated classification of the *Cyperus* clade. Therefore, two separate research lines were followed:

1. To understand the origin and nature of the taxonomically important, but conflicting characters that were used to delimitate the segregate genera. As *Pycreus* is characterised by a laterally compressed dimerous pistil, the focus here is on understanding mechanisms of pistil evolution (fig. 1.28). For this research line a combination of **ontogeny and anatomical techniques** is used.
2. An elaborate **molecular phylogenetic study** was performed to reveal the relationships within the *Cyperus* clade, the positions of the segregate taxa and to evaluate their monophyly. In addition also the homoplasy of taxonomically important characters is addressed.

By combining the results from both research lines it is possible to reevaluate the taxonomic value of the pistil types in the *Cyperus* clade and consequently also the generic status of the segregate taxa.

However, to be able to select representative taxa for both research lines, an overview was necessary of all available generic and subdivisional names for the taxa of the *Cyperus* clade. Therefore a list of ca. 350 names was compiled with evaluation of validity and priority and typifications of names where necessary. In chapter 3, a part of the results published (Huygh *et al*., 2010; Larridon *et al*., 2011a; Reynders *et al*., 2011) are presented.
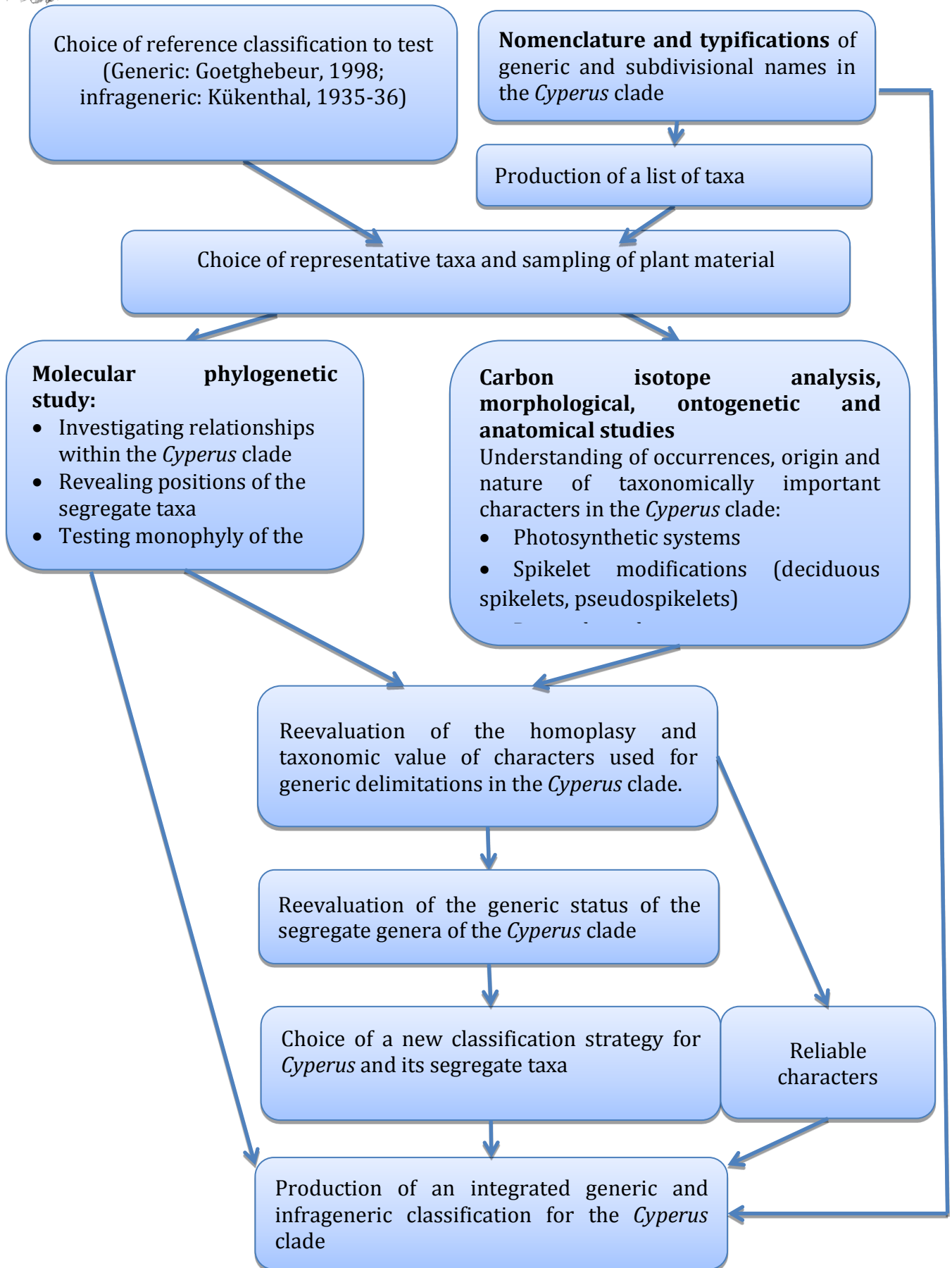
Fig. 1.26 Research strategy and main goals of the on the meso-scale for the joint research on *Cyperus* s.l. within the Research Group Spermatophytes, UGent.

Specific goals in the study of floral ontogeny and anatomy

Pycreus, Kyllinga, Queenslandiella and a few other sedges are characterised by a laterally compressed dimerous pistil, derived from an ancestral trimerous type (Goetghebeur, 1986). However, it was not clear how to interpret the origin and evolution of this pistil type and not surprisingly many authors subsequently considered this pistil type as a unique morphological phenomenon with high taxonomical value (l.c.). The latter opinion was also fed by observations of Blaser (1941a, b) and his interpretations of the anatomy of these pistils. With modern techniques in ontogenetic, anatomical and phylogenetic research, was possible to study the origin and evolution of these laterally compressed pistils and to reevaluate its taxonomical value. For the results of this study see chapters 4 and 5.

In the past decade, there has been an important research focus on floral and spikelet ontogenetic patterns of many different representatives of Cyperoideae (see e.g. Vrijdaghs, 2008, 2010). The large amount of data made it possible to derive general developmental models for the Cyperoideae. A first important hypothesis tested in the current thesis is:

a. Do the derived pistils of *Pycreus, Kyllinga* and *Queenslandiella* follow the same ontogenetic patterns as the other pistil types encountered in Cyperoideae? (see chapter 4)

Blaser (1941 a, b) studied the vascular patters of many cyperoid taxa and concluded that the laterally compressed pistils of *Pycreus* originated from important reorganizations of the different vascular bundles, following from the use of an acropetal developmental pattern. There is increasing evidence that development of vascular bundles is highly conserved among different lineages and organs in Angiosperms. The development of vascular bundles follow gradients of hormones (such as auxin) within the meristematic zones (Endress, 1994). It is therefore necessary to revisit the vascular patterns of *Pycreus* from a developmental point of view to test the following hypotheses (see chapter 5):

b. Are Blasers (1941) interpretations of pistil vasculature in *Pycreus* and subsequent conclusions on the evolutionary relationships of *Pycreus* within Cyperaceae valid?

c. Does the vascular development of the derived pistils of *Pycreus* and related taxa follow the general developmental pattern found throughout Angiosperms?

Specific goals in the molecular phylogenetic study

Previous molecular phylogenetic studies of Muasya *et al*. (2002) showed that *Pycreus*, *Kyllinga* and *Queenslandiella* are nested within the giant genus *Cyperus*. In previous morphological studies with a phylogenetic focus, *Cyperus* s.s. was compared as a single unit to the different genera of Cypereae (Bruhl, 1995). As many Cypereae genera were later found to be nested within *Cyperus*, Bruhl (1995) was not yet able to draw correct conclusions on their relationships. To investigate the relationships between *Pycreus, Kyllinga* and *Queenslandiella* and *Cyperus*, a molecular phylogenetic study with a broad dataset representing the different segregate taxa and sections of *Cyperus* s.l. was needed. Results from this study are presented in chapter 6.

In this context the following questions needed to be answered:

a. Is the paraphyletic nature of *Cyperus* s.s., found in previous studies, confirmed with the use of faster mutating markers such as ETS11f*, trnH-psbA* and *rpl32-trnL*?

b. Is *Pycreus* monophyletic?

c. Did the laterally compressed pistils of *Pycreus*, *Kyllinga* and *Queenslandiella* originate in a single evolutionary event?

1.5.3.2 Micro-scale: on infrageneric levels of *Pycreus*

The most recent revision of *Pycreus* goes back to Kükenthal (1935-36), who recognized 9 sections within this taxon. Infrageneric groups were largely based on the shapes of nutlet epidermal cells in combination with spikelet and glume characters. However, the taxonomic value of these characters had not yet been confirmed. In addition, the uncertain taxonomic status of *Pycreus* resulted in a complex nomenclature encompassing ca. 1500 names for only 120 accepted species. Therefore, a modern revision of *Pycreus* is required.

As highlighted above, priority was granted to the more fundamental questions on the evolution and relationships of *Pycreus* within the *Cyperus* clade (meso-scale). A reevaluation of the generic status of *Pycreus* and choice of a joint classification strategy for the *Cyperus* clade are also essential as a base for a new infrageneric treatment of *Pycreus*.

| Choice of reference classification to test (Generic: Goetghebeur, 1998; infrageneric: Kükenthal, 1935-36) | | **Nomenclature and typifications** of generic and subdivisional names in the *Cyperus* clade, including *Pycreus* |

Production of a list of taxa

Choice of representative taxa and sampling of plant material

Molecular phylogenetic study:
- Investigating relationships within the C$_4$ *Cyperus* clade
- Revealing positions of the segregate taxa including *Pycreus*
- Testing monophyly of the different segregate genera, with a focus on *Pycreus*

Ontogenetic and anatomical study:
Understanding of occurrences, origin and nature of taxonomically important characters in the *Cyperus* clade with a focus on modified pistils:
- Showing *Cyperus* and *Pycreus* species follow the standard ontogenetic pattern of Cyperoideae
- Understanding evolution of pistil modifications in Cyperoideae, including the laterally compressed pistils of *Pycreus*, *Kyllinga* and *Queenslandiella*

Reevaluation of the homoplasy and taxonomic value of characters used for generic delimitations in the *Cyperus* clade, including the derived pistils of *Pycreus*

Reevaluation of the generic status of the segregate genera of the *Cyperus* clade, including *Pycreus*

Choice of new classification strategy for *Cyperus* and its segregate taxa

Reliable characters

Production of an integrated generic and infrageneric classification for the *Cyperus* clade, including *Pycreus*

Fig. 1.27 Research strategy and specific goals on the meso-scale for *Pycreus* within the joint research on *Cyperus* s.l. of the Research Group Spermatophytes, UGent.

The research strategy (fig. 1.28) is similar to the strategy followed on the meso-scale (fig. 1.26 & 1.27). Infrageneric relationships and taxonomical value of the characters used in previous studies are reinvestigated using two research lines:

1. A detailed **molecular phylogenetic study** to reveal the relationships within *Pycreus*, the positions of the existing subdivisions and to evaluate their monophyly and also the possible homoplasy of taxonomically important

   characters. The results of the molecular phylogenetic study are embedded within chapter 6.

2. A **micromorphological study** of the nutlet epidermis. Nutlet epidermal cells show a remarkable variation within *Pycreus*. Many authors have used nutlet epidermal character states such as cell shapes and the presence/absence of silica bodies as the primary diagnostic characters for the different subdivisions in *Pycreus*. To reevaluate the taxonomic value of this characters and character states, an elaborate SEM study was performed investigating the nutlet epidermal cell walls of a range of species representing most sections previously recognized in *Pycreus*. The results of this study are included in chapter 7.

By combining the results from both research lines a reevaluation was aimed of the taxonomic value of nutlet epidermal cells in *Pycreus* and the taxonomic status of sections described in previous studies.

To be able to select representative taxa for both research lines an overview was necessary of all available infrageneric names published for *Pycreus*. A list of subdivisional names was compiled and is included in the publications on generic and subdivisional names in the *Cyperus* clade (see chapter 3). Evaluation of validity and priority of names and typifications of names was done where necessary. In addition, a preliminary list of specific and infraspecific names for *Pycreus* was compiled. This list includes ca. 1500 names that still need reevaluation of validity, priority and typifications (see electronic appendix).

Choice of reference classification to test (infrageneric: Kükenthal, 1936)

Nomenclature and typifications of names in *Pycreus*
- generic and subdivisional names
- specific and infraspecific names

Production of a list of taxa

Choice of representative taxa and sampling of plant material

Molecular phylogenetic study:
- Investigating infrageneric relationships in *Pycreus*
- Testing monophyly of existing subdivisions of *Pycreus*

(micro-) morphological study: Understanding of occurrences, origin and nature of taxonomically important characters in *Pycreus*
- Nutlet epidermal cells

Reevaluation of the taxonomic value of characters used for subdivisional delimitations in *Pycreus*

Implementation of the joint generic and subdivisional classification strategy for *Cyperus* s.l. on *Pycreus*

Reevaluation of the taxonomic status of the subdivisions of *Pycreus*

Studies towards an integrated revision of *Pycreus*:
- Reestablishment of *Pycreus* sect. *Tuberculati*
- Publication of necessary taxonomical changes (new combinations, etc.)

Reliable characters

Fig. 1.28 Research strategy and specific goals on the micro-scale.

Several smaller taxonomic case studies are included in chapter 8 and 9, these form smaller step stones towards a larger revision of the group:

a. Reestablishment of *P.* section *Tuberculati:* As a result from the nutlet epidermal study and morphological study of *Pycreus* species a discrepancy was discovered in Kükenthal's (1936) delimitation of his *C.* sect. *Muricati* and in the nomenclatural priority of the sectional names used. *Pycreus divulsus* (type of *P.* sect. *Tuberculati*) needed to be removed from the section and in addition *P. divulsus* ssp. *africanus* is moved to the specific level based on a detailed comparative study of both taxa. Results of this case study were published (Reynders & Goetghebeur, 2010; chapter 8).

b. Taxonomic changes: As a result of the joint decision to sink the segregate genera of the *Cyperus* clade into *Cyperus* s.l., several *Pycreus* species need to be combined into *Cyperus*. In addition, the synonymy of several African species is reevaluated and included as a summary of the taxonomic work performed on species level during this PhD project (Chapter 9).
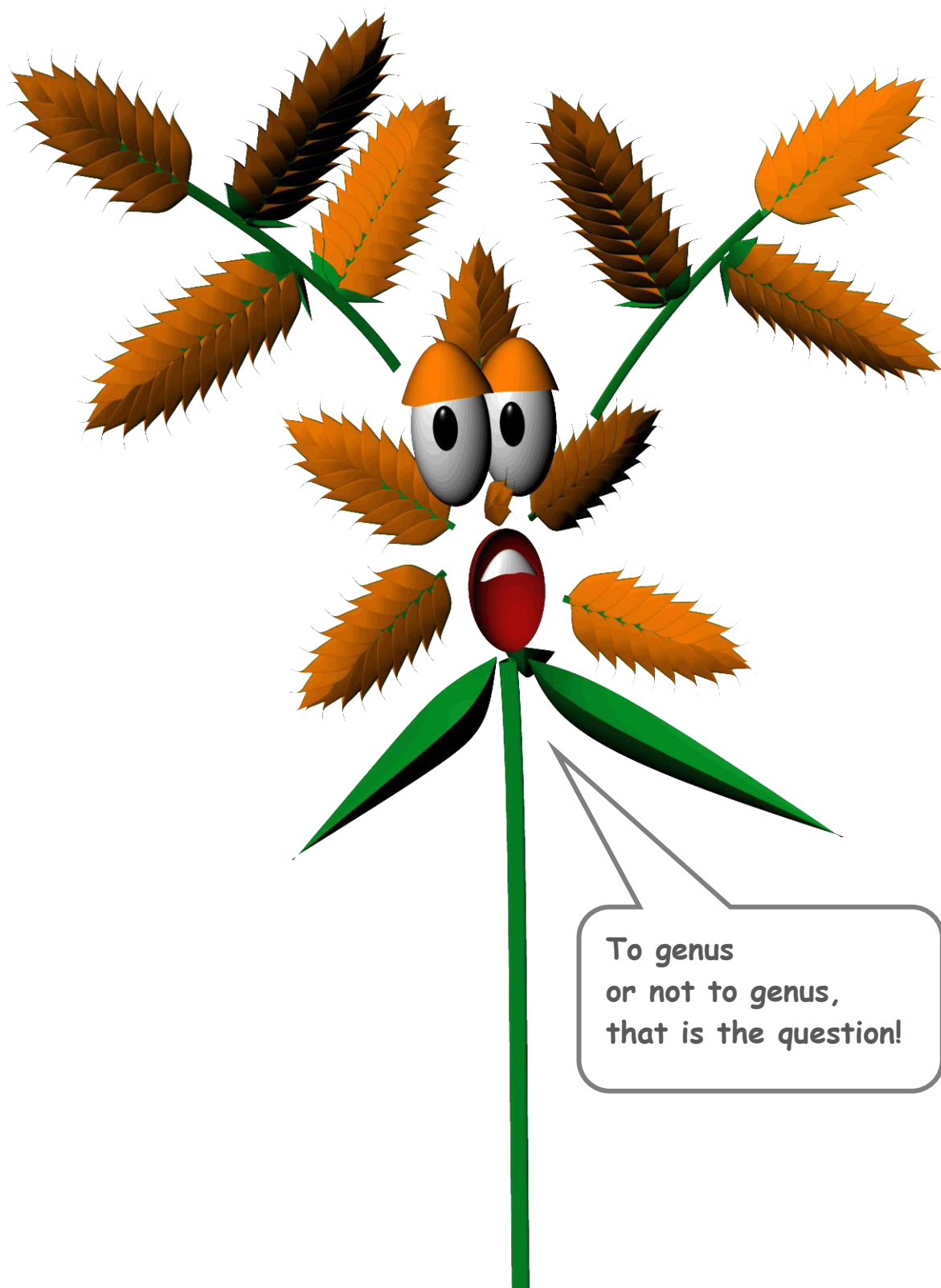
Fig. 1.29 *Pycreus* 'Cypertoon', wondering about its taxonomic future… Drawn in Rhinoceros 3D for a symposium poster. Quote inspired from Shakespeare's Hamlet (Act III scene 1). The question whether the segregate taxa of the *Cyperus* clade can be retained as separate genera or should be sunken into a broader circumscribed *Cyperus* forms one of the central questions of this PhD thesis.

2 Plant material & sampling

*"**S**omewhere, something incredible is waiting to be known."*

— Carl Sagan (1934-1996)

← Fig. 2.1 On a quest for sedges in Andringitra National Park, Madagascar. Picture taken by M. Reynders.

Spikelet theme: *Pycreus flavescens*

2.1 In this chapter…

In chapter 4, origin and types of plant material that are required in the different types of research are briefly discussed. In addition, a brief overview is given of the fieldwork executed during the course of this research and the collection of living Cyperaceae plants is addressed.

2.2 Plant material

2.2.1 Herbarium material

Cyperaceae are generally easy to dry and usually pest-free. When special attention is paid on the collection of complete plants, they provide good herbarium specimens where most characters are easily observable. Herbarium material still forms a most important source of information in plant taxonomy. Besides being a source of morphological and morphometric data, labels of specimens contain data on distribution, ecology, phenology, etc.

Well preserved herbarium specimens can also provide samples for molecular phylogenetic research, embryographical and SEM studies (micromorphology). The use of herbarium specimens for such samples is dependent on the policy of the herbaria where the specimens are deposited. Therefore, the current study mostly used samples of the GENT and BR herbaria that have a relatively more open policy for destructive sampling from herbarium material (except from type-specimens). In addition, some herbaria allow for DNA extractions within the proper research facilities of the herbarium, for example DNA extraction of specimens from K was performed in the Jodrell Laboratory of the Royal Botanic Gardens, Kew.

For the taxonomic parts of this PhD study, specimens have been studied mostly from Ghent University Herbarium (GENT), the National Botanic Garden of Belgium (BR), the Musée National d'Histoire Naturelle in Paris (P) and the Kew Herbarium (K). In addition also specimens were studied from the following herbaria: B, BM, CEBU, NY, TAN, UPS, WAG and YA (abbreviations according to Holmgren *et al*., 1900; underlined herbaria were visited during this study). Currently, many herbaria also provide access to an online catalogue and digital specimen consultation (e.g. BR, NY). In addition, large online databases have been made available through large scale international cooperation, providing digitalized (type) specimens (http://plants.jstor.org/ and http://www.tropicos.org/).

2.2.2 Fieldwork

Botanical fieldwork makes it possible to collect additional herbarium material , more particularly from rare species poorly represented in herbaria such as narrow endemics. In addition, sampling is done to obtain high quality samples for DNA extraction and ontogenetic studies. The latter methods are difficult or even impossible to perform on historic herbarium specimens. Both types of studies require different sampling techniques that are explained under 2.3. Attention has also been paid to the collection of living plants, which allows for more elaborate sampling and study after transplantation in a botanic garden. An overview of the fieldwork performed during the joint research project of the *Cyperus* clade within the Research Group Spermatophytes of Ghent University is given by Larridon (2011). A summary of this fieldwork can also be found in table 4.1.

With the ratifications of the Convention of Biodiversity, policies on collecting and transportation of plants have tightened. For most countries, collecting permits are required besides collaboration with local institutes and deposition of duplicate specimens in the herbaria of the cooperating institutes. Transportation regulations differ for the different kind of samples. Generally, for herbarium specimens and silica-gel samples, an export permit is sufficient as no Cyperaceae currently fall under the CITES regulations. For samples on alcohol, there are restrictions on the amount of alcohol that can be carried on board of aircrafts. Therefore, such samples need to be carried in small volumes or alternatively in wrapped alcohol soaked tissues sealed within a plastic bag. Policies for the transportation of living plants and soil are restricted by phytosanitary regulations. Sedges are generally not listed among the plants that fall within these regulations as few of them are cultivated.

Table 2.1 Overview of the fieldwork performed during the joint research project on *Cyperus* s.l.

Date	Country	Region	Team	# specimens	herbarium	Living plants	Silica-gel	ontogeny
18/12/2005 until 25/01/2006	Philippines	East Samar & Bohol	M. Reynders & B. Sabulao, in cooperation with DENR region 8	75	V	V	v	v
23/02/2007 until 09/03/2007	Cameroon	Mt Cameroun	W. Huygh, I. Larridon & M. Reynders, in cooperation with AETFAT & Limbe Botanic Gardens	50	V	V	v	
		Inselbergs near Yaounde and Highlands of the Northwest	W. Huygh & M. Reynders, In cooperation with AETFAT & l' Herbier National du Cameroun					
June- July 2009	Kenya		W. Huygh & I. Larridon, in cooperation with the East Africa herbarium of the National Museums of Kenya, Nairobi (M. Mbale) & Kenya Wildlife Service	212	V	v	V	v
01/04/2010 until 01/05/2010	Madagascar	Central North & East	W. Huygh & I. Larridon, in cooperation with l'Herbier National of Parc Botanique et Zoologique de Tsimbazaza, Université d'Antananarivo (J. Razanatsoa & J. Andriantiana) & Missouri Botanical Garden	350	v		v	v
		South-Central Highlands	W. Huygh, I. Larridon & M. Reynders, in cooperation with the University of Cape Town (A.M. Muasya), l'Herbier National of Parc Botanique et Zoologique de Tsimbazaza, Université d'Antananarivo (Andri) & Missouri Botanical Garden					
20/07/2010	Philippines	East Samar	M. Reynders & B. Sabulao, in cooperation with DENR region 8	6		V	v	v

2.2.3 A living Cyperaceae collection

In most living plant collections, sedges are poorly represented. Therefore, special attention was paid to the elaboration of the living sedge collection of the Ghent University Botanical Garden (BG Ugent) with new collections from fieldwork in the Philippines, Cameroon and Kenya (table 2.1, fig. 2.2) and with germination tests with seeds provided from other collections or seed banks (fig. 2.3).

To be able to provide a dynamic and continuous flow of plant samples from all different required ages (such as for ontogeny an anatomy) the living ex situ collection of Cyperaceae at the BG UGent is essential. In addition, this living collection has been used as a

source of leaf material for the extraction of high quality DNA for molecular studies. With a living collection, it is also possible to do experiments on the reaction of the phenotypes of different organs to different environmental factors as is illustrated in fig. 2.3. Finally, the living collection makes it possible to monitor the growth and flowering and provided optimal opportunities for photography at different stages of the life cycle of the plants studied (see chapter 5).

It is vital to maintain and extend these scientific reference collections for current and future research purposes. Currently, the Cyperaceae collection includes over 500 accessions representing 31 genera and 283 species (Larridon, 2011).

Fig. 2.2 Living ex citu collection of Cyperaceae in the Ghent University Botanical Garden. A. Young potted plants of *Paramapania parvibracteata* brought from the Philippines. B. Temperate region sedges. C. Tropical species collected during the expedition in the Philippines and Cameroon.
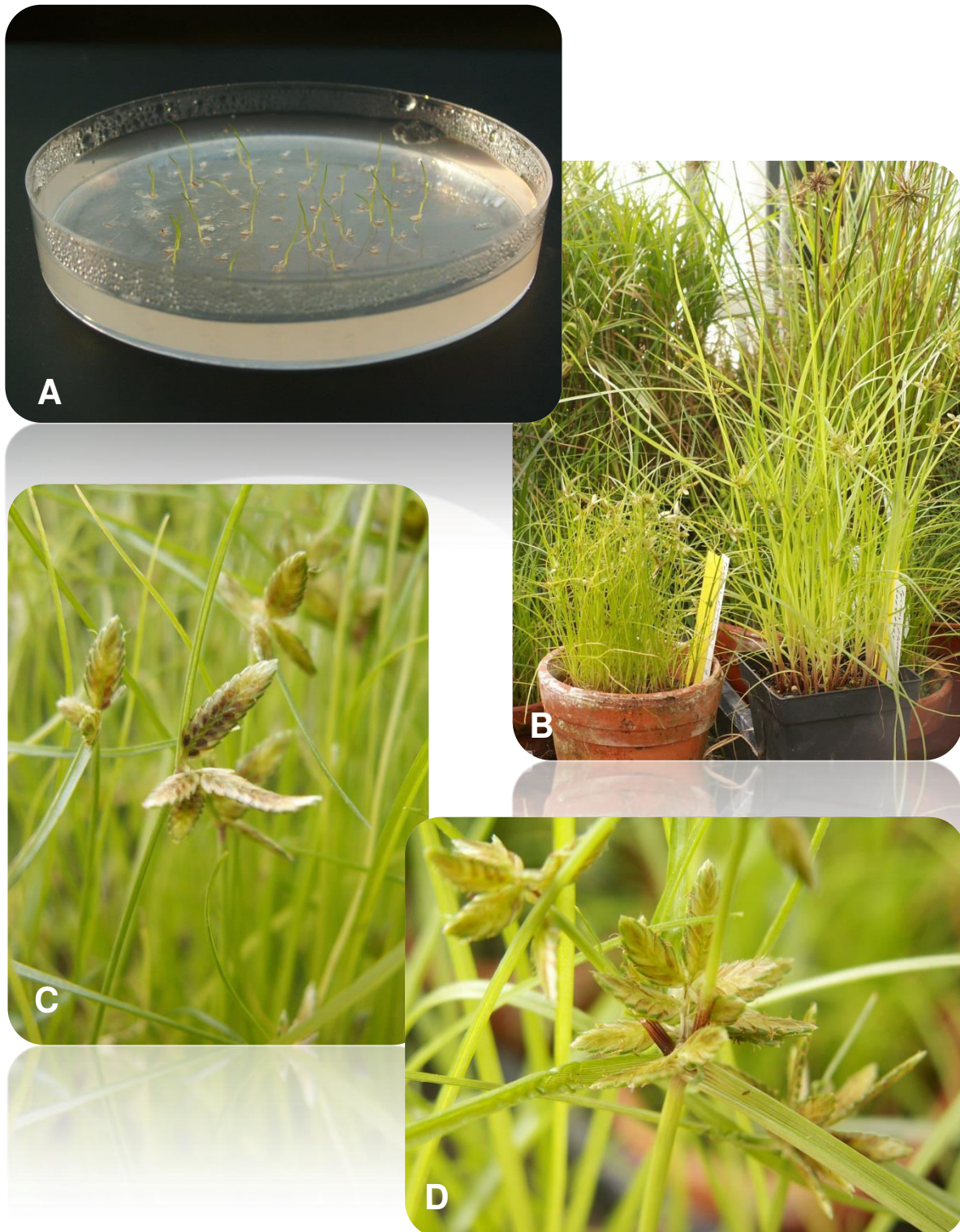
Fig. 2.3 Germination experiment in the Ghent University Botanical Garden. A. Seeds of *Pycreus flavescens* were germinated on a petri dish with 1% agar. B. The agar with seedlings was cut in half and transferred to two different pots. The left pot was watered only from the top, while the right pot was placed in a dish filled with water. Seedling subsequently developed different phenotypes. C. Phenotype of *P. flavescens* on a humid soil. D. Phenotype of *P. flavescens* on a wet soil.

2.3 Sampling

The availability of suitable plant material plays an essential role in the performance of modern plant taxonomic studies using a combination of many different techniques, such as morphology (complete living/dried plants), anatomy (living material, alcohol samples), ontogeny (alcohol samples of different stages), molecular phylogeny (fresh/ silica gel dried samples), embryography (mature nutlets) etc. This requires a variety of sample types (fig. 2.4) from a wide range of species from the group studied. Below, the most important types of samples used are listed.

2.3.1 Samples for micromorphological study and embryography

For micromorphological study of nutlets and glumes under a SEM, well preserved herbarium specimens provide a good source of samples. Mature fruit and glume samples from herbarium specimens are dry and do not need additional preparation before they are transferred to the stubs for SEM observation. For additional investigation of silica bodies, outer cell walls were removed following Goetghebeur & Van den Borre (1989). Mature fruits are also suitable for embryographical studies as sedge embryo's remain well preserved within the fruit. Dissection and treatment of embryo's was performed following the protocols of Van der Veken (1965).

2.3.2 Samples for molecular study

Molecular studies require samples containing high quality DNA. Fresh samples and fastly dried samples (e.g. in silica gel, fig. 2.4D) provide the best results for DNA extraction of sedges. In addition, silica-gel dried material allows for long term preservation of samples when stored away from light (Chase & Hillis, 1991). Silica-gel samples for this study were obtained by sampling from the living collection of the BG UGent, from field studies or from collaboration with colleagues. Dr. A.M. Muasya provided a large amount of samples of Cyperaceae collected during his fieldwork in Africa and Thailand. Additional material was collected during several expeditions in the Philippines, Cameroon, Kenya and Madagascar (see table 2.1).

2.3.3 Samples for ontogenetic and anatomical studies

For developmental and anatomical studies, samples freshly collected in the field or in botanic gardens are essential. These samples are stored in ethanol 70% or in FAA (5 % Formaldehyde 5% Acetic acid 90% Alcohol), fig. 2.4E.

Collecting in the field has the advantage that rare species or species that are difficult to cultivate can be sampled when collections for herbarium are made. However, for some taxa (e.g. Cariceae, Sclerieae, etc.) it is difficult to collect a full series of growth stages that are required to produce complete ontogenies. In addition, there are often logistic problems such as availability of ethanol, transportation restrictions for large quantities, etc.

In contrast, sampling from an ex situ living collection such as in the BG UGhent, makes it possible to collect samples from different developmental stages and to revisit the plants when needed. Therefore, for the ontogenetic and anatomical studies presented in chapter 4 and 5, almost all samples were collected from the Ghent University Botanical Garden.

2.3.4 Collecting living Cyperaceae plants

Collection and transportation of living Cyperaceae is challenging. Good results have been achieved during our fieldwork by carrying the plants in sealed ziplock bags (Fig. 2.4C). Therefore, roots and rhizomes surrounded with the original humid soil are put as soon as possible after collection in separate bags. Leaves and culms are shortened to fit in the closed bags. The plants need to be exposed to light (not full sun) as often as possible to keep them vital. In this way, most Cyperaceae can be stored easily for up to two weeks. Upon transplantation to pots in the botanic garden, roots and covering soil have to be left undisturbed. When washing off the soil before international transportation, as was done with the samples from the Philippines of 2006, success of growth was limited in comparison with the samples form Cameroun or the Philippines in 2010, where original soil had been left on. Especially annual species, but also perennial species such as *Lipocarpha chinensis*, *Scleria* sp., *Fuirena umbellata, Paramapania parvibracteata, Scirpodendron ghaeri,* etc., have higher survival rates when soil is not removed., Perennial species of *Cyperus, Pycreus, Kyllinga* and *Fimbristylis* generally survived either method. Samples without soil or species from humid forest floors (e.g. *Paramapania*) suffer from dehydration after removal from the humid

environment in the sealed bags. Therefore, potted plants need to be sealed again within closed bags and slow acclimatization is required until new growth becomes visible.
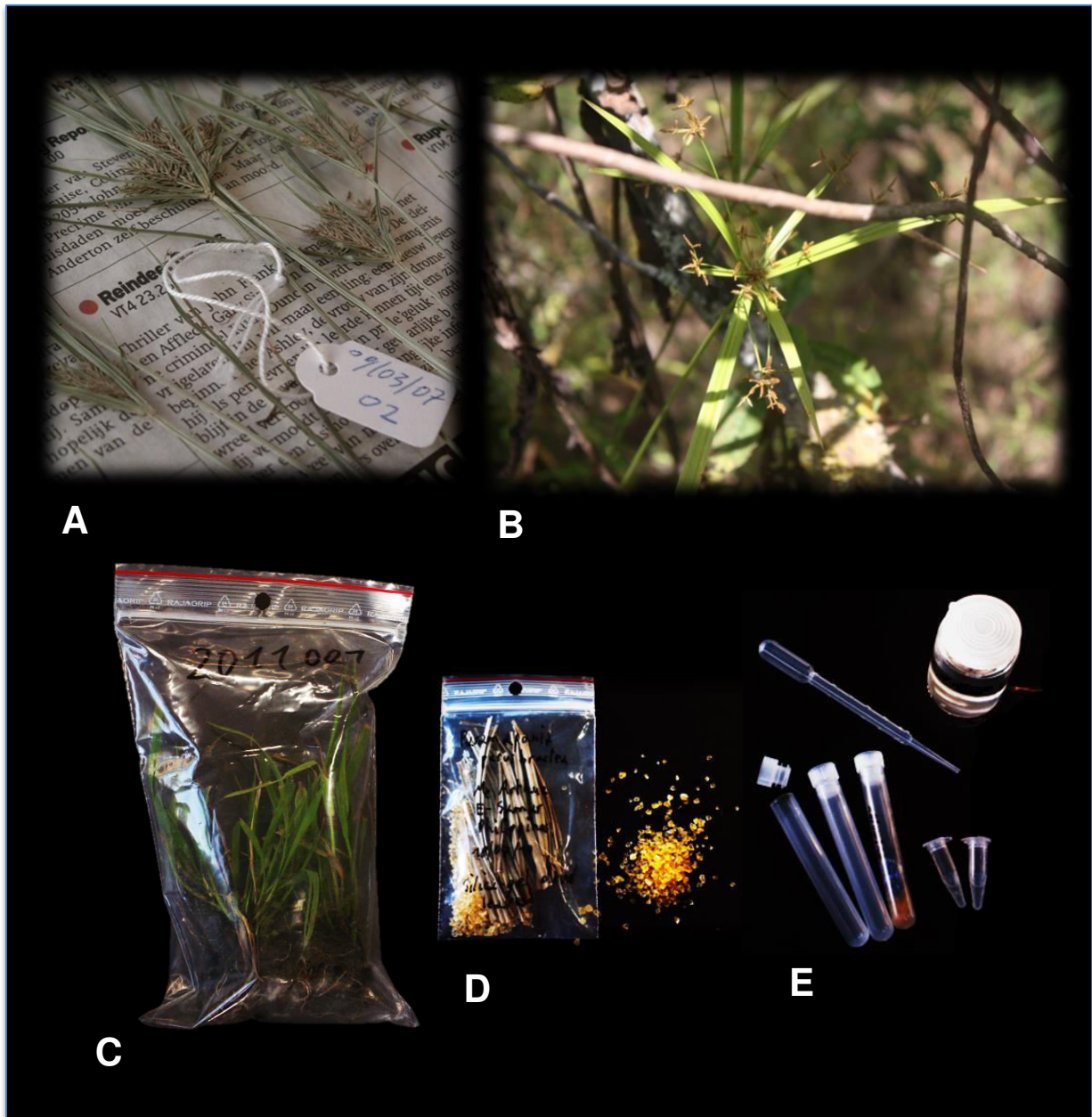
Fig. 2.4 Different samples and data collected during fieldwork on Cyperaceae. A. Herbarium specimens, B. In situ pictures. C. Living collection in zip-lock bag. D. Silica gel sample. E. Samples on ethanol 70% for ontogeny and anatomy.

3 Nomenclature

"Nomenclature, the other foundation of botany, should provide the names as soon as the classification is made... If the names are unknown knowledge of the things also perishes... For a single genus, a single name."

— Carolus Linnaeus (1707-1778)

← Fig. 3.1 *Pycreus holosericeus*, brought from Guiuan (Philippines) to the Botanical Garden of Ghent University. Picture taken by M. Reynders.

Spikelet theme: *Pycreus macrostachyos*

3.1 In this chapter…

A series of three papers was published presenting a survey of all names of genera and subdivisions of genera published for taxa now included in the Cyperus clade. In chapter 3, the introductions of the three papers are combined and subsequently, the nomenclature is treated of all names of genera and subdivisions of genera published for *Pycreus* taxa. In their PhD these, Isabel Larridon and Wim Huygh treat the names relating to C$_3$ *Cyperus* and the names relating to *Kyllinga* respectively.

1. Huygh, W., Larridon, I., Reynders, M., Muasya, A. M., Govaerts, R., Simpson, D.A. & Goetghebeur, P. (2010) Nomenclature and typification of names of genera and subdivisions of genera in Cypereae (Cyperaceae): 1. Names of genera in the *Cyperus* clade. *Taxon* 59: 1883–1890.

2. Larridon, I., Huygh, W., Reynders, M**.**, Muasya, AM., Govaerts, R., Simpson, DA., Goetghebeur, P. (2011) Nomenclature and typification of names of genera and subdivisions of genera in Cypereae (Cyperaceae): 2. Names of subdivisions in *Cyperus*. *Taxon* 60: 868–884.

3. Reynders, M., Huygh, W., Larridon, I., Muasya, AM., Govaerts, R., Simpson, DA., Goetghebeur, P. (2011) Nomenclature and typification of names of genera and subdivisions of genera in Cypereae (Cyperaceae): 3. Names of subdivisions in segregate genera of *Cyperus*. *Taxon* 60: 885–895.

The introduction presented below is a combination of the relavant parts of the intruductions of the published articles.

3.2 Abstract

The morphological diversity and the presence of several convergent evolutionary lineages in the tribe Cypereae (Cyperaceae) resulted in conflicting classifications. These conflicts do not only arise in the delimitation of genera and their subdivisions, but also in the use of similar subdivisional names for different species groups. This has resulted in the publication of ca. 350 names of genera and subdivisions of genera to accommodate the ca. 950 species in *Cyperus* and its segregate genera. This complex nomenclature has led to an accumulation of errors in the assessment of valid publication, priority and legitimacy, and in typifications in almost all existing taxonomic treatments for the group. Renewed interest in

the phylogeny and taxonomy of Cypereae reveals the need to evaluate the nomenclature of names of genera and of subdivisions of genera. In a series of three papers, types were designated where needed and priority and legitimacy of the names was evaluated. This series represent the first complete nomenclatural survey of the names of genera and subdivisions of genera in Cypereae. This is an essential step towards a modern classification of the tribe. Here a selection of names related to Pycreus are listed.

3.3 Introduction

3.3.1 Genera in Cypereae

The Cypereae tribe forms one of the most diverse lineages of Cyperaceae, and its species occur in almost all tropical wetland habitats. Cypereae include species, which are of both economic and ecological importance (e.g., *Cyperus papyrus*, *C. esculentus*; e.g., Boar & al., 1999; Junk & al., 2006). Originally, this tribe only included the large genus *Cyperus* and its obvious relatives characterised by spikelets with a distichous glume arrangement and flowers lacking perianth (Clarke, 1908; Kükenthal, 1935–1936). In Cypereae, several groups are recognizable based on morphological characters like presence of deciduous spikelets (e.g., *Courtoisina*, *Kyllinga*, *Mariscus*) or dimerisation of the pistils (e.g., *Juncellus*, *Kyllinga*, *Pycreus*, *Queenslandiella*). These groups have often been treated as separate genera. However, many of these genera, although described to distinguish species with distinctive characteristics, were only recognised for a short time and later included in *Cyperus* or one of its large segregate genera like *Kyllinga* and *Pycreus* (see Goetghebeur, 1989). Worth mentioning are the works of Palla (1905) and Rikli (1895) who used vegetative anatomy as key character for generic circumscription. Many other genera, phylogenetically belonging in Cypereae, have previously been included in other tribes such as Scirpeae due to the spiral glume placement (e.g., Bentham, 1878; Clarke, 1901–1902). Other genera had unclear affinities within the family and had been moved around between various tribes depending on the interpretations of their derived inflorescence morphologies (e.g., *Ascolepis*, *Lipocarpha*, *Remirea*; e.g., Nees, 1842; Kern, 1974). A major breakthrough in the circumscription of Cypereae arose from the studies of Van der Veken (1965) and Goetghebeur (1998), who showed that many other genera share a specific *Cyperus*-type embryo. This specific embryo type allows a natural delimitation of Cypereae, including also taxa with spirally arranged glumes or with highly specialized inflorescences. Recent molecular studies confirm this circumscription and reveal

that Cypereae largely consist of two well supported clades (Muasya & al., 2002, 2009a; Simpson & al., 2007: the '*Ficinia* clade' and the '*Cyperus* clade'. The predominantly South African *Ficinia* clade (155 spp.) consists of several taxa previously placed in the heterogeneous *Scirpeae* and even in *Chrysitricheae*: *Scirpoides*, *Hellmuthia*, *Ficinia* and *Isolepis* and two *Scirpus* species, i.e., *Scirpus falsus* and *Scirpus ficinioides*, which needed to be transferred to a new genus (Muasya & al., 2009a). The two latter species and *Hellmuthia* are the only members of Cypereae that still have perianth parts. The core *Ficinia* clade consists of a paraphyletic *Isolepis* including *Ficinia* (Simpson & al., 2007; Muasya & al., 2009a). *Desmoschoenus* has recently been transferred to *Ficinia* (Muasya & de Lange, 2010). Both *Isolepis* and *Ficinia* seem to contain species, which developed a distichous glume placement, while several taxa in the *Cyperus* have spirally arranged glumes, blurring generic delimitations within Cypereae (Muasya & al., 2002, 2006, 2007, 2009a,b).

The cosmopolitan *Cyperus* clade (950 spp.) is much more diverse. As in many large plant groups, such as *Peperomia* (Wanke & al., 2006), *Acacia* (Miller & Bayer, 2001; Ariati & al., 2006), and *Carex* (Starr & al., 1999), the taxonomic relationships and generic delimitations in the *Cyperus* clade are still largely unresolved (Goetghebeur, 1998; Muasya & al., 2009b). The *Cyperus* clade includes a paraphyletic *Cyperus* s.str. (696 accepted species; Govaerts & al., 2007, 2010) as the core genus, in which 13 segregate genera are nested (classification of Goetghebeur, 1998). The segregate genera, *Alinula*, *Androtrichum*, *Ascolepis*, *Courtoisina*, *Kyllinga*, *Kyllingiella*, *Lipocarpha*, *Oxycaryum*, *Pycreus*, *Queenslandiella*, *Remirea*, *Sphaerocyperus*, and *Volkiella*, have diverged significantly from typical *Cyperus* in vegetative, floral, and anatomical characters (Muasya & al., 2009b). The mutual relationships between these segregate genera and *Cyperus* s.str. are still unclear.

The complex taxonomy, large species number, and often convergent morphology resulted in many conflicting classifications of Cypereae (e.g., Clarke, 1893; Chermezon, 1919; Kükenthal, 1935–1936; Kern, 1974; Haines & Lye, 1983). Comparing these treatments, it appears that the same names have been used for different taxa (homonyms) and that similar species groups are known under various synonyms. This has lead to the accumulation of ca. 350 names to accommodate the 950 accepted species in *Cyperus* and its segregate genera. Previously, the lack of a survey of all published names lead to the adoption of many names that were contrary to priority or were illegitimate in almost all treatments of the group (e.g., Kükenthal, 1935–1936; Koyama, 1961; Kern, 1974; Haines & Lye, 1983; Goetghebeur, 1989;
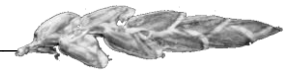
Väre & Kukkonen, 2006). In addition many citation errors have repeatedly occurred as Väre & Kukkonen (2006) already indicated for Nees's (1834) sections, which have often been incorrectly assigned to Kunth (1837). In total, 11% of all the intended names of genera and subdivisions of genera listed in this series of papers are not validly published and another 11% are superfluous and/or illegitimate after evaluation in accordance with the *International Code of Botanical Nomenclature* (*ICBN*; McNeill & al., 2006). Of the 67 intended names mentioned below, 9% are not validly published and 16% are superfluous and/or illegitimate.

3.3.2 History of infrageneric taxonomy of *Cyperus*

The morphological diversity and the presence of several convergent evolutionary lines in *Cyperus* result in various controversial classifications. Furthermore, there is no unanimity on the delimitation of the genus. Metcalfe (1971) already realised that to study relationships within Cyperaceae using only morphological characters is problematic: "The main difficulty about the classification of the Cyperaceae when the subject is approached solely along traditional lines is that the flowers are very small, the exact morphology of their parts is often obscure, and the morphology of the inflorescences is difficult to interpret." This leads to uncertain homologies and conflicting interpretations.

The first infrageneric classification of the genus (and of related genera which were later included in the genus) is that of Nees (1834). He was the first to apply a sectional division in the genus *Cyperus*, and created eight sections. Often, his sections have incorrectly been assigned to Kunth (1837) as stated by Väre & Kukkonen (2006). Bentham (1881) comments on Nees's work that "he created some confusion, as well by his usual tendency to raise species to the rank of genera, as by a want of reference to the original papers or work where Cyperaceae had been described, and by using a terminology occasionally founded on mistaken views of the homology of floral organs." Most genera created by Nees in Cyperaceae are no longer accepted as such.

Kunth (1837) greatly expanded our knowledge of Cyperaceae. He classified the species of *Cyperus* with a trifid style into 19 groups of unspecified rank, now usually treated at sectional level. He discussed the species of *Cyperus* with a bifid style separately. Kunth (1837) accepted *Mariscus* and *Kyllinga* as distinct genera. His main error was, according to Bentham (1881), "a tendency to give as characters rather what in theory we ought to see than what we actually do see, and, in his later works, to describe specimens rather than species".

Steudel (1854) divided *Cyperus* into three unnamed sections. The first section included species with a bifid style and corresponds to the genus *Pycreus*. His "Sectio II", split into 17 groups of unspecified rank, comprised the species of *Cyperus* with a trifid style. Many of these groups are now treated at sectional level. The third section held the species with uncertain affinities. Furthermore, Steudel (1854) recognised *Mariscus* and *Kyllinga* ("*Kyllingia*") as segregate genera.

Boeckeler (1868–77) studied Cyperaceae in the Berlin Herbarium. Like Steudel (1854), Boeckeler (1868) treated *Kyllinga* ("*Kyllingia*") as a separate genus, and included *Pycreus* at sectional level in the genus *Cyperus* alongside a section "*Eucyperus*". However, he did not accept *Mariscus* at generic rank. Boeckeler (1868) divided his section "*Eucyperus*" into 20 groups of unspecified rank, now usually treated at sectional level.

Clarke (1883, 1884, 1893, 1897, 1900, 1902, 1908), the founder of modern cyperology, originally considered *Cyperus* in a wide sense, but in his later publications he proposed the subdivision of *Cyperus* s.l. into seven distinct genera, i.e., *Cyperus*, *Courtoisina*, *Juncellus*, *Kyllinga*, *Mariscus*, *Pycreus*, and *Torulinium*.

The last complete revision of *Cyperus* was published in the generic monograph of Kükenthal (1935–36). His infrageneric classification is the one most commonly used at present. As in Valkenier Suringar's (1898) excellent revision of *Cyperus* in the Malaysian Archipelago, the genus is taken in its wide sense. Kükenthal (1935–36) divided *Cyperus* s.l. into six subgenera (*C.* subg. "*Eucyperus*", *Juncellus*, *Pycreus*, *Mariscus*, *Kyllinga*, and *Torulinium*), 61 sections and eight subsections. The sections were primarily delineated by the nature of branching of the compound inflorescence, extent of development of the rhizomes, and the number of stamens and carpels per flower. Obviously, many of Kükenthal's sections need revision before a natural classification of the genus can be proposed.

Van der Veken (1965) studied the embryos of 132 species of *Cyperus* belonging to different subdivisions of the genus. The uniformity of the embryos appears to support the wide concept of *Cyperus*. Furthermore, the study revealed the presence of embryos of the *Cyperus* type in many taxa previously placed near *Scirpus* (e.g., *Ascolepis*, *Ficinia*, *Isolepis*, *Lipocarpha*, *Kyllingiella*, and *Oxycaryum*). Since Van der Veken's (1965) publication, the inclusion of these genera in Cypereae led to a more natural circumscription of that tribe. All of these genera have, until now, been treated separately from *Cyperus*, except for the treatment of *Lipocarpha* under *Cyperus* by Koyama (1961). In his treatment of the genus, Kern (1974)

only accepted *C.* subg. *Cyperus*, *Pycreus* and *Kyllinga* at subgeneric rank, as he considered *Juncellus*, *Mariscus*, and *Torulinium* too ill defined to be recognised as subgenera.

Later publications are only regional studies of *Cyperus* (e.g., Kern, 1974; Haines & Lye, 1983). Although they present extensive knowledge of the genus, they are decidedly less valuable in assessing species relationships in a large genus such as *Cyperus*, the members of which have successfully utilized long-distance dispersal as well as more gradual short-distance migration and in situ diversification.

Goetghebeur (1989) discussed the problems in the lectotypification of names relating to the infrageneric classification of *Cyperus*. He specifically excluded all taxa with laterally compressed nutlets, i.e. *Kyllinga*, *Pycreus* and *Queenslandiella*, and several highly specialised taxa, i.e., *Alinula*, *Courtoisina*, *Kyllingiella*, *Oxycaryum*, *Remirea* and *Sphaerocyperus*, from *Cyperus* s.str. However, he included such segregates as *Anosporum*, *Galilea*, *Juncellus*, *Mariscus*, *Sorostachys* and *Torulinium*. Goetghebeur (1998) retained this perspective in his treatment of the family Cyperaceae.

3.3.3 Current views on the infrageneric classification of *Cyperus*

Cyperus s.str. is commonly divided into two units, determined by the alternative character states of an anatomical and of an inflorescence character set. The presence of Kranz anatomy, correlated with C_4 photosynthesis, has been used in the classification of *Cyperus* since Rikli (1895). The vegetative anatomy is eucyperoid or chlorocyperoid, characterizing "*C.* subg. *Eucyperus*" (Grisebach, 1846) and "*C.* subg. *Chlorocyperus*" (Schischkin, 1935). The inflorescence is composed of digitately clustered spikelets and is often condensed, or the inflorescence is an anthela composed of spikes of spikelets, characterising *C.* subg. *Pycnostachys* and *C.* subg. *Choristachys* (Clarke, 1893). As demonstrated by Goetghebeur (1989), the two classification systems are not completely congruent.

Either a few sections of subg. *Chlorocyperus* (with predominantly open inflorescence) exhibit a (partly) condensed and depauperate inflorescence (Kükenthal, 1935–36), or a few sections of the predominantly eucyperoid subgenus *Pycnostachys* reveal a chlorocyperoid type of anatomy (Druyts-Voets, 1970) linked to C4 photosynthesis. Inevitably, this means that at least one, or perhaps both of these presumed apomorphic character states (chlorocyperoid anatomy, condensed inflorescence) has or have evolved several times. This raises questions about the switch from C_3 to C_4 anatomy either having evolved repeatedly with no significant

morphological divergence or only once with subsequent convergence in the morphology of unrelated species. Clearly these are problems that cannot be resolved using morphological data alone. As already suggested by Raynal (1973) and Goetghebeur (1989), Soros & Bruhl (2000) confirmed that the chlorocyperoid anatomy type arose only once within *Cyperus*.

Soros & Bruhl (2000) stated that the C_4 photosynthetic pathway arose at least four separate times in Cyperaceae. However, in the tribe Cypereae only the chlorocyperoid anatomical variant occurs. Ongoing molecular research suggests that the *Cyperus* C_3 species form a paraphyletic group with a monophyletic C_4 subclade nested within the C_3 group (Muasya & al., 2002, 2009a; Simpson & al., 2007; Larridon & al., 2011b). On the other hand, Goetghebeur (1989) remarked that a multiple origin of the condensed inflorescence within *Cyperus* is much more probable. There are many instances of species exhibiting both inflorescence types (open vs. condensed), in Cyperaceae in general, and in *Cyperus* in particular.

Consequently, within *Cyperus* at least two main infrageneric groups have been recognised. An eucyperoid subgenus, uniting plants without the Kranz syndrome, and with an inflorescence composed of digitately clustered spikelets; and a chlorocyperoid subgenus, uniting plants with a chlorocyperoid type of Kranz syndrome, and an inflorescence composed of spikes of spikelets or condensed spikes. Within *Cyperus*, two subgenera thus circumscribed were recognised by Chermezon (1937), Raynal (1973), Tucker (1983) and Hooper (1985). However, Hooper (1985) also upheld *C.* subg. *Juncellus*, accommodating the chlorocyperoid species with a condensed inflorescence and dorsiventrally flattened nutlets. Even more subgenera were proposed by Haines & Lye (1983), who considered *Cyperus* in a broad sense, although in Lye's earlier work generic segregates such as *Anosporum*, *Sorostachys* (Lye, 1981b), *Kyllinga* (Lye, 1982), and *Pycreus* (Lye, 1981c) were recognised. Goetghebeur (1989) remarked that he failed to see the advantage of merging such easily recognisable taxa with obvious synapomorphies, like *Alinula*, *Ascolepis*, *Courtoisina*, *Kyllinga*, *Kyllingiella*, *Lipocarpha*, *Oxycaryum*, *Pycreus* and *Queenslandiella*. On the other hand Lye (1992) convincingly demonstrated the polyphyly of *Mariscus* within *Cyperus*. Most authors now accept *Mariscus* as an element of *Cyperus* (e.g., Goetghebeur, 1998; Simpson & Koyama, 1998). Goetghebeur (1989) proposed the recognition within *Cyperus* of only two subgenera, respectively composed of the eucyperoid and of the chlorocyperoid species, further arranged in sections. The recent molecular studies (Muasya & al., 2002, 2009a; Simpson & al., 2007; Larridon & al.,
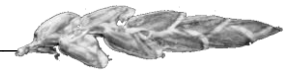
2013) reveal the paraphyletic character of *Cyperus* s.str. including several highly derived lineages, urging a redefinition of generic delimitations in Cypereae.

3.3.4 Segregate genera of *Cyperus*

The segregate genera represent roughly 25% of the diversity within the *Cyperus* clade and are circumscribed based on derived floral or inflorescence characteristics. Flower and, more specific, pistil variation are quite large within *Cyperus* (Kükenthal, 1935–36). From the basic trimerous pistil type, several dimerisations occurred in Cypereae. These dimerous pistils can be dorsiventrally flattened, as represented by several species scattered within the *Cyperus* clade (e.g., *Juncellus*, *Oxycaryum*), and are also common in many other Cyperaceae genera. In contrast, the laterally compressed pistils are restricted to three segregate genera of C$_4$ *Cyperus* (*Kyllinga*, *Pycreus*, *Queenslandiella*) (Goetghebeur, 1998).

All other segregate genera (except *Pycreus*) and the *Cyperus* s.str. species formerly included in *Mariscus* (Clarke, 1893) are characterised by a shift in seed dispersal units from a nutlet to a complete spikelet (Goetghebeur, 1998). Many of these species show strongly reduced and/or contracted inflorescences (for example: *Kyllinga*). *Alinula*, *Ascolepis*, *Lipocarpha*, and *Volkiella* are highly evolved Cypereae, in which partial inflorescences became functional spikelets (Palla, 1905; Raynal, 1973; Eiten, 1976; Goetghebeur, 1977). Spikelet organs like glumes gain new functions in seed dispersal, for instance, air captured around the nutlet facilitates dispersal over water (e.g., *Alinula*, *Ascolepis ampullacea*, *Lipocarpha*, *Remirea*), or development of wing-like structures for wind dispersal (e.g., *Ascolepis capensis*, *Courtoisina*, *Kyllinga squamulata*, *K. alata*) (Muasya & al., 2009b). In extreme cases these adaptations in seed dispersal unit causes the taxa in which they occur to lose most of the typical *Cyperus* characteristics.

The generic status of many of these segregates has long been under discussion, especially for the large segregate taxa *Juncellus*, *Kyllinga*, *Mariscus*, and *Pycreus*. The shifting opinions in the treatment of these taxa under *Cyperus* or as separate entities resulted in a complex generic and subdivisional nomenclature with approximately 350 generic and subdivisional names to contain the roughly 950 species present in the *Cyperus* clade. The subdivisional classifications of the segregate genera of *Cyperus* (especially *Kyllinga*, *Mariscus* and *Pycreus*) comprise 83 intended names of which 18% were not validly published and 5%

are found to be illegitimate after evaluation in accordance with the *International Code of Botanical Nomenclature* (*ICBN*; McNeill & al., 2006). The lack of overview and limited availabilities of literature sources in the past have led to the erroneous use of names of subdivisions of genera with regards to valid publication and legitimacy in almost all recent and past classifications of the group (e.g., Kükenthal, 1935–36; Chermezon, 1937; Kern, 1974; Haines & Lye, 1983).

The presence of this enormous diversity in floral, spikelet and inflorescence morphologies requires reassessment of generic delimitations and circumscriptions in Cypereae. Although a novel classification of this tribe is urgently needed, especially for *Cyperus* and its segregate genera, a well-resolved molecular phylogenetic hypothesis needs to be reconstructed first.

3.4 Materials and Methods

The typifications were made after careful review of the original descriptions of the taxa, examination of herbarium specimens, and of live specimens in the Ghent University Botanical Garden. They are founded on expertise in Cyperaceae obtained through long-standing research into the systematics of this family by the Research Group Spermatophytes of the Ghent University. References to Articles refer to the *International Code of Botanical Nomenclature* (*ICBN* , McNeill & al., 2006). References to accepted names refer to the *World Checklist of Cyperaceae* (Govaerts & al., 2007, 2010). The classification used is that of Goetghebeur (1998).

The names of subdivisions published in the segregate genera of C_4 *Cyperus* will be treated per genus in which they were originally included, i.e., *Ascolepis*, *Lipocarpha*, *Kyllinga*, *Mariscus*, *Pycreus* and *Scirpus*. For *Scirpus*, only those names of subdivisions encompassing species now placed in the *Cyperus* clade are included in this paper. The type of each name of genus or a subdivision of *Cyperus* of one of its segregate genera is indicated. If in the protologue only one validly published species name is cited or referred to (Art. 10.3 of the *ICBN*), the entry appears simply as "Type: …" Where a type has been selected later, the entry appears as "Lectotype" with a parenthetical reference to the publication in which the selection was made. Names that are homotypic, e.g., under Art. 7.5, are indicated appropriately. If, when first published, the name of a subdivision of *Cyperus* had been assigned to a generic subdivision of higher rank, that assignment is given in parentheses even
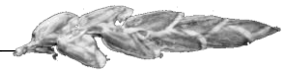
although it has no nomenclatural significance.

The large number of nomina nuda published by Chermezon are a consequence of the fact that Chermezon (1922, 1931) did not provide descriptions for his intended new names, and later Chermezon (1937) only provided a French diagnosis; therefore his names are not validly published (Art. 32.1(d) and 36.1).

Of the names of subdivisions of *Cyperus* with epithets that are derived from the epithet of a constituent species and hence typified under Art. 22.6 by the type of that species name, a significant number, when published, included the already established type of a name of another generic subdivision of the same rank, thereby making them superfluous and under a strict reading of Art. 52, also illegitimate. As Art. 22.6 establishes that such a name has a different type from that of the name that ought to have been adopted, automatic typification under Art. 7.5 does not apply. The resultant situation is very unsatisfactory in that perfectly appropriate epithets of subdivisions of *Cyperus* based on the names of familiar species, are blocked from use even when the taxon is circumscribed to exclude the type of any earlier name at that rank. One might suppose by analogy with superfluous names formed from legitimate basionyms (Art. 52.3) that such names would be available for use when the cause of the superfluity was removed, but a strict reading of Art. 52.3 does not permit this. As it would seem logical that the *Code* should extend the provisions of Art. 52.3 to such cases, which are particularly important in large genera such as *Cyperus*, we have noted the superfluous situation in the listings below, but have refrained from adding the word "illegitimate".

3.5 Names of genera related to *Pycreus.*

Chlorocyperus Rikli in Jahrb. Wiss. Bot. 27: 563. 1895, nom. illeg. (Art. 52.1) − Type: *Chlorocyperus polystachyos* ('*polystachyus*') (Rottb.) Rikli [≡ *Pycreus polystachyos* (Rottb.) P. Beauv. (*Cyperus polystachyos* Rottb.)] (Art. 7.5).

Rikli (1895) divided *Cyperus* L. into two new genera based on vascular anatomy. In the genus *Chlorocyperus*, Rikli (1895) placed all species with a chlorocyperoid C4 anatomy type, including *C. polystachyos* Rottb., the original type of *Pycreus* P. Beauv. (1816), making *Chlorocyperus* nomenclaturally superfluous and illegitimate (Art. 52.1).

= **Cyperus** L.

Cyperus L., Sp. Pl. 1: 44. 1753 – Lectotype (Britton, 1907: 6): *Cyperus esculentus* L.

Distimus Raf. in J. Phys. Chim. Hist. Nat. Arts 89: 105. 1819 – Lectotype (designated here): *Distimus flavescens* (L.) Raf. [≡ *Pycreus flavescens* (L.) P. Beauv. ex Rchb. (*Cyperus flavescens* L.)].
= **Pycreus** P. Beauv.

Pycreus P. Beauv., Fl. Oware 2: 48. 1816 – Type: *Pycreus polystachyos* (Rottb.) P. Beauv. (*Cyperus polystachyos* Rottb.)

Torreya Raf. in J. Phys. Chim. Hist. Nat. Arts 89: 105. 1819, nom. illeg. (Art. 53.1), non *Torreya* Raf. (*Dicot*.: *Lab*.; 1818) – Lectotype (designated here): *Torreya caespitosa* Raf. [= *Pycreus filicinus* (Vahl) T. Koyama (*Cyperus filicinus* Vahl)].

Originally, two specific names were listed in the protologue of *Torreya* Raf., i.e., *Torreya caespitosa* and *T. maritima* Raf. [≡ *Pycreus diander* (Torr.) C.B. Clarke]. Rafinesque first described a genus *Torreya* Raf. Belonging to the *Labiatae* in Amer. Monthly Mag. & Crit. Rev. 3: 356. 1818 (type: *T. grandiflora* Raf.). The following year he used "*Torreya*" again for a new genus in Cyperaceae, which is a later homonym and thus illegitimate (Art. 53.1). The genus *Torreya* Raf. belonging to the *Labiatae* has since been rejected in favour of *Torreya* Arn. in Ann. Nat. Hist. 1: 130. 1838, nom. cons. (*Taxaceae*).
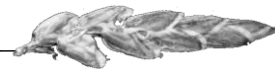= **Pycreus** P. Beauv.

3.6 Subdivisional names

3.6.1 Names of subdivisions under *Cyperus*

Cyperus (subg. *Pycreus*) sect. *Albomarginati* Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 359. 1936 – Type: *Cyperus albomarginatus* (Mart. & S chrad. e x N ees) S teud. [= *Pycreus macrostachyos* (Lam.) J. Raynal] (Art. 22.6).
This sectional epithet has not been combined in *Pycreus*.

"*Cyperus* subg. *Chlorocyperus* (Rikli) Schischk." in Komarov, Fl. URSS 3: 17. 1935, nom. inval. (Art. 22.2).

"*Cyperus* subg. *Chlorocyperus*" is not validly published (Art. 22.2), as it included *C. esculentus* which was selected as type of *Cyperus* (Britton, 1907). This proposed name was based on: *Chlorocyperus* Rikli, Jahrb. Wiss. Bot. 27: 563. 1895, nom. illeg. (see Huygh & al., 2010).

Cyperus sect. *Chrysanthi* (C.B. Clarke) J. Kern (see **Pycreus** **sect.** **Chrysanthi** C.B. Clarke; Reynders & al., 2011).

Cyperus L. **sect.** **Cyperus,** autonym automatically established by Nees in Linnaea 9: 284. 1834 (Art. 22.3) ≡ **Cyperus** L., Sp. Pl. 1: 44. 1753 (Huygh & al., 2010) ≡ **Cyperus** **subg.** **Cyperus,** autonym automatically established in Petermann, Deutschl. Fl. 11: 595. 1849 (Art. 22.3) ≡ *C.* subg. *Pterocyperus* Peterm., Deutschl. Fl. 11: 595. 1849 ≡ *Pterocyperus* (Peterm.) Opiz, Seznam: 80. 1852 ≡ *C.* sect. *Solubiles* C.B. Clarke in J. Linn. Soc., Bot. 20: 291. 1883 ≡ *C.* subg. *Choristachys* C.B. Clarke in Hooker, Fl. Brit. India 6: 605. 1893 ≡ *C.* sect. *Choristachys* (C.B. Clarke) C.B. Clarke" in Urban, Symb. Antill. 2: 22. 1900 ('*Choristachyae*') ≡ *C.* [unranked] *Alati* C.B. Clarke in Oliver, Fl. Trop. Afr. 8: 314. 1901 ('*Alatae*') ≡ **Cyperus** **subsect.** **Cyperus**, autonym automatically established by Nakai in Bot. Mag. (Tokyo) 26: 188. 1912 (Art. 22.3) – Lectotype: *Cyperus esculentus* L. (lectotype of *Cyperus* designated by Britton (1907) – lectotype of autonyms Art. 7.6 – lectotypes of *C.* subg. *Pterocyperus*, *C.* sect. *Solubiles*, *C.* subg. *Choristachys*, *C.* sect. *Choristachys* and *C.* [unranked] *Alati* designated here).

Because the lectotype of *Cyperus* was only designated by Britton (1907), Art. 22.2 does not apply on the names of subdivisions of *Cyperus* including *C. esculentus* published before 1907. Kern (1974) was the first to effectively use the autonyms of *Cyperus* at sectional and subgeneric rank. Väre & Kukkonen (2006) erroneously indicated *C. rotundus* L. as the type of *C.* sect. *Cyperus*. Five species were mentioned in the original description of *C.* subg. *Pterocyperus*, Opiz (1852) later established the genus *Pterocyperus* including only *C. esculentus* (Huygh & al., 2010).

Cyperus [unranked] *Eupycreus* Boeck. (see **Pycreus** P. Beauv. **ser. Pycreus**; Reynders & al., 2011)

Cyperus sect. *Flavescentes* Kük. (see **Pycreus ser. Zonati** (C.B. Clarke) C.B. Clarke; Reynders & al., 2011).

Cyperus (sect. *Pseudohaspani*) subsect. *Flavidi* Nakai in Bot. Mag. (Tokyo) 26: 188. 1912 – Type: *Cyperus flavidus* Retz. [= *Pycreus flavidus* (Retz.) T. Koyama] (Art. 22.6).

Nakai (1912) misinterpreted *C. flavidus* as being closely related to *C. haspan* L. and *C. tenuispica* Steud.; he mentioned the type of his *C.* sect. *Pseudohaspani*, i.e., *C. pseudohaspan* Makino [= *C. tenuispica* Steud.] as a synonym under *C. flavidus*. This subsection has not been combined in *Pycreus*.

Cyperus (subg. *Pycreus*) sect. *Fontinales* K ük. i n E ngler, Pflanzenr. IV, 20 (Heft 101): 340. 1936 – Type: *Cyperus fontinalis* (Cherm.) Kük. [≡ *Pycreus fontinalis* Cherm.] (Art. 22.6). This epithet has not been adopted for any ranked subdivision of *Pycreus*.

Cyperus sect. *Globosi* (C.B. Clarke) Kük. (see **Pycreus sect. Globosi** C.B. Clarke; Reynders & al., 2011).

Cyperus (subg. *Pycreus*) [unranked] *Isodiametrici* Kük. In Engler, Pflanzenr. IV, 20 (Heft 101): 327. 1936 – Lectotype (designated here): *Cyperus polystachyos* Rottb. [≡ *Pycreus polystachyos* (Rottb.) P. Beauv.].

Cyperus polystachyos is the type of the name *C.* subg. *Pycreus* (P. Beauv.) J. Carey in which *C.* [unranked] *Isodiametrici* is included. Kükenthal (1936) used this name at a level between subgenus and section to replace *Pycreus* [unranked] *Puncticulati* C.B. Clarke and *P.* subg. *Reticulati* C.B. Clarke (both characterised by nutlets with isodiametric epidermal cells). Although this name is validly published under Art. 35.3, its epithet has not been adopted in any ranked subdivision of *Pycreus*.

Cyperus [unranked] *Pseudopycreus* Boeck. (1868) is different from *C.* sect. *Pseudopycreus* (C.B. Clarke) Kük., nom. illeg. (1936).
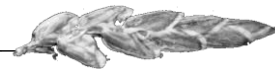
Cyperus sect. *Lancei* Kük., nom. superfl. (see ***Pycreus* sect. *Lancei*** L.K. Dai; Reynders & al., 2011).

Cyperus sect. *Latespicati* Kük. (see *Pycreus* sect. *Latespicati* (Kük.) L.K. Dai; Reynders & al., 2011).

Cyperus (subg. *Pycreus*) sect. *Muricati* Kük. Nom. Superfl. in Engler, Pflanzenr. IV, 20 (Heft 101): 394. 1936, nom. superfl. – Type: *Cyperus muricatus* Kük. [≡ *Pycreus muricatus* (Kük.) Napper] (Art. 22.6).

When published, *Cyperus* sect. *Muricati* included the type of *Pycreus* sect. *Tuberculati* Cherm. (1919) and is therefore superfluous. However, Reynders & Goetghebeur (2010) misinterpreted Art. 52.3 in stating that the name *C.* sect. *Muricati* can be used when *C. divulsus* Ridl. [≡ *Pycreus divulsus* (Ridl.) C.B. Clarke] is excluded.

Cyperus sect. *Platystachyi* (Kunth) C.B. Clarke (see ***Cyperus* sect. *Hymenolepides*** Nees).

Cyperus sect. *Polystachyi* (C.B. Clarke) Kük., nom. illeg. (see ***Pycreus*** P. Beauv. **ser. *Pycreus***; Reynders & al., 2011).

Cyperus sect. *Propinqui* (C.B. Clarke) Kük. (see ***Pycreus* sect. *Propinqui*** C.B. Clarke; Reynders & al., 2011).

Cyperus (sect. *Pycreus*) [unranked] *Pseudopycreus* Boeck. (see *Cyperus* sect. *Laevigati* Kük.).

Cyperus sect. *Pseudopycreus* (C.B. Clarke) Kük., nom. illeg. (see ***Kyllinga* subg. *Pseudopycreus*** C.B. Clarke; Reynders & al., 2011).

Cyperus sect. *Pumili* Kük. (see ***Pycreus* sect. *Pumili*** (Kük.) L.K. Dai; Reynders & al., 2011).

Cyperus sect. *Pycreus* (P. Beauv.) Griseb., *Cyperus* subg. *Pycreus* (P. Beauv.) J. Carey (see ***Pycreus*** P. Beauv. **ser. *Pycreus***; Reynders & al., 2011).

Cyperus sect. *Rhizomatosi* Kük. (see **Pycreus sect. Rhizomatosi** (Kük.) J. Raynal; Reynders & al.,
2011).

Cyperus (subg. *Pycreus*) sect. *Sulcati* Kük., nom. illeg. Non *C.* [unranked] *Sulcati* Boeck. (see
Pycreus sect. Vestiti C.B. Clarke; Reynders & al., 2011).

Cyperus sect. *Vestiti* (C.B. Clarke) J. Kern, nom. illeg. (see **Pycreus sect. Vestiti** C.B. Clarke;
Reynders & al., 2011).

Cyperus (subg. *Pycreus*) [unranked] *Zonati* (see **Pycreus ser. Zonati** (C.B. Clarke) C.B. Clarke;
Reynders & al., 2011).

3.6.2 Names of subdivisions of *Pycreus*

The name *Pycreus* is an anagram of *Cyperus* illustrating the close relationship of the
two taxa (Beauvois, 1816). *Pycreus* is the largest satellite genus of *Cyperus* s.l. Although
opinions vary on the validity of its generic status, *Pycreus* has always been treated as a
recognizable unit. The taxon only differs from the *Cyperus* C$_4$ species in its laterally
compressed pistils with only two style branches, a character that *Pycreu*s shares with *Kyllinga*
and *Queenslandiella*, both with deciduous spikelets. The first authors who published an
extensive treatment of Cyperaceae considered *Pycreus* at the subgeneric level within *Cyperus*
including those *Cyperus* species with two style branches and a dorsiventrally compressed pistil
and also *Cyperus hyalinus* Vahl, which is now treated as *Queenslandiella*. These authors
recognised two informal groups within the subgenus or section *Pycreus* of *Cyperus* based on
this difference in the pistils. Boeckeler (1868) divided the species into *C.* [unranked] *Eupycreus*
containing the species with laterally compressed pistils and *C.* [unranked] *Pseudopycreus*
containing the species with dorsiventrally positioned pistils. Later authors, such as Clarke and
Kükenthal, removed the latter group from *Pycreus* and treated it as a separate genus or
subgenus *Juncellus*.

Subdivisional classification of *Pycreus*, whether it is considered as a separate genus or
as a subgenus of *Cyperus*, started with Boeckeler (1868) who arranged the species in the two

previously mentioned groups of unspecified rank based on the position of the pistils: *C.* [unranked] *Eupycreus* and *Pseudopycreus*.

Clarke (1897) recognised two groups of unspecified rank, *P.* [unranked] *Zonati* and *Puncticulati*, based on the differences in shapes of the nutlet epidermal cells. In 1900, he formally described these groups as *Pycreus* ser. *Zonati* C.B. Clarke and "*P.* ser. *Reticulati* C.B. Clarke", which appear in Clarke's publication of 1908 at the subgeneric level. The latter subgenus was established to unite his former group *P.* [unranked] *Puncticulati* with *P. nigricans* (Steud.) C.B. Clarke (and relatives), which he originally placed in *P.* [unranked] *Zonati*. Within *P.* subg. *Reticulati* Clarke simultaneously described six sections. Chermezon (1919) treated Clarke's subgenera at sectional level as *Pycreus* sect. *Zonati* and *P.* sect. *Puncticulati* adding a third section *P.* sect. *Tuberculati* to accommodate the unique species *P. divulsus* (Ridl.) C.B. Clarke.

Finally, Kükenthal (1936) treated *Pycreus* as a subgenus of *Cyperus* and placed Clarke's subgenera as the rankless taxa *C.* [unranked] *Isodiametrici* (incorporating Clarke's *P.* subg. *Reticulati* and *P.* sect. *Puncticulati*) and *C.* [unranked] *Zonati*. At sectional level, Kükenthal only retained three of Clarke's sections and divided the species from the other sections into nine new sections. Kükenthal's classification was later adopted unchanged for the New World species by Corcoran in 1941.

Kern (1974) treated five of the existing sections in the *Flora Malesiana*. He retained two of Kükenthal's sections and transferred two of Clarke's sectional epithets into *Cyperus* to replace two of Kükenthal's later synonyms. He replaced *C.* sect. *Polystachyi* by *C.* sect. *Pycreus*, but he did not mention that Grisebach had already published the latter in 1846.

"*Pycreus* sect. *Capitati* Nakai" in Bot. Mag. (Tokyo) 26: 201. 1912, nom. inval. (Art. 22.2).

Pycreus sect. Chrysanthi C.B. Clarke in Bull. Misc. Inform. Kew, Addit. Ser. 8: 95. 1908 ('*Chrysanthae*') ≡ *Cyperus* (subg. *Pycreus*) sect. *Chrysanthi* (C.B. Clarke) J. Kern, Fl. Males., ser. 1, 7(3): 648. 1974 – Type: *Pycreus chrysanthus* (Boeck.) C.B. Clarke (Art. 22.6).

Clarke (1908) includes six species in this section, which share yellow to reddish spikelets and a compound anthela.

Pycreus* sect. *Colorati C.B. Clarke in Bull. Misc. Inform. Kew, Addit. Ser. 8: 95. 1908 ('*Coloratae*') – Lectotype (designated here): *Pycreus nigricans* (Ridl.) C.B. Clarke.

The characters of *P. nigricans* fit very well the description of this section (Clarke, 1908). Clarke (1908) included 21 species in this section based mainly on their brown to very dark glume colour: "Spiculae castaneae aut atrae, vel castaneo-tinctae (in *P. spissiflora*, fuscentes)."

Pycreus sect. *Flavescentes* (Kük.) T.V. Egorova (see ***Pycreus* ser. *Zonati*** (C.B. Clarke) C.B. Clarke).

Pycreus* sect. *Globosi C.B. Clarke i n Bull. M isc. Inform. Kew, Addit. Ser. 8: 95. 1908 ('*Globosus*') ≡ *Cyperus* (subg. *Pycreus*) sect. *Globosi* (C.B. Clarke) Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 352. 1936 ≡ *P.* sect. *Umbellati* Nakai in Bot. Mag. (Tokyo) 26: 202. 1912 ('*Umbellata*'), nom. illeg. (Art. 7.5, Art. 52.1) – Type: *Pycreus globosus* Rchb. [= *P. flavidus* (Retz.) T. Koyama] (Art. 22.6).

Pycreus* sect. *Lancei L.K. Dai in Tang & Wang, Fl. Reipubl. Popularis Sin. 11: 164. 1961 ('(Kük.) L.K. Dai') ≡ *Cyperus* (subg. *Pycreus*) sect. *Lancei* Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 330. 1936, nom. superfl. – Type: *Pycreus lanceus* (Thunb.) Turrill (*Cyperus lanceus* Thunb.) (Art. 22.6) [= *P. nitidus* (Lam.) J. Raynal].

When published, *Cyperus* sect. *Lancei* included the type of *Pycreus* sect. *Chrysanthi* C.B. Clarke (1908). Kükenthal (1936) should have used Clarke's epithet for this section. However, according to Art. 52.3 the name *C.* sect. *Lancei* can be used when *Cyperus chrysanthus* Boeck. [≡ *Pycreus chrysanthus* (Boeck.) C.B. Clarke] is excluded. Dai (1961) did not include *Pycreus chrysanthus* when he combined *Cyperus* sect. *Lancei* into *Pycreus* as *P.* sect. *Lancei*.

Pycreus sect. *Latespicati* (Kük.) L.K. Dai in Tang & Wang, Fl. Reipubl. Popularis Sin. 11: 172. 1961 ≡ *Cyperus* (subg. *Pycreus*) sect. *Latespicati* Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 388. 1936 – Type: *Pycreus latespicatus* (Boeck.) C.B. Clarke (*Cyperus latespicatus* Boeck.) (Art. 22.6) [= *P. diaphanus* (Schrad. ex Roem. & Schult.) S.S. Hooper & T. Koyama].

= *Pycreus* sect. *Monocephali* Nakai.

Pycreus sect. *Monocephali* Nakai in Bot. Mag. (Tokyo) 26: 201. 1912 – Type: *Pycreus setiformis* (Korsh.) Nakai (≡ *Cyperus setiformis* Korsh.) [= *P. diaphanus* (Schrad. Ex Roem. & Schult.) S.S. Hooper & T. Koyama].

"*Pycreus* sect. *Polystachyi* C.B. Clarke" in Bull. Misc. Inform. Kew, Addit. Ser. 8: 94. 1908 ('*Polystachyae*'), nom. inval. (Art. 22.2).

Pycreus sect. *Propinqui* C.B. Clarke in Bull. Misc. Inform. Kew, Addit. Ser. 8: 95. 1908 ('*Propinquae*') ≡ *Cyperus* (subg. *Pycreus*) sect. *Propinqui* (C.B. Clarke) Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 342. 1936 – Type: *Pycreus propinquus* Nees [= *P. lanceolatus* (Poir.) C.B. Clarke] (Art. 22.6).

Pycreus sect. *Pumili* (Kük.) L.K. Dai in Fl. Reipubl. Popularis Sin. 11: 169. 1961 ≡ *Cyperus* (subg. *Pycreus*) sect. *Pumili* Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 375. 1936 – Type: *Cyperus pumilus* L. [≡ *Pycreus pumilus* (L.) Nees]. "*Pycreus* [unranked] *Puncticulati* C.B. Clarke" in Thiselton- Dyer, Fl. Cap. 7: 155. 1897 ('*Puncticulatae*'), nom. inval. (Art. 22.2) – "*Pycreus* sect. *Puncticulati* Cherm." in Ann. Mus. Colon. Marseille, ser. 3, 7(2): 66. 1919, nom. inval. (Art. 22.2).

Pycreus P. Beauv. ser. *Pycreus,* autonym automatically established by C.B. Clarke in Urban, Symb. Antill. 2: 15 1900 (Art. 22.3) ≡ *Pycreus* P. Beauv., Fl. Oware 2: 48. 1816 ≡

Cyperus sect. *Pycreus* (P. Beauv.) Griseb., Spic. Fl. Rumel. 2: 419. 1846 ≡ *C.* subg. *Pycreus* (P. Beauv.) J. Carey in Gray, Manual: 517. 1847 ≡ *C.* (sect. *Pycreus*) [unranked] *Eupycreus* Boeck. in Linnaea 35: 437. 1868 ≡ *Pycreus* subg. *Pycreus,* autonym automatically established by C.B. Clarke in Bull. Misc. Inform. Kew, Addit. Ser. 8. 1908 (Art. 22.3) ≡ *Pycreus* sect. *Pycreus,* autonym automatically established by C.B. Clarke in Bull. Misc. Inform. Kew, Addit. Ser. 8: 94. 1908 (Art. 22.3) ≡ *C.* (subg. *Pycreus*) sect. *Polystachyi* Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 363. 1936, nom. illeg. (Art. 52.1) – Type: *Pycreus polystachyos* (Rottb.) P. Beauv. (type of *Pycreus* – type of autonyms Art. 7.6 –

type of *C.* subdivisions *Pycreus* Art. 7.4 – lectotype of *C.* [unranked] *Eupycreus* designated here – type of *C.* sect. *Polystachyi* Art. 22.6).

Cyperus polystachyos Rottb. [≡ *P. polystachyos*] is the type of the name *C.* sect. *Pycreus* in which *C.* [unranked] *Eupycreus* is included. Boeckeler (1868) i ncluded all species with bifid stigmas in *C.* sect. *Pycreus*. He divided it into two subgroups of unspecified rank: *Eupycreus* having laterally compressed pistils and *Pseudopycreus* having dorsiventrally compressed pistils. The latter group was later excluded from *Pycreus*. Although the epithet in Boeckeler's unranked subdivision of *Cyperus* begins with the prefix Eu-, Art. 21.3 does not apply here as it was not the generic name *Cyperus* to which it was prefixed.

"*Pycreus* ser. *Reticulati* C.B. Clarke" in Urban, Symb. Antill. 2: 15. 1900 ('*Reticulatae*'), nom. inval. (Art. 22.2) – "*Pycreus* subg. *Reticulati* C.B. Clarke" in Bull. Misc. Inform. Kew, Addit. Ser. 8: 94. 1908 ('*Reticulatae*'), nom. inval. (Art. 22.2).

Pycreus* sect. *Rhizomatosi (Kük.) J. Raynal in Adansonia, ser. 2, 16(4): 403. 1977 ≡ *Cyperus* (subg. *Pycreus*) sect. *Rhizomatosi* Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 327. 1936 – Type: *Cyperus rhizomatosus* (C.B. Clarke) Kük. (Art. 22.6) [≡ *Pycreus rhizomatosus* C.B. Clarke]. *Pycreus* sect. *Sulcati* L.K. Dai, nom. illeg. (see ***Pycreus* sect. *Vestiti*** C.B. Clarke).

Pycreus* sect. *Tuberculati Cherm. in Ann. Mus. Colon. Marseille, ser. 3, 7(2): 65. 1919 – Type: *Pycreus divulsus* (Ridl.) C.B. Clarke.

Pycreus divulsus is the only species included in this section by Chermezon (1919). The name is based on the tuberculate nutlets, which are unique for this species. Although most of the sections described by Chermezon in 1919 are nomina nuda, he added a remark to *P.* sect. *Tuberculati*, describing its difference with the existing sections of *Pycreus*, which is acceptable as diagnosis. For more detailed information see Reynders & Goetghebeur (2010).

Pycreus sect. *Umbellati* Nakai, nom. illeg. (see *Pycreus* sect. *Globosi* C.B. Clarke).

Pycreus* sect. *Vestiti C.B. Clarke in Bull. Misc. Inform. Kew, Addit. Ser. 8: 94. 1908 ('*Vestitae*') ≡ *Cyperus* (subg. *Pycreus*) sect. *Sulcati* Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 379. 1936, nom. illeg. (Art. 52.1) ≡ *P.* sect. *Sulcati* L.K. Dai in Tang & Wang, Fl. Reipubl.

Popularis Sin. 11: 170. 1961, nom. illeg. (Art. 52.1) ≡ *C.* (subg. *Pycreus*) sect. *Vestiti* (C.B. Clarke) J. Kern, Fl. Males., ser. 1, 7(3): 646. 1974, nom. illeg. (Art. 53.4) − Lectotype (Kern, 1974): *Cyperus sanguinolentus* Vahl [≡ *Pycreus sanguinolentus* (Vahl) Nees].

Clarke (1908) included five species in this section. *Pycreus divulsus* differs from the others in having strongly tuberculate nutlets and a simply spicate inflorescence which does not fit the description of this section by Clarke (1908): "Culmus basi decumbentes" nor to "Umbella simplex aut monocephala". *Pycreus atronervatus* has multinerved glumes in contrast to the other species. Kükenthal (1936) used the epithet *Sulcati* instead of the epithet *Vestiti* when treating this section in *Cyperus*, although Clarke's epithet has priority (Art. 11.4). *Cyperus* sect. *Sulcati* Kük. is illegitimate since it is a later homonym of *C.* [unranked] *Sulcati* Boeck. (Art. 35.3 and Art. 53.4). However, the transfer of *Pycreus* sect. *Vestiti* C.B. Clarke to *Cyperus* by Kern (1974) as *C.* sect. *Vestiti* also creates an illegitimate name under Art. 53.4 since *Cyperus* (subg. *Mariscus*) subsect. *Vestiti* Kük. (1936) is an older homonym.

Pycreus [unranked] *Zonati* C.B. Clarke in Thiselton-Dyer, Fl. Cap. 7: 155. 1897 ('*Zonatae*') ≡ ***P. ser. Zonati*** (C.B. Clarke) C.B. Clarke in Urban, Symb. Antill. 2: 15. 1900 ('*Zonatae*') ≡ ***P. subg. Zonati*** (C.B. Clarke) C.B. Clarke in Bull. Misc. Inform. Kew, Addit. Ser. 8: 94. 1908 ('*Zonatae*') ≡ ***P. sect. Zonati*** (C.B. Clarke) Cherm. in Ann. Mus. Colon. Marseille, ser. 3, 7(2): 83. 1919 ≡ *Cyperus* (subg. *Pycreus*) [unranked] *Zonati* (C.B. Clarke) Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 327. 1936 ≡ *C.* (subg. *Pycreus*) sect. *Flavescentes* Kük. in Engler, Pflanzenr. IV, 20 (Heft 101): 395. 1936 ≡ *P.* sect. *Flavescentes* (Kük.) T.V. Egorova in Novosti Sist. Vyssh. Rast. 35: 36. 2003 − Type: *Pycreus flavescens* (L.) P. Beauv. ex Rchb. (≡ *Cyperus flavescens* L.) (lectotype of *Pycreus* [unranked], subg., sect., & ser. *Zonati* designated here − type of *C.* sect. *Flavescentes* and *P.* sect. *Flavescentes* Art. 22.6).

Clarke (1897) published *P.* [unranked] *Zonati* including *P. flavescens*, *P. rehmannianus* C.B. Clarke and *P. macranthus* (Boeck.) C.B. Clarke. *Pycreus flavescens* is the only species mentioned when he published *P.* ser. *Zonati* (Clarke, 1900). *Pycreus flavescens* is a widespread species and it fits very well to the description given by Clarke (1897) "Superficial cells of the nut longitudinally oblong; nut often appearing zonate by reason of the narrow ends of the cells running into an undulating or broken horizontal line". Kükenthal (1936) used the epithet *Zonati* for a group of unspecified rank under *Cyperus* subg. *Pycreus* and divided it into three

new sections: *C.* sect. *Flavescentes*, *Latespicati* and *Muricati*. As Kükenthal placed *P. macranthus* in his *C.* sect. *Latespicati*, he did not include in *C.* sect. *Flavescentes*, "all elements eligible as type under Art. 10.2" (Art. 52.2) of *C.* sect. *Zonati*, and so his name is legitimate, although now superfluous by the typification here of the names based on *C.* unranked *Zonati* C.B. Clarke.

3.7 Acknowledgements
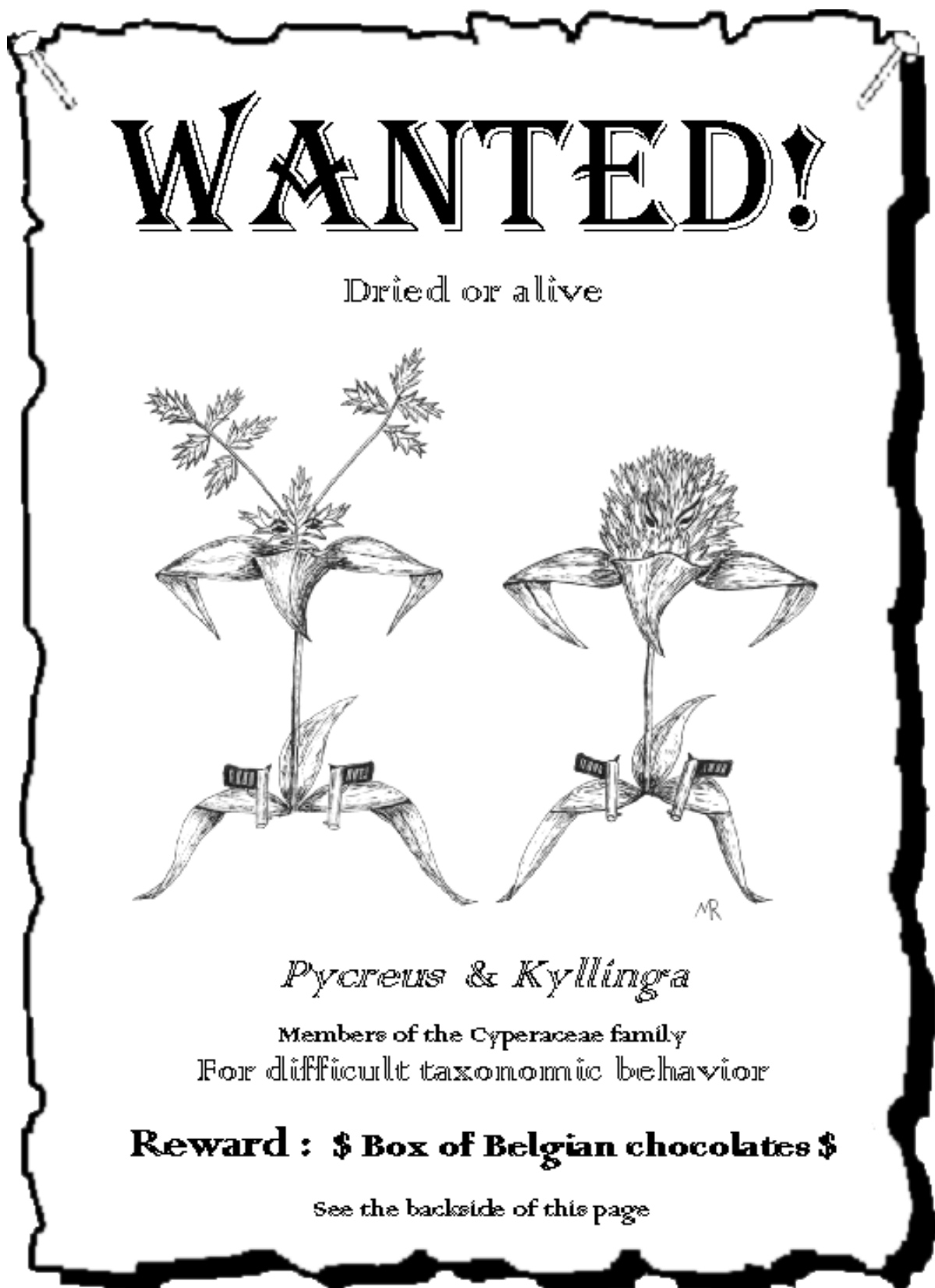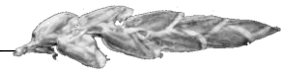
Fig. 3.2 Flyer distributed on the XVIIth International Botanical Congress in Vienna, 2005. Illustration by M. Reynders.
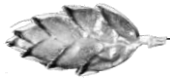
4 Development of spikelets and flowers

*"If we knew what it was we were doing,
it would not be called research, would it?"*

— *Albert Einstein (1979-1955)*

← Fig. 4.1 *Pycreus sanguinolentus* in the Ghent University Botanic Garden supplied wonderful results on floral vasculature.

Spikelet theme: *Pycreus pauper*

4.1 In this chapter…

Chapter 4 includes the results of an ontogenetic and anatomical study of floral and spikelet ontogeny in *Cyperus* and *Pycreus*. These results have been published as the following article:

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

Remark: The circumscription for the gynoecium development used in the article Is somewhat outdated. Here follows a more correct phasing: In Cyperoideae, the gynoecium originates from an annular gynoecium wall primordium, (previously described as ovary wall primordium, surrounding a central, floral-apical meristematic zone (previously described as 'ovule primordium') from which later on a single ovule originates. At early developmental stage, r-the raising, bag-like gynoecium wall surrounds the developing ovule, thus forming an immature open ovary. With the gynoecium wall further raising and forming a single style, the initially open ovary is gradually closed.

4.2 Abstract

Pycreus, Kyllinga, and *Queenslandiella* cluster together with *Cyperus* within the *Cyperus* s. lat. clade, one of the two large clades in Cypereae. However, in contrast with *Cyperus*, they have laterally flattened pistils/nutlets. *Pycreus, Kyllinga* and *Queenslandiella* form morphologically well circumscribed independent genera. In the context of a broader systematic project to work out a well-supported, evolution based taxonomy for *Cyperus* s. lat., we present in this paper general morphological and developmental data of species of *Pycreus* in comparison with three species of *Cyperus*, including *C. laevigatus* with dorsiventrally flattened nutlets. Freshly collected material was investigated using scanning electron microscopy (SEM) and light microscopy (LM). Special attention was given to spikelet and gynoecial development. SE micrographs of all species studied show an indeterminate rachilla with distichously arranged glumes, each subtending a bisexual flower. In spikelets of *C. capitatus* and *P. pumilus*, the proximal glume sometimes subtends a lateral spikelet instead of
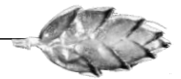
a flower. In the species of *Pycreus* studied, each flower sits in a cavity formed by the growth of the rachilla, which is congenitally fused with the wings of the glume of the higher, alternate flower. Glumes appear successively, each soon forming a flower primordium in its axil, which develops according to a general cyperoid ontogenetic pattern. In *Pycreus*, the stigma branches grow out from dorsiventrally positioned primordia. During gynoecium development, a hypogynous stalklet (gynophore) appears in all species studied. In spikelets of *Pycreus*, the rachilla and wings of the glumes are congenitally fused and consequently develop with epicaulescent displacements of the glumes resulting in typical spikelets with flowers in cavities. In spikelets of *Cyperus*, a similar though less pronounced development results in spikelets with zigzagging rachilla. The particular positions of the stigma branches in *C. laevigatus* and *Pycreus* are explained by the development of the gynoecium from an annular primordium, which facilitates shifts in localisation of the stigma primordia. Though we consider the combination of the typical spikelet ontogeny and the independently originated laterally flattened nutlets to be strong arguments in favor of a genus *Pycreus*, a phylogenetic confirmation that the taxon is monophyletic is an absolute, until now unfulfilled, condition. Moreover, the consequences for the giant genus *Cyperus* must be taken in consideration.

4.3 Introduction

4.3.1 Taxonomical data of *Cyperus* s.lat.

According to molecular phylogenetic studies in Cyperaceae (Muasya *et al.,* 2009a), the subfamily Cyperoideae comprises most of the cyperaceous genera, including the derived Cypereae clade (corresponding to Cypereae sensu Goetghebeur, 1998). Within this clade, *Cyperus* and allied genera, called *Cyperus* s. lat., form a subclade that is sister to a *Hellmuthia-Scirpoides-Isolepis-Ficinia* clade (Muasya *et al.,* 1998, 2001b, 2009a; Simpson *et al.,* 2007, fig. 4.2). Based on the embryological study of Van der Veken (1964) and corroborated by more recent molecular phylogenetic studies (Muasya *et al.,* 2002, 2009a, 2009b; Simpson *et al.,* 2007), several smaller satellite genera appear to be nested *within* the *Cyperus* s. lat. clade, such as among others, *Kyllinga*, *Queenslandiella* and *Pycreus*. Each of these is characterised by specialised inflorescence and flower morphologies. *Kyllinga* can be distinguished by its reduced spikelets and flowers with laterally flattened ovaries, *Pycreus* by flattened spikelets and flowers with laterally flattened ovaries, and *Queenslandiella* by deciduous spikelets

(formerly placed in *Mariscus*) and flowers with laterally flattened ovaries (Goetghebeur, 1986). In 1998, Goetghebeur wrote: "*Pycreus* and *Kyllinga*, plus some highly specialized smaller taxa are often excluded [from *Cyperus* s. lat.] and recognized at the generic level. Authors who include these taxa into *Cyperus* s. lat. mostly maintain them on the subgeneric level." Moreover, the more derived part of *Cyperus* s. lat., including *C. capitatus*, *C. laevigatus*, and *Pycreus*, consists of genera with C_4 photosynthesis and Kranz anatomy (fig. 4.2). Since *Kyllinga, Pycreus* and *Queenslandiella* are not sister taxa, we hypothesize independent and multiple origin of the laterally flattened pistil.

4.3.2 Inflorescence morphology in *Cyperus* s.lat. and *Pycreus*

The inflorescence in Cyperoideae is a compound inflorescence, essentially a panicle of spikelets with the main axis[1] called a culm. The ultimate branch in a cyperoid inforescence is always a lateral spikelet, consisting of a rachilla and spirally to distichously placed glumes, each subtending (or not) a bisexual (most Cyperoideae) or unisexual (Cariceae) flower. Lateral spikelets are subtended by a bract and have a prophyll (Goetghebeur, 1998). Terminal spikelets end the culm or a branch of it as a (co)florescence sensu Troll (1964; see Weberling, 1992), and as a consequence it is separated from its prophyll by the length of the culm/lateral branch, which constitutes also the axis of the terminal spikelet (Haines & Lye, 1983; Goetghebeur, 1998). In *Cyperus* s. lat., spikelets are distichously organised, which can be considered as a synapomorphy though a number of reversals to terete spikelets are recorded (Muasya *et al.,* 2001b). In *Pycreus*, the inflorescence is antheloid with as well the culm as lateral branches ending with a terminal spike of flattened spikelets. In *Pycreus*, the inflorescence is antheloid with as well the culm as lateral branches ending with a terminal spike of flattened spikelets.

Vrijdaghs *et al.* (2010) showed that cyperoid spikelets, including several, mostly distichously organised controversial ones that by some authors were interpreted as sympodial (e.g. Celakovsky, 1887; Kern,1962; Zhang *et al.,* 2004), have an indeterminate rachilla and can be considered to be an open spike as cited by Weberling (1992). Guarise & Vegetti (2008: 41) reported that in *Cyperus* section *Luzuloidei*, fascicles of spikelets occur, "which can be serial, prophyllar, or mixed". Serial fascicles of spikelets are mainly found in the distal part of the florescence and paraclades, the latter being a repetition of the main inflorescence's structure.

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[1] The main axis of Monocots is also often referred to as a 'scape'
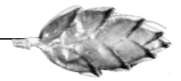
A serial fascicle of spikelets is subtended by a single bract. Guarise & Vegetti (2008: 55) also mentioned a 'torsion' within the spikelets: "some spikelets appear with the glumes in the same plane as the pherophyll and prophyll, or in an intermediate position". Several species in *Cyperus* s. lat., formerly grouped together in *Mariscus*, have dehiscent spikelets. Haines (1967: 57) reported a 'pulvinus' or swelling body at the base of lateral spikelets in *Cyperus tenuis* Sw., stating "But at the attachment of the prophyll, and probably a part of the prophyll, is a pulvinus which adjusts the position of both the branch and the umbel bract that subtends it". Haines & Lye (1983: 17) mentioned "a callus is developed at the prophyll base, swelling of this callus causing divergence of the shoot".
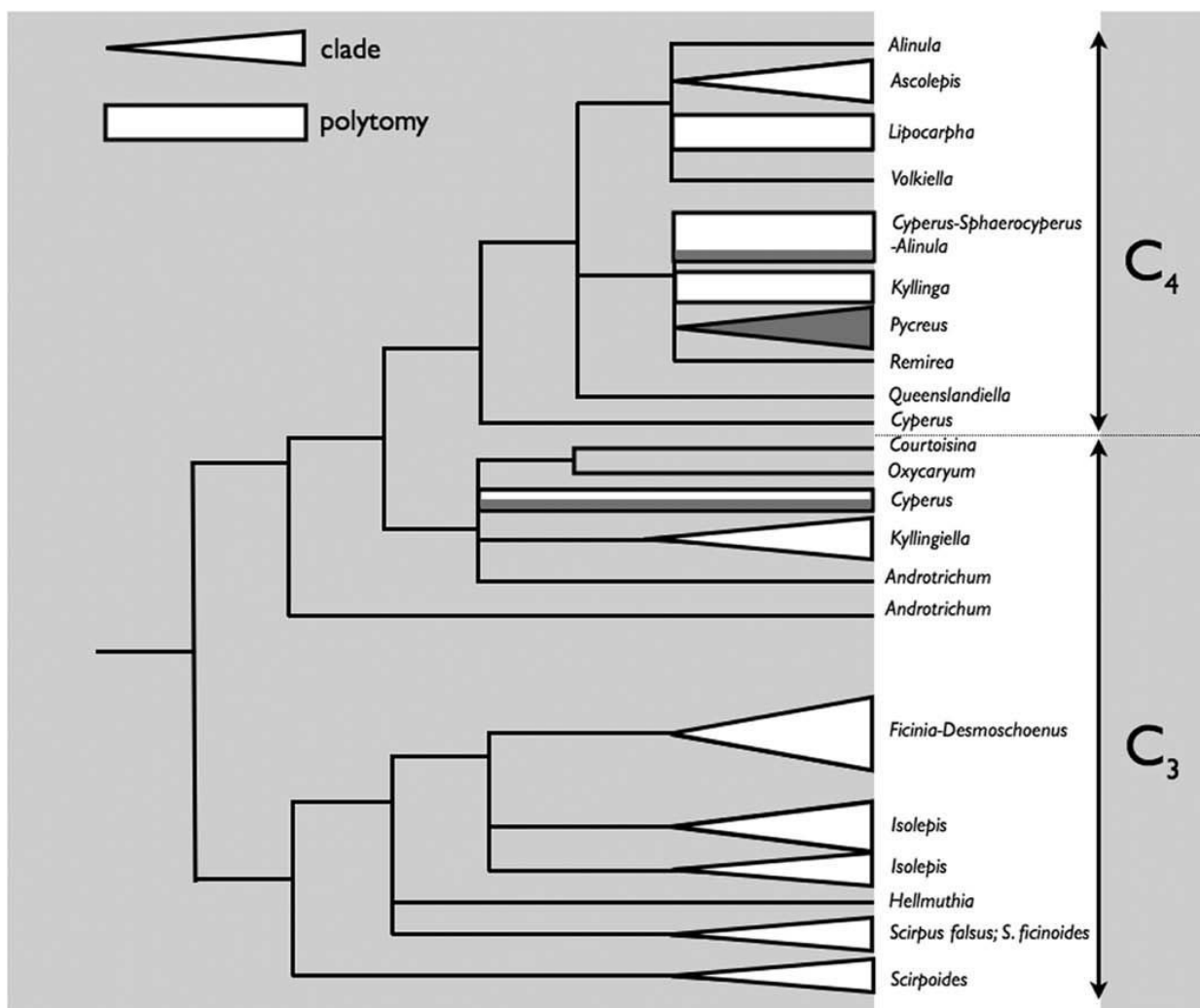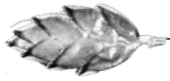

Fig. 4.2 Simplified cladogram of Cypereae based on Muasya *et al.,* (2009a). In dark grey, taxa of which species were used in this study. *Cyperus luzulae* is a C$_3$ species, whereas *C. capitatus* and *C. laevigatus* are C$_4$ species.

4.3.3 Floral morphology and development in *Cyperus* s.s. and *Pycreus*

Flowers in Cyperoideae either have a perianth (3 + 3 parts or less[2]) of varying size and shape or lack a perianth as observed in most species of Cypereae (e.g. Goetghebeur, 1998, Muasya *et al.*, 2009b). The androecium in most Cyperoideae is haplostemonous with usually three stamens with basifix and introrse anthers (Bruhl, 1991; Vrijdaghs *et al.*, 2005a), resulting from the reduction of the inner staminal whorl (Takhtajan, 1997). However, particularly in *Cyperus* s. lat., the number of stamens can be reduced to two or one (Haines & Lye, 1983). In the first developmental stages in cyperoid flowers, the stamens grow faster than the gynoecium (Vrijdaghs *et al.*, 2005a), but at maturity of the flower, the stigma branches usually are functionally active before the pollen grains are released (Goetghebeur, 1998). In many species of *Cyperus*, an apiculus or connective crest is formed on the top of the anthers (Haines & Lye, 1983).

The pistils in flowers within the *Cyperus* s. lat. clade vary from triangular with three stigma branches to dorsiventrally or laterally compressed with only two stigma branches. Raynal (1966) studied some African *Cyperus* species (e.g. *C. meeboldii* Kük., *C.. clavinux* C.B.Clarke, *C.. laterriticus* J. Raynal) with triangular nutlets and a single stigma branch. Most species with a dorsiventrally flattened pistil were often classified in a separate taxon (*Juncellus*) by several authors (e.g. Clarke, 1893; Kükenthal, 1936; Podlech, 1960). Already Clarke expressed some doubt: "This species [*Juncellus pustulatus*] has differentiated itself into *Juncellus*, but has not broken its connection with *Cyperus* entirely yet." (Clarke, 1901: 308). In some former *Juncellus* species, even within single specimens, the flowers can have both trigonous and dorsiventrally compressed nutlets (e.g. *Cyperus alopecuroides* Rottb., *C. pustulatus* Vahl, *C. pygmaeus* Rottb.). The polyphyletic dispersion of the *Juncellus* species was confirmed by many other authors (e.g. Goetghebeur, 1986; Muasya *et al.*, 2002). Therefore, a separate genus *Juncellus* is no longer recognised. Moreover, dorsiventrally flattened pistils can also be found in diverse other cyperoid genera such as *Dulichium arundinaceum* (L.) Britton, *Eleocharis*, *Fimbristylis*, *Nemum*, and *Carex*.

On the other hand, laterally compressed pistils are restricted to three genera, *Pycreus, Kyllinga* and *Queenslandiella*. Blaser (1941) showed that the laterally flattened pistil in *Pycreus* concurs with new vascular patterns. Several authors based the subdivisional classification within *Pycreus* among others on the morphology of the fruit wall epiderm cells (e.g. Clarke,

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[2] Or sometimes more (e.g. *Coleochloa, Eriophorum, Dulichium*, ect.)

1897; Chermezon, 1919; Kükenthal, 1936). Clarke (1897: 155) described the epiderm cells of nutlets in his "Zonatae" as follows: "Superficial cells of the nut longitudinally oblong; nut often appearing zonate by reason of the narrow ends of the cells running into an undulating or broken horizontal line." In several species, these cells contain silica bodies, though according to Metcalfe (1971), they have little taxonomical value.

4.3.4 Aims

This study represents the first results in a broader project in which the *Cyperus* s. lat. clade is investigated in analogy with our earlier study of the *Hellmuthia-Scirpoides-Isolepis-Ficinia* clade, which resulted in several publications (Muasya *et al.,* 2009a, 2009b; Vrijdaghs *et al.,* 2005b, 2006a, 2006b, 2009). By combining molecular phylogenetic data, anatomy, morphology and spikelet/floral ontogeny, our goal is to clarify the evolution of *Cyperus* s. lat. and the position of the so-called 'satellite genera' within it. In this paper, we present and discuss original SEM and LM images of the morphology and spikelet/floral development in species of *Pycreus*, which were selected based on our preliminary phylogenetic data and compared with two *Cyperus* C_4 and one *Cyperus* C_3 species (respectively *C. laevigatus, C. capitatus*, and *C. luzulae)*, starting from the hypothesis that *Pycreus* can be considered to be a genus of its own. Of the three genera with laterally compressed pistils, the mainly African genus *Pycreus* (±120) was chosen to be examined first, because it is the largest one.

4.4 Materials and Methods

4.4.1 Plant material

Inflorescences of the species studied were collected in the field and at the Ghent University botanical garden (table 4.1) and subsequently fixed in FAA (70% ethanol, acetic acid, 40% formaldehyde, 90/5/5). Spikelets and floral buds were dissected in 70% ethanol under a Wild M3 (Leica Microsystems AG, Wetzlar, Germany) stereo microscope equipped with a cold-light source (Schott KL1500; Schott-Fostec LLC, Auburn, NY, USA).

Since in *Cyperus* s. lat. most spikelets have many and a variable amount of flowers, and consequently in order to avoid the use of abstract numbers, (flower subtending) glumes are numbered from young (1) to old (x).
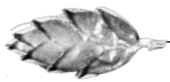
Table 4.1 Species of Cypereae (Cyperaceae) studied and voucher data.

taxa	Collector and origin	voucher
Cyperus capitatus Poir.	Goetghebeur, Sep. 2004, HBUG 2003-1782 (w)	PG10744
Cyperus laevigatus L.	Goetghebeur, Sep. 2004, HBUG1997-1237 Reynders, Nov. 2007, HBUG2003-1192	PG10202
Cyperus luzulae Rottb.	Vrijdaghs, HBUG1900-3306	AV05
Pycreus bipartitus C.B. Clarke	Reynders, Nov. 2004, HBUG 2003-0327 (s)	
Idem (fig.4.13)	Laegaard, Ecuador	Laegaard 101015 (GENT)
Pycreus flavescens (L.) P.Beauv.ex Rchb.	Reynders, Jul. 2007, HBUG2005-0401 (s)	
Idem (fig.4.13)	Muasya, 2005, Kenya	AM2585
Pycreus pelophylus (Ridl.) C.B.Clarke	Musili, 2005, Kenya	PM029
Idem (fig. 4.13)	Reekmans, Burundi	Reekmans 2547 (GENT)
Pycreus polystachyos subsp. holocericeus (Rottb.) P. Beauv.	Reynders, Jul. 2007, HBUG 2006-1258 (w)	
Idem (fig.4.13)	Lewalle, Burundi	Lewalle 6290 (GENT)
Pycreus pumilus (L.) Nees	Muasya, 2005, Kenya	AM2150
Idem (fig. 4.13)	Reekmans, Burundi	Reekmans 5795 (GENT)
Pycreus sanguinolentus (Vahl) Nees	Reynders, Jul. 2007, HBUG2006-1753 (w)	

4.4.2 Scanning electron microscopy (SEM)

The prepared material was washed twice with 70% ethanol for 5 minutes and then placed in a mixture (1/1) of 70% ethanol and DMM (dimethoxymethane) for 5 minutes. Subsequently, the material was transferred to 100% DMM for 20 min, before it was CO2 critical point dried using a CPD 030 critical point dryer (BAL-TEC AG, Balzers, Liechtenstein). The dried samples were mounted on aluminium stubs using Leit-C and coated with gold with a SPI-ModuleTM Sputter Coater (SPI Supplies, West-Chester, PA, USA). Images were obtained on a Jeol JSM-6360 (Jeol, Tokyo) at the Laboratory of Plant Systematics (K.U. Leuven).

4.4.3 Light microscopy (LM)

Samples were prepared in ethanol 70% and subsequently gradually transferred to ethanol 100%. Then, the samples were transferred to LR White Resin, hard grade (London Resin Company Ltd, Reading, England) in a graded LR White Resin/ethanol series using solutions of 25/75, 50/50, 75/25, 100/0 resin/ethanol 100% for at least 5 h each. Next, the samples were placed in a closed capsule filled with fresh resin, and hardened at 60°C during

48 h. Sections of 2 μm were made with a rotation microtome (Microm HM360 Waldorf, Germany) and subsequently stained with 0,1% toluidine blue. The stained sections were fixed on microscopy slides using Eukitt© quick hardening mounting medium (Fluka Chemie GmbH, Switzerland). Observations were done with a light microscope (Leitz Dialux 20, Van Hopplynus, Brussels, Belgium) equipped with a camera (PixeLINK PL-B622CF, Ottawa, Canada) with specially developed software (Microscopica v1.3, Orbicule, Leuven, Belgium).

4.5 Results

The development and morphology of spikelet and flower in *Cyperus* and *Pycreus* are described below.

4.5.1 *Cyperus*: spikelet structure

In all species studied, the spikelet consists of a open axis (rachilla) and many distichously arranged glumes, each subtending a bisexual flower (figs 4.3A, 4.4A, 4.5A & B). Glumes develop fast, the older glumes not only protecting the flower they subtend, but also the apical part of the spikelet (figs 4.3A, B & D, 4.4A, 4.5A, B & M). Mature glumes have lateral wings, which partially envelop the rachilla and alternate, lower flower (figs 4.3D & I, 4.4P, 4.5A, C & L, fig. 4.14). The basal part of glume and wings is congenitally fused with the rachilla (fig. 4.14B–E). In *C. luzulae*, mature glumes have conspicuous prickles at the distal side, as well as high numbers of stomata (fig. 4.3M & N).

4.5.2 *Cyperus*: floral ontogeny

A new glume originates below the rachilla apex, forming a rim-like primordium (figs 4.3A & D, 4.4A, 4.5A–C). Soon, a flower primordium appears in the axil of the glume. The flower primordium expands laterally, forming a stamen primordium at each side, followed by a third abaxial one (figs 4.3A, B & D, 4.4A & B, 4.5B–D). In *C. luzulae*, usually there is a single, lateral stamen primordium (fig. 4.3A, B & D). Simultaneously, the floral apex becomes convex (figs 4.3B & D, 4.4B, 4.5D) and starts differentiating into an annular ovary primordium surrounding a central ovule primordium (figs 4.4C–E, 4.5E). Subsequently, the ovary wall grows up from the base, enveloping the ovule (figs 4.3C & D, 4.4C–E, 4.5E). On its top, one abaxial and two adaxial stigma primordia appear (figs 4.3C & D, 4.4F & G). The stigma primordia grow out into three papillose stigma branches (figs 4.3D–F & K, 4.4G–J). In *C.*
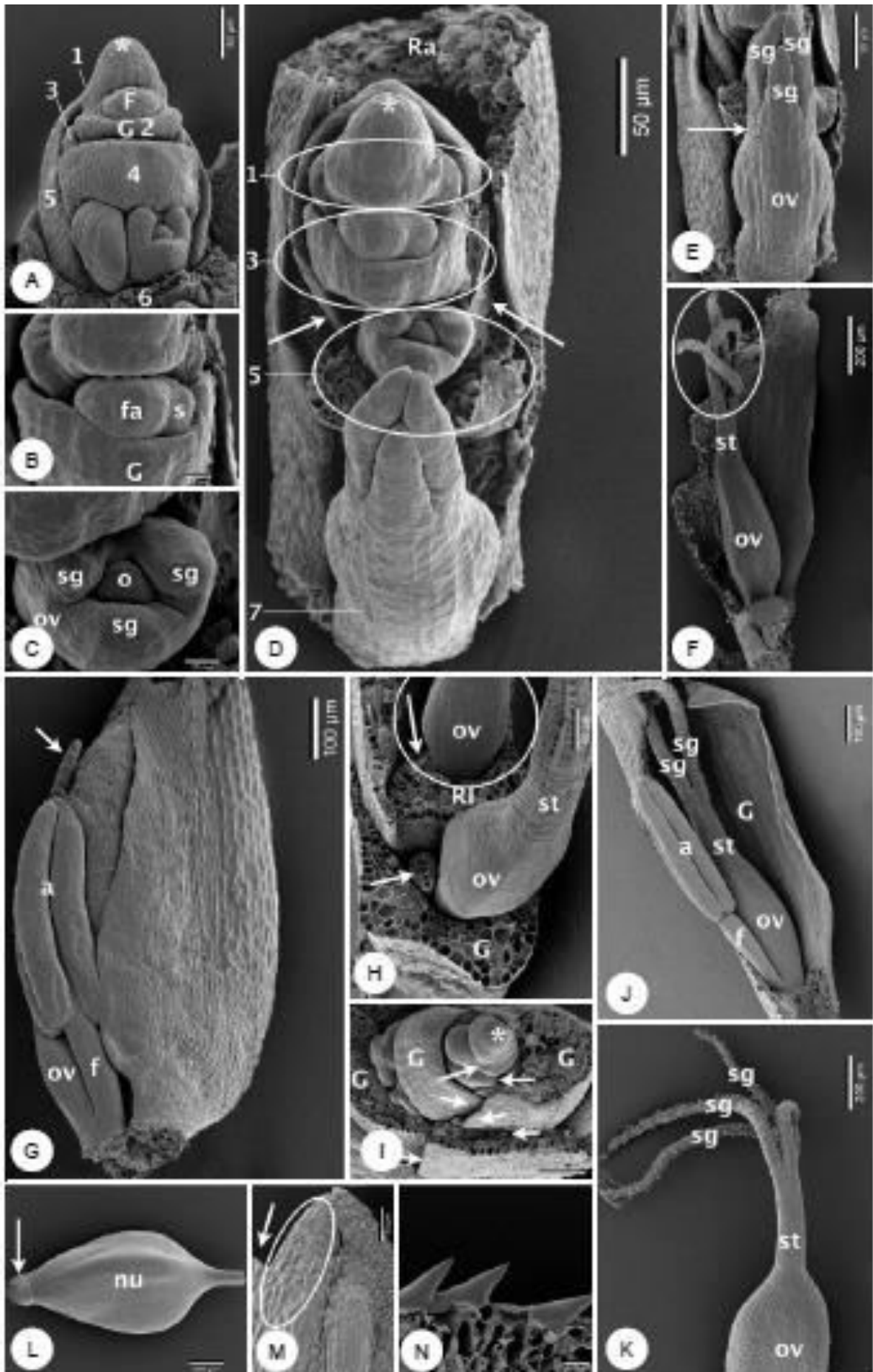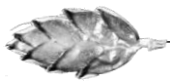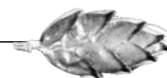
laevigatus, only two laterally positioned stigma primordia appear, which results in a dorsiventrally flattened ovary (fig. 4.5F–L & O). In *C. capitatus*, samples with four stigma branches occur (fig. 4.4H). Meanwhile, the ovary wall continues its growth, forming a single style (figs 4.3E & F, 4.4I & J, 4.5I–K). Simultaneously with the development of the ovary, the stamen primordia differentiate into filament and anther (figs 4.3G, 4.4F–J, 4.4P, 4.5F–H). Until this stage, the development of the stamens is as fast as or faster than the development of the pistil (figs 4.3G, 4.4G–I, 4.4P, 4.5E–H). However, at the later floral developmental stages style and stigma branches elongate faster, so that eventually they protrude above the stamens and even the glume (figs 4.3J, 4.4J, 4.5J–M). Meanwhile, the base of each pollen sac becomes papillose (figs 4.3J, 4.4K, 4.5K), and on the top of the anther an apiculus is formed (figs 4.4K, 4.5K). The ovule primordium develops into an anatropous bitegmic ovule, and within the locule, in a zone around the micropyle, hairs appear (figs 4.4M, 4.5N). In C. *capitatus*, the anther of the mature stamen becomes spiralised (fig. 4.4L). The nutlets of *C. laevigatus* and *C. luzulae* have a hypogynous stalklet, also called gynophore (figs 4.3L, 4.5O). The nutlet in *C. laevigatus* is dorsiventrally flattened (figs 4.5O, 4.13I).

← Fig. 4.3 *Cyperus luzulae*, SE micrographs of floral ontogeny. A, lateral view of the rachilla apex, with six istichously placed glumes at successive developmental stages (numbered 1–6 from young to older; '1', '3' and '5' show the wings of alternately positioned glumes); B, lateral view of glume 2 and a flower primordium in its axil; C, apical view of developing ovary wall surrounding a central ovule primordium with two adaxial and one abaxial stigma primordium; D, lateral view of a developing bractless spikelet belonging to a spikelet fascicle subtended by a common bract (not visible here) with flowers at successive developmental stages (encircled) and numbered from 1 (distal flower) to 7 (proximal flower). Arrows shows the wings of glume 4, which is also visible as the glume protecting the rachilla apex. The main axis, indicated as rachis, actually belongs to another, older spikelet in the fascicle; E, lateral view of a developing gynoecium. A single style appears (arrowed); F, lateral view of a developing ovary, with three stigma branches becoming papillose (encircled); G, lateral view of a part of a spikelet. Proximally, a developing flower with elongating stamen, and a stigma branch protruding above it (arrowed). At right hand side a glume with a wing enveloping the rachilla and a part of the stamen of the alternate flower; H, apical view of a part of a spikelet with removed glumes and stamens (arrowed); I, apical view of the distal part of a spikelet, with some glumes removed (arrows indicate the wings of the glumes); J, adaxial view of a developing flower; K, detail of developing style and stigma branches; L, nutlet with gynophore (arrowed); M, detail of apical part of a glume, with numerous stomata (encircled) and prickles (arrowed); N, detail of prickles. Abbreviations: a, anther; F, flower primordium; f, filament; fa, floral apex; G, glume; nu, nutlet; o, ovule primordium; ov, ovary wall (primordium); R1, rachilla; Ra, rachis; s, stamen primordium; sg, stigma (primordium); st, style; *, rachilla apex.
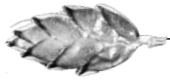
4.5.3 *Pycreus*: spikelet structure

The spikelet in all species studied consists of an indeterminate rachilla and many distichously arranged glumes, each subtending a bisexual flower (figs 4.6A & B, 4.8A, 4.9A & B, 4.10A & B, 4.11A & B). The basal part of glume and wings is fused with the rachilla (fig. 4.14F–I). At later stages, each flower stands in an alcove-like cavity (figs 4.6B, 4.6J, K & M, 4.7A, C & E, 4.8B & C, 4.9A & B, 4.10B & C, 4.11B & C, fig.13). In *P. pumilus*, a glume like bract in proximal position alternating with the prophyll subtends a lateral axis, which develops into a spikelet (fig. 4.6A & J), instead of a flower. In *P. pelophilus, P. polystachyos* and *P. sanguinolentus*, an adaxial swelling body[3] can be seen at the base of the spikelet (figs 4.7G, 4.8H, 4.10L). The spikelet of *P. polystachyos* has a long first internode or epipodium, which is enveloped by the tubular sheath of the spikelet prophyll (fig. 4.8G & H). The developing glumes at the apical part of the spikelet envelop the apex of the rachilla with a bonnet-like mucro (figs 4.6B, 4.8A, 4.10A).

→ Fig. 4.4 *Cyperus capitatus*, SE micrographs of floral ontogeny. A, apical view of the rachilla apex, and two flower primordia (arrowed) at early stages of development; B, differentiating flower primordium with three stamen primordia and a floral apex; C, annular ovary primordium surrounding a central ovule primordium (encircled), and three stamen primordia; D, idem as in 'C', with the ovary wall growing up from the base; E, position of a flower at early developmental stage with respect to the rachilla; F, apical view of developing flower, with the ovary wall enveloping the ovule (two adaxial stigma primordia and an abaxial one appear); G, lateral-adaxial view of developing flower (lateral stamen is removed); H, adaxial view of a developing flower, with one lateral stamen removed. The four (!) stigma primordia are growing out (encircled); I, adaxial view of a developing flower (arrow indicates single style); J, developing gynoecium and a single stamen, with stigma branches protruding high above the stamen (encircled); K, developing stamen before the elongation starts, with apiculus (left upper corner inset) and papillose cells at the bases of the pollen sacs (right hand side inset); L, elongated, withered stamen, with spiralised anther; M, ovule with obturator hairs covering the micropyle (arrowed); N, nutlet, with withered style still present; O, distal part of a culm, in the transition zone between florescence and lateral branches. These are spiro-tristichously positioned and each subtended by a bract, whereas in the terminal spikelet (florescence), the glumes are distichously arranged. This explains the position of the proximal glume-like bracts subtending a rudimentary spikelet; P, middle-apical part of spikelet with two developing flowers (encircled) and the wings of the glumes of the higher, opposite flower (arrows).

Abbreviations: a, anther; f, filament; fa, floral apex; G, glume; nu, nutlet; o, ovule (primordium); ov, ovary wall (primordium); Rl, rachilla; s, stamen primordium; sg, stigma (primordium); st, style; *, rachilla apex.

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[3] This swelling body belongs to the basal part of the prophyl
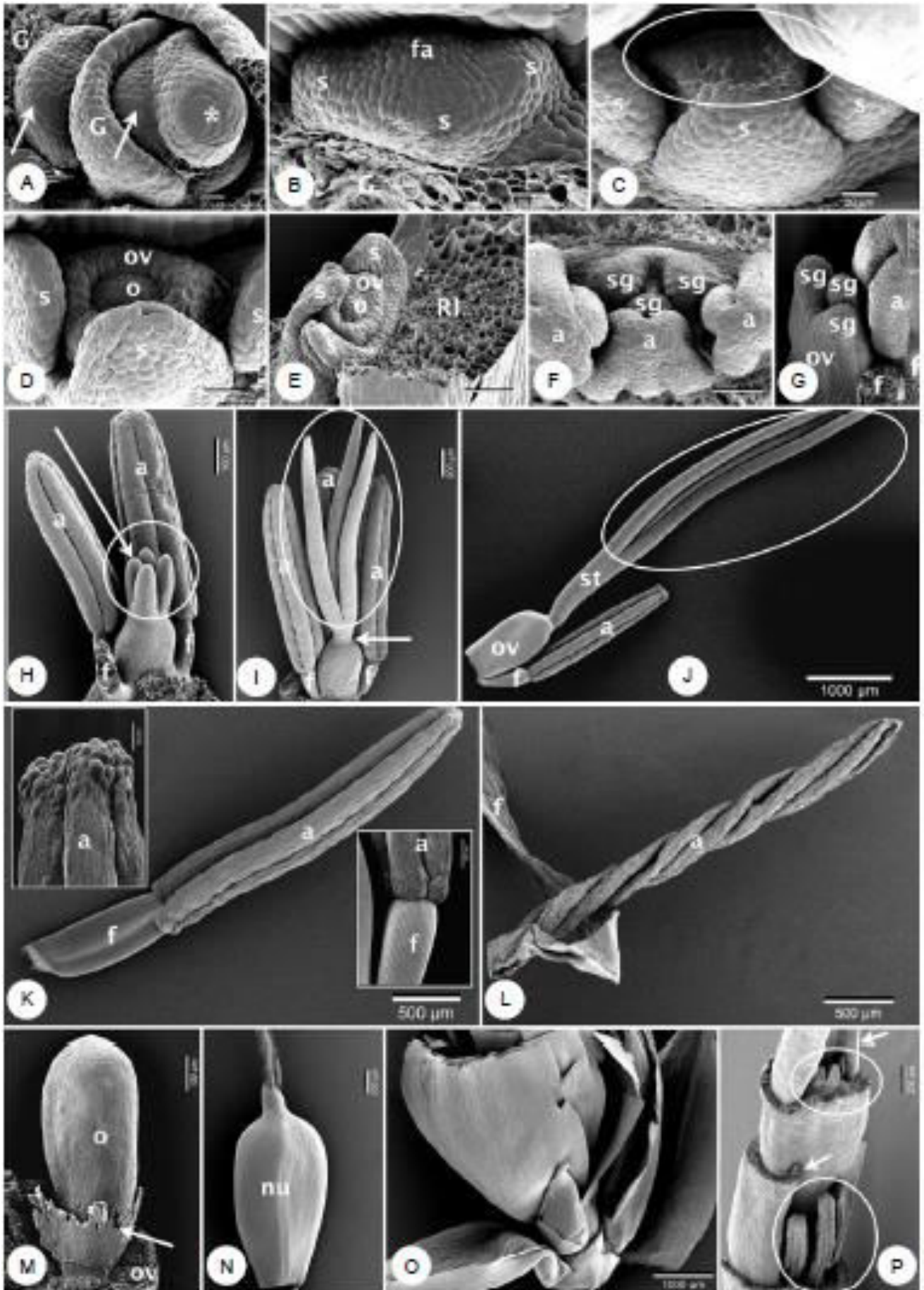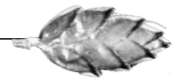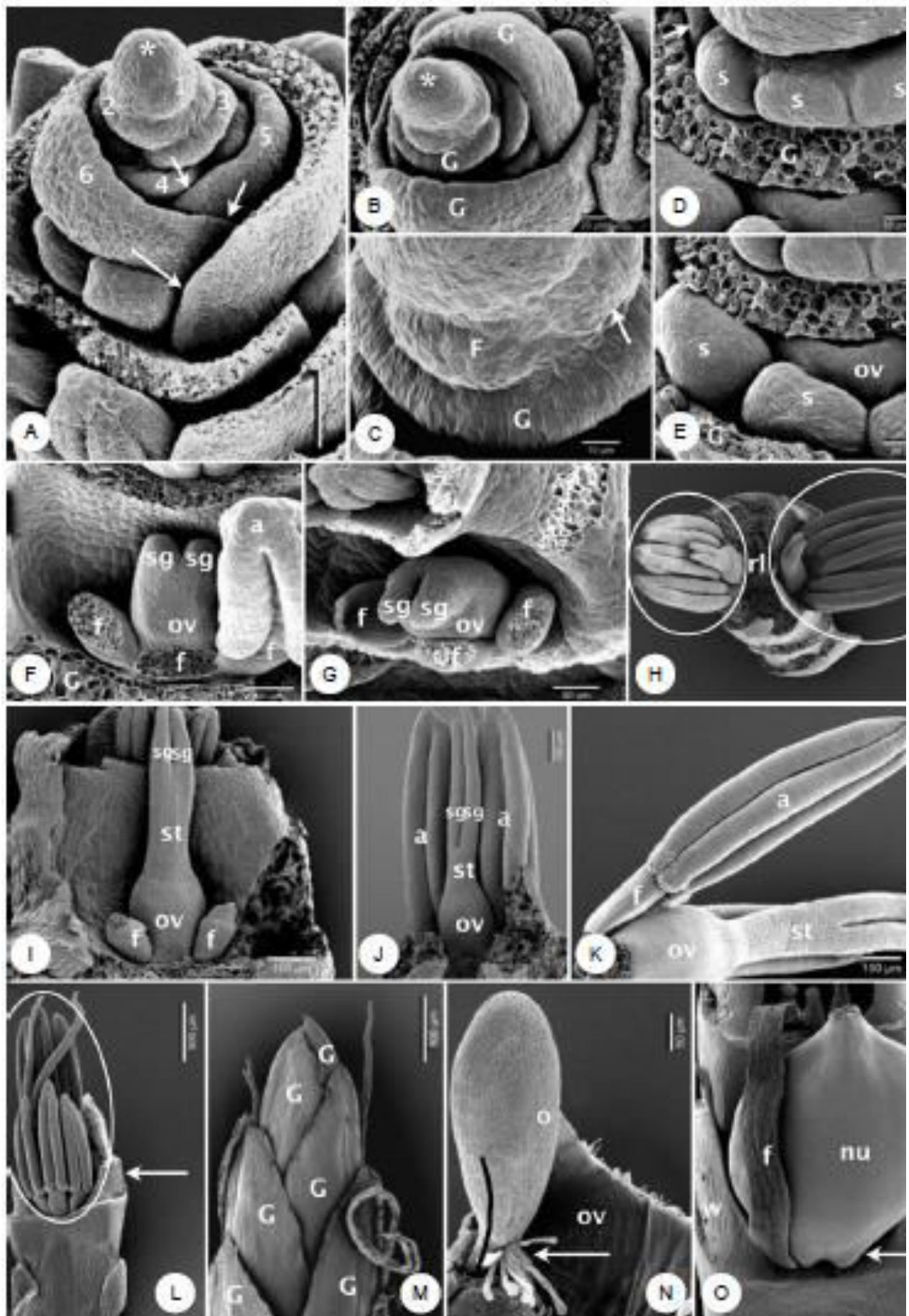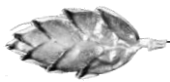
4.5.4 Pycreus: floral ontogeny

Glumes originate below the indeterminate spikelet apex (figs 4.6A & B, 4.8A, 4.9A & B, 4.10A & B, 4.11A & B), forming a 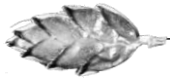rim-like primordium, of which the edges partially envelop the alternate, lower flower primordium (figs 4.6C, 4.6H & J, 4.8B, 4.9B). Soon after the formation of a new glume primordium, a flower primordium appears in its axil (figs 4.6B & C, 4.8B, 4.9B, 4.10B & C, 4.11B). With the glume developing, the flower primordium expands laterally, forming two lateral stamen primordia, followed with some delay by a third abaxial one (*P. flavescens*, *P. sanguinolentus*; figs 4.10C–E, 4.11B & C). In species with flowers with two stamens, no abaxial stamen primordium is formed (*P. bipartitus*, *P. pelophilus, P. polystachyos*; figs 4.8B, 4.9B–D). In flowers with only one stamen, the flower primordium expands laterally, forming only one stamen primordium (*P. pumilus*; fig. 4.6B–D). Simultaneously with the formation of the stamen primordia, a floral apex appears (figs 4.9C, 4.10D). Next, the floral apex differentiates into an annular ovary primordium, surrounding a central ovule primordium (figs 4.6E, 4.7A, 4.9D, 4.10E & F, 4.11C & D). The ovary wall primordium grows up from the base, gradually enveloping the central ovule (figs 4.6F & H, 4.7A & B, 4.9D & E, 4.10F, 4.11E). At this stage, on the top of the ovary wall two dorsiventrally positioned stigma primordia appear (figs 4.6G, I & J, 4.7C, 4.8C, 4.9E, 4.10G, 4.11F).

← Fig. 4.5 *Cyperus laevigatus*, SE micrographs of floral ontogeny. A, lateral view of a spikelet apex with flower subtending glumes at successive stages of development, numbered '1' (youngest) to '6' (oldest). The wings of each glume envelop partially the alternate, lower flower (arrowed); B, apical-abaxial view of spikelet apex with developing glumes; C, detail of very young glume subtending a flower primordium, and a wing of the alternate, higher glume (arrowed); D, differentiating flower primordium with three stamen primordia, and a part of the floral apex; E, developing flower with ovary wall growing up, and three stamen primordia beginning to differentiate; F, apicalabaxial view of a developing flower. Two laterally positioned stigma primordia are growing out on the top of the ovary wall, which envelops the ovule. Filaments and anthers are well developed; G, apical view of developing flower. The two stigma primordia are growing out; H, apical view of a transversally cut spikelet, with two alternating flowers at intermediary developmental stages (encircled); I, abaxial view of a developing flower; J, adaxial view of a developing flower; K, detail of a developing stamen, with apiculus (arrowed); L, abaxial view of a developing flower (encircled) in a tranversely cut spikelet. The subtending glume is removed. The wings of the higher, opposite flower can be seen (arrowed); M, apical part of a spikelet, with several, distichously placed glumes, and protruding style branches; N, lateral view of an ovule, with funiculus (black line) and obturator hairs covering the micropyle (arrowed); O, dorsiventrally flattened nutlet with a hypogynous stalklet or gynophore (arrowed).

Abbreviations: a, anther; F, flower primordium; f, filament; G, glume; nu, nutlet; o, ovule primordium; ov, ovary wall (primordium); Rl, rachilla; s, stamen primordium; sg, stigma (primordium); st, style; *, rachilla apex.
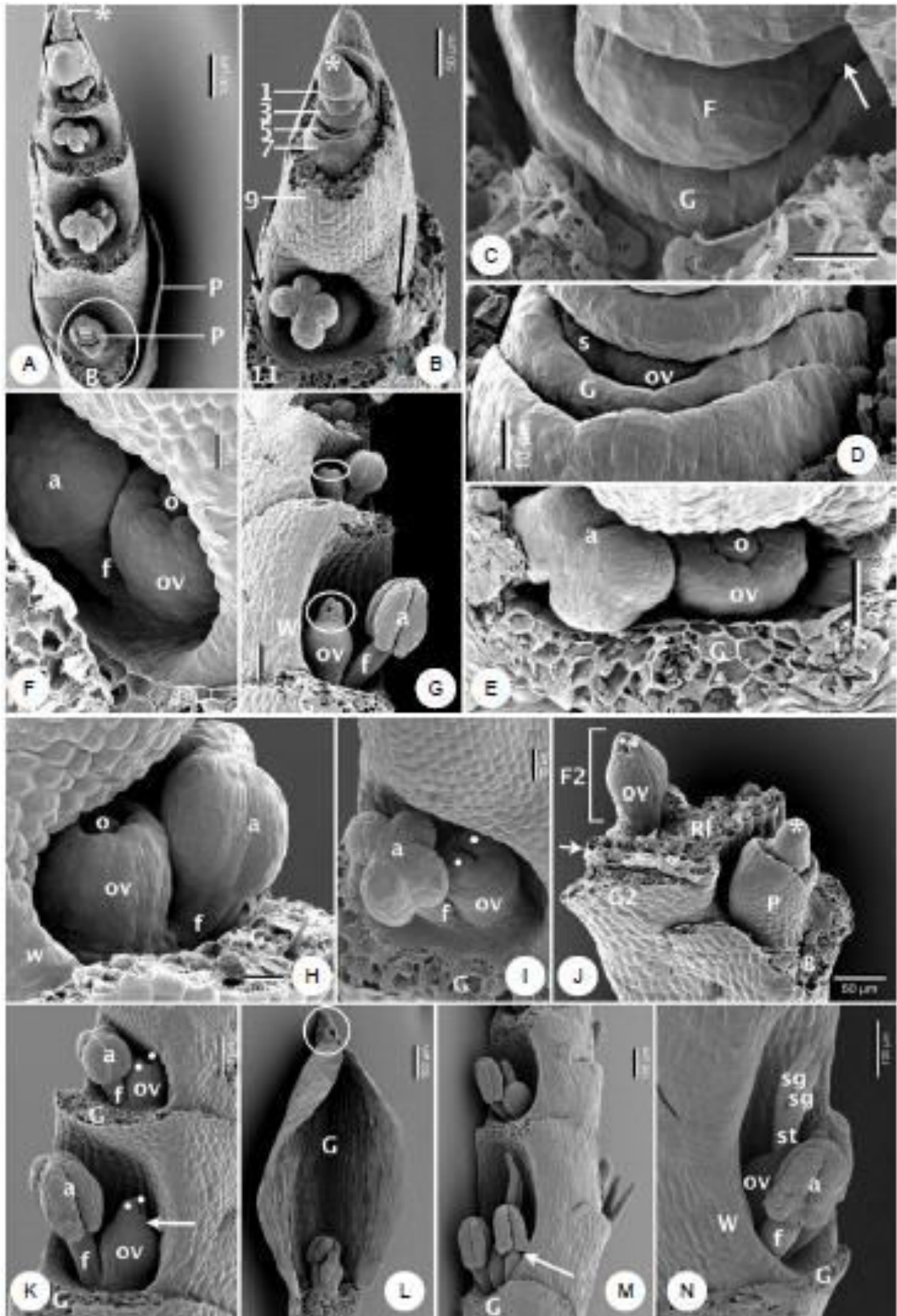
The development of the adaxial stigma primordium is sometimes slightly delayed with respect to the abaxial one (figs 4.9E & F, 4.10G–I, 4.11F–H, 4.12A & B). In *P. bipartitus*, the early adaxial stigma primordium sometimes splits (figs 4.9G & H). Subsequently, the growing ovary wall develops a single style without distinct style base, while the stigma primordia grow out into two papillose stigma branches (figs 4.6G & K, 4.6D–F, 4.7D & E, 4.8G & H, 4.9I, 4.10G & H, 4.11A & B). In *P. sanguinolentus*, at this stage, an annular constriction appears in the apical part of the ovary (fig. 4.10K).

→ Fig. 4.6 *Pycreus pumilus*, SE micrographs of floral ontogeny. A, lateral view of a branched spikelet with proximally a glume-like bract subtending a secondary spikelet (encircled). The prophylls (P) of the main and secondary spikelet are parallel to each other. All visible flowers have a single stamen; B, detail of a spikelet apex with 11 glumes, each subtending a flower (primordium), numbered 1–11 from young '1' to older '11'. In flower 11, the wings (arrowed) of the opposite, higher glume (number 10, only partially visible) form the walls of an alcove-like cavity in which the flower develops; C, detail of a young glume with flower primordium. At the right hand side, the wing of the alternate, superior glume is visible (arrowed); D, differentiating flower with primordia of stamen and ovary wall; E, flower with developing stamen and early gynoecium. The ring primordium of the ovary wall surrounds the central ovule primordium; F, developing flower with ovary wall growing up from the base, and stamen with distinct filament and anther; G, lateral-abaxial view of part of a spikelet with three flowers

→ Fig. 4.9 *Pycreus bipartitus*, SE micrographs of floral ontogeny. A, apical view of a spikelet apex with glumes/flowers at different developmental stages, numbered from young '1' to older '5'. Encircled is a proximal developing flower, with the ovary wall enclosing the ovule, and two developing stamens; B, spikelet apex with very young glume subtending a yet undifferentiated flower primordium. Arrows indicate wings of two superposed glumes at the other side of the spikelet; C, differentiating flower primordium with two lateral stamen primordia and a conspicuous floral apex; D, developing flower with the two stamens removed. The ovary wall is enveloping the central ovule; E, apical view of a developing flower. Two dorsiventrally oriented stigma primordia originate on the top of the ovary wall; F, lateral view of a developing flower. On the top of the anthers, an apiculus appears (arrowed); G, lateral view of a developing flower. A single style appears, with the stigma primordia growing out into stigma branches (encircled). The adaxial stigma primordium is split into two (arrowed); H, detail of stigma primordia with splitted adaxial one (encircled), and apiculus (arrowed); I, developing ovule with the micropyle nearly bent back over 180° (arrowed). The funiculus is indicated with a black line; J, mature flower. Stigma branches are encircled; K, lateral view of mature flower. The gynoecium/nutlet has a hypogynous stalklet or gynophore (arrowed).

Abbreviations: a, anther; F, flower primordium; f, filament; G, glume; o, ovule primordium; ov, ovary wall (primordium); s, stamen primordium; sg, stigma (primordium); st, style; te, outer tegument; ti, inner tegument; *, rachilla apex.
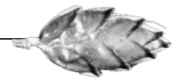
Fig. 4.7 *Pycreus pelophilus*, SE micrographs of floral ontogeny. A, transverse section in the distal part of a spikelet, with two flowers at early developmental stages. In the flower below, two stamen primordia and an annular ovary primordium surrounding a central ovule primordium are visible. In the upper flower, the stamen primordia start differentiating into anther and filament (not visible), and the annular ovary primordium grows up from the base; B, growing ovary wall enclosing the central ovule; C, apical-abaxial view of a developing flower and part of a tranverse section through the distal part of the rachilla. Two dorsiventrally positioned stigma primordia appear on the top of the ovary wall. In between the glume and the flower it subtends, two wings of the alternate, higher flower (removed) partially envelop the flower (arrowed). The wings are fused with the rachilla; D, abaxial view of a developing flower. A single style appears. The anthers are shorter than the filaments; E, lateral view of the middle part of a spikelet with removed glumes. Two developing flowers are visible, each partially envelopped by the wings of the higher, opposite glume (arrowed); F, abaxial view of a semi-mature flower; G, lateral view of the proximal part of a spikelet, with spikelet subtending bract, prophyll of the spikelet, proximal glume and proximal flower (encircled), partially hidden by the wing of the next glume. At the base of the prophyll, a swelling body or pulvinus is visible (arrowed); H, detail of the connective stalklet (arrowed) in between filament and anther.

Abbreviations: a, anther; B, bract; co, connective; f, filament; Fp, proximal flower primordium; G, glume; Gp, proximal glume; o, ovule primordium; ov, ovary wall (primordium); P, prophyll; ps, pollen sac; Ra, rachis; Rl, rachilla; s, stamen primordium; sg or white dot, stigma primordium; st, style; W, wing; *, rachilla apex.
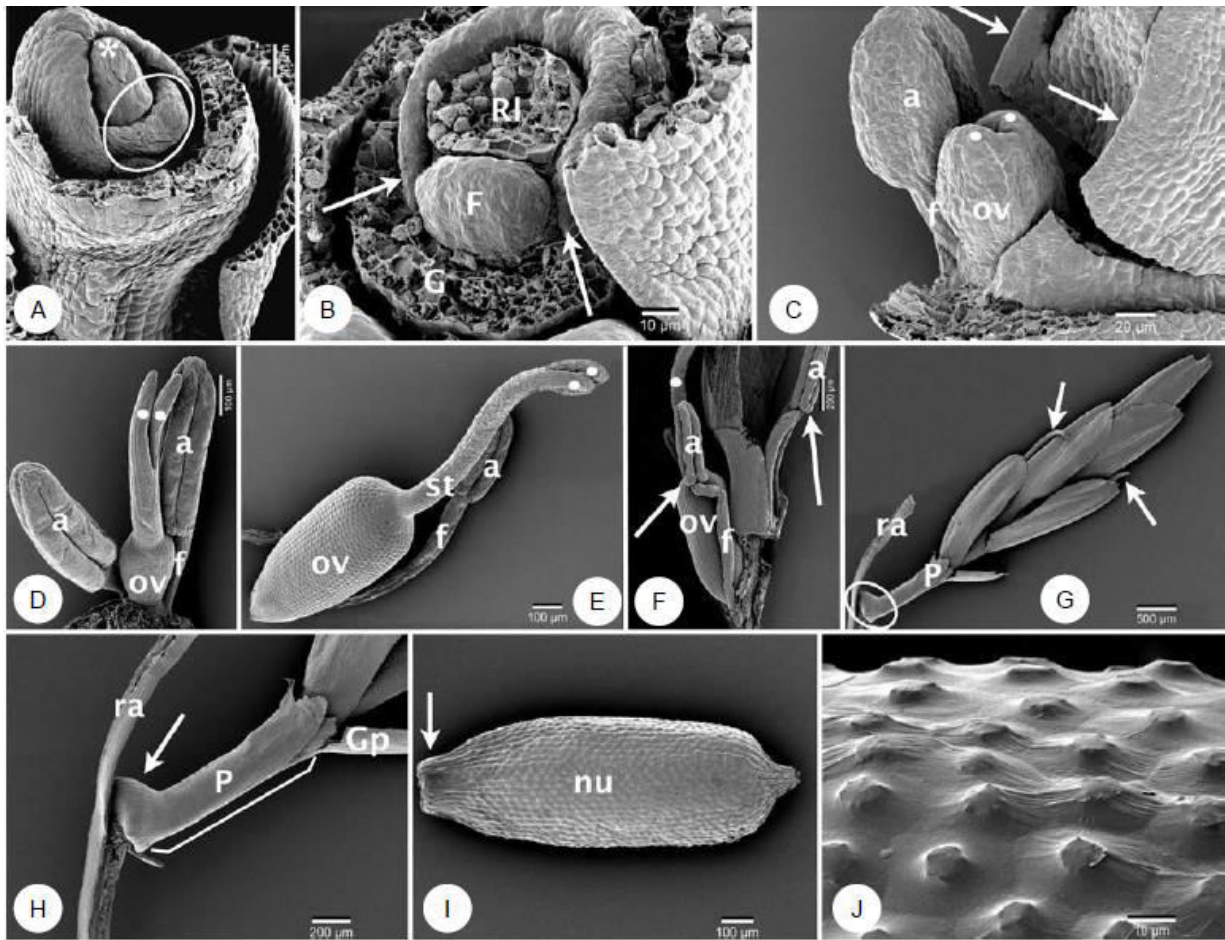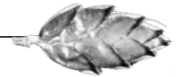
Fig. 4.8 *Pycreus polystachyos*, SE micrographs of floral ontogeny. A, rachilla apex and first glume (encircled); B, transverse section in the apical part of the spikelet, showing a newly formed glume with wings (arrowed) partially enveloping the alternate, lower flower primordium. The glume subtending this flower primordium is removed; C, lateral view of a developing flower. The wings (arrowed) of the alternate, higher flower contribute to its protection. The ovary wall is enveloping the ovule, and two dorsiventrally positioned stigma primordia appear. The two stamen primordia are differentiating into filament and anther; D, developing flower. A single style is formed, and the stigma primordia grow out into stigma branches; E, semi-mature flower. The anther becomes shorter than the filament; F, lateral view of a part of a spikelet with two semi-mature flowers. The cells at the bases of the pollen sacs become papillose; G, entire spikelet, with a long first internode enveloped by a sheath-like prophyll. At the base of the prophyll, a swelling body is present (encircled). Stigma branches protrude above the glumes (arrowed); H, detail of the first internode (white bar) and spikelet prophyll, with a conspicuous swelling body. The spikelet subtending bract is removed. (I) Nutlet with hypogynous stalklet or gynophore; J, detail of the surface of the nutlet, with tabular silica-bodies.

Abbreviations: a, anther; F, flower primordium; f, filament; G, glume; Gp, proximal glume; nu, nutlet; ov, ovary wall (primordium); P, prophyll; Ra, rachis; Rl, rachilla; st, style; white dot, stigma (primordium); *, rachilla apex.

Meanwhile, the stamen primordia have developed into introrse stamens with basifixed anthers with longitudinal slits (figs 4.6G, H, I & K, 4.7C–F, 4.8C–F, 4.9E–G, H & J, 4.10G & H–J, 4.11E & F, 4.12A & B). In semi-mature flowers of *P. flavescens*, and *P. sanguinolentus*, the anthers are as long as or longer than the filaments (figs 4.10J, 4.12A & B), whereas in *P. bipartitus, P. pelophilus, P. polystachyos* and *P. pumilus* the anthers are relatively short with respect to the filaments (figs 4.6K–N, 4.7D–G, 4.8E & F, 4.9J & K). In *P. pelophilus* and *P. pumilus*, a short connective stalklet appears between filament and anther (figs 4.6M, 4.7G & H). In all species studied, the cells at the base of the pollen sacs in developing anthers become more or less papillose (figs 4.6M & N, 4.7H, 4.8F, 4.9G & K, 4.10J, 4.11A & B). An apiculus is absent or remains rudimentary, with the apical cells becoming papillose (e.g. in *P. bipartitus*. fig. 4.9H). Maturing gynoecia and nutlets have a hypogynous stalklet or gynophore (e.g. figs 4.8I, 4.9K, 4.12C & D, 4.13A, C, E & G–I). In *P. polystachyos* and *P. pumilus*, the cells of the nutlet wall each contain a conspicuous tabular silica body (figs 4.8I & J, 4.13A–F), in *P. polystachyos* often with microsatellites around its top. In *P. pelophilus*, similar cells only occur in the center of each lateral side (fig. 4.13C & D). In *P. flavescens*, the epidermal cells of the mature nutlet become longitudinally elongated (zonate cells) pushing up the transverse cell walls, which gives the nutlet its typical wrinkled appearance (fig. 4.12D & E, 4.13H).

→ Fig. 4.9 *Pycreus bipartitus*, SE micrographs of floral ontogeny. A, apical view of a spikelet apex with glumes/flowers at different developmental stages, numbered from young '1' to older '5'. Encircled is a proximal developing flower, with the ovary wall enclosing the ovule, and two developing stamens; B, spikelet apex with very young glume subtending a yet undifferentiated flower primordium. Arrows indicate wings of two superposed glumes at the other side of the spikelet; C, differentiating flower primordium with two lateral stamen primordia and a conspicuous floral apex; D, developing flower with the two stamens removed. The ovary wall is enveloping the central ovule; E, apical view of a developing flower. Two dorsiventrally oriented stigma primordia originate on the top of the ovary wall; F, lateral view of a developing flower. On the top of the anthers, an apiculus appears (arrowed); G, lateral view of a developing flower. A single style appears, with the stigma primordia growing out into stigma branches (encircled). The adaxial stigma primordium is split into two (arrowed); H, detail of stigma primordia with splitted adaxial one (encircled), and apiculus (arrowed); I, developing ovule with the micropyle nearly bent back over 180° (arrowed). The funiculus is indicated with a black line; J, mature flower. Stigma branches are encircled; K, lateral view of mature flower. The gynoecium/nutlet has a hypogynous stalklet or gynophore (arrowed).

Abbreviations: a, anther; F, flower primordium; f, filament; G, glume; o, ovule primordium; ov, ovary wall (primordium); s, stamen primordium; sg, stigma (primordium); st, style; te, outer tegument; ti, inner tegument; *, rachilla apex.

4.5.5 Anatomical data

Cross sections were made at different levels through developing spikelets of *Cyperus laevigatus* (fig. 4.14A–E) and *Pycreus flavescens* (fig. 4.14F–I). Figure 4.14A serves as a key to symbols for 4.14B–I. Cross sections at the basal part of a flower in *C. laevigatus* (fig. 4.14B–D) and *P. flavescens* (fig. 4.14F–H) reveal that glume and rachilla are fused below the level where the filaments are clearly distinguishable. Cross sections at anther level show a separate glume and rachilla (fig. 4.14E & I). In both species, a cross section through the rachilla at internode level is butterfly-shaped (fig. 4.14E & I).
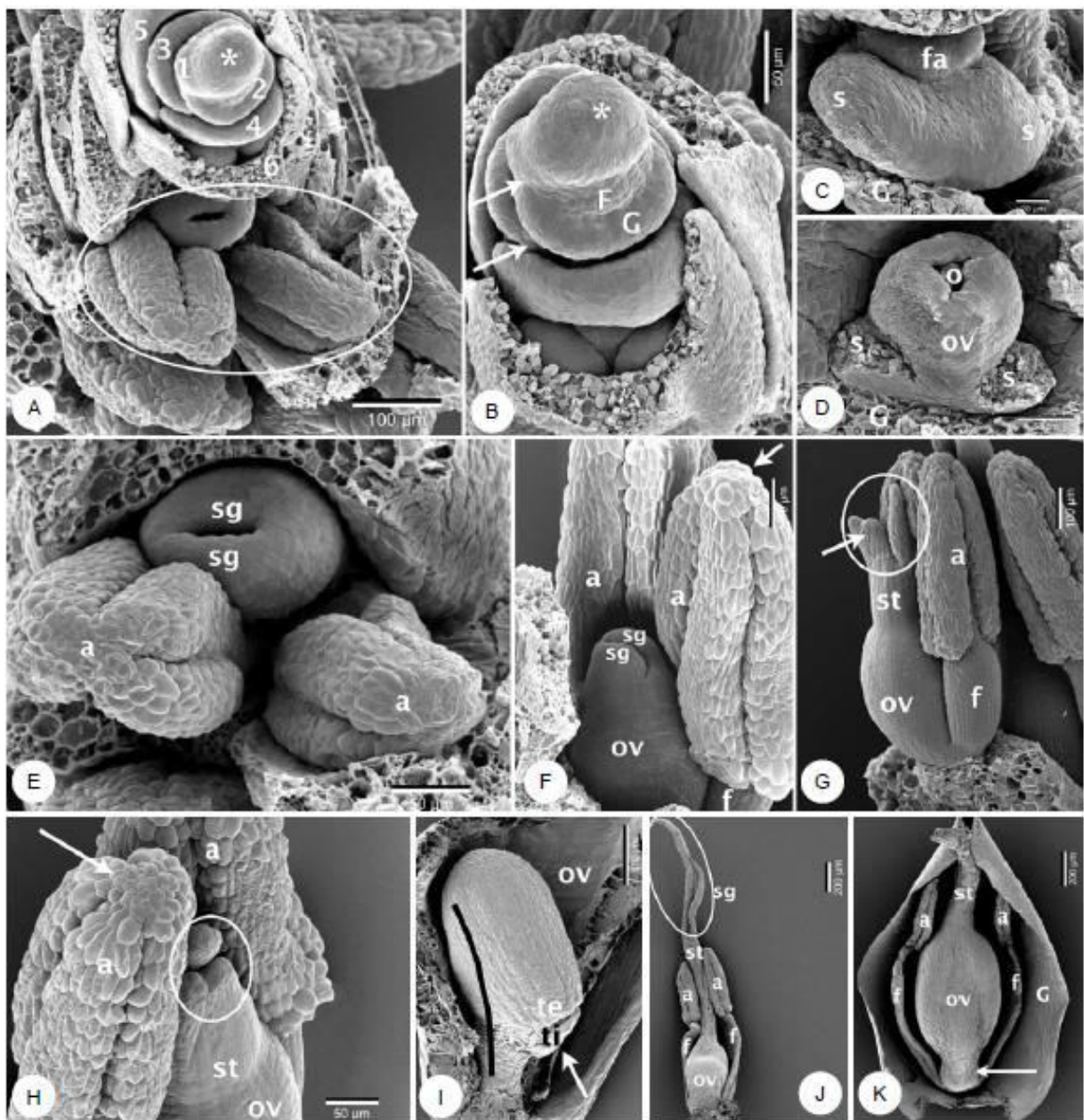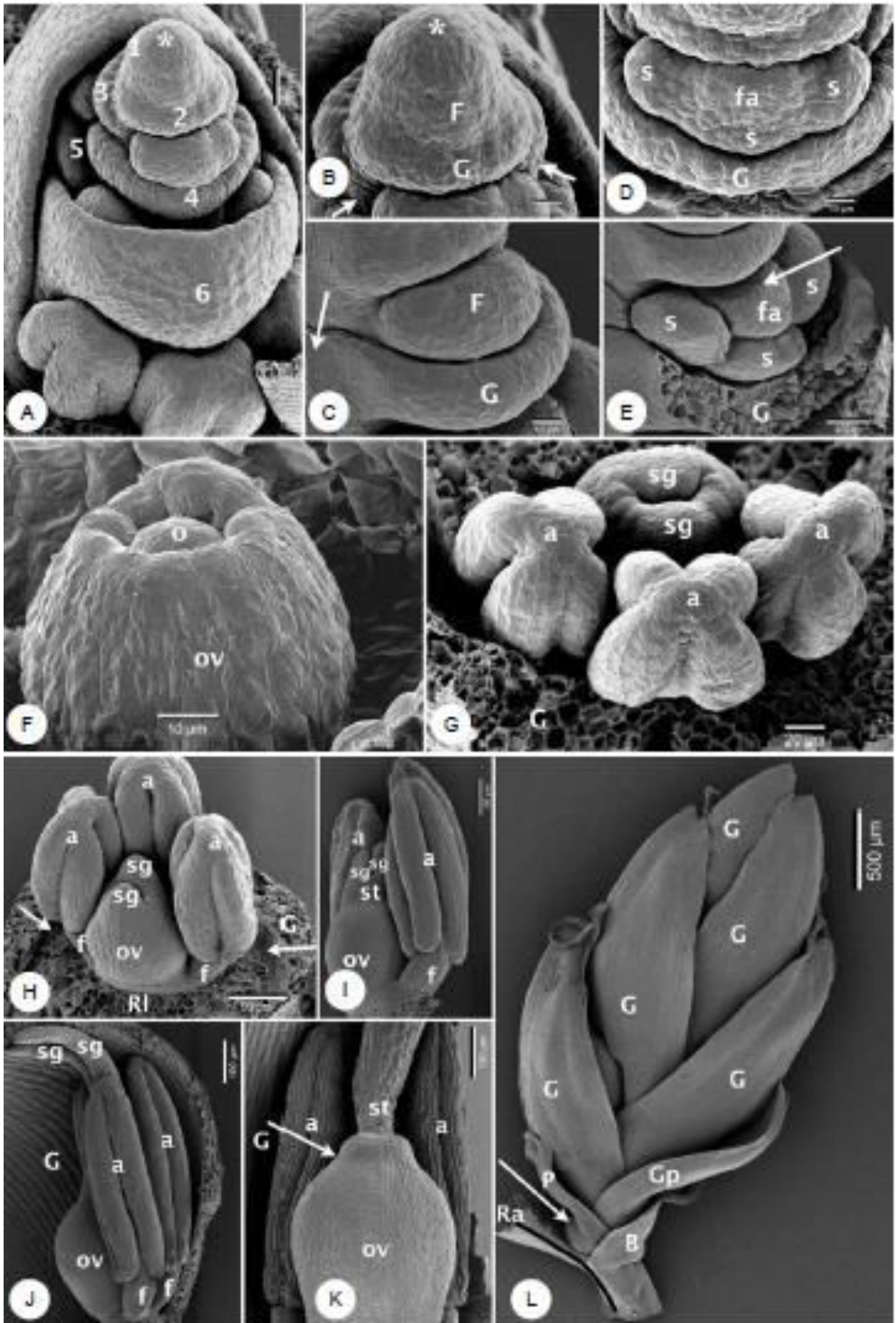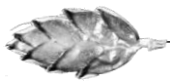
4.6 Discussion

4.6.1 Spikelet development and morphology

In all our observations presented here on *Cyperus* and *Pycreus*, the spikelet consists of an indeterminate rachilla and numerous, acropetally developing glumes, each subtending a single flower. This concurs with our earlier observations in a wide range of cyperoid species (Vrijdaghs *et al.,* 2006a, 2007, 2010). In Cypereae, a lateral spikelet (which is defined as 'ultimate branch' and hence should not have any ramification within it) is not always clearly distinguishable from a branched partial inflorescence; in some species, a secondary spikelet instead of a flower is formed in the axil of a glume (e.g. in *Hellmuthia*; Vrijdaghs *et al.,* 2006b). This was also observed in *Ficinia* (Muasya, unpubl. res.), *Cyperus* (figs 4.3D, 4.4E), and *Pycreus* (fig. 4.6A & J). Therefore, in strict sense, in such cases a glume subtending a secondary spikelet should be called 'glume-like bract', and the rachilla of the main spikelet 'rachis'. In *C. luzulae*, spikelets belong to a serial fascicle of spikelets (fig. 4.3D), in which several spikelets originate in the axil of a common bract, the one above the other, as described by Guarise & Vegetti (2008: fig. 4.9). We also observed similar spikelet clusters in *C. eragrostis* Lam. (both belonging to the section *Luzuloidei*; Denton, 1978). In all C_3 species studied, the position of the spikelet prophyll of rather distally on the rachis positioned spikelets is shifted in comparison

← Fig. 4.10 *Pycreus sanguinolentus*, SE micrographs of floral ontogeny. A, apical part of a spikelet, with glumes/flowers at successive developmental stages, numbered from young '1' to older '6'; B, detail of rachilla apex with a young glume primordium with undifferentiated flower primordium. The wings of the alternate glume reach the underlaying flower (arrowed); C, detail of a glume and flower primordium, which is expanding laterally. The wings of the glume envelop partially the rachilla (arrowed); D, differentiating flower primordium, with two lateral and a slightly delayed abaxial stamen primordium, and a floral apex. E, developing flower. The floral apex is starting to form an annular ovary primordium (arrowed); F, developing ovary, with ovary wall enveloping the central ovule; G, apical view of a developing flower. Two dorsiventrally positioned stigma primordia are visible on the top of the ovary wall; H, apical-adaxial view of a developing flower. The wings (arrowed) of the opposite, higher flower (not in the image) envelop partially the lateral stamens; I, lateral view of a developing flower. A single style appears; J, lateral view of a developing flower. The stigma branches are growing out; K, lateral view of semi-mature flower, one lateral stamen is removed. An annular constriction around the apical part of the ovary is formed (arrowed); L, entire spikelet. At the base of the prophyll, a conspicuous swelling body or pulvinus is visible (arrowed).

Abbreviations: a, anther; B, bract; f, filament; fa, floral apex; G, glume; Gp, proximal glume; o, ovule primordium; ov, ovary wall (primordium); P, prophyll; Ra, rachis; Rl, rachilla; s, stamen primordium; sg, stigma (primordium); st, style; *, rachilla apex.

with the plane determined by the distichous arrangement of the spikelet's other glumes. In C_4 species, such torsion has not been observed. Similar observations are reported by Guarise & Vegetti (2008).

In *C. capitatus*, the culm ends in a terminal spikelet (florescence) in which the glumes are distichously placed. Below the florescence, lateral branches, each subtended by a bract, are spiro-tristichously positioned (fig. 4.4O). This allows us to interpret the proximal glume-like bracts as bracts subtending a lateral spikelet, positioned out of the plane determined by the higher distichously placed glumes of the terminal spikelet. In the transition zone between florescence and the lower part of the culm with lateral branches, primordia in the axil of a bract have a high flexibility to become flower or lateral axis. This flexibility to determine a given, yet undetermined primordium in the axil of a glume(-like bract) also explains the presence of secondary spikelets in spikelets of e.g. *Pycreus pumilus* (fig. 4.6A & J; Vrijdaghs *et al.,* 2010). The glumes in all species studied are winged, with the wings of one glume partially enveloping the opposite, lower flower. In both *Cyperus* and *Pycreus*, the basal part of the glume including (part of) the wings is congenitally fused with the rachilla (figs 4.7C, 4.8B, 4.14) and grows up with it. This is most obvious in *P. pumilus*, whereas in *P. pelophilus* (fig. 4.7) and *P. flavescens* (fig. 4.11), a large part of the wings grows free from the rachilla. Consequently, the main part of the glume and the flower primordium it subtends are epicaulescently displaced to a more apical position (actually, until the next node) on the rachilla. As a result, the rachilla itself is winged along the common growth zone (Vrijdaghs *et al.,* 2010). In *Pycreus*, this epicaulescent metatopic displacement is more pronounced than in species of *Cyperus* or other Cyperoideae, resulting in the typical alcove-like cavities along the rachilla, of which the lateral walls consist mainly of the wings of the opposite, higher glume (fig. 4.14). In *Pycreus*, the glumes often have a prolonged midvein or mucro, which becomes cap-shaped, protecting the rachilla apex (e.g. figs 4.6B, 4.8A, 4.10A). At the adaxial lower part of prophylls of both inflorescence branches and spikelets in *P. pelophilus, P. polystachyos* and *P. sanguinolentus*, an adaxial swelling body can be seen (figs 4.7G, 4.8H, 4.10L). We also observed it in other Cypereae, such as *C. luzulae* (Reynders, unpubl. res.) and *Kyllinga* Rottb. (Huygh, University of Ghent, Belgium, and Vrijdaghs, unpubl. res.). These observations allow confirming Haines' (1967) suggestion that the swelling body or pulvinus is part of the prophyll.

4.6.2 Floral ontogeny and fruit morphology

The floral ontogenetic pattern in *Cyperus* and *Pycreus* is similar to the pattern observed by us in many other Cyperoideae (e.g. Vrijdaghs *et al.,* 2005, 2009). However, there is no formation of perianth primordia, which is a common feature for *Cyperus* s. lat. and Cypereae. However, in the *Ficinia-Isolepis* clade, two southern African species previously named as *Scirpus* (*S. falsus* and *S. ficinioides*) were added, as well as the formerly mapanioid *Hellmuthia.* These three species are the only recorded Cypereae with remnants of a perianth (Simpson *et al.,* 2003, Vrijdaghs *et al.,* 2006, Muasya *et al.,* 2009a, 2009b).

In *Pycreus*, the number of stamens is highly variable, with basic number three as in most other Cyperoideae (e.g. figs 4.10D, E, G & H, 4.11C, E & F). Kükenthal (1936) reported that nearly half of the 72 species he recognized in *Pycreus* have a constant number of two stamens instead of three. In these cases it is the abaxial stamen that does not develop (e.g. figs 4.7, 4.9A–G). In some species, the number of stamens can also vary within the species, and even within a single plant (e.g. *P. pumilus*, fig. 4.6M). We observed a tendency to delay the formation of the abaxial stamen or to reduce it completely in various other cyperoid genera, such as *Eriophorum*, *Scirpoides* (Vrijdaghs *et al.,* 2005a), *Fuirena* (Vrijdaghs *et al.,* 2004), *Ficinia* and *Isolepis* (Vrijdaghs *et al.,* 2005b). From from an annular ovary primordium. We believe that the organizational freedom resulting from the congenital fusion of the carpels into an annular ovary primordium made laterally flattened nutlets like in *Pycreus*, as well as dorsiventrally flattened nutlets like in *C. laevigatus*, possible.

In *P. bipartitus*, at early developmental stages, two adaxial stigma branches can occur (fig. 4.9G). Haines & Lye (1983) also reported the presence of three stigma branches in some specimens of *P. nigricans*. It is tempting to interpret these observations as an argument to state that the adaxial stigma branch in *Pycreus* resulted from the fusion of the two ancestral lateral ones. However, how to explain the presence of four stigma branches in *C. capitatus* (fig. 4.4H)? Therefore, we consider these particular structures rather as developmental accidents; the meristematic zones from which the stigma branches originate (we call them stigma primordia because they are not carpel tips, though we do not exclude that they are homologous with carpel tips) can be splitted (dédoublement). In *P. flavescens*, the development of the adaxial stigma branch at early developmental stages is slightly delayed with respect to the abaxial one (figs 4.11, 4.12A). This too might be explained by a temporary lack of space. In *P. sanguinolentus*, an apical constriction of the ovary appears at semi-mature
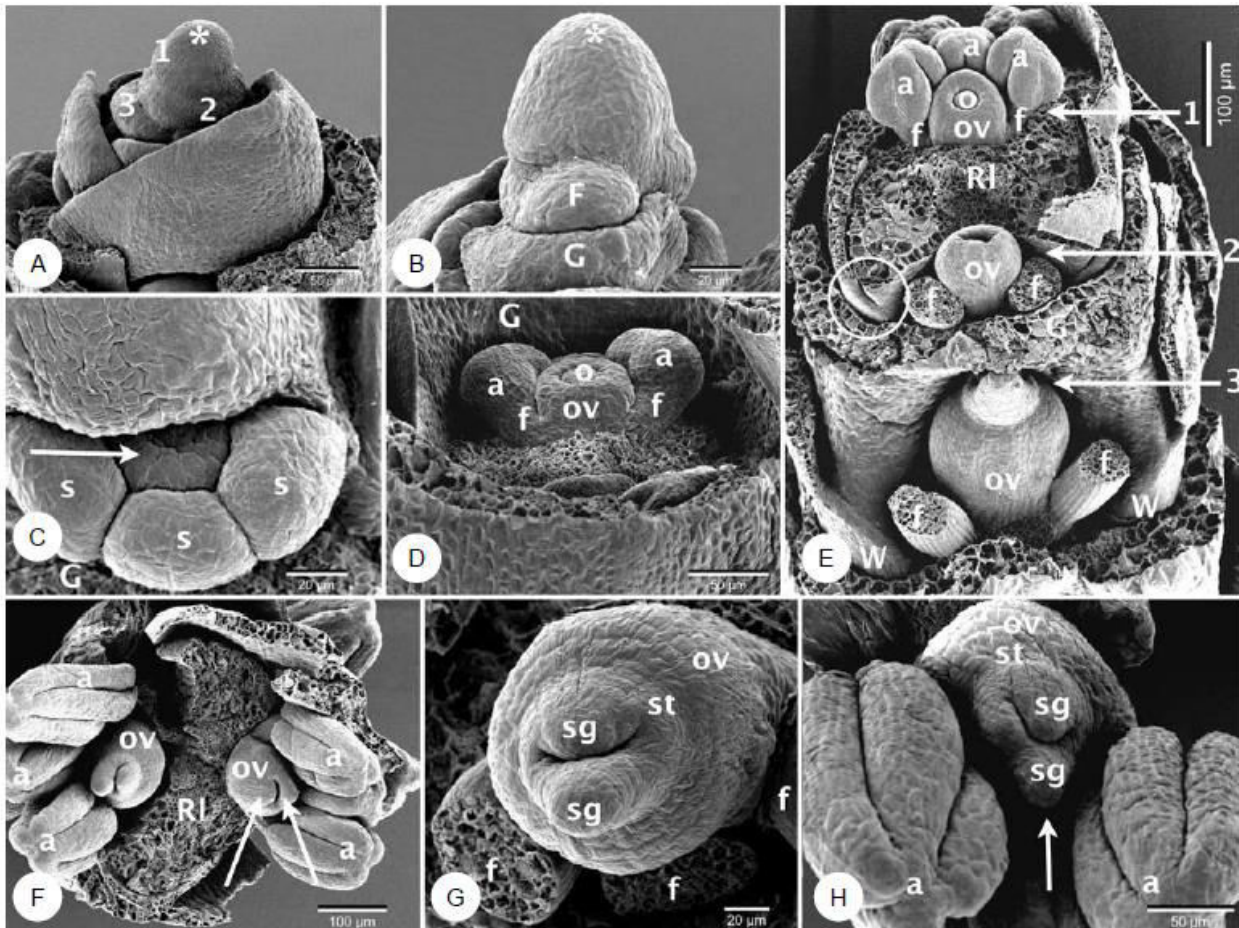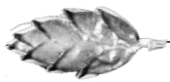
Fig. 4.11 *Pycreus flavescens*, SE micrographs of floral ontogeny. A, lateral view of a spikelet apex, with glumes/flowers at successive developmental stages numbered from young to older 1–3; B, rachilla apex with young glume subtending a flower primordium; C–D, differentiating flower primordium with one abaxial and two adaxial stamen primordia, and with the floral apex differentiating into an annular ovary primordium (arrowed) surrounding a central ovule primordium. In 'D', the stamen primordia start differentiating into filament and anther; E, transverse section through the rachilla, with three flowers (1, youngest; 3, oldest) at different developmental stages. Flower '1' is shown from an adaxial viewpoint, flowers '2' and '3' from an abaxial viewpoint, each with removed stamens. In flower '2', the adhesion of the wings of the subtending glume of flower '1' to the rachilla can be seen (encircled); F, apical view of a transverse section through the rachilla, with two flowers. The right hand one is less developed, with two dorsiventrally stigma primordia appearing on the top of the ovary wall (arrowed); G–H, apical view of a developing gyncoecium. A single style appears, and the stigma primordia grow out, the adaxial one (arrowed) delayed with respect to the abaxial stigma primordium.

Abbreviations: a, anther; F, flower primordium; f, filament; G, glume; o, ovule primordium; ov, ovary wall (primordium); s, stamen primordium; sg, stigma (primordium); W, wing; *, rachilla apex.
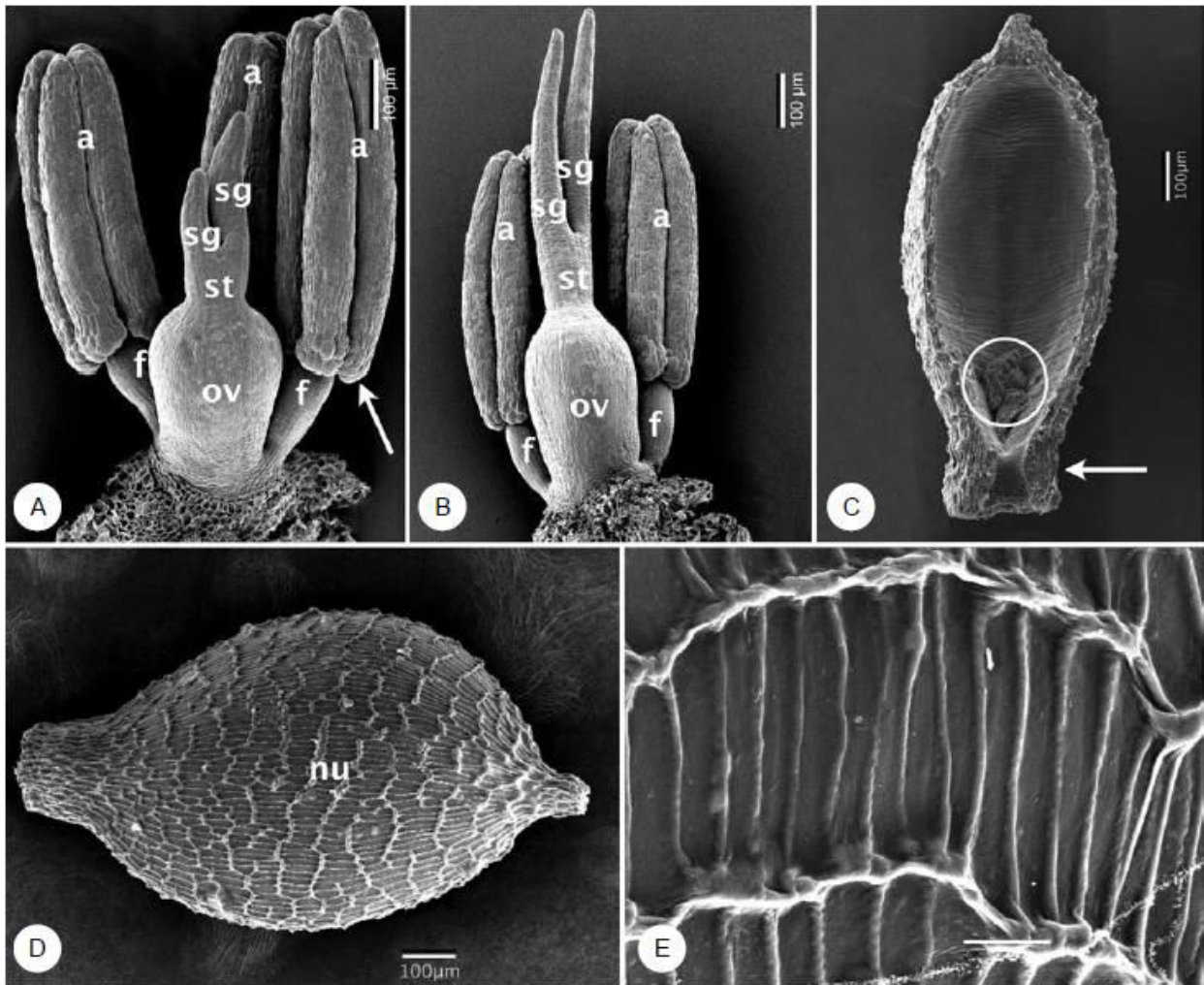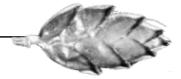
Fig. 4.12 *Pycreus flavescens*, SE micrographs of floral ontogeny. A–B, lateral-adaxial view of developing flower. In 'B', the delay of the development of the adaxial stigma branch diminishes; C, longitudinal section of a fruit wall with rests of the obturator hairs, and a hypogynous stalklet or gynophore (arrowed); D, nutlet; E, detail of the fruit wall. Abbreviations: a, anther; f, filament; nu, nutlet; ov, ovary wall; sg, stigma (primordium); st, style.

stage. We observed a similar phenomenon in *Fuirena abnormalis* C.B.Clarke (Vrijdaghs *et al.,* 2004). In maturing flowers of several *Cyperus* and *Pycreus* species, a rudimentary hypogynous stalklet or gynophore appears (e.g. figs 4.3E these observations, we may deduce that the reduction of the abaxial stamen occurred independently in different Cyperoid clades. In all cases, this tendency can probably be explained by a limited spacial freedom to develop the three stamens. *Pycreus pumilus*, with its highly compacted spikelets and flowers with usually one, sometimes two stamens, clearly illustrates this. Moreover, in stamens of flowers of *P. pelophilus* and *P. pumilus*, a 'connective stalklet' appears in semi-mature stamens. Similar observations were made in other Cypereae (e.g. *Kyllinga* and *Oxycaryum*; Vrijdaghs, unpubl. res.). We admit that this 'connective stalklet' acts as an articulation allowing the anther to bend over for better pollen dispersal by the wind.
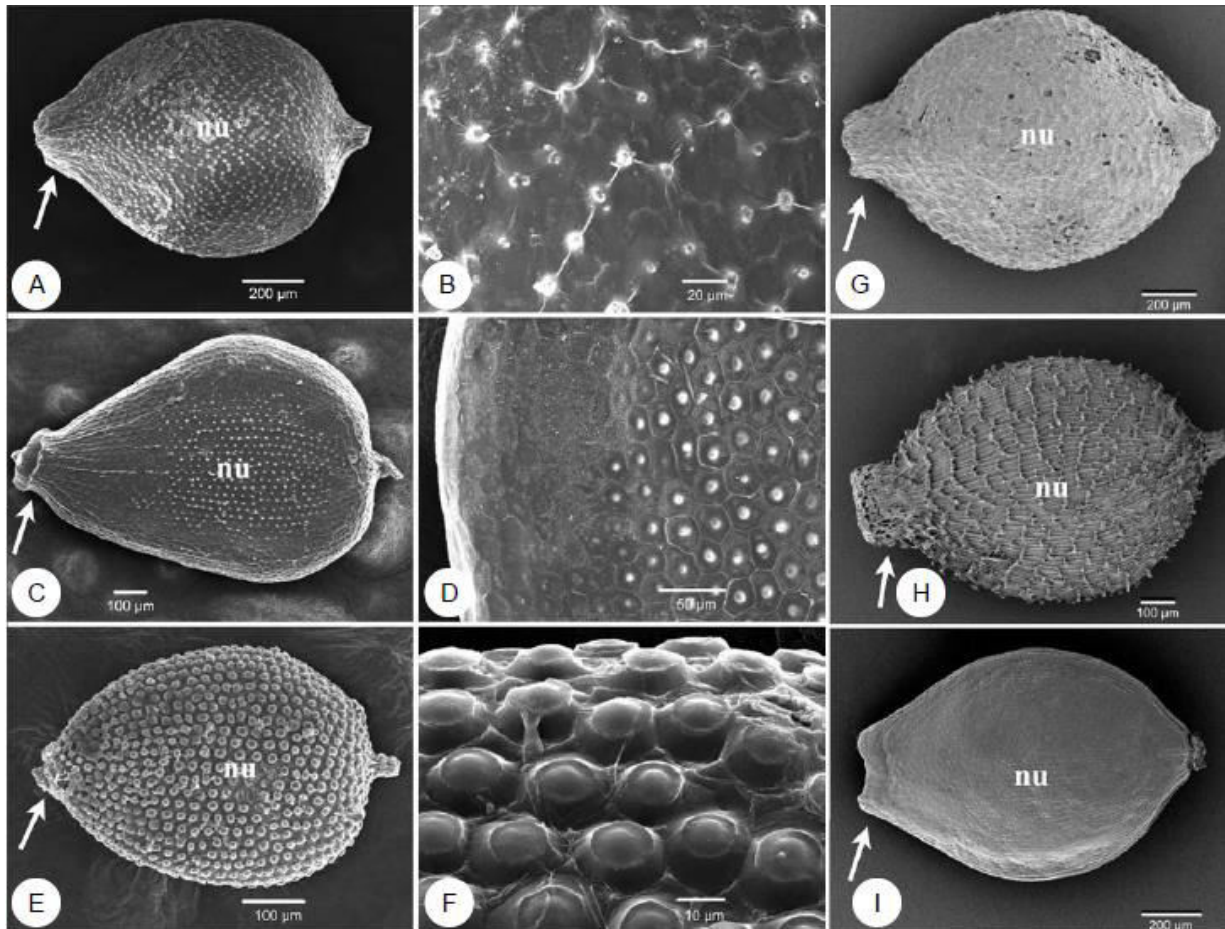
Fig. 4.13 SE micrographs of mature nutlets in *Pycreus* (A–H) and *Cyperus* (I). A, *P. bipartitus*, lateral view of a nutlet with a gynophore (arrowed); B, *P. bipartitus*. Detail of the fruit wall epidermis with cells with small conical silica bodies; C, *P. pelophilus*, lateral view of a nutlet with a gynophore (arrowed); D, *P. pelophilus.* Detail of the fruit wall epidermis with cells with each a tabular silica body; E, *P. pumilus*, lateral view of a nutlet with a gynophore (arrowed); F, *P. pumilus.* Detail of the fruit wall epidermis with small cells, each filled with a tabular silica body; G, *P. sanguinolentus*, lateral view of a nutlet with a hypogynous stalklet or gynophore (arrowed); H, *P. flavescens*, lateral view of a nutlet with a gynophore (arrowed). The epidermis consists of zonate (longitudinally elongated) cells; I, *Cyperus laevigatus*. Dorsiventral view of a nutlet with a gynophore (arrowed). Abbreviation: nu, nutlet.

As in all other Cyperoideae studied by us, the gynoecium in the species of *Cyperus* and *Pycreus* presented here are formed from an annular ovary primordium surrounding a central ovule primordium. Since the ovary wall in Cyperoideae is not resulting from a postgenital fusion of three distinct carpel primordia but growing up from an annular ovary primordium, new possibilities arise in organizing the vascularisation of the gynoecium and consequently also for its morphology, such as the positions and number of the stigmas. In *Pycreus*, only two stigma branches are formed, positioned dorsiventrally, which results in laterally flattened

gynoecia/nutlets (figs 4.6–11). Similar pistils also occur in *Kyllinga* and *Queenslandiella*. However, molecular phylogenetic data (Muasya *et al.,* 2009a) show that these genera form different clades within *Cyperus* s. lat., which suggests that this feature evolved independently in each of the three genera characterized by it. Also in *C. laevigatus*, only two, though laterally positioned stigma primordia appear, resulting in a dorsiventrally flattened pistil/nutlet (fig. 4.5F–H). Goetghebeur (1986) suggested that such a pistil, which also occurs in other *Cyperus* species and other cyperoid genera such as *Blysmus*, *Dulichium*, *Eleocharis* and *Fimbristylis*, results from the reduction of the abaxial carpel and a fusion of the two remaining adaxial carpels. However, each attempt to explain the *Pycreus* type pistil using the carpel concept fails. Moreover, in strict sense, carpels are not present in cyperoid Cyperaceae since the ovary originates from an annular ovary primordium. We believe that the organizational freedom resulting from the congenital fusion of the carpels into an annular ovary primordium made laterally flattened nutlets like in *Pycreus*, as well as dorsiventrally flattened nutlets like in *C. laevigatus*, possible.

In *P. bipartitus*, at early developmental stages, two adaxial stigma branches can occur (fig. 8G). Haines & Lye (1983) also reported the presence of three stigma branches in some specimens of *P. nigricans*. It is tempting to interpret these observations as an argument to state that the adaxial stigma branch in *Pycreus* resulted from the fusion of the two ancestral lateral ones. However, how to explain the presence of four stigma branches in *C. capitatus* (fig. 3H)? Therefore, we consider these particular structures rather as developmental accidents; the meristematic zones from which the stigma branches originate (we call them stigma primordia because they are not carpel tips, though we do not exclude that they are homologous with carpel tips) can be splitted (dédoublement).

In *P. flavescens*, the development of the adaxial stigma branch at early developmental stages is slightly delayed with respect to the abaxial one (figs 10, 11A). This too might be explained by a temporary lack of space. In *P. sanguinolentus*, an apical constriction of the ovary appears at semi-mature stage. We observed a similar phenomenon in *Fuirena abnormalis* C.B.Clarke (Vrijdaghs et al. 2004). In maturing flowers of several *Cyperus* and *Pycreus* species, a rudimentary hypogynous stalklet or gynophore appears (e.g. figs 2E& L, 4.5O, 4.8I, 4.9K). This also occurs in other genera in Cypereae, such as *Ficinia*, *Isolepis*, and *Scirpoides* (Vrijdaghs *et al.,* 2005a, 2006b).

Fruit wall epiderm cells in *Pycreus pelophilus*, *P. pumilus* and *P. polystachyos* have (at least partially) similar, tabular silica-bodies (fig. 4.13C–F). The fruit wall epiderm cells in *P. flavescens* are zonate and do not have silica-bodies (fig. 4.12D & E, 4.13H). *Pycreus bipartitus* has fruit walls with isodiametric epiderm cells with small conical silica bodies (fig. 4.13A & B), which is also reported in *P. sanguinolentus*, though we did not observe this in nutlets from herbarium specimens (fig. 4.13G). According to Metcalfe (1971), only the few neither conical nor tabular silica-bodies found in some species might have systematic value.
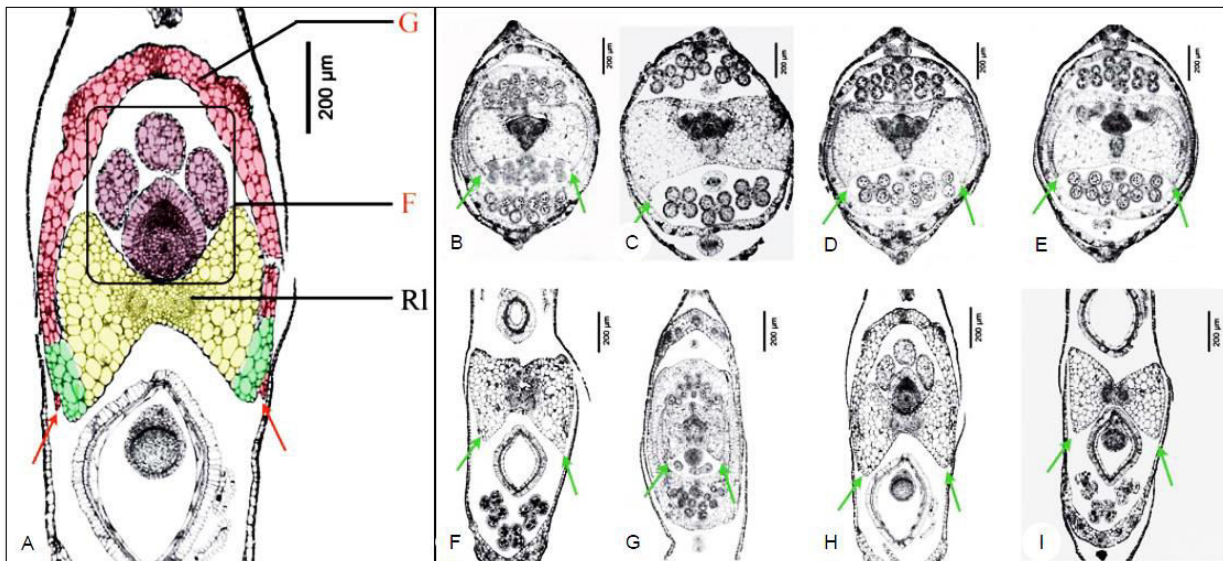
Fig. 4.14 A, Key for B–I; LM image of a cross section through a spikelet of *Pycreus flavescens*, at the height of the filaments. The green coloured areas are the fusion zones between rachilla and wings of a glume. The section of this glume and the flower in its axil (three filaments and centrally the gynoecium) are coloured in red. The wing tips are also coloured in red, and arrowed. The rachilla is coloured in yellow. In each section shown in figure B–I a similar glume with the flower it subtends can be observed, as well as a fusion zone of wings and rachilla; B–I, LM images of cross sections through the spikelet at different levels in *Cyperus laevigatus* (B–E) and *Pycreus flavescens* (F–I); B & F, cross sections through the basal level of a glume and its flower. In *P. flavescens*, the fusion of glume and rachilla (green arrow) is less complete than in *C. laevigatus*; C,D, G & H, cross sections at filament level; E & I, cross sections at anther level (or internode). Here, the glume is free from the rachilla. Abbreviations: F, flower; G, glume; Rl, rachilla.
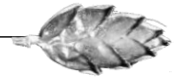
4.7 Conclusions

The spikelet ontogeny and morphology in the *Cyperus* and *Pycreus* species studied concurs with our observations in many other Cyperoideae that cyperoid spikelets consist of an indeterminate rachilla and many glumes which usually subtend (or not) a bisexual flower (Cariceae and sclerioid Cyperaceae not included). However, in Cypereae, proximal bracts of the spikelet may axillate a secondary spikelet. We consider this phenomenon to be a result of the flexibility plants have to activate different developmental patterns (to become a flower, a spikelet or a vegetative axis[4]) in yet undetermined primordia. Spikelets in *Cyperus* s. lat. have a typical zigzagging morphology, resulting from a congenital fusion of the rachilla and the wings of the glumes, which causes epicaulescent growth of the glumes with the rachilla. The particular morphology of a spikelet in *Pycreus* results from a pronounced epicaulescent growth of the glumes with the rachilla. The floral ontogeny in all species studied occurs according to the general Cyperoid floral ontogenetic pattern, though no perianth primordia are formed. The pistil, as it originates from an annular primordium, gets more organisational freedom, which is illustrated by the two dorsiventrally positioned stigma branches in *Pycreus*, as well as the two laterally positioned stigma branches in species with dorsiventrally flattened nutlets, such as *C. laevigatus*. Only on condition that in cladistic analysis *Pycreus* would appear as a monophyletic taxon, we think that the combination of 1) its particular spikelet ontogeny resulting in a '*Pycreus*-type' spikelet, 2) the laterally flattened ovaries/ nutlets which originated independently in the evolution from other taxa with similar ovaries, are strong arguments to consider this taxon to be a genus on its own. However, we also realise that this would make *Cyperus* paraphyletic.

4.8 Acknowledgements

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[4] c.f. Pseudovivipary found in e.g. *Eleocharis*
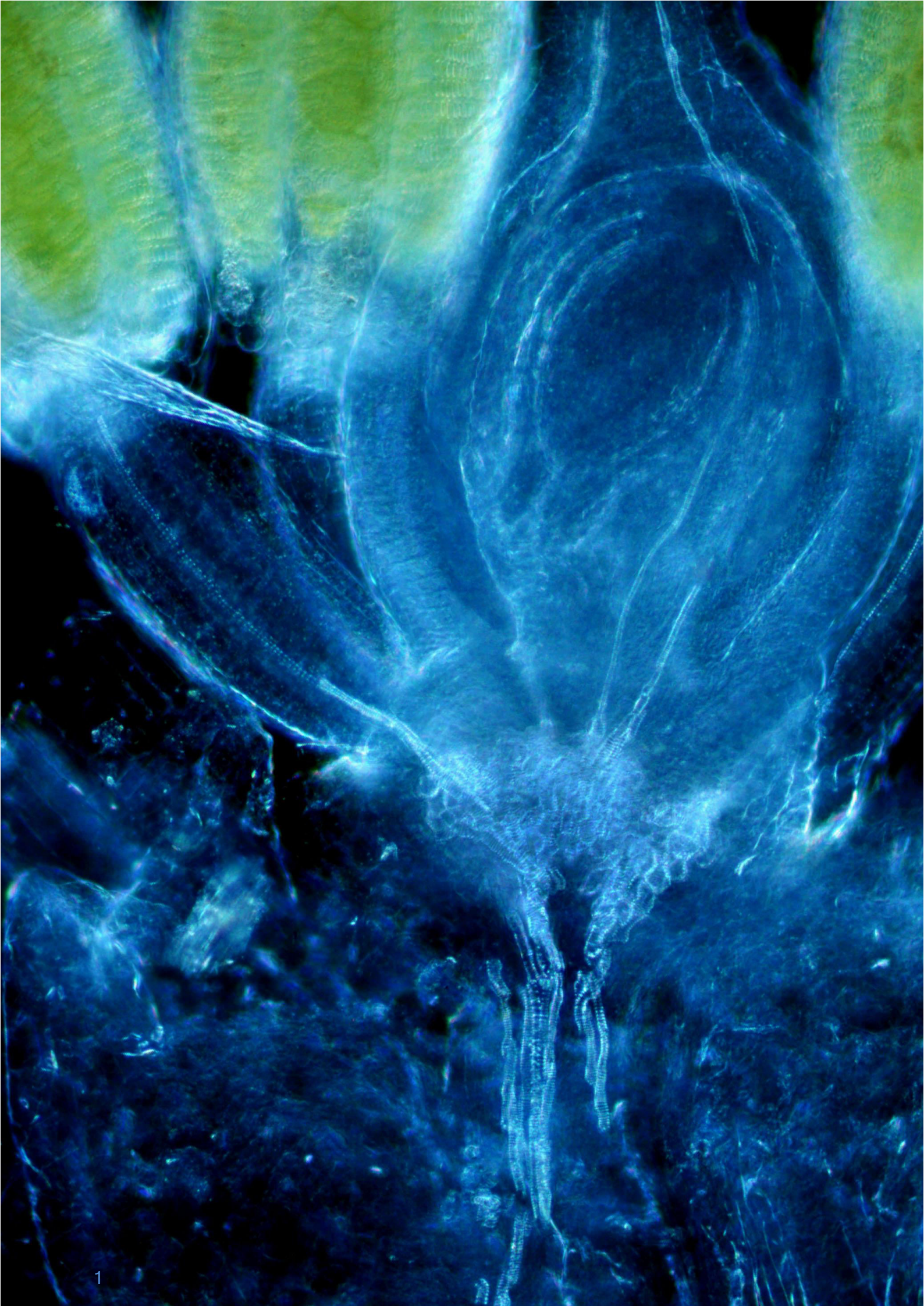
5 Pistil evolution

"The most exciting phrase to hear in science, the one that heralds the most discoveries, is not "Eureka!" (I found it!) but 'That's funny...'"

— Isaac Asimov (1920-1992)

← Fig. 5.1 Dark field microscopy picture of the vasculature within the base of a flower of *Pycreus sanguinolentus.*

Spikelet theme: *Pycreus bipartitus*

5.1 In this chapter…

In chapter 5 pistil evolution with a focus on the origin of derived pistils in Cyperoideae is reviewed with presentation of additional data from ontogeny and anatomy. The results presented have been published as the following article:

1. Reynders, M. & Vrijdaghs, A., Larridon, I., Huygh, W., Leroux, O., Muasya, AM., Goetghebeur, P. (2012). Gynoecial anatomy and development in Cyperoideae (Cyperaceae, Poales): congenital fusion of carpels facilitates evolutionary modifications in pistil structure. *Plant Ecology and Evolution* 145(1): 1-30.

Remark: The circumscription for the gynoecium development used in the article Is somewhat outdated. Here follows a more correct phasing: In Cyperoideae, the gynoecium originates from an annular gynoecium wall primordium, (previously described as ovary wall primordium, surrounding a central, floral-apical meristematic zone (previously described as 'ovule primordium') from which later on a single ovule originates. At early developmental stage, r-the raising, bag-like gynoecium wall surrounds the developing ovule, thus forming an immature open ovary. With the gynoecium wall further raising and forming a single style, the initially open ovary is gradually closed.

5.2 Abstract

In Cyperaceae, the single ovuled, usually triangular gynoecia are widely considered to have a basic number of three carpels, often reduced to two, resulting in dimerous pistils. However, laterally flattened dimerous pistils cannot be explained by any existing carpel reduction theories, because a single stigma in median position replaces the two adaxial stigmata. This paper presents a comparative study of the ontogenetic and anatomical adaptations facilitating the origin of new pistil forms in Cyperoideae, focusing on modified gynoecia. It includes a reevaluation of Blaser's (1941) anatomical studies in Cyperaceae. We aim to test Blaser's hypothesis that is based on an acropetal developmental model of the floral vasculature and the general Cyperoid ontogenetic model of Vrijdaghs *et al.,* (2009), which states that cyperoid ovaries originate from an annular primordium. SEM, dark field and phase contrast microscopy were used to study the development of flowers ans vascular

bundles in Cyperoideae. All cyperoid pistils studied develop according to a cyperoid floral ontogenetic pattern, in which carpel primordia are congenitally fused. In *Pycreus sanguinolentus* (and other species), separate procambial initiation zones were observed in both the flower receptacle and separate floral primordia, which connect (or not) at later developmental stages. The presence of an annular ovary primordium instead of individual carpel primordia, combined with the bidirectional development of the pistil vasculature liberate the developing gynoecium from the structural constraints proper to a typical carpellate organisation. Procambial initiation zones in the receptacular vascular plexus and in individual floral primordia constitute the basis for the formation of a flexible vascular system in cyperoid flowers. Moreover the development of the ovary and ovule are decoupled. Consequently, in Cyperoideae the acquired developmental freedom of the pistil resulted in various adaptations.

5.3 Introduction

5.3.1 Cyperoideae and their flowers

Cyperaceae, the third largest family in monocots, are characterised by their small, easily dispersible nutlets with a single ovule, which greatly contributed to their worldwide success. Whereas previously four subfamilies were considered (Muasya *et al.,* 1998, Simpson *et al.,* 2007), currently two main clades have been recognised as the only subfamilies of Cyperaceae, namely Cyperoideae and Mapanioideae (fig. 5.2). At tribal and generic levels, the classification of Goetghebeur (1998) is still in use (Govaerts *et al,.* 2007).

In Mapanioideae, the reproduction units (flowers or synanthia?) are enveloped by two lateral scales (e.g. Kern, 1974). The reproductive units are controversial because of the presence of scales in between a terminal gynoecium and the more proximally situated stamens. Until now, only one complete floral ontogeny has been done, on *Exocarya* Benth. (Richards *et al.,* 2006), unfortunately without much attention to the development of the gynoecium. From our current knowledge it becomes more and more clear that the whole floral organisation in mapanioids is fundamentally different from the one in cyperoids (Richards *et al.,* 2006, Simpson *et al.,* 2003, 2007, Vrijdaghs 2006). Therefore a thorough comparative study in Cyperoideae stands on its own. In Cyperoideae, flowers are typically monocotyledonous (e.g. Rudall & Bateman 2004) with a trimerous perianth, a trimerous androecium and a trimerous, superior gynoecium, or they are considered to be derived from

this pattern (e.g. Goetghebeur 1998, Vrijdaghs *et al.,* 2009). A cyperoid flower usually originates in the axil of a subtending bract, called glume, with the glumes and their flowers being organized in spikelets (e.g. Haines & Lye, 1983, Goetghebeur, 1998, Vrijdaghs *et al.,* 2009, 2010).
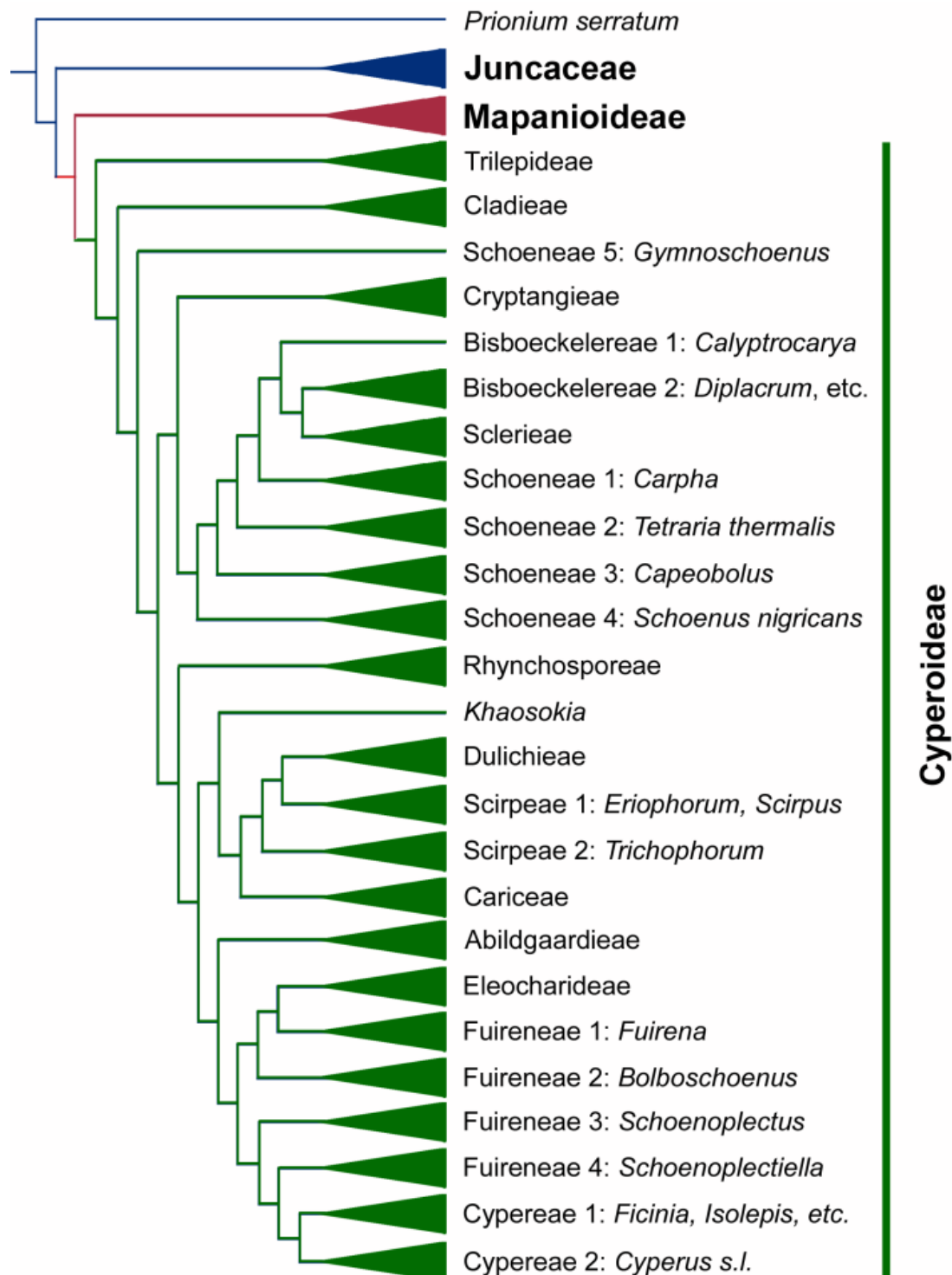
Fig. 5.2 Simplified cladogram of Cyperaceae, adapted from a strict consensus tree from Muasya et al. (2009a).
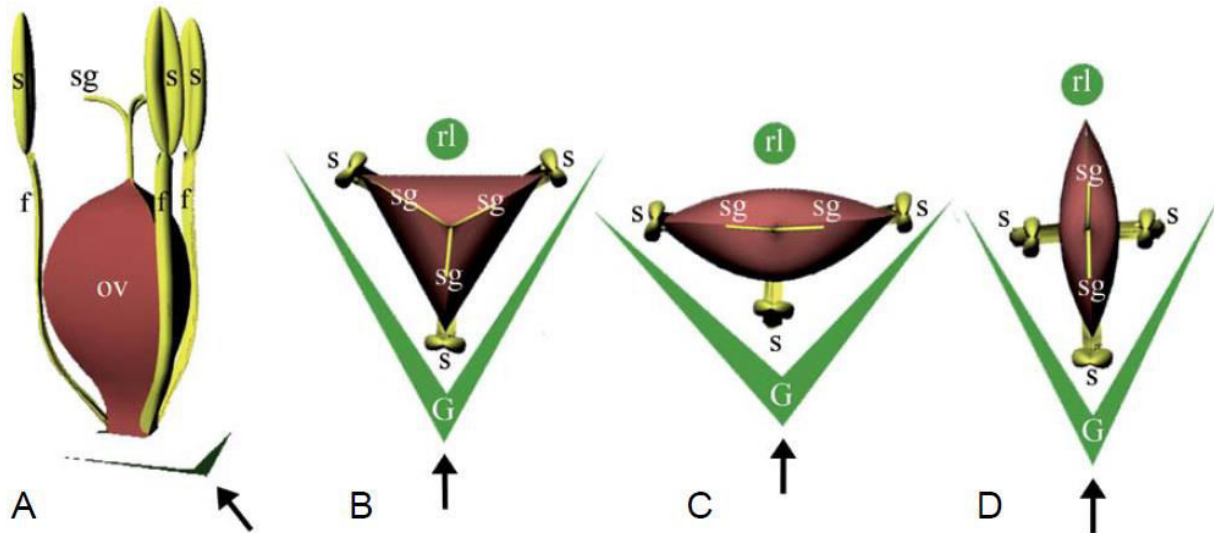
Fig. 5.3 Overview of the pistil types present in Cyperoideae, which are studied in this paper. A, lateral-abaxial view of a typical flower in Cyperoideae (without perianth); B, apical view of a flower with a trimerous gynoecium; C, apical view of a flower with a dorsiventrally flattened dimerous gynoecium; D, apical view of flower with a laterally flattened dimerous gynoecium.

Abbreviations: f, filament; G, glume; Rl, rachilla; s, stamen; sg, stigma. A black arrow indicates the abaxial side of each flower.

5.3.2 Aims of this study

This paper presents an overview, strictly limited to the Cyperoideae, of existing anatomical and ontogenetic data of the gynoecium, complemented with new anatomical and floral ontogenetic evidence. For the first time, old and new anatomical and floral ontogenetic observations are integrated in order to test two existing hypotheses outlined in the following paragraphs: (1) Blaser's (1941a, 1941b) anatomy-based interpretation of vascularisation in Cyperoideae, especially for the gynoecium wall in *Pycreus*, where he assumed lateral bundles took over the function of the dorsal bundles (see chapter on pistil vascularisation for more details), and (2) the scirpoid floral ontogenetic model of Vrijdaghs *et al.,* (2009) in which the pistil of all Cyperoideae is explained to originate from a ring primordium. In the anatomical study, special attention was given to procambial initiation patterns, which have not yet been studied in cyperoid flowers, and to evaluate Blaser's conclusions in the context of recent molecular phylogenetic hypotheses (Muasya *et al.,* 2002, Larridon *et al.,* 2011a) especially concerning dimerous flattened pistils.

Moreover, to illustrate two fundamentally different developmental patterns that result in coenocarpous gynoecia (i.e. gynoecia with fused carpels, Weberling, 1992), cyperoid gynoecium development is compared with gynoecial development in a relatively distantly related monocot, *Sansevieria trifasciata* Prain (included in *Dracaena*, Ruscaceae, Asparagales, Stevens 2001 onwards). *Sansevieria* was chosen as a highly illustrative monocotyledonous species with an ovary developing from individual carpel primordia that fuse postgenitally.

Finally, our conclusions are presented as a developmental model for the gynoecium and its vascularisation, giving a possible explanation for the variation of pistils found in Cyperoideae.

5.3.3 Pistil modifications in Cyperoideae

As in other monocots, in Cyperaceae, a trimerous gynoecium can be considered as the plesiomorphic condition (Endress, 1995). Usually, species of Cyperoideae have two lateral stigma branches at the adaxial side and one at the abaxial side (fig. 5.3A & B). While tetramerous, hexamerous and octamerous pistils occur in some tribes, dimerisation of pistils is more widespread within the family (Goetghebeur, 1998). In sedges, derived pistils have often been used for specific and generic delimitations, but their systematic value is controversial (e.g. Goetghebeur, 1998, Muasya *et al.,* 2009b).

In Cyperoideae, two main types of dimerous pistils occur: either dorsiventrally (fig. 5.3C) or laterally flattened pistils (fig. 5.3D), causing bilateral floral symmetry. A dorsiventrally flattened pistil has laterally positioned stigma branches (fig. 5.3C). This type occurs in almost all cyperoid tribes sensu Goetghebeur (see table 5.2). In Mapanioideae, this pistil type is also common (Kern, 1974). Based on early anatomical and ontogenetic studies, many authors suggested that dorsiventrally flattened pistils could be explained by a reduction of the abaxial carpel (e.g. Baillon, 1893, Schumann, 1890, Snell, 1936, Goetghebeur, 1986). Although often used in generic circumscriptions, the taxonomic value of the dorsiventrally compressed dimerous pistil should be handled with caution (Goetghebeur, 1986). This is illustrated by some species such as *Cyperus alopecuroides* Rottb., *Kyllingiella polyphylla* (A.Rich.) Lye, *Eleocharis variegata* (Poir.) C.Presl. and *Schoenoplectus corymbosus* (Roth ex Roem. & Schult.) J.Raynal, which can have both trimerous and dorsiventrally flattened dimerous pistils within a single spikelet (Haines & Lye, 1983, Larridon *et al.,* 2011b). In other

Table 5.1 List of cyperoid genera that include species with derived pistils based on Goetghebeur (1998). Abbreviations: D, distichous; dors., dorsally compressed; lat., laterally compressed; S, spiral; X, the most common situation; x, the less common situation; (x), observed as a rare variation within species that normally have another type of pistils.

Taxonomy		Gynoecium: number of stigmata							Glume placement
Clade (Muasya et al., 2009a)	Tribe and genus (Goetghebeur, 1998)	2 lat.	2 dors.	3	4	6	8	9	
Abildgardieae	Abildgardieae Lye								
	Bulbostylis Kunth		x	X					S
	Fimbristylis Vahl		X	X					S
	Nemum Desv. ex Ham.		X	x					S
Bisboeckelereae 1	Bisboeckelereae Pax ex L. T. Eiten								
	Calyptrocarya Nees		X						D
Cariceae	Cariceae Kunth ex Dumort.								
	Carex L.		x	X	x	(x)			S
	Kobresia Willd.		x	X					S
Cryptangieae	Cryptangieae Benth.								
	Exochogyne C.B. Clarke	X							D
Cypereae	Cypereae Dumort.								
	Ascolepis Nees ex. Steud.		x	X	(x)				S
	Cyperus L. s.s.		x	X					D/s
	Ficinia Schrad.		x	X					S
	Isolepis R.Br.		x	X					S
	Kyllinga Rottb.	X							D
	Lipocarpha R.Br.		x	X					S
	Oxycaryum Nees		X						S
	Pycreus P.Beauv.	X							D
	Queenslandiella Domin	X							D
Dulichieae	Dulichieae Rchb. ex J. Schultze-Motel								
	Blysmus Panz. ex Schult.		X						D
	Dulichium Pers.		X						D
?	*Sumatroscirpus* Oteng-Yeb.		X	(x)					S
Eleocharideae	Eleocharideae Goetgh.								
	Eleocharis R.Br.		x	X					S
	Websteria S.H.Wright		X						D
Fuireneae 2	Fuireneae Rchb. ex Fenzl								
	Bolboschoenus (Asch.) Palla		x	X					S
Fuireneae 3 & 4	*Schoenoplectus* (Rchb.) Palla		x	X					S
Rhynchosporeae	Schoeneae Dumort								
	Pleurostachys Brongn.		X						S/d
	Rhynchospora Vahl	x	X						D
Schoeneae 3	*Cladium* P.		(x)	X					D
Schoeneae 1	*Cyathochaeta* Nees		X						S/d
	Evandra R.Br.						X		S
	Gahnia J.R.Forst. & G.Forst.			X	(x)				S
	Lepidosperma Labill.			X	(x)				S
	Neesenbeckia Levyns					X	(x)		D
	Schoenus L.		(x)	X					D
	Tetraria P.Beauv.			X	x			(x)	D
?	*Trachystylis* S.T.Blake		X						S
Scirpeae 1	Scirpeae Kunth ex Dumort.								
	Amphiscirpus Oteng-Yeb.		X						S
	Scirpus L.		x	X					S

taxa, the dimerous, dorsiventrally flattened pistil state is constant such as in *Cyperus laevigatus* and *Eleocharis minuta* (Haines & Lye, 1983).

Laterally flattened pistils are less common and their origin has been more controversial. In this pistil type, the two stigma branches have median positions (fig. 5.3D). According to Goetghebeur (1986), reduction of one of the (two adaxial) carpels is not likely since there is no trace of the then expected asymmetry. Laterally flattened pistils have only been recorded in three cyperoid tribes: Cypereae (*Pycreus*, ±120 spp.; *Kyllinga*, ± 80 spp.; *Queenslandiella*, 1 sp.), Cryptangieae (*Exochogyne* Clarke [accepted name = *Lagenocarpus amazonicus*]) and Rhynchosporeae (*Rhynchospora rubra* subsp. *rubra* (Lour.) Makino) (Goetghebeur, 1998). Goetghebeur (1986) postulated a single origin of laterally flattened pistils for *Kyllinga*, *Pycreus* and *Queenslandiella* (Cypereae). However, a putative homology[1] of the laterally flattened dimerous pistils in *Kyllinga*, *Pycreus* and *Queenslandiella* has not been confirmed by molecular phylogenetic hypotheses (Muasya *et al.,* 2001, 2002, 2009a). Moreover, the recognition of these three taxa as genera (e.g. Goetghebeur, 1998) is controversial (e.g. Haines & Lye, 1983, Tucker *et al.,* 2002, Muasya *et al.,* 2009b). They are nested in a paraphyletic *Cyperus* (together with several other segregate 'genera'), in a polytomy of the clade of the *Cyperus* s.l. species using C_4 photosynthesis (C_4 *Cyperus* clade) (Muasya *et al.,* 2002, 2009a, Besnard *et al.,* 2009, Larridon *et al.,* 2011a). Despite their obvious close relationships with *Cyperus*, these three taxa are generally considered as well circumscribed entities (whether considered as separate genera or at subgeneric level in *Cyperus*), based on, among others, the presence of laterally flattened pistils in each of them. In expectance of a more elaborate molecular study on C_4 *Cyperus* and its segregate genera, we chose here to follow the classification of Goetghebeur (1998) and Govaerts *et al.,* (2007) for the currently accepted names of the taxa. To be complete, it must be mentioned that some authors were not convinced of the systematic value of laterally flattened pistils for generic delimitation (e.g. Koyama, 1961).

Finally, in several Cyperoideae taxa, apparent monomerous pistils can be found. In most of these cases the style is strongly elongated bearing very short stigma branches at its top (e.g. *Rhynchospora* sect. *Haplostylis* and *Cyperus* sect. Anosporum, Haines & Lye, 1983).

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[1] Several authors such as Goetghebeur (1986) supposed laterally flattened dimerous pistils had originated in a single evolutionary event (phylogenetic homology).

In *Cyperus meeboldii* and relatives (e.g*. C. holostigma*, *C. clavinux*), an unbranched style has also been observed (Raynal, 1966).

5.3.4 The carpel concept and its application to Cyperoideae

Goetghebeur (1986) pointed out that laterally flattened pistils in Cyperoideae are difficult to explain as resulting from the reduction of one of the two adaxial carpels. Instead, the two adaxial carpels seem to be replaced by a single carpel in median position. This novel position is remarkable since in most angiosperm groups, carpel positions appear to be highly conserved (Endress, 1995). In addition, Vrijdaghs *et al.,* (e.g. 2005a, 2005b) showed that the cyperoid ovary originates from an annular ovary primordium. In this context, we think it useful to recapitulate the carpel concept and its application in Cyperoideae.

Von Goethe (1790, in Miller, 1949: 256) was the first to see the carpel as a reproductive phyllome: "Earlier I tried to make as clear as possible that the various plant parts developed in sequence are intrinsically identical despite their manifold differences in outer form. It should come as no surprise that I also intend to explain the structure of the female parts in the same way." Goethe did not suggest carpels to be derived from leaves, but rather that leaves and all floral parts are lateral appendages of the stem, reflecting an archetypical leaf-like structure, called 'Blatt' by Goethe and from the end of the 19th century called 'phyllome' (Arber, 1937). The word 'carpellum' dates from the early 19th century, from the same period in which de Candolle (1827) hypothesised carpels to be structures derived from sporangium-bearing leaves. Goebel (1888) described a gynoecium as being formed by macrosporophylls, bearing ovules at the inner or ventral side of the leaf margins. This view became 'classic' with Engler (1900–1968), who adopted it for all angiosperms. In the course of the 19th and 20th centuries, several alternative floral hypotheses arose, such as the pseudanthium hypothesis (e.g. Wettstein, 1935) or the gonophyll hypothesis (Melville, 1969), consequently suggesting other interpretations of carpels. As a summary, carpels can be seen as either (1) homologous with leaves (phyllomes) and thus formed laterally on an axis and the ovules formed upon them (phyllospory) or (2) leaf-axis structures, with the ovules being formed on an axis subtended by a carpel (stachyospory). Endress (2001) added that a carpel can also be a compound organ with an ovule producing part and a leaf component, or a totally new organ. Leins & Erbar (2011) consider carpels to be megasporophylls and describe them as comparable with a hollow cylinder, through which an inclined section is made above the base

of the cylinder and consequently defining a circular basal zone or ascidiate zone, and distally an open zone called plicate. In ovaries consisting of several carpels, the dorsal side of it always forms the ovary wall, whereas placentas/ovules are formed at the ventral side of the carpels, usually at the margins (axile placentation) or situated more centrally at the ventral side of the carpels and hence at the inner side of the ovary wall (laminal placentation).

In Cyperaceae, Payer (1857: 698) described the origin of the gynoecium "dans les *Scirpus* et les *Eriophorum*" as carpellate: "trois autres bourrelets qui leur [stamen primordia] sont superposés et qui sont les rudiments du pistil", with postgenital fusion of these carpel primordia: "...ils sont promptement réunis à leur base, de façon à produire un sac ovarien" (Payer, 1857: 699). Until the last decade, Payer was the only author who did a thorough and complete floral ontogenetic investigation in Cyperoideae. In the course of the 20th century, floral ontogenetic data were only sporadically published (e.g. Schumann, 1890, Barnard, 1957, Schulze-Motel, 1959, Mora, 1960, Mora-Osejo, 1987, Bruhl, 1991). Since Payer (1857), in Cyperaceae-Cyperoideae, the ovary has always been described as tricarpellate, unilocular with one basal, anatropous and bitegmic crassinucellar ovule (e.g. Goetghebeur, 1998). However, according to Vrijdaghs *et al.*, (e.g. 2009), in Cyperoideae, the ovary rises from an annular ovary primordium, enveloping the single, central ovule.

→ Fig. 5.4 3D reconstructions of the vascular bundle patterns within the base of different Cyperaceae flowers as interpreted by Blaser (1941a, 1941b). Vascular patters are grouped according to the pistils types (A–D) and the presence or absence of lateral bundles within the gynoecium wall (1–2). Vascular traces towards perianth parts are omitted. Bundles, which continue into the different floral organs, are arrowed, rudimentary bundles are not arrowed. The larger models are shown from a lateral-abaxial position and the abaxial position is always indicated with a small black arrow. A smaller inlay adds an apical view of each separate model (with staminal traces removed). Colour codes and circumscription for the different vascular bundles: purple, receptacular bundles, these bundles connect the vascular tissue of the flower with the stele; yellow, staminal bundles; green, dorsal bundles, running in the carpels and continuing in the stigmata; blue, ventral bundles, usually two bundles for each carpel that run towards the placentation of the ovule; orange, lateral bundles, running in the wall of the gynoecium in some taxa; red, central and ovular bundles connecting the ventral bundles to the ovule. A, trimerous pistil type; B, dorsiventrally flattened dimerous pistil with the abaxial receptacular bundles still contributing to the vascularisation of the pistil, however the abaxial dorsal bundle is reduced; C, dorsiventrally flattened dimerous pistil, the abaxial receptacular bundle does not contribute to the vascularisation of the pistil; D, the situation in the laterally compressed pistils of *Cyperus rivularis* (= *Pycreus bipartitus*) where Blaser (1941a) interpreted the bundles within the gynoecium wall to be lateral bundles (D1). D2 shows a hypothetical, alternate interpretation of these bundles to be interpreted as dorsals. See table 1 for a list of taxa and their vascularisation types as observed by Blaser (1941 a, 1941b).

Chapter 5
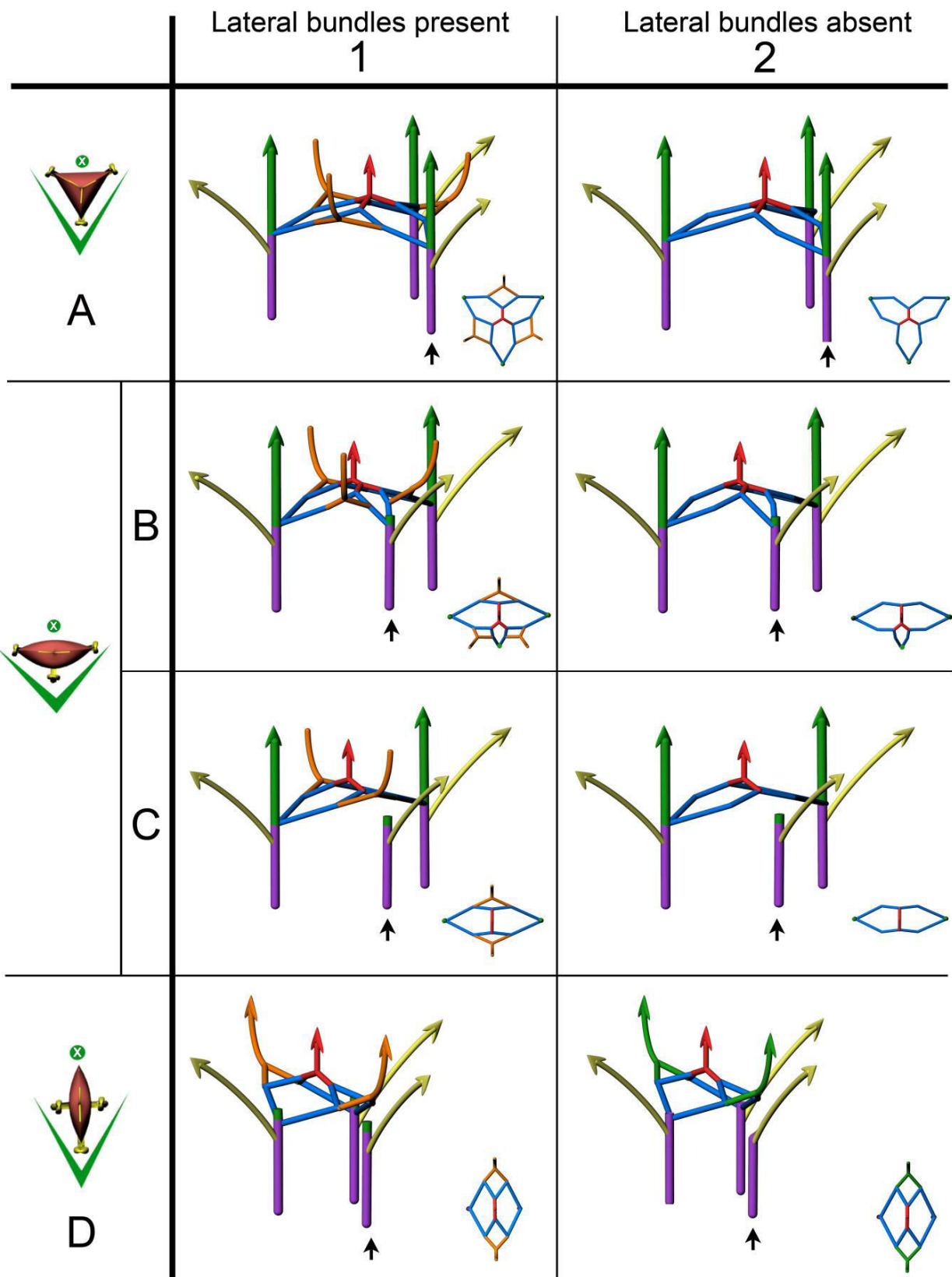
5.3.5 Recent insights in the development of (floral) primordia

Gould (2002) linked classic morphology with evolutionary developmental biology (evo-devo), suggesting that members of the floral whorls, including the gynoecium, originate from phyllome primordia that are 'empty', undetermined structures, getting identity through the functioning of developmental regulator genes/programs, such as the ABC model of Coen & Meyerowitz (1991). According to Endress (2006), annular primordia often result in a decoupling of the development of the concerned organ type from the neighboring floral organs, with alterations in time (sequence of floral organ appearance) and number. In *Eriophorum*, Vrijdaghs *et al.,* (2005a) observed that congenital fusion of the perianth primordia into a massive perigonial primordium resulted in a new kind of perianth consisting of many perianth hairs. The development of a pappus in some Asteraceae from fused sepal primordia is a similar situation (see Harris, 1995).

5.3.6 Pistil vascularization in Cyperoideae

In angiosperms, a carpel is most often vascularised by three vascular bundles: a midvein (or dorsal carpellary bundle), running towards the stigma and two marginal veins (or ventral carpellary bundles), which connect to the placentae and ovules (e.g. Dickison 2000). In Cyperoideae, only the dorsal bundles run within the ridges of the gynoecium wall towards the stigmata (fig. 5.4). Saunders (1937) and Snell (1936) both remarked that the vascular system within the receptacle is highly disorganised. However, since this vascular plexus connects towards the ovule, its bundles were interpreted as ventral bundles by all previous authors. Therefore, the ventral bundles of each carpel have been described to branch off already below the gynoecium and fuse in the centre of the receptacle to supply the single basal ovule (indicated as central bundle) (Saunders, 1937, Snell, 1936, Blaser, 1941a, 1941b) (fig. 5.4). Sometimes, adjacent ventral bundles fuse and continue shortly in the sides of the gynoecium wall (indicated as lateral bundles) (Blaser, 1941a, 1941b) (fig. 5.4, orange bundles). In an elaborate study of a wide spectrum of genera in Cyperaceae, Blaser (1941a, 1941b) used the vascularisation of *Scirpus* as a prototype from which all other patterns were derived (for details see fig. 5.4 and table 5.3). Based on the frequent presence of vestigial ventral and dorsal vascular bundles belonging to the hypothetical abaxial carpel (fig. 5.4B), Blaser (1941a, 1941b) concluded that dorsiventrally flattened pistils originated by reduction of the abaxial carpel.

Concerning the laterally flattened type, Blaser (1941a) observed that vascularisation patterns in pistils of *Pycreus bipartitus* (Torr.) C.B.Clarke, *P. lanceolatus* (Poir) C.B.Clarke and *P. diander* (Torr.) C.B.Clarke include bundles towards the stigma branches, which connect to the ventral branches of the two adaxial main bundles entering the flower (fig. 5.4D1). He interpreted these bundles as lateral bundles, stating: "In this species (*Pycreus bipartitus*), the dorsal bundles, which are characteristically midribs of foliar carpels, form none of the vascular supply of the pistil. The ventrals, in forming the ovule supply, are shortened as usual and the supply to the styles is assumed by the lateral bundles. The laterals are seen as vestiges in many Cyperaceae but are well developed here" (Blaser, 1941a: 547; figs 5.4D1 & 5.24C). In contrast, the vascularisation patterns in flowers of *Kyllinga* and *Queenslandiella* were not yet clear. Blaser (1941a) also included *Kyllinga* pumila Michx. in his study but surprisingly concluded that its vascular pattern is similar to the vascularisation of trimerous *Cyperus* pistils.

Based on his anatomical studies in mature plants, Blaser (1941a, 1941b) assumed the vascular bundles running in the gynoecial wall of *Pycreus* up to the stigma branches to be homologues of the lateral branches found in scirpoid taxa. In *Cyperus*, lateral branches are absent and as a consequence Blaser postulated a separate origin of *Pycreus* from an ancestor with lateral bundles.

In order to correctly address homology questions and evolutionary interpretations of vascularisation patterns it is necessary to have an idea of how these vascular strands develop (Pizzolato, 2000). In Cyperaceae, procambial initiation has so far only been studied in the leaves and culms of *Cladium mariscus* (L.) Pohl (Fisher, 1971). The vascular ontogeny in this species concurs with patterns found in other monocots, such as *Tradescantia zebrina* Bosse (Commelinaceae, Pizzolato 2006), in which differentiation of veins within the leaves is bidirectional and starting from several separate procambial initiation points, in a later stage these merge with each other and with the older vascular bundles of the culms (e.g. Dickison 2000). Similar patterns have also been observed in the formation of reticulations between the main veins within the leaves of *Arabidopsis* Heyhn. (Scarpella *et al.,* 2006). Merging of remote procambial strands from different plant organs seems to be the basic pattern in the development of the floral vascularisation found in angiosperms (e.g. Endress, 1994). According to Aloni (2004), auxin plays a leading role in procambial initiation and polar auxin transport from primordia is a controlling factor in both phyllotactic (Reinhardt *et al.,* 2003) and leaf venation patterning (Scarpella & Meijer 2004, Scarpella *et al.,* 2006), resulting in self-

regulated development of vascular bundles where needed. Before these modern insights were generally accepted, vascularisation development in angiosperm flowers had been assumed to be acropetal (e.g. Grégoire, 1938).

5.3.7 The ovule

Cyperaceae are characterised by unilocular ovaries with a single basal ovule (e.g. Goetghebeur, 1998). According to Snell (1936), the presence of a single basal ovule could be interpreted as a final reduction state of a free central placentation. Van der Veken (1965) was the first to show in a dorsiventrally oriented longitudinal section through a semimature gynoecium, that hairs occur within the locule, around the micropylar zone of the ovule, which grow into the micropyle. He suggested that these hairs have a pollen tube guiding function and called them therefore 'obturator hairs'. Several authors reported outgrowth of funicular cells into a 'funicular obturator' (e.g. Coan *et al.,* 2008). Based on its 'glandular nature' observed in *Bulbostylis*, Gonzalez & López (2010) suggested an integumentary origin of the obturator. While Bouman (1984) considers the obturator to be degenerating after fertilisation, Gonzalez & López (2010) observed lignification of the obturator after fertilisation and persistence in the mature fruit.

Table 5.2 List of genera studied by Blaser (1941a, 1941b) with the reference of the corresponding models of the floral vascularisation shown in fig. 5.4.

Genus	Model (fig. 5.4)
Bolboschoenus (Asch.) Palla (as *Scirpus* L.)	A1/B1
Carex L.	A2/B2
Cyperus L.	A2
Dulichium Pers.	C1
Eleocharis R.Br. (as *Heleocharis* T.Lestib.)	A2/C2
Eriophorum L.	A1
Fimbristylis Vahl	A2/B2
Lipocarpha R.Br.	A2/B2
Pycreus P. Beauv. (as *Cyperus* L.)	D1
Rhynchospora Vahl	B1/B2/C1/C2
Schoenoplectus (Rchb.) Palla (as *Scirpus* L.)	A1/B1

5.4 Materials and methods

5.4.1 Plant material

Spikelets and flowers of 108 species from 34 cyperoid genera (fig. 5.2) were examined at early and mature stages (see appendix 1 of Vrijdaghs *et al.,* 2010), of which only a representative selection of illustrative examples is presented here (table 5.4). Numbering of glumes and subtended flowers was done from most recently originated (1) to oldest (n), in order to avoid abstract numbers in spikelets with many and/or a variable number of (flower subtending) glumes. Partial inflorescences were collected in the field or in botanical gardens (table 5.4) and immediately fixed in FAA (70% ethanol, acetic acid, 40% formaldehyde, 90 : 5 : 5). Spikelets were dissected in 70% ethanol under a Wild M3 stereo microscope (Leica Microsystems AG, Wetzlar, Germany) equipped with a cold-light source (Schott KL1500, SchottFostec LLC, Auburn, NY, USA).

5.4.2 Scanning electron microscopy

To prepare the material for critical-point drying, it was washed twice with 70% ethanol for 5 min. Next it was placed in a mixture (1:1) of 70% ethanol and DMM (dimethoxymethane) for 5 min. The material was then transferred for 20 min to pure DMM. Critical-point drying was done using liquid CO_2 with a CPD 030 critical-point dryer (BAL-TEC AG, Balzers, Liechtenstein). The dried samples were mounted on aluminium stubs using Leit-C. For SEM observation,
the material was coated with gold via a SPI-ModuleTM Sputter Coater (SPI Supplies, West-Chester, PA, USA). SEM images were obtained with a JEOL JSM-6360 (JEOL Ltd, Tokyo, Japan) at the Laboratory of Plant Systematics (K.U.Leuven), or with a JEOL JSM-5800 LV scanning electron microscope at the National Botanical Garden of Belgium in Meise.

5.4.3 Bleaching

Flowers and spikelet tips were dissected and bleached with a 5% sodium hypochlorite (NaClO) solution for 15–60 min. Subsequently, samples were washed for the same period in distilled water and mounted on slides. Cleared up samples were studied under dark field, a technique using indirect light which is scattered by the object, enhancing contrast between different tissues in the sample and makes the xylem vessels to light up. Dark field images were recorded with a Nikon Eclipse E600 microscope, equipped with a Nikon digital camera

DXM1200. To enhance sharpness in depth, optical section images were taken from the samples and manually combined in Adobe Photoshop® CS 8.0 (Adobe Systems Inc., San Jose, USA).

Table 5.3 Species studied and voucher data. Abbreviations: HBUG, Ghent University Botanical Garden, Belgium; KDTN-Leuven, Kruidtuin, botanical garden of the town of Leuven, Belgium; Ptk-K.U.Leuven, botanical garden of the Institute of Botany of the K.U.Leuven, Belgium.

Taxa	Collected by	Origin	Voucher
Cyperus haspan L.	Reynders M.	HBUG 2006-1243 (wild origin Philippines)	20061243 (GENT)
Cyperus laevigatus L.	Reynders M.	HBUG 2002-0878 (wild origin Zimbabwe)	20020878 (GENT)
Dulichium arundinaceum (L.) Britton	Goetghebeur P.	HBUG 2002-1303 (P)	PG 9914 (GENT)
Eriophorum latifolium Hoppe	Vrijdaghs A.	KDTN-Leuven	AV 04
Lagenocarpus amazonicus (C.B. Clarke) H. Pfeiff.	Aparecida da Silva M., Proença C., Cardoso E. & Paixao J.P.	Brazil	1986 (GENT)
Kyllinga microbulbosa Lye	Muasya A.M.	Kenya	AM 2658 (EA)
Kyllinga nemoralis (J.R.Forst. & G.Forst.) Dandy ex Hutch. & Dalziel	Reynders M.	HBUG 2006-1238 (wild origin Philippines)	20061238 (GENT)
Pycreus bipartitus (Torr.) C.B.Clarke	Reynders M.	HBUG 2005-0801 (S)	20050801 (GENT)
Pycreus flavescens (L.) P.Beauv. ex Rchb.	Reynders M.	HBUG 2005-0401 (S)	20050401 (GENT)
Pycreus sanguinolentus (Vahl) Nees	Reynders M.	HBUG 2007-1753 (wild origin China)	20071753 (GENT)
Queenslandiella hyalina (Vahl) Ballard	Muasya A.M.	Mombassa, Kenya	AM 2189 (EA)
Rhynchospora corymbosa (L.) Britton	Reynders M.	HBUG 2007-1418 (wild origin Cameroon)	20071418 (GENT)
Sansevieria trifasciata Prain.	Goetghebeur P.	HBUG 1900-1241	19001241 (GENT)
Scirpus sylvaticus L.	Vrijdaghs A.	Ptk-K.U.Leuven	AV 02

5.4.4 Anatomy

Entire spikelets were fixed overnight in FAA (50% ethanol, 5% acetic acid and 5% commercial formalin in distilled water). Dehydration was performed using a 50%, 70%, 85% and 94% ethanol series. After the last alcohol step, the tissue was infiltrated using a mixture of Technovit® 7100 liquid (Heraeus, Kulzer, Wehrheim, Germany) (2-hydroxymethylmeth-acrylate) and Hardner I (dibenzoylperoxide), which was diluted to 30%, 50% and 70% with ethanol 94%. To enhance infiltration, samples were placed under vacuum during 24 h. The infiltrated samples were transferred to a 100% infiltration liquid for 48 h. Next, the samples

were embedded according to Leroux *et al.,* (2007) to obtain an optimal orientation of the samples within the resin. Transverse and longitudinal sections were cut at 5 µm with a rotation microtome (Minot, 1212, Leitz Wetzlar, Germany), equipped with a holder for disposable Superlap Knives (Adamas Instrumenten, Netherlands). The sections were collected on water drops on slides, which were subsequently dried on a hot plate at 40°C and stained with a 0.05% [w/v] aqueous solution of toluidine blue O (Meck, Darmstadt, Germany, C.I. No. 52040) and 0.1% [w/v] Na2B4O7, and subsequently mounted with DePeX (Gurr, BDH Laboratory, Poole, U.K.). LM images were made with a Nikon Eclipse E600 microscope, equipped with a Nikon digital camera DXM1200 (Nikon, Tokyo, Japan). All anatomical images were taken using the phase contrast II position of the microscope, which creates a dark field effect under a magnification of 200x. Phase contrast adds contrast between the different stained tissues and makes xylem bundles to light up in bright blue when colored with a toluidine blue O solution. Slices are shown for the first distal flower with a fully developed vascular system.

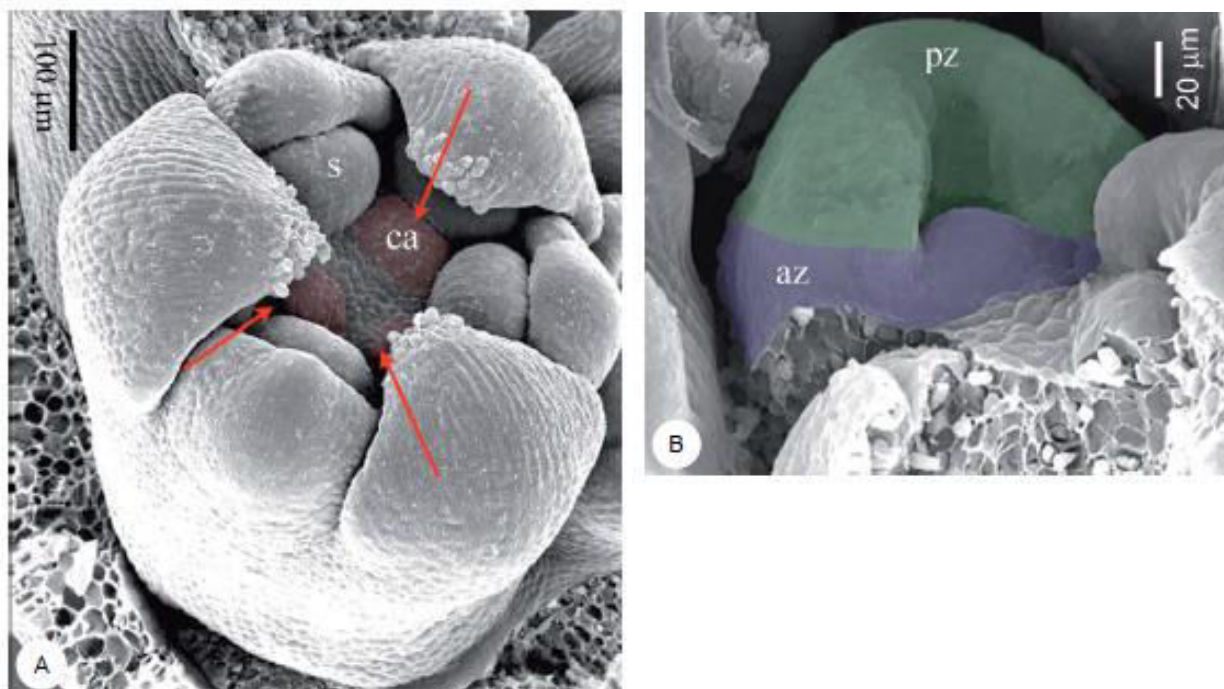Fig. 5.5 SEM images of developing gynoecia in *Sansevieria trifasciata*. A, flower at early developmental stage in *S. trifasciata*. In red and arrowed, carpel primordia alternating with the stamen primordia; B, detail of a developing carpel in *S. trifasciata*, with a plicate zone (purple) and an ascidiate zone (green). Abbreviations: az, ascidiate zone; ca, carpel primordium; pz, plicate zone; s, stamen primordium.

5.4.5 3D diagrams

The 3D diagrams as shown in figs 5.3, 5.4, 5.23, 5.24 & 5.25 were manually drawn in Rhinoceros 3D® (Mc Neel, Seattle, USA) and were based on the data of Blaser (1941a, 1941b) and our new data.

5.5 Results

5.5.1 An illustration of individual carpel formation in flowers of *Sansevieria*

At early developmental stages, *Sansevieria trifasciata* has three individual carpel primordia. These grow out into carpels in which a plicate zone and an ascidiate zone can be distinguished (fig. 5.5A & B).

5.5.2 Cyperoid gynoecium development

In *Scirpus sylvaticus*, an undifferentiated flower primordium is positioned in the axil of a glume (fig. 5.6A). The different floral whorls appear with apically an annular ovary wall primordium surrounding a central ovule primordium (fig. 5.6B). The ovary wall grows up from the ring primordium, forming a single style and three stigma branches developing from three stigma primordia on the top of the ovary wall, two lateral-adaxial and a single abaxial one. In some individuals, four stigma branches are formed like in this example of *Scirpoides holoschoenus* (fig. 5.6D). A detailed observation of the differentiation of the ovary wall primordium and ovule primordium at the floral apex in *Scirpus sylvaticus* shows that both primordia originate simultaneously, after the formation of the stamen primordia, and also simultaneously with the formation of the perianth primordia (fig. 5.7A–D). In species with dimerous, dorsiventrally flattened gynoecia, such as *Dulichium arundinaceum*, an annular ovary wall primordium surrounding a central ovule primordium is formed in the same way as in *S. sylvaticus* (fig. 5.8A & B). Two lateral stigma primordia appear on the top of the ovary wall, growing out into two stigma branches (fig. 5.8C & D). The development of dimerous, laterally flattened gynoecia only differs in the dorsiventral position of the two stigma primordia on the top of the ovary wall as illustrated in *Pycreus bipartitus* (fig. 5.9A & B), *Kyllinga microbulbosa* (fig. 5.9C & D) and *Queenslandiella hyalina* (fig. 5.9E & F). In *Pycreus flavescens* and *P. sanguinolentus* a delay in the development of the adaxial stigma branch was observed with respect to the abaxial stigma branch (figs 5.20A & 5.21A). In *Lagenocarpus amazonicus*, the ribs of the mature nutlets are in the symmetry plane formed by the spikelet

bract, prophyll and glumes of the spikelet for which we can confirm these to be laterally compressed dimerous pistils (fig. 5.10A). Several nutlets were observed with three instead of two ribs (fig. 5.10B–D), in such case, the second adaxial rib has sometimes only partially developed.
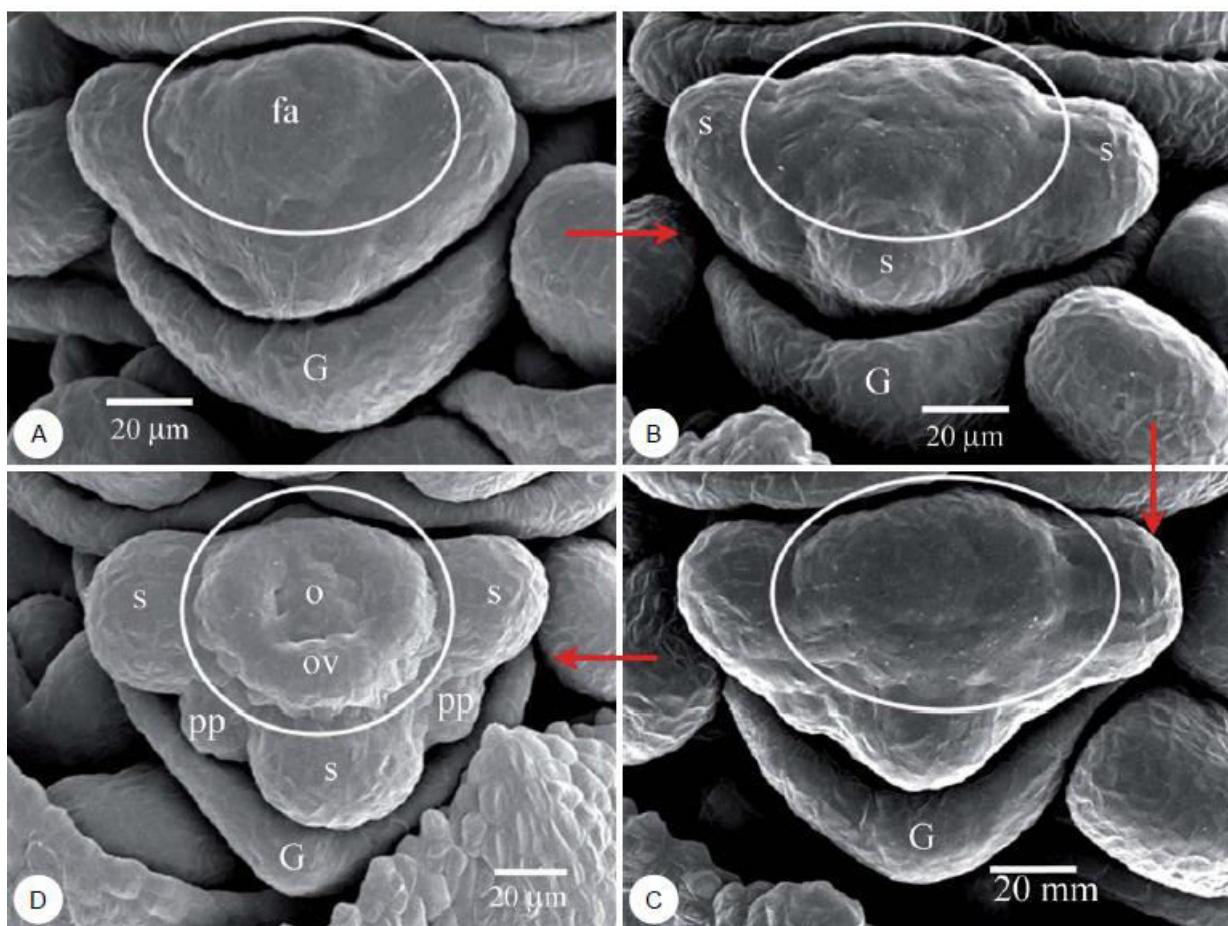
Fig. 5.6 SEM images of the early development of the trimerous gynoecium in *Scirpus sylvaticus* (A–C) and of a developing gynoecium in *Scirpoides holoschoenus* (D). A, flower primordium in the axil of a developing glume; B, early stage in gynoecium development with an annular ovary wall primordium (red) around a central ovule primordium (amber); C, developing gynoecium with three growing stigma branches; D, developing gynoecium with four stigma branches (arrowed).

Abbreviations: a, anther; f, filament; o, ovule primordium; ov, ovary wall primordium; sg, stigma primordium; st, style.
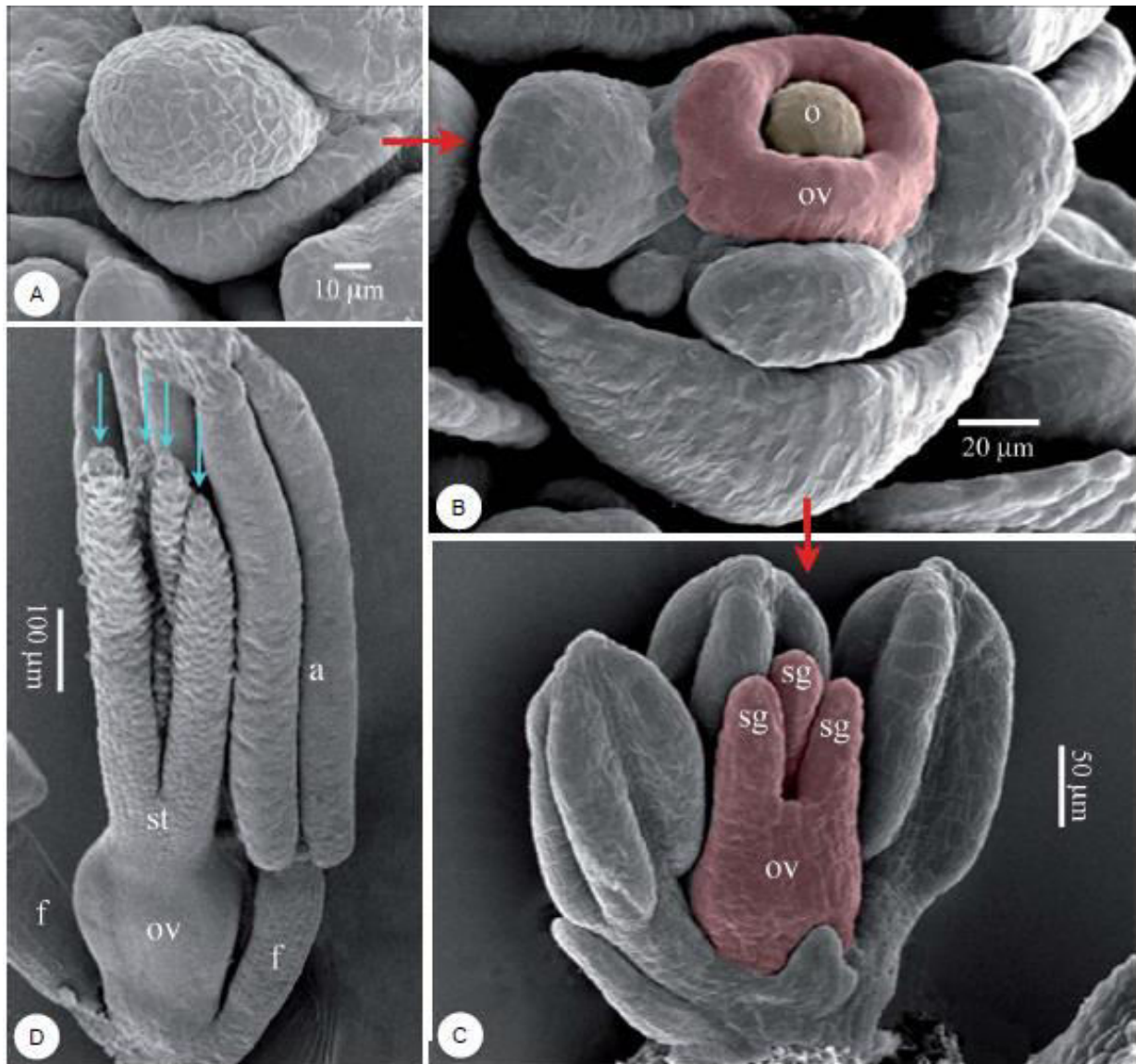
Fig. 5.7 SEM images of the earliest differentiation steps of the floral apex in *Scirpus sylvaticus* into annular ovary wall primordium and ovule primordium. A, flower primordium with perianth and stamen primordia and yet undifferentiated floral apex (encircled); B–C, an annular ovary wall primordium is being formed (encircled). B is a lateral-abaxial view, and C is an apical view on the same developmental stage; D, a central ovule primordium becomes visible, surrounded by the annular ovary wall primordium (encircled). The primordia of the other floral whorls also become more prominent.

Abbreviations: fa, floral apex; G, glume subtending flower; o, ovule primordium; ov, ovary wall primordium; pp, perianth part primordium; s, stamen primordium.
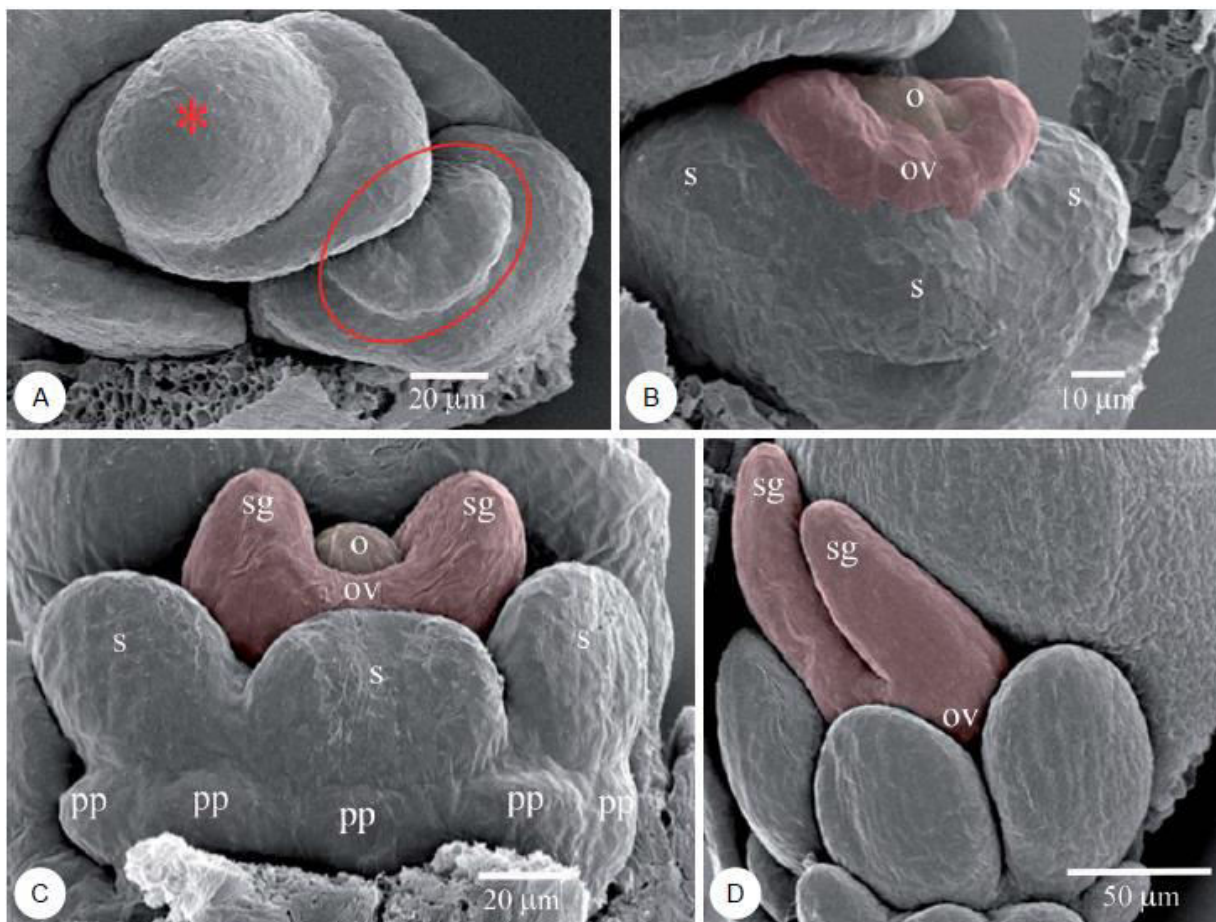
Fig. 5.8 SEM images of the development of the gynoecium in *Dulichium arundinaceum,* a species with a dorsiventrally flattened dimerous gynoecium. A, distal part of a spikelet with a developing flower with floral apex differentiating into annular ovary wall primordium surrounding a central ovule primordium (encircled in red); B, the annular ovary wall primordium (in red) surrounding a central ovule primordium (in amber). The stamen primordia are also visible; C, rising ovary wall with two laterally oriented stigma primordia (in red) surrounding the central ovule primordium (amber). All primordia of the other floral whorls are visible; D, the ovary wall encloses the ovule, and the stigma branches grow out.

Abbreviations: o, ovule; ov, ovary wall (primordium); pp, perianth part primordium; s, stamen primordium; sg, stigma primordium; asterisk (*), apex of the rachilla.

5.5.3 Development of the vascular system within spikelets and flowers

In cleared up spikelets and flowers, the annular xylem vessels are visible using a dark field light microscope (figs 5.11–5.14). The combination of bleaching and dark field microscopy forms a fast and cheap technique enabling the study of vascular developmental patterns in flowers and spikelets of Cyperoideae. However, the quality of the images obtained by this technique was variable. Species with flattened spikelets, which are continuously

producing new flowers and species with strongly reduced floral parts, such as *Pycreus* sp., were the most convenient to interpret.

For spikelets of *Pycreus sanguinolentus*, *P. flavescens* and *Queenslandiella hyalina*, xylem vascular elements appear first within the rachilla and glumes (figs 5.11A, 5.13A & 5.20A). The vascular bundles within the rachilla of the *Pycreus* species studied run in two opposite laterally positioned vascular zones (figs 5.11A & 5.15B, 5.16). In contrast, the rachilla in *Cyperus laevigatus* contains three groups of vascular bundles (fig. 5.12C, 5.18B) and a single group is present in *Kyllinga nemoralis* (fig. 5.15A, 5.17).

In the flowers, vascular bundles start to develop only after all floral organs have been initiated (figs 5.11A & 5.12B, 5.13). At this stage, the stigma primordia are developing on the top of the ovary wall and the developing ovule starts to turn towards the abaxial base. The development of the xylem vessels is initiated within the receptacle of the flowers (fig. 5.11A). From there, connections to the vascular bundles of the rachilla (receptacular bundles) and to the different floral organs are formed (fig. 5.11A, 5.13C). In the *Pycreus* and *Queenslandiella* species studied, only two receptacular bundles are present (figs 5.11A, 5.13A & 5.15B, 5.17C), whereas in the receptacle of the *Cyperus* species studied, three bundles are observed (figs 5.12B & 5.15C).

Stamens are the first floral organs in which vascular bundles originate (staminal bundles, figs 5.11A & 5.12B, 5.13A) and these subsequently connect to the developing bundles in the receptacle, which are at this stage not yet fully connected with the vascular traces of the rachilla. Within the receptacle of *Cyperus laevigatus* and *C. haspan*, a single trace branches off from each main receptacular bundle towards the stamen in the corresponding position. In *Pycreus* and *Queenslandiella*, there is no abaxial receptacular bundle and the vascular bundle of the abaxial stamen connects to both adaxial bundles (figs 5.11A, 5.13C & 5.15B, 5.16). Subsequently and only after the stamens are fully vascularised and connections between vascular bundles in the rachilla and receptacle are made, vascularisation appears in the ovary wall and ovule (figs 5.11A, 5.12B & 5.20A). Meanwhile, the receptacular vascular network becomes denser towards adaxial and abaxial positions and towards the centre (figs 5.11, 5.12C & 5.15B, 5.16). In *C. laevigatus*, there are no connections between the abaxial receptacular bundle and the receptacular plexus (fig. 5.12C). In all species studied, the positions of the dorsal vascular bundles within the ovary wall are in line with the positions of the stigma primordia. Consequently, in *Cyperus haspan*, the ovary wall has two lateral and

one abaxial dorsal bundles (fig. 5.12D), while in *C. laevigatus*, only the two lateral ones are present (figs 5.12B–C & 5.15C, 5.18B), in both species dorsal bundles run in the prolongation of the main receptacular bundles. Both in *Cyperus haspan* and *C. laevigatus* (figs 5.12 & 5.15C, 5.18) lateral bundles seem to be absent. *Rhynchospora corymbosa* is characterised by a very long style (fig. 5.14A), bearing two very short stigma branches at its top (fig. 5.14B). Within the whole gynoecium and style two dorsal vascular bundles run in lateral positions (fig. 5.14C), revealing the dorsiventrally flattened, dimerous nature of these pistils. In *Pycreus* and *Kyllinga*, a single adaxial and a single abaxial bundle are observed (figs 5.11A–B & 5.15A–B, 5.16 & 5.17), which do not run in the prolongation of the two main receptacular bundles but merge with the vascular plexus in the receptacle. The ventral bundles that run towards the centre fuse to form the central bundles, eventually connecting to the developing ovule vascular bundle (figs 5.11B, 5.12C & 5.15B, 5.16). In *C. laevigatus*, this connection appears to be eccentric (fig. 5.12C). The vascular bundles of the ovary wall (dorsal bundles) and ovule (ovule bundles) independently connect to the vascular bundles of the receptacle (figs 5.11B, 5.12C–D & 5.15B, 5.16). Meanwhile, the xylem vessel elements of the vascular bundles within the rachilla, glume and receptacle are become denser (figs 5.11A–B & 5.12C, 5.13B).

In *Kyllinga nemoralis*, the vascular bundles are concentrated into two laterally situated zones as they enter in the receptacle of the flower (fig. 5.15A, 5.17B). These soon split into two sets of three separate centres (fig. 5.15A, 5.17C). These centres expand abaxially and adaxially (fig. 5.15A, 5.17D–F) until eventually a ring of vascular bundles is formed (fig. 5.15A, 5.17G). Subsequently the staminal traces and the adaxial dorsal bundle are branching off from the bundle ring (fig. 5.15A, 5.17G) followed by the abaxial dorsal bundle and several traces, which eventually fuse in the centre to form the ovule vascular bundle (fig. 5.15A, 5.17). The position of the abaxial ovary wall bundle is not on the symmetry plane formed by the rachilla and glume (fig. 5.15A, 5.17).

Xylem elements differ in length and width among different organs. Rachilla and glume xylem bundles consist of long elements and the vascular tissue of the receptacle consists of a dense network of many short vessels, which results in a vascular plexus. The xylem bundles within the floral organs consist of only one or few long and narrow annular vessels (figs 5.11A–B & 5.12C, 5.13A).
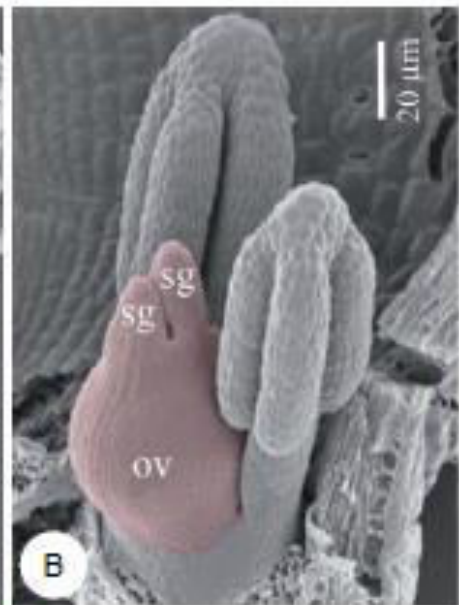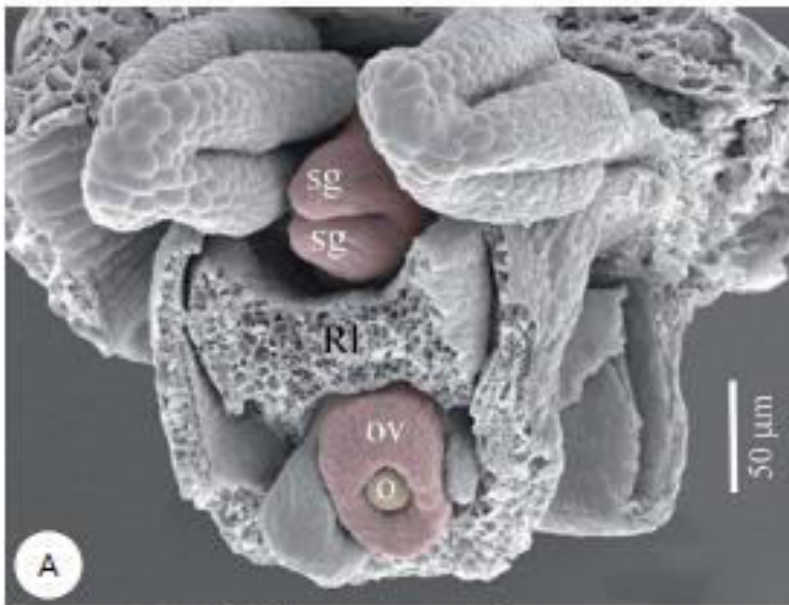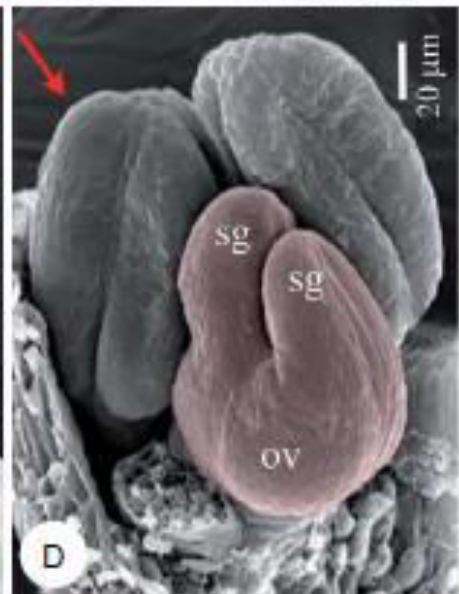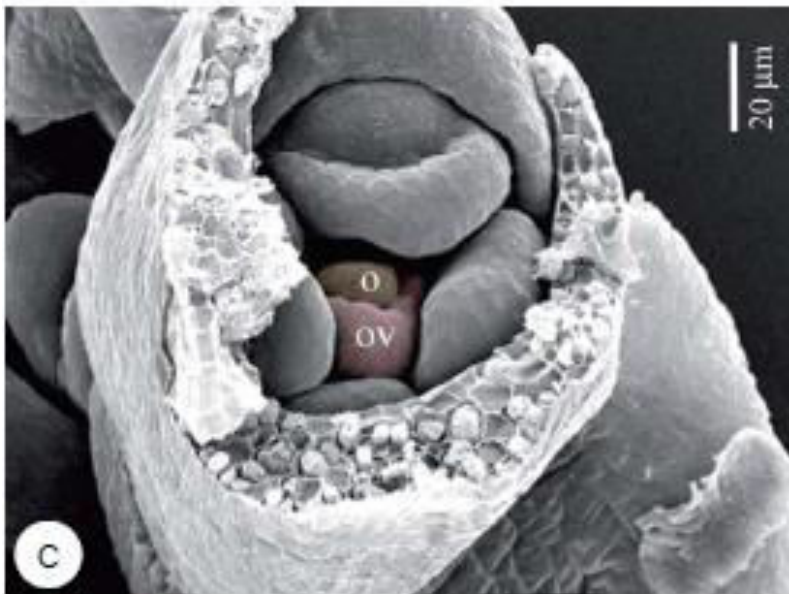
5.5.4 Ovule development

The ovule primordium is formed from the apex of the flower primordium, simultaneously with the annular ovary wall primordium (figs 5.6–5.9 & 5.19A). Once the developing ovary wall encloses it, the ovule primordium starts to differentiate, (figs 5.11A, 5.19B & C). Subsequently and in successive order the interior and exterior teguments are formed, defining the micropylar zone (figs 5.19B, 5.20A & 5.21A). At this stage, thre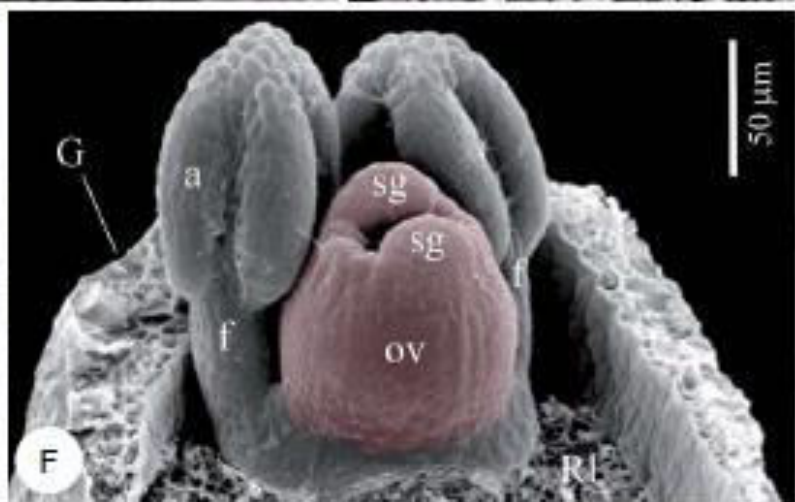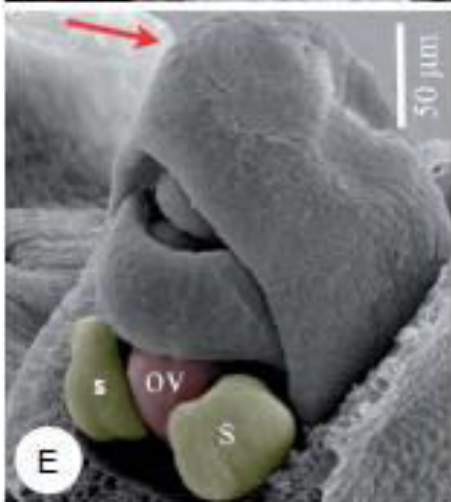e layers in the ovary wall start to differentiate (figs 5.19B & 5.20A). Meanwhile the ovule primordium grows out cylindrically, bending so that the micropylar zone forms an angle of 90°, directed to the abaxial side of the flower (figs 5.19B & 5.21C). When reaching this stage, the xylem of the vascular traces of pistil and ovule becomes visible (fig. 5.20A). The bending concurs with an elongation, continuing until the micropyle is turned over 180° and positioned against the basal-abaxial part of the funiculus (figs 5.19C & 5.21D). Meanwhile, at the basal and ventral (abaxial) part of the funiculus, numerous 'obturator hair' primordia originate (figs 5.19C & 5.21B–D). They develop fast in the direction of the micropyle, often sticking together to form a kind of cover, which closes the micropyle (figs 5.15A–C, 5.16A, 5.17, 5.18A, 5.19C, 5.21C & D).

← Fig. 5.9 SEM images of the development of the gynoecium in *Pycreus bipartitus* (A–B), *Kyllinga microbulbosa* (C–D) and *Queenslandiella hyalina* (E–F), species with laterally flattened gynoecia. A, apical view of a middle part of a spikelet in *P. bipartitus* with the distal part of it removed. Two alternate flowers at different developmental stages are visible. The in the image lower (youngest) flower has a still open, developing gynoecium with annular ovary wall (red) surrounding a central ovule primordium (amber). In the upper (oldest) flower, the ovary wall (red) envelops totally the ovule, and two dorsiventrally situated stigma branch primordia are growing out; B, developing flower in *P. bipartitus* with laterally flattened, developing pistil (red); C, developing flower with annular ovary wall primordium (red) surrounding a central ovule primordium (amber) in *K. microbulbosa*; D, developing gynoecium in *K. microbulbosa* with two dorsiventrally placed stigma branches. The red arrow indicates the abaxial stamen. The left lateral stamen is removed; E, lateral view of the distal part of a developing spikelet in *Q. hyalina*. Proximally, a developing flower is visible with two lateral stamens (yellow) and an ovary (red). The rachilla apex is hidden by older bonnet-shaped glume (arrowed); F, adaxial view of a developing flower in *Q. hyalina*. The ovary wall (red) is rising from the base and enveloping the central ovule. Two dorsiventrally positioned stigma primordia are visible on the top of the ovary wall. The stamen primordia have developed into anther and filament.

Abbreviations: a, anther; f, filament; G, glume; o, ovule; ov, ovary wall (primordium); Rl, rachilla; s, stamen primordium; sg, stigma primordium.
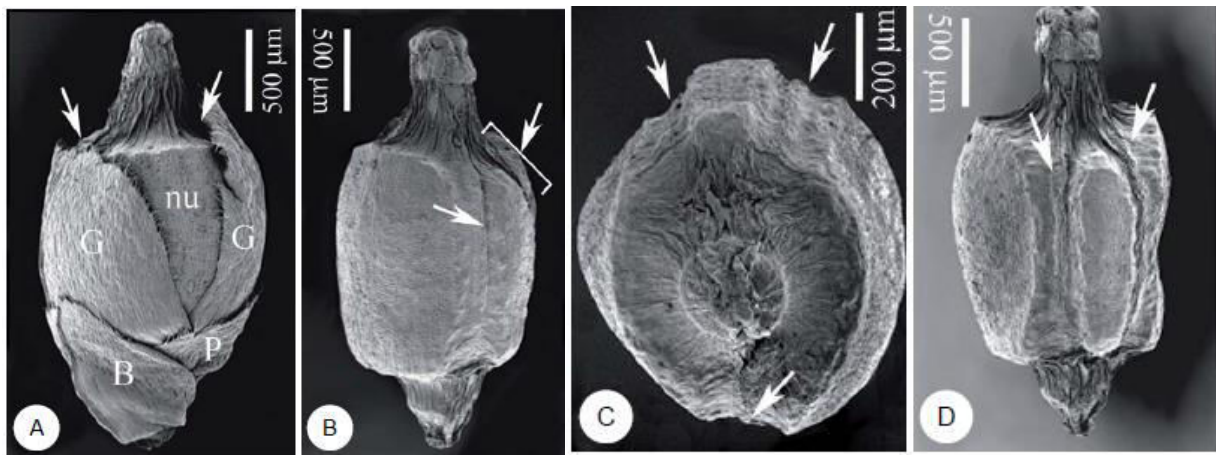
Fig 5.10 SEM pictures of mature spikelets and nutlets in *Lagenocarpus amazonicus*, a species with laterally compressed dimerous nutlets. A, lateral view of a spikelet showing the ridges of a typical nutlet to lie in the symmetry plane formed by the bract, prophyll and glumes; B, lateral adaxial view on an atypical nutlet showing one fully developed ridge and one partially developed ridge at the adaxial side of the nutlet. The abaxial ridge is not visible; C, apical view of an atypical nutlet with one abaxial and two adaxial ridges; D, adaxial view of the same nutlet as in C showing two fully developed adaxial ridges. Abbreviations: B, spikelet bract; G, glume; nu, nutlet; P, prophyl. Arrows indicate the positions of the ridges of the different nutlets.

5.6 Discussion

5.5.1 Gynoecial ontogeny: congenitally fused carpels

According to Payer (1857) and never previously tested (Vrijdaghs *et al.,* 2009), a cyperoid gynoecium originates from individual carpel primordia, which fuse postgenitally (as can be observed in e.g. *Sansevieria*, fig. 5.5). However, our results show that in the earliest developmental stages of the gynoecium in all species studied, no individual carpel primordia are present (e.g. fig. 5.6–5.9). Instead, the ovary wall originates as a ring primordium surrounding the central ovule primordium. This annular ovary wall primordium grows up as a bag-like structure, as also observed by Payer (1857: 699), who called it a 'sac ovarien'. We agree with Payer (same page) that the development of the stigma branches originates from "deux ou trois bourrelets primitifs", two or three primitive bulges on the top of the rising ovary wall, which we call stigma primordia since they are distinct meristematic zones positioned upon a structure with different nature (the ovary wall) and determined to grow out as stigma branches. In summary, in all cyperoid species studied, the floral apex consists of a fusion of the floral axis and the (three) carpels. From this tissue, both the annular ovary wall primordium, and the single, centrally positioned ovule primordium originate. The carpel tips (stigma primordia), from which the stigma branches develop subsequently can be considered
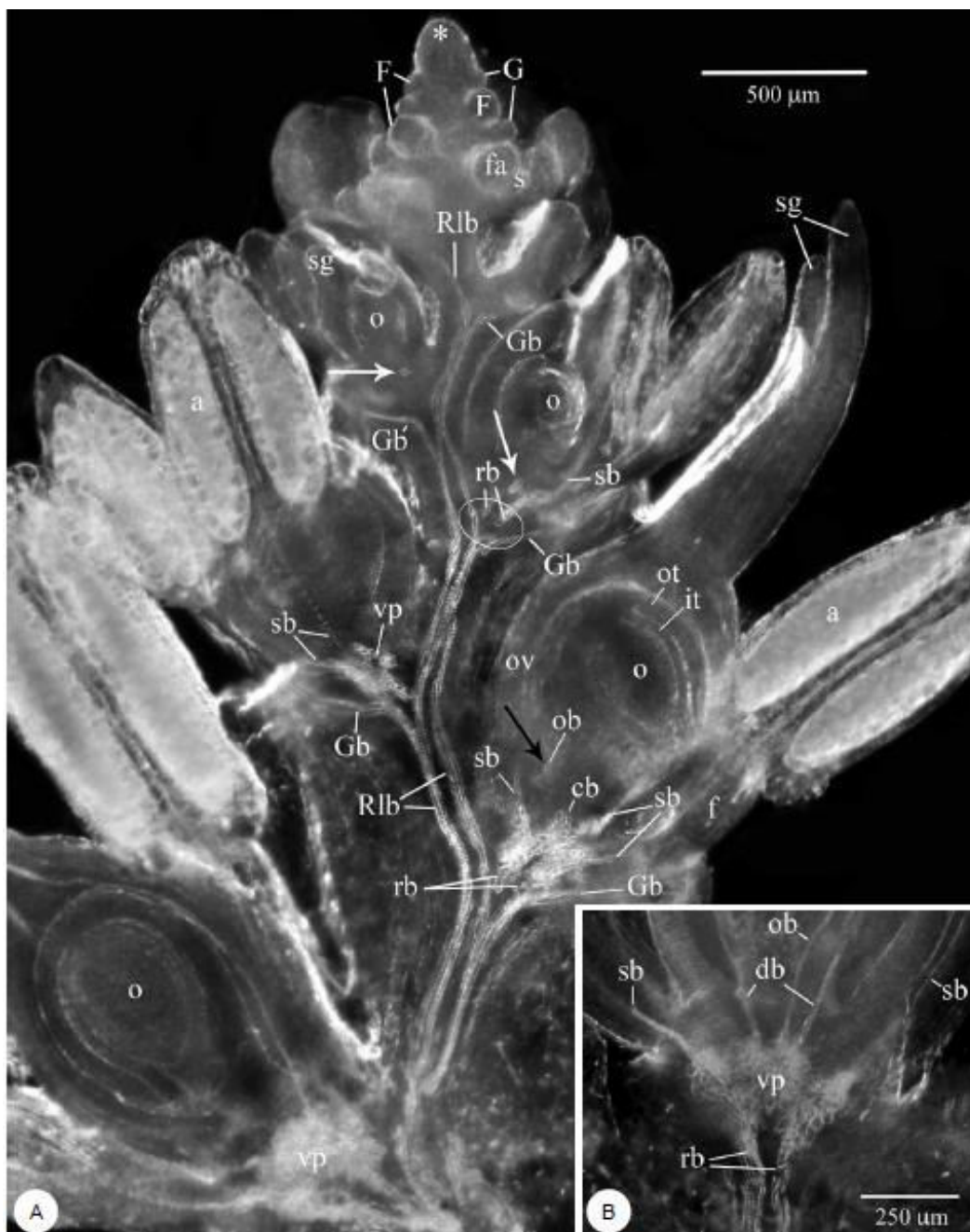
Fig. 5.11 Dark field images of a cleared up spikelet in *Pycreus sanguinolentus*, with laterally compressed dimerous gynoecia. Proximate glumes are removed. A, lateral view of a spikelet showing the gradual development of the vascular system within consecutive flowers; B, adaxial view of the base of a flower with all vascular traces developed. Abbreviations: a, anther; cb, central vascular bundle; db, dorsal vascular bundle; F, flower (primordium); f, filament; fa, flower apex; G, glume (primordium); Gb, glume vascular bundle; it, inner tegument; o, ovule (primordium); ob, ovule vascular bundle; ot, outer tegument; ov, ovary wall; rb, main receptacular vascular bundle; Rlb, rachilla vascular bundle; s, stamen (primordium); sb, staminal vascular bundle; sg, stigma primordium; vp, vascular plexus (ventral bundles); asterisk (*), apex of the rachilla. White arrows indicate procambial initiation points within the receptacle. The black arrow indicates developing vascular connections of the ovule. A white ellipse indicates the developing vascular bundles within the base of the receptacle, which are still unconnected with the rachilla vascular bundles.
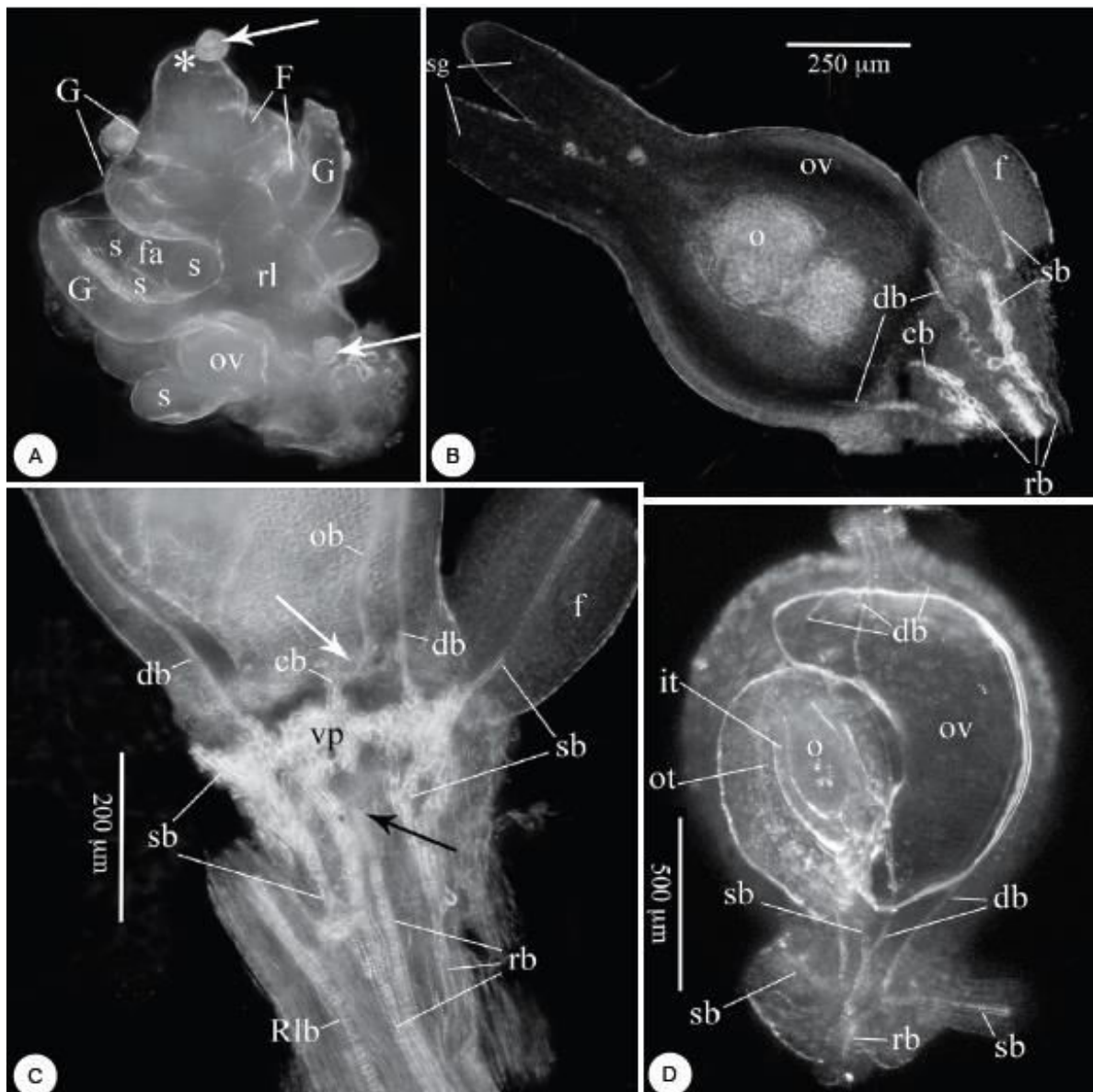
Fig. 5.12 Dark field images of cleared up spikelets and flowers in *Cyperus laevigatus* (A–C) that has dorsiventrally flattened dimerous gynoecia and *Cyperus haspan* (D), with a trimerous gynoecium. A, lateral view of a spikelet tip of *C. laevigatus* showing consecutive developing flowers. Vascular bundles have not yet originated. White arrows indicate pollen grains; B, adaxial view of an immature flower of *C. laevigatus* showing development of the vascular bundles towards the ovary wall, ovule and stamens; C, adaxial view of a mature flower of *C. laevigatus* with densification of vascular bundles within the receptacle. Vascular bundles of all floral organs have differentiated. The white arrow shows the eccentric connection of the ovular vascular bundle with the central vascular bundle. A black arrow indicates the unconnected abaxial receptacular bundle; D, young fruit of *C. haspan* in lateral view showing the three dorsal bundles within the ovary wall. Abbreviations: cb, central vascular bundle; db, dorsal vascular bundle; F, flower primordium; f, filament; fa, flower apex; G, glume (primordium); it, inner tegument; o, ovule (primordium); ob, ovule vascular bundle; ot, outer tegument; ov, ovary wall; ovb, ovary wall vascular bundle; rb, main receptacular vascular bundle; rl, rachilla; Rlb, rachilla vascular bundle; s, stamen (primordium); sb, staminal vascular bundle; sg, stigma primordium; vb, ventral bundle; asterisk (*), apex of the rachilla.
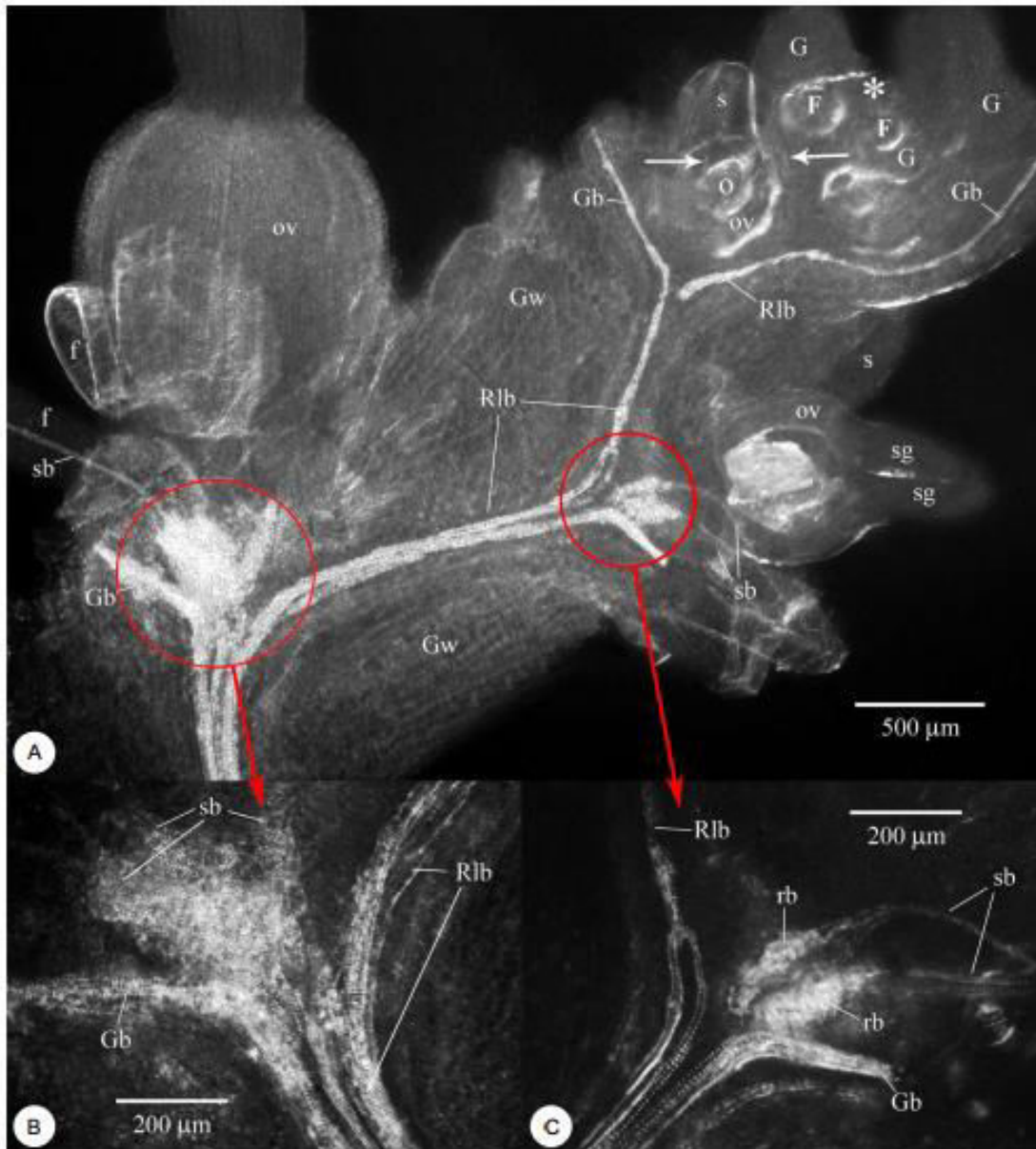
Fig. 5.13 Dark field image of a cleared up developing spikelet in *Queenslandiella hyalina*, with laterally compressed dimerous gynoecia. Proximate glumes are removed. A, lateral view of a spikelet tip showing the gradual development of the vascular system within consecutive flowers. The vascular traces of the ovary wall and ovule have not yet developed. Zones encircled in red are enlarged in B & C; B, detail of the base of a young flower showing a densification of the vascular bundles within the receptacle. Receptacular bundles are connected with the vascular system of the rachilla; C, detail of the base of a young flower showing development of the vascular system towards the stamens. Receptacular bundles are not yet connected to the vascular system of the rachilla. Abbreviations: db, dorsal vascular bundle; f, filament; F, flower (primordium); G, glume (primordium); Gb, glume vascular bundle; Gw, glume wing attached to the rachilla; o, ovule; ov, ovary wall; rb, main receptacular vascular bundle; Rlb, rachilla vascular bundle; s, stamen (primordium); sb, stamen vascular bundle; sg, stigma primordium; asterisk (*), apex of the rachilla. White arrows indicate developing vascular traces within the rachilla and stamens.

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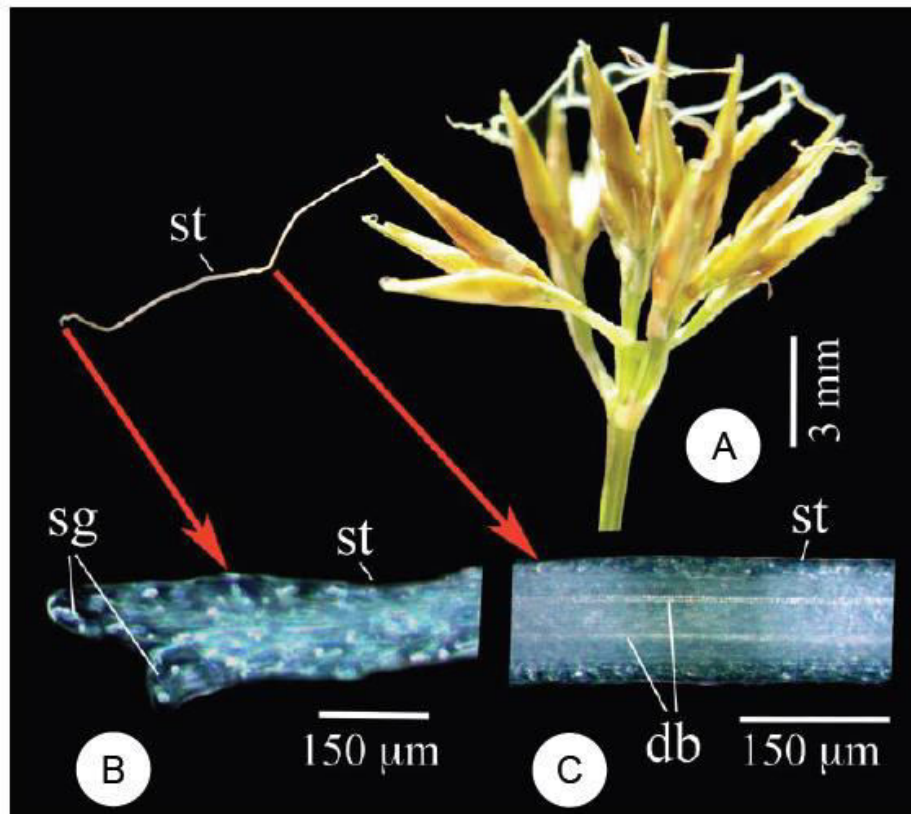
Fig. 5.14 Pseudomonomery in *Rhynchospora corymbosa*, which has dorsiventrally flattened dimerous gynoecia. A, part of an inflorescence showing the long and single styles emerging from the tips of the spikelets. Red arrows indicate the parts of the style from which details are shown in B & C; B, dark field microscopy picture of a cleared up tip of the style showing the presence of two stigma rudiments; C, dark field microscopy picture of a cleared up middle part of the style showing two dorsal vascular bundles.

Abbreviations: db, dorsal bundle; sg, stigma; st, style.

as ontogenetic 'witnesses' of the carpellary origin of the ovary wall. Only when the stigma primordia originated on the top of the rising ovary wall, the positions of the original carpels become clear. Subsequently, vascular traces are initiated in these primordia, which will connect with the stele and give form to the ribs of the gynoecium/fruit. As carpel positions are quite well conserved (Endress, 1995, 2001), it is not surprising that in the majority of Cyperoideae, the pistil is triangular with three stigma branches. However, the annular origin of the ovary wall gives it de facto new freedom of organisation since the stigma primordia are no longer linked to the rigid positions of individually developing carpels as found in most other monocots (fig. 5.22). We hypothesise that in Cyperoideae, the acquired organisational freedom might be reflected in the derived, dimerous pistils in at least some of the most

recent, rapidly radiated taxa in the C_4 clade of the giant genus *Cyperus* with laterally flattened gynoecia, such as *Pycreus*, *Kyllinga* and *Queenslandiella* (fig. 5.9) (Larridon *et al.,* 2011b).

5.6.2 Consequences of the presence of an annular ovary primordium

In our opinion, the observations of Endress (2006) and Vrijdaghs *et al.,* (2005a) are examples of how congenital fusion of individual primordia into an annular primordium creates the condition to develop something totally new. Also for the Cyperoid pistils, several consequences of the presence of the annular pistil primordium can be identified especially involving alterations in the amount and positions of stigma primordia.

Decoupling of gynoecium wall and ovule – Firstly, in Cyperoideae, the development of ovary and ovule seem to be decoupled. This is already visible in the earliest stages of pistil, where the ovule starts developing already before the ovary has closed above it (fig. 5.23). Ovules, which appeared in the evolution much earlier than carpels, are to be considered as individual organs (Endress 2006). While in most angiosperms ovules are superimposed on carpels, the development of carpels and ovules appear to be decoupled in Cyperoideae. Similar patterns in early ovary development have been reported for other angiosperm families with syncarpous fruit types and basal uniovulate placentation such as Asteraceae (Harris, 1995), Chenopodiaceae (Flores Olvera *et al.,* 2008, 2011) and advanced Poaceae (Philipson, 1985). Vascular evidence and other developmental characteristics will be discussed in a separate chapter on ovules.

Dedoublements and polymerisations – As the stigma branches are supposed to grow from meristimatic zones in the carpel tips (stigma primordia), their number (in Cyperaceae usually three) reflects the number of original carpels. However, due to the congenital fusion of the carpel primordia, the number of stigma branches does not necessarily reflect anymore the original number of carpels. Moreover, splitting of a given primordium (dedoublement) is a common phenomenon, which can also result in deriving numbers of stigma branches. E.g. during our studies, we observed specimens with four stigma branches instead of three in *Cyperus capitatus* Vand. (Vrijdaghs *et al.,* 2011) and *Scirpoides holoschoenus* (fig. 5.6D). In a similar way, in taxa with laterally flattened dimerous pistils, we frequently found flowers with three instead of two stigma branches (e.g. in *Pycreus bipartitus* and *P. flavescens*, Vrijdaghs *et al.,* 2011).
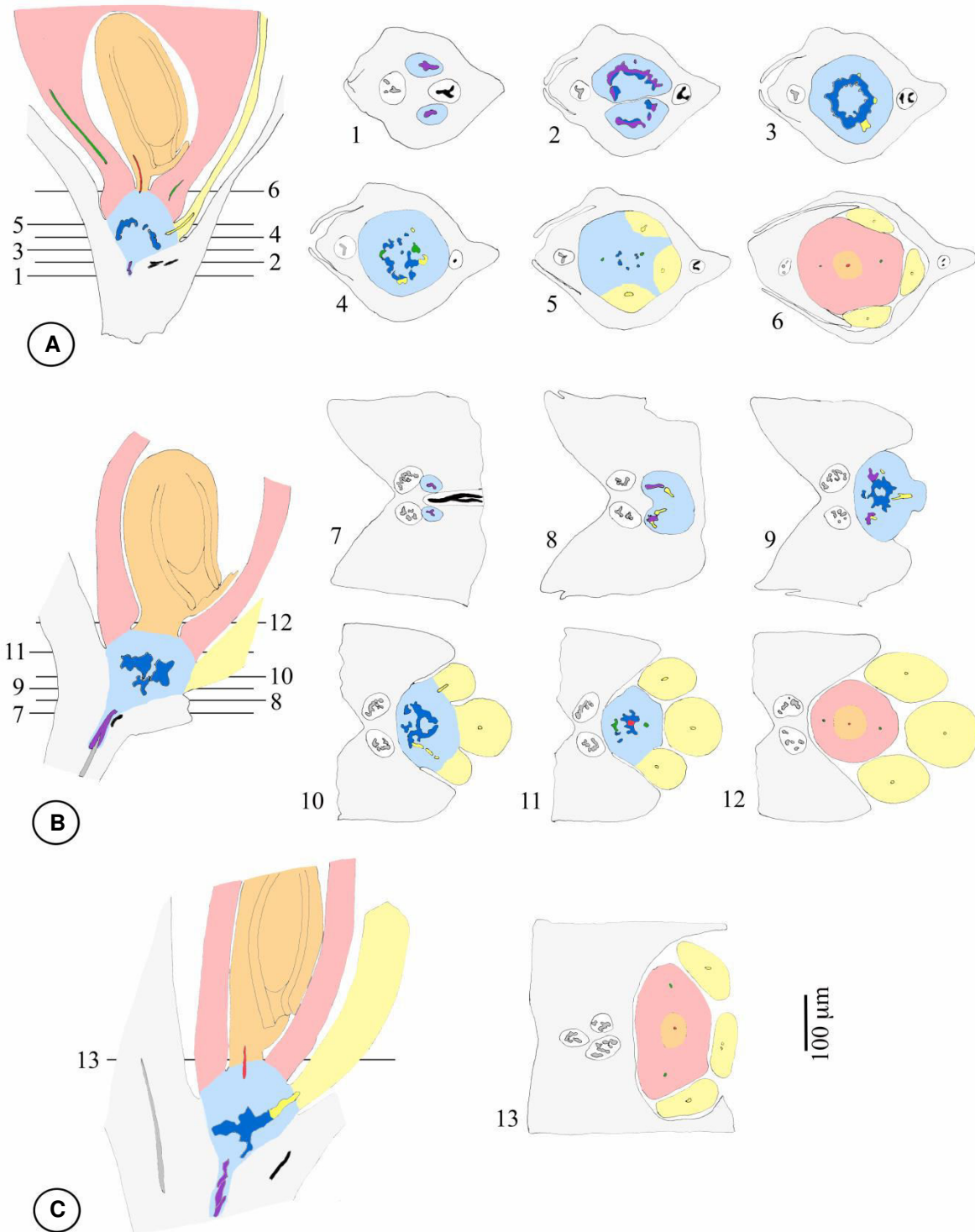
Fig. 5.15 Diagrams of sections through some Cyperoid flowers based on phase contrast microscopy pictures. A, *Kyllinga nemoralis*, with laterally compressed dimerous pistils. B, *Pycreus flavescens*, with laterally compressed dimerous pistils. C, *Cyperus laevigatus*, with dorsiventrally compressed pistils. The first diagram of each series shows a longitudinal section, subsequent transversal slices are indicated with black lines and numbered on the diagrams. Colour codes floral organs: pale blue, receptacle; pale green, gynoecium wall; pale red, ovule; pale yellow, stamens; grey, rachilla and glume. Colour codes vascular bundles: black, glume bundle; blue, receptacular plexus (ventral bundles); green, dorsal bundles; grey, receptacular bundle; red, ovule bundle; purple, main receptacular bundle; yellow, stamen bundle.

In *Lagenocarpus amazonicus* (fig. 5.10B–D), we observed nutlets with three instead of two ribs, in that case one of the adaxial ribs sometimes only partially developed. In our opinion this is rather a reversal to the plesiomorphic trimerous state. In several Cyperoid species (see table 5.3), such as *Carex dolichostachya* Hayata (described by Hayata (1921) as a separate genus *Diplocarex* Hayata) and *C. concinnoides* Mack. (Snell, 1936), populations with four to six stigma branches were reported. In addition many rhynchosporoid species show a constant number of four (*Tetraria* p.p., *Tetrariopsis*), six (*Neesenbeckia*) or even eight (*Evandra*) to nine (*Tetraria p*.p.) stigma branches (Goetghebeur, 1998). Also in Mapanioideae, Kern (1974) reported that six stigma branches occur often in *Chrysitrix* L. and in *Paramapania* Uittien (Mapanioideae) he observed tetramerous pistils, most often in species which have normal trimerous pistils. In *Paramapania gracillima* (Kük. & Merr.) Uittien, tetramerous pistils are the dominant type (Kern, 1974). The multiplication of the number of stigmas has not yet been clarified and may be part of a larger polymerisation phenomenon in these taxa. This 'polymerisation phenomenon' is reflected in the entire floral organisation, including perianth and androecium as well as the culm. It seems logical that the tetra- and octamerous pistils in the taxa mentioned above, are most probably polymerizations of dimerous pistils. Nevertheless we believe both dedoublements and polymerizations to be facilitated by congenitally fused carpels.

Facilitation of pistil dimerisations – Reductions in carpel numbers are common in Poales and usually, these are explained by reduction or fusion of carpels (e.g. Philipson, 1985). Where reduction tendencies occur, a carpel (predestined to be reduced) first becomes sterile (e.g. in Eriocaulaceae, Ronse Decraene *et al.,* 2002) and due to the rigid position of the carpel primordia, reduction of an adaxial carpel mostly results in an asymmetric gynoecium. Dimerisation may also result from a fusion between two of the three carpels, of which at least one is mostly sterile. The presences of multiple dorsal bundles are generally considered as proof of such a fusion product (e.g. Linder, 1992). This widely adapted classic carpel theory is difficult to apply for dimerous Cyperoid genera since all carpels are congenitally fused. We believe the ring primordium facilitates dimerisations since these can be established by a simple loss of one of the stigma primordia together with all vascular traces at this side of the gynoecium wall, instead of a gradual reduction of a complete carpel. Especially dorsiventrally flattened dimerous pistils can be explained by the loss of the abaxial stigma primordium (e.g. *C. laevigatus*, figs 5.12 & 5.15C). The multiple origins of this pistil type within many different
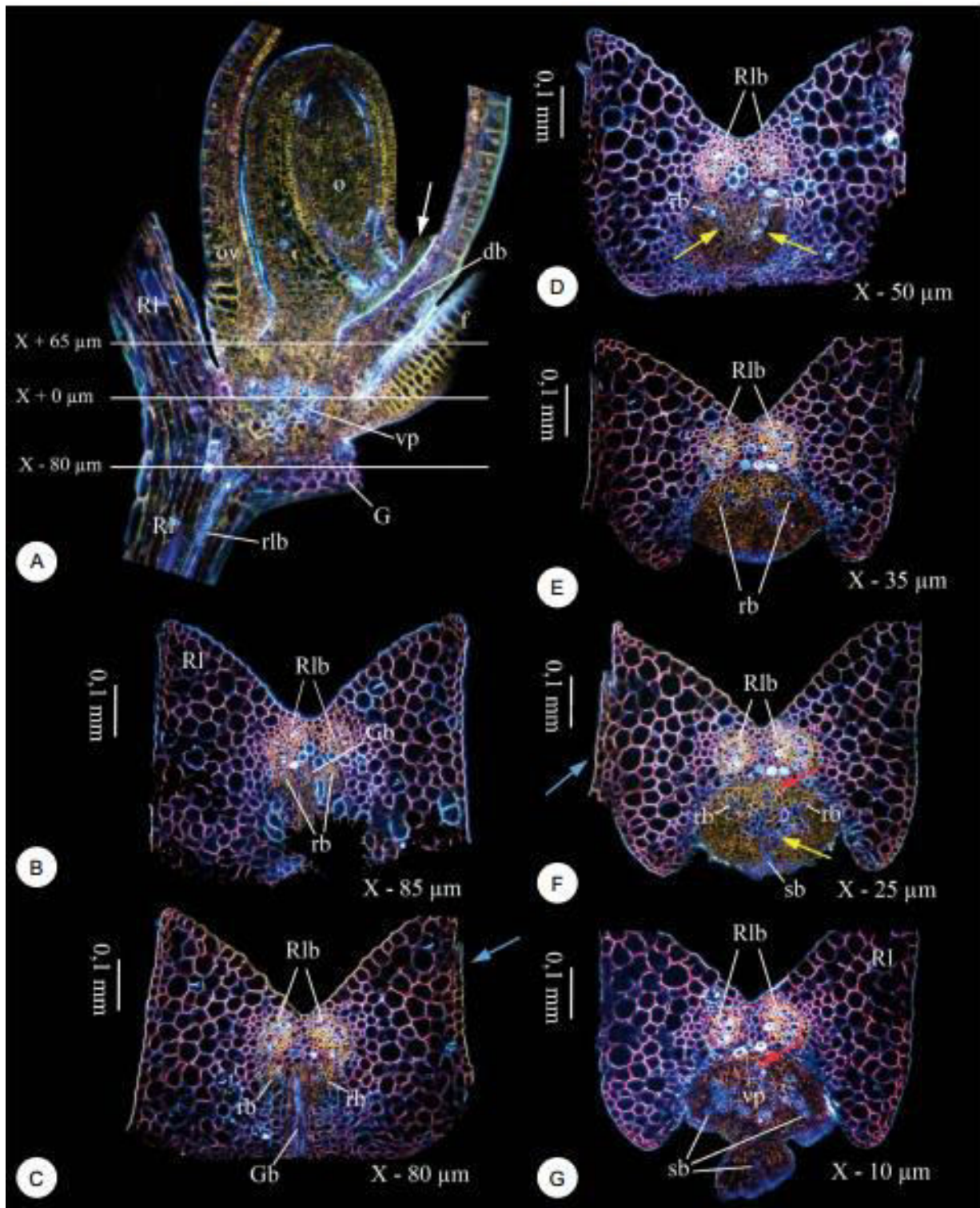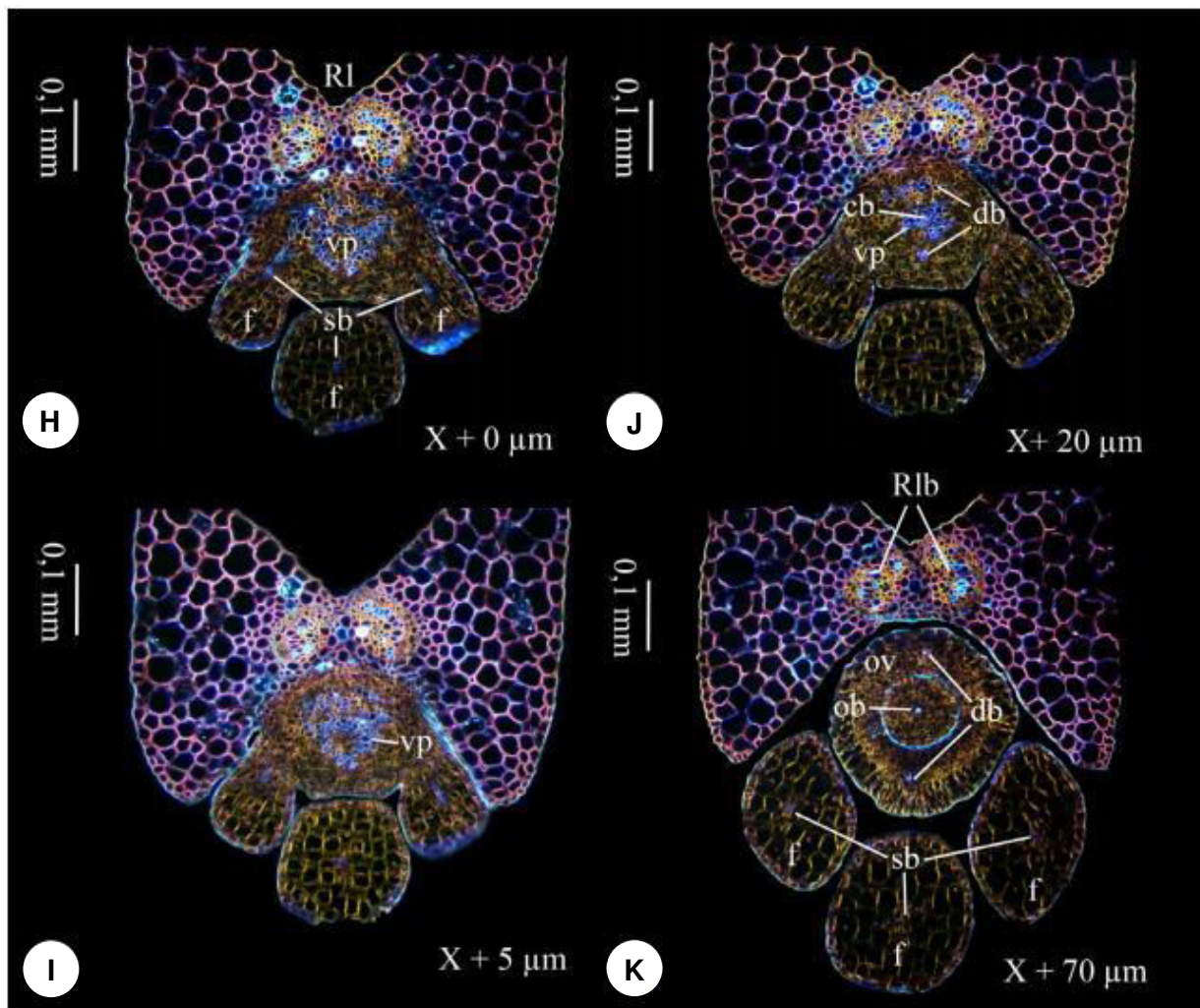
Fig. 5.16 Phase contrast pictures of sections through a flower of Pycreus flavescens, with laterally compressed dimerous gynoecia. A, radial section of a spikelet node showing a single flower in lateral view. White lines indicate several positions of transverse sections. The white arrow indicates the obturator hairs; B–K, subsequent transverse sections trough a spikelet node showing the vascularisation of the receptacle. Blue arrows show glume wings attached to the rachilla by epicaulescent growth (see Vrijdaghs *et al.,* 2010). Yellow arrows indicate the connection of the abaxial staminal trace to both receptacular bundles. Red arrows show ventral bundles towards the adaxial pole.

Abbreviations: cb, central bundle; db, dorsal vascular bundle; f, filament; G, glume; Gb, glume vascular bundle; o, ovule; ob, ovule bundle; rb, main receptacular vascular bundle; Rl, rachilla; Rlb, rachilla vascular bundle; sb, staminal vascular bundle; vp, vascular plexus (ventral bundles).
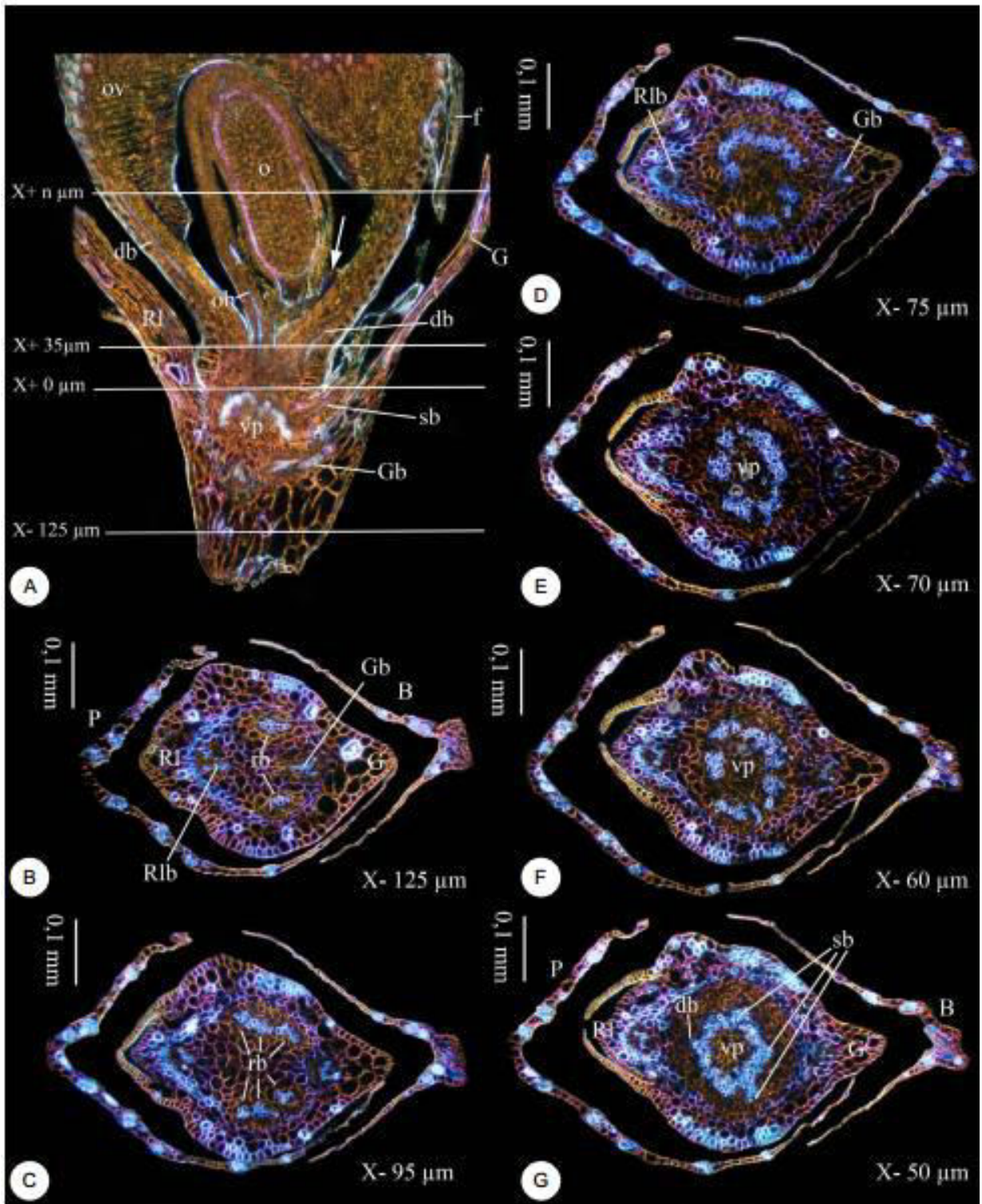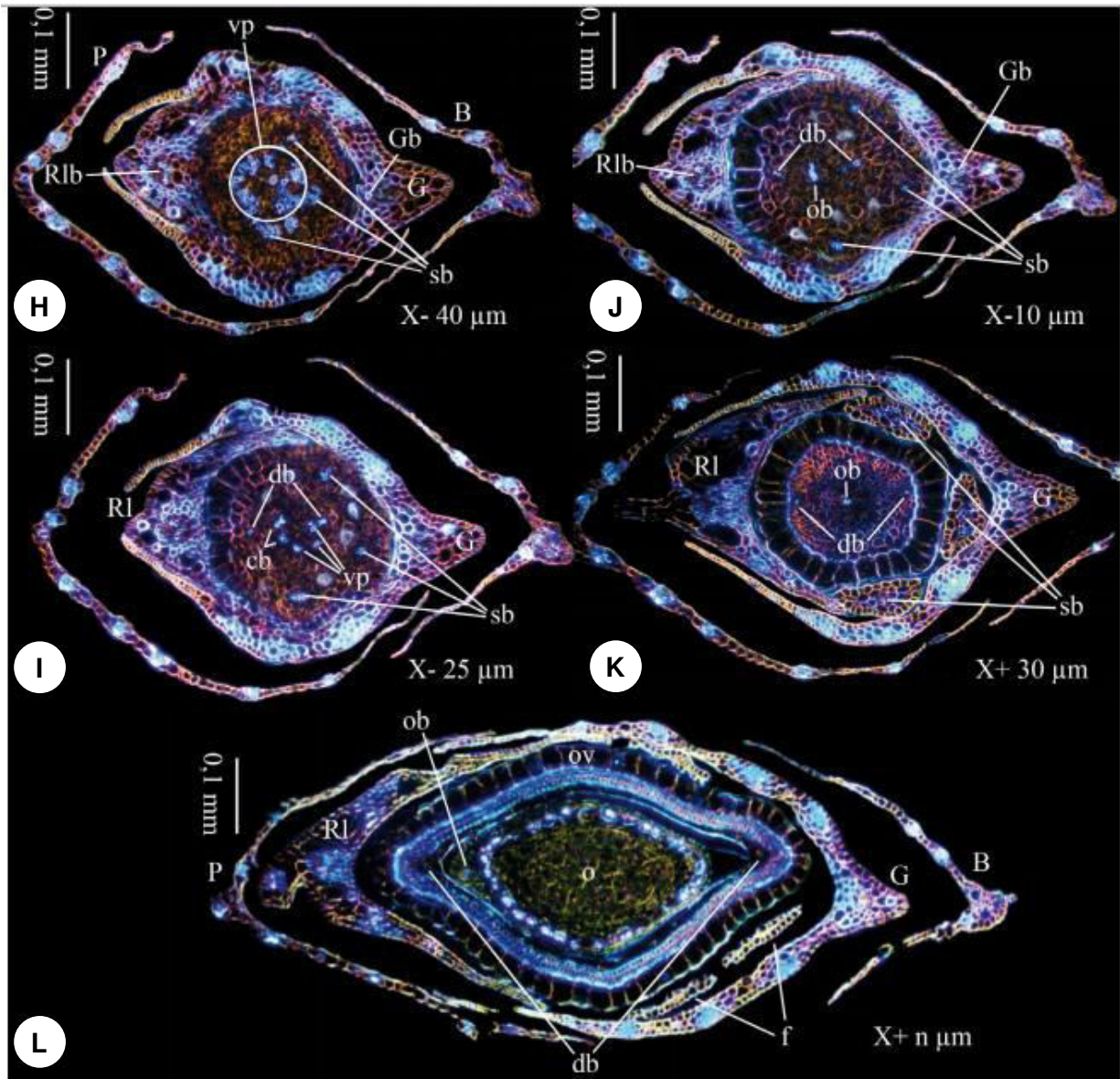
Fig. 5.17 Phase contrast pictures of sections through a flower of *Kyllinga nemoralis*, with laterally compressed dimerous gynoecia. A, radial section of a spikelet node showing the single flower in lateral view. White lines indicate several positions of transverse sections; B–L, subsequent transverse sections trough a spikelet node showing the vascularisation of the receptacle.

Abbreviations: B, (spikelet) bract; cb, central vascular bundle; db, dorsal vascular bundle; f, filament; G, glume; Gb, glume vascular bundle; o, ovule; ob, ovule vascular bundle; ov, ovary wall; P, (spikelet) prophyll; rb, main receptacular vascular bundle; Rl, rachilla; Rlb, rachilla vascular bundle; sb, staminal vascular bundle; vp, vascular plexus (ventral bundles).
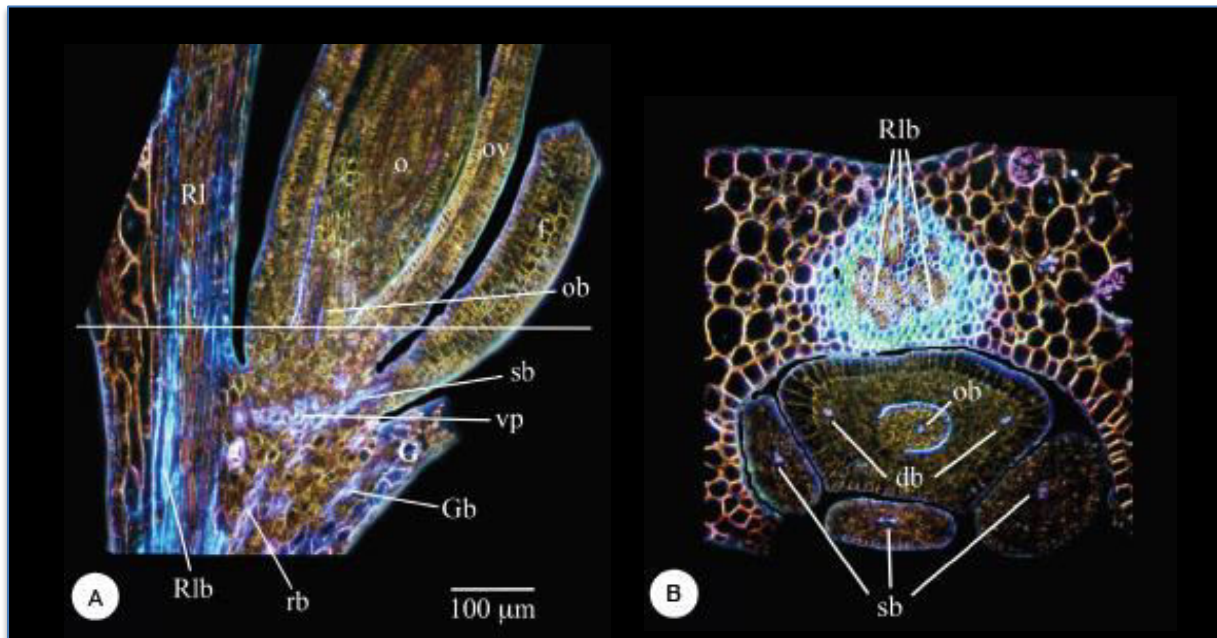
Fig. 5.18 Phase contrast pictures of sections through a spikelet of *Cyperus laevigatus*, with dorsiventrally compressed dimerous gynoecia. A, radial section of a spikelet node showing one flower in lateral view. The white lines indicate the transverse section in B; B, transverse section trough a spikelet node showing the vascularisation of the rachilla and the floral organs.

Abbreviations: db, dorsal vascular bundle; f, filament; G, glume; Gb, glume vascular bundle; o, ovule; ob, ovule vascular bundle; ov, ovary wall; rb, main receptacular vascular bundle; Rl, rachilla; Rlb, rachilla vascular bundle; sb, staminal vascular bundle; vp, vascular plexus (ventral bundles).

genera, representing almost all Cyperoid tribes (table 5.3), corroborates the relative ease in which this pistil type can be derived from a trimerous pistil due to the presence of congenitally fused carpels (fig. 5.22D). The situation in laterally flattened pistils seems to be more complex since it involves stigmata in novel positions (fig. 5.22C). Therefore, Goetghebeur (1986) remarked that it was not possible to explain the origin of laterally compressed pistils as a result from simple carpel reductions. However, now we understand the laterally compressed pistil as a result of newly acquainted organisational freedom due to (1) the invention of annular ovary primordia and (2) the initiation of floral vessels in the different floral primordia. Subsequently, the initiated vessels grow to and connect with the stele. Probably, spacial pressure in compact inflorescences/spikelets triggers in *Pycreus* the gynoecium adaptation from trimerous to dimerous(reduction in number of stigma branches) and laterally compressed.

Alterations in stigma positions – In most monocots, carpels develop as independent units that may (or not) fuse postgenitally with adjacent carpels (fig. 5.5). Consequently, at later developmental stages, their positions remain strongly conserved and this is even still the case for all trimerous Cyperaceae. However, in the laterally flattened dimerous pistils, this pattern is lost since they have two carpels in median positions (fig. 5.22C). Remarkably, both adaxial stigmata encountered in a regular cyperoid pistil are now replaced by a single stigma in an intermediate position. These pistils should therefore be considered as an exception of Endress' (1995) observation concerning well-conserved carpel positions in angiosperms.

Blaser (1941a) explained the nature of this pistil type by reorganisation of the vasculature. However, our observations (summarised in fig. 5.24A, B & C) falsifies Blaser's theory, which is further explained in the chapter on anatomical evidence. We believe that due to the organisational freedom in a congenitally fused carpel complex, after loss of one of the adaxial stigma primordia, the remaining stigma primordium develops in a more optimal position concerning the available space on the ring primordium with respect to the other stigma primordia, which is in the case of *Pycreus*, *Kyllinga* and *Queenslandiella*, opposite to the abaxial carpel (figs 5.9, 5.15A–B & 5.22C). This is the organisational freedom that we assume to be a result from congenital fusion of carpels. In cases where an additional adaxial stigma primordium is formed in a few individual flowers as a developmental error, these shift again out of the intermediate position (e.g. *Pycreus*, Vrijdaghs *et al.,* 2011, *Lagenocarpus amazonicus*, fig. 5.10B–D) into a spatially more optimal position. A similar situation in which a single carpel in an intermediate position where originally two carpels were present has also been described in Eriocaulaceae and hypothesised to have resulted from the fusion of sterile carpels and their dorsal bundles (Ronse Decraene *et al.,* 2002). We can assume congenitally fused carpels of Cyperoideae (which are decoupled from the ovule) can behave in a similar way as fused sterile carpels in other taxa. However, our findings on vascular development (see chapter on anatomical evidence) do not support the idea of fusion of two dorsal bundles to a single bundle in an intermediate position but support our theory that stigma primordia can shift to more optimal positions in some complexes of fused capels after loss of additional stigma primordia.

All dimerous pistils found in Cyperoideae can thus be explained by a combination of a fusion step (congenital fusion of all the carpels, resulting in a new kind of primordium, the annular ovary primordium) and a reduction in number of stigma primordia, which implies a

corresponding reduction in number of dorsal bundles. While theoretically a ring primordium would allow for any novel position of the stigmata, in the case of dimerous pistils only two types can be distinguished in which the pistils are either in lateral (dorsiventrally flattened type) or median (laterally flattened type) positions, which are the only positions congruent with the symmetry plane of the flowers and their subtending bract (figs 5.3 & 5.22). In the following chapter we discuss some underlying mechanisms that could help to explain these patterns of pistil dimerisations in Cyperoideae.
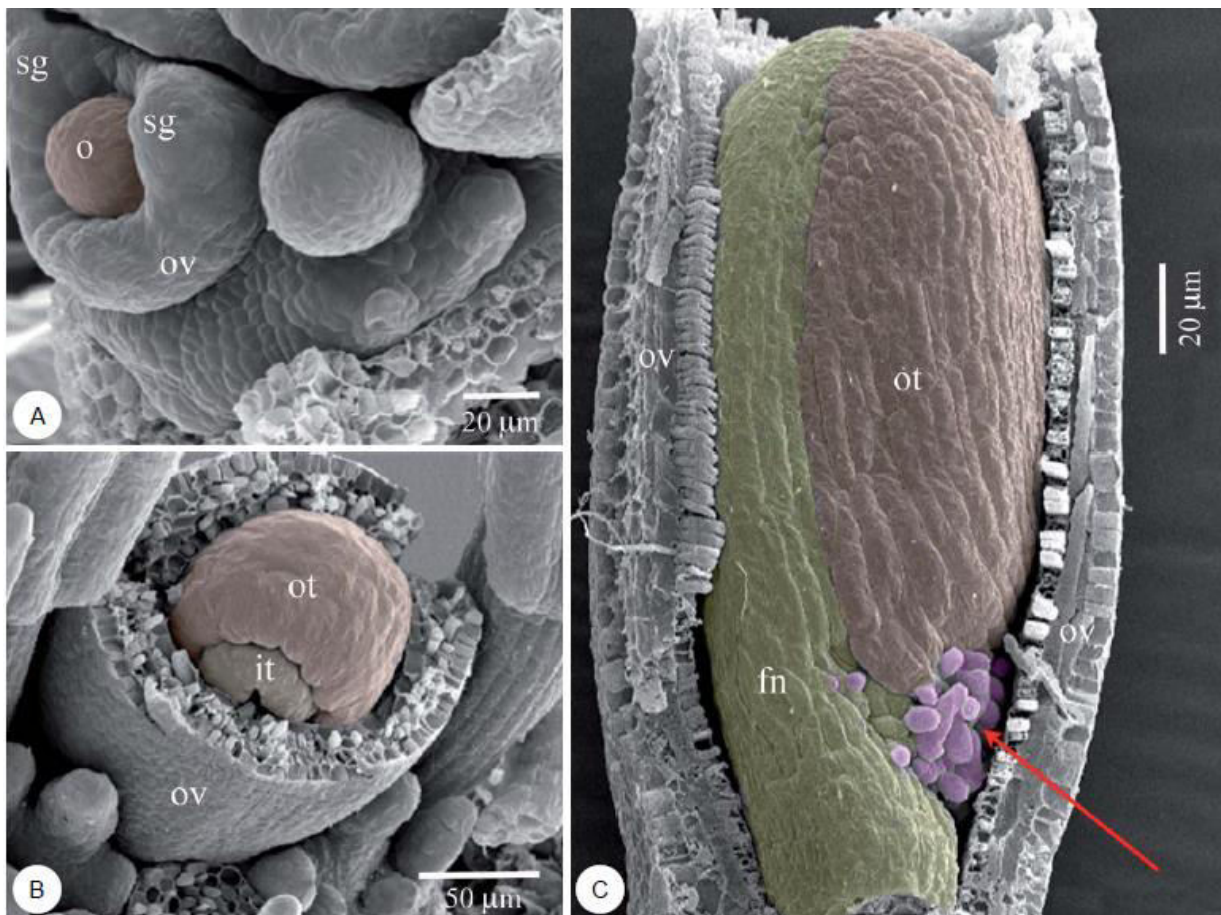
Fig. 5.19 SEM images of the development of the ovule in *Eriophorum latifolium* (A–B) and *Dulichium arundinaceum* (C). A, ovule primordium surrounded by the annular ovary wall primordium; B, developing ovule with outer integument (red) and inner integument (amber); C, longitudinal view of a mature, anatropous ovule with the funiculus (yellow), outer integument (red) and obturator hairs (purple) growing upon the funiculus and covering the micropylar zone (arrowed).

Abbreviations: fn, funiculus; it, inner integument; o, ovule; ot, outer integument; ov, ovary wall (primordium); sg, stigma primordium.

5.6.3 Underlying mechanisms for pistil dimerisations in Cyperoideae

Spatial pressures – In Poales, reduction of the number of carpels has often been interpreted to be a consequence of spatial pressures that the pistil suffers during its development, especially in taxa that bear dense spikelets such as Restionaceae (Ronse Decraene *et al.,* 2002) and Poaceae (Philipson, 1985). In Cyperoideae, only part of the variation can be explained in this way. Dorsiventrally flattened pistils are quite common in Cyperaceae (table 5.2) and often appear to be correlated with taxa bearing dense spikelets with restricted developmental space for the flowers in the abaxial direction, e.g. *Mapania* Aubl., *Nemum* (Larridon *et al.,* 2008). One could also try to understand dimerous, laterally flattened pistils as a result of lateral pressures caused by the two lateral stamens, forcing the adaxial carpel that remains after reduction into a median position. However, we would then expect the connection of the dorsal bundles to the ventral plexus to reflect the original position of the remaining carpel and therefore to be asymmetric, which is not the case (fig. 5.15A–B, 5.16 & 5.17). Moreover, at early developmental stages of the flower, there is an equally strong spatial pressure on the median adaxial part of the ring primordium by the rachilla and the higher glume. In the species of *Pycreus* and *Queenslandiella* studied, this spatial limitation even results in a delay of the development of the adaxial stigma branch with respect to the abaxial stigma (figs 5.9B, 5.20A & 5.21A) (Vrijdaghs *et al.,* 2011), even before the developing stamens become large enough to interfere with the development of the stigma branches. In addition, the wings of the alternating glume, which are attached to the rachilla by epicaulescent growth (Vrijdaghs *et al.,* 2009), push the developing stamens towards the abaxial position (fig. 5.9E). In *Kyllinga*[2], *Lagenocarpus amazonicus* and *Rhynchospora rubra* subsp. *rubra* spikelets only bear a single flower and are aggregated in very dense florescences. Possibly spatial limitations played a more important role in these taxa than in *Pycreus* and *Queenslandiella*. From a spatial point of view trimerous pistils still seem to be the most advantageous in most Cyperoid spikelets since stigma primordia are in optimal positions to grow up easily in the spaces between the different overlapping glumes of the spikelet.

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[2] Few *Kyllinga* species have spikelets with more than one maturing flower
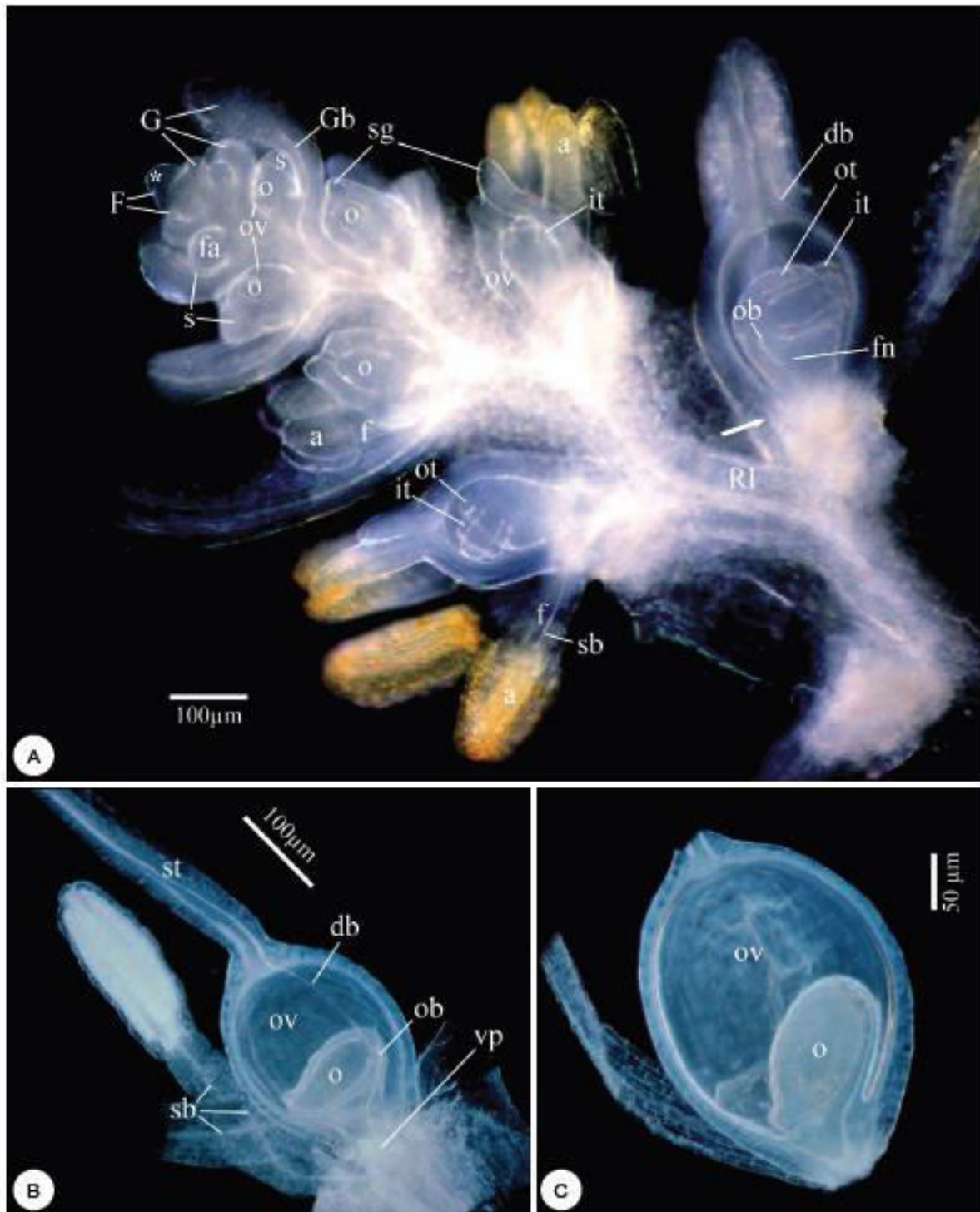
Fig. 5.20 Dark field microscopy image of cleared up spikelets and flowers in *Pycreus flavescens*. A, lateral view of a spikelet with proximal glumes removed, showing the early development of the gynoecium and ovule in the subsequent flowers. A white arrow indicates a developing dorsal bundle; B, lateral view of a flower just before anthesis with fully developed vascular system; C, lateral view of a flower after anthesis.

Abbreviations: a, anther; db, dorsal vascular bundle; f, filament; F, flower (primordium); fa, flower apex; fn, funiculus; G, glume (primordium); Gb, glume vascular bundle; it, inner tegument; o, ovule (primordium); ob, ovule bundle; ot, outer tegument; ov, ovary wall (primordium); Rl, rachilla; sb, staminal vascular bundle; st, style; vp, vascular plexus; asterisk (*), apex of the rachilla.

Zygomorphy of the spikelet – All cases of laterally compressed dimerous pistils seem to be linked with distichously arranged spikelets (table 5.2). In other taxa, such as *Rhynchospora*, *Dulichium, Websteria* (now in *Eleocharis*) and *Cyperus* there may be a link of dorsiventrally compressed pistils with the presence of distichously placed glumes. This apparent correlation of floral zygomorphy with the presence of distichously organised spikelets may be part of an underlying pattern of bilateral symmetry of the complete spikelet as a functional flowering unit. Cyperoid spikelets (and sometimes whole inflorescences) tend to take over floral function (e.g. Vrijdaghs *et al.,* 2009). In wind pollinated grasses and sedges, this often goes together with a reduction of floral parts (Rudall & Bateman 2004). As an example, the loss of abaxial stamens occurs frequently with bilateral floral symmetry (Rudall & Bateman 2004). Vrijdaghs *et al.,* (2011) observed that in most Cyperoideae with a reduction of the number of stamens, the abaxial stamen disappears first. This is particularly the case in *Pycreus*, which has zygomorphic gynoecia (Kükenthal, 1936). Pressures from surrounding organs fail as an explanation for the frequent loss of the abaxial stamen in *Pycreus*. Possibly, in *Pycreus*, there is a connection with the absence of abaxial main bundles within rachilla and receptacle (fig. 5.15B, 5.16). In addition, we also observed a dimerisation of the vascular system at the level of the rachilla in all *Pycreus* and *Queenslandiella* species studied (figs 5.11, 5.13, 5.15B, 5.16) However, the stigma primordia, and hence also the pistil vascularisation develop only after the formation of the vascular system within the rachilla (fig. 5.11). The number of dorsal bundles entering a flower equals the number of bundles within the rachilla. This can be explained by procambial initiation occurring in the centre of the receptacle. Subsequently, connections with the rachillar bundles are made (fig. 5.25). In their study of the wandering carpel mutant (wcr) of *Zea mays* (Poaceae), Irish *et al.,* (2003) hypothesise that both spikelet polarisation/orientation and floral symmetry are regulated by changes in a same factor. Several other authors assumed that zygomorphic development in flowers and leaves is the result of the inhibition of growth in the adaxial part of the floral meristem, which is induced by the shoot meristem (e.g. Wardlaw, 1949, Luo *et al.,,* 1996). These hypotheses may help to understand the apparently higher frequency of pistil and other dimerisations in taxa with distichously organised spikelets in Cyperaceae and Poaceae.

We can conclude that congenital fusion of carpels allowed several separate origins of laterally flattened pistils within Cyperoideae. Moreover, our study shows this pistil type originated much easier than previously assumed when using classic carpel reduction theories.
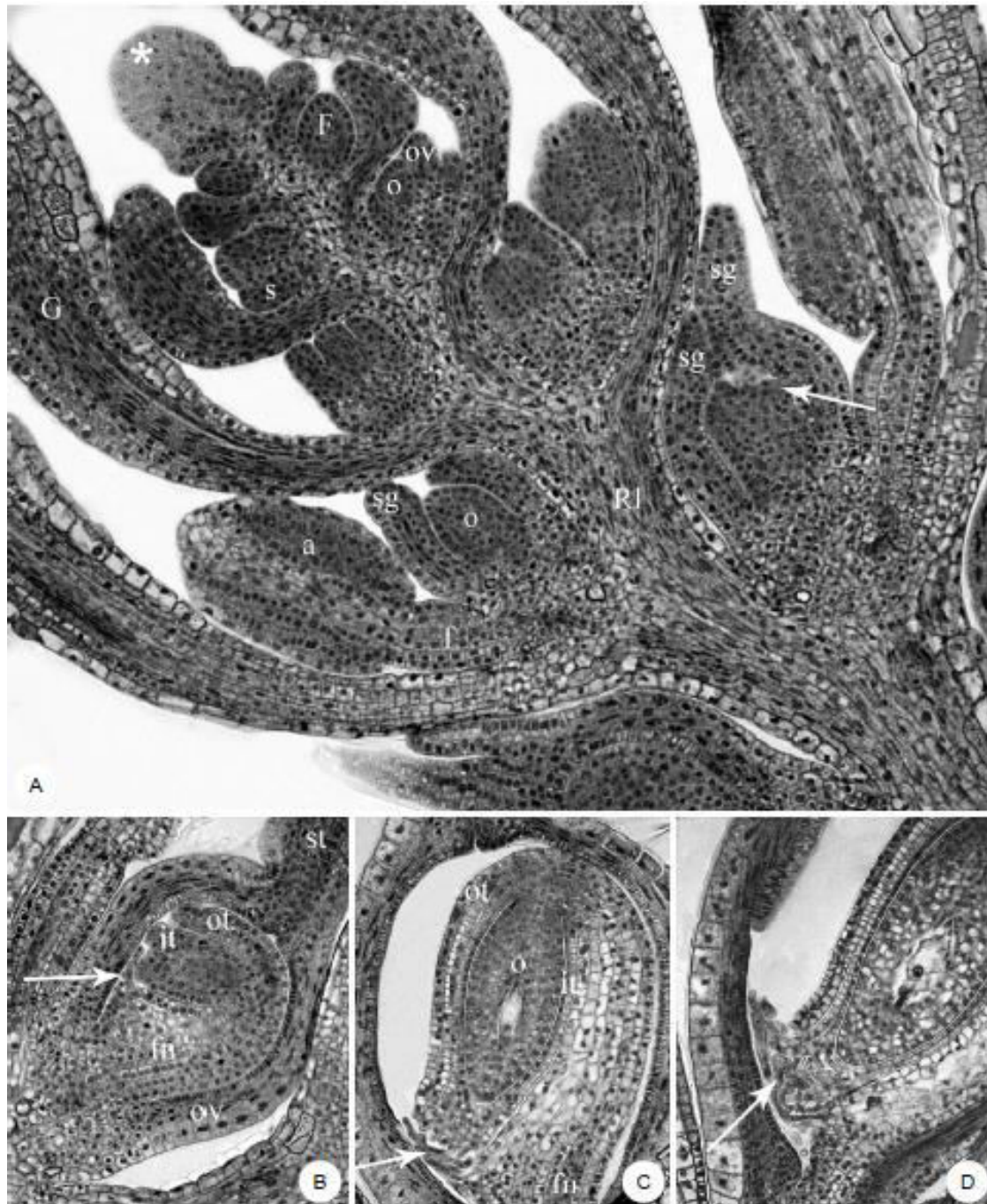
Fig. 5.21 Light microscopic images of sections through spikelets and gynoecia of *Pycreus sanguinolentus*. A, section through the central part of a spikelet showing a lateral view of the early development of the gynoecium and ovule in the subsequent flowers. A white arrow indicates the development of the inner tegument when the ovule tip starts to bend towards the abaxial side; B, older developmental stage showing a bending ovule with obturator hairs growing from the abaxial side of the funiculus (arrowed); C, lateral view of a mature ovule, fully bended ovule in which the obturator covers the micropylar zone; D, detail of the obturator of a mature ovule (arrowed) showing the obturator hairs growing into the micropyle.

Abbreviations: a, anther; f, filament; F, flower (primordium); fn, funiculus; G, glume (primordium); it, inner tegument; o, ovule (primordium); ot, outer tegument; ov, ovary wall (primordium); Rl, rachilla; sg, stigma primordium; st, style; asterisk (*), apex of the rachilla.
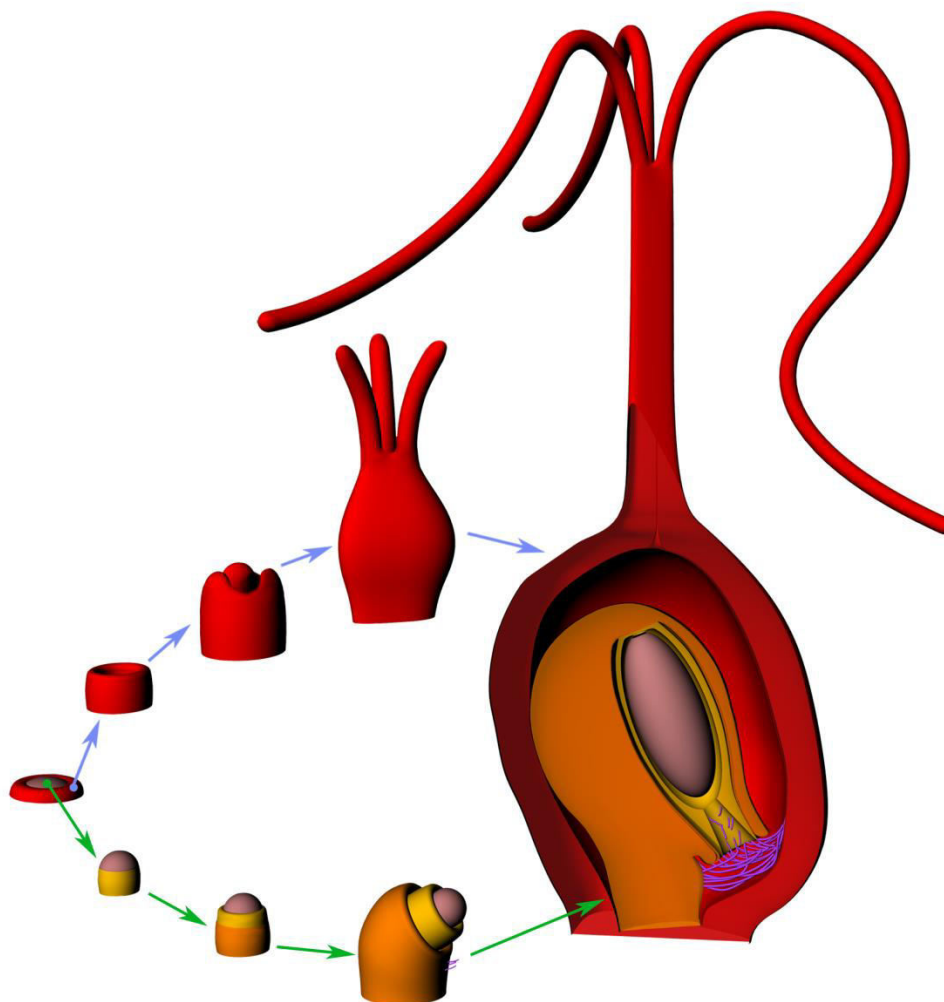
Fig. 5.22 Series of models representing the decoupling in the development of the ovary wall (upper series) and of the ovule (lower series) in Cyperoideae.

Colour codes: red, ovary wall; reddish brown, ovule tip; yellow, inner tegument; orange, outer tegument and funiculus; purple, obturator hairs.

Therefore we might have to face the possibility of multiple origins of laterally flattened pistils even within Cypereae, which is however still waiting for molecular confirmation.

5.6.4 Anatomical argumentations, integration and comparison of our data with Blaser's (1941a, 1941b)

Bidirectional development of the floral vascular system – At early developmental stages of flowers of *Pycreus sanguinolentus* and *Queenslandiella hyalina*, we found vessel initiations at several separate procambial zones within the rachilla and the base of the different floral organ primordia (fig. 5.11, 5.13). The development of vascular bundles in the

species studied concurs with the basic patern found in culms and leaves of *Cladium* (Fisher, 1971) and in angiosperms in general (Endress, 1994, Dickison 2000), therefore we think that the formation of a vascular system by the merging of remote procambial strands from different organs in the plant may be the general pattern in all Cyperaceae. The formation of vascular connections with nearby main bundles seems to be regulated by signals from developing primordia resulting in the formation of vascular bundles where necessary. This is reflected in the sequence of initiation of the vascular tissue of the floral organs and their connections to the receptacular bundles (ventral bundles/receptacular plexus), which follow the same order as the sequence of appearance of the floral primordia, starting with the stamens (figs 5.11–5.13 & 5.25).
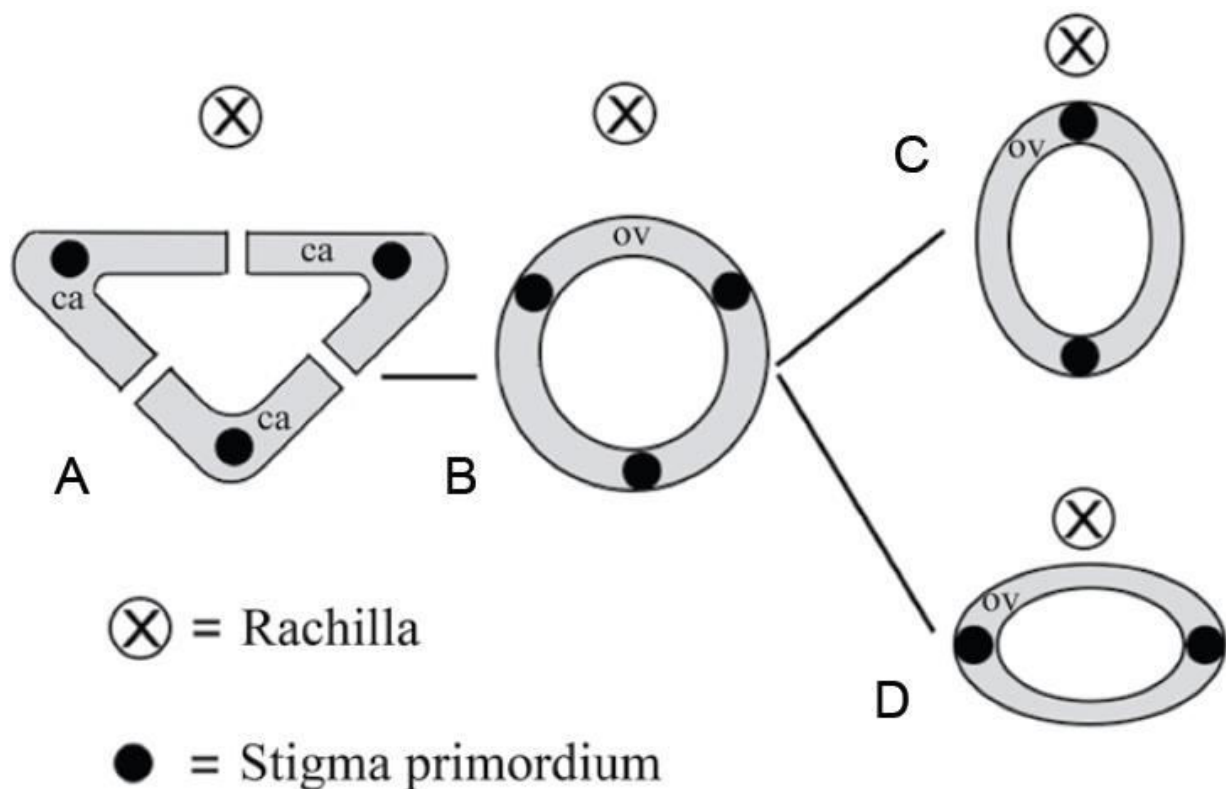
Fig. 5.23 Schematic representation of: A, a tricarpellate gynoecium; B, a trimerous gynoecium developing from an annular ovary wall primordium and gynoecia derived from B; C, laterally flattened with dorsiventrally positioned stigma primordia; D, dorsiventrally flattened with laterally positioned stigma primordia.

The presence of procambial initiation points within the receptacle and the formation of connections induced by the primordia of the different floral organs explains the connection of the gynoecial wall vascular traces in all pistil types to the central plexus within the

receptacle. It also explains that in species, which lack the abaxial receptacular bundle, the abaxial staminal bundle connects to both adaxial bundles (e.g. *Pycreus sanguinolentus*, fig. 5.11A, *Pycreus flavescens*, figs 5.15B & 5.24F, 5.16D–G, *Rhynchospora macrostachya* Torr. ex A.Gray, Blaser, 1941b). Concerning this case we should remark that Blaser (1941a) described an abaxial receptacular bundle in *Cyperus rivularis* (= *Pycreus bipartitus*), which branches towards the abaxial stamen and subsequently disappears. In *P. diandrus*, a species without abaxial stamen, he also observed only two receptacular bundles entering the flowers. Both *P. bipartitus* and *P. diandrus* are closely related to *P. sanguinolentus* (Clarke, 1908, Kükenthal, 1936), which is included in this study. Next, our model predicts the amount and positions of dorsal bundles of the flower, which connect to the corresponding bundles within the rachilla, and it explains the existence of a dense network of rather disorganized and short vessels within the floral receptacles (e.g. figs 5.11B, 5.12C & 20F). Moreover, this concurs with the dissimilarities in xylem vessels we observed within the receptacle and floral organs. Vascular traces within the receptacle consist of a large amount of rather disorganised short vessels in comparison with the traces within the floral organs, which mostly consist of few, long and narrow annular vessels (e.g. fig. 5.11A). Finally, pistil and ovular vascular bundles in all samples studied branch off within the receptacle to form independent bundles before entering the gynoecia, which corroborates the presence of separate primordia for the ovule and ovary wall from the start of the differentiation of the floral apex (figs 5.23 & 20).

In the receptacle of the Cyperoideae studied we observed a strong density of vascularisation (figs 5.11–5.17), which confirm the observations of Saunders (1937) and Snell (1936). In contrast with Blaser (1941a, 1941b), these authors considered the vascularisation within the receptacle to be disorganised. In our opinion, the dense and disorganised nature of the vascularisation within the receptacles of Cyperoideae reflects the presence of the annular primordium. This vascular plexus of the receptacle might be interpreted as an adaptation to fruit dispersal, since it position concurs with the abscission zone of the mature nutlets.

To conclude, our observations suggest an ontogenetic pattern for the vascular system in Cyperoideae, which appears to be formed from different initiation zones from which the growing vessels find each other (fig. 5.25). In contrast, Blaser (1941a) followed an acropetal model (e.g. Grégoire, 1938) to understand the development of a vascular system in Cyperaceae, in despite of the fact that he reported unconnected vascular supply in rudimentary abaxial style branches of dimerous *Schoenoplectus* species and that he logically

suggested a bidirectional development of the vascular system within the receptacle, rather than acropetal development.

Dorsiventrally flattened dimerous pistils – As in most taxa with dorsiventrally compressed pistils, we observed that in *C. laevigatus* the abaxial stigma branch disappears together with all vascular traces at this side of the ovary wall (figs 5.12 & 5.15C, 5.18). Blaser (1941a), reasoning from an acropetal vascularisation model, supported the idea of the loss of the abaxial carpel in *Schoenoplectus*, based on the presence of unconnected bundles within rudimentary abaxial style branches. However, traces originate in the floral organ primordia to subsequently connect with the stele. In this case, the connection with the stele was not made, but the presence of unconnected bundles can indeed be interpreted as an indication of an original third carpel. In many taxa with dorsiventrally compressed pistils, the ovule is still vascularised with bundles from the abaxial receptacular trace, which were interpreted by Blaser (1941a) as vestigial bundles of the abaxial carpel (see fig. 5.4B). However, in our opinion, the ovule is connected to the rachillar plexus, independently from the dorsal bundles of the carpels (figs 5.11–5.12, 5.15 & 5.25). In the species studied, the number and positions of the main receptacular bundles reflect the number and positions of vascular bundles within the rachilla of the spikelet, rather than the number and positions of the carpels (fig. 5.15).

➜   Fig. 5.24 Series of diagrams contrasting Blaser's (1941*a,b*) model (A--C) with our current interpretation (D--F) of the vasculature in laterally compressed dimerous pistils: A, Blaser's (1941a, b) model of the vasculature in a primitive sedge (e.g. *Scirpus*) in which lateral bundles are present in the sides of the gynoecium wall, which connect to the ventral bundles; B, Blaser's (1941a, b) model of a *Cyperus* flower, where lateral bundles are absent; C, Blaser's (1941a, b) interpretation of a *Pycreus* flower, which has laterally flattened dimerous pistils. Since the vascular bundles that run within the gynoecium wall towards the stigmata connect with the ventral bundles, Blaser (1941a) interpreted these as lateral bundles, which took over the function of the dorsals. Subsequently he postulated separate origins of models B and C from A (black arrows). A red arrow indicates an abaxial receptacular bundle connecting towards the abaxial anther, which Blaser (1941a) reported in his study; D, This model corresponds to model A. However, our current study shows the vasculature within the receptacle is highly disorganised and is here shown as a vascular plexus, which corresponds to what Blaser (1941a, b) indicated as ventral bundles; E, Same as model B with ventral bundles shown as a vascular plexus; F, Same as model C with ventral bundles shown as a vascular plexus. Vascular traces within the ovary wall are interpreted as dorsal bundles and thus as homologous to these in models D & E. This corroborates with the origin of *Pycreus* from a *Cyperus* ancestor (black arrows). A red arrow indicates the absence of an abaxial dorsal bundle in *Pycreus* and connection of the vascular bundle of the abaxial stamen to both adaxial receptacular bundles, as observed in this study.
Colour codes: purple, receptacular bundles; yellow, staminal bundles; green, dorsal bundles; blue, ventral bundles; red, central and ovule bundles; orange, lateral bundles.
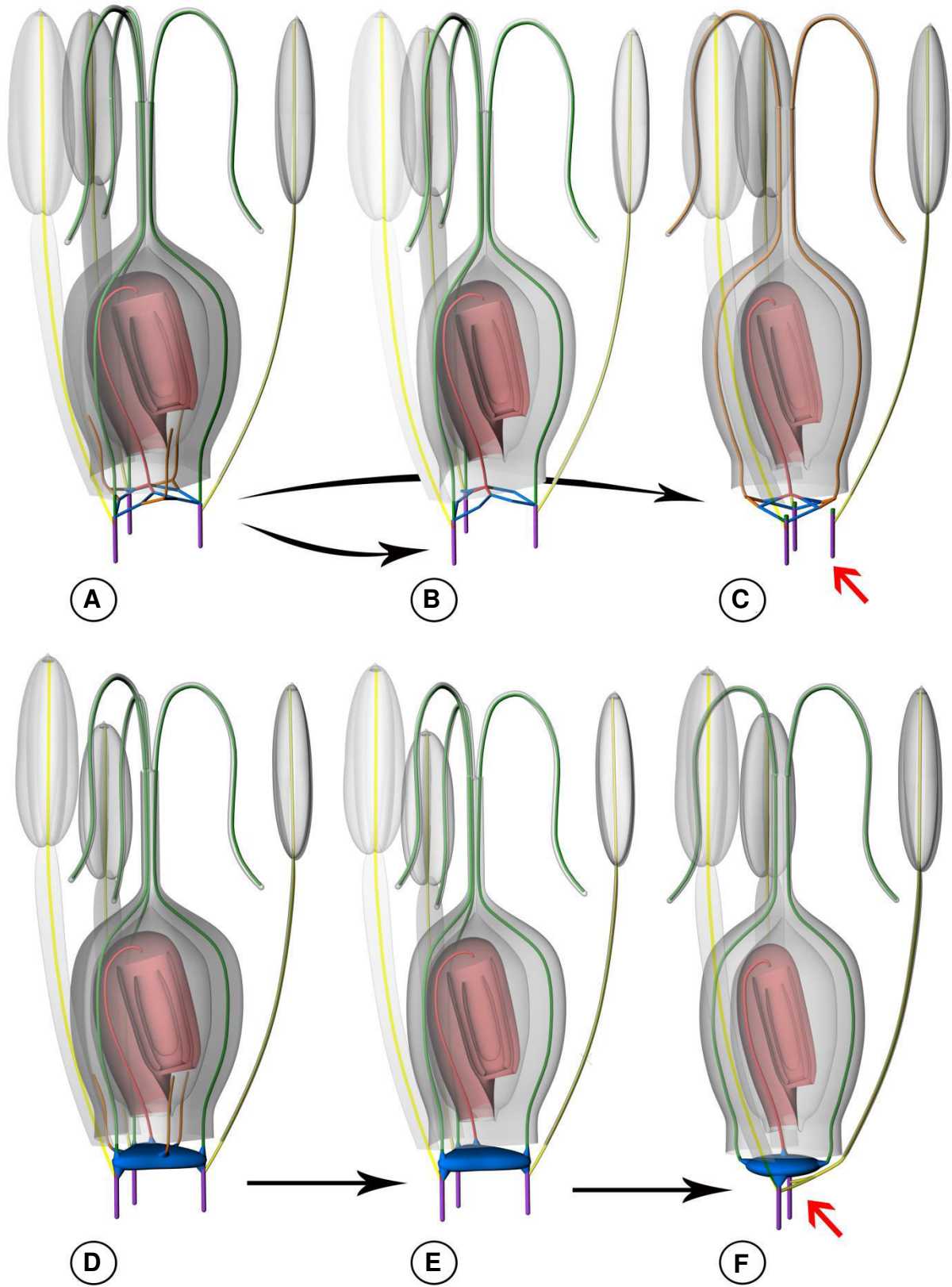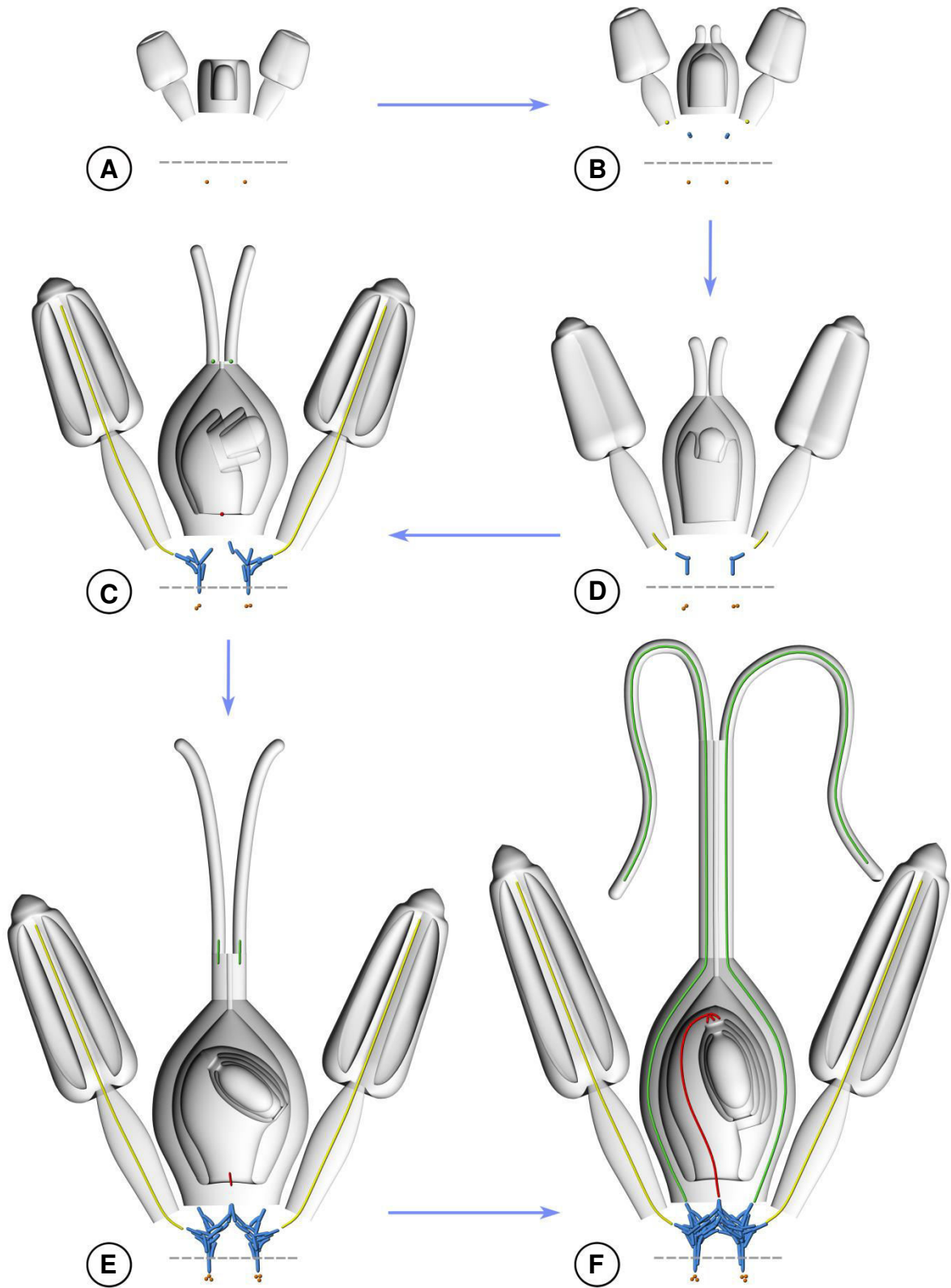
As a conclusion, general patterns of vascularisations of unrelated taxa with dorsiventrally flattened pistils can be similar (see table 5.2) since they all reflect the same underlying general pattern of pistil ontogeny.

Laterally flattened pistils – The origin of laterally compressed pistils required developmental freedom of the pistil, which is present as an annular primordium in several recently evolved lineages of Cyperoideae. Moreover, floral vascular patterns are a reflection of the floral ontogeny (fig. 5.25). In the next paragraphs, we discuss the vascular evidence supporting our theory on alterations of stigma positions but falsifying alternative hypotheses on homology (Blaser, 1941a) or fusion (Ronse Decraene *et al.,* 2002) of vascular bundles. In addition, a discussion of some specific observations in taxa with laterally compressed pistils is presented here.

The observed vascular ontogeny and recent phylogenetic studies (Muasya *et al.,* 2002, Larridon *et al.,* 2011a) imply the homology of the bundles within the ovary wall of *Cyperus* and *Pycreus* (fig. 5.24D–F). This is in contrast with Blaser (1941a), who, based on the connections of the bundles, interpreted the pistil bundles in *Cyperus* as dorsal bundles and the ones in

← Fig. 5.25 Series of diagrams showing the development of organs and vasculature of a hypothetical cyperoid flower with a dorsiventrally flattened dimerous pistil and two stamens in adaxial positions: A, At the stage of the annular gynoecium wall primordium surrounding an ovule primordium the vascular traces of the rachilla start to differentiate; B, Once all floral organs are initiated (including the stigma primordia) several procambial initiation points originate within the receptacle and from the base of the stamens; C, In this stage the stamens are strongly developing. Connections are made between the procambial initiation points within receptacle with those of the stamens and towards the vascular traces of the rachilla. Meanwhile the stigma primordia are elongating and the first integument is formed on the ovule primordium that starts to bend; D, Stamens and their vascular system are fully differentiated. On the tip of the ovule primordium the second integument is formed. Additional procambial initiation points are formed from the base of the ovule and the stigma branches. Meanwhile, the vascular traces within the receptacle become denser and branch towards the different primordia of the gynoecium; E, Procambial initiation points from the base of the stigma branches differentiate further within the elongating pistil and stigmata. The ovule vascular traces also start differentiating in this stage; F, All floral organs and their vascular traces are completed and these all have formed connections with the now very dense vascular plexus within the receptacle.

Diagrams are shown from an abaxial viewpoint and a black dotted line indicates the hypothetical border between the rachilla and the flower. Colour codes: grey: floral organs; orange: Vascular bundles within the rachilla, here seen in transversal section; blue: vascular traces within the rachilla (= receptacular, ventral and central bundles); green: vascular traces within the gynoecium wall (= dorsal bundles) and red: vascular traces within the ovule.

Pycreus as lateral bundles (fig. 5.24A–C). In Blaser's (1941a) view, the dorsal bundles within the ribs of the ovary are a continuation of the main vascular bundles within the receptacle after all bundles towards other floral organs and the ventral bundles have branched off (figs 5.4A–C & 5.24A & B). However, in *Pycreus*, *Kyllinga*, and *Queenslandiella*, stigma branches connect to the vascular plexus (ventral bundles) within the receptacle (figs 5.4D, 5.11, 5.15A–B, 5.16, 5.17 & 5.24C) and do not form a continuation of the main vascular bundles in the receptacle. Therefore, Blaser (1941a) saw the vascular bundles in the ovary wall of *Pycreus* as lateral bundles (figs 5.4D1 & 5.24C), comparable with those he found in the scirpoid taxa (figs 5.4A1 & 5.24A). As these bundles continue to the style branches, Blaser assumed that they took over the function of the dorsals. In contrast, in the scirpoid taxa, the lateral bundles usually run within the sides of the triangular ovary wall and end below the style (fig. 5.24A). However, the time gap between the connecting of the receptacular plexus with the rachillar bundles and the connecting of the receptacular plexus with the vascular bundles of the pistil, suggests that both vascular systems are independent and thus do not necessarily need to converge. Also the vascular bundles from the different organs connect independently to the receptacular vascular plexus. Since lateral bundles are lacking in all *Cyperus* species studied by Blaser and the authors of the current study (figs 5.4A1 & 5.12) and taking the most recent phylogenetic hypothesis about *Cyperus* into consideration, with *Pycreus*, *Kyllinga* and *Queenslandiella* nested within the C_4 *Cyperus* clade (Muasya *et al.,* 2002, 2009a, Besnard *et al.,* 2009, Larridon *et al.,* 2011b), there are no reasons to assume homology of the ovary wall bundles in *Pycreus* and the lateral bundles in scirpoid taxa. The development of the vascularisation in angiosperms tends to allow the formation of vessels 'wherever needed', as the main vascularisation systems are determined by the positions of organ primordia, from where newly initiated bundles connect with existing vascular bundles (Endress, 1994). Consequently, the vascular bundles in the ovary in e.g. *Pycreus*, *Kyllinga* and *Queenslandiella* are therefore to be regarded as dorsal bundles similar to those found in ovaries of other Cyperoideae.

Secondly, although all carpels are congenitally fused, we found no evidence in *Pycreus* and related taxa for a further fusion of the two adaxial stigmata and their dorsal bundles to form a single stigma and a dorsal bundle in intermediate position. The latter theory was applied by some authors to explain similar cases in African Restionaceae, where transitional series are known in which two dorsal bundles are present in a pair of fused sterile carpels,

while in the more derived situation only a single dorsal bundle is present in an intermediate position (Linder, 1992, Ronse Decraene *et al.,* 2002). It is more parsimonious to assume a developmental reorganisation of the fused carpel complex resulting in an ovary with a single, intermediately positioned stigma primordium from which a corresponding dorsal bundle is initiated.

Next, Blaser (1941a) included *Kyllinga pumila* in his study, concluding that its vascular pattern is similar to the vascularisation in trimerous *Cyperus* flowers. However, *Kyllinga* pistils are dimerous and logically our observations do not confirm those of Blaser (1941a). Possibly the latter were based on misidentified material. In the rachilla of *Kyllinga nemoralis*, we observed that the vascularisation is concentrated into a single V-shaped bundle at a level above the branching of the first flower (fig. 5.15A). This can be explained by the strong reduction of the spikelet in most *Kyllinga* species, in which only one flower is functional. Vascular bundles entering the flowers of *K. nemoralis* run into two laterally positioned centres, comparable with the two bundles entering the flowers in the *Pycreus* species studied. However, in *Kyllinga nemoralis* these two bundles expand and split into three smaller bundles each, which then fuse again into an annular bundle before splitting off staminal and gynoecial traces (fig. 5.15A, 5.17). These observations for *K. nemoralis* concur with the observations of Blaser (1941a) for *Cyperus retrorsus* Champ. in which he observed six separate bundles entering the flowers. These six bundles fuse two by two to form the dorsal bundles of the flower. Possibly, this is due to the position of the flowers studied within the spikelet. In *K. nemoralis* as well as in *C. retrorsus*, only the most proximal flowers of the deciduous spikelets are functional. Also in other groups with strongly reduced or condensed spikelets, vascular connections of different structures within the spikelets tend to interfere, making correct interpretations of the original vascularisation almost impossible, e.g. *Carex* (Snell, 1936), *Scleria* P.J.Bergius (Blaser, 1941b). This adaptive nature of vascular development makes vascular patterns in our opinion less reliable for the study of evolutionary relationships between different taxa in Cyperoideae.

Pseudomonomerous pistils – Finally, in both *Rhynchospora* and *Cyperus*, lineages arose in which the style is elongated with reduced the stigma branches. Such pistils are often called pseudomonomerous gynoecia (Dickison 2000). In these cases, remnants of the stigma primordia are still visible on the top of the style and two or three dorsal bundles can be observed within the style (fig. 5.14).

Vascular connections of the ovule – The vascularisation of the ovule connects to the central vascular plexus within the receptacle independently from the dorsal bundles of the gynoecial wall (fig. 5.25). This also reflects the developmental reorganisation of the gynoecium. The central position of the ovule results in an equal contribution of vascular connections (ventral bundles) from all vascular poles within the receptacle instead of a polarisation towards a single carpel. As discussed by Snell (1936) and Blaser (1941b), this reflects a vascularisation supply typical of axile or free central placentae from which the situation in Cyperoideae can be interpreted as a final reduction stage. Blaser (1941b) observed in some species that the ovule is asymmetrically positioned, with the funiculus inserted at the adaxial side of the locule and the micropyle bent back at the abaxial side. In *Cyperus laevigatus*, we observed an asymmetric connection of the ovule vascular bundle with the central vascular bundle coming from the receptacle (fig. 5.12C). However, ontogenetically, in all species studied by us, the ovule primordium is centrally positioned. Therefore, we assume that the asymmetric position of the ovule along the adaxial-abaxial axis is due to the bending of the ovule, which initially is atropous and subsequently turns to become anatropous.

5.6.5 The Cyperoid ovule

Reduced ovule numbers – Apparently, the congenital fusion of the carpel primordia allows a new developmental organisation with as a symptom the central, basal ovule (fig. 5.23). We can agree with Snell's (1936) interpretation of a central basal ovule as a 'reduction' as far as we can see a reduction trend in Juncaceae where in *Luzula* DC. the ovary wall also originates from an annular primordium (Vrijdaghs *et al.,* 2006, unpubl. res.). In contrast to Cyperaceae, in *Luzula* the originally carpellary structure of the gynoecium is still noticeable in the presence of the three basal ovules. This tendency apparently reaches its maximum in Cyperaceae with the single, basal ovule, which is no longer linked to a carpellary structure (fig. 5.23). However, in our opinion, 'reduction' here rather means reorganisation of the development the ovary, accompanied by a simplification. According to Linder & Rudall (2005) reduced ovule number is often associated with aggregated inflorescences.

Is there a link with pseudomonad pollen? – Kress (1981) suggested that while it could be advantageous to have simultaneous fertilisation of multiple ovules within the same ovary by a pollen unit (four at once for tetrads), such advantage is lost when only a single ovule is

present. McGlone (1978) discussed this hypothesis for Styphelioideae (Ericaceae) where such parallels between ovule number and tetradmonad reductions exist. A similar link might exist between respectively single ovuled pistils and pseudomonad pollen (Cyperaceae), and multi-ovuled pistils and tetrad pollen (Juncaceae, Thurniaceae). However, this comparison does not consider the difference in pollination strategy between sedges (wind) and Styphelioideae (insect), which are known to have different effects on the natural selection of successful floral and pollen morphologies. Char *et al.,* (1973) assumed pseudomonads are advantageous in wind pollination due to the smaller pollen size, which might explain the situation in sedges but not in Styphelioideae (McGlone, 1978). In addition, tetrad polled might still be advantageous for single ovuled gynoecia since it maintains a possibility of selection of the fittest member of the pollen tetrad.

Placentation – Blaser (1941b) also mentioned a distinct spine-like projection along one side of the ovule towards the top of the ovary of *Bolboschoenus robustus* (Pursch) Soják, which he interpreted to be remnants of a placental column. However, since the ovary wall rises from an annular primordium, and since the development of the ovule occurs independently of the development of the ovary wall, we consider it (in contrast to Blaser, 1941b) impossible to find remnants of carpellary structures, which could only occur in a developing gynoecium resulting from postgenital fusion of (morphologically reduced) carpels, quod non. The term 'central placenta', however, can be used in the meaning of centrally positioned region of adhesion of the ovule, following Leins (2000: 100), who defined a placenta as: "Im weitesten Sinne ist die Plazenta der Gewebeteil eines Karpells (oder der Blütenachse), der die Samenanlagen hervorbringt [In the widest sense, the placenta is the part of the tissue of a carpel or of the floral axis, which produces the ovules] ".

Obturator hairs – The recent observations of Coan *et al.,* (2008) in *Rhynchospora* and *Hypolytrum* Rich. ex Pers., of Gonzalez & López (2010) in *Bulbostylis*, as well as our observations in 37 mostly African species in Scirpeae, Fuireneae, Eleocharideae, Abildgaardieae, Cypereae, Cariceae, Schoeneae and Trilepideae sensu Goetghebeur (1998) about intralocular hairs confirm the observations of Van der Veken (1965). In all species studied, obturator hairs originate at the basal-abaxial side of the funiculus and subsequently grow towards the micropyle where they stick together, thus closing the micropyle. Observed variation appeared to be totally random and hence of no systematic value.

5.7 Conclusions: Understanding pistil diversification in Cyperoideae, an integrated developmental model

Integrating our anatomical, floral ontogenetic data and recent functional insights makes it possible to present a developmental model for the gynoecium in Cyperoideae to understand all variations of its essentially trimerous morphological Bauplan.

(1) The ovary originates from an annular ovary primordium (Vrijdaghs *et al.,* 2009) – During the earliest ontogenetic stages, the annular ovary primordium grows upwards to form a bag-like structure. At this stage, the primordium can be considered as an 'empty box' (Gould 2002), with no other developmental determination than growing upwards. Next, in most cyperoid species, two adaxially positioned and one abaxially positioned stigma primordia (according to the conservative positions of the original carpel tips) are formed on the top of the cylindrical ovary wall. However, other numbers and positions of stigma primordia are possible. We believe that congenital fusion of carpels allows shifts in positions of stigma primordia to novel, more optimal positions with respect to the available space (fig. 5.22).

(2) Signaling from primordia causes bidirectional origin of the vascular system in the pistil (Endress, 1994) – Vessel initiation zones are present in the stigma primordia (fig. 5.25). From there, the vessels grow to the receptacular plexus, to be connected with the stele. These vessels constitute the ribs of the pistil. Consequently, the number and positions of the stigma primordia determine the future shape of the pistil, which develops initially as a bag-like structure, and subsequently typically assumes a triangular shape, or a derived dimerous, dorsiventrally or laterally flattened shape, or a polymerous shape.

(3) Annular primordia facilitate decoupling of the development of a whorl with respect to the neighboring whorls (Endress 2006) – The annular ovary primordium and central ovule primordium differentiate simultaneously from the floral apex. The development of the ovary wall and ovule appear as two distinct phenomena (fig. 5.23). Ovary wall and ovule vascular traces show independent connections with the receptacular plexus, thus reflecting the ontogenetic separation of the annular ovary wall primordium and ovule primordium.

5.8 Acknowledgements

P. polystachyos

P. nuerensis

P. intactus

P. pelophylus

100

P. rhizomatosus

100

100

P. macrostachyos

P. pumilus

98

P. longistolon

51

P. katangensis

P. gracillimus

P. smithianus

P. pseudodiaphanus

P. cataractarum

86

98

100

P. divulsus

76

P. nitidus

P. juncelliformis

65

P. flavidus

92 **97**

P. rehmanianus

P. flavescens

65

P. niger

P. melas

P. sanguineosquamatus

P. xantholepis

P. lanceolatus

100

P. micromelas

78

P. macranthus

99

P. nigricans

P. sanguinolentus

P. pauper

P. bipartitus

P. capilifolius

97

P. mundtii

92

P. melanacme

P. megapotamicus

72

P. reductus

100

57

64

100

99

6 Molecular phylogenetic hypothesis of C$_4$ *Cyperus*

"The most beautiful experience we can have is the mysterious - the fundamental emotion which stands at the cradle of true art and true science."

— Albert Einstein (1979-1955)

← Fig. 6.1 Stylized cladogram of *Pycreus* species based on a maximum parsimony analysis of the ETS1f marker. Bootstrap values are indicated on the separate nodes, species are represented by a spikelets photographed from the GENT and BR herbaria. This image was presented on a poster on the XVIII AETFAT congress, Yaounde, Cameroon.

Spikelet theme: *Pycreus nervulosus*
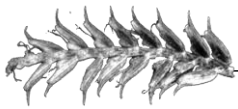
6.1 In this chapter…

The results presented in this chapter are published in the following paper:

Larridon I, Bauters K, Reynders M, Huygh W, Muasya AM, Simpson DA, Goetghebeur P. 2013. Towards a new classification of the giant paraphyletic genus *Cyperus* (Cyperaceae): phylogenetic relationships and generic delimitation in C$_4$ *Cyperus*. Botanical Journal of the Linnean Society 172(1): 106-126.

The original aim for this chapter was for it to be first authored by M. Reynders. However due to limitations in time the original draft was fine-tuned by I. Larridon and published, while K. Bauters updated the cladistic analysis. The contributions of M. Reynders consist mainly of the lab work on the ETS1f marker and DNA extractions of most taxa presented here, important parts of the discussion and the production of fig 6.3.

6.2 Abstract

Maximum likelihood and Bayesian inference analyses of nuclear ribosomal DNA (ETS1f) and plastid DNA (*rpl32-trnL, trnH-psbA*) sequence data are presented for "C$_4$ *Cyperus*" (Cyperaceae). The term "C$_4$ *Cyperus*" encompasses all species of *Cyperus* s.l. that use C$_4$ photosynthesis linked with chlorocyperoid vegetative anatomy. Sampling comprises 107 specimens of 104 different taxa, including many of the subdivisions of C$_4$ *Cyperus* s.s. and all C$_4$ segregate genera (*Alinula, Ascolepis, Kyllinga, Lipocarpha, Pycreus, Queenslandiella, Remirea, Sphaerocyperus* and *Volkiella*). According to our results, C$_4$ *Cyperus* is a well-supported monophyletic clade nested in C$_3$ *Cyperus*. Despite the lack of resolution along the backbone of the C$_4$ Cyperus clade and for some internal branches, several well-supported clades can be distinguished. The first clade in C$_4$ *Cyperus* is formed by *Cyperus cuspidatus* and *C. waterloti*. Other recognizable and well-supported clades correspond to segregate genera, i.e. *Ascolepis, Lipocarpha* including *Volkiella*, and *Kyllinga*. Species of C$_4$ *Cyperus* s.s. form a core grade in which the C$_4$ segregate genera are embedded. *Pycreus*, the largest segregate genus composed of c. 120 species, is not monophyletic since it includes several C$_4$ species of *Cyperus* s.s.. This study establishes a phylogenetic framework for revising classification and character evolution in *Cyperus* s.l.
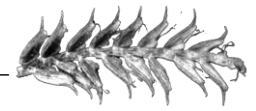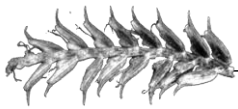
6.3 Introduction

6.3.1 Cypereae and the *Cyperus* clade

Cyperaceae (the sedge family) have an almost cosmopolitan distribution and play a dominant role in wetland vegetation. The many reductions and convergences in inflorescences of Cyperaceae have impeded evolutionary reconstruction (homology questions; e.g. Bruhl, 1991; Vrijdaghs *et al.*, 2009, 2010; Muasya *et al.*, 2009b) and classification (e.g. Clarke, 1908; Kükenthal, 1935-36; Kern, 1974; Haines & Lye, 1983; Bruhl, 1995; Goetghebeur, 1998). Based on recent molecular phylogenetic studies, Cyperaceae consist of two main clades corresponding to subfamilies Cyperoideae and Mapanioideae (Simpson *et al.*, 2003, 2007; Muasya *et al.*, 2009a). In Cyperoideae, two clades stand out due to their extraordinary species diversity: (1) the clade corresponding to the predominantly temperate tribe Cariceae (c. 1950 spp.); and (2) the clade corresponding to the mainly tropical tribe Cypereae (c. 1120 spp.). Together they cover nearly three fifths of species diversity in Cyperaceae (Govaerts *et al.*, 2012).

Recent molecular phylogenetic studies of Cyperaceae (Simpson *et al.*, 2003, 2007; Muasya *et al.*, 2009a) showed Cypereae sensu Goetghebeur (1998) to be monophyletic, but the generic delimitations in Cypereae remained controversial (Muasya *et al.*, 2009b). In the past, Cypereae were circumscribed as having spikelets with distichous glumes and reduced, perianthless flowers (e.g. Kükenthal, 1935-36). However, neither the distichy of the glumes nor the absence of a perianth could be regarded as phylogenetically informative characters (e.g. Vrijdaghs *et al.*, 2006; Muasya *et al.*, 2009a, b; in press). Currently, Cypereae are circumscribed by the presence of a Cyperus-type embryo or the similar Ficinia-type embryo (Van der Veken, 1965; Goetghebeur, 1998; Muasya *et al.*, 2009a, b). The presence of various combinations of characters (e.g. reduced flowers, reduced and/or contracted inflorescences) and convergent morphologies led to the misinterpretation of the relationships of many lineages of Cypereae. A number of taxa (belonging especially to *Erioscirpus* Palla, *Ficinia* Schrad, *Hellmuthia* Steud., *Isolepis* R.Br., *Kyllingiella* R.W.Haines & Lye, *Oxycaryum* Nees, *Scirpoides* Séq.) were allocated to various tribes in Cyperaceae, including Scirpeae, Rhynchosporeae, Hypolytreae and Schoeneae (e.g. Kunth, 1837; Nees, 1842; Steudel, 1854-55; Clarke, 1908). However, extensive anatomical (Kranz anatomy), embryographical and molecular phylogenetic studies (e.g. Van der Veken, 1965; Goetghebeur, 1986, 1998; Bruhl,
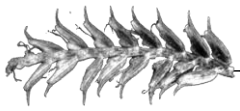
1995; Simpson *et al.*, 2003, 2007; Muasya *et al.*, 2001a, 2002, 2009a, b; Larridon *et al.*, 2011a, b; Yano *et al.*, 2012) revealed that these genera are closely related to *Cyperus* L. Consequently, reinterpretation of the morphological characters of these genera in the context of Cypereae is required.

Based on molecular phylogenetic studies (e.g. Simpson *et al.*, 2007; Muasya *et al.*, 2009a), two clades are recognized in Cypereae: (1) the *Ficinia* clade and (2) the *Cyperus* clade. The first, smaller clade (c. 160 spp.) consists of several genera with a mainly southern African distribution, a ficinoid habit (hemicryptophytes, culm scapose, inflorescence capitate and appearing pseudolateral with main involucral bract being stem-like) and mostly spiral glumes. The basalmost branches include species with perianth parts (*Dracoscirpoides* Muasya, *Erioscirpus, Hellmuthia*; Vrijdaghs *et al.*, 2006; Muasya *et al.*, 2012; Yano *et al.*, 2012). Prior to the embryographical study of Van der Veken (1965), most of these genera had been classified in or near *Scirpus* L.

The second, larger, pantropical clade (c. 950 spp.), with mostly distichous glumes, comprises a paraphyletic *Cyperus* s.s. as the core genus (c. 700 spp.) in which at least 12 segregate genera are nested (Goetghebeur, 1998; Govaerts *et al.*, 2012; see Table 6.1). The branch leading to *Androtrichum* (Brongn.) Brongn. (two species) appears to be at the base of the *Cyperus* clade (Muasya *et al.*, 2002, in press), but this needs further confirmation. Although molecular phylogenetic studies revealed that all these genera are nested in *Cyperus* (e.g. Muasya *et al.*, 2002; Larridon *et al.*, 2011a), there has been considerable discussion about whether to include these taxa into *Cyperus*. Contemporary treatments either recognize the segregate genera as separate from *Cyperus* (e.g. Bruhl, 1995; Goetghebeur, 1998;

→ Fig. 6.2 Morphological diversity in C$_4$ *Cyperus*. A. *Cyperus cyperoides* (L.) Kuntze with strongly contracted spikes of spikelets in an anthelate inflorescence. B. *Pycreus polystachyos* (Rottb.) P.Beauv. with spikes of spikelets in an anthelate inflorescence. C. *Cyperus laevigatus* L. with a reduced inflorescence consisting of only a few sessile spikelets in a pseudolateral inflorescence. D. *Lipocarpha chinensis* (Osbeck) J.Kern with three sessile pseudospikelets. E. *Cyperus capitatus* with a capitate inflorescence. F. *Kyllinga polyphylla* Willd. ex Kunth with a capitate inflorescence of reduced, deciduous spikelets. G. *Cyperus ustulatus* A.Rich. with contracted spikes of spikelets in an anthelate inflorescence. H. *Cyperus waterloti* Cherm. with an inflorescence of digitately clustered spikelets. Photographs A--G taken by M. Reynders in the Ghent University Botanical Garden, H taken by W. Huygh at Cirque Rouge near Mahajanga, Madagascar.
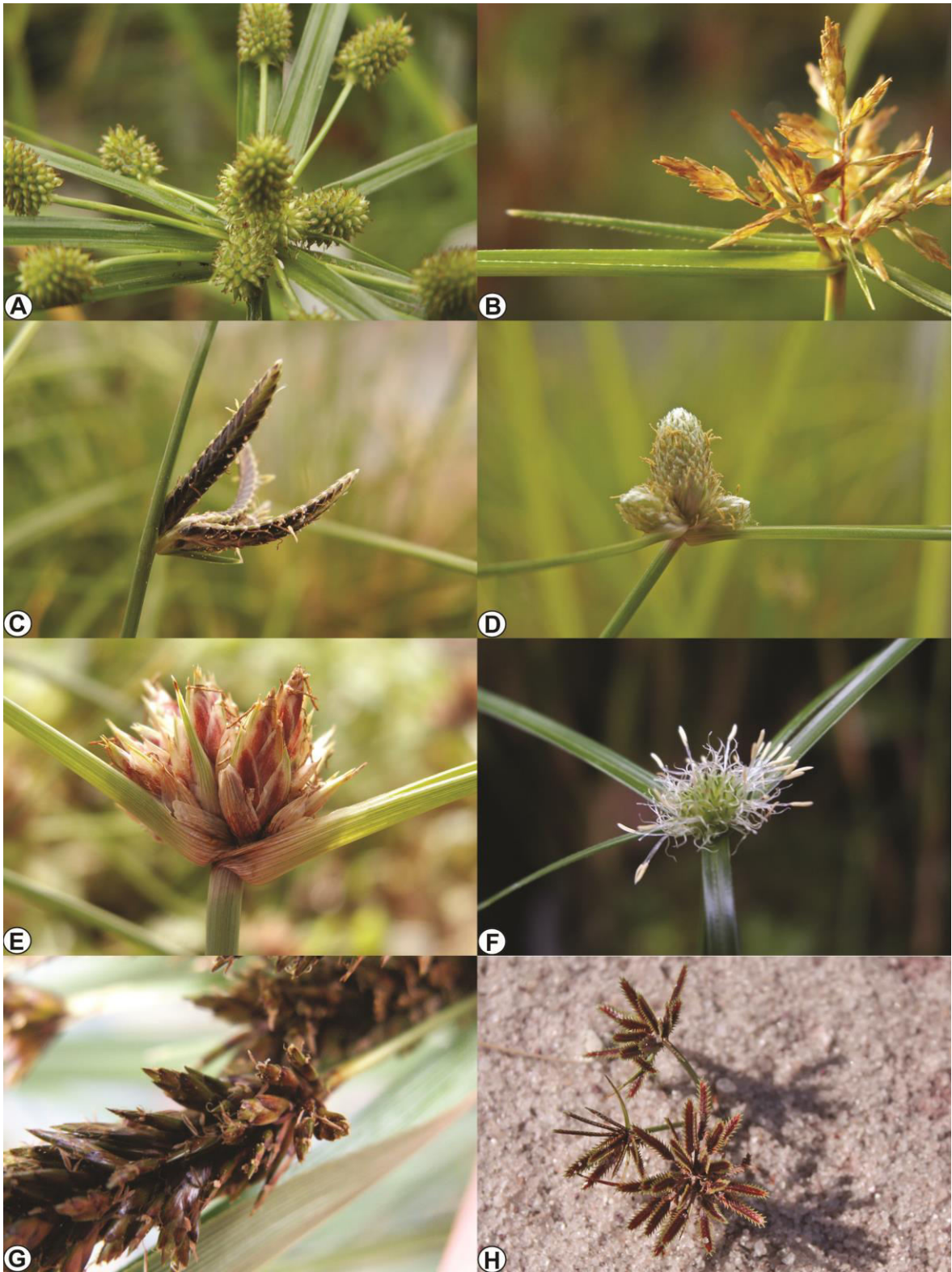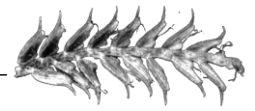
Govaerts *et al.*, 2007, 2012) or merge them into *Cyperus* at an infrageneric rank (e.g. Kükenthal, 1935-36; Haines & Lye, 1983; Lye, 1997).

The *Cyperus* clade includes a grade of branches characterized by C$_3$ photosynthesis (C$_3$ *Cyperus*, c. 190 spp.), which were well-resolved in a combined analysis of ETS1f, trnH-psbA and rpl32-trnL (Larridon *et al.*, 2011a). In C$_3$ *Cyperus*, most sections of the classification according to Kükenthal (1935-36) were confirmed. Larridon *et al.* (2011b) included the C$_3$ segregates *Courtoisina* Soják, *Oxycaryum* and *Kyllingiella* in *Cyperus*, supported by molecular data, combined with morphology, embryography, ontogeny and anatomy.

Nested in C$_3$ *Cyperus* is a highly diverse clade (C$_4$ *Cyperus*, c. 760 spp.) with the C$_4$ photosynthetic pathway as a synapomorphy (e.g. Muasya *et al.*, 2001b, 2002, 2009a, in press; Besnard *et al.*, 2009; Larridon *et al.*, 2011a). The nine C$_4$ segregate genera represent c. 30 % of diversity in the C$_4$ *Cyperus* clade. Figure 7.2 shows some of the morphological diversity of C$_4$ *Cyperus* lineages. They are generally considered as well-delimitated entities (e.g. Goetghebeur, 1998) and are circumscribed by a combination of morphological characters including inflorescence and spikelet morphology, unit of dispersal and nutlet orientation (e.g. Muasya *et al.*, 2009b; Vrijdaghs *et al.*, 2011; Reynders *et al.*, 2012; Fig. 6.3). However, the mutual relationships of the taxa in C$_4$ *Cyperus* still need to be determined.

6.3.2 Paraphyly and modern classification strategies

With the advancement of molecular phylogenetic research, species relationships and evolutionary patterns in giant genera provide new and valuable opportunities to study evolutionary processes. Often, these giant genera appear to contain derived lineages that have, up to now, been considered as separate genera (e.g. *Acacia* Mill., Miller & Bayer, 2001; *Carex* L.; Starr & Ford, 2009; *Croton* L., Berry *et al.*, 2005; *Euphorbia* L., Steinmann & Porter, 2002; *Salvia* L., Walker *et al.*, 2004). The development of new classifications, encompassing the concept of monophyly for these large paraphyletic entities and their segregate genera has been highly challenging. Three main strategies can be implemented: (1) splitting; (2) accepting paraphyletic taxa; and (3) lumping. Splitting paraphyletic taxa into a large number of small genera has been proposed for a number of large genera (e.g. *Acacia*; Maslin, Miller & Seigler, 2003). The decision on where to split needs to be based on a well-resolved phylogenetic hypothesis, and there are challenges to identifying diagnostic characters for the segregate entities and controversies about name application (*Acacia*; e.g. Moore *et al.*, 2010, 2011;
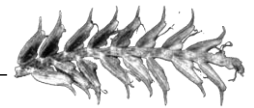
Smith & Figueiredo, 2011; Thiele *et al.*, 2011). A second, less popular, strategy is a classification in which various segregate genera are upheld which are themselves monophyletic, but remain part of a paraphyletically circumscribed giant genus. The use of paraphyletic genera has been defended by some authors (e.g. Brummitt, 1996; Brummitt & Sosef, 1998), but has been strongly opposed by others (e.g. Nelson, Murphy & Ladiges, 2003). The third and most popular strategy when dealing with paraphyletic giant genera is lumping all the segregates into a broader circumscribed genus (e.g. in *Euphorbia*; Steinmann & Porter, 2002). A negative consequence of lumping is that it can become difficult to describe clearly the giant genus as a whole.

Table 6.1 The genera in Cypereae currently accepted by Govaerts *et al.* (2012), plus the recently published genus *Dracoscirpoides* (Muasya *et al.*, 2012) and the recent phylogenetic novelty *Erioscirpus* (Yano *et al.*, 2012). The segregate genera using the C$_4$ photosynthetic pathway are underlined. The taxa indicated by an asterisk were recently included in *Cyperus* (Larridon *et al.*, 2011b)

Cypereae		
***Ficinia* clade**	**?**	***Cyperus* clade**
Dracoscirpoides Muasya (3 spp.)	*Androtrichum* (Brongn.) Brongn.	*Alinula* J.Raynal (4 spp.)
Erioscirpus Palla (2 spp.)		*Ascolepis* Nees ex Steud., (22 spp.)
Hellmuthia Steud. (1 sp.)		*Courtoisina* Soják (2 spp.)*
Ficinia Schrad. (75 spp.)		*Kyllinga* Rottb. (74 spp.)
Isolepis R.Br. (76 spp.)		*Kyllingiella* R.W.Haines & Lye (4 spp.)*
Scirpoides Ség. (4 spp.)		*Lipocarpha* R.Br. (36 spp.)
		Oxycaryum Nees (1 sp.)*
		Pycreus P.Beauv. (114 spp.)
		Queenslandiella Domin (1 sp.)
		Remirea Aubl. (1 sp.)
		Sphaerocyperus Lye (1 sp.)
		Volkiella Merxm. & Czech (1 sp.)
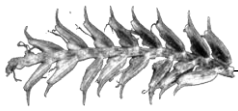
6.4 OBJECTIVES

In the present study, molecular phylogenetic data of the *Cyperus* clade were analysed: (1) to determine the mutual relationships of the taxa (i.e. genera, sections, species) included in C$_4$ *Cyperus*; (2) to test whether the segregate genera and infrageneric taxa in C$_4$ *Cyperus* (Kükenthal, 1935-36; Govaerts *et al.*, 2012) are monophyletic; and (3) to examine the most suitable classification strategy for C$_4$ *Cyperus*. Papers documenting the necessary nomenclatural/taxonomical changes based on the results presented in this paper and more detailed studies of several of the larger C$_4$ segregates will be published elsewhere. This study is part of a larger research project aiming at recircumscribing *Cyperus* as a monophyletic unit and to create a new infrageneric classification of the genus supported by both molecular and morphological data.

6.5 MATERIALS AND METHODS

One hundred and seven samples from 104 different taxa were used for this study. Sixty-seven sequences from 23 species were used from a previous study (Larridon *et al.*, 2011a). The other 213 sequences from 81 different taxa were newly generated for this study. The samples with species names, voucher information, origin and GenBank accession numbers for the sequences, are given in Appendix 3. Taxa within *Cyperus* were selected to represent a broad morphological and geographical range and to include a wide range of the traditionally recognized sections, subgenera and segregate genera. As this study assesses relationships above the rank of species, multiple species samples and infraspecific taxa were generally not used. The outgroup taxa were selected based on the results of previous molecular phylogenetic analyses of Cypereae by Muasya *et al.* (2002, 2009a) and Larridon *et al.* (2011a). Taxonomic information for most taxa mentioned (such as author, place and date of publication, synonyms, distribution) follows Govaerts et al. (2007, 2012). The molecular phylogenetic hypothesis obtained was compared with the classification of Kükenthal (1935-36). Detailed information on the nomenclature of generic and subdivisional names of the *Cyperus* clade (including the synonymy of the names used by Kükenthal) is given in Huygh *et al.* (2010), Larridon *et al.* (2011c) and Reynders *et al.* (2011).

Samples were either of wild origin, mostly collected during recent field expeditions (silica-dried), or sampled from plants from the Ghent University Botanical Garden. Additional
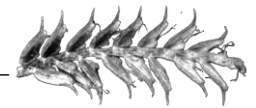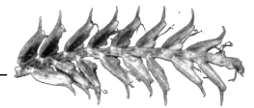
dried leaf samples were selected from herbarium specimens (GENT, BR). The DNA extraction protocol, markers (ETS1f, rpl32-trnL and trnH-psbA) and methods for PCR amplification and sequencing and obtaining alignments used in this study follow Larridon *et al*. (2011a).

Phylogenetic hypotheses were produced using maximum likelihood (ML) and Bayesian inference (BI) analyses. All analyses were first performed on the single marker data sets (ETS1f, rpl32-trnL, trnH-psbA). Since no conflicting clades with a significant confidence value were revealed, a combined data set was constructed and analyzed. The latter was subdivided into three partitions, corresponding to the single markers. The program RAxML v7.2.8 (Stamatakis, 2006) was used to execute the Rapid Bootstrapping algorithm for 500 replicates combined with a ML search, using the GTRCAT model (Stamatakis, Hoover & Rougemont, 2008). Model parameters were optimized for each partition when analyzing the combined dataset.

Bayesian phylogenetic (BI) analyses were carried out in MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). For the analysis, MrModeltest v2.3 (Nylander, 2004) was used to determine the model that best fits the data, applying the Akaike Information Criterion. For the combined data set, a model was determined for each partition. This method is referred to as the BI method. Four independent, parallel runs of one cold and three heated chains were run for 30 million generations each. Trees and parameter estimates were saved every 1000 generations. The analyses were run on a high performance computer at Ghent University (Stevin Supercomputer Infrastructure, ICT Department). Convergence, associated likelihood values, effective sample size values and burn-in values of the different runs were verified with Tracer v1.5 (Rambaut & Drummond, 2007). Calculation of the consensus tree and the posterior probability (PP) of clades was based upon the trees sampled after the chains converged. Trees were drawn using FigTree v1.3.1 and Adobe Photoshop CS3.

→ Fig. 6.3 3D reconstruction of the spikelet evolution in the *Cyperus* clade. The illustrations were drawn in Rhinoceros 3D® (Mc Neel, Seattle, USA) by M. Reynders. The basic *Cyperus* spikelet with distichous glumes developed several times independently into lineages with spiral glumes. Also deciduous spikelets originated several times and from there different reduction lineages can be identified resulting in single-flowered spikelets. In the extreme situation the bracts subtending the spikelets behave like glumes bearing the strongly reduced spikelets. Difficult interpretation of the latter resulted in the classification of these taxa among various Cyperaceae tribes before their affinity with *Cyperus* had been resolved.
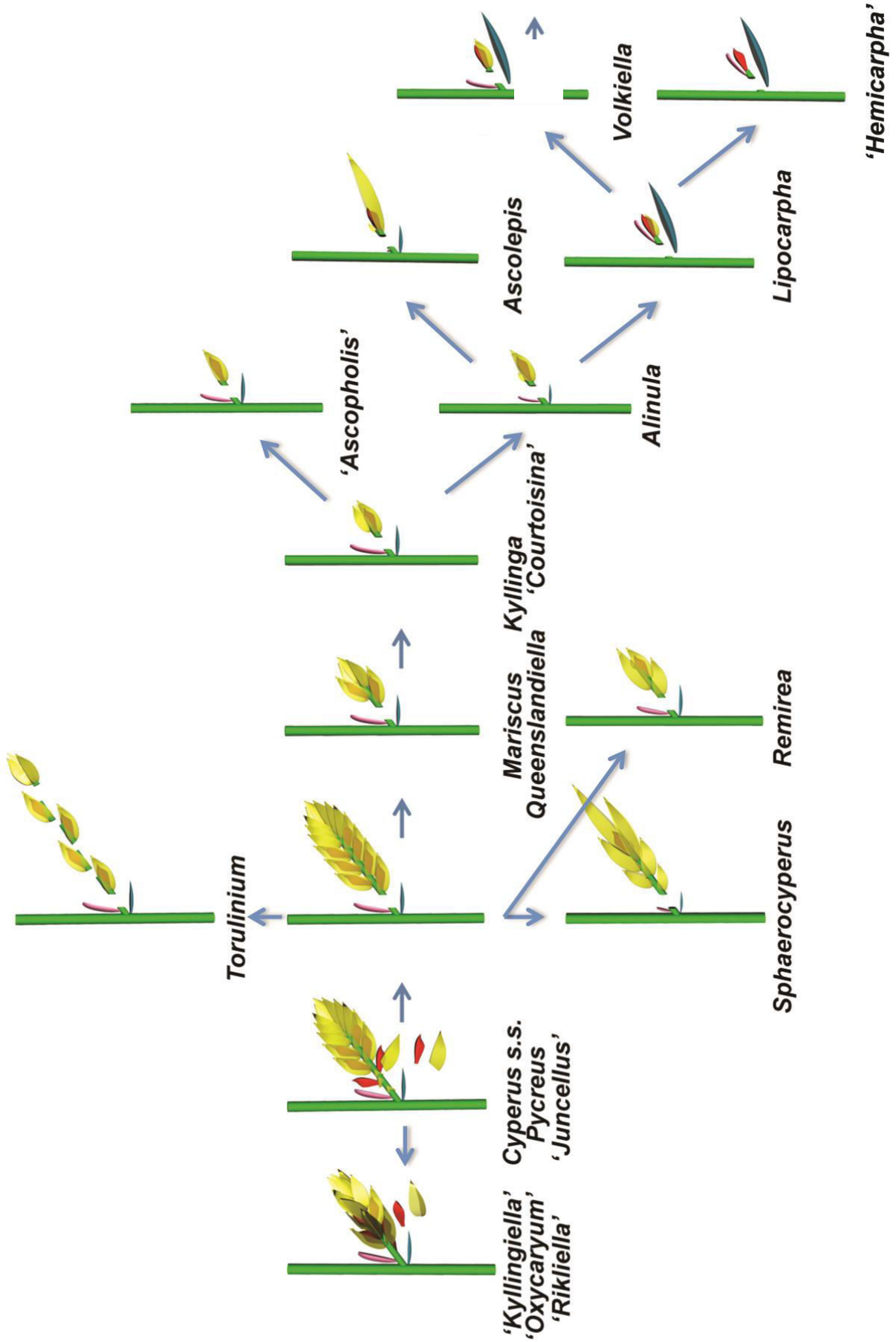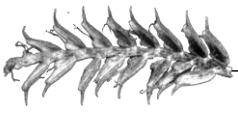
6.6 RESULTS

6.6.1 Sequence alignments

After alignment and application of Gblocks v0.91b (Castresana, 2000), the ETS1f alignment included 105 sequences of 953 bases, the rpl32-trnL alignment 94 sequences of 1334 bases and the trnH-psbA alignment 81 sequences of 1364 bases. The concatenated data set included 108 sequences and the Gblocks program retained 57% or 2101 characters of the original alignment. Most excluded regions came from the ETS1f region.

6.6.2 Phylogenetic analysis

The three single-locus ML analyses revealed nearly identical topologies and bootstrap values. As expected, the clades supported by single-locus analyses, received greater support in the multi-locus ML-analysis. In the various analyses, only minor conflicts concerning the position of some C_4 *Cyperus* spp. in the backbone of the C_4 *Cyperus* clade were detected. Most nodes in the backbone of this clade had little or no support.

The three single-locus BI analyses did not significantly differ in tree topologies. The multi-locus BI topologies did not differ from the multi-locus ML tree, except for some of the C_4 *Cyperus* spp. in the main polytomy as mentioned above for the ML analyses. Evaluation of the multi-locus BI analysis output showed that the four runs converged on similar log likelihood (-23908) and parameter values. The burn-in value for all runs was determined at 3 million generations. The effective sample size (ESS) for the likelihood value of the combined runs consisted of 1397.38 uncorrelated samples.

Figure 7.4 shows the 50% majority consensus multi-locus BI tree with the associated PP values and the bootstrap values of the multi-locus ML tree. Only bootstrap values above 75% and posterior probabilities above 0.85 are shown.

6.7 DISCUSSION

6.7.1 Affinities with C₃ *Cyperus*

In Cypereae, the *Cyperus* clade is sister to the *Ficinia* clade, here used as outgroup represented by species of *Scirpoides, Isolepis* and *Ficinia* (Fig. 6.4). The *Cyperus* clade is strongly supported as monophyletic, but includes several lineages which are currently recognised at the generic level. As in Larridon *et al*. (2011a), C₃ *Cyperus* spp. form a grade at the base of *Cyperus* (Fig. 6.4; Table 6.2). The clade sister to the C₄ *Cyperus* clade is formed by *Cyperus* section *Leucocephali* Cherm. ex Kük. sensu Larridon *et al*. (2011b) (Fig. 6.4). Although the species of this section use C₃ photosynthesis (e.g. Bruhl & Wilson, 2007; Larridon *et al*., 2011a), they occur in open grassland habitats which are generally dominated by species using C₄ photosynthesis. This suggests that the species of *C.* section *Leucocephali* have characters (e.g. geophytic hemicryptophytes, resprouting immediately at start of wet season and dying back on onset of dry season, photosynthesis in at high temperatures and irradiation) which make them fitter to survive in these habitats than most other C₃ *Cyperus* spp.

Table 6.2 C₃ *Cyperus* species included in the phylogeny and the sections they represent

Species	Section
Cyperus haspan	*Cyperus* section *Haspani* (Kunth) C.B. Clarke
Cyperus luzulae	*Cyperus* section *Luzuloidei* (Kunth) C.B. Clarke
Cyperus pectinatus	*Cyperus* section *Anosporum* (Nees) Pax
Cyperus alternifolius and *C. marginatus*	*Cyperus* section *Alternifolii* (Kunth) C.B. Clarke
Cyperus spiralis	*Cyperus* section *Leucocephali* Cherm. ex Kük.

6.7.2 C₄ *Cyperus* radiation

Our molecular phylogenetic hypothesis shows very short branch lengths for most of the C₄ *Cyperus* clade when compared to the C₃ *Cyperus* grade and the deepest nodes of the C₄ *Cyperus* clade, suggesting a rapid diversification of the clade. Endress (2011, p.370) wrote: "Many structural innovations originated in several clades of Angiosperms and in special cases could become key innovations, which likely were hotspots of diversification". The evolution of C₄ photosynthesis in Cypereae can be considered as a key innovation, being the cause of a burst of speciation due to: (1) increased fitness in drier habitats (Besnard *et al*., 2009); (2) optimised nitrogen-uptake; and (3) improved resistance to higher irradiance, fire and chemical stress caused by salt and heavy metals (Li, Wedin & Tieszen, 1999; Stock *et al*., 2004). Based
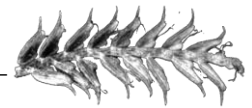
on our results and on literature and herbarium data on the distribution of species, we hypothesise that the evolution of the C_4 photosynthetic pathway in Cypereae occurred in East Africa. This region, particularly present-day Tanzania, is the centre of diversity for C_4 *Cyperus* spp. In addition, all segregate lineages and most sections are represented in the East African flora. Outside Africa, the *Cyperus* clade is either represented by widespread species or by taxa which evolved locally due to smaller radiations originating from dispersal events.

6.7.3 Affinities in C_4 *Cyperus*

The basal nodes -- Several early[1] branches of the C_4 *Cyperus* clade are strongly supported (Fig. 6.4). The first subclade, also retrieved in previous studies (e.g. Muasya *et al.*, 2002, in press, Larridon *et al.*, 2011a), is represented by *Cyperus cuspidatus* Kunth (and its Malagasy relative *C. waterlotii* Cherm.). Kükenthal (1935-36) placed the species of this clade in *C.* section *Amabiles* C.B.Clarke. Although homogeneous, this section is only held together by characters which probably represent the plesiomorphic condition in C_4 *Cyperus*, such as spikelets arranged in digitate clusters (as in many C_3 *Cyperus* spp. vs. generally spikes of spikelets in C_4 *Cyperus*) and multi-nerved glumes with an excurrent mucro. Species of *Cyperus* section *Amabiles, Aristati* Nees and *Rupestres* C.B.Clarke show similar characters. After the *C. cuspidatus* clade, the next branches of our molecular phylogenetic hypothesis include species of the segregates *Alinula* J.Raynal, *Ascolepis* Nees, *Lipocarpha* R.Br., *Queenslandiella* Domin and *Volkiella* Merxm. & Czech, and of *C.* section *Rupestres* (i.e. *C. rupestris* Kunth and *C. meeboldii* Kük.). The relationship between the two species of *C.* section *Rupestres* is strongly supported in our analysis. Taxonomically, this section is well circumscribed by several synapomorphies, such as swollen stem bases and a tendency to reduced flowers, each with a single stigma branch and a single stamen. The exact position of its corresponding clade remains to be confirmed, but its position among the early branches of the C_4 *Cyperus* clade seems acceptable.

Queenslandiella -- The monotypic *Queenslandiella* is currently recognised as a separate genus, based on its laterally flattened, dimerous gynoecia and its deciduous spikelets. *Queenslandiella* has multi-nerved glumes with an excurrent mucro suggesting it is a early branching lineage of C_4 *Cyperus* (Fig. 6.4). When dried, it has a strong curry odour, a character it shares with *C. squarrosus* L., another species showing many of the presumed plesiomorphic

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[1] Early emerging clades

characters of the clade. *Cyperus squarrosus* falls among the basal nodes in an ITS analysis of *Cyperus* (Reid, unpubl. data).

Alinula – According to the current circumscription, *Alinula* includes four species (Goetghebeur, 1998; Govaerts *et al.*, 2012). Only one species, *Alinula paradoxa* (Cherm.) Goetgh. & Vorster, is included in this study (Fig. 6.4). From a morphological point of view, this species significantly differs from the other three. In our opinion, the current circumscription of *Alinula* does not represent a natural group. Haines & Lye (1983), who treated *Alinula* as a subgenus in *Cyperus*, included *A. paradoxa* in *Cyperus* subgenus *Fimbricyperus* Lye separate from the other *Alinula* spp. In our molecular phylogenetic hypothesis, *A. paradoxa* clusters among the early branches of the C$_4$ *Cyperus* clade. More research is needed to reveal its exact relationships. *Alinula lipocarphioides* (Kük.) J.Raynal has been shown to be closer to *Lipocarpha* (Muasya *et al.*, 2009a, in press).

Ascolepis-Lipocarpha clade -- A well-supported clade in our molecular phylogenetic hypothesis includes the genera *Ascolepis, Lipocarpha* and *Volkiella* (Fig. 6.2), which are all characterised by strongly reduced deciduous spikelets grouped into pseudospikelets (spikes of spikelets). Our results confirm that *Ascolepis* and *Lipocarpha* are closely related, as already observed by Muasya *et al*. (2002). Their relatively early branching position in C$_4$ *Cyperus* is corroborated by the presence of a small, weakly differentiated *Cyperus*-type embryo that is also common in C$_3$ *Cyperus* and in the early branches of the *Ficinia* clade (Reynders, unpubl. data). *Lipocarpha* appears paraphyletic, including *Ascolepis* and *Volkiella* (Fig. 6.4). The first diverging branch is formed by *Lipocarpha kernii* (Raymond) Goetgh. and *L. rehmannii* (Ridl.) Goetgh. (Fig. 6.3), formerly placed in a separate genus *Rikliella* J.Raynal. Although these species strongly resemble *Lipocarpha*, prophyll and glumes have not been observed around the flower. Therefore, Goetghebeur & Van de Borre (1989) interpreted *Rikliella* as a highly evolved lineage of *Lipocarpha*. However, on the basis of our phylogenetic trees, it is unclear whether the partial inflorescences should be interpreted as pseudospikelets or as true spikelets with spiral glumes (which occur in at least three other lineages of the *Cyperus* clade; Muasya *et al.*, in press). Sister to this clade is a clade comprising *Ascolepis* and *Lipocarpha* s.s. (Fig. 6.4). *Ascolepis* spikelets are characterised by a single large glume subtending a flower and the loss of the spikelet prophyll. In *Lipocarpha* s.s., the first branching clade is formed by *Lipocarpha micrantha* (Vahl) G.C.Tucker (Fig. 6.4), which is characterised by a reduction of the glume. This clade is followed by the rest of *Lipocarpha* s.s. which also includes the monotypic
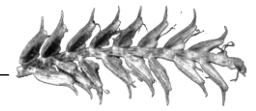
Volkiella (Fig. 6.4*). Volkiella* possesses both a spikelet prophyll and a glume and is included in a subclade with *Lipocarpha albiceps* Ridl. and *L. comosa* J. Raynal (Fig. 6.4). These two *Lipocarpha* spp. are characterised by a well-developed, firm and often dark coloured prophyll which falls off the rachis separately from the flower and its glume. In other *Lipocarpha* spp. the prophyll is hyaline and falls off together with nutlet and glume. *Volkiella* shares the more rigid prophyll with the two abovementioned species. *Volkiella disticha* Merxm. & Czech is in many aspects a special, highly derived species differing from *Lipocarpha* by the distichous arrangement of the spikelets on the rachis. A more elaborate study of *Lipocarpha* integrating molecular phylogeny and morphology will be presented in another paper (Bauters *et al.*, submitted).

The hard polytomy -- The vast majority of C_4 *Cyperus* spp. are included in an unresolved polytomy (Fig. 6.4), which can also be found in all previous molecular phylogenetic studies (e.g. Muasya *et al.*, 2002, 2009a, b). As it has not been possible to resolve this polytomy, even when using fast mutating plastid and nuclear markers, additional markers need to be tested as well as other techniques based on next generation sequencing (e.g. Harrison & Kidner, 2011). However, in our molecular phylogenetic study several subclades and the relationships between some taxa are strongly supported (Fig. 6.4). These taxa are discussed below.

C_4 *Cyperus* s.s. – One subclade of C_4 *Cyperus* s.s. which is strongly supported in our molecular phylogenetic hypothesis (Fig. 6.4) contains species belonging to *Cyperus* sections *Papyri* (Willd.) Thouars (*C. papyrus* L., *C. dives* Delile, *C. alopecuroides* Rottb.) and *Rotundi* C.B.Clarke (*C. rotundus* L., *C. longus* L., *C. endlichii* Kük., *C. rigidifolius* Steud.). These species are all characterised by a narrowly to broadly winged rachilla with deciduous or persistent wings. Several other sections which are not represented in the current analysis, i.e. *Cyperus* sections *Brevifoliati* C.B.Clarke, *Exaltati* (Kunth) C.B.Clarke and *Fastigiati* Kük., share these characters. *Cyperus compressus* L. (*C.* section *Compressi* Nees) also clusters in this clade (Fig. 6.4).

→ Fig. 6.4 Phylogenetic hypothesis for the *Cyperus* clade: 50% majority consensus multi-locus BI tree with the associated PP values and the bootstrap values of the multi-locus ML tree. Only bootstrap values > 75% and posterior probabilities > 85% are shown.
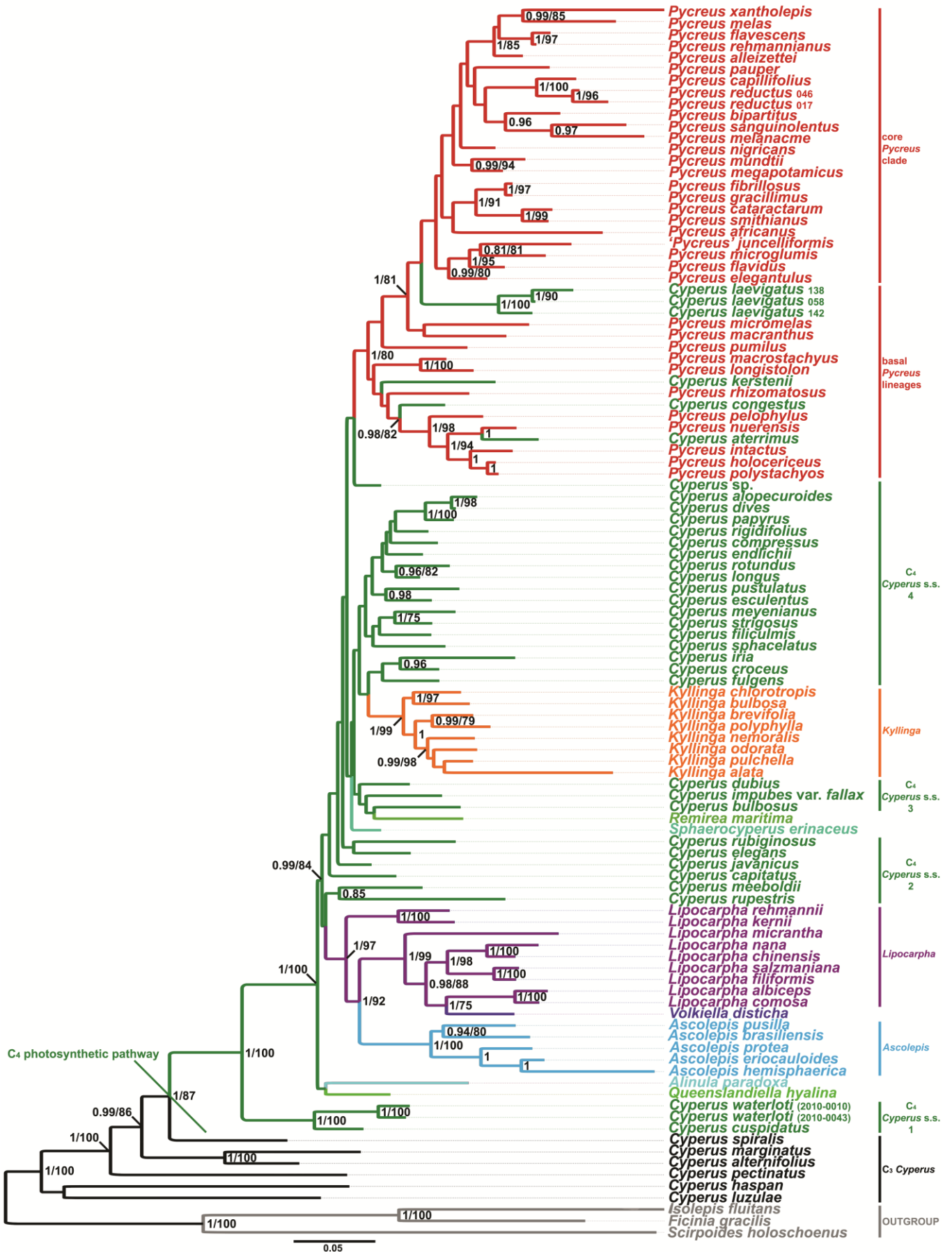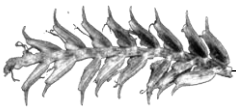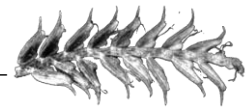
Kyllinga -- *Kyllinga* Rottb. forms a strongly supported monophyletic clade (Fig. 6.4). There is weak support for the *Kyllinga* clade as sister to a clade including *Cyperus iria* L., *C. croceus* Vahl and *C. fulgens* C.B.Clarke. *Kyllinga* is delimited by the combination of a head-like inflorescence, deciduous spikelets and laterally flattened gynoecia. Three subclades can be recognised in the current molecular phylogenetic hypothesis (Fig. 6.4). A detailed molecular phylogenetic study of *Kyllinga*, including AFLP data, is being prepared (Huygh, Bauters, Larridon, Reynders, Muasya, De Riek, Simpson & Goetghebeur, unpubl. data).
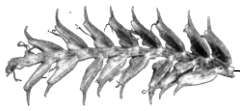
Remirea and *Sphaerocyperus* -- The monotypic genera *Remirea* Aubl. and *Sphaerocyperus* Lye remain unresolved in C_4 *Cyperus* (Fig. 6.4). Both taxa are characterised by a series of empty scales below the flower-bearing glume. For this reason affinities with Schoeneae or Rhynchosporeae had been suggested (Fenzl, 1836: p. 144; Bentham, 1883: p. 1038; Ridley, 1884: p. 165; Pax, 1888: p. 116; Baillon, 1894: p. 377; Clarke, 1901-02: p. 267; Kükenthal, 1944: p. 200-209). Additionally, *Remirea* has corky rachilla internodes.

Pycreus -- *Pycreus* P.Beauv. is here retrieved as a paraphyletic entity including several *Cyperus* spp. (Fig. 6.4). In *Pycreus*, relationships are poorly resolved although good resolution is obtained for some smaller clades of related species. Furthermore, one large clade is well-supported and contains the majority of the sections and species in addition to *Cyperus laevigatus* L. (Fig. 6.4). This clade is referred to as the 'core *Pycreus* clade'.

The *Pycreus* species which are not included in the core *Pycreus* clade all belong to four of Kükenthal's (1935-36) sections, namely *Cyperus* section *Albomarginati* Kük., *C.* section *Lancei* Kük, nom. superfl., *C.* section *Polystachyi* (C.B.Clarke) Kük., nom. illeg., *C.* section *Pumili* Kük. and *C.* section *Rhizomatosi* Kük. Their mutual relationships remain unresolved, but their position outside the core *Pycreus* clade can be justified since the species in these sections possess plesiomorphic characters [2] in contrast to the species in the core *Pycreus* clade (Table 6.3).

Among the early branching lineages, two smaller clades are well-supported (Fig. 6.4). *Pycreus longistolon* (Peter & Kük.) Napper and *P. macrostachyos* (Lam.) J.Raynal are strongly supported together. Kükenthal (1935-36) classified *P. longistolon* in *C.* section *Lancei*, nom. superfl., a section which appears artificial since the species only share rather large and dark glumes. *Pycreus macrostachyos* was included in *C.* section *Albomarginati* [as *Cyperus albomarginatus* (Mart. & Schrad. ex Nees) Steud.]. Including *P. longistolon* in *C.* section

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[2] Such as multinerved glumes with an excurrent mucro, pioneering growth strategy, ect.

Albomarginati seems appropriate in view of the overall habit of the plants (except for the stolons), the large dimensions of the spikelets, glumes and nutlets and the wide, hyaline glume margins. However, the last character is less conspicuous than in *P. macrostachyos*. Another well-resolved subclade corresponds to *C.* section *Polystachyi*, nom. illeg., and is characterised by typically elongated nutlets and a winged rachilla. *Pycreus pelophilus* C.B.Clarke is an exception in having broad nutlets. Nevertheless, it was placed in this section by Kükenthal (1935-36) and this relationship is confirmed here (Fig. 6.4).

The strongly supported inclusion of *Cyperus aterrimus* (Fig. 6.4) in the early branching lineages of *Pycreus* is noteworthy since this species has triangular nutlets, a different inflorescence and overall larger dimensions of the glumes and nutlets compared to *Pycreus*. *Cyperus aterrimus* Hochst. ex Steud. is strongly supported as sister of *Pycreus nuerensis* (Boeckeler) S.S.Hooper, which it resembles in its growth form, dark coloured inflorescence and Afromontane distribution. *Cyperus kerstenii* Boeckeler and *C. congestus* Vahl also appear to be associated with the early branching *Pycreus* lineages, although without support. We found no morphological characteristics to support this relationship, especially since both species have deciduous glumes, a character which does not occur in *Pycreus*. The presence of species with triangular nutlets in *Pycreus* suggests a reversion of the dimerisation of the gynoecium. Recently, Vrijdaghs (2006) and Reynders *et al*. (2012) showed that gynoecia in Cyperoideae originate from an annular primordium on which stigma primordia originate. This offers more flexibility for the positioning of stigma branches with respect to the restrictions previously assumed based on the anatomical studies by Blaser (1941a, b).

The core *Pycreus* clade only includes *Pycreus* spp., except for *Cyperus juncelliformis* Peter & Kük. and *C. laevigatus*. *Cyperus juncelliformis* is a true *Pycreus*, but its name has never been combined into *Pycreus*. Therefore, its name is mentioned as '*Pycreus' juncelliformis* in figure 7.4. The association of *C. laevigatus* with the core *Pycreus* clade seems to be strong. It was verified by including three separate samples of *C. laevigatus*, and this relationship also occurred in the analyses of the three markers separately (Reynders, unpubl. data). Whereas *Pycreus* is characterised by laterally flattened dimerous gynoecia, *C. laevigatus* has dorsiventrally flattened dimerous gynoecia. This might either represent an intermediate state between a trimerous *Cyperus* ancestor and *Pycreus* or a derived state from a *Pycreus* ancestor. Moreover, the vascularisation pattern in the rachilla of *C. laevigatus* differs from the

pattern in rachillas of several *Pycreus* spp. studied by Reynders *et al*. (2012). Shared characters of *C. laevigatus* and *Pycreus* are the rather glossy glumes and their ecology.

ETS1f sequences of the species in the core *Pycreus* clade (except *C. laevigatus*) show a large duplication of 140 bp, which is a strong additional argument that this represents a natural group. In the core *Pycreus* clade, several species clusters are resolved (Fig. 6.4). *Pycreus flavidus* (Retz.) T.Koyama clusters with '*Pycreus*' *juncelliformis*, corresponding to Kükenthal's (1935-36) *Cyperus* section *Globosi* (C.B.Clarke) Kük. The inclusion of *P. niger* (Ruiz & Pav.) Cufod. is morphologically supported by the similar nutlets and the shape of the glumes. In contrast, the inclusion of *P. flavescens* (L.) P.Beauv. ex Rchb. subsp. *microglumis* Lye is remarkable and needs further investigation. Morphologically, the species cluster of *P. capillifolius* (A.Rich.) C.B.Clarke and *P. reductus* Cherm. shows resemblances to *C.* section *Globosi,* but this relationship remains unresolved in the current study. Also, species of *C.* section *Sulcati* Kük., nom. illeg., are distributed between two clades, although the species of this section all share peculiar glumes with a furrow on both sides*. Pycreus sanguinolentus* (Vahl) Nees and *P. bipartitus* (Torr.) C.B.Clarke are smaller representatives of this section, whereas *P. mundtii* Nees and *P. megapotamicus* (A.Dietr.) Nees are taller plants with long culms with spaced leaves that form floating mats on open water. The clustering of *P. melanacme* Nelmes with this section needs further investigation, since this is in many ways a rather distinct therophytic species.

Pycreus africanus (S.S.Hooper) Reynders, *P. smithianus* (Ridl.) C.B.Clarke, *P. cataractarum* C.B.Clarke, *P. fibrillosus* (Kük.) Cherm. and *P. gracillimus* Chiov. form a well-resolved clade. *Pycreus africanus* belongs to *P.* section *Tuberculati* Cherm. (Reynders & Goetghebeur, 2010). *Pycreus smithianus* and *P. cataractarum* share many characters such as a contracted inflorescence, straight rachilla, bright white glumes and a Guineo-Congolean distribution, with a preference for habitats by running water. Kükenthal (1935-36) included both species in *Cyperus* section *Propinqui* (C.B.Clarke) Kük. *Pycreus fibrillosus* and *P. gracillimus* both have a plant base covered with fibrous remains of old leaf sheaths, an inflorescence reduced to only a few spikelets, a flexuous rachilla and a Zambesian distribution in Afromontane habitats. These species were respectively placed in *C.* section *Propinqui* and *C.* section *Latespicati* Kük. by Kükenthal (1935-36) based on their pale versus dark glumes. As this character seems to depend on altitude (many species of *Cyperus* s.l. growing above 2000

m have dark-coloured glumes), it is not considered reliable for sectional delimitation. Therefore, these two sections are likely to be polyphyletic.

A final strongly supported clade contains species belonging to *Cyperus* section *Latespicati* (*Pycreus alleizettei* Cherm.) and *C.* section *Flavescentes* Kük. nom.superfl. (*P. flavescens, P. rehmanianus* C.B.Clarke) sensu Kükenthal (1935-36). *Pycreus xantholepis* Nelmes, a tall therophyte, shares its yellow glume colour and nutlet shape with *P. alleizettei* and *P. flavescens*. The inclusion of *P. melas* (Ridl.) C.B.Clarke [*C.* section *Globosi* (C.B. Clarke) Kük.] needs further investigation, as this species is morphologically distinct.

Table 6.3 Comparison between the noncore *Pycreus* species and the core *Pycreus* clade (with the exception of *Cyperus laevigatus*)

Character	Noncore species	Core *Pycreus* clade
Glumes	Multi-nerved	Midrib with only three nerves
Mucro	Usually present, excurrent	Not present or rarely shortly excurrent
Anthela	Well-developed with long and narrow spikelets, often with second-order branches	Often condensed or reduced, especially in therophytic species
Nutlet epidermal cells	Isodiametric	Isodiametric to strongly elongate
Ecology	Mostly opportunistic and lowland concentrated.	Often very specialised
Distribution and habitat	Widespread and common on roadsides and rice fields	Narrow distribution, occurring in high-altitude bogs, salt marshes, floating on open water, etc.

7.7.4 Re-evaluating the generic status of the segregate lineages

In this section of the paper, we re-evaluate the generic status of the segregate lineages based on the currently available knowledge about these taxa. This is a combination of morphological, anatomical, ontogenetic and, embryographical, … data, and the results of previous and current molecular phylogenetic studies.

Alinula -- Goetghebeur & Vorster (1988) included four species in this genus. A species from eastern Africa was originally described as *Ficinia lipocarphioides* Kük. based on the presence of a hypogynous disc around the base of the fruit. However, after studying its
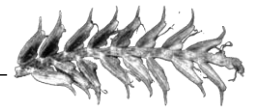
inflorescence morphology and chlorocyperoid anatomy, Raynal (1973) hypothesised that the species was intermediate between *Ascolepis* and *Mariscus* Vahl, and eventually placed it in a new genus *Alinula* (Raynal, 1977). Three more species were added to *Alinula*, after a complex taxonomical trajectory (e.g. Goetghebeur, 1977; Voster, 1978; Haines & Lye, 1983). In our opinion, the current circumscription of *Alinula* does not represent a natural group, although *A. lipocarphioides, A. malawica* (J.Raynal) Goetgh. & Vorster and *A. peteri* (Kük.) Goetgh. & Vorster show clear morphological affinities, such as the presence of pseudospikelets. However, since pseudospikelets also occur in other, more distantly related taxa of Cypereae (e.g. *Ascolepis* and *Lipocarpha*), their presence is, in our opinion, insufficient for generic delimitation. Moreover, *A. lipocarphioides* has been shown to be nested in the *Lipocarpha* clade (Muasya *et al.*, in press).

Ascolepis -- The head-like inflorescence of *Ascolepis* consists of clusters of single-flowered spikelets, sometimes with a rudimentary second glume. Typically the spikelet prophyll does not develop, but the only glume subtending the single flower is always well-developed and larger than the bract which subtends the spikelet. In other species, the glume encloses the flower completely and wings are often developed, possibly for wind dispersal. In other species, the glume is strongly elongated and/or brightly coloured, which gives the inflorescence heads an Asteraceae-like appearance (e.g. as in *A. protea* Welw.), suggesting insect pollination. Raynal (1973) postulated the origin of *Ascolepis* from a mariscoid ancestor. However, Goetghebeur (1980) argued that, although glume and nutlet are shed together in *Ascolepis*, the rachilla remains fixed on the rachis in contrast to *Mariscus*. *Mariscus* was an artificial genus grouping together members of Cypereae with deciduous spikelets. Our results concur with Muasya *et al*. (2002) in resolving *Ascolepis* and *Lipocarpha* as sister taxa. Morphological differentiation in these two taxa shows that similar functional inflorescences originated in both groups using different organs (e.g. *A. protea* vs. *L. comosa*). In *Lipocarpha*, the spikelet bract is strongly developed, whereas the glume subtending the flower is reduced. In *Ascolepis*, the spikelet bract is rudimentary, whereas the glume subtending the flower is strongly developed. Because of the morphological diversity of the inflorescence, rachilla and glumes among the different subgroups in *Ascolepis*, Goetghebeur (1986) considered the possibility that *Ascolepis* is a complex of convergent lineages which developed a similar inflorescence Bauplan. A more thorough molecular investigation of *Ascolepis* is needed to test the monophyly of this taxon.

Kyllinga -- *Kyllinga* is characterised by the combination of laterally flattened gynoecia, deciduous spikelets with a reduced number of flowers and capitate inflorescences. The close relationship of *Kyllinga* with *Cyperus* has always been acknowledged and various authors have treated *Kyllinga* at the subgeneric level in *Cyperus* (e.g. Kükenthal, 1935-36; Haines & Lye, 1983). However, *Kyllinga* has always been considered a homogeneous, natural entity, as illustrated by the fact that several authors have maintained Kyllinga as a separate genus while lumping *Mariscus, Pycreus, Torulinium* Desv. ex Ham. and *Juncellus* C.B.Clarke in *Cyperus* (Lye, 1972, 1982; Tucker, 1983). The monophyly of *Kyllinga* is confirmed by our results where it is retrieved as a strongly supported clade (Fig. 6.4). Since (1) *Kyllinga* is nested in C$_4$ *Cyperus*, (2) capitate inflorescences with reduced, deciduous spikelets (i.e. pseudospikelets) are encountered in various lineages in C$_4$ *Cyperus*, such as *Cyperus* section *Bulbocaules* (C.B.Clarke) Kük., *Ascolepis, Lipocarpha* and *Remirea* and (3) laterally flattened gynoecia also occur in *Pycreus* and *Queenslandiella* which are not immediately related, there are in our opinion no sufficient arguments to warrant generic status for *Kyllinga*.

Lipocarpha -- *Lipocarpha* spp. generally have a highly specialised inflorescence consisting of a spike of highly reduced spikelets with each spikelet, subtended by a bract, containing an abaxial prophyll and an adaxial glume subtending the flower. A few *Lipocarpha* spp. have lost the glume subtending the flower, although some rudiments of it remain visible (Goetghebeur & Van den Borre, 1989). These species were originally classified in a separate genus *Hemicarpha*, based on the reduction of the glume and the presence of a pseudolateral inflorescence (Nees & Arnott, 1834). Since the type species of *Hemicarpha* Nees, *H. isolepis* Nees (accepted name: *L. hemisphaerica* (Roth) Goetgh.), does not show this reduction, *Hemicarpha* was synonymised with *Lipocarpha* (Goetghebeur & Van den Borre, 1989). *Lipocarpha micrantha*, which belongs to this group, is sister to all other *Lipocarpha* spp. studied, including *Volkiella*.

Haines & Lye (1971, 1983) and Goetghebeur & Van den Borre (1989) considered *Rikliella* to represent a final reduction step of a *Lipocarpha* spikelet in which the spikelet prophyll and glume subtending the flower are lost, resulting in a perfect pseudospikelet with flowers in the axil of the spikelet bracts. *Hemicarpha* was indicated as the transitional stage between *Lipocarpha* and *Rikliella*. *Hemicarpha* and *Rikliella* are no longer recognised at generic level (Goetghebeur & Van den Borre, 1989; Govaerts *et al*., 2012). Our results place the two species of *Rikliella* (*L. rehmannii* and *L. kernii*) on a separate, strongly supported
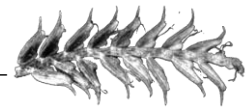
branch and not as a specialised lineage of *Lipocarpha*. This questions previous interpretations of its inflorescence Bauplan.

As in *Alinula, Ascolepis* and *Kyllinga*, we do not consider the presence of pseudospikelets enough to warrant generic status for *Lipocarpha*. Furthermore, in this study, *Lipocarpha* is found to be paraphyletic, containing *Ascolepis* and *Volkiella*. A more detailed study of *Lipocarpha* and *Rikliella* will be published elsewhere (Bauters *et al*., submitted).

Pycreus -- *Pycreus* is the largest segregate genus in C$_4$ *Cyperus.* Furthermore, it is morphologically and ecologically diverse. The close relationship between *Cyperus* and *Pycreus* has never been doubted since *Pycreus* only differs from *Cyperus* s.s. in its laterally flattened gynoecia. These gynoecia also occur in *Kyllinga* and *Queenslandiella*, which, in contrast to *Pycreus*, also have deciduous spikelets. The generic status of these taxa has always been controversial and their status strongly correlated with the taxonomic value granted to laterally flattened gynoecia. From our results, it is evident that taxa with laterally flatttened gynoecia are not sister groups, and *Kyllinga* is strongly supported as separate entity. Therefore we can conclude that there have been multiple independent origins of lateral gynoecia in Cypereae.

Our current molecular phylogenetic study includes species representing all 13 sections of Kükenthal (1935-36). Although relationships between the different sections remain poorly resolved, several patterns need further attention. *Pycreus* is not monophyletic, since species that Kükenthal (1935-36) included in *Cyperus* sections *Albomarginati, Polystachyi*, nom. illeg., *Pumili* and *Rhizomatosi* are found in the main C$_4$ *Cyperus* polytomy (Fig. 6.4). Many species of these sections share several plesiomorphic characters which also occur in C$_4$ *Cyperus*, whereas species in the core *Pycreus* clade show more evolved character states (see Table 6.3). As in *Kyllinga*, we do not consider laterally flattened gynoecia sufficient to maintain *Pycreus* at the generic level, especially as it resolved as polyphyletic in the present study.

Queenslandiella -- *Queenslandiella* is a third taxon nested in the C$_4$ *Cyperus* polytomy, which is characterised by laterally flattened gynoecia. It shares the open inflorescence with *Pycreus* (which is the plesiomorphic condition in C$_4$ *Cyperus*). However, it was most often considered to be related to *Kyllinga* with which it shares deciduous spikelets, and keeled and multi-nerved glumes (Chermezon, 1919; Ballard, 1932, 1933; Koyama, 1977). The species has always been placed in or near *Cyperus*. However, even when included in *Cyperus*, it was most
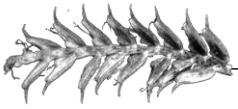
often retained in its own section or subgenus (Kern, 1974; Govindaralaju, 1975; Haines & Lye, 1983).

As with the other specialised, short lived and monotypic segregate lineages, *Queenslandiella* has also accumulated many peculiar characters which isolate it from the other C_4 *Cyperus* taxa. These characters include the large proportions of glumes and nutlets compared to most other *Cyperus* spp., vegetative anatomy (Govindaralaju, 1975) and embryo type (Van der Veken, 1965). Several *Cyperus* spp. have been considered closely related to *Queenslandiella*, including *Cyperus soyauxii* Boeckeler, which has similar deciduous spikelets with similar glumes and a similar embryo (Kükenthal, 1936; Van der Veken, 1965) but has trimerous pistils (Goetghebeur, 1986). Lye (1983) described *Cyperus micromariscus* Lye, which is only known from its type collection in Tanzania. This plant also has an open inflorescence with deciduous spikelets and laterally flattened pistils comparable to *Queenslandiella* but differs in the small glumes and nutlets and different habit. Therefore Lye (1983) assumed a different origin of this species and placed it in its own *Cyperus* subgenus *Micromariscus* Lye (Haines & Lye, 1983). The relationship of *Queenslandiella* to both *C. soyauxii* and *C. micromariscus* need further confirmation. As for the segregates above, we do not consider the specialised characters of *Queenslandiella* sufficient to warrant recognition at generic level.

Remirea -- *Remirea* is another monotypic entity with special adaptations to its coastal habitat. It is characterised by a capitate inflorescence with deciduous spikelets. Each spikelet contains a few empty glumes at the base and a corky rachilla which envelops the fruit. For these reasons, it had been classified among Rhynchosporeae (Fenzl, 1836; Bentham, 1883; Pax, 1888; Baillon, 1894; Clarke, 1901-02; Kükenthal, 1944; Haines & Lye, 1983). However, Nees (1834) had already placed *Remirea* correctly in Cypereae. After Kunth (1837) gave a correct interpretation of the spikelet, this opinion was followed by Chermezon (1922), Kern (1958, 1974), Oteng-Yeboah (1975), Hooper (1983) and Goetghebeur (1986, 1998).

Remirea is nested in the main C_4 *Cyperus* polytomy similar to *Sphaerocyperus* (Fig. 6.4), which also has empty glumes in the lower part of the spikelets. The relationship between these two taxa remains unclear. However, we do not believe empty glumes at the base of the spikelets to be sufficient as a generic character considering that other links with C_4 *Cyperus* are clear. The corky rachilla is also observed in *Cyperus odoratus* L. (formerly in the genus *Torulinium* Desv. ex Ham.), a species with multiple flowers in which the rachilla breaks up into
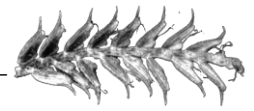
individual segments. The affinity between *Remirea* and *C. odoratus* needs further investigation.

Sphaerocyperus -- The deciduous spikelets of the monotypic *Sphaerocyperus* have six or seven distichously arranged glumes of which only one bears a maturing nutlet. The sole species has variously been placed in *Actinoschoenus* Benth., *Cyperus, Schoenus* L. and *Rhynchospora* Vahl before it was described as a separate genus *Sphaerocyperus* (Lye, 1972). Like *Remirea*, we consider the empty glumes as insufficient to retain this taxon as a separate genus nested in a paraphyletic *Cyperus* with which it shares clear morphological affinities.

Volkiella -- *Volkiella* is a rare monotypic taxon from south-western Africa (mainly Namibia) and can be seen as an extremely specialised lineage adapted to psammophytic habitats. When described, *Volkiella* was considered to be intermediate between *Cyperus* and *Lipocarpha* (Merxmüller & Czech, 1953). The relationship with *Lipocarpha* was explained by the similar presence of the two "floral scales" ("hypogynen Skalen") of which the correct interpretation was not yet clear, but the relationship with *Cyperus* was assumed based on the distichous placement of the "Glumae" which are in fact the spikelet bracts and thus not homologues of the glumes in *Cyperus* and other sedges. This initial interpretation was followed by Van der Veken (1965) and Raynal (1973), but was later correctly interpreted by Goetghebeur (1986, 1998). As in several other lineages such as *Ascolepis, Lipocarpha* and *Alinula, Volkiella* shows highly derived pseudospikelets with a Bauplan comparable to that of *Lipocarpha* possessing a spikelet bract, a spikelet prophyll, a proximal glume subtending the single flower and a spikelet bract larger than the glume. Peculiarly, in *Volkiella*, the spikelets are distichously arranged on the spike axis whereas this position is spiral in all other C_4 *Cyperus* spp. Although *Volkiella* shows an abundance of autapomorphic, derived characters which isolate it from all other C_4 *Cyperu*s spp., it is nested in *Lipocarpha* and should thus be sunk into *Cyperus* together with *Lipocarpha*.

7.7.5 Basis for a modern classification of *Cyperus*

From the current and previous molecular phylogenetic analyses it is evident that the classification of Goetghebeur (1998) in Cypereae can no longer be upheld without accepting paraphyletic genera. Although most of the segregate genera are morphologically well-circumscribed, the rapid diversification of the *Cyperus* clade has resulted in several nested paraphyletic entities (e.g. the genus *Volkiella* is nested in the genus *Lipocarpha*, which is

nested in the group of C$_4$ *Cyperus* spp. formerly known as *Mariscus*, and C$_4$ *Cyperus* is, in turn, nested in C$_3$ *Cyperus*). Moreover, most morphological characteristics used for the delimitation of the different genera related to *Cyperus* appear to have a high level of homoplasy in the *Cyperus* clade (e.g. spiral glumes, dorsiventrally flattened dimerous pistils, deciduous spikelets, pseudospikelets; Fig. 6.3). Subsequently, different combinations of the same sets of these morphological characters have been used to circumscribe most taxa.

Larridon *et al*. (2011a, b) placed the C$_3$ segregate genera in C$_3$ *Cyperus* based on a well-resolved phylogenetic hypothesis combined with morphological, embryographical, ontogenetic and anatomical data. In that paper, a classification for the *Cyperus* clade was suggested in which two subgenera are recognised. Although *Cyperus* subgenus *Anosporum* (Nees) C.B.Clarke (C$_3$ *Cyperus*) is currently circumscribed as a paraphyletic entity (Larridon *et al*., 2011a, b), the single origin of C$_4$ photosynthetic pathway, a clear apomorphy for the C$_4$ *Cyperus* clade, forms a sufficiently strong argument for the use of an evolutionary approach restricted to the subgeneric level in *Cyperus*. For the lower level classification a cladistic approach was followed in circumscribing only monophyletic sections and subsequently the segregate genera will be included in existing or new sections in *Cyperus*.

This classification can be extended to include the different taxa of the C$_4$ *Cyperus* clade (*Cyperus* subgenus *Cyperus*). However, since most segregate genera are nested in a hard polytomy with many species from different sections of *Cyperus* s.s. and since the lower level relationships in several segregate genera are poorly resolved, it is currently premature to build a new sectional classification for the largest part of *Cyperus* subgenus *Cyperus*. A joint international effort will be necessary to expand the current phylogenetic studies with more DNA markers and taxa. This will then serve as a basis for the growing modern classification of the giant genus *Cyperus*.

7.8 Conclusions

From the data presented here we conclude that the *Cyperus* clade consists of a paraphyletic C$_3$ *Cyperus* and a well-supported monophyletic C$_4$ *Cyperus* clade. Nine segregate genera are nested in C$_4$ *Cyperus*, i.e. *Alinula, Ascolepis, Lipocarpha, Kyllinga, Pycreus, Queenslandiella, Remirea, Sphaerocyperus* and *Volkiella*, most of which are monophyletic. Because they are nested in the *Cyperus* clade and as a consequence of the multiple origins of the characters used to circumscribe them, we suggest including all nine C$_4$ *Cyperus* segregate
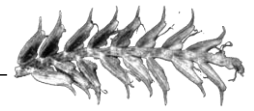
genera into a more broadly circumscribed *Cyperus*. This study establishes a phylogenetic framework for future studies of the different C$_4$ *Cyperus* sections and segregates and for the taxonomic inclusion of the C$_4$ segregate genera into *Cyperus* s.l.

7.9 Acknowledgements

7 Nutlet micromorphology

"*Of course in science there are things that are open to doubt and things need to be discussed. But among the things that science does know, evolution is about as certain as anything we know.*"

— *Richard Dawkins (1941 -   )*

← Fig. 7.1 Nutlet epidermal cells of *P. micromelas*.

Spikelet theme: *Pycreus flavidus*

7.1 In this chapter…

Chapter 7 shows the results of a micromorphological study of the nutlet epidermis in *Pycreus***.** The SEM observations are combined with the results of the molecular phylogenetic study of chapter 6 to reevaluate the diagnostic value of nutlet characters and character states.

This study of the variation in nutlet epidermal cells in *Pycreus* was performed in collaboration with Ľuboš Majeský (Palacký University in Olomouc, Czech Republic), who worked at the Spermatophytes Research Group in 2004, during an internship.

The results of this chapter are currently unpublished.

7.2 Abstract

Pycreus is the largest of the so called segregate genera nested within the C_4 subclade of *Cyperus*. Variation in small structures as glumes and nutlets seems to be remarkably large in *Pycreus*. In the past variation in nutlet epidermis has already been used as a key characteristic in the subgeneric taxonomy of the genus. Generally two main types had been distinguished: isodiametric and elongated nutlet epidermal cells. To be able to re-evaluate the value of the variation in nutlet epidermis for subgeneric taxonomy in *Pycreus*, the nutlet surface is studied here using SEM. In addition to the shapes of the cells, also the variation in silica bodies has been studied. Next to isodiametric and elongates cells, several species show a variety of cells with intermediate elongations. Of silica bodies three main types have been observed, each with several subtypes. In some species silica bodies were reduced or even absent. It is very hard to draw conclusions on homoplasy since the infrageneric relationships are poorly resolved. However, isodiametric cells with a single tabular silica body seem to be common among the noncore species, while isodiametric to zonate cells with silica bodies with a knobby apex or with silica bodies lacking are related to the core clade. Among the species of several sections of the classification of Kükenthal there seems to be a variation rather than a single cell type.

7.3 Introduction

7.3.1 The nutlet epidermis, a key diagnostic character in *Pycreus*?

Recent molecular studies (Muasya *et al*., 2002; Larridon *et al*., 2011a, 2013) all show the Cyperaceae genus *Pycreus* to be nested within the C$_4$ *Cyperus* along with several other previously recognised genera. Moreover, *Pycreus* is not confirmed as a monophyletic entity as only one subclade is supported, which is sister to *Cyperus laevigatus*. Since the position of the other species remains unresolved, it is not yet clear whether *Pycreus* represents a paraphyletic or polyphyletic taxonomical entity. However, it is clear that a generic status separately from *Cyperus* can no longer be maintained. Previously, *Pycreus* was considered to be a well circumscribed taxon, characterised by its laterally flattened dimerous pistils in combination with fruits and glumes that are deciduous separately from the rachilla at maturity (Goetghebeur, 1998).

Adaptation to several similar habitats resulted in many similarities in growth forms and morphological adaptations of *Pycreus* species. In the past, this was the source for many misclassifications and several heterogeneous sections (e.g. Kükenthal, 1935-36). The variation in small structures as glumes and nutlets is remarkably large in *Pycreus*. The variation in nutlet epidermis was used as a key character in the subgeneric taxonomy of the group (see chapter 5). Generally, authors distinguished two main types of nutlet epidermal cells: isodiametric cells (cells which have more or less the same length and width) and zonate cells (strongly elongated cells). Usually isodiametric cells also possess silica bodies in the centre of each cell, which makes the nutlet surface appear to be puncticulate. The elongated cells of the zonate type on the other hand cause the short walls between the cells to become uplifted from the nutlets surface, resulting in a fringed appearance. There should be remarked that Chermezon (1937) described a third type, with a tuberculate nutlet surface (for *P. divulsus*). However this type is, unlike the previous groups, not caused by the shape of the epidermal cells (Reynders & Goetghebeur, 2010; see chapter 8), therefore, it is not further considered here. Although the classification of Kükenthal (1935-36) using either isodiametric or zonate cell types has the appearance of being clear cut, there is also a grey zone of cell types with intermediate forms between isodiametric and zonate cells (in most cases lacking silica bodies). These were first classified by Clarke (1894) together with the zonate cells in the *Reticulati*, but later removed and added to the *Puncticulati* to form the *Isodiametrici*. Kükenthal took over the latter

strategy but Mariën (1969) suggested to classify them in a third (but unpublished) group 'Mixtae'. For sectional delimitations, both Kükenthal (1935-36) and Mariën (1969) opted that each section should contain only species with the same type of nutlet epidermal cells.

To re-evaluate the value of the variation in nutlet epidermis for subgeneric taxonomy in *Pycreus*, the nutlet surface is studied here using scanning electron microscopy. This allows for a much more detailed view of the cells shapes and also of the variations in silica bodies, which have previously not yet been recorded in detail for *Pycreus*. In a detailed study of *Lipocarpha,* there appeared to be a large variation of silica bodies (Goetghebeur & Van den Borre, 1989), even within one single species. In Lipocarpha, these appeared to have only a moderate taxonomical value.

The following research questions will be addressed in this chapter:

1. Which characters and character states can be defined for the nutlet epidermis?
2. Can these character (states) be useful for subgeneric taxonomy?
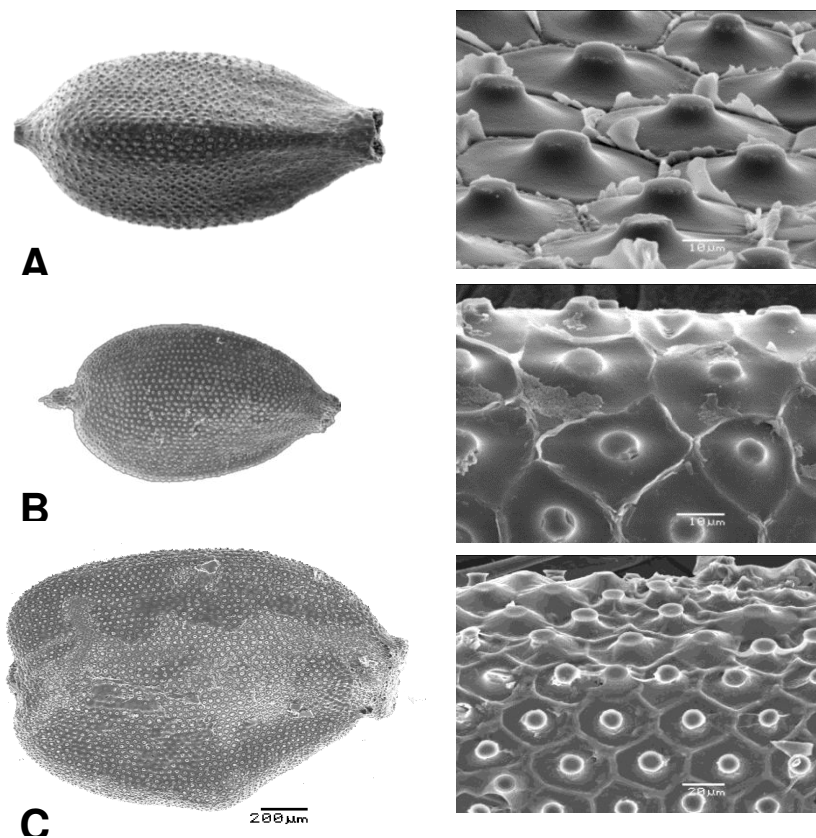
Fig. 7.2 Examples of isodiametric cells from three lineages of C_4 *Cyperus*: A. *Cyperus longus* (Coppejans 5696). B. *Kyllinga polyphylla* (Lewalle 8399). C. *Queenslandiella hyalina* (Kilian & Lobin 2097).

Table 7.1 Specimens studied. The classification in groups and sections occurred according to Kükenthal (1935-36) (ISD = Isodiametrici group, Rhi-Rhizomatosi, Fon-Fontinales, Prp-Propinqui, Lan-Lancei, Glo-Globosi, Alb-Albomarginati, Pol-Polystachyi, Pum-Pumili, Sul-Sulcati, ZON = Zonati group, Lat-Latespicati, Mur-Muricati, Fla-Flavescenti). In the right column the numbers of the pictures displayed in this chapter are given for each specimen. Abbreviations c and n respectively indicate only the picture of the epidermal cells or complete nutlet are given for that specimen. For all other cases bot nutlets and cells are shown from the same specimen.

SPECIES	COUNTRY	COLLECTOR	NUMBER	SECTION	Fig.
Cyperus longus	Kenya	Coppejans	5696	/	7.2A
Kyllinga polyphylla	Burundi	Reekmans	8399	/	7.2B
Pycreus africanus	Congo	Léonard	4156	ZON/Mur	7.10E
Pycreus atribulbus	S. Africa	Browning	172	ISD/Pol	7.3H
Pycreus betschuanus	S. Africa	Reid	794	ISD/Lan	7.4H
Pycreus bipartitus	Ecuador	Laegaard	101015	ISD/Sul	7.5D
Pycreus capillifolius	Burundi	Reekmans	8811	ISD/Glo	7.4F
Pycreus cataractatum	Cameroon	de Wilde	1452	ISD/Prp	7.8D c
Pycreus cataractatum	Zambia	Richards	2143	ISD/Prp	7.8D n
Pycreus demangei	Angola	Hess	52/1983	?	7.9E c
Pycreus demangei	Angola	Hess	52/1835	?	7.9E n
Pycreus divulsus	Madagascar	Perrier de la Bâthie	13052	ZON/Mur	7.10D
Pycreus flavescens	S. Africa	Reid	1079	ZON/flav	7.7H
Pycreus flavidus var. nilagiricus	China	Clemens	1345	ISD/Glo	7.4A
Pycreus fontinalis	Zambia	Robinson	3705	ISD/Fon	7.6B
Pycreus gracillimus	Zambia	Robinson	4182	ISD/Lan	7.8B
Pycreus intactus	Senegal	Vanden Berghen	2554	ISD/Pol	7.3J
Pycreus intactus	S. Africa	Reid	559	ISD/Pol	7.3J
Cyperus juncelliformis	Zaire	Malaisse & Goetghebeur	409	ISD/Prp	7.4D
Pycreus lanceolatus	Burundi	Reekmans	4907	ISD/Prp	7.7C
Pycreus lanceolatus	Burundi	Reekmans	5280	ISD/Prp	7.7D
Pycreus longistolon	Tanganyika	Bullock	3630	ISD/Lan	7.3A
Pycreus macrostachyos	Burundi	Reekmans	4337	ISD/Alb	7.3B
Pycreus megapotamicus	Argentina	Goetghebeur	4826	ISD/Prp	7.5E
Pycreus melanacme	Congo	Schmitz	1294	ISD/?	7.5F
Pycreus melas	Zimbabwe	Brain	8945	ISD/Glo	7.4G
Pycreus micromelas	Zambia	Robinson	2310	ISD/?	7.6A
Pycreus mortonii	Burundi	Reekmans	10342	ISD/Prp	7.7B
Pycreus mundtii	Rwanda	Bouxin & Radoux	1178	ISD/Sul	7.5B
Pycreus muricatus	S. Africa	Browning	633	ZON/Mur	7.10A
Cyperus nervulosus	Australia	Wilson	3336	ISD/Pum	7.3C
Pycreus niger subsp. elegantulus	Rwanda	Bouxin	909	ISD/Prp	7.4B
Pycreus nigricans	Uganda	Greenway &Eggeling	7115	ISD/Lan	7.9B
Pycreus nitidus	Burundi	Reekmans	8814	ISD/Lan	7.9A
Cyperus oakfortensis	S. Africa	Taylor	117	ISD/Lan	7.5A

Pycreus overlaetii	Zambia	Robinson	4651	ZON/Fla	7.7E
Pycreus pauper	Cameroon	Raynal	12334	ZON/Mur	7.9D
Pycreus pelophilus	Burundi	Reekmans	2547	ISD/Pol	7.3F
Pycreus poikilostachys	Congo	Malaisse	11551	ISD/Glo	7.4C
Pycreus polystachyos	Burundi	Lewalle	6290	ISD/Pol	7.3G
Pycreus pseudohildebrandtii	Kenya	Coppejans	5699 A	ISD/Pol	7.3I
Pycreus pumilus	Burundi	Reekmans	4383	ISD/Pum	7.3D
Pycreus reductus	Madagascar	Dhondt	11	ISD/Rhi	7.4E
Pycreus rhizomatosus	Madagascar	Gerau&Dumetz	5259	ISD/Rhi	7.3E
Pycreus sanguinolentus	Mississippi /USA/	Carter	11545	ISD/Sul	7.5C
Pycreus scaettae	Congo	Troupin	618	ISD/Prp	7.8A
Pycreus smithianus	Burundi	Reekmans	8057	ISD/Prp	7.8C n
Pycreus smithianus	Burundi	Reekmans	2399	ISD/Prp	7.8C c
Pycreus tener	Brazil	Irwin	15171	ISD/Prp	7.7A
Pycreus unioloides	Zambia	Robinson	3339	ISD/Lan	7.7F
Pycreus vicinus	Madagascar	Dhondt	4	ZON/Mur	7.7G
Pycreus waillyi	Congo	Witte	6170	ZON/Lat	7.9F
Pycreus xantholepis	Congo	Germain	6874	ISD/Fon	7.7I
Pycreus aff. xantholepis	Zambia	Robinson	1299	ISD/Fon	7.7J
Pycreus zonatus	Zambia	Robinson	3449	ZON/Mur	7.9C
Queenslandiella hyalina	Somalia	Kilian&Lobin	2097	/	7.2C

7.4 Materials and methods

7.4.1 Plant material

Nutlets used for this study were obtained from herbarium specimens from GENT and BR. All *Pycreus* species studied are mainly of (central) Africa and Madagascar, except to a few species from the Americas and two from Asia. From each specimen, two to ten mature nutlets were selected from each specimen, which can be recognised by a dark grey to black colour. We took care to have represented all sections according to Kükenthal (1935-36) in his *Cyperus* subgenus *Pycreus* with at least one specimen.

7.4.2 Acid treatment

Without any treatment, silica bodies are visible as cone-like structures in most *Isodiametrici*. When the fruit ripens this outer cell walls sometimes spontaneously disappears, or dries around the silica bodies. When the outer wall of the epidermis is removed, the silica cones and sometimes also satellites and other tubular structures, become visible. In some species the silica bodies (if present) cannot be seen without removal of the outer cell walls.

In this study, we used a modified technique from Goetghebeur and Van den Borre (1989). To remove the cell walls, the samples were transferred to 1,5 ml eppendorf tubes with 1M sulphuric acid and 1M acetic acid (1:9) and subsequently treated with an ultrasonic cleaner during 30 minutes. One half of the samples was used for acid treatment while the other were left untreated.
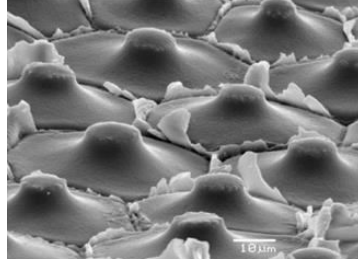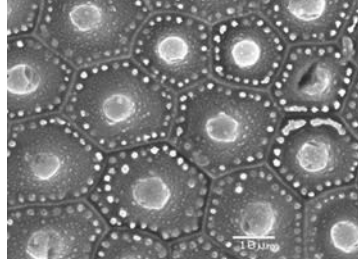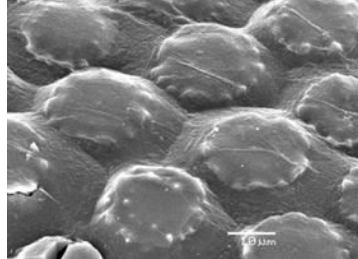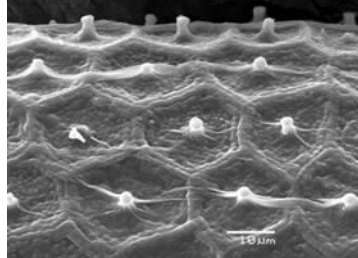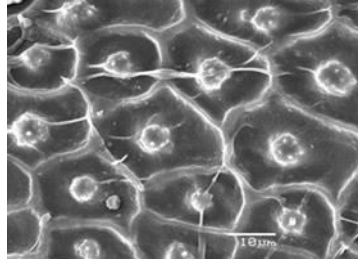
7.4.3 SEM study

For SEM observation, two nutlets (one treated and one untreated) of each specimen were transferred to aluminum stubs using Leit-C and coated with gold with a SPI-Module™ Sputter Coater (SPI Supplies, West-Chester, PA, USA). Images were obtained using a JEOL JSM-5800 LV (JEOL, Tokyo) scanning electron microscope at the National Botanic Garden of Belgium in Meise. Pictures were taken of the complete nutlet (treated, untreated), a detailed picture of the cells at the surface of treated/untreated nutlets and a picture of surface from lateral view.

For the description of silica bodies, Ollendorf (1992) produced a categorisation of silica bodies in sedges based on the variations in the shape and top of the central silica body, the plate and satellites. Each character state is subsequently coded by a letter, which results in a string of 5-6 characters. However, the resulting code is difficult to interpret and to compare among species as small variations give different letters in the code (e.g. Majesky, unpublished data). Therefore, in the current study, we categorized silica bodies into different broader morphotypes and several subtypes (table 7.2).

Table 7.2 Overview of the main silica body types that have been observed among *Pycreus* species.

Picture	Type + characters	Species
Type 1: tabular with smooth apex		
	Type 1a: • central tabular sb • no satellites • plesiomorphic state	Common: • outgroup species • noncore clades • core clade: only observed in *P. micromelas* and sometimes also in *P. capillifolius*
	Type 1b: • central tabular sb • 1 row of satellites around the central silica body • derived from 1a	Restricted to *P. rhizomatosus*
	Type 1c: • central tabular sb • Satellites around the fringe of the top of the central body • derived from 1a	Restricted to *P. polystachyos* & *P. atribulbus*
	Type 1d: • narrow central tabular sb • no satellites • derived from 1a	Restricted to *P. hildebrandtii*
Type 2: rounded with knobby apex		
	Type 2a: • central sb rounded with knobby apex • no satellites • derived from 1a	Common: • core clade • most often in species with isodiametric cell type

	Type 2a reduced form: • central sb rounded but small • top with only a few knobs • no satellites • derived from 2a	Common: • core clade • most often in species with intermediate to zonate cell types
	Type 2b: • central sb rounded with knobby apex • small cone shaped satellites around the central sb • derived • derived from 2a	Restricted to *P. reductus* and *P. capillifolius*
	Type 2c: • central sb rounded with sharply pointed knobs • small pits around central sb • derived from 2a	Restricted to *P. melas* and *P. betschuanus*
	Type 2d: • cental sb rounded with knobs • central sb uplifted • small rounded satellites around central sb • derived from 2a	Restricted to *P. poikilostachys*

Type 3: cone shaped

	Type 3a: • low cone shaped central sb • small knobs only on apex • derived from 2a	Restricted to *P. megapotamicus*
	Type 3b: • high cone shaped central sb • smooth • derived from 2a or 3a	Restricted to *P. melanacme*

Table 7.3 Overview of species studied with mentioning of phylogenetic group (noncore clades, core clade or outgroup), sectional classification of Kükenthal (x indicates the species was not known by Kükenthal, 1935-36), the current most likable sectional position, the cell type (iso= isodiametric= as wide as long, int= intermediate= longer than wide but less than twice as long as wide, zon= zonate= more than twice as long as wide, main silica body type observed in this study. Colour codes help to recognize patterns between the different columns.

SPECIES	Phylogenetic position	Cell type	Silica body type	section Kükenthal	Current section
Cyperus longus	Outgroup	iso	Type 1a		
Kyllinga polyphylla	Outgroup	iso	Type 1a		
Queenslandiella hyalina	Outgroup	iso	Type 1a		
Pycreus longistolon	Noncore *Pycreus*	iso	Type 1a	*Lancei*	*Albomarginati*
Pycreus macrostachyos	Noncore *Pycreus*	iso	Type 1a	*Albomarginati*	*Albomarginati*
Pycreus nervulosus	Noncore Pycreus	iso	Type 1a	*Pumili*	*Pumili*
Pycreus pumilus	Noncore Pycreus	iso	Type 1a	*Pumili*	*Pumili*
Pycreus rhizomatosus	Noncore Pycreus	iso	Type 1b	*Rhizomatosi*	*Rhizomatosi*
Pycreus pelophilus	Noncore Pycreus	iso	Type 1a(r)	*Polystachyi*	*Pycreus*
Pycreus atribulbus	Noncore *Pycreus*	iso	Type1c	*Polystachyi*	*Pycreus*
Pycreus intactus	Noncore *Pycreus*	iso	Type 1a	*Polystachyi*	*Pycreus*
Pycreus polystachyos	Noncore Pycreus	iso	Type 1c	*Polystachyi*	*Pycreus*
Pycreus pseudohildebrandtii	Noncore *Pycreus*	iso	Type 1d	*Polystachyi*	*Pycreus*
Pycreus micromelas	Core *Pycreus*	iso	Type1a	*x*	*Sect. nov.1*
Pycreus niger subsp. elegantulus	Core Pycreus	iso	Type 2a(r)	*Propinqui*	*Globosi1*
Pycreus flavidus var. nilagiricus	Core *Pycreus*	iso	Type 2a	*Globosi*	*Globosi1*
Cyperus juncelliformis	Core *Pycreus*	iso	Type 2a	*Propinqui*	*Globosi1*
Pycreus poikilostachys	Core Pycreus	iso	Type 2d	*x*	*?*
Pycreus capillifolius	Core *Pycreus*	iso	Type 2b	*Globosi*	*Globosi2*
Pycreus reductus	Core *Pycreus*	iso	Type 2b	*x*	*Globosi2*
Pycreus melas	Core *Pycreus*	iso	Type 2c	*Globosi*	*Globosi3*
Pycreus betschuanus	Core *Pycreus*	int	Type 2c	*Lancei*	*Globosi3?*
Cyperus oakfortensis	Core *Pycreus*	iso	Type 2a	*Lancei*	*Sulcati*
Pycreus mundtii	Core *Pycreus*	iso	Type 2a	*Sulcati*	*Sulcati*
Pycreus megapotamicus	Core *Pycreus*	iso	Type 3a	*Propinqui*	*Sulcati*

Pycreus sanguinolentus	Core Pycreus	iso	Type 2a(r)	*Sulcati*	*Sulcati*
Pycreus bipartitus	Core *Pycreus*	iso	Type 2a(r)	*Sulcati*	*Sulcati*
Pycreus melanacme	Core *Pycreus*	iso	Type 3b	*x*	*Sulcati*
Pycreus africanus	Core *Pycreus*	int	absent	*x*	*Tuberculati*
Pycreus divulsus	Core *Pycreus*	int	absent	*Muricati*	*Tuberculati*
Pycreus gracillimus	Core *Pycreus*	int	Type 2a	*Latespicati*	*Propinqui2*
Pycreus scaettae	Core Pycreus	iso/int	Type 2a	*Propinqui*	*Propinqui2*
Pycreus smithianus	Core Pycreus	int	Type 2a	*Propinqui*	*Propinqui2*
Pycreus cataractarum	Core *Pycreus*	zon	absent	*Propinqui*	*Propinqui2*
Pycreus mortonii	Core *Pycreus*	iso	Type 2a(r)	*x*	*Zonati*
Pycreus tener	Core *Pycreus*	iso	Type 2a(r)	*x*	*Zonati*
Pycreus lanceolatus	Core *Pycreus*	iso/int	Type 2a(r)/ absent	*Propinqui*	*Zonati*
Pycreus unioloides	Core Pycreus	int/zon	absent	*Lancei*	*Zonati*
Pycreus overlaetii	Core Pycreus	zon	absent	*x*	*Zonati*
Pycreus vicinus	Core Pycreus	zon	absent	*Latespicati*	*Zonati*
Pycreus flavescens	Core *Pycreus*	zon	absent	*Flavescentes*	*Zonati*
Pycreus aff. xantholepis	Core Pycreus	int	Type2a(r)	*x*	*Zonati?*
Pycreus xantholepis	Core Pycreus	int	absent	*x*	*Zonati?*
Pycreus fontinalis	Core *Pycreus*	int	absent	*Fontinales*	*Fontinales*
Pycreus nitidus	Core Pycreus	iso	Type 2a	*Lancei*	*?*
Pycreus nigricans	Core Pycreus	int	Type 2a	*Lancei*	*Colorati*
Pycreus pauper	Core Pycreus	zon	Type 2a	*Muricati*	*Colorati?*
Pycreus zonatus	Core Pycreus	zon	Type 2a	*Muricati*	*Colorati?*
Pycreus muricatus	Core *Pycreus*	zon	absent	*Muricati*	*Colorati?*
Pycreus demangei	Core *Pycreus*	int	absent	*x*	*?*
Pycreus waillyi	Core Pycreus	zon	absent	*x*	*?*

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7.5 Results

7.5.1 The nutlet epidermis in *Cyperus*, *Kyllinga* and *Queenslandiella*

In the *Cyperus, Kyllinga* and *Queenslandiella* species studied, the nutlet epidermal cells appear to be more or less isodiametric (Fig. 7.2). Each cell contains a single tabular silica body (Type 1a; table 7.2).

7.5.2 Variation in the shapes of nutlet epidermal cells in *Pycreus*

In our study, we found a large variation in the ornamentation of the cells with silica bodies and in the shape of the nutlet epidermal cells. The shape of the cells is from regular or irregular hexagonal (like a honeycomb), over more or less irregular polygonal, to elongated cells, which in some sections give a transversely wrinkled aspect to the nutlets. In some cases irregular cells or mixtures of the previous types were observed.

All outgroup species studied (Fig. 7.2) as well as the species from the noncore sections possess isodiametric cells. In the core *Pycreus* clade, all types can be found (see table 7.3).

7.5.3 Variation in silica bodies in *Pycreus*

There is a large variation in shapes and in the presence of appendages or satellites around the central silica body. Although variation seems to linked to single species, some more general types can be found. We distinguish between three main types of silica bodies, each with several derived types (table 7.2). Table 7.3 gives an overview of the different character states and occurrence of these silica body types among the different *Pycreus* species.

Most species with more or less hexagonal cells (isodiametric type) have silica bodies. Silica bodies also occur in some species with slightly to strongly elongated cells. Several species with intermediate to elongated cells lack silica bodies. Species without si-bodies occur in several non-related species clusters for which the loss of silica bodies probably occurred several times. Some species show strongly reduced silica bodies, in this case the silica bodies are often lacking in at least part of the cells (indicated with r in table 7.3). Many species in the core clade also seem to possess short cell walls that are raised.
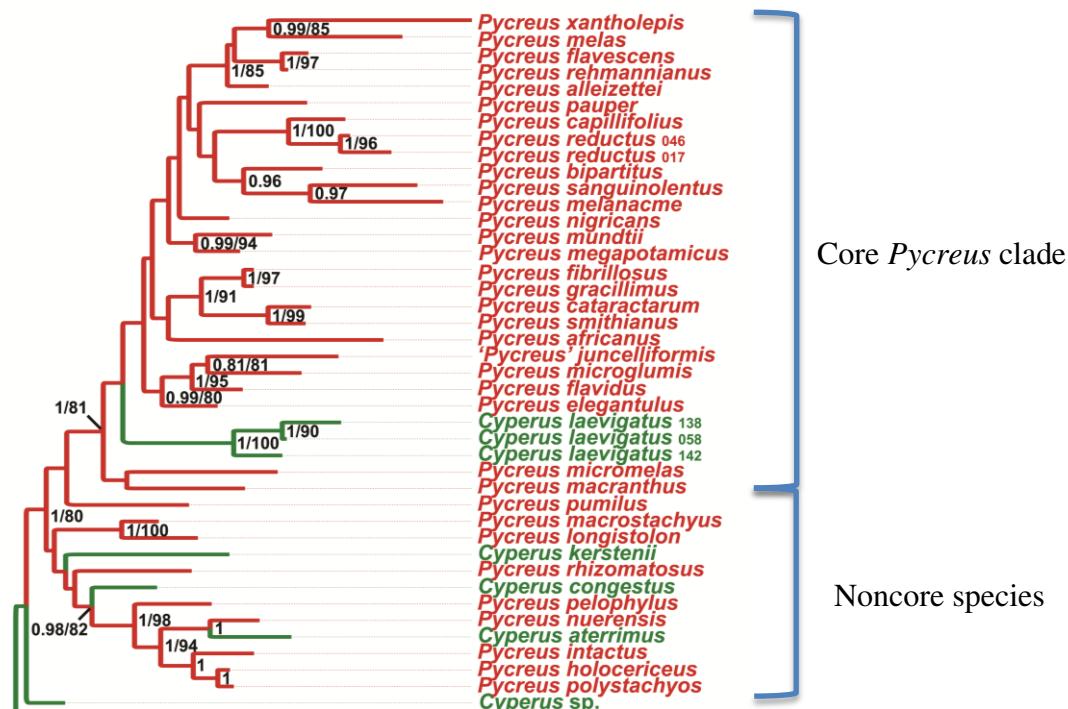
Fig. 7.3. Part of the phylogenetic hypothesis for the *Cyperus* clade: 50% majority consensus multi-locus BI tree (Larridon *et al.*, 2013).

7.6 Discussion

7.6.1 Which characters and character states can be defined for the nutlet epidermis and where do they occur?

7.6.1.1 Nutlet epidermal cell shapes

In *Pycreus,* shapes in nutlet epidermal cells show a large variation when compared to the most of the C_4 *Cyperus* clade, where epidermal cells of the nutlet are generally isodiametric/hexagonal. In several *Pycreus* species, cells are extremely elongated, giving the nutlets a fringed appearance. In some other species, we confirm the observations of Mariën (1969), who observed intermediate cell lengths between the isodiametric and zonate types.

The isodiametric type is considered to form the primitive state in *Pycreus* as it can be found throughout *Cyperus* and is also the most common type in other sedge genera, while elongated cells are restricted to certain species of *Pycreus*. Molecular phylogenic studies showed several species fall out of the core *Pycreus* clade, corresponding to sections as

Albomarginati, Pumili, Rhizomatosi and *Polystachyi* (Larridon *et al.,* 2013)*.* All species of these sections have isodiametric nutlet epidermal cells.

In contrast among the species in the core clade the whole range from isodiametric to strongly elongated cells can be found. The core *Pycreus* clade contains, next to *Cyperus laevigatus*, which has in contrast to *Pycreus* species, dorsiventrally flattened dimerous pistils, species classified by Kükenthal (1935-36) in the sections *Sulcati, Globosi, Fontinalis, Lancei, Propinqui, Flavescentes, Latespicati* and *Muricati*. Although Kükenthal (1935-36) classified the five first of these sections in the *Isodiametrici* and the other three in the *Zonati*, Mariën (1969) placed the *Fontinales, Propinqui* and *Lancei* in his "*Mixtae*" since these exhibit intermediate cell types. As, within *Cyperus* s.l., intermediate and zonate nutlet epidermal cells have only been described for several *Pycreus* species, these most likely represent derived character states.

Molecular phylogenetic relationships within the core clade are currently still poorly resolved (Larridon *et al.*, 2013; see chapter 6). Therefore, it is impossible to get a clear overview on the evolution of the nutlet epidermal characters in *Pycreus* and pointless to optimize the different character states on the phylogenetic hypothesis. However, some smaller species clusters are supported in the molecular phylogenetic hypothesis. Several of these species clusters show a mix of different types of nutlet epidermal cells, such as the relationships between *P. fibrillosus, P. gracillimus, P. smithianus* and *P. cataractarum*. Among these species, there seems to be a gradient in the elongation of the nutlet epidermal cells correlated with a loss of silica bodies. This most likely represents a group in which nutlet cell elongation originated independently from other groups with zonate cell types. Also, *P. flavescens* an *P. rehmannianus* (*Flavescentes*), which have zonate cells, are strongly supported with species with intermediate cells such as *P. xantholepis* (unplaced) or isodiametric cells such as *P. melas* (*Globosi*) and *P. alleizettei* (*Propinqui*). Hooper and Raynal (1969) described several species such as *P. mortonii* and *P. overlaetii*, which show morphological links between *P. flavescens* (*Flavescentes*) and species such as *P. unioloides* (*Lancei*) and *P. lanceolatus* (*Propinqui*). These putative relationships however need further confirmation from molecular phylogenetic studies. However, the presence of a mix of cell types with various degrees of elongation within several separate subclades might indicate zonate cells probably originated multiple times within the core *Pycreus* clade.
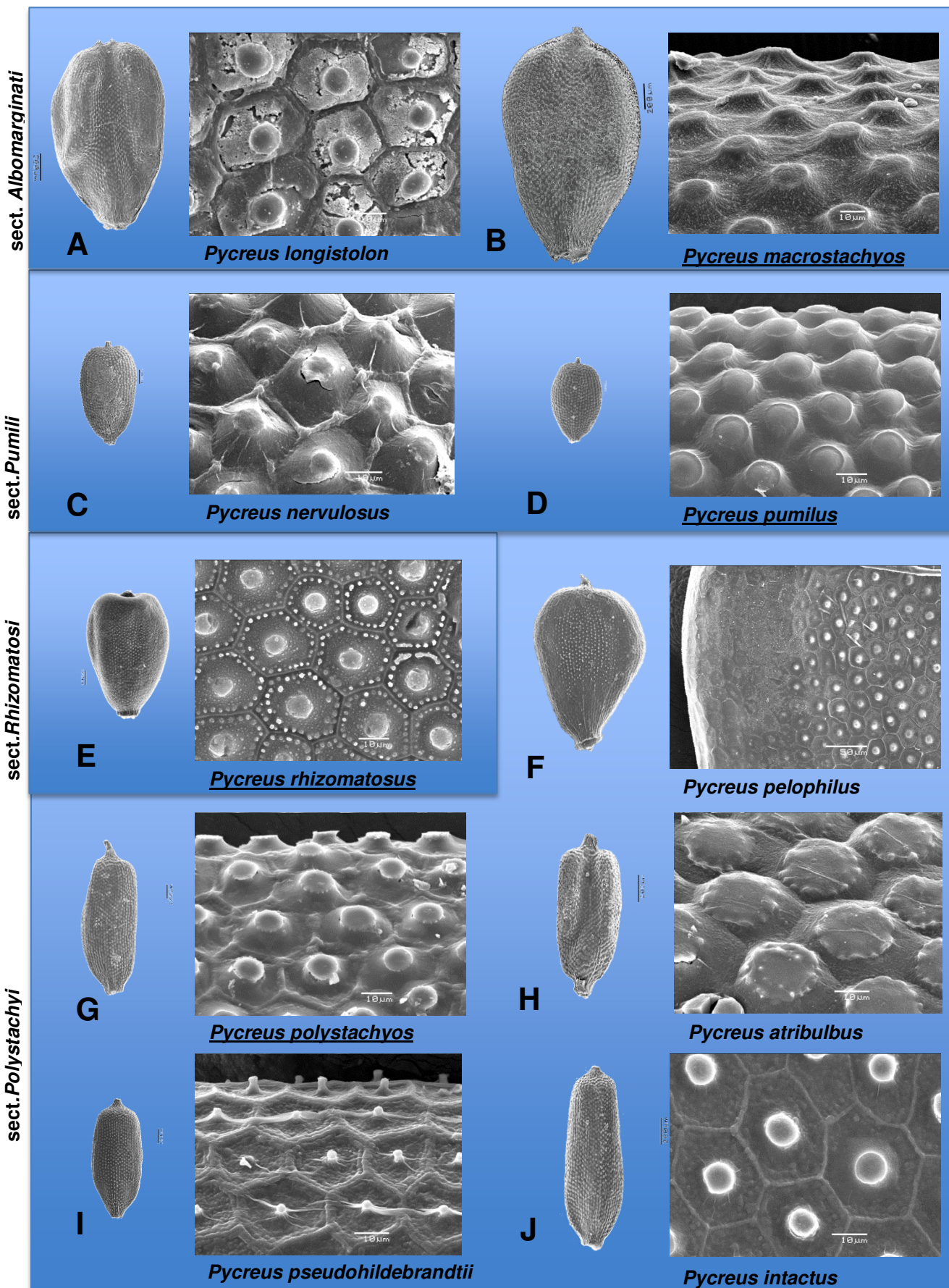
Fig. 7.4 SEM pictures of nutlets and epidermal cells of species of the noncore sections. Sectional names according to Kükenthal (1935-36). Names of type species are underlined.
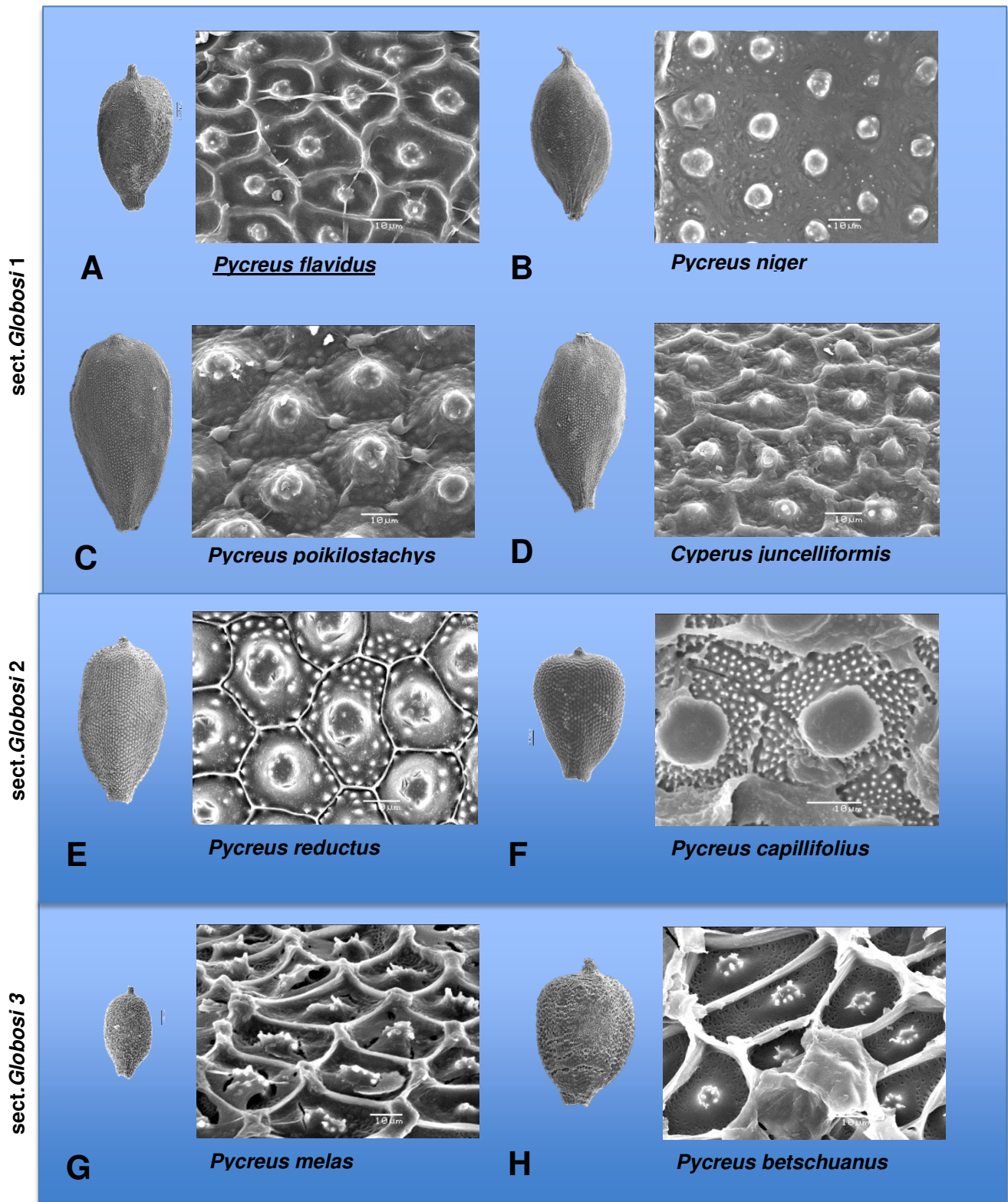
Fig. 7.5 SEM pictures of nutlets and epidermal cells of species of the core clade part 1. Species belonging to sect. *Globosi* of Kükenthal (1935-36), and relatives according to the molecular phylogenetic analysis of Larridon *et al*. (2013).
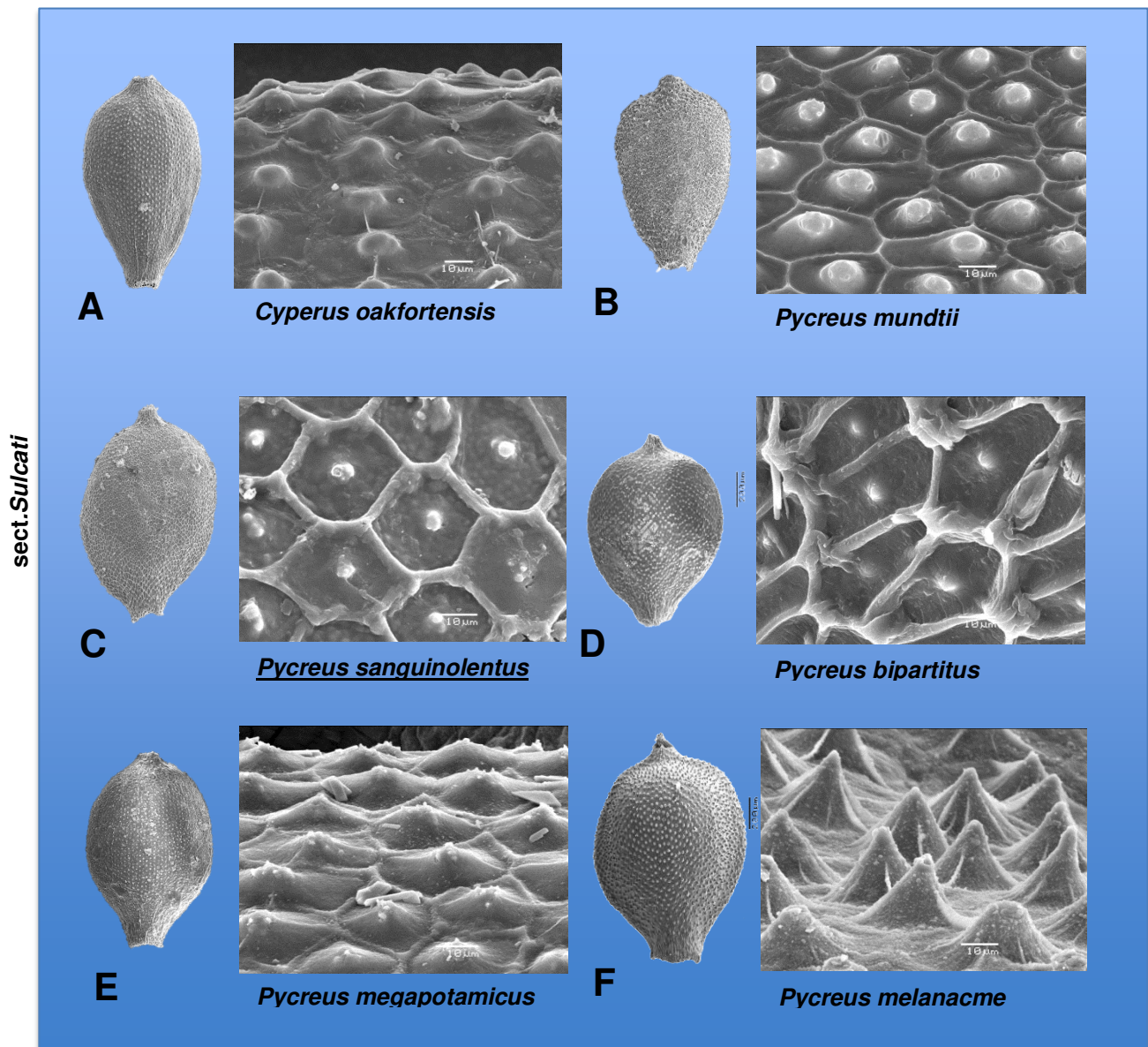
Fig. 7.6 SEM pictures of nutlets and epidermal cells of species of the core clade part 2. Species belonging to sect. *Sulcati* of Kükenthal (1935-36), and relatives according to the molecular phylogenetic analysis of Larridon *et al*. (2013).

7.6.1.2 The variation in silica bodies

In the noncore lineages of *Pycreus* (representing the sections *Albomarginati, Pumili, Rhizomatosi* and *Polystachyi),* almost all species show isodiametric nutlet epidermal cells with one tabular to rounded central silica body. The type with a single tabular silica body is also very common throughout *Cyperus* s.l. (see fig. 7.2; Haines & Lye, 1981). In *P*. sect. *Pumili* the cells are very small and subsequently, they are completely filled with the silica body. A few species of the *Polystachyi* have satellites around the top of the central silica body (e.g. *P. polystachyos*). Only two species were found with derived morphologies: *P. hildebrandtii* shows very narrow silica bodies of the tabular type which are only around 2 μm wide while silica bodies are typically around 10 μm wide. Also, *P. rhizomatosus* shows a derived state as it possesses one row of satellites along the edges of the cells. This type slightly resembles the one observed in for example *P. reductus* (core clade), but the latter has satellites which are irregularly placed and a central silica body with a knobby apex.

In the core clade, we found three main types of silica bodies (see table 7.2): a first type with tabular silica bodies (type 1a), a second type with rounded silica bodies with a knobby apex (type 2) and a third type with cone shaped silica bodies (type 3).

P. micromelas has tabular silica bodies (type 1a) within small isodiametric cells with undulating cell walls. Although the type of *P. micromelas* resembles the one observed in *P. pumilus* and *P. nervulosus*, this similarity is probably due to a reduction in size of the nutlet in all these small therophytic species and correlated reduction of the diameter of the epidermal cells while the silica bodies retained their general width of around 10 μm. As *P. micromelas* accumulated several peculiar characters, Mariën (1969) proposed to accommodate this species in a new (currently still unpublished) section '*Angustispicati*'.

Knobby silica bodies (Type 2) have been observed in many species previously classified in the sections *Globosi, Sulcati, Propinqui, Lancei* or *Muricati.* Consequently, they form the most common type within *Pycreus*. We observed this type not only in species with strictly isodiametric cells (e.g. *P. flavidus, P. mortonii*) but also in elongated (e.g. *P. fibrillosus, P. nigricans, P. nitidus*) to zonate cell types (e.g. *P. zonatus*). The number of knobs on the silica bodies is very variable and in many species the silica bodies are reduced in size. Usually, the cell walls between the cells are raised. A few species clusters such as the *P. capillifolius + P. reductus* clade show derived morphologies, with satellites around the central silica body (Type

2b). The relationship of *P. capillifolius* and *P. reductus* has been confirmed with molecular data, however their relationship with the remainder of the species is unclear. Kûkenthal (1935-36) placed them in the section *Globosi*. Moreover, *P. melas* and *P. betschuanus* both show sharply pointed knobs on the silica body in combination with pores in the noncore plate (Type 2c) (Fig. 7.4 G-H). *Pycreus melas* had also been classified in the section *Globosi* but molecular studies it clusters with *P. xantholepis*, which is however a very different species..

The *Sulcati* share several apomorphies such as glumes with a paler depressed zone on each side of the midrib and the presence of decumbent culms in the perennial species. From the current study, we can observe a rather strong homogeneity in the nutlet epidermal cells. Although molecular phylogenetic relationships are currently only weakly supported, except for some species clusters, the *Sulcati* probably represent a monophyletic group.

In many species silica bodies have been lost. Most often this is associated with species having elongated (e.g. *P. divulsus, P. fontinalis, P. demangei*) to zonate cells (*P. flavescens, P. waillyi,.…*), which are reprentatives of the sections *Tuberculati, Fontinales, Flavescentes* and *Latispicati.* Both on morphological and molecular phylogenetic grounds, we found no arguments to assume that these species form a natural entity.

The third type, with the cone shaped silica bodies, is restricted to *P. melanacme* and *P. mesapotamicus*. In the latter species the silica body has a knobby surface while the ones of *P. melanacme* have a smooth surface. *P. melanacme* is a therophyte with very large glumes and nutlets. It shows an accumulation of derived characters and it cannot easily be related to any of the existing sections. Subsequently, Mariën (1969) suggested to accommodate the species in its own (unpublished) section '*Variegati*'. Our molecular study resolves this species along with *P. sanguinolentus* and *P. bipartitus* (both *Sulcati*) while *P. megapotamicus* is sister to *P. mundtii* (also *Sulcati*).

sect.*Propinqui* 1 -- *Flavescentes*
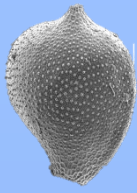
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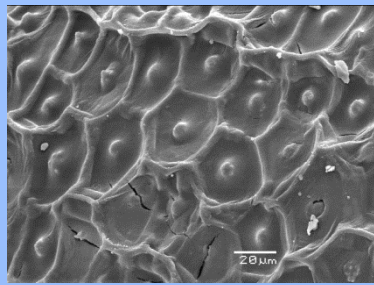
Pycreus tener

B
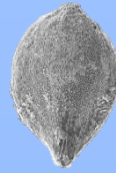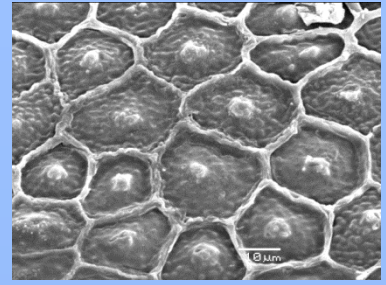
Pycreus mortonii

C
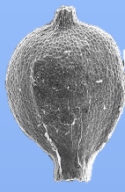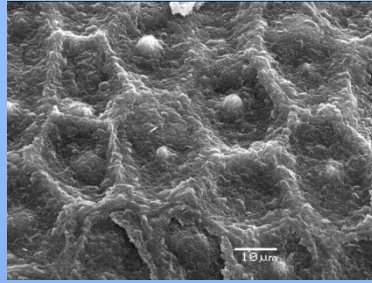
Pycreus lanceolatus

D
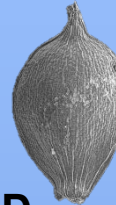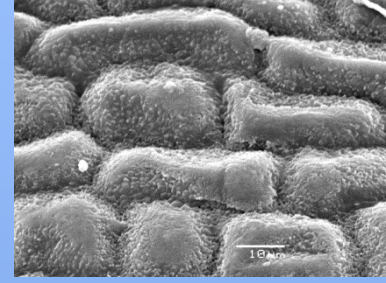
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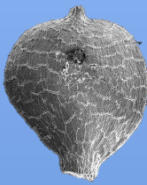
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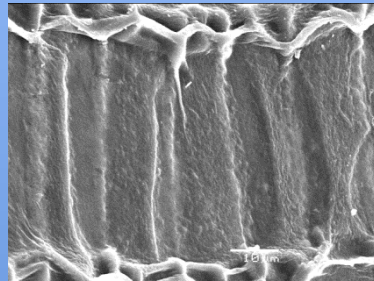
Pycreus overlaetii
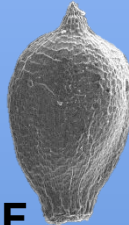
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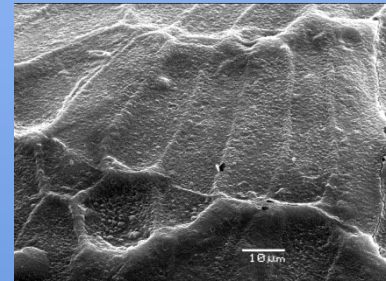
Pycreus unioloides
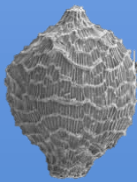
G
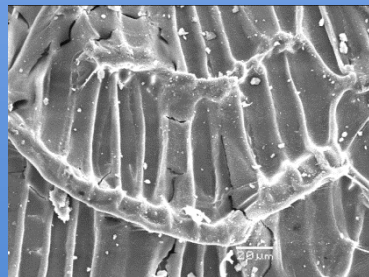
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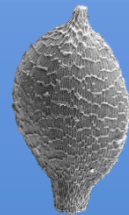
H
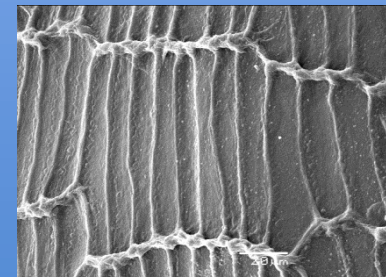
Pycreus flavescens

I
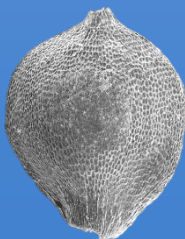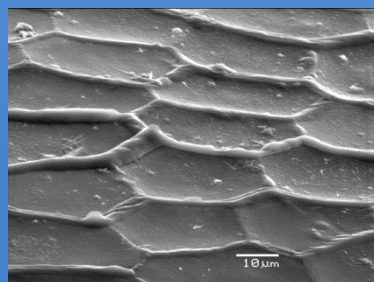
Pycreus xantholepis
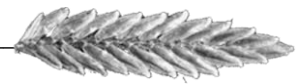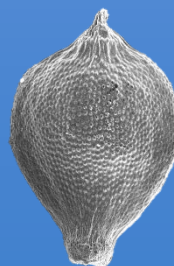
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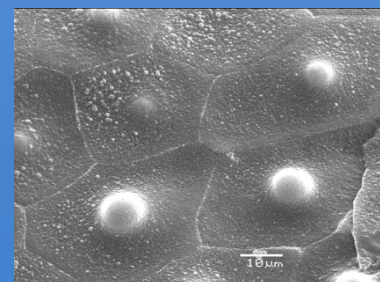
Pycreus aff. xantholepis
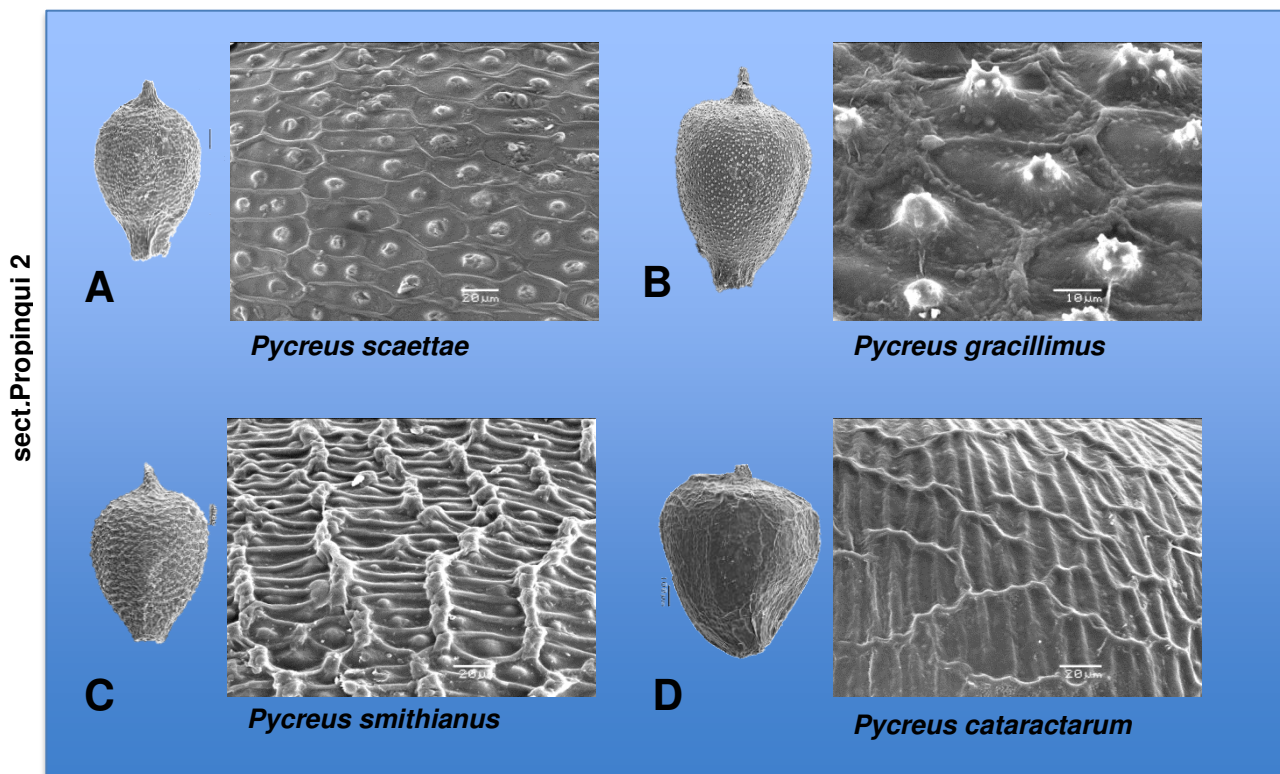
Fig. 7.7 SEM pictures of nutlets and epidermal cells of species of the core clade part 5. Species belonging to sect. *Propinqui* of Kükenthal (1935-36), according to the molecular phylogenetic analysis of Larridon *et al.* (2013) these form a strongly supported clade independent of the other species formerly placed in the sect. *Propinqui.*

← Fig.7.8 SEM pictures of nutlets and epidermal cells of species of the core clade part 4. Species belonging to sect. *Propinqui* and *Flavescentes* of Kükenthal (1935-36). Most species are linked by intermediate morphologies, therefore, they most likely form a natural group.
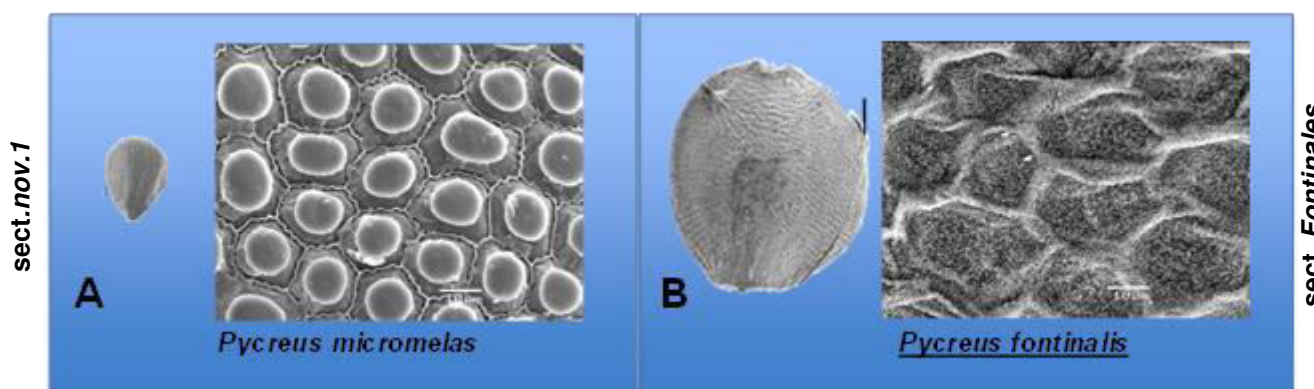
Fig. 7.9 SEM pictures of nutlets and epidermal cells of species of the core clade part 3. *Pycreus micromelas* is currently unplaced. *P. fontinalis* was classified in a monotypic section *Fontinales* by Kükenthal (1935-36). Relationships of these species remain unclear.
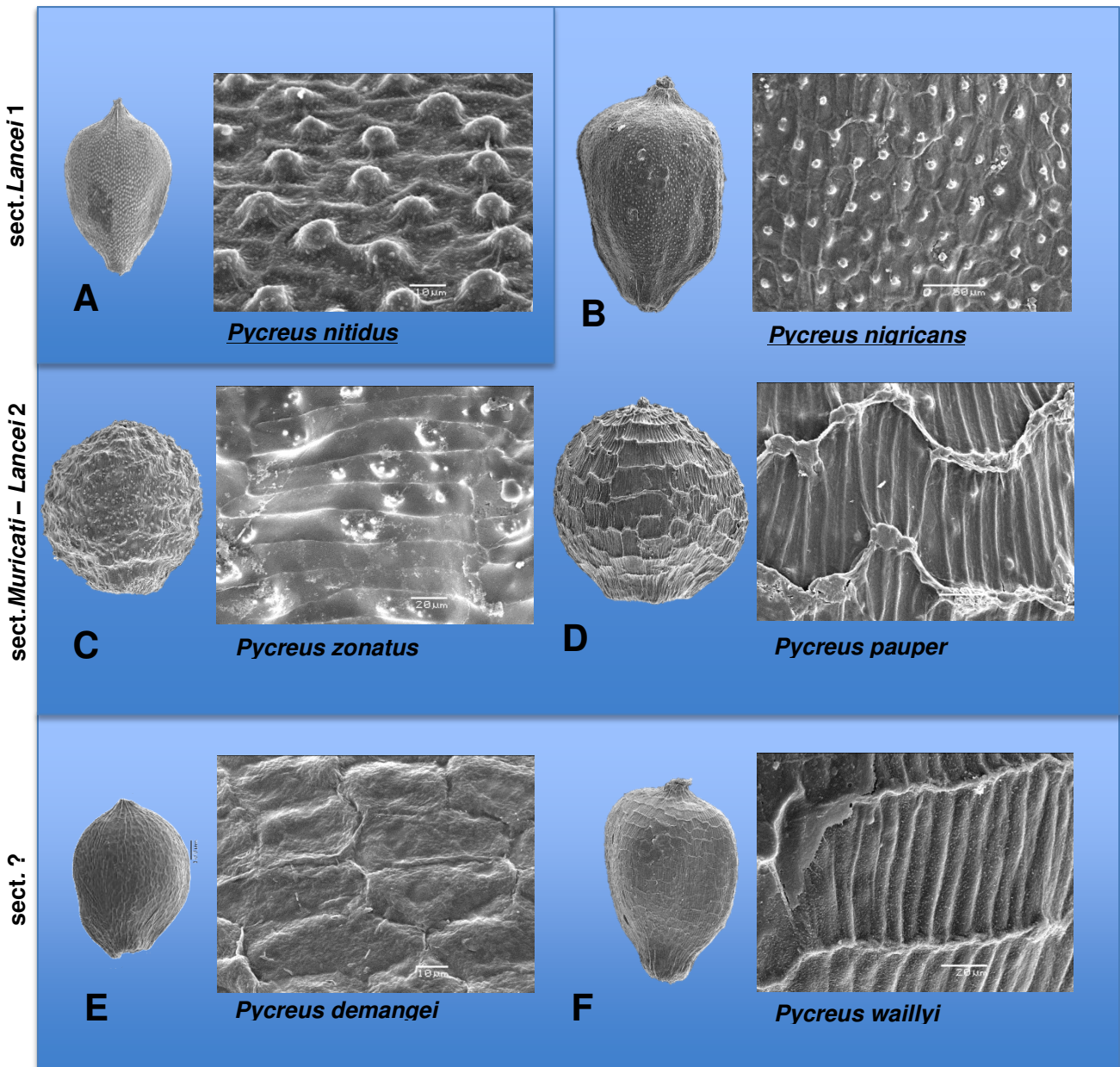
Fig. 7.10 SEM pictures of nutlets and epidermal cells of species of the core clade part 6. Species belonging to sect. *Lancei* and *Muricati* of Kükenthal (1935-36). The position of *P. demangei* and *P. waillyi* is still unclear.

7.6.2 Can nutlet characters be useful for subgeneric delimitations in *Pycreus*?

The shape of the nutlet epidermal cells has been used as the most important characters for sectional delimitations by Clarke (1908), Kükenthal (1935-36) and Chermezon (1937) who recognised two types of cells: isodiametric or zonate. Consequently, this also influenced the species concept in *Pycreus* as a single species could only possess a single type of epidermal cell type.

We distinguish three character states of nutlet epidermal cells in *Pycreus*: The isodiametric type with cells that are as long as wide; an intermediate type with cells slightly longer than wide to twice as long as wide and the zonate type which has cells of more than twice longer than wide. In reality the length of the cells represents a range rather than separate categories. Also Mariën (1969) observed intermediate cells in several species, which he classified in a third category 'Mixtae'. In some species such as *P. permutatus* he observed a wide range in the elongation of the nutlet epidermal cells. In a few resolved subclades we also observed a variation in the elongation of the epidermal cells among related species such as in the clade formed by *P. cataractarum, P. smithianus, P. gracillimus* and *P. fibrillosus*. In our molecular phylogenetic analysis *P. flavescens* subsp. *microglumis*, a taxon with zonate cells, is strongly supported with several species with isodiametric cells (*P. juncelliformis, P. flavidus* and *P. elegantulus*, all thre of which had been placed in different sections by Kükenthal (1935-36). Pycreus flavescens and *P. rehmanianus*, two taxa with zonate cells assumed to be related to *P. flavescens* ssp. *microglumis* by Lye (Haines & Lye, 1981) cluster elsewhere together with other species with different elongations of nutlet epidermal cels. Nevertheless, many small subclades of strongly related species show the same character state of nutlet epidermal cells (e.g. *P. macrostachyos* and *P. longistolon*; *P. reductus* and *P. capillifolius*; *P. cataractarum* and *P. smithianus*; …).

Concerning silica bodies, we distinguish three main character states with several subtypes. Tabular silica bodies and silica bodies with a rounded and knobbed apex are the most common character states. Generally the first type only occurs in isodiametric cells, predominantly in the noncore clades while the rounded and knobbed type occurs in the core clade, in cells with different shapes. Several subtypes were observed that are mostly restricted to a single or a few species. In other species with intermediate or zonate cells the silica bodies are very small or absent.

As a conclusion, the poorly resolved molecular phylogenetic analysis of *Pycreus* currently does not allow us to do a full optimisation of the nutlet characters and character states to evaluate e homoplasy. However, from our observations in well resolved smaller clades we can conclude the different character states of both the cells shapes and silica bodies are not reliable as primary character for sectional delimitations. In some cases these character states might concur with smaller groups of species or with single species, in other clades this is not the case.

When better resolved phylogenetic hypothesis become available in the future optimisation of the different character states on the nutlet epidermis would be useful to investigate homoplasy. As shapes of nutlet epidermal cells has also been used for species delimitations, a future study of infraspecific variation is necessary to reevaluate the value for species delimitations and this might bring new insights to the taxonomy of sever taxonomically complex species clusters in *Pycreus*.

7.7 Conclusions

In *Pycreus*, nutlet epidermal cells show a large variation in shapes and silica bodies. While the shape of these cells was used in the past as a strong character for subgeneric classification in *Pycreus*. In our opinion its taxonomic value is rather limited since the different states of cell types are not linked to different subclades. However they might still be useful for certain species clusters of species.

Silica bodies are present in diverse forms in *Pycreus*. The noncore branches generally show the tabular type found commonly in C_4 *Cyperus* and elsewhere in Cyperaceae while in the core *Pycreus* clade, the type with a knobby apex is the most common. We assume the taxonomical value is rather limited except for a few species clusters that possess derived types.

7.8 Acknowledgements
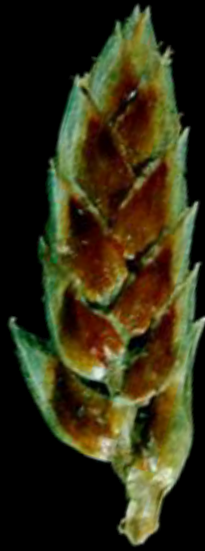
8 Reestablishment of
Pycreus sect. *Tuberculati*

"*The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.*"

— *William Bragg (1862 - 1942)*

← Fig. 8.1 Inflorescence of *Pycreus divulsus*, a Malagassy endemic. Photographed near Lac Froid, Ankaratra, Madagascar.

Spikelet theme: *Pycreus africanus*

8.1 In this chapter…

During the study of the nutlet epidermis we found the wavy nutlets of *Pycreus divulsus* are significantly different than the ones of other members of the section *Muricati*, where Kükenthal (1935-36) placed *P. divulsus*. This was already known by Chermezon who originally placed the species in *P*. sect. *Tuberculati*. These findings gave rise to the case study presented below, which was also published as the following article:

Reynders, M. & Goetghebeur, P. 2010. Reestablishment of *Pycreus* section *Tuberculati* (Cyperaceae). Blumea 55(3):226-230.

8.2 Abstract

In the latest treatment by Kükenthal (1936), who considered *Pycreus* Beauv. as a subgenus of *Cyperus* L., *C. divulsus* Ridl. was put in *Cyperus* sect. *Muricati* Kük. This name is however nomenclaturally a later name for *Pycreus* sect. *Tuberculati* Cherm., which has *P. divulsus* (Ridl.) C.B. Clarke as type (Chermezon 1919). The latter epithet should thus be used for correctly naming the section.

Besides this a SEM study of the nutlet epidermal cells of the species placed by Kükenthal in the section *Muricati* reveals that the wavy nutlets of *P. divulsus* are not formed by extremely elongated epidermal cells as in the other species of this section. Since the species also differs from all other *Pycreus* species in its inflorescence characteristics it is most appropriate to reserve the section *Tuberculati* for *P. divulsus* and relatives.

More recently a second subspecies, *P. divulsus* subsp. *africanus* Hooper, was described from the African mainland based on the completely smooth nutlets and the presence of three instead of two stamens, but having a similar inflorescence (Hooper 1972). The two taxa are easily distinguishable without any intermediates so the species level seems to be more appropriate for the younger taxon. We here reestablish *Pycreus* sect. *Tuberculati* Cherm. Both species are fully described and an illustration for *P. africanus* (Hooper) Reynders comb. nov. is added.

8.3 Introduction

The genus *Pycreus* Beauv. consists of around 120 mainly African species, all characterized by their combination of indehiscent spikelets with distichous glumes and laterally compressed pistils with only two style branches. Along with several other genera, *Pycreus* is nested within the C$_4$ clade of *Cyperus* (Muasya et al. 2001, 2002), showing many typical characteristics of this clade, such as an anthela composed of spikes and the chlorocyperoid anatomy (Bruhl & Perry 1995; Soros & Bruhl 2000).

Pycreus divulsus (Ridl.) C.B. Clarke is an annual Madagascan endemic differing from the other *Pycreus* species by its reduced simply spicate inflorescence: a few large spikelets, each sessile in the axil of a bract and arranged in a single spike. Besides this the internodes of the main axis are elongated (Hooper 1972), (Fig. 8.1, 8.3.a-b). Inflorescence reductions are quite common in *Pycreus* and related genera, and can be found in either annual species from seasonal habitats (e.g.. *P. melanacme* Nelmes, *P. pauper* (Hochst. ex Rich.) C.B. Clarke, *P. atrorubidus* Nees from the Soudano-Zambezian floristic region) or in perennial species with dense fibrous culm bases, living in extreme habitats such as high altitude mountains (e.g. *P. gracillimus* Chiov.) or frequently burnt vegetations (*P. fibrillosus* (Kük.) Cherm., *P. diloloensis* Kük. ex Cherm.). The combination of reduction and elongation of the internodes however is rather unique for *P. divulsus*.

Not only the inflorescence makes this plant very peculiar among other *Pycreus* species, also the fruits show special characteristics and lead to several controversial classifications. At the time of its publication (Ridley, 1884), a subgeneric classification for *Pycreus* was not yet available; he related the species to *C. intermedius* Steudel and *C. stramineus* Nees since as he stated both latter rarely show signs of an elongation of the main axis but in a very much less degree. Clarke (1908) was the first to establish a detailed infrageneric classification of *Pycreus*. *Pycreus divulsus* was put in *P.* subgenus *Reticulatae* C.B. Clarke, which is characterized by (nearly) isodiametric nutlet epidermal cells, in contrast to his second subgenus Zonati, which has strongly elongated nutlet epidermal cells. On the sectional level Clarke placed *P. divulsus* together with *P. sanguinolentus* (Vahl) Nees, *P. atronervatus* (Böck.) C.B. Clarke, *P. mundtii* Nees and *P. atropurpureus* C.B. Clarke in *P.* sectio *Vestitae* C.B. Clarke, from which it differs however in having a completely different habit, inflorescence, different nutlets and glumes. It was Chermezon (1919) who remarked the difficulties of classifying *Pycreus divulsus* among the

other known species and, based on the unique tuberculated nutlets of the species, he established a new section *Tuberculati.* Chermezon treated Clarke's two subgenera on the sectional rank as well, resulting in a classification with three sections. Kükenthal (1936), who considered *Pycreus* as a subgenus of *Cyperus* L., finally synonymized *P.* sect. *Tuberculati* Cherm. with *Cyperus* (*Pycreus*) sect. *Muricati* Kük. within the rankless placed *Zonati* C.B. Clarke. This new section contains, next to *C. divulsus*, three other species: *C. pauper* (Hochst. ex Rich.) C.B. Clarke*, C. zonatissimus* Kük. and *C. muricatus* Kük.. All these species are characterized by turgid nutlets with a strongly wavy or muricate surface. In his key, Kükenthal places *C. divulsus* most closely to *C. pauper,* which is also an annual species with rather large glumes and nutlets and a reduced inflorescence.

More recently Hooper (1972) identified several African collections as approximating *Pycreus divulsus* based on the presence of a simply spicate inflorescence. At first they were thought to be introductions of the Madagascan species on the African mainland. Considering the remote collections of this species all over tropical African and the clearly different nutlets (smooth vs. tuberculate) and three vs. two anthers, the African specimens were described as *P. divulsus* subsp. *africanus* Hooper. The distinction between the two taxa is however clear-cut and very easy observed, so the species level seems more appropriate for the African taxon. Both taxa are poorly known and often unidentified in the visited herbaria. A key and illustrations are added to overcome this problem in the future. The nutlet epidermis of these taxa was studied with SEM to evaluate their position in the Kükenthal (1936) classification.

8.4 Materials and methods

In order to compare the nutlet epidermis, SEM pictures were taken from mature nutlets of representative specimens from GENT and BR herbaria (Table 5.1). The following species were photographed: *Pycreus divulsus* (Ridl.) C.B. Clarke*, Pycreus africanus* (Hooper) Reynders comb. nov.*, Pycreus muricatus* (Kük.) Napper*, Pycreus pauper* (Hochst. ex Rich.) C.B. Clarke and *Pycreus zonatus* Cherm.

Representative specimens of *P. divulsus* and *P. africanus* from K, P and BR herbaria were studied and a distribution map was created with Arcview GIS 3.2.

References to Articles refer to International Code of Botanical Nomenclature (McNeill et al., 2006).

Table 8.1 Specimens used in the SEM study of nutlet epidermal cells.

Taxa	Collector	Nr.	Herbarium	Country
Pycreus				
P. muricatus	Browning	633	GENT	South Africa
P. zonatus	Robinson	5102	GENT	Zambia
P. pauper	Taylor	9184	BR	Tanzania
P. divulsus	Perrier de la Bâthie	13052	BR	Madagascar
P. africanus	Léonard	4156	BR	D.R. Congo

8.5 Results and discussion

8.5.1 The section *Tuberculati* Chermezon

Cyperus (*Pycreus*) sect. *Muricati* Kük. was established to unite the *Pycreus* species with turgid and muricate nutlets (Kükenthal 1936). It can be automatically typified under Art. 22,6 by the type of the name of the species from which the subdivisional epithet was derived, i.e., *C. muricatus* Kükenthal. Although this section contains *C. divulsus,* which is the type of the in 1919 established *P.* sect. *Tuberculati* Cherm., he placed the latter in synonymy. Kükenthal's name should therefore be considered as a later synonym for the sect. *Tuberculati* Cherm. and thus becomes unavailable for future use (Art. 11.4).

As Kükenthal (1936) noticed, the nutlets of *Pycreus divulsus* resemble those of the other members of the section in their wavy aspect. SEM pictures from the nutlets of *Pycreus divulsus, Pycreus pauper, Pycreus muricatus* and *Pycreus zonatus* however clearly show a difference in the shape of the nutlet epidermal cells. *P. pauper, P. muricatus* and *P. zonatus* all have strongly elongated epidermal cells and due to this elongation, the tangential walls of the epidermal cells are lifted resulting in the strongly wavy aspect of the nutlets (Fig. 8.2a-c). In other *Pycreus* species as for example *P. flavescens* this elongation is less expressed and only resulting in narrow transverse frills on the nutlets surface. The nutlet epidermal cells of *P. divulsus* in contrast are isodiametric or only slightly elongated, as already correctly observed by Clarke (1908) (see Fig. 8.2d-e).
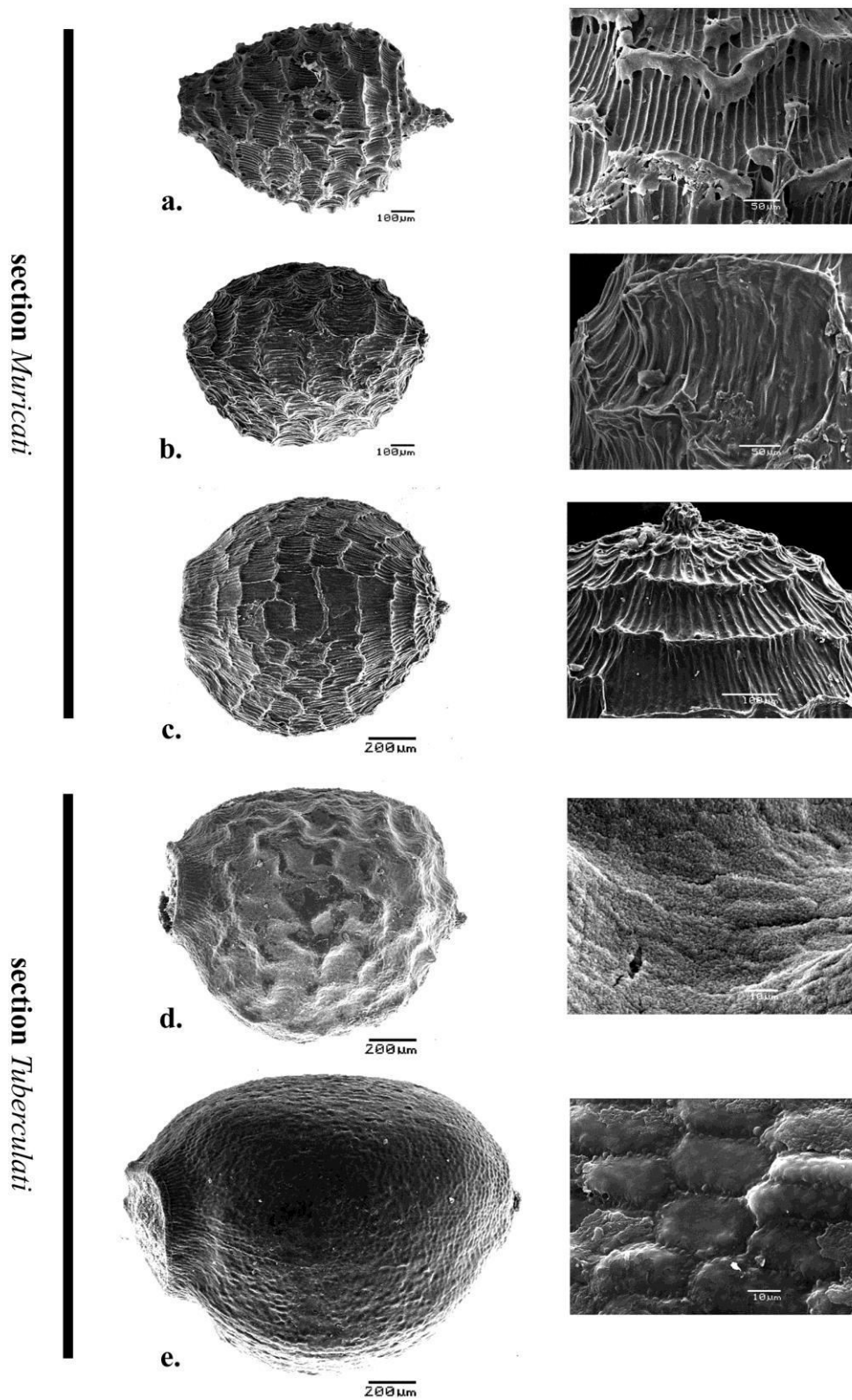
Fig. 8.2 SEM pictures of the nutlets in the section *Muricati* Kükenthal and the section *Tuberculati* Chermezon, on the left lateral views of mature nutlets, on the right details of the nutlet epidermis of: a. *P. muricatus* (Browning 633, GENT), b. *P. zonatus* (Robinson 5102, GENT), c. *P. pauper* (Taylor 9184, BR), d. *P. divulsus* (Perrier de la Bâthie 13052, BR) and e. *P. africanus* (Léonard 4156, BR).

Hereby we can conclude that the classification of *Pycreus divulsus* in the 'Zonati' and esteemed relationships with the other members of *C.* (*Pycreus*) sect. *Muricati* by Kükenthal (1936) was based on superficial similarities and the name *P.* sect. *Tuberculati* Cherm. should be reserved for *P. divulsus* and relatives.

8.5.2 Formal taxonomy

Pycreus sect. *Tuberculati* Cherm., Ann. Mus. Colon. Marseille XXVII, 3 ser. 7 (1919) : 65. – Type:
> *Pycreus divulsus* (Ridl.) C.B. Clarke.
>
> = *Cyperus* (*Pycreus*) sect. *Muricati* Kük. (1936) 394, nom.illeg.
>
> Note: The section comprises *Pycreus* species characterized by a simply spicate inflorescence and large, asymmetrically turgid nutlets (abaxial side most swollen) with a smooth to tuberculate surface. The section is automatically typified by *P. divulsus*, the only species in the section at the time of its description.

1 a   Nutlets strongly tuberculate, 1.2--1.5 mm long; plants from Madagascar

P. divulsus

   b   Nutlets smooth, 1.5--1.9 mm long; plants from tropical Africa

P. africanus

Pycreus divulsus (Ridl.) C. B. Clarke in T. A. Durand & H. Schinz, Consp. Fl. Afric. 5: 536 (1894).

> ≡ *Cyperus divulsus* Ridl. (1884), 128. – Type: Madagascar, Centre: Betsiléo, in Sümpfen, 2/1881, Hildebrandt 4020 (K holo: K000363055!, M iso: M0106884!, M iso: M0106885!, P iso: P00459905!, P iso: P00459906!, P iso: P00459907!).
>
> = *Cyperus paucispiculatus* Boeck. (1884), 497-498. – Type: see *C. divulsus* and discussion below.

Note: In January 1884, Ridley published *Cyperus divulsus* based on Hildebrandt 4020 from central Madagascar. In September of the same year Boeckeler however described, independently from Ridley, *Cyperus paucispiculatus* Boeck. based on the same collection. Clarke (1894) subsequently synonymized *C. paucispiculatus* with *C. divulsus* and in later studies only the latter name has been used. Unfortunately Chermezon (1919) was unaware of

Boeckelers earlier name when he used *C. paucispiculatus* Cherm. nom. illeg. for a new Madagascan taxon and placed it in its own *P.* sect. *Paucispiculati* Cherm. The latter species clearly does not belong to *Pycreus* and is therefore not related to *Pycreus divulsus,* although both share a reduction in the number of spikelets. *C. paucispiculatus* Cherm. is a younger homonym of *C. paucispiculatus* Boeck. and thus illegal. A new name for this taxon has been given (Larridon *et al.* 2008).

Pycreus divulsus is a rare species found scattered, from central to east Madagascar (Fig. 8.1, 8.4). Although it occurs also near sea level it is mainly a medium altitude species. Its habitat is quite variable from moist grassland to weedy gardens.

Annual herbs of 7--25 cm high, with triangular and glabrous culms of 0.4--0.8 mm wide, often curved. Leaves basal, 0.6--1.2 mm wide, canaliculate to flat, scabrid near the tip; sheaths pale. Anthela simple and reduced to a terminal spike with 2 to 4 sessile and suberect spikelets, the spikelets widely spaced from each other; Bracts 3—4, leafy, at the base of each single spikelet, 1.5--7 cm long, erect. Spikelets narrow elliptic, suberect, 6--15 mm long (at approximately 7--10 mm from the top the fruits are ripe and the glumes are falling off) and 3--4 mm wide with 6--20 flowers; rachilla straight, pale. Glumes ovate, with a narrow acute tip, 2.3--3.9 mm long and 1.2--1.5 mm wide, shiny castaneous, with a narrow hyaline undulating border, keel green with 3 nerves; imbricate. Stamens 2, anthers linear, with a short reddish connective. Nutlets broadly elliptic to almost globose, 1.2--1.5 mm long and 1--1.1 mm wide, strongly swollen, black and shiny, apiculate, the surface strongly tuberculate; epidermal cells irregular.

Reference specimens:

MADAGASCAR: Central, Betsileo, in Sümpfen, 1156m, 2/1881, Hildebrandt 4020 (K!, M!, M!, P!, P!, P!); Tananarive, marécage, Perrier de la Bâthie 2677b (P!), jardins, Perrier de la Bâthie 13052 (P!, BR!), bord de route, Perrier de la Bâthie 17606 (P!), 04/1922, Waterlot 495 (P!, P!); ca.5 km S of Tananarive centrum, in grassland in edge of pool, 31/03/1971, K. A. Lye 5932 (K!, P!); Antsirabe, Perrier de la Bâthie 2730 (P!), 1926 (P!); Forêt d'Analamazaotra: fonds humides vers 900 m, 1/10/1912, Viguier et Humbert 949 (P!); Region de l'est, Tamatave, 11/1906, d'Alleizette 1380 (P!); Tamatave province, E of Moramanga, Andasibe, Perinet reserve, open area on trail trough forest, 5/03/1988, D.A. Simpson 88/109a (K!); s.l., Baron 5641; s.l. donné par l' Academie Malgache (R. Lambinon) 910 (P!, P!).

Pycreus africanus (Hooper) Reynders comb. nov.

≡ *Pycreus divulsus* subsp. *africanus* S.S.Hooper (1972) 579. – Type: Cameroon, Gaudua, eastern foothills of the Gotel Mountains, march, 17 July 1969, J.Br. Hall 1381 (K holo!, P iso!).

Note: *Pycreus africanus* (Hooper) Reynders is a rare species known from several remote locations in tropical Africa (Fig. 8.4). Most collections are from moist grassland on medium altitudes except the collection in Sierra Leone which is from near the coast. In Ethiopia the species could be confused with *Pycreus pauper* which can be found in the same habitats (e.g. Robertson in Mooney 7548a & b (K), mixed collection). The latter is also an annual species with a reduced inflorescence and large spikelets and nutlets. It however differs from *P. africanus* in having a rather capitate inflorescence, black tipped glumes and nutlets with elongated epidermal cells as shown in fig. 1.c.

Annual herbs of 6.5--38 cm high, with triangular and glabrous culms of 0.7--1.1 mm wide (Fig. 8.3.a.). Leaves basal, 0.8--2 mm wide, scabrid near the tip; sheaths pale with many small red dots. Anthela simple and reduced to a terminal spike with 2--4 sessile and suberect spikelets, the lower spikelets often 5--7 mm lower than the others (Fig. 8.3.b.); Bracts 3--4, leafy, 1.4--9.8 cm long, erect. Spikelets narrowly elliptic, suberect, 4--15 mm long and 2.5--4.4 mm wide with 4--18 flowers; rachilla strait, pale. Glumes oblong elliptic, with a narrow acute tip, 3.1--4.2 mm long and 1.1--1.5 mm wide, golden, brownish tinged and with many small red dots, hyaline border wider towards the tip, keel green with 5 nerves; slightly imbricate (Fig. 8.3.g--h.). Stamens 3, anthers oblong, 0.5 mm long (Fig. 8.3.j.). Nutlets broad elliptic, 1.5--1.9 mm long and 1--1.2 mm wide, strongly swollen (nearly round on section), black and shiny, the surface smooth (Fig. 8.3.d--f.); epidermal cells irregular (Fig. 8.3k.).

Reference specimens:

SIERRA LEONE: Freetown, Tower hill, in grass on dry gravel hillside, 2/11/1930, F.C. Deighton 1868 (K!, P!).

NIGERIA: Plateau province, near Farin Rua between William kamp and Marbai, short grass on bank of river in open situation, 24/08/1968, Hall J. Br. 652 (K!).

CAMEROON: Gaudua, eastern foothills of the Gotel Mountains, march, 17/07/1969, Hall J. Br. 1381 (K!, P!); Manengouba mts. Base, Nkongsamba, 900m, 30/09/1971, A.J.M. Leeuwenberg 8527 (K!).

ETHIOPIA: Midwest Ethiopia, Mattu near Gore, open grassland, 1500m, 23/10/1958, Robertson in Mooney 7548a (K!); Wollega region, 138 km on Ghimbi-Asosa road, sloping short grassland, 16/09/1975, M.G. Gilbert &
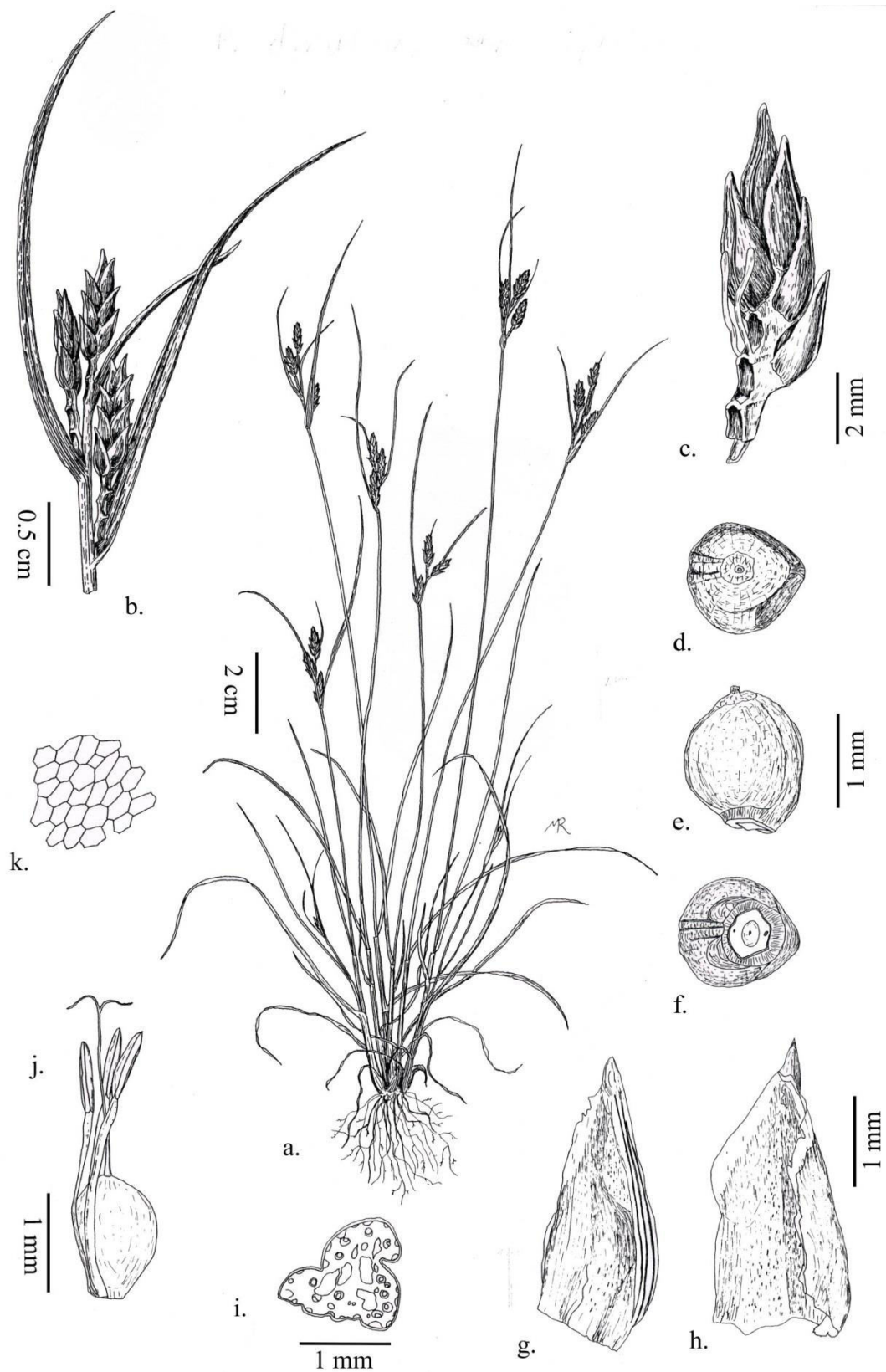
Fig. 8.3 *Pycreus africanus* (Hooper) Reynders comb. nov.; a. habit, b. inflorescence, c. spikelet, d.
nutlet upper view, e. nutlet lateral view, f. nutlet basal view, g. & h. glumes, i. transverse section culm,
j. flower, k. detail nutlet epidermal cells. Drawing based on Leonard 4156 (BR!).

M. Thulin 796 (K!, P!); Hippo pool on Jimma river, c. 5 km North-west of Jimma on Addia Abala road, Kaffa province, 22/10/1973, Ash 2225 (K!); Illubabor region, 38 km north of Tepi, along the new road to Gore, 1900m, meadow in moist *Pouteria adolfifriderici* (Engl.) A. Meeuse - *Schefflera abyssinica* (Hochst. ex A. Rich.) Harms forest, 16/11/1995, I. Friis, S. Bidgood, P. Host, Dessalegn Desissa & Shigulte Kebede 7164 (K!).

CONGO: Kivu region, zone de Mwenga, Collectivité Luindi, Localité Kilimbwe, house yard in grass, 1300m, 13/11/1977, Takako Yamada 134 (K!); Walungu, Kabare territory, Savanne á *Eragrostis* Wolf, 05/1959, Léonard 4156 (BR!).

ZAMBIA: Namwala, heavily grazed mixed grassland, light sandy soil in a mixed woodland area, 17/04/1963, H.J. van Rensburg 2014 (K!).
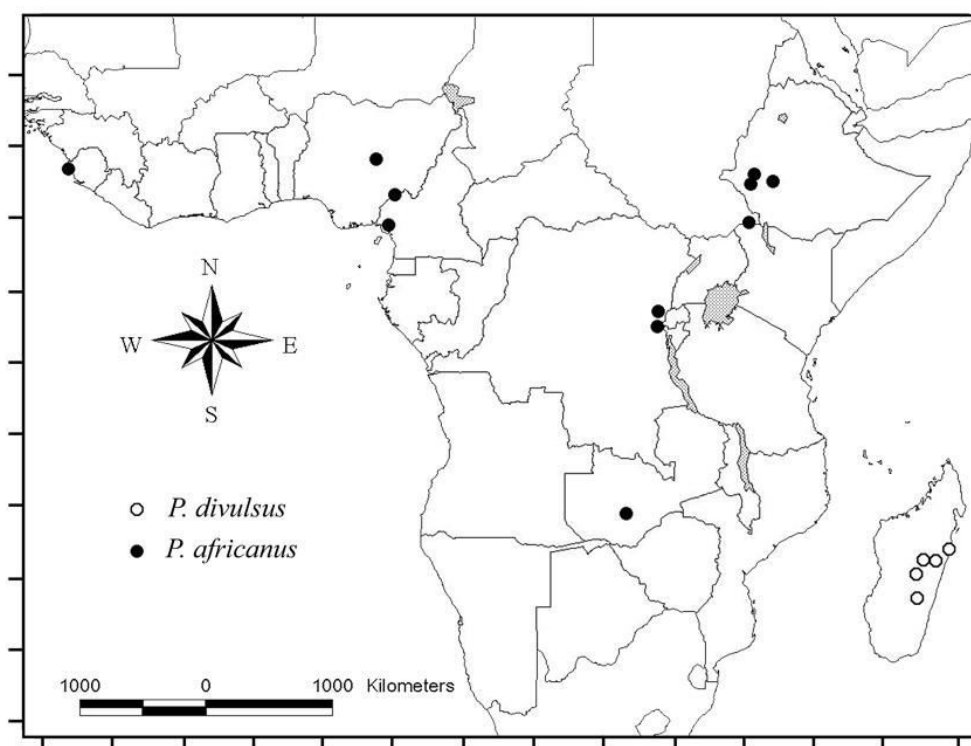
Fig. 8.4 Distribution of *Pycreus* sect. *Tuberculati* Cherm. based on the specimens cited in the text.

8.3.6 Acknowledgements

9 Taxonomic changes

"Everything must be made as simple as possible. But not simpler."

— *Albert Einstein (1979-1955)*

Detail of the inflorescence of *Pycreus nitidus,* Ranomafana national park, Madagascar. Picture taken by M. Reynders.

Spikelet theme: *Pycreus fontinalis*

9.1 In this chapter....

Implementation of the new classification strategy for *Cyperus* s.l. on the segregate taxa requires several taxonomic changes. In chapter 9, taxonomic changes are presented for *Pycreus* names that need to be combined in *Cyperus* in addition with several new or reviewed synonymisations.

The results of this chapter will be included in a joint publication:

Larridon I., Bauters K., Huygh W., Reynders M. & Goetghebeur P. (submitted). Taxonomic changes in C_4 *Cyperus* (Cypereae, Cyperoideae, Cyperaceae): reducing the sedge genera *Ascolepis*, *Kyllinga* and *Pycreus* into *Cyperus* sensu lato. *Phytotaxa*.

9.2 Abstract

Recent molecular phylogenetic and morphological studies reveal the need to sink several segregate genera within a broadly circumscribed *Cyperus*. In this paper several names for African species of *Pycreus*, the largest of these segregates, are formally combined into *Cyperus*, some of which require a new name. Of several currently accepted species, the taxonomic status is doubtful and their synonymy is discussed.

9.3 Introduction

9.3.1 Research context: A growing classification for *Cyperus* s.l.

The tribe Cypereae forms the second most species-rich tribe of Cyperaceae and its largest genus *Cyperus* forms the most diversified sedge genus in the tropics and in addition it represents probably one of the most ecologically important angiosperm genera in tropical wetlands. Cypereae have complex compound inflorescences, in which many adaptations such as reductions and contractions have occurred, complicating evolutionary reconstruction and classification. With the development of molecular phylogenetics (Simpson et al., Muasya et al. 2002, 2009a; Larridon et al. 2013) there has been revival of interest in the classification of this tribe and a need for re-evaluation of the applicability of the morphological characteristics used for generic delimitation in this tribe (Muasya *et al*, 2009b; Huygh *et al*, 2010; Larridon *et al,* 2011, 2013; Reynders *et al*, 2011).
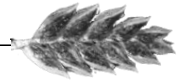
Cyperus s.l. is found to be paraphyletic, including 13 segregate genera (following the classification of Goetghebeur, 1998, accepted by Govaerts *et al.*, 2007). Several supernested paraphyletic entities have been identified (e.g. *Cyperus* subgenus *Anosporum* < *Cyperus* subgenus. *Cyperus* (< *Mariscus*) < *Lipocarpha* < *Volkiella*). Most characteristics that have been used for generic delimitation in the tribe have been found to have originated multiple times such as switches from spiral to distichous glume arrangements and vice-versa, deciduous spikelets, pistil dimerisations and condensations of the inflorescence. Due to the complexity of the relationships within *Cyperus* s.l. a classification with inclusion of the segregate lineages in a broadly circumscribed *Cyperus* seems currently to be the best classification strategy.

Larridon *et al.* (2011b) proposed two subgenera under *Cyperus*, based on the photosynthetic type: (1) *Cyperus* subgenus *Anosporum* (C_3 photoynthesis, paraphyletic) and *Cyperus* subg. *Cyperus* (C_4 photosynthesis, monophyletic). A new sectional classification for *C.* subgenus *Anosporum* could already be presented based on a well-resolved phylogeny for the part of *Cyperus* using C_3 photosynthesis. The segregate lineages *Courtoisina, Oxycaryum* and *Kyllingiella* have formerly been sunken into different new or existing sections of *Cyperus* along with some intermediate species of *Cyperus* (Larridon *et al.*, 2011b). Larridon *et al.* (2013) initiated the merging of the different segregate lineages of the C_4 *Cyperus* clade. Bauters *et al.* (submitted) managed to propose a new sectional classification under *Cyperus* for the species formerly placed in *Lipocarpha, Ascolepis* and *Volkiella*. Also for the monophyletic *Kyllinga* a new subsectional classification is in preparation (Huygh *et al.*, in prep). An overview of all published generic and subdivisional names is available for the Cyperus clade (Huygh *et al*, 2010; Larridon *et al.*, 2011, Reynders *et al.*, 2011) and serves as the nomenclatural base in our efforts to construct a modern classification of the giant genus *Cyperus*.

9.3.2 *Pycreus,* segregate lineage(s) of *Cyperus*

Pycreus (approx. 120 sp.) forms the largest of the segregate genera nested within *Cyperus*. Yet with the description of the genus *Pycreus* Beauv., its relationship with *Cyperus* L. was the subject of speculation, as illustrated by the name '*Pycreus',* being an anagram of *Cyperus* (Palisot de Beauvois, 1816). Many cyperologists were not convinced of the generic status of *Pycreus* and kept the species under *Cyperus* sensu lato (e.g. Kunth 1837, Steudel 1854, Boeckeler 1868, Kükenthal 1935, Haines & Lye 1983). During their careers some authors
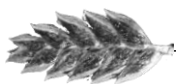
even changed their opinion from *Pycreus* to *Cyperus* (e.g. Govindaralaju 1974 vs 1991 , Lye 1981 vs 1983) or the other way around (e.g. Clarke 1884 vs 1894).

The key diagnostic feature separating *Pycreus* from *Cyperus* s.s. are its remarkable laterally flattened dimerous pistils that can be recognised by two stigma branches in median position. In a broad study of the evolution of pistils in Cyperoideae we showed the combination of a ring wall primordium and the adaptive development of the floral vasculature allowed multiple origins of laterally compressed pistils within this subfamily. *Pycreus* shares these pistils with both *Kyllinga* and *Queenslandiella* (two other segregates of the C_4 *Cyperus* clade, separated from *Pycreus* by their deciduous spikelets). Our ontogenetic theory would even allow for multiple origins of the laterally compressed pistils within *Cyperus* but the phylogenetic relationship between these three taxa is currently still unresolved.

As was expected (Goetghebeur, 1986), phylogenetic research revealed that *Pycreus* is consistently nested within the C_4 clade of *Cyperus* along with several other genera as *Kyllinga* and *Queenslandiella* (Muasya & al. 2002a, 2002b, 2009; Larridon *et al*. 2011b, 2013), a relationship that is confirmed by morphological and anatomical data (Haines & Lye 1983, Bruhl & Perry 1995, Soros & Bruhl 2000). Modern molecular techniques show that the paraphyletic nature of large genera is a common phenomenon in flowering plants (e.g. Miller & Bayer 2001) and there is a growing opinion favouring the maintenance of paraphyletically circumscribed taxonomic entities. Unfortunately the different lineages of *Pycreus* are not resolved in the recent molecular studies. Several lineages are nested in the main *Cyperus* polytomy as are most segregate genera, while the crown group of *Pycreus* is monophyletic with *Cyperus laevigatus* (a species with dorsiventrally flattened pistils) strongly supported as sister. This implies a paraphyletic (with a reversal to a pistil state common among many different lineages of *Cyperus*) or even polyphyletic (multiple origins of laterally flattened pistils) nature of *Pycreus*. Since the monophyletic status of *Pycreus* as segregate lineage of *Cyperus* is not confirmed, it is currently impossible to maintain an evolutionary classification for this clade in treating *Pycreus* as a separate genus beside a paraphyletic circumscribed *Cyperus* s.s. as had been suggested by Goetghebeur (1986, 1998) and followed by many recent authors (Muasya *et al*, 2009b; Hoenselaar *et al*., 2010; Reynders & Goetghebeur, 2010; Govaerts *et al*., 2007). A decision on the taxonomic status and subgeneric level of *Pycreus* (treatment on the sectional level with the current sections on the subsectional level or
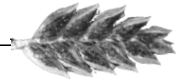
treatment as several different sections under *Cyperus*) and its current sections is currently impossible and awaits better-resolved phylogenies.

9.3.3 Aims of this chapter

Although at this stage it is not possible to propose a new subdivisional classification for *Pycreus* under *Cyperus*, the formal inclusion of *Pycreus* is needed in the light of the classification strategy for *Cyperus* s.l.. As both *Pycreus* and *Cyperus* have always been considered to be closely related, most names already have homotypic synonyms under both genera. Only few names need still to be transferred to *Cyperus* and most of these consist of fairly recent names for African or Asian species with a limited geographical range. Since the type collections are difficult to access little attention is given here to the Asian species pending an urgent need of confirmation of the taxonomic status of these species. Especially the Indian taxa described by Govindaralaju (e.g. 1990), who applied an extremely narrow morphological species concept, need closer study. For example Prasad (2009) synonymised five of these species with *P. malabaricus*. Also in the *P. pumilus* complex, similar re-evaluations are needed for the Indian species. Collections of the African species are better accessible (K, P, BR, B, Jstor Plants, 2011) for which it was possible to evaluate their taxonomical status here. Next also *P. decumbens* Koyama (1976) may fall within the variability range of *P. mundtii* (a species widespread in Africa). As *P. decumbens* is only known from a few locations along the Amazon river, it may represent introductions of *P. mundtii*. Anyhow both species are strongly related and this needs further investigation before *P. decumbens* can be transferred to *Cyperus*.

In this chapter new combinations or new names are listed for African taxa we consider to be valid species. In addition several species names needed to be synonymised (see 9.4.2).
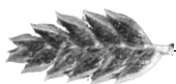
9.4 Results & discussion

9.4.1 New combinations in *Cyperus*

***Cyperus africanus* (S.S.Hooper) Reynders, comb. nov.** Basionym: *Pycreus divulsus* subsp. *africanus* Hooper (1972: 579) ≡ *Pycreus africanus* (S.S.Hooper) Reynders (2010: 227). Type: CAMEROON, Gaudua, foothills of Gotel Mountains, 17 July 1969, *J.B. Hall 1381* (holotype K!, isotype P!).

Description: — Reynders *et al.* (2010).

***Cyperus acaulescens* Reynders & Goetghebeur nom. nov.** ≡ *Pycreus acaulis* Nelmes, Kew Bull. 10: 91 (1955), non *Cyperus acaulis* Steudel (1842: 599). Type: MALAWI, Nyika Plateau, Kaulime Pond, 27 June 1952, *G. Jackson 870* (holotype K!, isotype BR!).

Description: — Nelmes (1955 :91)

***Cyperus neocooperi* Reynders nom. nov.** ≡ *Pycreus cooperi* C.B.Clarke in W.H.Harvey & auct. suc. (eds.), Fl. Cap. 7: 160 (1897). Synonyms: — *Cyperus cooperi* (C.B.Clarke) Kük., Bot. Not. 1934: 68 (1934), nom. illeg. non *Cyperus cooperi* (C.B.Clarke) K.Schum. (1900: 328). Type: SOUTH AFRICA, 17 January 1861, *T. Cooper 912* (holotype K!).

Description: — C.B.Clarke (1897: 160), Kükenthal (1936).

Notes : — Kükenthal (1936) placed this species in his very heterogenous section *Lancei* based on the rather large and dark glumes. Blackish glumes are very common among *Cyperus* species growing on higher altitudes (see e.g. Haines & Lye 1983)1 and originated most likely multiple times within the genus.

***Cyperus okavangensis* (Podlech) Reynders comb. nov.** Basionym: *Pycreus okavangensis* Podlech, Mitt. Bot. Staatssamml. München 3: 522 (1960). Type: NAMIBIA, [Rundu], 11 May 1939, *O.H. Volk 1966* (holotype M!, isotype PRE!).

Description: — Podlech (1960: 522), Kükenthal (1965)
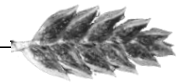
Cyperus poikilostachys (Nelmes) Reynders comb. nov. Basionym: *Pycreus poikilostachys* Nelmes, Kew Bull. 6: 320 (1951 publ. 1952). Type: ZAMBIA, 24 January 1938, *E. Milne-Redhead 4311* (holotype K!, isotypes BR!, K!, PRE!).

Description: — Nelmes (1952)

Cyperus poikilostachys var. heterochrous (Nelmes) Reynders comb. nov. Basionym: *Pycreus heterochrous* Nelmes, Kew Bull. 6: 321 (1951 publ. 1952). Type: ZAMBIA, Mwinilunga District, Matonchi Farm, 0.5 mile South of farm, 24 January 1938, *E. Milne-Redhead 4309* (BR!, P!, isotype PRE!).

Description: — Nelmes (1952: 321)

Note : — Among the *Milne-Redhead* from Zambesia a few new species had been described simultaneously by Nelmes, among which *P. poikilostachys* and *P. heterochrous*. The latter two species only differ in their glume colour as *P. poikilostachys* has dark brown glumes and *P. heterochrous* has pale reddish brown glumes. As both are sympatric with an absence of individuals showing intermediate glume colors, Nelmes (1938) considered both color variants as different species. Glume color alone is now generally considered to be unreliable for species delimitation in *Cyperus* (Goetghebeur, 1986). In addition, as known from classic Mendelian genetics, it is possible that different color variants can be present in the same populations without the presence of intermediates (dominant-recessive inheritance instead of intermediate inheritance). Different colour variants of a single species are usually treated on the variety rank or below.

Cyperus poikilostachys var. poikilostachys (autonym, automatically established here)

Cyperus scaettae (Cherm.) Reynders comb. nov. Basionym: *Pycreus scaettae* Cherm., Rev. Zool. Bot. Africaines 24: 295 (1934). Type: CONGO, 1930, *H. Scaetta 58M* (syntype BR!, isosyntype BR !, isosyntype K!) ; Scaetta 2418 (syntype BR !, isosyntype P !)

Description: — Chermezon (1934: 295)
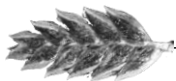
Notes : — *Pycreus scaettae* belongs to a group of species from Zambesian Africa showing thick accumulations of fibers (remains of leaf sheaths) surrounding the bases of the culms and most often inflorescences are reduced to only a few spikelets set in a single spike. With the description of the different taxa showing these characteristics, relatively less attention has been made to spikelets, glume and nutlet morphology than to the striking culm bases and glume color. In addition communication and consultation of type material seemed to have been rather limited in the short period these taxa have been described, combined and synonymised by different authors (Chermezon, 1932, 1933, 1934; Kükenthal, 1921, 1936). Subsequently species separations and especially synonymisations were not very clear from the beginning and several misinterpretations arose in later publications (e.g. Haines & Lye, 1983). Comparison of spikelet characteristics shows that *P. scaettae* clearly differs from *P. fibrillosus* (Fig. 8.13 b-c, f-g).

Cyperus scaettae var. **vanderystii** **(Cherm.)** **Reynders** **comb.** **nov.** ≡ *Pycreus vanderystii* Cherm., Rev. Zool. Bot. Africaines 24: 296 (1934). Synonyms: -- *Cyperus fibrillosus* var. *vanderystii* (Cherm.) Kük. in H.G.A.Engler (ed.), Pflanzenr., IV, 20(101): 348 (1936). TYPE: CONGO, Vanderyst 16469 (syntype BR!); Vanderyst s.n. (syntype BR!).

Description: — Chermezon (1934: 296)

Notes: *Pycreus vanderystii* is clearly related to *P. scaettae*, which was already remarked by Chermezon (1934), in the description of both taxa in the same publication. Both taxa have the same habit, spikelet and glume shapes. The rachilla is straight. *P. vanderystii* only differs in its overall larger dimentions of the plant and of the glumes and a yellowish glume color. Since there is an overlap in size between both taxa, which are also sympatric, the variety level seems to be the most appropriate rank for *P. vanderystii*. As discussed under *C. scaettae*, *C. fibrillosus* is clearly different by its flexuous rachilla and different glumes. Therefore also *P. vanderystii* needs to be removed from synonymy with the latter taxon.

Cyperus scaettae var. **scaettae** autonym established here.
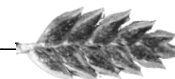
9.4.2 Notes on synonymy of several African species

P. sanguineosquamatus has not yet been combined into *Cyperus*. However, in our opinion this name represents a species that needs to be placed in synonymy of an older name. Therefore, a new combination into *Cyperus* would be superfluous. Also, the recently described *Cyperus sumbawangensis* is here considered as a synonym.
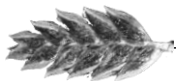
Pycreus sanguineosquamatus Van der Veken Bull. Jard. Bot. État Bruxelles 24: 145 (1955).
Type: CONGO, May 1939, *H. Bredo 2750* (holotype BR!, isotypes BR!, C!, NY!, P!, PRE!).

= ***Cyperus fontinalis* (Cherm.) Kük.** in H.G.A.Engler (ed.), Pflanzenr., IV, 20(101): 341 (1936).
Basionym: -- *Pycreus fontinalis* Cherm., Bull. Soc. Bot. France 67: 327 (1920 publ. 1921).

Notes: — *Pycreus fontinalis* is a rare species only known from its type localities near the hot water sources of Antsirabe, Madagascar. *P. sanguineosquamatus* is described on material from salt marshes of volcanic origin in the region of Shaba (Congo). With the description of the latter species, Van der Veken already noted the similarity of this taxon with *P. fontinalis*. However *P. fontinalis* was circumscribed by Chermezon and Kükenthal by having a tiny rhizome. As Van de Veken interpreted the material from Congo as therophytic, both species were assumed to be separated by their growth form. However when comparing the type material of both taxa, this distinction of growth forms is in our opinion no more than a matter of interpretation as no striking differences could be observed between the plant bases of the Congolean and Malagasy material. In addition glumes and nutlets (the taxonomically most important characteristics in this group of *Cyperus*) are very similar. Type localities of *Cyperus fontinalis* in Antsirabe seem to be destroyed due to exploitation of the hot water from the springs by the local communities and the species may even be locally extinct (field observations in Madagascar, April 2010). Also the salt marshes in southern Congo are exploited. The salt marshes of this region are of volcanic origin and contain a large amount of endemic plant species that are adapted to the mineral rich soils (Symoens, 1953) such as several short lived (endemic) *Pycreus* and *Bulbostylis* (Cyperaceae with C4 photosynthesis!). Considering the strong local geographic distribution of the species (two known localities) with

a possible extinction from Antsirabe and a continuing human exploitations of both localities, we advise a IUCN Critically Endangered status for *Cyperus fontinalis*.

Cyperus sumbawangensis (Hoenselaar) Lye, Lidia 7: 97 (2011). Basionym: -- *Pycreus sumbawangensis Hoenselaar, in Fl. Trop. E. Afr., Cyp.: 297 (2010).* Type: -- TANZANIA, Ufipa District: Sumbawanga, Richards 3452A

= ***Cyperus gracillimus*** (Chiov.) Kük. in H.G.A.Engler (ed.), Pflanzenr., IV, 20(101): 393 (1936). Basionym: -- *Pycreus gracillimus* Chiov., Ann. Bot. (Rome) 13: 58 (1914). Type: Bovone 75, Katanga, Kayoyo, supfige ebene.

Notes: -- In the Flora of Tropical East Africa, *P. sumbawangensis* is indicated for high altitude pastures from Burundi and W & SW Tanzania. It is characterised by its culm bases covered with fibers and its reduced anthela with a single spike of blackish spikelets. In our opinion no differences can be found between *C. sumbawangensis* and the numerous central African specimens in the BR and GENT herbaria, identified as *C. gracillimus*. Unfortunately we were not able to locate the type specimen in any of the Italian herbaria, most likely the specimen has been lost. Also Kükenthal (1936), who combined the species into *Cyperus*, indicated that he did not see the type specimen. However, the protologue of the species by Chiovenda is clearly speaking about culm bases covered with fibrous remains and black spikelets. Since the Bovone material is from the same habitat and region as the other specimens under this name in BR (high altitude mountains from Katanga and the southern Albertine rift), they undoubtedly represent the same species.
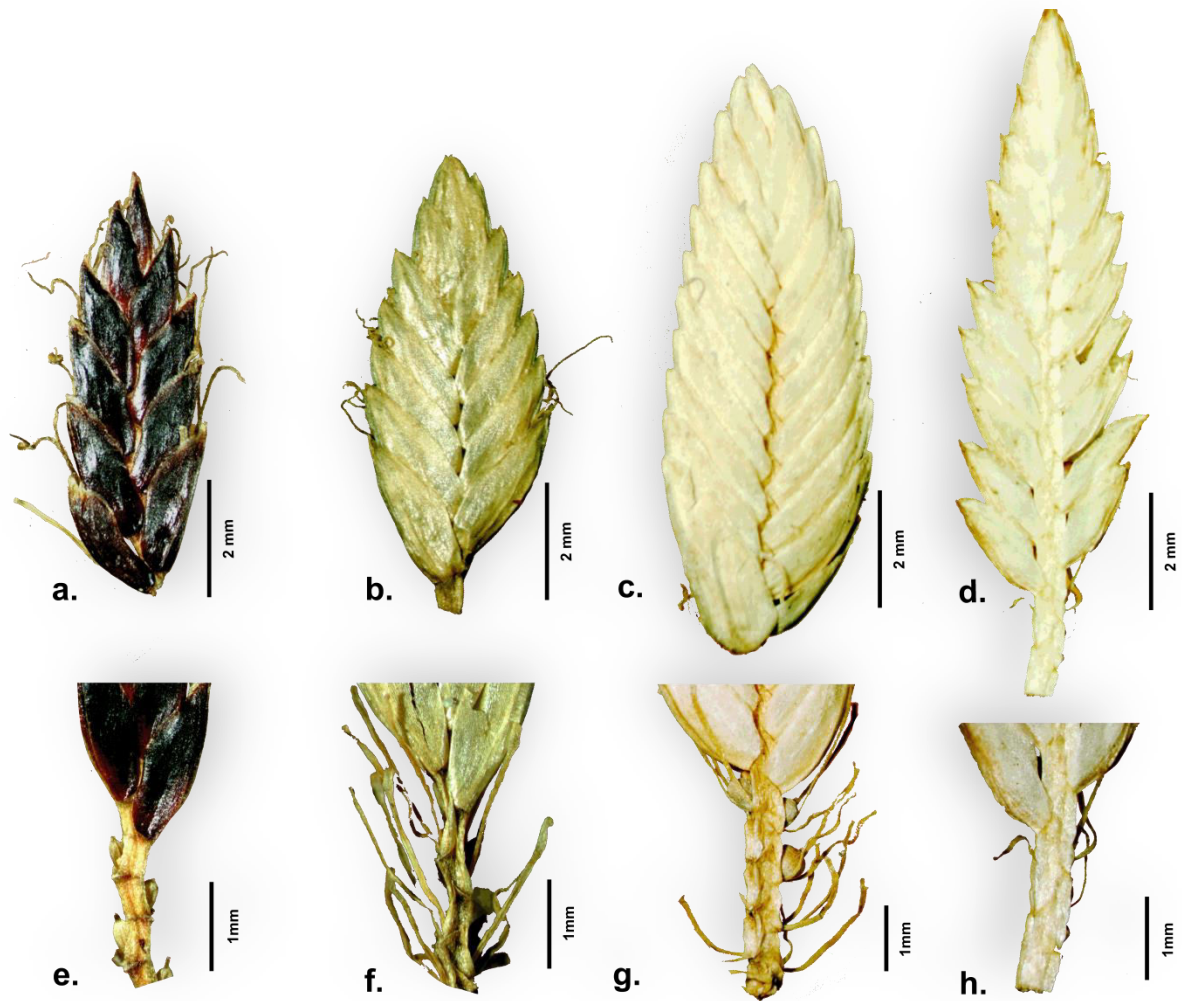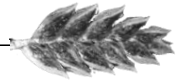
Fig 8.13 Comparision of spikelets of different fibrous *Pycreus* species clearly showing the difference between *P. fibrillosus* (b & f) and *P. scaettae* (c & g). *P. fibrillosus* has a flexuous rachilla which is hardly visible between the glumes. Lower glumes mostly have 1-2 additional nerves on their wings. These characteristics are in common with *P. gracillimus* (a & e). *P. scaettae* has larger spikelets with strongly imbricate glumes and a straight rachilla which is visible between the glumes which are characterisitcs in common with *P. smithianus* (d & h).

Beach

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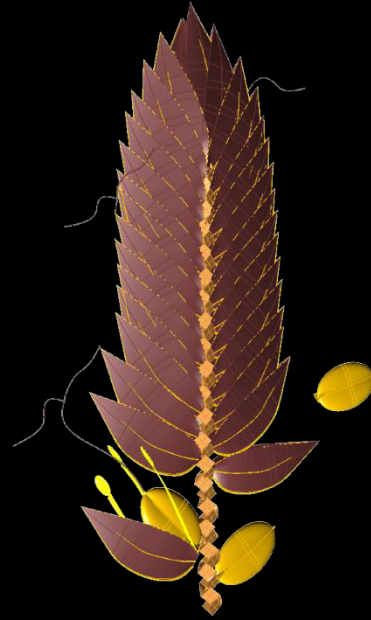
10 General discussion
& future prospects

"An expert is a person who has made all the mistakes that can be made in a very narrow field."

— Niels Bohr (1885-1962)

10.1 In this chapter…

Chapter 10 brings together the key results obtained during this PhD research project and gives a critical view on future strategies for the research of the taxonomy in C_4 *Cyperus* and *Pycreus*.

10.2 State of the art

10.2.1 Meso-scale

The *Cyperus* clade comprises ca. 950 species and forms the most important sedge genus in the tropics. Among the 800 species that use C_4 photosynthesis, 9 segregate lineages (accepted in the classification of Goetghebeur, 1998) are nested in C_4 *Cyperus*. Their taxonomical status needs to be reevaluated, especially since there is a conflict in the taxonomical importance of the characters used for the delimitation of the different segregate taxa.

By combining an elaborate molecular phylogenetic study with morphology, anatomy and floral developmental investigations, we unraveled relationships and evolutionary patterns in C_4 *Cyperus*. Consequently, the taxonomical value of delimitating characters and the generic status of the segregate lineages was reevaluated, with special attention to the taxonomic status of *Pycreus*.

10.2.1.1 Nomenclature and typifications

Firstly, a nomenclatural overview was made of all generic and subdivisional names related to the *Cyperus* clade sensu Muasya *et al.* (2009a). Types were designated where necessary and priority and legitimacy of names were evaluated. The resulting list of ca. 350 names was published in a series of tree articles (Huygh *et al.*, 2010; Larridon *et al.*, 2011; Reynders *et al.*, 2011). In this thesis, the names for taxa relating to *Pycreus* are presented. This list of published names served as a basis to select important taxa (e.g. types) for our molecular phylogenetic studies and to be able to use the correct names for the subdivisions in our modern classification of the *Cyperus* clade.

10.2.1.2 Phylogenetic relationships in C$_4$ *Cyperus*, including *Pycreus*

In our molecular phylogenetic study, we included 107 specimens of 104 different taxa, representing all segregate genera (based on Goetghebeur, 1998 and Muasya *et al.*, 2002) and many sections of *Cyperus* s.s. from the classification of Kükenthal (1935-36). For *Pycreus*, representatives of all 13 sections from the treatment of Kükenthal (1935-36) were included.

From the combined molecular phylogenetic analysis using ETS1f, *trnH-psbA* and *rpl32-trnL* markers we confirm a single origin of C$_4$ *Cyperus* within a grade of *Cyperus* species using C$_3$ photosynthesis. The diversity found in the C$_4$ *Cyperus* clade, in combination with short branch lengths and poorly resolved relationships observed in our and other analyses, all suggest a fast diversification or radiative burst after the origin of C$_4$ photosynthesis as a key innovation. C$_4$ photosynthesis allowed colonization of new niches with more solar irradiation, periods of desiccation, limitations of nutrients and/or environmental stress (e.g. high salinity).

Among the early emerging clades of the C$_4$ *Cyperus* clade, in the main polytomy and also the early emerging species in more derived clades, there are many species that have a pioneering growth strategy which can be assumed to be the primitive state. Many of such pioneer species show plesiomorphic characters such as more or less digitately clustered spikelets, glumes with a well-developed mucro and multiple nerves on both sides of the midrib. Among early emerging lineages, the sections *Amabiles* and *Rupestres* are represented together with several segregate lineages as *Queenslandiella* and *Alinula paradoxa*. The taxa with pseudospikelets; *Lipocarpha* s.l.*, Volkiella* and *Ascolepis,* form a single well resolved clade. All other C$_4$ *Cyperus* sections and the segregate genera *Remirea, Sphaerocyperus, Kyllinga* and *Pycreus* are nested within a large hard polytomy. *Kyllinga* is monophyletic while the status of *Pycreus* remains unclear (para- or polyphyletic). Among the sections of *Cyperus* s.s. nested within this polytomy, better resolutions were obtained for the sections *Papyri* and *Exaltati,* which contain tall species with a competitive ecological strategy and are all characterised by a winged rachilla.

10.2.1.3 Pistil evolution and the origins of laterally compressed pistils

From the combined investigation of the anatomy and ontogeny of the different pistil types in Cyperoideae (including several Cypereae an *Pycreus* species) and their vascularisation we can present the following developmental model for the gynoecium in Cyperoideae:

(1) Development of the gynoecium: The ovary originates from an annular gynoecium wall primordium (Vrijdaghs *et al.,* 2009) – During the earliest ontogenetic stages, the annular gynoecium wall primordium grows upwards to form a bag-like structure. Next, in most cyperoid species, two adaxially positioned and one abaxially positioned stigma primordia (according to the conservative positions of the original carpel tips) are formed on the top of the cylindrical ovary wall. However, other numbers and positions of stigma primordia are possible. We believe that the formation of a ring primordium from congenitally fused carpels allows shifts in positions of stigma primordia to novel, more optimal positions with respect to the available space (fig. 6.35).

(2) Development of the vascular bundles: Vessel initiation zones are present in the stigma primordia (fig. 6.38). From there, the vessels grow to the receptacular plexus, to be connected with the stele. These vessels constitute the ribs of the pistil. Consequently, the number and positions of the stigma primordia determine the future shape of the pistil, which develops initially as a bag-like structure, and subsequently typically assumes a triangular shape, or a derived dimerous, dorsiventrally or laterally flattened shape, or a polymerous shape.

(3) The development of the ovary wall and ovule appear as two distinct phenomena (also in time; fig. 6.36). Ovary wall and ovule vascular traces show independent connections with the receptacular plexus, thus reflecting the ontogenetic separation of the annular gynoecium wall primordium and ovule primordium.

This model allows us to understand all variations of the essentially trimerous morphological Bauplan of the Cyperoid gynoecium. The derived character states, such as laterally flattened dimerous pistils, were made possible through a synapomorphy of Cyperoideae, namely the gynoecium wall annular primordium, which reduced the constrains of a carpellary organization. Our observation on *Lagenocarpus amazonicus* suggest laterally flattened dimerous pistils originated from the loss of one adaxial stigma primordium and subsequent shift of the remaining pistil primordia into a spatially more optimal position allowed by the presence of the annular gynoecium wall primordium.

Unfortunately, it has not yet been possible to fully resolve the relationships of *Pycreus, Kyllinga* and *Queenslandiella* within the C$_4$ *Cyperus* clade. However, in none of the phylogenetic analyses *Pycreus*, *Kyllinga* and *Queenslandiella* cluster together. *Queenslandiella* is resolved among the early branches of the C$_4$ *Cyperus* clade, separate from *Pycreus* and

Kyllinga, which are nested in the main polytomy. This implies at least a separate origin of character state of laterally flattened pistils for *Queenslandiella*, which is congruent with its general morphology with respect to *Pycreus* and *Kyllinga* species. Secondly, *Pycreus* is not monophyletic. As the noncore clades are not resolved it is not clear whether *Pycreus* is to be considered as para- or polyphyletic. However, the core clade of *Pycreus* is consistently associated with *Cyperus laevigatus* as sister group, a species characterised by a dorsiventrally flattened pistil. From our observations of the anatomy and morphology of the pistil it is unlikely that the dorsiventrally compressed pistils have originated from a reversal from the laterally flattened character state. We prefer the interpretation as a new origin of a dorsiventrally compressed dimerous pistil in one clade (loss of the abaxial stigma primordium) and a complementary origin of a laterally compressed pistil in the sister clade (loss of one of the adaxial stigma primordia). Clearly, the ring wall primordium as an independent ontogenetic zone reaches an optimum in C_4 *Cyperus* which allows the variation in ovary structure that can be found in the clade.
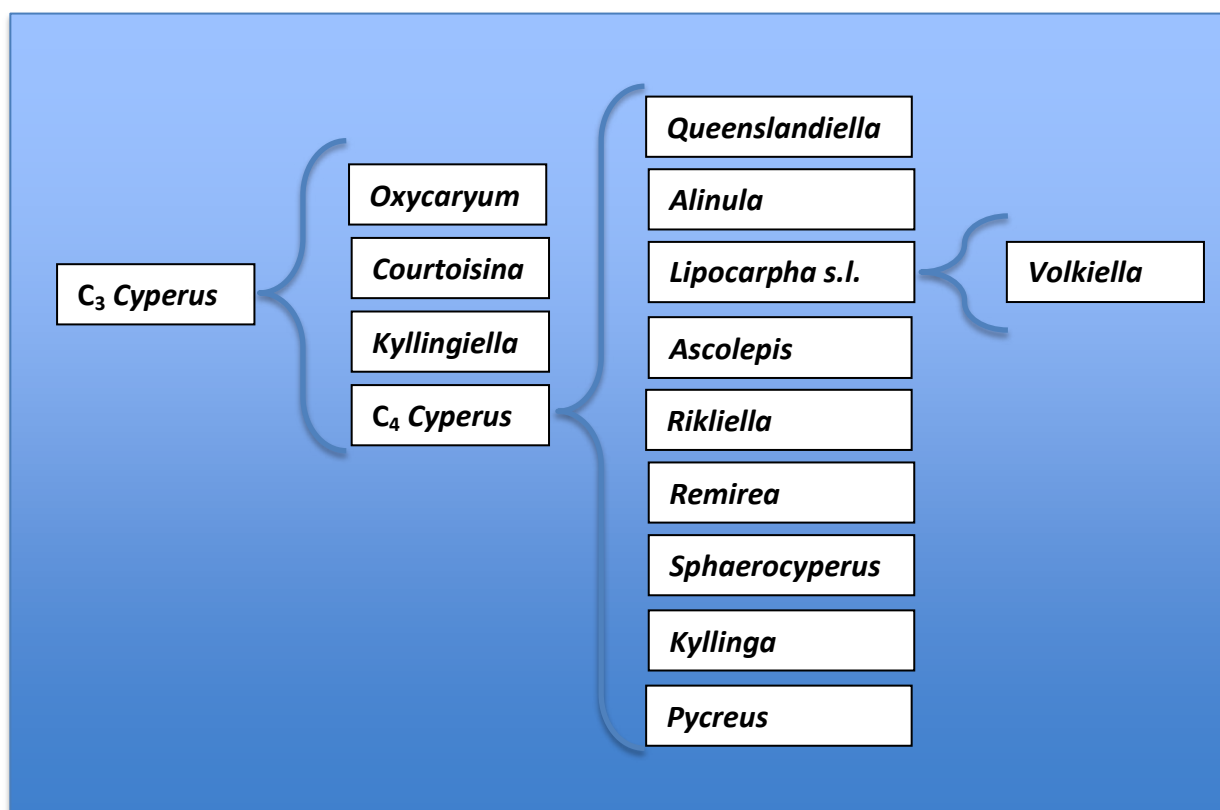
Fig. 10.2 Supernested paraphyletic entities in the *Cyperus* clade as found in our molecular studies. Four levels have been recognized. Possibly the *Lipocarpha* subclade can be additionally nested within *Alinula*.

10.2.1.4 An integrated classification strategy for *Cyperus* s.l.

The development of a modern classification of *Cyperus* s.l. is challenging due to the following reasons:

1. The *Cyperus* clade appears to be a story of supernested paraphyletic entities (Fig. 10.2), which makes it difficult to translate existing taxonomic delimitations of genera and subdivisions into a classification that uses exclusively monophyletic taxa.

2. With inclusion of the derived lineages it is difficult to circumscribe *Cyperus* s.l. morphologically (Muasya et al., 2009b). The generic characters traditionally used for *Cyperus* s.l. are not valid for all segregate lineages (e.g. spirally glumes reappeared multiple times in *Cyperus* while distichously placed glumes also originated in a few members of the *Ficinia* clade).

3. The C_4 *Cyperus* clade remains largely unresolved (Fig. 7.4), even with the use of fast evolving markers. Although the sequences obtained show high mutation levels, a phylogenetic signal was insufficiently detected. This makes it difficult to draw conclusions on relationships within *Cyperus* and more specific on the mutual relationships of the different segregate taxa of *Cyperus*.

As a consequence, although a decade ago, the first molecular findings already confirmed the paraphyletic nature of *Cyperus*, the generic classification of Goetghebeur (1998) remained largely accepted for pragmatic reasons. Currently we obtained better resolution at least for parts of the *Cyperus* clade such as the species using C_3 photosynthesis (including *Courtoisina*, *Oxycaryum* and *Kyllingiella*) and for the early emerging subclades of the C_4 *Cyperus* clade, including the taxa with pseudospikelets (*Alinula, Ascolepis*, *Lipocarpha* and *Volkiella*). In addition, we have now a much better understanding of the spikelet and floral ontogeny of *Cyperus* s.l. This allows us, even with missing data, to set out a new classification strategy for *Cyperus* s.l.

Two main classification strategies for resolving the taxonomies of paraphyletic taxa and their segregates are commonly accepted: lumping and splitting. Both methods use a cladistic approach in which taxa have a monophyletic circumscription. For giant genera, lumping is the most often applied strategy since with splitting we would end up with various smaller but morphologically undistinguishable genera (cfr. *Euphorbia*, *Horn* et al., 2012). Both methods have advantages and also several disadvantages such as an under- or over-estimation of the morphological diversity present in the group (Fig. 10.3). Consequently, we

hesitated to apply one of these classification strategies on the complete *Cyperus* clade (especially concerning the taxa with pseudospikelets). Therefore, we considered a third (but controversal) so called 'evolutionary classification strategy' (see table 10.1), which allows for the use of paraphyletic genera (e.g. Brummitt, 2006; Hörandl, 2006). Hörandl & Stuessy (2010) proposed to use characters that play a major role in evolutionary success (adaptations) as basis for placing segregate groups on the same taxonomic level as the paraphyletic rest group. These characters can be objectively selected trough patrocladistic analysis (Stuessy & König, 2008). Herein, a statistical comparison of the branch lengths of different clades form the most important clustering criterion (Pommier *et al.*, 2009). A strong shortening of branch lengths in comparison to the branch lengths of the outgroup clades often indicates a radiative burst. In table 10.1 we give a brief overview of advantages and disadvantages of each classification strategy.

Fig. 10.3 Cypertoon of the largely unresolved phylogenetic relationships in C$_4$ *Cyperus*, a challenge for taxonomists working on a modern classification for the group. Drawn by M. Reynders.

Table 10.1 Overview of different classification strategies that could be applied on *Cyperus* s.l.

Strategy	Phylogenetic status of taxa		Arguments
Splitting (cladistic)	monophyletic	Pro	- Monophyletic taxa
			- Taxa with derived morphologies are recognised as separate entities.
		Contra	- Could create several morphologically hardly separable groups (overestimation of diversity).
Lumping (cladistic)	monophyletic	Pro	- Monophyletic taxa
		Contra	- Several segregate taxa are highly atypical and hard to recognize as belonging to the higher taxon (underestimation of diversity).
			- Very broad generic concept, difficult to circumscribe as a group
			- More complex infrageneric taxonomy, many taxonomic changes
Evolutionary	mono or paraphyletic	Pro	- Morphological/ evolutionary diversity is reflected by different generic names
			- Generic circumscriptions often straightforward
			- Conservative regarding taxonomy and nomenclature, which is especially advantageous for widely known taxa (e.g. with economic importance).
		Contra	- Generic circumscriptions sometimes based on plesiomorphic characters
			- Paraphyletic taxa are generally not accepted
			- Segregate taxa must show highly adaptive characters (altered speciation rates), with low levels of homoplasy.
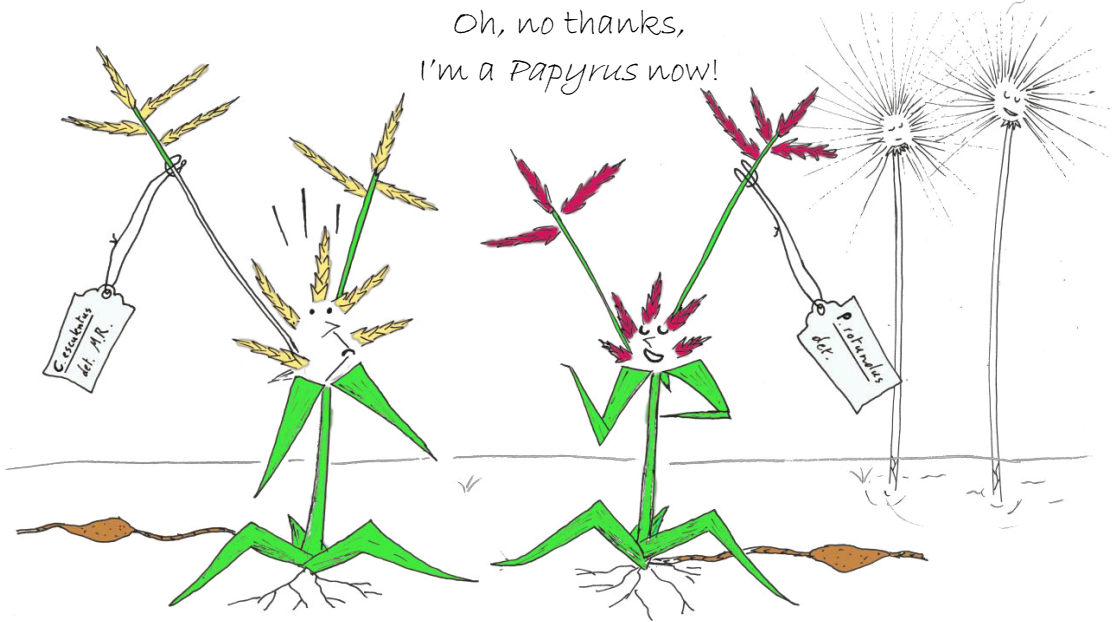
And suddenly, with the pass of a splitter, the yellow nutsedge realised its taxonomical future could once become very lonesome...
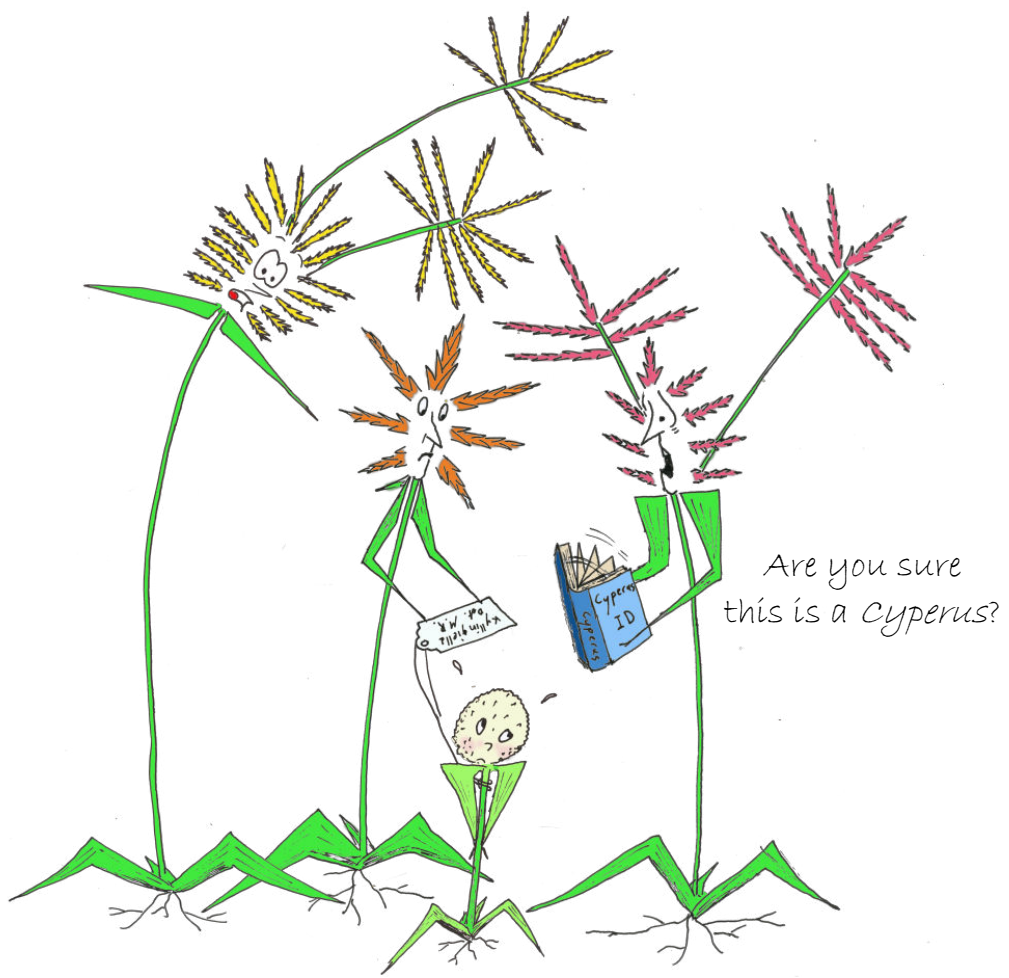
The lumper's effect....

Fig. 10.4 Cypertoon showing disadvantages of classifications strategies as splitting and lumping. Drawn by M. Reynders.

We use a combination of molecular phylogenetics, anatomy, morphology and ontogeny to evaluate monophyly of the segregate taxa of *Cyperus* and the taxonomical value of the characters that have been used for generic delimitations in the *Cyperus* clade. Table 10.2 shows an overview of these characters with an evaluation of the taxonomical value in three categories (low, moderate, high). Evaluation of the taxonomical value was based on two criteria:

1. The estimated minimum number of origins of this character state based on result of the molecular study. A single origin means low levels of homoplasy and a very high taxonomical value, while multiple origins point to high homoplasy and low taxonomical value.

2. The presence of intermediate characters or species which show both plesiomorphic and apomorphic states. This indicates divergence of the different taxonomical groups has not yet been fully completed. Subsequently, the taxonomical value of this character is rather low.

In addition, also the evolutionary importance of the character state is evaluated. This depends on the ecological advantage the character state provides to the taxon (adaptiveness, e.g. advantages in seed dispersal, pollination,…). In a cladogram, this evolutionary importance is often reflected in an alteration in branch lengths (fast divergence) and success of the clade (species richness). As already mentioned above, according to Hörandel & Stuessy (2010), evolutionary significant characters are essential to consider application of an evolutionary classification.

Fig. 10.5 shows our decision tree for further selection of the most appropriate classification strategy or combination of classification strategies for the *Cyperus* clade. This selection is mainly based on our evaluation of the taxonomical value of the derived characters of the taxon, the evaluation of its evolutionary value, the possibility to split the paraphyletic rest genus into recognisable units and the possible (nomenclatural) disadvantage of lumping the segregate taxa.
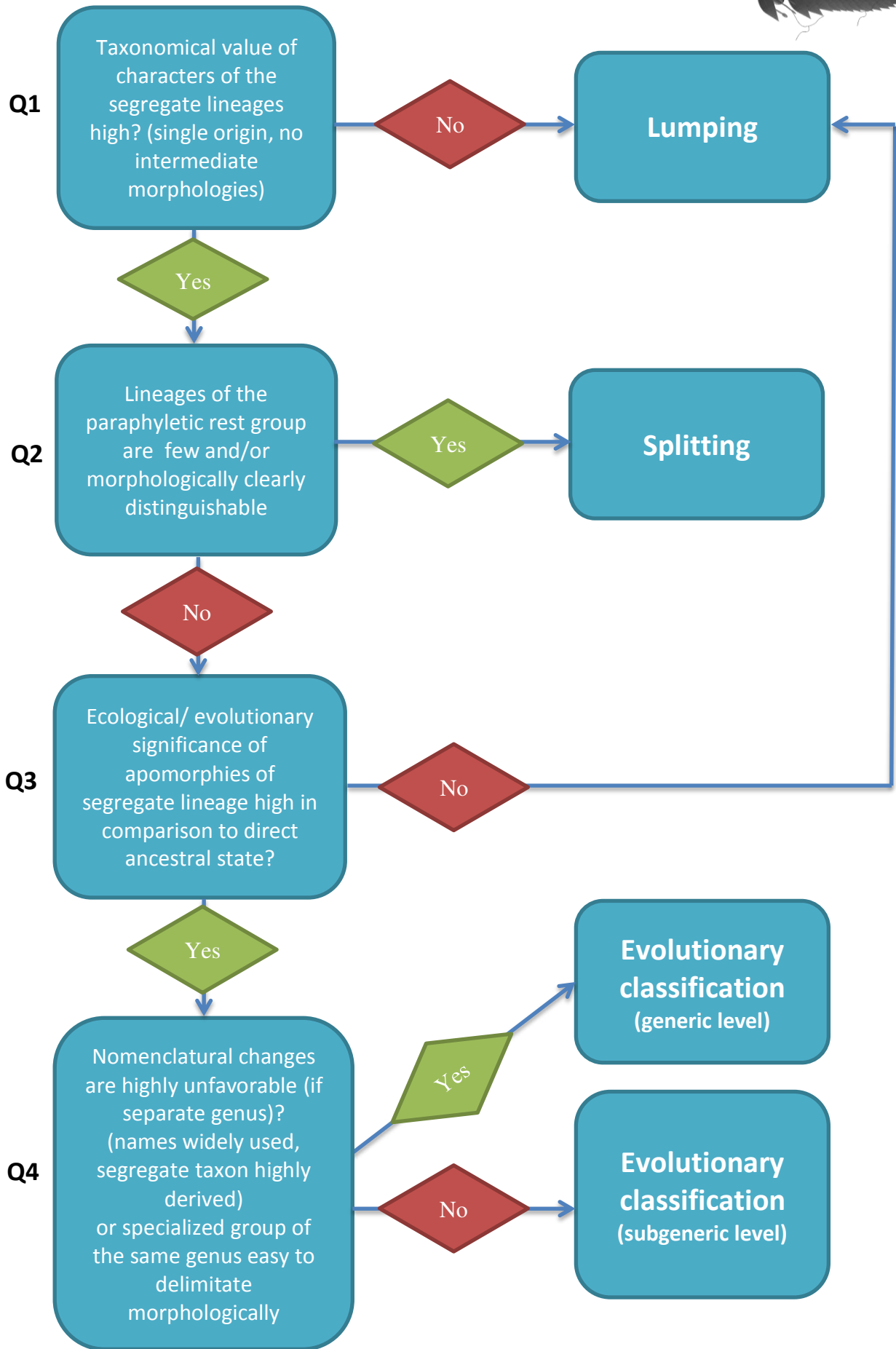
Fig. 10.5 Decision tree for the choice of the most appropriate classification strategy for specialised lineages that are nested within a paraphyletic genus.

Table 10.2 Evaluation of the taxonomical value of derived characters used for the delimitation of the segregate genera of the *Cyperus* clade. The evaluation of the taxonomical value is based on the number of origins of the derived characters and presence of taxa with transitional morphologies. Evolutionary significance of the character state can be seen as an additional but not essential criterion. Scoring: Number of origins (1=low, 2=moderate,>2 high); Transitional stages present (no=low; yes=high); Taxonomic value (number of origins and or transitional stages high= low taxonomic value, number of origins moderate and transitional stages low/unknown= moderate taxonomic value, number of origins low and transitional stages low/unknown= high taxonomic value). *Unknown means no transitional stages have been observed so far, however in most cases sister taxa are not yet known.

Character	Taxa	Number of origins	Transitional stages present	Taxonomic (diagnostic) value	Ecological/ evolutionary significance (adaptive)
C₄ photosynthesis	C₄ *Cyperus* clade, including: *Alinula, Ascolepis, Lipocarpha, Volkiella, Queenslandiella, Kyllinga, Pycreus, Remirea, Sphaerocyperus*	1 (low)	Unknown*	**high**	high (key innovation)
Spiral glume placement	*Oxycaryum, Kyllingiella, Cyperus pygmaeus*	3 (high)	yes	**low**	low
Deciduous spikelets	*Courtoisina, Cyperus deciduus,* C₄ *Cyperus p.p., Queenslandiella, Kyllinga, Remirea, Sphaerocyperus*	>3 (high)	yes	**low**	moderate

Character	Taxa	Number of origins	Transitional stages present	Taxonomic (diagnostic) value	Ecological/ evolutionary significance (adaptive)
Pseudospikelets	*Alinula, Lipocarpha s.l., Ascolepis, Volkiella*	2 (moderate)	Unknown*	**moderate**	moderate
Empty glumes	*Remirea, Sphaerocyperus*	1-2 (moderate)	Unknown*	**moderate**	low
Headlike inflorescence	C$_3$ *Cyperus p.p.*, *Kyllingiella*, C$_4$ *Cyperus p.p.*, *Lipocarpha*, *Ascolepis*, *Volkiella*, *Remirea*, *Sphaerocyperus*, *Kyllinga*, *Pycreus p.p.*	>6 (high)	yes	**low**	moderate
Dorsiventrally compressed pistils	*Oxycaryum*, C$_4$ *Cyperus p.p.*, *Lipocarpha p.p.*, *Ascolepis p.p.*	>5 (high)	yes	**low**	low
Laterally compressed pistils	*Pycreus, Kyllinga, Queenslandiella*	>2 (high)	Unknown*	**low**	low

Table 10.3 Application of the decision tree (Questions 1-4) on the different segregate lineages of the _Cyperus_

clade based on the evaluation of taxonomical and evolutionary value presented in table 10.2.

Taxon	Nested in	Q1	Q2	Q3	Q4	Strategy
C$_4$ _Cyperus_	C$_3$ _Cyperus_	yes	no	yes	no	Evolutionary classification (subgeneric level)
Alinula	C$_4$ _Cyperus_	no	/	/	/	lumping
Courtoisina	C$_3$ _Cyperus_	no	/	/	/	lumping
Kyllinga	C$_4$ _Cyperus_	no	/	/	/	lumping
Kyllingiella	C$_3$ _Cyperus_	no	/	/	/	lumping
Lipocarpha clade (including Ascolepis and Volkiella)	C$_4$ _Cyperus_	yes/no	no	no	/	lumping
Oxycaryum	C$_3$ _Cyperus_	no	/	/	/	lumping
Pycreus	C$_4$ _Cyperus_	no	/	/	/	lumping
Queenslandiella	C$_4$ _Cyperus_	no	/	/	/	lumping
Remirea	C$_4$ _Cyperus_	yes/no	no	no	/	lumping
Sphaerocyperus	C$_4$ _Cyperus_	yes/no	no	no	/	lumping

In applying the decision tree on the evaluation of the taxonomic value of the different derived characters, only C$_4$ photosynthesis stands out with a high taxonomic value. The lineages using C$_3$ photosynthesis form a grade. Unfortunately, morphological variation in this group is too low to assign a separate generic status to each of the lineages within the grade. Therefore, splitting is not the appropriate classification strategy. However, an evolutionary classification (see table 10.1) is electable for this node. The evolutionary significance of the origin of C$_4$ photosynthesis is scored high since it clearly gives the plants an ecological advantage which resulted in fast diversification (short branch lengths and large species diversity). However, as morphological characters other than the shift in photosynthesis system are not correlated fully with these groups, it is not favourable to grant a separate generic status to C$_3$ and C$_4$ groups and nomenclatural changes are not favorable for these groups (the type species of _Cyperus_ sits in the large C$_4$ _Cyperus_ polytomy). We thus opted to use a paraphyletically circumscribed subgenus _Anosporum_ to accommodate the C$_3$ species. While the C$_4$ clade, including its segregate lineages, is classified in _C_. subg. _Cyperus_. We did not perform a patrocladistic analysis (Hörandl & Stuessy, 2010) to objectively test our decision on the use of a paraphyletically circumscribed _Cyperus_ subgenus _Anosporum_. However, our

analysis of the *Cyperus* clade (Larridon *et al.*, 2011, 2013) shows a change in branch lengths indicating the evolutionary significance of the origin of C$_4$ photosynthesis.

The proposed classification implies lumping of 12 segregate genera sensu Goetghebeur (1998) either due to a low evaluation of the taxonomic value from our study or lower evolutionary significance of the derived characters. Some characters, such as deciduous spikelets might have advantages in seed dispersal while others, such as headlike inflorescences might play a role in pollination (creating secondary spikelets or possibly even adaptations towards insect pollination). However, these characters are not necessarily linked with species rich clades. It was not possible yet to evaluate shifts in branch lengths since most of the C$_4$ *Cyperus* clade is still unresolved.
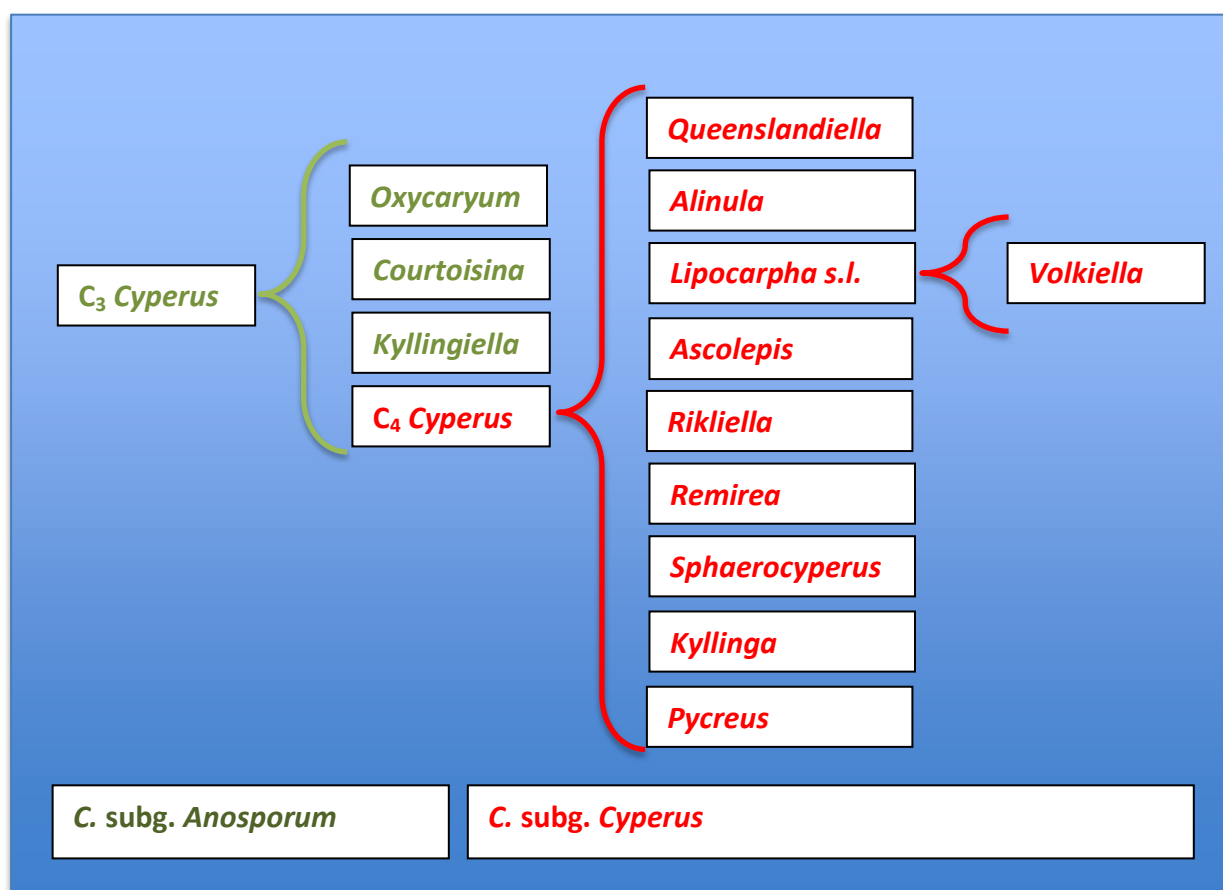
Fig. 10.6 Subgeneric classification sensu Larridon *et al*. (2011a). Lineages with C$_3$ photosynthesis are classified in a paraphyletically circumscribed *C.* subg. *Anosporum*. While the taxa with C$_4$ photosynthesis form *C.* subg. *Cyperus*.
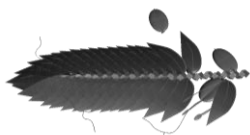
From our results, the lumping of *Pycreus* in *Cyperus* subg. *Cyperus* was straightforward. With our findings that laterally compressed pistils probably originated in multiple evolutionary events in C$_4$ *Cyperus* and that *Pycreus* is not monophyletic and is nested within *Cyperus*, there are no arguments left to maintain *Pycreus* on a separate generic level.

A next step is to choose for the appropriate infrageneric level for each segregate lineage. This requires a well resolved species level phylogeny which has been accomplished for C$_3$ *Cyperus* where *Oxycaryum, Courtoisina* and *Kyllingiella* are placed in three sections along with several transitional species (Larridon *et al.,* 2011a). Also for the *Lipocarpha* clade, a new sectional classification is in construction (Bauters *et al.,* submitted). *Kyllinga* has been shown to be monophyletic and consequently, it can be considered also as a separate section. A subsectional classification is also proposed by Huygh *et al.* (in prep). However, for most of the C$_4$ *Cyperus* clade, current molecular phylogenetic hypothesis are not resolved well enough to be able to reevaluate the delimitations of existing sections and to propose a new classification.

10.2.2 Micro-scale

10.2.2.1 Phylogenetic relationships within *Pycreus*

From our combined analysis of ETS1f, *trnH-psbA* and *rpl32-trnL* markers we found that *Pycreus* species do not form a monophyletic group and that *Pycreus* is nested within the large polytomy of C$_4$ *Cyperus*. It is not yet clear whether the *Pycreus* species form a paraphyletic or polyphyletic group.

Relationships between the species belonging to the sections *Rhizomatosi, Albomarginati, Pumili* and *Polystachyi* are not resolved within the large C$_4$ *Cyperus* polytomy. However, throughout our different molecular analyses they seem to be consistently (but without strong support) associated with the core *Pycreus* clade. In contrast, in the recent analysis of Reid (in press) using ITS, these species seem to cluster among the early emerging lineages of the C$_4$ *Cyperus* clade, away from the core *Pycreus* clade. Only relationships within the section *Polystachyi* have high support. Among these noncore sections many plesiomorphic characters states can be observed such as multinerved glumes, glumes with a mucro, strongly branched inflorescences, a pioneering growth strategy (many weeds in rice fields) and a preference for lowland (or even coastal habitats). Among these noncore sections several *Cyperus* species seem to be clustering. However, since relationships are poorly resolved we

cannot make any speculations about relationships. Moreover, in our previous analysis including only ETS1f, *Ascolepis* species tended to cluster among these noncore *Pycreus* species as well. However, *Cyperus aterrimus* is resolved among the species of section *Polystachyi* and resembles the high altitude species. Further investigation is needed to confirm this possible relationship, which would also implicate a reversal from laterally compressed dimerous pistils to trimerous pistils.

The remainder of the *Pycreus* species form a well-supported clade representing the sections *Globosi, Sulcati, Lancei, Propinqui, Fontinales, Flavescentes, Latespicati* and *Muricati*. This clade is consistently supported as sister to *Cyperus laevigatus,* which is a species with dorsiventrally compressed dimerous pistils. Species belonging to these sections show more diversified and derived characters such as reduced or contracted inflorescences, glumes with only a trinerved midrib, a more continental dispersal up to high altitude mountains. Within this core clade relationships are still poorly resolved.

10.2.2.2 Which characters and character states can be defined for the nutlet epidermis in *Pycreus* and what is their value for infrageneric classification?

The following characters and character states were observed in *Pycreus*: Isodiametric cells can be found throughout sedges and *Cyperus*, among the noncore clades of *Pycreus* and also in several species belonging to the core clade. Within several subclades of the core *Pycreus* clade, a variation range in the elongation of the nutlet epidermal calls can be found. From our analysis of silica bodies we conclude that two main types occur among the *Pycreus* species. The latter are restricted to small entities of 1-2 species. The first main type is the tabular silica body which is the most widespread type in sedges and also the plesiomorphic condition in C_4 *Cyperus*. Noncore sections of *Pycreus* also possess this type or a derived condition of it. In the core clade we only observed tabular silica bodies in *P. micromelas* and in some specimens of *P. capillifolius* (central silica body variable in this species). However, most species of the core clade possess the second main type which is characterised by a rounded central silica body with a knobby apex. We observed this latter type in species with isodiametric, intermediate and zonate nutlet epidermal cells. In many species with intermediate to zonate cells silica bodies are very small or absent. In addition, six derived types can be distinguished that are restricted to only a few species.

The taxonomic value of nutlet epidermal characters and character states was reevaluated by combining our results from molecular phylogenetic analysis with the SEM observations of the nutlet epidermis cells and variation of silica bodies. Optimization of the characters and character states on the cladogram was not possible since the molecular phylogenetic hypothesis is poorly resolved. However, observations from several smaller, well resolved clades indicate nutlet epidermal cell schapes and silica bodies are not reliable as primary characters for infrageneric classification in *Pycreus*. Variations in the shape of nutlet epidermal cells were used as the most important character for subgeneric classifications in *Pycreus* (Clarke, 1897; Chermezon, 1937; Kükenthal, 1935-36).

There is an urgent need for a better resolved molecular phylogenetic hypothesis of the group before further conclusions can be drawn on relationships based on nutlet epidermal characters. This is necessary to be able to readjust sectional boundaries.

10.2.2.3 What remains of the classification of Kükenthal (1935-36)?

Kükenthal (1935-36) used 12 sections to accommodate the *Cyperus* species with laterally compressed dimerous pistils without deciduous spikelets. From our nomenclatural analysis it became clear many of the subdivisional names used by Kükenthal (1935-36) are superfluous as they hold types of older names from the older but less elaborate classifications of Clarke (1908) and Chermezon (1937). In addition the nutlet epidermis does not seem to be reliable for subdivisional taxonomy in *Pycreus* for which sectional delimitations need to be redefined. The two groups *Isodiametrici* and *Zonati* therefore can no longer be upheld; neither can the unpublished group 'Mixtae' of Mariën (1969).

Cyperus pars *Isodiametrici* – Isodiametric nutlet epidermal cells represent the plesiomorphic condition in *Cyperus*, the noncore sections of *Pycreus* as well as several species within the core clade. It is therefore not useful to maintain a group based on this character in future classifications.

Noncore sections:

Cyperus sect. *Rhizomatosi* – This section holds two endemic species from coastal sand dunes of Madagascar. The species are characterised by multinerved glumes with a short mucro which are plesiomorphic characters in C_4 *Cyperus*. The phylogenetic position of

the section is unresolved among the noncore sections of *Pycreus*. *C. rhizomatosus* has peculiar silica bodies on its nutlets. As Raynal (1977a) already pointed out, this is most likely a natural group.

Cyperus sect. *Pumili* – In our opinion, this section contains five small annual species and has been characterised by Kükenthal (1935-36) mainly on the presence of truncate glumes with a well-developed mucro. However, this character is considered to be the plesiomorphic condition in the genus since many taxa nested in the C$_4$ *Cyperus* polytomy share this character along with multinerved glume wings which are still present in several species of the section *Pumili* (*P. compressiformis and P. nervulosus*). Also, this section comprises species restricted to lowland conditions. Not surprisingly it clusters along the noncore sections of *Pycreus*. Currently only *P. pumilus* was included in the molecular phylogenetic analysis. Although many plesiomorphic characters are present, based on the annual growth form and very small nutlets and glumes, we consider this as a natural group. The sections shows strong allopatric speciation around the Indian Ocean. Most species have narrow distributions while only *P. pumilus* is pantropical. The four species Govindaralaju (1991) described for India all seem to belong to the strongly related *P. pumilus* and *P. nervulosus*.

Cyperus sect. *Albomarginati* – Kükenthal (1935-36) included three species in this section based on the very large glumes with a wide hyaline edge. *Cyperus macrostachyos* and *C.tremulus* are now considered as subspecies of the same species. In our molecular study, *C. longistolon* is strongly supported in this section which is itself one of the noncore lineages of *Pycreus*. Kükenthal (l.c.), previously classified the latter species in his section *Lancei*. Both *C. longistolon* and *C. puncticulatus* have glumes with a long straight mucro, which is a plesiomorphic state in C$_4$ *Cyperus*, also the preference for lowland habitats of all species is typical for the noncore sections of *Pycreus*. The overall large dimensions of the plants, glumes and nutlets form a character of all species, which seem to form a natural group.

Cyperus sect. *Polystachyi* (= *C*. sect. *Pycreus*) – This section clearly forms a natural group as several synapomorphies are shared by most of the species such as elongated nutlets and narrow spikelets with a winged rachilla. *Cyperus pelophilus* forms an atypical species as it has broad nutlets with only silica bodies in the central cells. However,

our molecular results confirm Kükenthal's (1935-36) inclusion of this species in the section. Only *C. niger* is transferred to *C.* sect. *Globosi*. Most species of the section are lowland taxa except for a few species that are specialized in high altitude habitats. Our molecular analysis shows well resolved species level relationships within this section. *Cyperus aterrimus* (a species with a trimerous nutlet) also clusters in this clade, which needs further investigation.

Core clade sections :

Cyperus sect. *Globosi* – Kükenthal (l.c.) based this section on species with narrow spikelets with more or less spaced flowers and brown to reddish-brown glumes. This section lacks clear apomorphies. Additionally, glume tips seem to be rounded with a narrow hyaline border and inflorescences appear pseudolateral in the species originally placed in this section. However, the three species originally included by Kükenthal (*C. flavidus, C. capillifolius* and *C. melas*) do not cluster together in our molecular phylogenetic analysis and, in addition, they all show different silica body types for which they not seem to be directly related. However, *C. flavidus* (the type species of the section) clusters together with *C. niger* and *C. juncelliformis*, which is consistent in the silica body types. *C. capillifolius* is strongly related to *C. reductus* (which only differs in the shape of its nutlet). The position of *C. melas* is unclear, its peculiar silica body type strongly resembles the one we observed in *C. betschuanus*, which might indicate a close relationship. More recently, *C. micromelas* was described, which resembles *C. melas* but has much smaller glumes and nutlets and another type of silica bodies. It does not seem to be related to any of the species mentioned above. In conclusion, characters Kükenthal (l.c.) used for delimitation of this section seem to be based on convergent morphologies from different lineages of annual species with reduced habit and inflorescences. Further investigation is needed to be able to decide whether additional (sub) sections should be created for the accommodation of the different small clusters of annual species with derived morphologies.

Cyperus sect. *Sulcati* – Although relationships within this section are currently unresolved, the sulcate glumes and decumbent culms with often strongly spaced leaves form clear synapomorphies for the species in this section. *Cyperus atronervatus* from Ethiopia bears multiple nerved glumes (a assumed plesiomorphic state among pycreoid species). The South African *C. oakfortensis* is very resembling this latter species

(except for the multinerved glumes) and clearly possesses the sectional characters. Therefore, it should be transferred from sect. *Lancei* where it was originally placed by Kükenthal (1935-36). As all species of the section show isodiametric cells, this section might represent an early emerging lineage within the core pycreoid clade.

Cyperus sect. *Lancei* – Kükenthal (l.c.) based this group on the possession of broad spikelets with large densely imbricate glumes. Nutlet epidermal cells are clearly visible but not zonate. In our analysis, *Lancei* do not hold together as a natural group. Several species need to be transferred to other sections as *C. longistolon* (*Albomarginati*) and *C. oakfortensis* (*Sulcati*). The yellow-glumed species (*C. unioloides* and *C. chrysanthoides*) are probably more related to the *Propinqui* and *Flavescentes*. The group of *C. nigricans* most likely is related to the Latispicati and Muricati, however this needs further investigation since DNA extraction from material of these three sections did not work well on herbarium specimens. Species of these groups are linked to Mediterranean regions (India-South China, Afromontane or South Africa). If relationships are confirmed the sectional name *Colorati* would be the correct name to be used.

Cyperus sect. *Propinqui* – This section seems to be rather heterogeneous as Kükenthal (l.c.) used it to accommodate species with moderate glume length and an overall rather pale glume color. Several morphological groups can be recognised that correspond to different clades. *C. lanceolatus* (type) and relatives such as *C. alleizettei* are most likely linked to the *Flavescentes* (see discussion there). *Cyperus smithianus* is not surprisingly strongly related to *C. cataractarum*, both have white glumes and are linked to fast running rivers, Kükenthal (l.c.) placed both species in different sections sice the nutlet epidermal cells in *C. cataractarum* seem to be more elongated. Both are supported together with the species with fibrous culms bases (*C. fibrillosus, C. scaettae*) and need to be accommodated together in a new group. Finally, there are a few species that do not resemble either of both previous groups and need to be placed elsewhere (e.g. C*. juncelliformis* needs to be transferred to *Globosi*).

Cyperus sect. *Fontinales* – Kükenthal (l.c.) created this section to accommodate *C. fontinalis,* a species with a very peculiar morphology and ecology (hot water springs). It was first only known from Madagascar but later also described from southern Congo (as *P.*
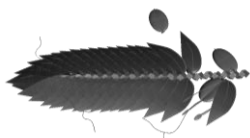
sanguineosquamatus). Relationships of this species with other pycreoid species are still unclear.

Cyperus pars *Zonati* – From our molecular phylogenetic analysis, combined with a detailed SEM study of nutlet epidermis variation in *Pycreus* we can conclude zonate cells probably originated several times within the core clade of *Pycreus*. In addition, many species show intermediate elongations of the nutlet epidermal cells. Subsequently, it is difficult to sharply delimitate a zonate type. Therefore nutlet epidermal cells are insufficient for the delimitation of sections.

Cyperus sect. *Flavescentes* (= *C.* sect. *Zonati*) – This section was delimited with the combination of zonate cells and small glumes. The section seems to be quite homogeneous in growth form, glume color (yellowish to reddish tinges) and nutlet shape. Several species are difficult to distinguish as many transitional morphologies are present. Therefore, this section probably forms a natural but only recently diverging group. The ETS1f sequences of *C. flavescens* and *C. rehmannianus* for example only differ in a single nucleotide pair. Aside of the elongated cells there seem to be no other morphological similarities between the *Flavescentes* and the two other sections with zonate cells (*Muricati* and *Latespicati*). However, our molecular phylogenetic hypothesis suggests (although with low support) a possible link with *C. lanceolatus* (*Propinqui*) and relatives. Morphologically there are several similarities between both sections and also with species such as *C. unioloides* and *C. chrysanthoides*. In our opinion, these species should be accommodated in a single (sub)section. During the 20th century Hooper (e.g. 1972) described many species which form morphological bridges between these groups of species such as *C. mortonii, C. diaphanus, C. overlaetii.* Most likely, these species form a grade at the base of the *Flavescentes* with an evolutionary series in the reduction of the inflorescence and glume sizes linked with an evolution from a perennial growth form with stolons to annual species. In addition, there seems to be a series of increasing elongation of the nutlet epidermal cells. As there was a general agreement on granting a high taxonomic value to the shapes of nutlet epidermal cells in *Pycreus* many species and subtaxa have been delimitated based on small variations in nutlet epidermal cells. As this is considered to form a taxonomical difficult group, species

boundaries should be reinvestigated with a lower valuation of the nutlet epidermal characters.

Cyperus sect. *Latispicati* – Relationships between species originally classified in this section are currently still unclear. For most species, it was not possible to extract good DNA from herbarium specimens. Most likely, this section will not hold as a monophyletic group since it currently contains many different species with broad spikelets.

Cyperus sect. *Muricati* – Nutlet epidermal cells seem to be extremely elongated in *C. pauper*, *C. muricatus* and *C. zonatissimus* and subsequently, they most likely form a natural group. Kükenthal (l.c.) also included *C. divulsus*, which also has knobby nutlets. However, this is not caused by strong elongation of the cells. Chermezon (1934) already created a separate section for the latter species (C. sect. *Tuberculati*), which we reestablished, adding a second species.

10.3 Future prospects

10.3.1 Macro-scale: Towards an integrated classification of Cyperoideae and Cypereae

During the last decades research on Cyperaceae and its different tribes has been in an acceleration. Some of the most remarkable findings have been made in the Cypereae, the crown tribe in sedge evolution, which holds both some of the most recognizable and some of the most enigmatic species of the family.

Cyperus is not the only paraphyletic taxonomic entity that came to light during the family-wide molecular studies. Several tribes such as Schoeneae and Fuireneae form grades around more specialized tribes of the classification of Goetghebeur (1998). Currently, Cypereae are circumscribed by their *Cyperus* type (and *Ficinia* type)embryo as single delimiting character. Also other tribes are mainly based on the embryo types… and several of these types represent plesiomorphic states. However, as different branches in the backbone phylogeny contain only a single genus, there has been a strong hesitation for creating additional tribes in order to fit everything into a formal cladistic classification. Instead, there in a general tendency to maintain the taxa from the tribal and generic classifications of Goetghebeur (1998) for pragmatic reasons and to opt for a clade based non formal classification (e.g. Schoeneae 5, Fuireneae 3, …).

For the *Cyperus* clade, we opted to follow this strategy for a long period. However, with more profound study of evolutionary processes (through ontogeny, anatomy, molecular

phylogenetics, …), we made the decision to evaluate the different classification options more profoundly through a reevaluation of the taxonomic value of morphological characters that have been used for delimitating taxa in Cyperoideae. From the elaborate ontogenetic studies of flowers and spikelets (e.g. Vrijdaghs et al., 2011; Reynders & Vrijdaghs et al., 2012) in the subfamily, we learned that an important increase of flexibility of primordia opened various possibilities for diversification and adaptations within Cyperoideae and this has inevitably also lead to high levels of homoplasy. In addition, the high levels of paraphyly and revelation of the true affinities of many enigmatic taxa (e.g. *Hellmuthia*, …) has led to a fading of generic circumscriptions (e.g. see Muasya et al. 2009b: 'What's a genus in Cypereae'). We can say the same about tribes: What's a tribe in Cyperoideae? Therefore, also on tribal level the need arises to reevaluate the taxonomical and also the evolutionary value of the characters linked to the different nodes of the Cyperaceae backbone phylogenetic hypothesis. Subsequently, we can select the most appropriate classification strategy for each group of taxa.

On the level of Cypereae, a similar story can be told about *Ficinia* and *Isolepis* which are currently kept under separate generic names. A profound analysis of the taxonomic and evolutionary value of characters might help to decide on the most appropriate classification strategy once a more robust phylogeny is available for *Ficinia*.

10.3.2 Meso-scale: The C$_4$ *Cyperus* radiation: what next?

10.3.2.1 Molecular Phylogenetic analysis

While for *Cyperus* subgenus *Anosporum,* we have a good outline of feasibility and choice of molecular markers to fill the gaps in the current knowledge, for *Cyperus* subgenus *Cyperus* there is still a long way to go. A better resolution of the large polytomy is necessary to be able to produce a full modern revision of *Cyperus*. Therefore, screening for additional markers could be performed. However, the probability of solving the hard polytomy may be rather limited even when using fast mutating markers. For example the ETS1f region shows a large amount of variation while clades remained poorly resolved. As the C$_4$ radiation probably happened very quickly, the mutations that occurred during that period are few with respect to the complete sequence. Subsequently statistical support is very low on the level of the radiation and phenomena such as long-branch attraction might influence the topology and support phylogenetic reconstruction. Additional data mining of the existing sequences with

cleaning of sequences, the use of other alignment criteria or methods of analysis might also improve the support of several relationships.

For several parts of C$_4$ *Cyperus,* we expect possibilities for further investigation based on the current combination of markers:

The early emerging branches could be resolved more profoundly by using the methodology of Bauters *et al.* (submitted), used to resolve relationships within the *Lipocarpha* subclade. This involves species of the sections *Amabiles, Monocephali (= Rupestres), Arenarii, Aristati, Minuti* and *Pygmaei.* Also, species originally classified by Kükenthal (1935-36) in the sections *Elegantes* (*Glutinosi*) and '*Dichostylis*', which appear to contain a mix of species with C$_3$ and C$_4$ photosynthesis are possibly linked to the early emerging clades. Next, of *Alinula* only one species has been included so far (*A. paradoxa*). Several authors already suggested the other three species represent a different taxon than *A. paradoxa* (e.g. Haines & Lye, 1983), a relation with the *Lipocarpha* clade might be possible for these tree species. Finally, Goetghebeur (1986) suggested a possible link between *Queenslandiella hyalina* and the rare African species *Cyperus soyauxii,* which is worth investigation.

Several sections seem to cluster consistently together with reasonable resolution of species level relationships. For example, the sections *Papyri, Dives, Exaltati* and *Rotundi.* These sections consist mostly of tall to very tall species which all share a rachilla with deciduous wings. This gives morphological support to their relationships. Several of the species have economical/cultural importance or are notorious weeds for which there have already been profound molecular studies such as AFLP (usually below species level). A more elaborate phylogenetic analysis with a focus on a modern revision of these sections could be performed by one or two MSc Students.

10.3.2.2 Morphology/ontogeny (spikelets)

Deciduous spikelets, which occur multiple times across both C$_3$ and C$_4$ groups of *Cyperus* form an intriguing phenomenon. Although, their (higher level) taxonomical value seems to be rather limited in Cypereae, deciduous spikelets seem to be of considerable adaptive value as they play a role in more specialised seed dispersal (certainly when other adaptations such as wings or corky thickened zone are added). In Cariceae, they might even be interpreted as a key innovative structure (utriculi), which has led to the enormous success of the tribe. In the *Cyperus* clade, several other adaptations (such as reduction of the amount

maturing fruits to one/spikelet and contraction of the complete inflorescences into a headlike inflorescence) seem often to be linked to species with deciduous spikelets.

With a combination of ontogenetic and anatomical techniques focusing on processes around the abscission zones, origins of deciduous spikelets could be better understood. Also, the link with the epicaulescent growth of the glume wings onto the rachilla (which is especially well represented in the *Cyperus* clade) would be an interesting research topic.

10.3.3 Micro-scale: Research strategy for a revision of *Pycreus*

Since phylogenetic relationships in *Pycreus* are still largely unresolved, it is a this stage not possible to produce a modern infrageneric revision for the pycreoid species of *Cyperus*. Current subdivisions are mostly based on patterns in nutlet epidermal cells in combination with length and color of glumes and shapes of the spikelets. However, we showed several species groups to be very heterogeneous for these characters. Subsequently, it is not possible to interpret these correctly without a solid molecular phylogenetic background.

Within several subclades, it was possible to resolve species level relationships in the current molecular analysis. For these, it seems realistic to perform more detailed analysis with more species and more samples for each species involved, using the existing markers. The following groups could be studied as two separate Ms thesis projects:

1. *C.* sect. *Pycreus* (*C.* sect. *Polystachyi* nom. illeg.) : Although the position of this group is still unclear among the noncore pycreoid lineages, species relationships are very well supported in our previous analysis, which includes 5 species originally placed by Kükenthal (1935-36) in this section. The group is also morphologically distinct by its elongated nutlets and clearly winged rachilla. In our molecular analysis *Cyperus aterrimus* seems to be nested within this group. However, this needs further investigation. *C. polystachyos* (which is also the type species of *Pycreus*) is a pantropical species with many local variations. Kükenthal (1935-36) has sunken many taxa into *C. polystachyos*. However, several of these (e.g. *C. holosericeus*, *C. thouarsii*, …) have distinct characters and ecologies. This hidden diversity needs to be reinvestigated in a molecular phylogenetic analysis. It should be possible to reinvestigate this group in more detail in a molecular phylogenetic analysis. DNA extraction from herbarium specimens works reasonably well for the species of this

group, except for *C. aethiops*. The existing DNA markers can be used for the molecular analysis.

2. *Cyperus fibrillosus* and relatives: In our molecular phylogenetic analysis, *C. fibrillosus, C. gracillimus, C. smithianus* and *C. cataractarum* form a well-supported subclade within the core *Pycreus* clade. Kükenthal (1935-36) classified these species among the sections *Lancei, Propinqui* and *Flavescentes,* based on differences in glume size and color and nutlet epidermal cell type. As the type species of these three sections are not related to this clade, a new subdivion might be needed for the subclade of *C. fibrillosus*. We also included one specimen from Ghana in our molecular study (Jongkind 2424), which was misidentified as *P. pseudodiaphanus* in the GENT herbarium. It most likely represents a new species. We did not publish it yet since it consists of only a single and immature specimen. Most likely also other species such as *C. scaettae, C. permutatus* and *C. diloloensis* belong to this group. However, all attempts to isolate high quality DNA from herbarium material of these species failed so far. Freshly collected or silica gel dried samples might yield better results. Species are all African and most of the species missing in the current molecular analysis can be found in the Zambesian part of Congo (Katanga/ Shaba). In addition, several species with fibrous culm bases such as *C. permutatus* and *C. smithianus* (including *C. fluminalis*) are very morphologically diverse, and currently have unclear species limits. These species are still poorly known and often misinterpreted as we discussed in chapter 8.3. Additional material from throughout the Soudano-Zambesian region should be investigated in regard to this species group as there could be some hidden diversity and poorly known taxa (e.g. *C. segmentatus*).

As already addressed under C$_4$ *Cyperus*, further search of new molecular markers and optimization of the analysis methods are needed to be able to obtain a better resolved phylogeny for the pycreoid species of *Cyperus*. We suggest to study a select group of species that are nested within the large polytomy in order to test a larger range of markers. For some sections the currently used markers yield enough support in our molecular study. Of these sections, more profound analysis can be performed and revisions can be made.

10.3.4 Patterns in ecology and speciation

Giant genera such as *Cyperus* form a living lab for the study of evolutionary mechanisms and patterns such as "niche conservatism" (see e.g. Wiens *et al*. 2010). Several studies already showed in Angiosperms, niches are generally strongly conserved within certain lineages. Even with the poorly resolved molecular phylogenies, numerous examples can be given of niche-conservatism among related species in *Cyperus* (see table 10.5).

In the past these types of characters have often been considered as taxonomically less relevant since they evolve in parallel in unrelated lineages. However, within lineages of related species they might be of higher taxonomical importance. A more detailed study of characters linked to niche preferences (e.g. growth form and ecological strategy) might add additional relevant data to phylogenetic and taxonomic studies on *Cyperus* s.l.

Table 10.5 Examples of niche conservatism among strongly related species of *Cyperus* (*Pycreus).*

	Species cluster	Ecology	Distribution
1	*Cyperus divulsus*	Wet pastures on medium altitudes	Madagascar
	Cyperus africanus	Wet pastures on medium altitudes	Africa
2	*Cyperus macrostachyos*	Lowland pioneer	Africa & New world
	Cyperus puncticulatus	Lowland pioneer	India to Australia
3	*Cyperus rhizomatosus*	Coastal sand dunes	SE Madagascar
	Cyperus pervillei	Coastal sand dunes	NW Madagascar
4	*Cyperus cataractarum*	Edges of fast running streams	W Central Africa
	Cyperus smithianus	Edges of fast running streams	E Central Africa
	Cyperus fluminalis	Edges of fast running streams	East Africa to Madagascar

10.4 Conclusions

We recognize two subgenera in *Cyperus*: *C.* subg. *Anosporum*, comprising the species using C_3 photosynthesis and *C.* subg. *Cyperus* comprising the species with C_4 photosynthesis. The first subgenus is paraphyletically circumscribed while the latter represents a strongly diversified monophyletic clade. Unfortunately molecular phylogenetic relationships remain poorly resolved for large parts of *C.* subgenus *Cyperus*. The segregate genus *Pycreus*, along with 11 other segregate genera, is now included in *Cyperus*. *Pycreus* species have been recognised by their remarkable laterally compressed dimerous pistils. With investigations of ontogeny and anatomy we showed that *Cyperus* and *Pycreus* follow the general developmental patterns of flowers and spikelets found throughout Cyperoideae. The presence of an annular gynoecial wall primordium allowed for the development of stigma primordia in novel positions in Cyperoideae, formation of vascular bundles is adaptive and follows the development of the primordia. In addition our molecular phylogenetic research shows multiple origins for laterally compressed pistils in *Cyperus* and does not support *Pycreus* as a monophyletic group.

On the subdivisional levels of *Pycreus,* relationships remain poorly resolved. Several sections remain in the main C_4 *Cyperus* polytomy while the more derived species form a well-supported clade, which is sister to *Cyperus laevigatus*. Also, within the core clade relationships are poorly resolved. From a SEM study of nutlet epidermis we conclude that characters such as cell shapes and silica bodies and their different character states are not useful for subdivisional classification of pycrioid species. A better resolved molecular phylogenetic hypothesis is needed to draw further conclusions on the correlations of nutlet epidermal character states with certain clades.

A long term goal is to establish a modern revision of *Pycreus* with application of the joint classification strategy obtained from the meso-scale collaboration on the *Cyperus* clade. However, this requires better understanding of the relationships between the different species. As a contribution, several smaller case studies are presented: Section *Tuberulati* Chermezon is reestablished is this study and relevant new names and combinations of *Pycreus* species into *Cyperus* are presented with discussion of several critical synonymies.

11 References

*"N*ature is earlier than man, but man is earlier than natural science.*"*

— Carl Friedrich von Weizsäcker (1912-2007)

← Fig. 11.1 *Cyperus micrantherus* endemic species in the Andringitra National Park, Madagascar. Picture taken by M. Reynders.

Spikelet theme: *P. nitidus*

Aloni R. 2004. The induction of vascular tissue by auxin. In: Davies P.J., ed. *Plant Hormones: Biosynthesis, Signal Transduction, Action*! Dordrecht: Kluwer Academic Publishers, 471–492.

Angiosperm Phylogeny Group 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III, *Botanical Journal of the Linnean Society* 161 (2): 105–121, doi:10.1111/j.1095-8339.2009.00996.x

Arber A. 1937. The interpretation of the flower: a study of some aspects of morphological thought. Biological Reviews 12: 157– 184. http://dx.doi.org/10.1111/j.1469-185X.1937.tb01227.x

Ariati S.R., Murphy D.J., Udovicic F. & Ladiges P.Y. 2006. Molecular phylogeny of three groups of acacias (*Acacia* subgenus *Phyllodineae*) in arid Australia based on the internal and external transcribed spacer regions of nrDNA. *Syst. Biodivers.* 4: 417–426.

Baas J.H. & Handerson H.E. 1889. *Outlines of the History of Medicine and the Medical Profession. J.H. Vail, New York.*

Baillon H. 1893. Notes organogéniques et organographiques sur les *Carex. Bulletin de la Société Linnéenne de Paris* 2: 1097–1101.

Baillon H. 1894. Cyperaceae. *Histoire des plantes 12 (Cyperaceae).* Paris: Hachette, 335-382

Barnard C. 1957. Floral histogenesis in the Monocotyledons 2. The Cyperaceae. *Australian Journal of Botany* 5: 115–128. http://dx.doi.org/10.1071/BT9570115

Bauters K. 2011. Phylogeny, ontogeny and taxonomy of *Ascolepis, Lipocarpha* & *Volkiella*. MSc. Thesis. Ghent University, Belgium.

Bauters K., Reynders M., Larridon I., Huygh W. & Goetghebeur P. 2010. Two New Species of *Cyperus* (Cyperaceae) from the Zambezian Region of Africa. *Novon* 20: 133-138.

Bauters K., Larridon I., Reynders M., Huygh W., Asselman P., Vrijdaghs A., Muasya A.M., Goetghebeur P. (submitted) Infrageneric classification of *Lipocarpha* (Cypereae, Cyperoideae, Cyperaceae): based on morphological, developmental and molecular evidence. *Phytotaxa.*

Beauvois P. (de) 1816. *Flore d'Oware et de Bénin, en Afrique*, vol. 2, part 15. Paris: de l'imprimerie de Fain.

Beerling D.J. & Osborne C.P. 2006. The origin of the savanna biome. *Global Change Biology* 12: 2023-2031.

Bentham G. 1878. *Flora australiensis*, vol. 7. London: Reeve.

Bentham G. 1881. Notes on *Cyperaceae*. *J. Linn. Soc., Bot.* 18: 281–360.

Bentham G. 1883. Cyperaceae. In: Bentham G., Hooker J., eds. *Genera plantarum* 3. London: Reeve, 1037-1073.

Berry P.E., Hipp A.L., Wurdack K.J., Van Ee B. & Riina R. 2005. Molecular phylogenetics of the giant genus *Croton* and tribe Crotoneae (Euphorbiaceae *sensu stricto*) using ITS and *trnL-trnF* DNA sequence data. *American Journal of Botany* 92: 1520-1534.

Besnard G., Muasya A.M., Russier F., Roalson E.H., Salamin N. & Christin P.A. 2009. Phylogenomics of C_4 Photosynthesis in Sedges (Cyperaceae): Multiple Appearances and Genetic Convergence. Mol Biol Evol 26: 1909–1919. http://dx.doi.org/10.1093/molbev/msp103

Blaser H.W. 1941a. Studies in the morphology of the Cyperaceae 1. Morphology of the flowers A. Scirpoid genera. *American Journal of Botany* 28: 542–551.

Blaser H.W. 1941b. Studies in the morphology of the Cyperaceae. 1. Morphology of flowers B. Rhynchosporoid genera. *American Journal of Botany* 28: 832–838.

Boar R.R., Harper D.M. & Christopher S.A. 1999. Biomass Allocation in *Cyperus papyrus* in a Tropical Wetland, Lake Naivasha, Kenya. *Biotropica* 31: 411–421.

Boeckeler O. 1868–77. Die Cyperaceen des Königlichen Herbariums zu Berlin. *Linnaea* 35: 397–612, 36: 271–512, 691–768, 37: 1–142,520–647, 38: 223–544, 39: 1–152, 40: 327–452, 41: 145–356.

Boeckeler O. 1870. Die Cyperaceen des Königlichen Herbariums zu Berlin. Die Cypereen, Scirpeen und Hypolytreen. *Linnaea* 36: 385–512.

Boeckeler O. 1884. Neue Cyperaceen. Bot. Jahrb. Syst. 5: 497--521.

Bouman F. 1984. The ovule. In: Johri B.M., ed. *Embryology of Angiosperms*: 123–157. Berlin, Springer.

Britton N.L. 1907. The sedges of Jamaica (*Cyperaceae*). *Bull. Dept. Agric. Jamaica* 5, Suppl. 1: 1–19.

Brongniart, A.T. (de) 1834. *Voyage Autour de Monde, Phanerogamie*. Paris.

Bruhl J.J. 1991. Comparative development of some taxonomically critical floral/inflorescence features in Cyperaceae. *Australian Journal of Botany* 39: 119–127.

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

Bruhl J.J. & Perry S. 1995. Photosynthetic pathway related ultrastructure of C_3, C_4 and C_3-like and C_3–C_4 intermediate sedges (Cyperaceae), with special reference to Eleocharis. *Australian Journal of Plant Physiology* 22: 521--530.

Bruhl J.J. & Wilson K.A. 2007. Towards a comprehensive survey of C_3 and C_4 photosynthetic pathways in Cyperaceae. In: *Monocots III/Grasses IV. Aliso 23.* Claremont, CA: Rancho Santa Ana Botanic Garden, 99-148.

Brummitt R.K. 1996. In defence of paraphyletic taxa. In: van der Maesen L.J.G., van der Burgt X.M., van Medenbach de Rooy J.M., eds. *The biodiversityof African plants, Proceedings XIVth AETFAT Congress, 22-27 August 1994, Wageningen, The Netherlands*. Dordrecht: Kluwer, 371-384.

Brummitt R. K. 2006. Am I a bony fish? *Taxon* 55: 268–269.

Brummitt R.K. & Sosef M.S.M. 1998. Paraphyletic taxa are inherent in Linnaean classification - a reply to Freudenstein. *Taxon* 47: 411-412.

Castresana J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17: 540-552.

Celakovsky L. 1887. Ueber die ährchenachtige Partial-inflorescenzen der Rhynchosporeen. *Berichte der Deutschen Botanischen Gesellschaft* 5(1): 148–152.

Char M.B.S., Cheluviah M.C., Ganesh O. (1973) Pseudomonad pollen development in Styphelieae (Epacridaceae) and Cyperaceae, an interpretation. Journal of Palynology 9: 185–191.

Chase M. W. & H. H. Hillis 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.

Chase M.W., Soltis DE., Olmstead RG., Morgan D., Les DH., Mishler BD., Duvall MR., Price RA., Hills HG., Qiu Y-L, Kron KA., Rettig JH., Conti E., Palmer JD., Manhart JR., Sytsma KJ., Michaels HJ., Kress WJ., Karol KG., Clark

WD., Hedren M., Gaut BS., Jansen RK., Kim K-J., Wimpee CF., Smith JF., Furnier GR., Strauss SH., Xiang Q-Y., Plunkett GM., Soltis PS., Swensen SM., Williams SE., Gadek PA., Quinn CJ., Eguiarte LE., Golenberg E., Learn GH., Graham SW., Barrett SCH., Dayanandan S. & Victor A. Albert VA. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. *Annals of the Missouri Botanical Garden* 80: 528–580.

Chermezon H. 1919a. Révision des Cypéracées de Madagascar. 1. *Ann. Mus. Colon. Marseille* ser.3, 7 : 29--87.

Chermezon H. 1919b. Un genre nouveau des Cyperacées. *Bulletin du Musée National d'Histoire Naturelle (Paris)* 25: 60-63.

Chermezon H. 1920. *Cyperus* nouveaux de Madagascar. *Bulletin de la Société Botanique de France* 66: 338- 353.

Chermezon H. 1922. Révision des Cypéracées de Madagascar. *Ann.Mus. Colon. Marseille*, ser. 3, 10(1): 1–62.

Chermezon H. 1931. Les Cypéracées de Haut-Oubangui. *Arch. Bot.Mem.* 4, Mém. 7: 1–56.

Chermezon H. 1932. Sur quelques Cypéracées du Congo belge. Rev. Zool. Bot. Afr. 22(1) : 63-71.

Chermezon H. 1933. Cypéracées nouvelles du Gabon. II. Bull. Soc. Bot. France 80 : 506-509.

Chermezon H. 1934. Cypéracées nouvelles du Congo belge.   Rev. Zool. Bot. Afr. 24(3) : 294-299 ('1933').

Chermezon H. 1935a. Cypéracées nouvelles du Congo belge. 1. Bull. Jard. Bot. Etat Brux. 13(4) : 277-283.

Chermezon H. 1935b. Cypéracées récoltées par M. Humbert au Congo belge oriental. Bull. Soc. Bot. France 82(5-6): 334-346.

Chermezon H. 1937. Cypéracées. Pp. 1–335 in: Humbert, H. (ed.), *Flore de Madagascar*, vol. 29. Tananarive: Imprimerie officièle.

Chiovenda 1914. Le piante raccolte dal Dott. Bovone nel Catanga. *Annali di Botanica* (Rome) 13: 58.

Christin P.A., Salamin N., Muasya A.M., Roalson E.H., Russier F. & Besnard G. 2008. Evolutionary switch and genetic convergence on *rbcL* following the evolution of C_4 photosynthesis. *Molecular Biology and Evolution* 25 : 2361–2368.

Clarke C.B. 1883. *Cyperus.* Pp. 279–296 in: Baker J.G., ed. Contributions to the flora of Madagascar. — Part 3. *J. Linn. Soc., Bot.* 20: 237–304.

Clarke C.B. 1884. On the Indian species of *Cyperus*; with remarks on some others that specially well illustrate the subdivisions of the genus. *J. Linn. Soc., Bot.* 21: 1–202, pl. 1–4.

Clarke C.B. 1893. *Cyperaceae*. Pp. 585–672 in: Hooker, J.D. (ed.), *Flora of British India*, vol. 6, part 19. London: Reeve.

Clarke C.B. 1894. Cyperaceae. In Durand T. & Schinz H., eds. *Conspectus Florae Africae* 5: 526--692. Jardin Botanique de l'Etat, Bruxelles.

Clarke C.B. 1897. *Cyperaceae*. Pp. 149–192 in: Thiselton-Dyer W.T., ed. *Flora capensis*, vol. 7. London: Reeve.

Clarke C.B. 1900. *Cyperaceae*. Pp. 8–169 in: Urban I., ed. *Symbolae antillanae*, vol. 2, part 1. Leipzig: Fratres Borntraeger.

Clarke C.B. 1901–1902. *Cyperaceae*. Pp. 266–524 in: Thiselton-Dyer W.T., ed. *Flora of Tropical Africa*, vol. 8. Ashford: Reeve.

Clarke C.B. 1905. Cyperaceae. In: Pilger R., ed. *Beiträge zur Flora der Hylaea nach der Sammlungen von E. Ule. Verhandlungen des Botanischen Vereins der Provinz Brandenburg und die angrenzenden Lander* 47: 101.

Clarke CB. 1908. New genera and species of Cyperaceae. *Kew Bulletin of Miscellaneous Information, Additional Series* 8: 1–196.

Coan I., Alves M.V. & Scatena V.L. 2008. Comparative study of ovule and fruit development in species of *Hypolytrum* and *Rhynchospora* (Cyperaceae, Poales). *Plant Systematics and Evolution*. 272: 181–195. http://dx.doi.org/10.1007/s00606-007-0636-9

Coen E.S. & Meyerowitz E.M. 1991. The war of the whorls: genetic interactions controlling flower development. *Nature* 353: 31–37. http://dx.doi.org/10.1038/353031a0

Colla 1836. pub. Herb. Pedem. 5: 532.

Corcoran M.L. 1941. A revision of the subgenus *Pycreus* in North and South America. *Biol. Ser. Catholic Univ. Amer.* 37: 1–68.

Couvreur T.L.P., Forest F., & Baker W.J. 2011. Origin and global diversification patterns of tropical rain forests: inferences from a complete genus-level phylogeny of palms. *BMC Biology* 2011, 9:44. doi:10.1186/1741-7007-9-44

Dai L.K. 1961. *Pycreus.* Pp. 162–173 in: Tang T. & Wang F.T., eds., *Flora Reipublicae Popularis Sinicae*, vol. 11. Beijing: Science Press.

de Candolle A.P. 1827. *Organographie Végétale*. Paris, Deterville.

Denton M.F. 1978. A Taxonomic treatment of the *Luzulae* group of *Cyperus*. *Contributions from the University of Michigan Herbarium* 11(4): 197–271.

Dhooge, S., Goetghebeur P. & Muasya A.M. 2003. *Zameioscirpus*, a new genus of Cyperaceae from South Amerca. *Plant Syst. Evol*. 2431–2: 73–84.

Dickison W.C. 2000. *Integrative plant anatomy*. San Diego, Academic Press.

Dodoens R. 1554. *Cruydeboeck.* Plantijn, Antwerp.

Druyts-Voets E. 1970. Types van stengel- en bladstrukturen in het genus *Cyperus* L. *Natuurw. Tijdschr., Antwerp.* 52: 28–49.

Durand T. & Schinz H. 1894. *Conspectus Florae Africae 5:* 526 – 692. Jardin Botanique de l'Etat, Bruxelles.

Edwards G.E. & Voznesenskya E.V. 2011. C_4 photosynthesis: Kranz forms and single-cell C_4 in terrestrial plants. In: Raghavendra A.S. & Sage R.F., eds. *C_4 photosynthesis and related CO_2 concentrating mechanisms.* Dordrecht, The Netherlands: Springer; 2011. p. 29-61.

Eiten L.T. 1976. Inflorescence units in the *Cyperaceae*. *Ann. Missouri Bot. Gard.* 63: 81–112.

Endlicher S.F.L. 1836. *Genera plantarum secundum ordines naturales disposita*, vol. 2. Vienna: Fr. Beck.

Endress P.K. 1994. *Diversity and evolutionary biology of tropical flowers.* Cambridge, Cambridge Tropical Biology Series, Cambridge University Press.

Endress P.K. 1995. Major evolutionary traits of monocot flowers. In: Rudall P.J., Cribb P.J., Cutler D.J., Humphries C.J., eds. *Monocotyledons: Systematics and Evolution*: 43–79. Kew, Royal Botanic Gardens.

Endress P.K. 2001. Origins of flower morphology. *Journal of Experimental Zoology*: Molecular and Developmental Evolution 291: 105–115. http://dx.doi.org/10.1002/jez.1063

Endress P.K. 2006. Angiosperm floral evolution: Morphological developmental framework. *Advances in Botanical Research* 44: 1–61. http://dx.doi.org/10.1016/S0065-2296(06)44001-5

Endress PK. 2011. Evolutinary diversification of the flowers in angiosperms. *American Journal of Botany* 98: 370-396.

Engler H.G.A., ed. 1900–1968. Das Pflanzenreich. Regni vegetabilis conspectus. Berlin, Engelmann.

Escudero M., Hipp A.L., Hansen T.F., Voje K.L. & Luceño M. 2012. Selection and inertia in the evolution of holocentric chromosomes in sedges (*Carex*, Cyperaceae). The new Phytologist 195(1):237-47. doi: 10.1111/j.1469-8137.2012.04137.x.

Farr E.R., Leussink J.A. & Stafleu F.A. 1979. *Index nominum genericorum (Plantarum)*. Regnum Vegetabile 100. Utrecht: Bohn, Scheltema & Holkema.

Farr E.R. & Zijlstra G., eds. 1996+. *Index nominum genericorum (Plantarum)*. http://botany.si.edu/ing/ (accessed 16 June 2010).

Fenzl E. 1836. Cyperaceae. In: Endlicher S., ed. *Genera plantarum* 2. Wien: Beck, 109-119.

Fisher J.B. 1971. Inverted vascular bundles in the leaf of Cladium (Cyperaceae). *Botanical Journal of the Linnean Society* 64: 277–293. http://dx.doi.org/10.1111/j.1095-8339.1971.tb02148.x

Flores Olvera H., Smets E. & Vrijdaghs A. 2008. Floral and inflorescence morphology and ontogeny in *Beta vulgaris*, with special emphasis on the ovary position. *Annals of Botany* 102: 643–651. http://dx.doi.org/10.1093/aob/mcn140

Flores-Olvera H., Vrijdaghs A., Ochoterena H. & Smets E. 2011. The need to re-investigate the nature of homoplastic characters: an ontogenetic case study of the 'bracteoles' in Atripliceae (Chenopodiaceae). *Annals of Botany* 108: 847–865. http://dx.doi.org/10.1093/aob/mcr203

Frodin D.G. 2004. "History and concepts of big plant genera". *Taxon* 53 (3): 753–776. doi:10.2307/4135449

Gaertner J. 1788. *De fructibus et seminibus plantarum*, vol. 1. Stuttgart.

Ghamkhar K., Marchant A., Wilson K.L. & Bruhl J.J. 2007. Phylogeny of Abildgaardieae (Cyperaceae) inferred from ITS and trnL-F data. *Aliso*. 23: 149–164.

Goebel K. 1888 Über den Bau der Ärchen und Blüten einiger Javanischer Cyperaceen. *Annales du Jardin Botanique de Buitenzorg* 7: 120–140.

Goetghebeur P. 1977. Studies in *Cyperaceae* 1. Taxonomic notes on *Ascolepis* and *Mariaculus*, a new genus of the tribe Cypereae. *Bull.Jard. Bot. Natl. Belg.* 47: 435–447.

Goetghebeur P. 1980. Studies in *Cyperaceae* 2. Contributions towards a revision of the mainly African genus *Ascolepis* Nees ex Steudel.*Adansonia*, ser. 2, 19: 269–305.

Goetghebeur P. 1986. *Genera Cyperacearum. Een bijdrage tot de kennis van de morfologie, systematiek en fylogenese van de Cyperaceae-genera*. PhD Thesis, Groep Plantkunde, Rijksuniversiteit Gent, Belgium

Goetghebeur P. 1989. Studies in *Cyperaceae* 9. Problems in the lectotypification and infrageneric taxonomy of *Cyperus. Bull. Soc. Roy. Bot. Belgique* 122: 103–114.

Goetghebeur P. 1998. Cyperaceae. In: Kubitzki K., ed. *The families and genera of vascular plants 4*. *Flowering plants - Monocotyledons*. Berlin: Springer-Verlag, 141–190.

Goetghebeur P. & Vorster P. 1988. Studies in Cyperaceae 7. The genus *Alinula* J.Raynal: a reappraisal. *Bulletin du Jardin Botanique National de Belgique* 58: 457-465.

Goetghebeur P. & Van den Borre A. 1989. Studies in Cyperaceae 8. A revision of *Lipocarpha*, including *Hemicarpha* and *Rikliella*. *Wageningen Agricultural University Papers* 89-1: 87 p., 42 fig.

Gonzalez A.M. & López M.C. 2010. Development and morphology of the gynoecium and nutlet in two South-American Bulbostylis (Cyperaceae) species. *Flora – Morphology, Distribution, Functional Ecology of Plants* 205: 211–220. http://dx.doi.org/10.1016/j.flora.2009.02.002

Gould S.J. 2002. *The structure of evolutionary theory*. Cambridge, Belknap Press of Harvard University Press.

Govaerts R. & Simpson D.A., with Bruhl J., Egorova T., Goetghebeur P. & Wilson K. 2007. *World Checklist of Cyperaceae. Sedges.* Kew Publishing, Royal Botanic Gardens, Kew: xiii + 765 p.

Govaerts R., Simpson D.A., Goetghebeur P., Wilson K.L., Egorov, T. & Bruhl J. 2010. *World checklist of Cyperaceae.* Kew: The Board of Trustees of the Royal Botanic Gardens, Kew. http://www.kew.org/wcsp/monocots/ (accessed 20 May 2010).

Govindarajalu E. 1978. The Systematic Anatomy of South Indian Cyperaceae *Cyperus* subgenus *Pycreus*. *Adansonia* 18, 95-128.

Govindarajalu E. 1979. Studies in Cyperaceae: XVI. Novelties in *Cyperus* Linn. and their vegetative anatomy. *Proceedings of the Indian academy of sciences* 88: 229-242.

Govindarajalu E. 1991. Cyperaceae Indiae australis precursores : new species and combinations in *Pycreus* Beauv. *Proceedings of the Indian academy of sciences* 100(6) (1990) : 423-433, 4 fig., key

Grégoire V. 1938. La morphogénèse et l'autonomie morphologique de l'appareil floral 1. Le carpelle. *La Cellule* 47: 287–452.

Grisebach A.H.R. 1846. *Spicilegium florae Rumelicae*, vol. 2. Brunswick: F. Vieweg et filium.

Grisebach A.H.R. 1864. *Flora of the British West Indian Islands*. London: Reeve.

Guarise N.J. & Vegetti A.C. 2008. The inflorescence structure of *Cyperus* L. section *Luzuloidei* Kunth. (Cyperaceae). *Plant Systematics and Evolution* 271: 41-63.

Haines R.W. 1967. Prophylls and branching in Cyperaceae. *Journal of the East African Natural History Society* XXVI (1): 51-70

Haines, R.W. & Lye, K.A. 1971. Studies in African Cyperaceae 4. Lipocarpha R.Br., Hemicarpha Nees and Isolepis R. Br. Bot. Not.124: 473–482.

Haines R.W., Lye K.A. 1983. *The sedges and rushes of East Africa*. Nairobi: East African National History Society.

Harper D. "Taxonomy". *Online Etymology Dictionary*. Retrieved April 18, 2011.

Harris E.M. 1995. Inflorescence and floral ontogeny in Asteraceae: A synthesis of historical and current concepts. *Botanical Review* 61: 93–278. http://dx.doi.org/10.1007/BF02887192

Harrison N. & Kidner C.A. 2011. Next-generation sequencing and systematics: what can a billion base pairs of DNA sequence data do for you? *Taxon* 60: 1552-1566.

Hayata B. 1921. *Iconum Plantarum Formosanarum* 10. Taiwan, Bureau of Productive Industries.

Hennig W. 1950. Grundziige einer Theorie der phylogenetischen Systematik. Deutscher Zentralverlag, Berlin.

Hillis D.M., Moritz C. & Mable B.K., eds. 1996. *Molecular Systematics*, 2nd edn, Sinauer, Sunderland, MA, pp. 407–514.

Hoenselaar K., Verdcourt B. & Beentje H.J. 2010. Cyperaceae. in Beentje H.J., ed. *Flora of Tropical East Africa.,* Kew Publishing, UK.

Holm L.G., Plucknett D.L., Pancho J.V., & Herberger J.P. 1977. *The world's worst weeds, distribution and biology*. East-West Center, University Press of Hawaii, Honolulu. 609 pp.

Holm L.G., Plucknett D.L., Pancho J.V., & Herberger J.P. 1978. *Cyperus rotundus L. Cyperaceae, sedge family*. FAO Plant Protection Bulletin 26:73-92.

Holmgren P.K., Holmgren N.H. & Barnett L.C. 1990. *Index Herbariorum. Part 1: The Herbaria of the World*. New York Botanical Garden, Bronx, NY.

Hooper S.S. 1972. New taxa, names and combinations in Cyperaceae for the "Flora of West Tropical Africa". *Kew Bull*. 26(3): 577--583.

Hooper SS. 1983. *Remirea* or *Mariscus*? - New support for a monotypic genus in Cyperaceae. *Kew Bulletin* 38: 479-480.

Hooper S.S. 1985. *Cyperaceae*. Pp. 1682–1710 in: Meikle R.D., ed., *Flora of Cyprus*, vol. 2. Kew: Bentham-Moxon Trust.

Holttum RE. 1948. The spikelet in Cyperaceae. Bot. Rev. 14(8): 525-541.

Hörandl E. 2006. Paraphyletic versus monophyletic taxa— evolutionary versus cladistic classifications. *Taxon* 55:564–570.

Hörandl E. & Stuessy T.F. 2010. "Paraphyletic groups as natural units of biological classification". *Taxon* 59 (2): 345–350.

Horn J.W., van Ee B.W., Morawetz J.J., Riina R., Steinmann V.W., Berry P.E. & Wurdack K.J. 2012. Phylogenetics and the evolution of major structural characters in the giant genus Euphorbia L. (Euphorbiaceae), *Molecular Phylogenetics and Evolution* 63(2): 305-326, ISSN 1055-7903, http://www.sciencedirect.com/science/article/pii/S1055790311005483

Huygh W., Schouppe D., Larridon I., Simpson D.A. & Goetghebeur P. *Kyllinga cataphyllata* (Cyperaceae), a new species from the highlands of West and Central Africa. *Blumea* 55 (3): 291-293.

Huygh W., Larridon I., Reynders M., Govaerts R., Simpson D.A. & Goetghebeur P. Nomenclature and typification of names of genera and subdivisions of genera in *Cypereae* (*Cyperaceae*): 1. Names of genera in the *Cyperus* clade. *Taxon* 59: 1883–1890.

Irish E.E., Szymkowiak E.J. & Garrels K. 2003. The wandering carpel mutation of *Zea mays* (Gramineae) causes misorientation and loss of zygomorphy in flowers and two-seeded kernels. *American Journal of Botany* 90: 551–560. http://dx.doi.org/10.3732/ajb.90.4.551

Integrated Taxonomic Information System (ITIS), http://www.itis.gov. Accessed 20/12/2012.

Janssen T. & Bremer K. 2004. The age of major monocot groups inferred from 800+ *rbcL* sequences. *Journal of the Linnean Society* 146(4): 385-398. DOI: 10.1111/j.1095-8339.2004.00345.x

Jaubert H.F. & Spach E. 1844. *Illustrationes plantarum orientalium*, *ou Choix de plantes nouvelles ou peu connues de l'Asie occidentale*. Paris: Roret.

Judd W.S., Campbell C.S., Kellog E.A., Stevens P.F. & Donoghue M.J. 2007. Taxonomy. In *Plant Systematics - A Phylogenetic Approach, Third Edition*. Sinauer Associates, Sunderland.

Junk W.J., Brown M., Campbell I.C., Finlayso, M., Gopal B., Ramberg L. & Warner B.G. 2006. The comparative biodiversity of seven globally important wetlands: A synthesis. *Aquatic Sci.* 68: 400–414.

Jussieu (de) A.l. 1789. *Genera Plantarum, secundum ordines naturales disposita juxta methodum in Horto Regio Parisiensi exaratam, anno MDCCLXXIV*. Paris.

Kern J.H. 1952. Notes on Malaysian *Cyperaceae*. *Reinwardtia* 2: 97–130.

Kern J.H. 1962. New look at some Cyperaceae mainly from the tropical standpoint. *Advancement of Science* 19: 141–148.

Kern J.H. 1974. Cyperaceae. Pp. 435–753 in: Van Steenis C.G.G.J., ed. *Flora Malesiana*, ser. 1, vol. 7, part 3. Leiden: National Herbarium.

Kneucker A. 1903. Allgemeine Botanische Zeitschrift F R Systematik, Floristik, Pflanzengeographie Etc Volume Jahrg. 9.

Koyama T. 1961. Classification of the family *Cyperaceae* 3. *Quart. J.Taiwan Mus.* 14: 159–194.

Koyama T. 1976 New Cyperaceae from Tropical America. *Bull. Nat. Sci. Mus. Tokyo* Ser. B (Bot.) 2(4) : 167-176, 5 fig.

Koyama T. 1982. The genus *Lipocarpha* R. Br., its morphology and systematic position in the family Cyperaceae. *Acta Phytotax. Geobot*. 33:218-226.

Koyama T. 1985. Cyperaceae. Pp. 125–405 in: Dassanayake, M.D. & Fosberg, F.R., eds. *A revised handbook to the flora of Ceylon*, vol. 5. New Delhi: Amerind Publishing.

Kress W.J. 1981. Sibling competition and evolution of pollen unit, ovule number, and pollen vector in angiosperms. *Systematic Botany* 6: 101–112. http://dx.doi.org/10.2307/2418541

Kükenthal G. 1935-1936. Cyperaceae – Scirpoideae – Cypereae. In Engler A., ed. *Das Pflanzenreich, Regni vegetabili conspectus:* IV, 20(101), 671 p. H.R. Engelmann (J. Cramer) Stuttgart.

Kükenthal G. 1944. Vorarbeiten zu einer Monographie der Rhynchosporoideae 14. *Feddes repertorium Specierum Novarum Regni Vegetabilis* 53: 187-219.

Kukkonen I. 1984. On the inflorescence structure in the family *Cyperaceae. Ann. bot. fenn*. 21: 257-264.

Kunth C.S. 1837. *Enumeratio plantarum*, vol. 2, *Cyperographia synoptica*. Stuttgart & Tübingen: sumtibus J.G. Cottae.

Lanjouw J., Baehni C.H., Merrill E.D., Rickett H.W., Robyns W., Sprague T.A. & Stafleu F.A., eds. 1952. *International code ofbotanical nomenclature: Adopted by the Seventh International Botanical Congress Stockholm, Sweden, July 1950.* Regnum Vegetabile 3.Utrecht: I.U.B.S.

Lanjouw J., Baehni C.H., Robyns W., Ross R., Rousseau J., Schoppf J.M., Schultze G.M., Smit, A.C., De Vilmorin R. & Stafleu F.A., eds. 1952. *International code of botanical nomenclature: Adopted by the Ninth International Botanical Congress Montreal, Canada, August 1959.* Regnum Vegetabile 20. Utrecht: I.U.B.S.

Larridon I. 2011. Exploring giant genera and their satellites: tales from the C$_3$ *Cyperus* universe. Ghent University. Gent.

Larridon I., Reynders M & Goethebeur P. 2008a. Novelties in *Nemum* (Cyperaceae). *Belgian Journal of Botany* 141: 157-177.

Larridon, I., Reynders M. & Goetghebeur P. 2008b. *Cyperus limiticola*, a New Name for a Madagascan *Cyperus* (Cyperaceae). *Novon* 18(2): 187--188.

Larridon I., Reynders M., Huygh W., Bauters K., Van de Putte K., Muasya A.M., Boeckx P., Simpson D.A., Vrijdaghs A., Goetghebeur P. 2011a. Affinities in C$_3$ *Cyperus* lineages (Cyperaceae) revealed using molecular phylogenetic data and carbon isotope analysis. *Botanical Journal of the Linnean Society* 167: 19–46. http://dx.doi.org/10.1111/j.1095-8339.2011.01160.x

Larridon I., Reynders M., Huygh W., Bauters K., Vrijdaghs A., Leroux O., Muasya A.M., Simpson D.A. & Goetghebeur P. 2011b. Taxonomical changes in C$_3$ *Cyperus* (Cyperaceae) supported by molecular data, morphology, embryology, ontogeny and anatomy. *Plant Ecology and Evolution* 144: 327–356. http://dx.doi.org/10.5091/plecevo.2011.653

Larridon I., Huygh W., Reynders M., Muasya A.M., Govaerts R., Simpson D.A. & Goetghebeur P. 2011c. Nomenclature and typification of names of genera and subdivisions of genera in *Cypereae*(*Cyperaceae*): 2. Names of subdivisions in *Cyperus*. *Taxon* 60: 868–884.

Larridon I., Bauters K., Huygh W., Reynders M. & Goetghebeur P. (submitted). Taxonomic changes in C$_4$ *Cyperus* (Cypereae, Cyperoideae, Cyperaceae): reducing the sedge genera *Ascolepis*, *Kyllinga* and *Pycreus* into *Cyperus* sensu lato. *Phytotaxa*.

Leebens-Mack J.H., Raubeson L.A., Cui L., Kuehl J.V., Fourcade M.H., Chumley T.W., Boore J.L., Jansen R.K. & de Pamphilis C.W. 2005. Identiying the basal angiosperm node in chloroplast genome phylogenies: sampling one's way out of the Felsenstein zone. *Molecular Biology and Evolution* 22(10):1948-1963.

Leins P. 2000. *Blüte und Frucht. Morphologie, Entwicklungsgeschichte, Phylogenie, Funktion, Ökologie*. Stuttgart, SchweizerbartScience Publishers.

Leins P. & Erbar C. 2010. *Flower and fruit. Morphology, ontogeny, phylogeny, function and ecology.* Stuttgart, Schweizerbart Science Publishers.

Leroux O., Van der Kinderen G. & Viane R.L.L. 2007. A sandwich-embedding method for oriented sectioning. *Journal of Microscopy* 227: 79–82.

Li M-R., Wedin D.A. & Tieszen L.L. 1999. C$_3$ and C$_4$ photosynthesis in *Cyperus* (Cyperaceae) in temperate eastern North America. *Canadian Journal of Botany* 77: 18–209.

Liebmann F.M. 1850. Mexicos Halvgraes. *Kongel. Danske Vidensk.Selsk. Skr., Naturvidensk. Math. Afd.* 5(2): 189–277.

Linder H.P. 1992. The structure and evolution of the female flower of the African Restionaceae. Botanical Journal of the Linnean Society 109: 401–425. http://dx.doi.org/10.1111/j.1095-8339.1992.tb00282.x

Linder H.P. & Rudall P.J. 2005. Evolutionary history of Poales. Annual Review of Ecology, Evolution, and Systematics 36: 107–124. http://dx.doi.org/10.1146/annurev.ecolsys.36.102403.135635

Linnaeus C. 1753. *Species plantarum*. Stockholm: Impensis Laurentii Salvii.

Lu S., Tong G., Long Y. & Feng H. 2006. Partial purification and characterisation of polyphenol oxidase from fresh-cut Chinese water chestnut. *Journal of food biochemistry* 30(2): 123-137.

Luo D., Carpenter R., Vincent C., Copsey L. & Coen E. 1996. Origin of floral asymmetry in Antirrhinum. *Nature* 383: 794–799. http://dx.doi.org/10.1038/383794a0

Lye KA. 1972. Studies in African Cyperaceae. 6. New species and combinations in *Kyllinga.* Bot. Not. 125: 217-219.

Lye K.A. 1981a. Studies in African *Cyperaceae* 18. Two new subgenera of *Cyperus*. *Nordic J. Bot.* 1: 57–61.

Lye K.A. 1981b. Studies in African *Cyperaceae* 19. The genera *Anosporum* Nees and *Sorostachys* Steudel. *Nordic J. Bot.* 1: 186–191.

Lye K.A. 1981c. Studies in African *Cyperaceae* 20. New taxa and combinations in *Pycreus* Beauv. *Nordic J. Bot.* 1: 617–622.

Lye K.A. 1982. Studies in African *Cyperaceae* 21. New taxa and combinations in *Kyllinga* Rottb. *Nordic J. Bot.* 1: 741–747.

Lye K.A. 1983. Studies in African *Cyperaceae* 25. New taxa and combinations in *Cyperus* L. *Nordic J. Bot.* 3: 213–232.

Lye KA. 1992. The history of the genus *Mariscus. Lidia* 3: 37–72.

Lye K.A. 1995. Cyperaceae. In: M. Thulin, ed. *Flora of Somalia* 4: 98-147, fig.

Lye KA. 1997. Cyperaceae. In: Edwards S, Demissew S & Hedberg I, eds. *Flora of Ethiopia and Eritrea* 6. Addis Ababa: Addis Ababa University, 391-511.

Lye KA. 2011. New combinations in *Cyperus*. *Lidia 7: 96-97.*

Lye K.A. & Cheek M. 2006. Studies in African *Cyperaceae* 32. *Cyperus rheophyticus* sp. nov. *Nordic J. Bot*. 24: 273–277.

Lye K.A. & Pollard B.J. 2006. Studies in African Cyperaceae 31. *Cyperus microcristatus*, a new species from Mt Kupe, Cameroon. *Nordic J. Bot.* 24: 269–272.

Manktelow M. 2010. History of Taxonomy. Lecture from Dept. of Systematic Biology, Uppsala University. http://atbi.eu/summerschool/files/summerschool/Manktelow_Syllabus.pdf

Marchand L. 1830. Derde verhandeling over de cryptogamische planten van het Groothertogdom Luxemburg. *Bijdr. Natuurk. Wetensch.* 5: 184–199.

Mariën W. 1969. Bijdrage tot de kennis van de Kongolese *Pycreus*-soorten (Cyperaceae). Verhandeling ingediend tot het behalen van de graad van Licenciaat in de wetenschappen (Groep plantkunde), Rijksuniversiteit Gent.

Maslin B.R., Miller J.T. & Seigler D.S. 2003. Overview of the generic status of *Acacia* (Leguminosae : Mimosoideae). *Australian Systematic Botany* 16: 1-18.

Mathieu G., Samain M.-S., Reynders M. & Goetghebeur P. 2008. Taxonomy of the *Peperomia* species (Piperaceae) with pseudo-epiphyllous inflorescences, including four new species. *Bot. J. Linn. Soc.* 157: 177-196.

Mayr E. (1982). *The Growth of Biological Thought*. Cambridge (Mass.): Belknap P. of Harvard U.P.

McGlone M.S. 1978. Pollen structure of the New Zealand members of the Styphelieae (Epacridaceae). *New Zealand Journal of Botany* 16: 91–101.

McNeill J., Barrie F.R., Burdet H.M., Demoulin V., Hawksworth D.L., Marhold K., Nicolson D.H., Prado J., Silva P.C., Skog J.E., Wiersema J.H. & Turland N.J., eds. 2006. *International Code of Botanical Nomenclature*

(Vienna Code) Adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005. Gartner Verlag, Rugell. [Regnum Veg. 146].

McNeill J., Barrie F. R., Buck W.R. et al., eds. 2012. *International Code of Nomenclature for algae, fungi, and plants (Melbourne Code), Adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011* (electronic ed.), Bratislava: International Association for Plant Taxonomy, retrieved 2012-12-20 .

Melville R. 1969. Leaf venation patterns and the origin of angiosperms. *Nature* 224: 121–125. http://dx.doi.org/10.1038/224121a0

Metcalfe C.R. 1971. *Anatomy of the Monocotyledons*, vol. 5, *Cyperaceae*. Oxford: Clarendon Press.

Miao R.H. 1991. A systematic taxonomy of *Lipocarpha* R. Br. From China. *Acta Sci. Nat. Univ. Sunyatseni* 30: 85–87.

Miller A.H. 1949. Some ecologic and morphologic considerations in the evolution of higher taxonomic categories. Pp. 84-88 *in* Mayr E. & Schuz E., eds. *Ornithologie als Biologische Wissenschaft.* Carl Winter/Universitätsverlag, Heidelberg.

Miller J.T. & Bayer R.J. 2001. Molecular phylogenetics of *Acacia* (*Fabaceae*: *Mimosoideae*) based on the chloroplast *matK* coding sequence and flanking *trnK* intron spacer regions. *Amer. J. Bot.* 88: 697–705.

Moore G., Smith G.F., Figueiredo E., Demissew S., Lewis G., Schrire B., Rico L. & van Wyk A.E. 2010. *Acacia*, the 2011 Nomenclature Section in Melbourne, and beyond. *Taxon* 59: 1188-1195.

Moore G., Smith G.F., Figueiredo E., Demissew S., Lewis G., Schrire B., Rico L., van Wyk A.E., Luckow M., Kiesling R. & Sousa M. 2011. The *Acacia* controversy resulting from minority rule at the Vienna Nomenclature Section: much more than arcane arguments and complex technicalities. *Taxon* 60: 852-857.

Mora L.E. 1960. Beitrage zur Entw 1 icklungsgeschichte und vergleichende Morphologie der 2 Cyperaceen. *Beiträge zur Biologie der Pflanzen* 35: 253–341.

Mora-Osejo L.E. 1987. Consideraciones sobre la naturaleza morfológica de las flores de algunos géneros de las Cyperaceae. *Revista de la Academia Colombiana de Ciencias Exactas, Fisicas y Naturales* 16: 23–35.

Muasya A.M. & de Lange, P.J. 2010. *Ficinia spiralis* (*Cyperaceae*), a new genus and combination for *Desmoschoenus spiralis*. *New Zealand J. Bot.* 48: 31–39.

Muasya A.M., Simpson D.A., Chase M.W. & Culham A. 1998. An assessment of suprageneric phylogeny in Cyperaceae using *rbcL* DNA sequences. *Plant Systematics and Evolution* 211: 257–271.

Muasya A.M., Simpson D.A., Chase M.W., Culham A. 2001a. A phylogeny of *Isolepis* (Cyperaceae) inferred using plastid rbcL and trnL–F sequence data. Systematic Botany 26: 342–353. DOI: 10.1043/0363-6445-26.2.342

Muasya A.M., Simpson D.A. & Chase M.W. 2001. Generic relationships and character evolution in *Cyperus* s.l. (Cyperaceae). *Syst. Geogr. Pl.* 71: 539--544.

Muasya A.M., Simpson D.A. & Chase M.W. 2002. Phylogenetic relationships in *Cyperus* s.l. (Cyperaceae) inferred from plastid DNA sequence data. *Bot. J. Linn. Soc*. 138 : 145--153, 1 fig.

Muasya A.M., Simpson D.A. & Smets E. 2006. *Isolepis tenella*, a new combination in *Cyperaceae*. *Novon* 16: 89–90.

Muasya A.M., Simpson D.A. & Smets E. 2007. *Isolepis levynsiana*, a new name for *Cyperus tenellus* (*Cyperaceae*). *Novon* 17: 59.

Muasya A.M., Simpson D.A., Verboom G.A., Goetghebeur P., Naczi R.F.C., Chase M.W. & Smets E. 2009*a*. Phylogeny of Cyperaceae based on DNA sequence data: current progress and future prospects. *Botanical Review*. 75: 2-21.

Muasya A.M., Vrijdaghs A., Simpson D.A., Chase M.W., Goetghebeur P. & Smets E. 2009*b*. What is a genus in Cypereae: Phylogeny, character homology assessment and generic circumscription in Cypereae. *Botanical review*. 75: 52-66.

Muasya A.M., Larridon I., Reynders M., Huygh W., Goetghebeur P., Cable S., Simpson D.A. & Gehrke B. 2011. The Cyperaceae in Madagascar show high diversification in high altitude forest and wetland habitats. *Plant Ecology and Evolution* (AETFAT proceedings). 144(3): 357-362.

Muasya A.M., Reynders M., Goetghebeur P., Simpson D.A. & Vrijdaghs A. 2012. *Dracoscirpoides* (Cyperaceae) - a new genus from Southern Africa, its taxonomy and floral ontogeny. *South African Journal of Botany* 72: 104-115.

Muasya A.M., Viljoen J-A., Dludlu M.N. & Demissew S. in press. Phylogenetic position of *Cyperus clandestinus* (Cypereae, Cyperaceae) clarified by morphological and molecular evidence. *Nordic Journal of Botany*.

Nagels A., Muasya A.M., Huysmans S., Vrijdaghs A., Smets E. & Vinckier S. 2009. Palynological diversity and major evolutionary trends in Cyperaceae. *Plant Systematics and Evolution* 277, 117–142.

Nakai T. 1912. *Cyperaceae-Cyperinae* Japono-Coreanae. *Bot. Mag. (Tokyo)* 26: 185–207.

Nees von Esenbeck C.G. 1834. Uebersicht der Cyperaceengattungen. *Linnaea* 9: 273–308.

Nees von Esenbeck C.G. 1842. Florae brasiliensis cyperographia. Pp. 1–226 in: von Martius, C.F.P., ed. *Flora brasiliensis*, vol. 2(1). Munich, Vienna & Leipzig.

Nelmes E. 1952. Notes on Cyperaceae. 26. New annual species of *Pycreus* from tropical Africa. *Kew Bull*. 6(3) : 319-321 ("1951")

Nelmes E. 1955. Notes on Cyperaceae. 36. New annual species of *Pycreus* from tropical Africa II. *Kew Bull*. 10 (1) : 91-92

Nelson G., Murphy D.J. & Ladiges P.Y. 2003. Brummitt on paraphyly: a response. *Taxon* 52: 295-298.

Nylander J.A.A. 2004. MrModeltest v2. Program distributed by the author. Uppsala University: Evolutionary Biology Centre.

Ollendorf L. 1992. Toward a classification sheme of sedge (Cyperaceae) phytoliths. *Phytolith Systematics* 5: 91.

Opiz F.M. 1852. *Seznam Rostlin Kveteny Ceske*. Prague.

Oteng-Yeboah A.A. 1977. Observations on the genus *Ascolepis*. *Notes Roy. Bot. Gard. Edinburgh.* 35: 391–397

Palla E. 1888. Zur Kenntnis der Gattung *Scirpus*. *Bot. Jahrb. Syst.* 10: 293–301.

Palla E. 1905. Über den morphologischen Wert der Blüte der Gattungen *Lipocarpha* und *Platylepis*. *Ber. Deutsch. Bot. Ges.* 23: 316–323.

Panigrahi G. 1985. Correct author citation for *Hypolytrum* Richard, LC and lectotypification of *Hypolytrum* and *Hypaelyptum* Vahl (*Cyperaceae*). *Taxon* 34: 510–512.

Pax F. 1886. Cyperaceae. In: Engler A. & Prantl K, eds. *Die natürlichen Pflanzen-familien* ed. 1, 2 (2). Leipzig:

Engelmann, 98-126.

Payer J.B. 1857. *Traitement d'organogénie compareé de la fleur*. Paris, Masson.

Pfeiffer L. 1874. *Nomenclator botanicus*, vol. 2. Kassel: Fischer

Phillipson W.R. 1985. Is the grass gynoecium monocarpellary? *American Journal of Botany* 72: 1854–1961. http://dx.doi. org/10.2307/2443612

Pizzolato T.D. 2000. A systematic view of the development of vascular systems in culms and inflorescences of grasses. In: Jacobs S.L.W. & Everett J., eds. *Grasses: Systematics and evolution*. Melbourne: CSIRO, 8–28.

Pizzolato T.D. 2006. Procambial initiation for the vascular system in the shoot of *Tradescantia zebrina* (Commelinaceae). *International Journal of Plant Science* 167:59–81. http://dx.doi.org/10.1086/497842

Plunkett G.M., Soltis D.E., Soltis P.S. & Brooks R.E. 1995. Phylogenetic relationships between Juncaceae and Cyperaceae: insights from rbcL sequence data. *Amer. J. Bot.* 82: 520–525.

Podlech D. 1960. Ueber einige Cyperaceen Südafrikas. *Mitteilungen der Botanischen Staatssammlung. München* 3: 521-530.

Pommier T., Canbäck B., Lundberg P., Hagström A. & Tunlid A. 2009. "RAMI: a tool for identification and characterization of phylogenetic clusters in microbial communities". *Bioinformatics (journal)* 25 (6): 736–742.

Porembski S. & Barthlott W. 2000. Inselbergs: Biotic Diversity of Isolated Rock Outcrops in Tropical and Temperate Regions. *Ecological Studies* 146, Springer-Verlag, Berlin.

Prasad V.P. 2009. Five New Synonyms of *Pycreus malabaricus* (Cyperaceae). *Rheedea* 19(1-2): 19-24.

Prata A.P., Camelbeke K., Reynders M., Goetghebeur P., Fedon I. & Huber O. 2007. *Bulbostylis medusae* (Cyperaceae), a new species from Venezuela. *Novon* 17: 67-71.

Prychid C.J. & Bruhl J.J. 2013. Floral ontogeny and gene protein localization rules out euanthial interpretation of reproductive units in *Lepironia* (Cyperaceae, Mapanioideae, Chrysitricheae). *Annals of Botany* 112(1):161-77. doi: 10.1093/aob/mct111

Rambaut A. & Drummond A.J. 2007. Tracer v1.4, Available from http://beast.bio.ed.ac.uk/Tracer.

Raynal J. 1966. Notes cyperologiques 4. Trois *Cyperus* africains a style indivis. *Adansonia* 6: 301–308.

Raynal J. 1972. Tropical African plants. 32. Cyperaceae. *Kew Bull*. 26(3) : 568

Raynal J. 1973. Notes cypérologiques: 19. Contribution à la classification de la sous-famille des *Cyperoideae*. *Adansonia*, ser. 2, 13(2): 145–171.

Raynal J. 1977a. Notes cypérologiques 29. Les *Pycreus* sect. *Rhizomatosi. Adansonia* sér. 2, 16(4):401-404.

Raynal J. 1977b. Notes cypérologiques 31. Mélanges nomenclaturaux (Cyperoideae). *Adansonia* sér. 2, 17: 43-47.

Reinhardt D., Pesce E.R., Stieger P., Mandel T., Baltensperger K., Bennett M., Traas J., Friml J. & Kuhlemeier C. 2003. Regulation of phyllotaxis by polar auxin transport. *Nature* 426: 255–260.

Reynders M. 2004. De Cyperaceae van Rwanda en Burundi: bijdrage tot een nieuwe flora. Ms. Thesis. Ghent University, Belgium.

Reynders M., Dhooge S. & Goetghebeur P. 2006. A new Central African species, *Cyperus vandervekenii* (Cyperaceae) from the sources of the Nile in Rwanda. *Novon* 16: 512–515.

Reynders M. & Goetghebeur P. 2010 Reestablishment of *Pycreus* sect. *Tuberculati* Cherm. *Blumea* 55: 226–230.

Reynders M., Huygh W., Larridon I., Govaerts R., Simpson D.A. & Goetghebeur P. 2011 Nomenclature and typification of names of genera and subdivisions of genera in *Cypereae* (*Cyperaceae*): 3. Names of subdivisions in segregate genera of *Cyperus*. *Taxon* 60: 885–895.

Reynders M. & Vrijdaghs A., Larridon I., Huygh W., Leroux O., Muasya AM. & Goetghebeur P. 2012. Gynoecial anatomy and development in Cyperoideae (Cyperaceae, Poales): congenital fusion of carpels facilitates evolutionary modifications in pistil structure. *Plant Ecology and Evolution* 145(1): 1-30.

Richards J.H. 2002. Flower and spikelet morphology in sawgrass, *Cladium jamaicense* Crantz (Cyperaceae). *Annals of Botany* 90: 361–367.

Richards J.H., Bruhl J.J. & Wilson K.L. 2006. Flower of Spikelet? Understanding the morphology and development of reproductive structures in *Exocarya* (Cyperaceae, Mapanioideae, Chrysitricheae). *American journal of Botany* 93: 1241–1250. http://dx.doi.org/10.3732/ajb.93.9.1241

Rickett H.W. & Stafleu F.A. 1959. Nomina generica conservanda et rejicienda spermatophytorum. *Taxon* 8: 213–243.

Ridley H.N. 1884. The Cyperaceae of the west coast of Africa in the Welwitsch herbarium. *Trans. Linn. Soc*. *Bot.*, 2 : 121-172, pl. 22--23.

Rikli M. 1895. Beiträge zur vergleichenden Anatomie der Cyperaceen mit besonderer Berücksichtigung der inneren Parenchymscheide. *Jahrb. Wiss. Bot.* 27: 485–580.

Ronquist F. & Huelsenbeck J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Ronse Decraene L.P., Linder H.P. & Smets E.F. 2002. Ontogeny and evolution of the flowers of South African Restionaceae with special emphasis on the gynoecium. *Plant Systematics and Evolution* 231: 225–258. http://dx.doi.org/10.1007/s006060200021

Rottbøll C.F. 1773. *Descriptionum et iconum rariores et pro maxima parte novas plantas illustrantium liber primus*. Copenhagen: sumptibus Societatis typographicae.

Rudall P.J. & Bateman R.M. 2004. Evolution of zygomorphy in monocot flowers: iterative patterns and developmental contraints. *New Phytologist* 162: 25-44.

Samain M.-S., Reynders M. & Goetghebeur P. 2006. *Kyllinga beninensis* (Cyperaceae), a new species from Bénin. *Novon* 16: 516–519.

Saunders E. 1937 Floral morphology: a new outlook with special reference to the interpretation of the gynoecium. Vol. 1. Cambridge, W. Heffer.

Scarpella E., Meijer A.H. 2004. Pattern formation in the vascular system of monocot and dicot plant species. *New Phytologist* 164: 209–242.

Scarpella E., Marcos D., Friml J. & Berleth T. 2006. Control of leaf vascular patterning by polar auxin transport. *Genes & Development* 20: 1015–1027.

Schischkin B.K. 1935. Cyperaceae and Juncaceae. Pp. 1–578 in: Komarov V.L., ed. *Flora unionis rerumpublicarum sovieticarum socialisticarum*, vol. 3. Leningrad: Institutum Botanicum Academiae Scientiarum URSS.

Shuping L.S., Snyman R.G., Odendaal J.P. & Ndakidemi P.A. 2011. Accumulation and Distribution of Metals in *Bolboschoenus maritimus* (Cyperaceae), from a South African River. *Water, Air & Soil Pollution*. 216(1-4, 319-328.

Schultze-Motel W. 1959. Entwicklungsgeschichtliche und vergleichend-morphologische Untersuchungen im Blütenbereich der Cyperaceae. *Botanische Jahrbuch der Systematischen Pflanzengeschichte und Pflanzengeographie* 78: 129–170.

Schumann K.M. 1890. Cyperaceae. In: Schumann K.M., ed. *Neue Untersuchungen über den Blüthenanschluss*: 37–55. Leipzig, Engelmann.

Simpson D.A. 1990. A revision of *Cyperus* sect. *Leucocephali*. *Kew Bull*. 45: 485–501.

Simpson D.A. 1992. A revision of the genus *Mapania*. Kew: Royal Botanic Gardens, Kew. 189 pp.

Simpson D.A. & Inglis C.A. 2001. Cyperaceae of economic, ethnobotanical and horticultural importance: a checklist. *Kew Bulletin* 56: 257-360.

Simpson D.A. & Koyama T. 1998. Cyperaceae. Pp. 345–387 in: Santisuk T. & Larsen K., eds. *Flora of Thailand*. Bangkok: Royal Forest Department.

Simpson D.A., Furness C.A., Hodkinson T.R., Muasya A.M. & Chase M.W. 2003. Phylogenetic relationships in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. *American Journal of Botany* 90: 1071–1086. http://dx.doi.org/10.3732/ajb.90.7.1071

Simpson D.A., Muasya A.M., Alves M., Bruhl J.J., Dhooge S., Chase M.W., Furness C.A., Ghamkhar K., Goetghebeur P., Hodkinson T.R., Marchant A.D., Nieuborg R., Reznicek A.A., Roalson, E.H., Smets, E., Starr, J.R., Thomas, W.W., Wilson, K.L. & Zhang, X. 2008 ('2007'). Phylogeny of *Cyperaceae* based on DNA sequence data—a new *rbcL* analysis. *Aliso* 23: 72–83.

Simpson D.A., Yesson C., Culham A., Cough C.A. & Muasya A.M. 2011. Climate change and Cyperaceae. In: Hodkinson T., Jones M., Waldren S. & Parnell J. (eds). Climate change, ecology and systematics. Cambridge University Press, pp. 439-456. http://centaur.reading.ac.uk/20419/

Skvortzow B.W. 1969. Siberian and Chinese fresh water diatoms new to science. *Bot. Not.* 122: 375–379.

Smith S.Y., Collinson M.E., Simpson D.A., Rudall P.J., Marone F. & Stampanoni M. 2009. Elucidating the affinities and habitat of ancient, widespread Cyperaceae: *Volkeria messelensis* gen. et sp. nov., a fossil mapanioid sedge from the Eocene of Europe. *American Journal of Botany* 96(8): 1506–1518.

Smith G.F. & Figueiredo E. 2011. Conserving *Acacia* Mill. with a conserved type: what happened in Melbourne? *Taxon* 60: 1504-1506.

Snell R.S. 1936. Anatomy of the spikelet and flowers of *Carex, Kobresia* and *Uncinia*. *Bulletin of the Torrey Botanical Club* 63: 277–295. http://dx.doi.org/10.2307/2480780

Sokal R.R. & Sneath P.H. 1963. *Principles of Numerical Taxonomy*. W.H. Freeman, San Francisco.

Soros C.L. & Bruhl J.J. 2000. Multiple Evolutionary Origins of C_4 Photosynthesis in the Cyperaceae. In Wilson K. & Morrison D.A., eds. *Monocots: Systematics and Evolution*. pp. 629--636. CSIRO Publishing & Royal Botanic Garden Kew: Melbourne.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

Stamatakis A., Hoover P. & Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web Servers. *Systematic Biology* 57: 758-771.

Starr J.R., Bayer R.J. & Ford B.A. 1999. The phylogenetic position of *Carex* section *Phyllostachys* and its implications for phylogeny and subgeneric circumscription in *Carex* (*Cyperaceae*). *Amer. J. Bot.* 86: 563–577.

Starr J.R. & Ford B.A. 2009. Phylogeny and evolution in Cariceae (Cyperaceae): current knowledge and future directions. *Botanical Review* 75: 110-137.

Steinmann V.W. & Porter J.M. 2002. Phylogenetic relationships in Euphorbieae (Euphorbiaceae) based on ITS and *ndhF* sequence data. *Annals of the Missouri Botanical Garden* 89: 453-490.

Steudel E.G. von. 1850. Über den gegenwärtigen Stand der Synopsis plantarum und eine neue Gattung der Gräser. *Flora* 33: 225–232.

Steudel E.G. von. 1854–1855. *Synopsis plantarum glumacearum*, vol. 2. *Cyperaceae*. Stuttgart: Metzler.

Stevens P.F. 2001 onwards. Angiosperm Phylogeny Website. Version 12, Juli 2012. http:/www.mobot.org/MOBOT/Research/APWeb/. [Accessed 30 Jun. 2011]

Stock W.D., Chuba D.K. & Verboom G.A. 2004. Distribution of South African C_3 and C_4 species of Cyperaceae in relation to climate and phylogeny. *Austral Ecology* 29: 313–319.

Strid A. 1974. On the typification of *Bobartia* L. *Taxon* 23: 422–424.

Stuessy T. F. & König Ch. 2008: Patrocladistic classification. Taxon 57, 594–601.

Takhtajan A. 1997. *Diversity and classification of flowering plants*. New York, Columbia University Press.

Thiele K.R., Funk V.A., Iwatsuki K., Morat P., Peng C.I., Raven P.H., Sarukhan J. & Seberg O. 2011. The controversy over the retypification of *Acacia* Mill. with an Australian type: a pragmatic view. *Taxon* 60: 194-198.

Thomas W.W., Araujo A.C., and Alves M.V. 2009. A Preliminary Molecular Phylogeny of the Rhynchosporae (Cyperaceae). *Botanical Review* 75:22-29.

Troll W. 1964. *Die Infloreszenzen, Typologie und Stellung im Aufbau des Vegetationskörpers*. Bd I, II, 1. Jena 1964/69, Germany

Tucker G.C. 1983. The taxonomy of *Cyperus* (*Cyperaceae*) in Costa Rica and Panama. *Systematic Botany Monographs* 2: 1–85.

Tucker G.C. 1987. The genera of *Cyperaceae* in the SE United States. *J. Arnold Arbor.* 68: 361–445.

Tucker G.C. 1994. Revision of the Mexican Species of *Cyperus* (Cyperaceae). *Systematic Botany Monographs* 43: 213.

Tucker G.C., Marcks B.G. & Carter J.R. 2002. *Cyperus*. In: Flora of North America Editorial Committee, eds. *Flora of North America*, vol. 23. New York and Oxford.

Turrill W.B. 1922. Notes on *Cyperaceae* 1. (*Pycreus pumilis* and *Pycreus hyalinus*). *Bull. Misc. Inform. Kew* 1922: 122–124.

Van der Veken P. 1955. Trois taxa nouveaux de Cypéracées du Congo belge. Bull. Jard. Bot. Etat Brux. 25(1) : 143-147, fig. 38-39.

Van Der Veken P. 1964. Bijdrage tot de systematische embryologie der Cyperaceae- Cyperoideae. PhD thesis, K.U. Leuven, Belgium.

Van der Veken P. 1965. Contribution à l' embryographie systématique 1 des *Cyperaceae-Cyperoideae*. *Bulletin du Jardin botanique de l'État à Bruxelles* 35: 285–354.

Vahl M. 1805. *Enumeratio plantarum*, vol. 2. Schubothe, Hauniae & Lipsiae: Typis N. Mölleri et Filii.

Valckenier Suringar J. 1898. *Het geslacht Cyperus*. Hugo Suringar, Leeuwarden.

Väre H. & Kukkonen I. 2006. Typification of names in *Cyperus* section *Arenarii* (*Cyperaceae*). *Nord. J. Bot.* 24: 279–294.

Verboom GA. 2006. A phylogeny of the schoenoid sedges (*Cyperaceae*: *Schoeneae*) based on plastid DNA sequences, with special reference to the genera found in Africa. Molecular Phylogenetics and Evolution 38: 79–89.

Verloove F. & Reynders M. 2007a. Studies within the genus *Paspalum* (Paniceae, Poaceae) in Europe: 1. *Paspalum distichum* subsp. *paucispicatum*, an overlooked taxon in France. *Willdenowia* 37: 199-204.

Verloove F. & Reynders M. 2007b. Studies within the genus *Paspalum* (Paniceae, Poaceae) in Europe – 2. The *Quadrifaria* group. *Willdenowia* 37: 423-430.

von Goethe J.W. 1790. *The metamorphosis of plants*. Cambridge, MIT Press (reprint 2009).

Voss E.G., Burdet H.M., Chaloner W.G., Demoulin V., Hiepko P., McNeill J., Meikle R.D., Nicolson D.H., Rollins R.C., Silva P.C. & Greuter W., eds. 1983. *International code of botanical nomenclature: Adopted by the Thirteenth International Botanical Congress Sydney, Australia, August 1981.* Regnum Vegetabile 111. Utrecht/Antwerp: Bohn, Scheltema & Holkema.

Vrijdaghs A. 2006. *A floral ontogenetic approach to homology questions in non-mapanioid Cyperaceae - Een bloemontogenetische benadering van homologie-vraagstukken bij niet-mapanioïde Cyperaceae.* PhD Thesis, K.U.Leuven, Belgium.

Vrijdaghs A., Goetghebeur P., Smets E. & Caris P. 2003. The orientation of the developing gynoecium of *Cladium mariscus* (L.) Pohl. In: Bayer C., Dressler S., Schneider J. & Zizka G., eds. *16th International Symposium Biodiversity and Evolutionary Biology, 17th InternationalSenckenberg Conference*. 1 Abstracts: 24. Palmarum Hortus Francofurtensis 7, Frankfurt-am-Main, Germany.

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

Vrijdaghs A., Goetghebeur P., Muasya A.M., Caris P. & Smets E. 2005*b*. 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. & Muasya A.M. 2006. The floral scales in *Hellmuthia* (Cyperaceae, Cyperoideae) and *Paramapania* (Cyperaceae, Mapanioideae): An ontogenetic study. *Annals of Botany* 98: 619–630.

Vrijdaghs A., Goetghebeur P., Smets E., Caris P. 2007. The *Schoenus* spikelet: a rhipidium? A floral ontogenetic answer. In J. T. Columbus, E. A. Friar, J. M. Porter, L. M. Prince, and M. G. Simpson [eds.], Monocots:

comparative biology and evolution—Poales. Rancho Santa Ana Botanic Garden, Claremont, California, USA. *Aliso* 23, pp. 204-209

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

Vrijdaghs A., Reynders M., Larridon I., Muasya A.M., 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. http://dx.doi.org/10.1093/aob/mcq010

Vrijdaghs A., Reynders M., Muasya A.M., Larridon I., Goetghebeur P. & Smets E. 2011. Spikelet and Floral Ontogeny in *Cyperus* and *Pycreus* (Cyperaceae). *Plant Ecology and Evolution* 144: 44–63. http://dx.doi.org/10.5091/plecevo.2011.436

Walker J.B., Sytsma K.J., Reutlein J. & Wink M. 2004. *Salvia* (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of *Salvia* and tribe Mentheae. *American Journal of Botany* 91: 1115-1125.

Wanke S., Samain M.-S., Vanderschaeve L., Mathieu G., Goetghebeur P. & Neinhuis C. 2006. Phylogeny of the genus *Peperomia* (*Piperaceae*) inferred from the *trnK/matK* region (cpDNA). *Pl. Biol.* 8: 93–102.

Wardlaw C. 1949. Experiments on organogenesis in ferns. *Growth* 9: 93–131.

Weberling F. 1992. *Morphology of flowers and inflorescences*. Ed. Cambridge University Press, Cambridge, UK

Weigel D., Meyerowitz A.M. 1994. The ABCs of floral homeotic genes. *Cell* 78: 203–209. http://dx.doi.org/10.1016/0092-8674(94)90291-7

Welwitsch F. 1869. Sertum Angolense. *Trans. Linn. Soc. London* 27: 1–94.

Wettstein R. 1935. *Systematische Botanik*. 4th Ed. Leipzig, Franz Deuticke.

Wiens JJ., Ackerly DD., Allen AP., Anacker BL., Buckley L.B., Cornell HV., Damschen EI., Davies TJ., Grytnes J-A., Harrison SP., Hawkins BA., Holt RD., McCain CM. & Stephens PR. 2010. Niche conservatism as an emerging principles in ecology and conservation biology. *Ecology Letters* 13: 1310–1324

Yano O., Ikeda H., Watson M.F., Rajbhandari K.R., Jin X-F, Hoshino T., Muasya A.M. & Ohba H. 2012. Phylogenetic position of the Himalayan genus *Erioscirpus* (Cyperaceae) inferred from DNA sequence data. *Botanical Journal of the Linnean Society* 170: 1-11.

Zhang X., Wilson K.L. & Bruhl J.J. 2004. Sympodial structure of spikelets in the tribe Schoeneae(Cyperaceae). *American Journal of Botany* 91: 24-3.

Appendix I Glossary

Below you can find the definition of the common and recurrent technical terms used in this dissertation. Most definitions are adopted from Larridon, 2011 and Henderson's Dictionary of Biological Terms (13th edition, edited by E. Lawrence, by Pearson Education, Prentice Hall).

abaxial: the side facing away from the axis

achene: one-seeded, dry, indehiscent fruit, developed from a single carpel, with the seed wall usually not fused with the fruit wall

acropetal (<-> basipetal): ascending; leaves, flowers, roots or spores developing successively along an axis so that the youngest are at the apex

acute: ending in a sharp point

adaptive radiation/ radiative burst: a process in which organisms diversify rapidly into a multitude of new forms, particularly when a change in the environment makes new resources available and opens environmental niches

adaxial: the side facing towards the axis

anatropous: inverted; ovule bent over so that hilum and micropile are close together

annular: having the shape of a ring

anthela: cymous inflorescence with a contracted central axis, subsequently the different lateral axes and subtending bracts stand close together forming an involucre

anther: pollen producing floral part

antrorse: directed forwards or upwards

apex: distal tip of an axis, usually with a meristematic zone from which new primordia originate

articulate: (1) joined; (2) separating easily at certain points

attenuated: gradually tapering to a point

basal (placentation): The placenta is at the base (bottom) of the ovary. Simple or compound carpel

basifixed (anthers): filaments fixed to the base of the anthers

bract: leaf bearing a (partial) inflorescence, e.g. involucral bracts, bracts subtending spikelets

bristle: long thin perianth part, often with scabrid edges

C$_3$ (photosynthesis): metabolic pathway for carbon fixation in photosynthesis, in which the first product of carbon fixation is a 3-carbon molecule

C₄ (photosynthesis): metabolic pathway for carbon fixation in photosynthesis, in which the first product of carbon fixation is a 4-carbon molecule

carina: ridge on bracts of certain grasses

carpel: the ovule and seed producing reproductive organ in flowering plants

chlorocyperoid (anatomy): type of Kranz anatomy found in Cypereae

clade: a group consisting of an ancestor and all its descendants; branch in a phylogenetic hypothesis

cladistic classification: classification using only monophyletic taxa

compressed: flattened transversely

concaulescence: metatopic displacement of a floral primordium with respect to the subtending glume with the growth of the next internode, because of the partial fusion between the flower primordium and apical growth zone of this axis, resulting in a separation of the flower and the flower subtending glume

congenital (carpel fusion): emerging primordia of different carpels are fused from their origin

contracted (inflorescence): shortening of internodes within a (partial) inflorescence

coriaceous/ corious (leaves): leathery

cuspidate: terminating in a sharp point

cymose: inflorescence formed by successive growth of axillary shoots after growth of main shoot in each branch has stopped

dambo: shallow wetlands dominated by grasses and sedges in central, southern and eastern Africa, particularly in Zambia and Zimbabwe

deciduous: falling at the end of growth period or at maturity

decumbent: with a creeping lower part

decurrent: having leaf base prolonged down stem as a winged expansion of rib

dimerous: consisting of two parts; applied on gynoecia: consisting of two carpels

distichous (leaves): arranged in two vertical or spiral rows along an axis; alternate leaves arranged so that 1st is more or less directly below 3rd and so on

epicaulescence: a metatopic displacement, sometimes occurring in distichously arranged spikelets where the glumes have wings, where a fusion zone of the wings of the glume and rachilla grows out with the rising rachilla, displacing the main part of the glume and the flower primordium in its axil to the next node (see Vrijdaghs *et al.*, 2010).

evolutionary (classification): classification using both monophyltic and paraphyletic taxa

ferruginous: rust-colored

filament: elongated part of a stamen that bears the anther

flexuous/ flexuose: curving in a zigzag manner

floral apex: central-distal zone of a flower primordium from which ovary and ovule primordia originate

foliaceous: (1) having the texture of a leaf ; (2) thin and leaf-like ; (3) bearing leaves

funiculus: the stalk attaching an ovule to the ovary wall in a flower or fruit

fuscous: of a dark, almost black, color

fusiform: spindle-shaped, tapering at both ends

geophyte: plant surviving unfavorable seasons with buds below the soil or water surface

giant genus: genus consisting of more than 600 species

glabrous: with a smooth even surface; without hairs

globose: spherical or globular

glume: bract subtending a flower, usually arranged in a spikelet; glumes can also be empty

gynoecium: a collective term for all carpels in a flower

halophyte: salt tolerating plant

helophyte: plant surviving unfavorable seasons with buds below the water surface but bearing at least a few stems or leaves above the water surface during favorable seasons

headlike (inflorescence): fully contracted inflorescence resembling a sphere of head

hemicryptophyte: plant surviving unfavorable seasons with buds near the soil surface

holotype: single specimen which bears the name of a newly described (lower) taxon and serves as primary reference specimen for identification of all other collections under that taxon; a holotype is designated with the description of the taxon

homologous structures: structures in different plants possibly but not necessarily having a different morphology and function, but positioned at the same place in a plant, and developing according to a similar developmental pattern. According to the phylogenetic approach, it is added that homologous structures originated from a common ancestral structure

hydrophyte: plant living completely submerged under the water surface for most of its life cycle or floating loosely on the water surface.

hypogynium: structure supporting the ovary in flowers of some sedges

inselberg: an isolated rock hill, knob, ridge, or small mountain that rises abruptly from a gently sloping or virtually level surrounding plain.

introrse: (1) turned inwards or towards the axis; (2) of anthers, opening towards the center of the flower

involucre: bracts surrounding a terminal inflorescence with contraction of at least the main axis

isodiametric: of a regular geometrical shape in such a way the sections taken from different angles all have about the same length

isotype: specimen belonging to the same collection as the holotype

key innovative structure: structure that is highly adaptive resulting in elevated levels of speciation

Kranz (anatomy): presence of a bundle sheath surrounding the vascular bundles in stems and leaves; this is predominat in plants using C_4 photosynthesis

lanceolate: slightly broad or tapering at base, and tapering to a point at tip, appl. leaves, bracts and spikelets

lax: loosely clustered

lectotype: primary type designated after description of a taxon and chosen from the specimens or lower taxa cited with the original description of the taxon

lustrous: shiny/ shining

membranaceous: having the consistancy or structure of a membrane

molecular (phylogenetics): hypothesis based on the comparison of DNA molecules

monad (pollen): a single individual that is free from other individuals, not united with them into a group; pollen grain consisting of a single cell and is free from other pollen grains from its formation

monophyletic: a taxon that forms a clade, meaning that it consists of an ancestral species and all its descendants

monotypic: taxon consisting of a single lower taxon

muticous: without a point or pointed process; blunt

natural classification: classification based on the evolutionary relationships of taxa, usually based on phylogenetic hypotheses

nervulose: having nerves

niche: term describing the way of life of a species; the place a species fills within an ecosystem

nodulose/ nodose: having knots or swellings

nutlet: small nut; dry, indehiscent fruit developed from unilocular superior ovary with the (usually single) seed wall not fused with the fruit wall

oblong: elongated, as in oval, elliptical

obturator hairs: hairs formed from the funiculus and covering the micropile (in sedges)

orbiculate: nearly circular in outline

ovary: part of the carpel or fused carpels surrounding the ovules

ovule: structure that gives rise to and contains the female reproductive cells

palynology: study of the pollen grains

pappus: modified calyx, the part of an individual disk, ray or ligule floret surrounding the base of the corolla, in flower heads of the plant family Asteraceae

paraphyletic: when a taxon or clade that consists of an ancestral species and only part of its descendants

patrocladistic analysis: analysis of the correlation between branch lengths within a clade and characteristics linked to the different nodes to detect evolutionary significant characteristics.

perianth: consists of the calyx (all sepals) and the corolla (all petals) of the flower

phyllospory: interpretation of a carpel as a leaf bearing the ovules

phylogeny: hypothesis about the evolutionary history of taxonomic groups

pistil: discrete unit of the gynoecium; a pistil can consist of either a single carpel (in a monocarpous or apocarpous gynoecium), or of several fused carpels (in a syncarpous gynoecium)

placentation: pattern of placement of the ovules within the gynoecium

plicate: folded or ridged

polyphyletic: when not all organisms in a taxonomical group share a single common ancestor

polytomy: many temporal based branches; a section of a phylogeny in which the evolutionary relationships cannot be fully resolved to dichotomies

postgenital (carpel fusion): primordia of different carpels fuse after they have emerged separately

primordium: visible group of cells from which a plant structure develops

procambium: group of cells from which vascular tissue develops

prophyll: first leaf on an axis, situated adaxially and usually bi-keeled

proliferous: (1) multiplying quickly, appl. bud-bearing leaves of inflorescences; (2) developing supernumery parts abnormally

protologue: the printed matter accompanying the first description of a name

proximal: closest to the base of the axis (opposed to terminal/ distal)

pseudanthium: (partial) inflorescence resembling a flower or fulfilling the function of a single flower

pseudomonad (pollen): pollen grain that originates as a tetrad (4 attached cells) but in which one cell dominates and the other three are abortive

pseudomonomerous (pistil): unilocular pistil that seemingly consists of a single carpel but in reality resulted from a fusion of several carpels

pseudospikelet/ secondary spikelet: (partial) higher order inflorescence resembling a spikelet or fulfilling the function of a spikelet; the original spikelets are reduced and covered by the spikelet bracts, which fulfill the function of the glumes

punctate: (1) dotted; (2) having surface covered with small holes, pores or dots ; (3) having a dot-like appearance

puncticulate: slightly punctate

rachilla: axis of a spikelet

rachis: axis with spikelets

ray: the stalk of a group of spikelets in an anthela

recaulescence: when bract grows up with the lateral axis it is subtending because of partial fusion of the bract with the growth zone of the lateral axis. Consequently, the free part of the bract develops at the distal side of the lateral axis, while its insertion point is at the relative main axis. From its base up to the free part of the bract, the lateral axis is winged.

reticulate: like a network

retrorse: turned or directed backwards

rugose: with many wrinkles or ridges

rugulose: slightly rugose

sanguineous: having the color of blood ; blood-red

satellite: smaller structure or taxon which accompanies a similar larger structure/taxon

scabrous/ scabrate: rough ; with a covering of stiff hairs, scales or points

scape: flower stalk arising at or under ground level

segregate (taxon): taxon nested within another paraphyletically circumscribed taxon

silica body: type of silica deposition (phytolith) in specialized epidermal cells of grasses and sedges; consists mainly of noncrystalline silicon dioxide

speciation: the evolution of new species

spicate: (1) having a flowerhead in the form of a spike; (2) bearing spikes

spicoid: flowering unit in Mapanioideae when interpreted as a synanthium

spike: a flowerhead with stalkless flowers or secondary small spikes (spikelets) of flowers borne alternately along a single axis

spikelet: a mainly functional flowering unit, consisting of an indeterminate spikelet axis or rachilla, with spirally tot distichously arranged glumes. The glumes subtend (or not) a flower. Lateral spikelets are subtended by a bract and have a prophyll

stachyospory: interpretation of a carpel as a structure of compound leaf and stem origin, bearing the ovules

stamen: pollen producing floral organ; consists of filament and anther

stigma: distal part of a carpel or of fused carpels that serves for pollen reception; in sedges formed from separate stigma primordia on top of the ovary wall

stipitate: stalked

stramineous: straw-colored

striate(d): marked by narrow parallel lines or grooves

style: narrow and elongated median part of a pistil bearing the stigma(ta) at is end; in sedges it is formed from the upper part of the ovary wall primordium

subulate: awl-shaped, i.e. narrow and tapering from base to a fine point, appl. leaves as of an onion

sulcate: grooved or furrowed

tabular: cone shape with a flattened top (table shaped)

taxon: group of organisms; unit of a classification with a certain rank

terete: nearly cylindrical in section, as stems

tetrad (pollen): pollen grain consisting of four attached cells of equal size

therophyte: plant surviving unfavorable conditions as a seed

tribe: taxonomical rank consisting of several related genera within a family

trigonal: triangular in cross-section

triquetrous/ triquetral: stem with three angles and three concave faces

trimerous: consisting of three parts; applied on gynoecia: consisting of three carpels

tristichous/ three-ranked: arranged in three vertical or spiral rows

truncate/ abrupt: terminating abruptly; as if tapering end were cut off

type: reference specimen of a certain taxon

unilocular (pistil): pistil formed from a single or more carpels but with only with a single chamber holding the ovules

wing: (1) The membranous outgrowth of certain fruits; (2) the flat membranous sides of the glumes; (3) A flange running down a stem or stalk as, for example on the rachilla of certain species

xylem: vascular tissue for the upward water transportation in plants

zonate: with elongated cells on the surface

Appendix II List of cited taxa

Abildgaardieae Lye
Acacia Mill.
Actinoschoenus Benth.
Afrotrilepis (Gilly) J.Raynal
Afrotrilepis pilosa (Boeck.) J.Raynal
Alinula J.Raynal
Alinula lipocarphioides (Kük.) J.Raynal
Alinula malawica (J.Raynal.) Goetgh. &
        Vorster
Alinula paradoxa (Cherm.) Goetgh. & Vorster
Alinula peteri (Kük.) Goetgh. & Vorster
Anarthriaceae D.F.Cutler & Airy Shaw
Androtrichum (Brongn.) Brongn.
Anosporum Nees
Arabidopsis Heyhn.
Ascolepis ampullacea J.Raynal
Ascolepis brasiliensis Benth. ex C.B.Clarke
Ascolepis capensis Ridl.
Ascolepis eriocaulides Nees ex Steud.
Ascolepis hemisphaerica Peter ex Goetgh.
Ascolepis Nees ex. Steud.
Ascolepis protea Welw.
Ascolepis pusilla Ridl.
Ascopholis C.E.C.Fisch.
Asparagales Bromhead
Asteraceae Bercht. & J.Presl
Bisboeckelereae Pax ex L. T. Eiten
Blysmus Panz. ex Schult.
Bolboschoenus (Asch.) Palla
Bolboschoenus robustus (Pursch) Soják
Bromeliaceae Juss.
Bulbostylis Kunth
Capeobolus J.Browning
Carex concinnoides Mack.
Carex dolichostachya Hayata
Carex L.
Cariceae Kunth ex Dumort.
Caricoidea Chandler
Carpha Banks & Sol. ex R.Br.
Centrolepidaceae Endl.
Chenopodiaceae Vent.
Chlorocyperus Rikli
Chrysitricheae Nees
Chrysitrix L.

Cladieae Nees
Cladium mariscus (L.) Pohl
Coleochloa Gilli
Commelinaceae Mirb.
Costularia C.B.Clarke ex Dyer
Courtoisina Soják
Croton L.
Cryptangieae Benth.
Cyperaceae Juss.
Cypereae Dumort.
Cyperoideae Kostel.
Cyperus L.
Cyperus acaulescens Reynders nom.nov.
Cyperus africanus (Hooper) Reynders
Cyperus alopecuroides Rottb.
Cyperus alternifolius L.
Cyperus aterrimus Hochst. ex Steud.
Cyperus buchholzii Boeck.
Cyperus bulbosus Vahl
Cyperus capitatus (Poir.) Vand.
Cyperus clavinux C.B.Clarke
Cyperus compressus L.
Cyperus congestus Vahl
Cyperus cooperi (C.B.Clarke) Kük nom.illeg.
Cyperus croceus Vahl
Cyperus cuspidatus Kunth
Cyperus cyperoides (L.) Kuntze
Cyperus deciduus Boeck.
Cyperus distans L.f.
Cyperus dives Delile
Cyperus dubius Rottb.
Cyperus elegans L.
Cyperus endlichii Kük.
Cyperus eragrostis Lam.
Cyperus esculentus L.
Cyperus filiculmis Vahl
Cyperus fulgens C.B.Clarke
Cyperus fuscus L.
Cyperus haspan L.
Cyperus impubes var. *fallax* (Cherm.) Kük.
Cyperus intermedius Steudel
Cyperus involucratus Rottb.
Cyperus iria L.

Cyperus javanicus Houtt.

Cyperus juncelliformis Peter & Kük.

Cyperus kerstenii Boeck.

Cyperus laevigatus L.

Cyperus laterificus Raynal

Cyperus longibracteatus (Cherm.) Kük.

Cyperus longus L.

Cyperus luzulae Rottb.

Cyperus malaccensis Lam.

Cyperus marginatus Thunb.

Cyperus meeboldii Kük.

Cyperus meyenianus Kunth.

Cyperus micrantherus Cherm.

Cyperus micromariscus Lye

Cyperus muricatus Kük.

Cyperus neocooperi Reynders nom.nov.

Cyperus nervulosus (Kük.) S.T. Blake

Cyperus oakfortensis Boeck. ex C.B.Clarke

Cyperus odoratus L.

Cyperus okavangensis (Podlech) Reynders

Cyperus papyrus L.

Cyperus pectinatus Vahl

Cyperus poikilostachys (Nelmes) Reynders

Cyperus poikilostachys var. *heterochrous*
      (Nelmes) Reynders

Cyperus pulchellus R.Br.

Cyperus pustulatus Vahl

Cyperus pygmaeus Rottb.

Cyperus reduncus Hochst. ex Boeck.

Cyperus retrorsus Champ.

Cyperus rigidifoius Steud.

Cyperus rivularis Kunth

Cyperus rotundus L.

Cyperus rubiginosus Hook.f.

Cyperus rufostriatus C.B.Clarke ex Cherm.

Cyperus rupestris Kunth

Cyperus scaettae (Cherm.) Reynders

Cyperus scaettae var. *vanderystii* (Cherm.)
      Reynders

Cyperus scariosus R.Br.

Cyperus sect. *Albomarginati* Kük.

Cyperus sect. *Alternifolii* (Kunth) C.B.Clarke

Cyperus sect. *Amabiles* C.B.Clarke

Cyperus sect. *Anosporum* (Nees) Pax.

Cyperus sect. *Aristati* Nees

Cyperus sect. *Brevifoliati* C.B.Clarke

Cyperus sect. *Bulbocaules* (C.B.Clarke) Kük.

Cyperus sect. *Compressi* Nees

Cyperus sect. *Courtoisina* (Sojàk) Larridon

Cyperus sect. *Exaltati* (Kunth) C.B.Clarke

Cyperus sect. *Fastigiati* Kük.

Cyperus sect. *Flavescentes Kük.* nom. superfl.

Cyperus sect. *Fusci* (Kunth) C.B.Clarke

Cyperus sect. *Globosi* (C.B.Clarke) Kük.

Cyperus sect. *Haspani* (Kunth) C.B.Clarke

Cyperus sect. *Lancei* Kük.

Cyperus sect. *Latispicati* Kük.

Cyperus sect. *Leucocephali* Cherm. ex Kük.

Cyperus sect. *Luzuloidei* (Kunth) C.B.Clarke

Cyperus sect. *Muricati* Kük. nom. illeg.

Cyperus sect. *Oxycaryum* (Nees) Larridon

Cyperus sect. *Papyri* (Willd.) Thouars

Cyperus sect. *Polystachyi* (C.B. Clarke) Kük.

Cyperus sect. *Propinqui* (C.B.Clarke) Kük.

Cyperus sect. *Pumili* Kük.

Cyperus sect. *Rhizomatosi* Kük.

Cyperus sect. *Rotundi* C.B.Clarke

Cyperus sect. *Rupestres* C.B.Clarke

Cyperus sect. *Sulcati* Kük., nom. illeg.

Cyperus soyauxii Boeck.

Cyperus sphacelatus Rottb.

Cyperus spiralis Larridon

*Cyperus squarrosu*s L.

Cyperus stramineus Nees

Cyperus strigosus L.

Cyperus subg. *Anosporum* (Nees) C.B.Clarke

Cyperus subg. *Chlorocyperus* (Rikli) Schischkin,
      nom. inval.

Cyperus subg. *Choristachys* C.B.Clarke

Cyperus subg. *Cyperus*

Cyperus subg. *Eucyperus* Griseb.

Cyperus subg. *Juncellus* Griseb.

Cyperus subg. *Micromariscus* Lye

Cyperus subg. *Pycnostachys* C.B.Clarke

Cyperus subgenus *Fimbricyperus* Lye

Cyperus sumbawangensis (Hoenselaar) Lye

Cyperus tenellus L.f.

Cyperus tenuis Sw.

Cyperus textilis Thunb.

Cyperus ustulatus A.Rich.

Cyperus vestitus Hochst. ex C.Krauss

Cyperus waterlotii Cherm.

Cyperus zonatissimus Kük.

Desmoschoenus Hook.f.
Diplocarex Hayata
Dracaena Vand. ex L.
Dracoscirpoides Muasya
Dracoscirpoides surculosa Muasya
Dulichieae Rchb. ex J. Schultze-Motel
Dulichium arundinaceum (L.) Britton
Dulichium Pers.
Ecdeiocoleaceae D.F.Cutler & Airy Shaw
Eleocharideae Goetgh.
Eleocharis acutangula (Roxb.) Schult.
Eleocharis R.Br.
Eleocharis variegata (Poir.) C.Presl
Ericaceae Juss.
Eriocaulaceae Martinov.
Eriocaulon L.
Eriophorum L.
Eriophorum latifolium Hoppe
Erioscirpus Palla
Eucyperus Rikli
Euphorbia L.
Evandra R.Br.
Exocarya Benth.
Exochogyne C.B.Clarke
Ficinia Schrad.
Ficinia gracilis Schrad.
Ficinia lipocarphioides Kük.
Fimbristylis dichotoma (L.) Vahl
Fimbristylis Vahl
Flagellariaceae Dumort.
Fuirena Rottb.
Fuirena abnormalis C.B.Clarke
Fuirena ciliaris (L.) Roxb.
Fuirena umbellata Rottb.
Fuireneae Rchb. ex Fenzl
Galilea Parl.
Gymnoschoenus Nees
Hellmuthia Steud.
Hemicarpha isolepis Nees
Hemicarpha Nees
Hypolytreae Nees ex Wight & Arn.
Hypolytrum Rich. ex Pers.
Isolepis R.Br.
Isolepis fluitans (L.) R.Br.
Isolepis levynsiana Muasya & D.A.Simpson
Joinvilleaceae Toml. & A.C.Sm.

Juncaceae Juss.
Juncellus C.B.Clarke
Juncellus pustulatus (Vahl) C.B.Clarke
Khaosokia D.A. Simpson
Kobresia Willd.
Kyllinga Rottb.
Kyllinga alata Nees
Kyllinga brevifolia Rottb.
Kyllinga bulbosa P.Beauv.
Kyllinga chlorotropis Steud.
Kyllinga nemoralis (J.R.Forst. & G.Forst.)
    Dandy ex Hutch. & Dalziel
Kyllinga odorata Vahl.
Kyllinga polyphylla Willd. ex Kunth
Kyllinga pumila Michx.
Kyllinga squamulata Vahl
Kyllingiella R.W.Haines & Lye
Kyllingiella polyphylla (A.Rich.) Lye
Lagenocarpus amazonicus (C.B. Clarke) H.
    Pfeiff.
Lipocarpha R.Br.
Lipocarpha albiceps Ridl.
Lipocarpha chinensis (Osbeck) J.Kern
Lipocarpha comosa J.Raynal
Lipocarpha filiformis (Vahl) Kunth
Lipocarpha hemisphaerica (Roth) Goetgh.
Lipocarpha kernii (Raymond) Goetgh.
Lipocarpha micrantha (Vahl) G.C.Tucker
Lipocarpha nana (A.Rich.) Cherm.
Lipocarpha rehmanii (Ridl.) Goetgh.
Lipocarpha salzmanniana Steud.
Lipocarpheae Chapm.
Luzula DC.
Luzula abyssinica Novikov.
Mapania Aubl.
Mapanioideae C.B.Clarke
Mariscus Scop.
Mayaca fluviatilis Aubl.
Mayacaceae Kunth
Microdracoides Hua
*Microdracoides squamosu*s Hua
Neesenbeckia Levyns
Nemum Desv. ex Ham.
Nemum angolense (C.B.Clarke) Larridon &
    Goetgh.
Nemum atracuminatum Larridon, Reynders &
    Goetgh.

Nemum capitatum S.S.Hooper ex Larridon & Goetgh.

Nemum raynalii S.S.Hooper ex Larridon & Goetgh.

Nemum spadiceum (Lam.) Desv. ex Ham.

Oxycaryum Nees

Paramapania Uittien

Paramapania gracillima (Kük. & Merr.) Uittien

Paramapania parvibractea (C.B.Clarke) Uittien

Peperomia Ruiz. & Pav.

Piperaceae Giseke

Poaceae Samhart

Poales Small

Prionium serratum Baill.

Pycreus P.Beauv.

Pycreus acaulis Nelmes

Pycreus africanus (S.S.Hooper) Reynders

Pycreus alleizettei Cherm.

Pycreus atribulbus (Kük.) Napper

Pycreus atronervatus (Boeck.) C.B. Clarke

Pycreus atropurpureus C.B.Clarke

Pycreus atrorubidus Nees

Pycreus betschuanus (Boeck.) C.B. Clarke

Pycreus bipartitus (Torr.) C.B.Clarke

Pycreus capillifolius (A.Rich.) C.B.Clarke

Pycreus cataractarum C.B.Clarke

Pycreus cooperi C.B.Clarke

Pycreus decumbens T.Koyama

Pycreus demangei J.Raynal

Pycreus diander (Torr.) C.B.Clarke

Pycreus diloloensis Kük. ex Cherm.

Pycreus divulsus (Ridl.) C.B.Clarke

Pycreus divulsus subsp. *africanus* S.S.Hooper

Pycreus elegantulus (Steud.)C.B.Clarke

Pycreus felicis J.Raynal

Pycreus ferrugineus (Boeck.) C.B. Clarke

Pycreus fibrillosus (Kük.) Cherm.

Pycreus flavescens (L.) P.Beauv.ex Rchb.

Pycreus flavescens subsp. *intermedius* (Rikli) Lye

Pycreus flavescens subsp. *microglumis* Lye

Pycreus flavidus (Retz.) T.Koyama

Pycreus flavidus var. *nilagiricus* (Hochst. ex Steud.) Karthik.

Pycreus fontinalis Cherm.

Pycreus gracillimus Chiov.

Pycreus heterochrous Nelmes

Pycreus hildebrandtii C.B.Clarke

Pycreus holosericeus Merr.

Pycreus intactus (Vahl) J. Raynal

Pycreus lanceolatus (Poir.) C.B.Clarke

Pycreus longistolon (Peter & Kük.) Napper

Pycreus macranthus (Boeck.) C.B. Clarke

Pycreus macrostachyos (Lam.) J.Raynal

Pycreus malabaricus C.B.Clarke

Pycreus megapotamicus (A.Dietr.) Nees

Pycreus melanacme Nelmes

Pycreus melas (Ridl.) C.B.Clarke

Pycreus micromelas Lye

Pycreus mortonii S.S.Hooper

Pycreus mundtii Nees

Pycreus muricatus (Kük.) Napper

Pycreus niger subsp. *elegantulus* (Steud.) Lye

Pycreus nigricans (Steud.) C.B.Clarke

Pycreus nitidus (Lam.) J.Raynal

Pycreus nuerensis (Boeck.) S.S.Hooper

Pycreus overlaetii Cherm. ex S.S.Hooper

Pycreus pauper (Hochst. ex Rich.) C.B. Clarke

Pycreus pelophilus (Ridl.) C.B.Clarke

Pycreus permutatus (Boeck.) Napper

Pycreus poikilostachys Nelmes

Pycreus polystachyos (Rottb.) P.Beauv.

Pycreus polystachyos subsp. *holosericeus* (Rottb.) P. Beauv.

Pycreus pumilus (L.) Nees

Pycreus reductus Cherm.

Pycreus rehmannianus C.B.Clarke

Pycreus rhizomatosus C.B.Clarke

Pycreus sanguineosquamatus Van der Veken

Pycreus sanguinolentus (Vahl) Nees

Pycreus scaettae Cherm.

Pycreus scaettae var. *katangensis* Cherm.

Pycreus sect. *Tuberculati* Cherm.

Pycreus sect. *Vestitae* C.B.Clarke

Pycreus smithianus (Ridl.) C.B.Clarke

Pycreus subg. *Reticulatae* C.B.Clarke

Pycreus sumbawangensis Hoenselaar

Pycreus tener C.B. Clarke

Pycreus unioloides (R.Br.) Urb.

Pycreus vanderystii Cherm.

Pycreus vicinus Cherm.

Pycreus waillyi Cherm.

Pycreus xantholepis Nelmes

Pycreus zonatissimus Cherm.

Pycreus zonatus Cherm.

Queenslandiella Domin

Queenslandiella hyalina (Vahl) Ballard

Rapateaceae Dumort.

Remirea Aubl.

Remirea maritima Aubl.

Restionaceae R.Br.

Rhynchospora Vahl

Rhynchospora colorata (L.) H.Pfeiff.

Rhynchospora corymbosa (L.) Britton

Rhynchospora macrostachya Torr. ex A.Gray

Rhynchospora rubra subsp. *rubra* (Lour.) Makino

Rhynchospora sect. *Haplostylis* (Nees) Benth.

Rhynchosporeae Wight & Arn.

Rikliella J.Raynal

Ruscaceae Sprengl. ex Hutch.

Salvia L.

Sansevieria trifasciata Prain

Schoeneae Dumort.

Schoenoplectiella Lye

Schoenoplectus (Rchb.) Palla

Schoenoplectus corymbosus (Roth ex Roem. & Schult.) J.Raynal

Schoenus nigricans L.

Scirpeae Kunth ex Dumort.

Scirpodendron Sprengl. ex Hutch.

Scirpodendron ghaerii (Gaertn.) Merr.

Scirpoides Ség.

Scirpoides holoschoenus (L.) Soják

Scirpus L.

Scirpus falsus C.B.Clarke

Scirpus ficinioides Kunth

Scirpus sylvaticus L.

Scleria P.J. Bergius

Scleria depressa (C.B.Clarke) Nelmes

Sclerieae Kunth ex Fenzl

Sorostachys Steud.

Sparganium L.

Sphaerocyperus Lye

Sphaerocyperus erinaceus (Ridl.) Lye

Styphelioideae Sweet

Tetraria   P.Beauv.

Tetraria thermalis (L.) C.B.Clarke

Tetrariopsis C.B.Clarke

Thurniaceae Engl.

Torulinium Desv. ex Ham.

Tradescantia zebrina Bosse

Trichophorum Pers.

Trilepideae Goetgh.

Trilepis Nees

Typha L.

Typhaceae Juss.

Uncinia Pers.

Volkeria messelensis S. Y. Smith, Collinson, Simpson, Rudall, Marone & Stampanoni

Volkiella Merxm. & Czech

Volkiella disticha Merxm. & Czech

Websteria S.H.Wright

Xyridaceae C.Agardh

Xyris Gronov. ex L.

Zea mays L.

Appendix III Sequences

List of the samples used in the molecular phylogenetic study (see chapter 6) with species names, voucher information (*leaf sample courtesy of the collector A.M. Muasya), origin and GenBank accession numbers for the sequences. In addition single marker trees are added at the end of this appendix. Allignments can be downloaded at: https://www.researchgate.net/profile/Marc_Reynders/contributions/

Taxon	Voucher (herbarium)	Origin	ETS1f	trnH-psbA	rpl32-trnL
Alinula paradoxa	Reid 1027 (GENT)	South Africa	HQ705964	-	HQ705894
Ascolepis brasiliensis	Larridon et al. 2010-0304 (GENT)	Madagascar	HE993954	HE993894	HE993685
Ascolepis eriocauloides	De Wilde s.n. (BR)	Congo	HE993955	HE993895	-
Ascolepis hemisphaerica	Reekmans 6729 (GENT)	Burundi	HE993956	-	-
Ascolepis protea	Malaisse & Kisimba 695 (GENT)	Congo	HE993957	HE993896	HE993686
Ascolepis pusilla	Malaisse & Goetghebeur 846 (GENT)	Congo	HE993958	HE993897	-
Cyperus alopecuroides	Hess 52/1581 (GENT)	Angola	HE993959	HE993898	HE993687
Cyperus alternifolius	Goetghebeur 11516 (GENT)	BG Ghent	HQ705948	HQ705818	HQ705878
Cyperus aterrimus	Muasya & Ramdhani 2722 (BOL)	South Africa	HE993960	HE993899	HE993688
Cyperus bulbosus	Laegaard et al. 17024 (GENT)	Senegal	HE993961	HE993900	HE993689
Cyperus capitatus	Goetghebeur 10744 (GENT)	BG Ghent	HE993962	HE993901	HE993690
Cyperus compressus	Reynders & Sabulao 15 (GENT)	Philippines	HE993963	HE993902	HE993691
Cyperus congestus	Goetghebeur 11988 (GENT)	BG Ghent	HE993964	HE993903	HE993692
Cyperus croceus	Rostad s.n. (GENT)	USA	HE993965	HE993904	-
Cyperus cuspidatus	Jongkind & Nieuwhuis 2847 (GENT)	Ghana	HQ705954	HQ705823	HQ705884
Cyperus dives	Muasya et al. 2529 (KA)	Kenya	HE993966	HE993905	HE993693
Cyperus dubius	Muasya & Muthama 1251 (EA)	Kenya	HE993967	-	HE993694
Cyperus elegans	Goetghebeur 5601 (GENT)	Cuba	HQ705959	HQ705827	HQ705889
Cyperus endlichii	Muasya & Knox 954 (EA)	Tanzania	HE993968	-	-
Cyperus esculentus	Goetghebeur 11303 (GENT)	BG Nantes, BG Ghent	HQ705960	HQ705828	HQ705890
Cyperus filiculmis	Carter 4355 (GENT)	Florida	HE993969	HE993906	HE993695
Cyperus fulgens	Goetghebeur 4329 (GENT)	South Africa	HE993970	HE993907	HE993696

Cyperus haspan	Muasya & Muthama 1269 (EA)	Kenya	HQ705927	HQ705803	HQ705803
Cyperus impubes var. fallax	Dhondt 9 (GENT)	Madagascar	HE993971	-	HE993697
Cyperus iria	Desmet 77/13 (GENT)	Burkina Faso	HE993972	HE993908	HE993698
Cyperus javanicus	Reynders & Sabulao 60 (GENT)	Philippines	HE993973	-	HE993699
Cyperus kerstenii	Muasya 984 (EA, K; Muasya et al., 2002)	Kenya	HQ705961	HQ705829	HQ705891
Cyperus laevigatus 053	Goetghebeur 10201 (GENT)	Morocco, BG Ghent	HE993975	HE993910	-
Cyperus laevigatus 138	Larridon et al. 2009-0033 (GENT)	Kenya	HE993974	HE993909	HE993700
Cyperus laevigatus 142	Goetghebeur 10202 (GENT)	Morocco, BG Ghent	HE993976	HE993911	HE993701
Cyperus longus	Farjon 217 (GENT)	Netherlands	HE993977	HE993912	HE993702
Cyperus luzulae	Van den Eynden 213 (GENT)	Ecuador	HQ705910	-	HQ705846
Cyperus marginatus	Larridon et al. 2009-0076 (GENT)	Kenya	HQ705949	HQ705819	HQ705879
Cyperus meeboldii	Kilian & Lobin 6848 (GENT)	Somalia	HE993978	HE993913	HE993703
Cyperus meyenianus	Fosberg 47227 (GENT)	Hawaii	HE993979	HE993914	HE993704
Cyperus papyrus	Goetghebeur 5866 (GENT)	BG Ghent	HQ705962	HQ705830	HQ705892
Cyperus pectinatus	Larridon et al. 2010-0265 (GENT)	Madagascar	HQ705936	HQ705810	HQ705869
Cyperus pustulatus	Porembski 624 (GENT)	Ivory Coast	HE993980	HE993915	HE993705
Cyperus rigidifolius	Samain 2005-001 (GENT)	Kenya	HE993981	HE993916	HE993706
Cyperus rotundus	Shaw 890 (K*)	Hong Kong (China)	HQ705963	HQ705831	HQ705893
Cyperus rubiginosus	Unknown s.n. (GENT)	Ecuador	HE993982	HE993917	HE993707
Cyperus rupestris	Laegaard 15909 (GENT)	Zimbabwe	HE993983	HE993918	HE993708
Cyperus sp.	Goetghebeur 5965 (GENT)	BG Ghent	HE993985	HE993920	HE993710
Cyperus sphacelatus	Goetghebeur 4908 (GENT)	Cameroon	HE993984	HE993919	HE993709
Cyperus spiralis	Muasya & Muthama 1247 (EA)	Kenya	HQ705953	HQ705822	HQ705883
Cyperus strigosus	BG 20051035G (GENT)	BG Poznan, BG Ghent	HE993986	HE993921	HE993711
Cyperus waterlotii	Larridon et al. 2010-0010 (GENT)	Madagascar	HQ705955	HQ705824	HQ705885
Cyperus waterlotii	Larridon et al. 2010-0043 (GENT)	Madagascar	HQ705956	HQ705825	HQ705886
Ficinia gracilis	Muasya 2713 (BOL)	South Africa	HQ705902	HQ705784	HQ705839
Isolepis fluitans	Muasya & Knox 3195 (EA)	Kenya	HQ705901	HQ705783	HQ705838
Kyllinga alata	Acocks 22902 (BR)	South Africa	HE993987	-	HE993712
Kyllinga brevifolia	Reynders and Sabulao	Philippines,	HE993988	HE993922	HE993713

	68 (GENT)	BG Ghent			
Kyllinga bulbosa	Goetghebeur 11989 (GENT)	BG Ghent	HE993989	-	HE993714
Kyllinga chlorotropis	Muasya & Gerhke 2606 (EA)	Kenya	HE993990	HE993923	HE993715
Kyllinga nemoralis	Goetghebeur 11518 (GENT)	Philippines, BG Ghent	HQ705965	HQ705832	HQ705895
Kyllinga odorata	Strong 3485 (GENT)	USA	HE993991	HE993924	HE993716
Kyllinga polyphylla	Beeckman Z35 (GENT)	Congo	HE993992	HE993925	HE993717
Kyllinga pulchella	Muasya & Knox 991 (EA)	Kenya		HE993926	HE993718
Lipocarpha albiceps	Hess 52/195 (GENT)	Angola	HE994025	HE993944	HE993748
Lipocarpha chinensis	Reynders & Sabulao 26A (GENT)	Philippines	HE994029	HE993948	HE993752
Lipocarpha comosa	Mincier 1027 (GENT)	Zambia	HE994028	HE993947	HE993751
Lipocarpha filiformis	Vanden Berghen 7913a (GENT)	Senegal	HE994030	HE993949	HE993753
Lipocarpha kernii	Laegaard 21195 (GENT)	Burkina Faso	HE994026	HE993945	HE993749
Lipocarpha micrantha	Luceño 186 (GENT)	Brazil	HE994032	HE993951	-
Lipocarpha nana	Larridon et al. 2010-0041A (GENT)	Madagascar	HE994031	HE993950	HE993754
Lipocarpha rehmannii	Larridon et al. 2010-0320 (GENT)	Madagascar	HE994027	HE993946	HE993750
Lipocarpha salzmanniana	Luceño 28 (GENT)	Brazil	HE994033	HE993952	-
Pycreus africanus	Leeuwenberg 8527 (GENT)	Congo	HE993994	HE993927	-
Pycreus alleizettei	Larridon et al. 2010-0299 (GENT)	Madagascar	HE993993	-	HE993719
Pycreus bipartitus	Goetghebeur 11990 (GENT)	BG Ghent	HE993995	HE993928	HE993720
Pycreus capillifolius	Muasya & Knox 999 (EA)	Kenya	HE993996	-	HE993721
Pycreus cataractarum	De Wilde 1452 (GENT)	Cameroon	HE993997	-	HE993722
Pycreus elegantulus	Unknown 348 (GENT)	Kenya	HE993998	HE993929	HE993723
Pycreus fibrillosus	Schmitz 7479 (GENT)	Congo	HE994005	-	HE993729
Pycreus flavescens	Goetghebeur 10224 (GENT)	BG Ghent	HE993999	HE993930	HE993724
Pycreus flavescens subsp. *microglumis*	Malaisse & Goetghebeur 390 (GENT)	Congo	HE994000	HE993931	-
Pycreus flavidus	Reynders & Sabulao 45 (GENT)	Philippines	HE994001	HE993932	HE993726
Pycreus gracillimus	Lewalle 2112 (GENT)	Burundi	HE994002	HE993933	-
Pycreus intactus	Reid 609 (GENT)	South Africa	HE994003	-	HE993727
Cyperus 'Pycreus' juncelliformis	Malaisse & Goetghebeur 409 (GENT)	Congo	HE994004	-	HE993728
Pycreus longistolon	Muasya & Knox 1027 (EA)	Kenya	HE994006	HE993934	HE993730
Pycreus macranthus	Edwards 1038 (GENT)	South Africa	HE994007		HE993731
Pycreus	Muasya with Kirika,	Kenya	HE994008	HE993935	HE993732

macrostachyos	Obunyali & Musili 2471 (EA)				
Pycreus megapotamicus	Goetghebeur 4826 (GENT)	Argentina	HE994009	-	HE993733
Pycreus melanacme	Richards 8409 (GENT)	Congo	HE994010	HE993936	
Pycreus melas	Robinson 3478 (GENT)	Zambia	HE994011	-	HE993734
Pycreus micromelas	Robinson 2310 (GENT)	Zambia	HE994012	-	HE993735
Pycreus mundtii	Muasya & Knox 1018 (EA)	Kenya	HE994013	HE993937	HE993736
Pycreus nigricans	Unknown 368 (GENT)	Kenya	HE994014	-	HE993737
Pycreus nuerensis	Muasya & Knox 940 (EA)	Tanzania	HE994015	HE993938	HE993738
Pycreus pauper	Milne-Redhead & Taylor 9184 (GENT)	Tanzania	HE994016	-	HE993739
Pycreus pelophilus	Muasya & Muthama 1263 (EA)	Kenya	HE994017	HE993939	HE993740
Pycreus polystachyos	Goetghebeur 11519 (GENT)	South Africa, BG Ghent	HQ705966	HQ705833	HQ705896
Pycreus polystachyos subsp. *holosericeus*	Reynders and Sabulao 64 (GENT)	Philippines, BG Ghent	-	-	HE993741
Pycreus pumilus	Muasya & Muthama 1264 (EA)	Kenya	HE994018	-	HE993742
Pycreus reductus	Dhondt 11 (GENT)	Congo	HE994020	HE993940	HE993744
Pycreus reductus	Larridon et al. 2010-0161 (GENT)	Madagascar	HE994019		HE993743
Pycreus rehmannianus	Muasya & Knox 1022 (EA)	Kenya		HE993941	HE993725
Pycreus rhizomatosus	Gereau & Dumetz 3259 (GENT)	Madagascar	HE994021	HE993942	-
Pycreus sanguinolentus	Kwika & Mundi 21 (GENT)	Kenya	HE994022	-	HE993745
Pycreus smithianus	Reekmans 7531 (GENT)	Burundi	HE994023	HE993943	HE993746
Pycreus xantholepis	Reekmans 9809 (GENT)	Burundi	HE994024	-	HE993747
Queenslandiella hyalina	Muasya 2490 (EA)	Kenya	HQ705967	HQ705834	HQ705897
Remirea maritima	Faden et al. 96/48 (K*; Muasya et al., 2002)	Tanzania	HQ705968	HQ705835	HQ705898
Scirpoides holoschoenus	Goetghebeur 11520 (GENT)	BG Porto, BG Ghent	HQ705900	HQ705782	HQ705837
Sphaerocyperus erinaceus	Faden et al. 96/358 (K*; Muasya et al., 2002)	Tanzania	HQ705969	HQ705836	HQ705899
Volkiella disticha	Müller & Giess 493 (GENT)	Namibia	HE994034	HE993953	HE993755

Fig. A.3.1. 50% majority consensus single marker ETS1f BI tree with the associated PP values
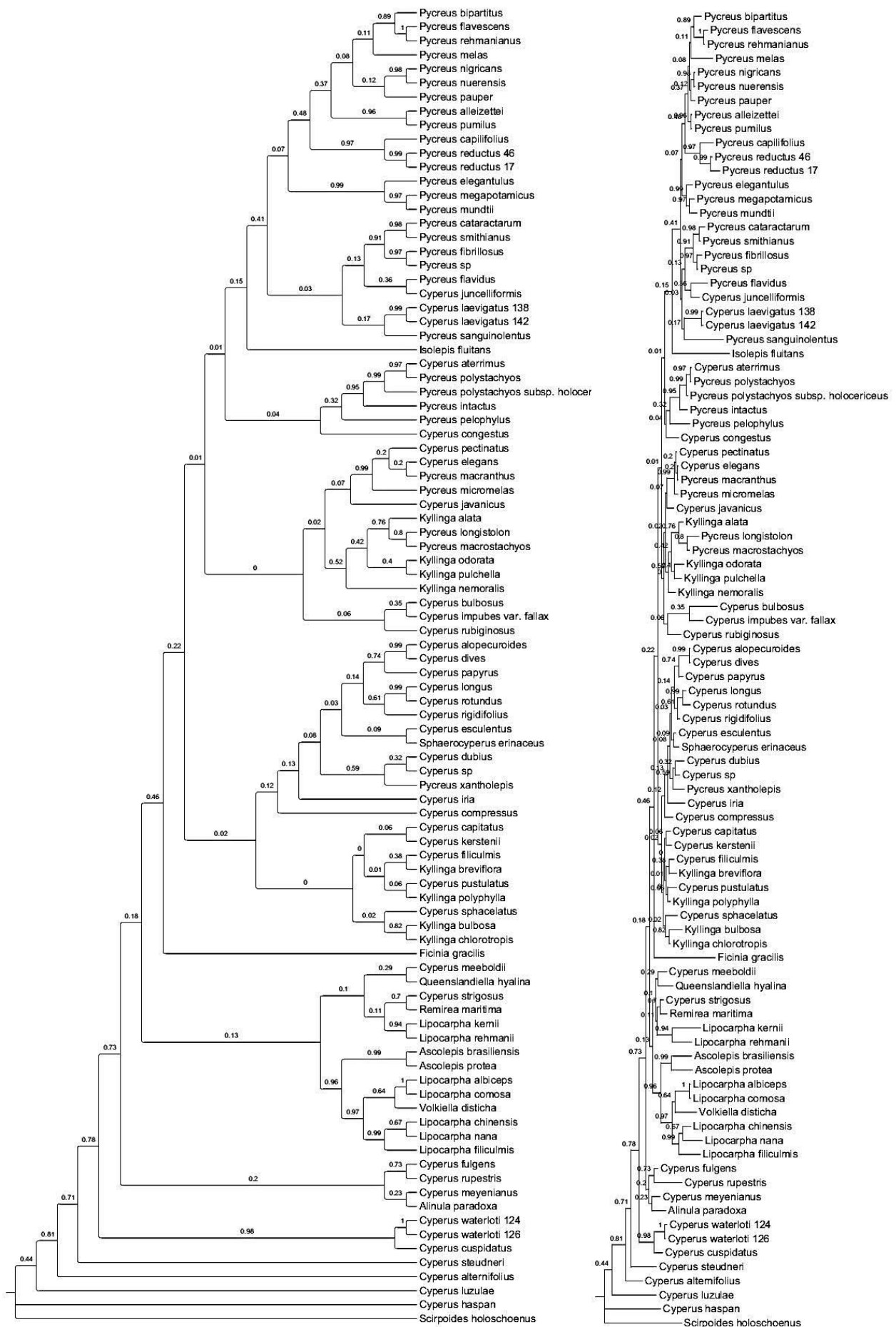
347

Fig. A.3.2. 50% majority consensus single marker *rpl32-trnL* BI tree with the associated PP values
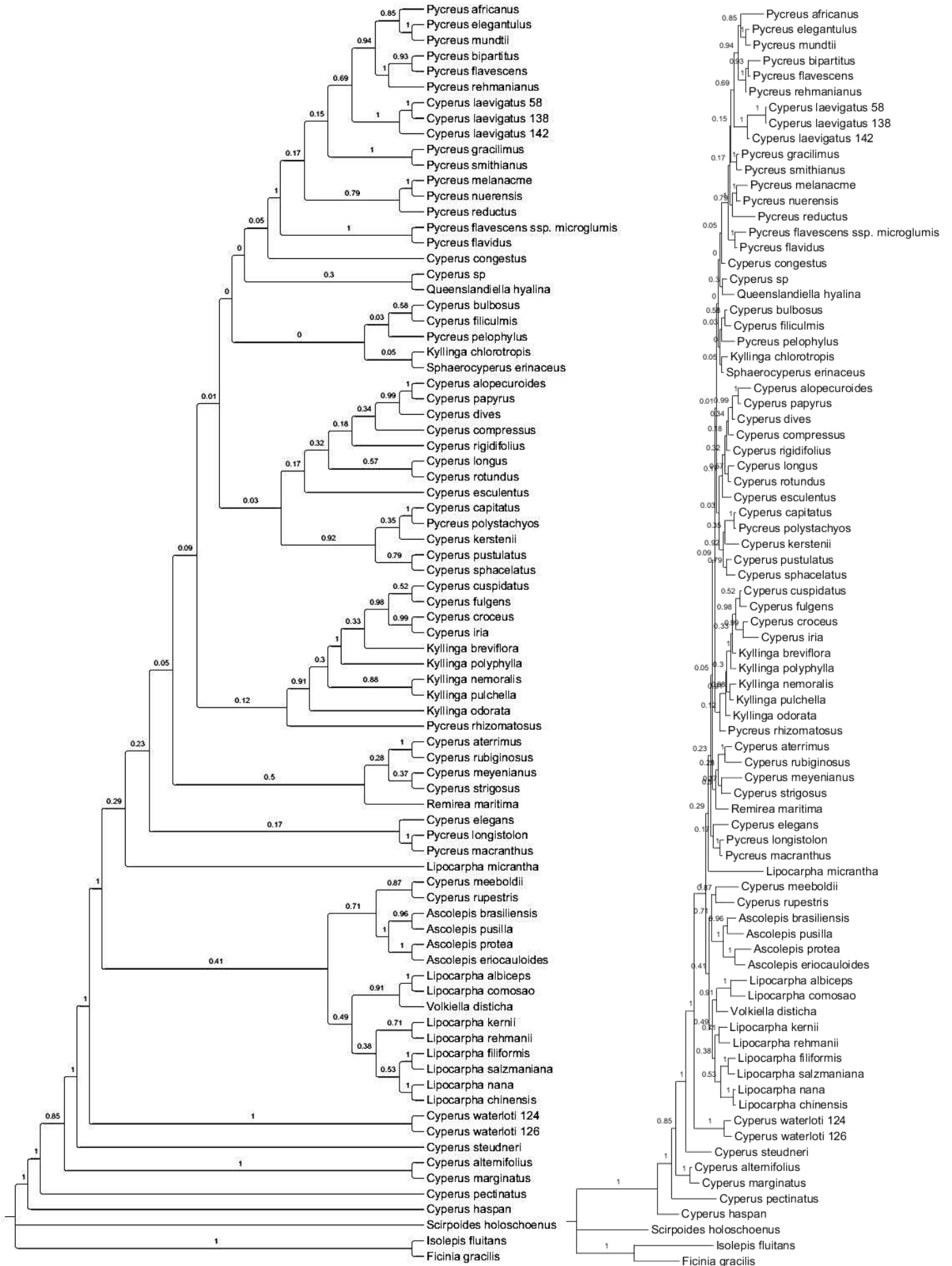
Fig. A.3.3. 50% majority consensus single marker *psbA - trnH* BI tree with the associated PP values

Appendix IV Curriculum Vitae

Personal data

Name:	**Marc Reynders**
Adress:	**J. de Bethunelaan 20,**
	9800 Deinze
Tel:	**09 329 07 89**
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ResearchGate: **Link: https://www.researchgate.net/profile/Marc_Reynders/**

Work experience

Period	Function	Organisation
16/01/2014 – current	Scientific assistant for the management of the greenhouse collections	**Agentschap Plantentuin Meise,** Meise
14/02/2013 – 15/01/2014	Ecology advisor	**Antea Group,** Antwerp
16/04/2012 – 19/12/2012	Lecturer	**Hogeschool Gent**, Ghent
	1st & 2nd Bachelor Landscape- and Garden Architecture: plant knowledge, botany, vegetation biology and green management.	School of Arts
01/10/2009 - 30/09/2011	Assistant	**Ghent University**, Ghent
	1. Education (50%) 2. Research (48%) 3. Service (2%)	Faculty of Sciences Research Group Spermatophytes:
01/10/2004 -30/09/2008	BOF-researcher	See website: www.spermatophytes.ugent.be
	1. Research (88%) 2. Education (10%) 3. Service (2%)	
12/10/2008 – 30/06/2009 07/11/2011 - 10/03/2012	Science teacher (Secondary school), several assignments	Flemish Ministry for Education

Education

Period	Subject	Shool
01/10/2004 – 18/10/2013	**PhD in sciences**, Biology	Ghent University, Ghent
	Dissertation: T*he challenging taxonomy and evolution of C4 Cyperus (Cyperaceae): A focus on Pycreus and its remarkable laterally flattened pistils.*	
01/10/2002 - 30/06/2004	**Master in Biology**, option Botany	Ghent University, Ghent
	Thesis: *De Cyperaceae van Rwanda en Burundi: bijdrage tot een nieuwe flora*	
	Degree: cum magna laude	
01/10/2000 - 30/06/2002	**Bachelor in Biology**	University of Hasselt, Diepenbeek
	Degree: cum maxima laude	
01/09/1994 - 30/06/2000	**Latin-Math-Sciences**	Heilig Grafinstituut, Bilzen
2005-2006	**Advanced Academic English**: writing skills – Natural Sciences	Universitair Talencentrum, Gent

Publications

PUBLICATIONS as first author

1. **Reynders, M.** & Vrijdaghs, A., Larridon, I., Huygh, W., Leroux, O., Muasya, AM., Goetghebeur, P. (2012). Gynoecial anatomy and development in Cyperoideae (Cyperaceae, Poales): congenital fusion of carpels facilitates evolutionary modifications in pistil structure. *Plant Ecology and Evolution* 145(1): 1-30.

2. **Reynders, M.,** Huygh, W., Larridon, I., Muasya, AM., Govaerts, R., Simpson, DA., Goetghebeur, P. (2011) Nomenclature and typification of names of genera and subdivisions of genera in Cypereae (Cyperaceae): 3. Names of subdivisions in segregate genera of *Cyperus*. *Taxon* 60: 885–895.

3. **Reynders, M.** & Goetghebeur, P. (2010) Reestablishment of *Pycreus* section *Tuberculati* (Cyperaceae). *Blumea* 55: 226–230.

4. **Reynders, M.**, Dhooge, S. & Goetghebeur, P. (2006) A new Central African species, *Cyperus vandervekenii* (Cyperaceae) from the sources of the Nile in Rwanda. *Novon* 16: 512–515.

PUBLICATIONS as second author

5. Muasya, AM., **Reynders, M.**, Goetghebeur, P., Simpson, DA., Vrijdaghs, A. (2012) Dracoscirpoides (Cyperaceae) – a new genus from Southern Africa, its taxonomy and floral ontogeny. *South African Journal of Botany*. 78(1) 104-112.

6. Larridon, I., **Reynders, M.,** Huygh, W., Bauters, K., Vrijdaghs, A., Leroux, O., Muasya, AM., Goetghebeur, P. (2011). Taxonomic changes in C₃ *Cyperus* (Cyperaceae) supported by molecular phylogenetic data, morphology, embryology, ontogeny and anatomy. *Plant Ecology and Evolution* 144(3): 327-356.

7. Larridon, I., **Reynders, M.,** Huygh, W., Bauters, K., Van de Putte, K., Muasya, AM., Boeckx, P., Simpson, DA., Vrijdaghs, A., Goetghebeur, P. (2011) Affinities in C₃ *Cyperus* lineages (Cyperaceae) revealed using molecular phylogenetic data and carbon isotope analysis. *Botanical Journal of the Linnean Society* 167: 19–46.

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

9. Bauters, K., **Reynders, M.**, Larridon, I., Huygh, W. & Goetghebeur, P. (2010) Two New Species of *Cyperus* (Cyperaceae) from the Zambezian Region of Africa. *Novon* 20: 133-138.

10. Vrijdaghs, A., **Reynders, M.,** Larridon, I., Muasya, A. M., 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.

11. Larridon, I., **Reynders, M.** & Goetghebeur, P. (2008) Novelties in *Nemum* (Cyperaceae). *Belg. J. Bot*. 141: 129-149.

12. Larridon, I., **Reynders, M.** & Goetghebeur, P. (2008) *Cyperus limiticola*, a New Name for a Madagascan *Cyperus* (Cyperaceae). *Novon* 18(2): 187-188.

13. Verloove, F. & **Reynders, M.** (2007) Studies within the genus *Paspalum* (Paniceae, Poaceae) in Europe – 2. The *Quadrifaria* group. *Willdenowia* 37: 423-430.

14. Verloove, F. & **Reynders, M.** (2007) Studies within the genus *Paspalum* (Paniceae, Poaceae) in Europe: 1. *Paspalum distichum* subsp. *paucispicatum*, an overlooked taxon in France. *Willdenowia* 37: 199-204.

15. Samain, M.-S., **Reynders, M.** & Goetghebeur, P. (2006) *Kyllinga beninensis* (Cyperaceae), a new species from Bénin. *Novon* 16: 516–519.

PUBLICATIONS as third author

16. Larridon I., Bauters K., Huygh W., **Reynders M.** & Goetghebeur P. Taxonomic changes in C₄ *Cyperus* (Cypereae, Cyperoideae, Cyperaceae): reducing the sedge genera *Ascolepis*, *Kyllinga* and *Pycreus* into *Cyperus* sensu lato. Submitted for publication in *Phytotaxa*.

17. Bauters K., Larridon I., **Reynders M.,** Huygh W., Asselman P., Vrijdaghs A., Muasya A.M., Goetghebeur P. Infrageneric classification of Lipocarpha (Cypereae, Cyperoideae, Cyperaceae): based on morphological, developmental and molecular evidence. Submitted for publication in Phytotaxa.

18. Larridon I, Bauters K, **Reynders M**, Huygh W, Muasya AM, Simpson DA, Goetghebeur P. (2013) Towards a new classification of the giant paraphyletic genus *Cyperus* (Cyperaceae): phylogenetic relationships and generic delimitation in C₄ *Cyperus*. *Botanical Journal of the Linnean Society* 172(1):106-126.

19. Muasya, AM., Larridon, I, **Reynders, M.**, Huygh, W., Goetghebeur P., Cable S., Simpson, DA., Gehrke, B. (2011). The Cyperaceae in Madagascar show high diversification in high altitude forest and wetland habitats. *Plant Ecology and Evolution* (AETFAT proceedings). 144(3): 357-362.

20. Larridon, I., Huygh, W., **Reynders, M.**, Muasya, AM., Govaerts, R., Simpson, DA., Goetghebeur, P. (2011) Nomenclature and typification of names of genera and subdivisions of genera in Cypereae (Cyperaceae): 2. Names of subdivisions in *Cyperus*. *Taxon* 60: 868–884.

21. Huygh, W., Larridon, I., **Reynders, M.**, Muasya, A. M., Govaerts, R., Simpson, D.A. & Goetghebeur, P. (2010) Nomenclature and typification of names of genera and subdivisions of genera in Cypereae (Cyperaceae): 1. Names of genera in the *Cyperus* clade. *Taxon* 59: 1883–1890.

22. Mathieu, G., Samain, M.-S., **Reynders, M.** & Goetghebeur, P. (2008) Taxonomy of the *Peperomia* species (Piperaceae) with pseudo-epiphyllous inflorescences, including four new species. *Bot. J. Linn. Soc*. 157: 177-196.

23. Prata, A.P., Camelbeke, K., **Reynders, M.**, Goetghebeur, P. & Huber, O. (2007) *Bulbostylis medusae* (Cyperaceae), a new species from Venezuela. *Novon* 17: 67-71.

Congresses and symposia

Period	Congress	Location
03/05/2005	PhD symposium Faculty of Sciences ABSTRACT ***Reynders,* M.,** P. Goetghebeur & L. Majesky. (2005) Nutlet anatomy, a key character for the taxonomy of Pycreus (Cyperaceae). In: Doctoraatssymposium Faculteit Wetenschappen, 3 mei 2005: Book of Abstracts: p. 113, Universiteit Gent. (poster; nomination best poster)	ICC International Convention Center, Ghent
17-23/07/2005	International Botanic Congress XVII	Vienna, Austria
29-30/09/2005	Africa's Great Rift: Diversity and Unity ABSTRACT **Reynders, M.,** Goetghebeur, P. (2005) A key to the Cyperaceae of Rwanda and Burundi. In: Africa's Great Rift: Diversity and Unity 29-30 September 2005. Book of Abstracts: Royal Academy for Overseas Sciences, Brussels. (poster)	Brussels
26/02/2007 – 02/03/2007	AETFAT XVIII congress ABSTRACTS **Reynders, M.,** Huygh, W., Muasya, AM., Goetghebeur P. (2007). Phylogeny and evolution of the mainly African genus *Pycreus* (Cyperaceae), based on molecular and morphological data. In: *XVIIIth AETFAT Congress, 26 February – 2 March 2007. Abstracts*: p.45. Yaoundé, Cameroon. 197 p. (poster) Huygh, W., **Reynders, M**., Muasya, M. Vrijdaghs, A. & P. Goetghebeur. (2007). Origin and evolution of the *Cyperaceae* with laterally compressed pistils. In: *XVIIIth AETFAT Congress, 26 February – 2 March 2007. Abstracts*: p.33. Yaoundé, Cameroon. 197 p. (talk) Larridon I., **Reynders, M.** Goetghebeur P. (2007). Systematic and phylogeny of the African genus Nemum (Cyperaceae). In: XVIII AETFAT congress abstracts: p. 35 Yaoundé. 197 p. (poster)	Yaoundé, Cameroon
24/04/2007	PhD symposium Faculty of Sciences ABSTRACTS **Reynders, M., Huygh, W.,** Muasya, AM., Goetghebeur P. (2007). Phylogeny and evolution of the mainly African genus *Pycreus* (Cyperaceae), based on molecular and morphological data. In: Doctoraatssymposium Faculteit Wetenschappen 4 april 20072007. Book of Abstracts: Universiteit Gent. (poster) Huygh, W., **Reynders, M.,** Muasya, M. Vrijdaghs, A. & P. Goetghebeur. (2007). Origin and evolution of	ICC International Convention Center, Ghent

	the *Cyperaceae* with laterally compressed pistils. In: Doctoraatssymposium Faculteit Wetenschappen 4 april 2007: Book of Abstracts: Universiteit Gent. (poster)	
11/08/2008 – 15/08/2008	The fourth International Conference on The Comparative Biology of the Monocotyledons (MONOCOTS IV) & The Fifth International Symposium on Grass Systematics and Evolution	Copenhagen, Danmark

ABSTRACTS

Reynders, M., Muasya, AM., Larridon I., Dludlu, MN., Huygh, W., Vrijdaghs, A., Simpson, DA., Goetghebeur P. (2008) Cypereae revisited – evidence from morphology and DNA data. In: Monocots IV, Abstracts: P. 51. Natural History Museum of Denmark, Copenhagen. 100p. (talk)

Huygh, W., **Reynders, M.,** Larridon, I., Muasya, AM., Vrijdaghs, A., Simpson, DA., Goetghebeur P. (2008) Kyllinga, Cyperaceae's worst nightmare? In: Monocots IV, Abstracts: P. 31. Natural History Museum of Danmark, Copenhagen. 100p. (talk)

Larridon I., **Reynders, M**., Huygh, W., Simpson, DA., Muasya, AM., Goetghebeur P. (2008). Taxonomy and evolution of the *Cyperus* species (Cyperaceae) with C_3 photosynthesis type. In: Monocots IV, Abstracts: P. 35. Natural History Museum of Danmark, Copenhagen. 100p. (talk)

25/04/2010 – 01/05/2010	AETFAT XIX congress	Antananarivo, Madagascar

ABSTRACTS

Reynders, M. & Vrijdaghs, A., Larridon, I., Huygh, W., Leroux, O., Muasya, AM., Goetghebeur, P. (2010). The Cypereae radiation: Pycreus highlighted (Cyperaceae). In XIXth AETFAT Congress – Madagascar, 25-30 April, 2010. Abstracts. Scripta Botanica Belgica 46: 445. National Botanic Garden of Belgium. 510p. (talk)

Reynders, M., Larridon I., Muasya, AM., Goetghebeur P. (2010). Do you understand the fibrillous Pycrei (Cyperaceae)? In XIXth AETFAT Congress – Madagascar, 25-30 April, 2010. Abstracts. Scripta Botanica Belgica 46: 444. National Botanic Garden of Belgium. 510p. (poster)

Huygh, W., Larridon, I., **Reynders, M**., Muasya, AM., Vrijdaghs, A., Simpson, DA., Goetghebeur P. (2010) Easy to see but hard to name! In XIXth AETFAT Congress – Madagascar, 25-30 April, 2010. Abstracts. Scripta Botanica Belgica 46: 218. National Botanic Garden of Belgium. 510p. (talk)

Larridon I., Huygh, W., **Reynders, M**., Vrijdaghs, A., Leroux, O., Boeckx, P., Simpson, DA., Muasya, AM., Goetghebeur P. (2010). The Cyperus Clade! In XIXth AETFAT Congress – Madagascar, 25-30 April, 2010. Abstracts. Scripta Botanica Belgica 46: 261. National Botanic Garden of Belgium. 510p. (talk)

16/09/2010 – 18/09/2010	Botanical Diversity: exploration, understanding and use	National Botanic Garden of Belgium, Meise

ABSTRACTS

Reynders, M., Larridon I., Huygh, W., Muasya, AM., Goetghebeur P. (2010). To genus or not to genus? Radiations and paraphyly in Cypereae! In: Botanical Diversity: exploration, understanding and use, 16-18 September 2010. Programme & Abstracts. Scripta Botanica Belgica 48:109-110. National Botanic Garden of Belgium. 110p. (poster)

Larridon, I., Huygh, W., **Reynders, M.,** Vrijdaghs, A., Leroux, O., Boeckx, P., Simpson, DA., Muasya, AM., Goetghebeur P. (2010). C_3 Cyperus (Cyperaceae). In: Botanical Diversity: exploration, understanding and use, 16-18 September 2010. Programme & Abstracts. Scripta Botanica Belgica 48:62. National Botanic Garden of Belgium. 110p. (poster)

Other abstracts:

1. Larridon I., Huygh, W., **Reynders, M**., Vrijdaghs, A., Boeckx, P., Goetghebeur P. (2009). The Cyperus clade. In: Doctoraatssymposium Faculteit Wetenschappen 2009: Book of Abstracts: p. 144. Universiteit Gent. 193p. (poster)

2. Vrijdaghs, A., **Reynders, M.,** Larridon, I., Muthama Muasya, A., Smets, E. & P. Goetghebeur. (2011). Metatopic displacements as an explanation for deriving morphologies in spikelets of *Cyperoideae*, *Cyperaceae*. <u>In</u>: Borsch, T., Giere, P., Hoffmann, J., Jahn, R., Löhne, C., Nordt, B. & M. Ohl (eds.), *BioSystematics Berlin, 21 – 27 February 2011, Programme and Abstracts*: 379-380. Botanic Garden and Botanical Museum Berlin-Dahlem, Freie Universität Berlin, Germany. 434 p. ISBN 978 3 921800 68 3

3. **Reynders, M.,** Muasya, AM., Larridon, I., Huygh, W., Vrijdaghs, A., Goetghebeur, P. (2011). Radiations and paraphyly in *Cyperus*: Challanging taxonomy of a giant genus. In: International Botanic Congress 2011, 23-30 July 2011, Abstracts: p. 248-249 , Melbourne, Australia.

4. Bruhl, JJ., Vrijdaghs, A., Prychid, C., **Reynders, M.** (2011). Mapaniids are critical to resolving floral and spikelet ontogeny in Cyperaceae. . In: International Botanic Congress 2011, 23-30 July 2011, Abstracts: p. 249 , Melbourne, Australia.

Fieldwork experience

Period	Purpose	Locations
18/12/2005 – 25/01/2006	Collection of living Cyperaceae + herbarium, sampling for ontogeny & DNA	Philippines: Samar & Bohol islands
23/02/2007 – 09/03/2007	Collection of living Cyperaceae & Peperomia + herbarium, sampling for ontogeny & DNA	Cameroon: Mt Cameroon, inselbergs near Yaoundé, highlands of the northwest
14/04/2010 – 01/05/2010	Collection of Cyperaceae: herbarium, sampling for ontogeny & DNA	Madagascar, central highlands

International herbarium visits

Period	Location	Purpose
12/01/2006	San Carlos University, Cebu, Philippines (CEBU)	Identifications.
22-24/08/2007	Royal Botanic Gardens of Kew, UK (K)	Identifications, registration of data, loan of material for taxonomic studies.
31/07-1/08/2007	Herbier National d'Histoire Naturelle, Paris (P)	Identifications, registration of data, loan of material for taxonomic studies.
28/02/2007	Herbier National, Yaoundé, Cameroun (YA)	Preparation of fieldwork, identifications, processing & deposit of collected materials
14/04/2010 – 01/05/2010	Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar (TAN)	Preparation of fieldwork, identifications, processing & deposit of collected materials

Skills

- Languages: Dutch: native speaker; English: fluent; French: well.
- Ms Office: Word, Exell, Powerpoint, Outlook, Access
- Specific software: vb. ArcView, ArcGis, BioEdit, Paup, PhyDe, …
- Scientific illustrations: photography, ink-drawing, cartoons, Photoshop, 3D graphics (Rhinoceros 3D).
- Molecular research: DNA extraction, PCR, AFLP, Nanodrop, gel-elektroforesis, ...
- Ecological fieldwork: floristic inventarisations, herborisation, vegetation study
- Species knowledge: Tropical sedges, flora of Belgium, indoor & outdoor ornamental plants, …

Extra curricular

- 1998-2000: Floristic fieldwork with the botany workgroup of LIKONA (Limburgse koepel voor Natuurstudie): participations to the 'black holes project' for the Atlas of the flora of Flanders and Brussels. Participation to nature management with the Jeugdbond voor Natuurstudie en Milieubescherming (JNM).

Appendix V Portfolio of botanical illustrations

A.5.1 In this appendix…

Appendix VI contains a portfolio of botanic ink drawings, published in several additional papers, which are indirectly or not linked to the main research topic of this dissertation.

Visual presentation adds value to plant characteristics, which are difficult to describe such as complex shapes and densities. Especially in large taxonomically complex groups, adequate illustrations form a useful tool for fast and reliable species identification.

In the first part, novelties in Cyperaceae are presented from in and outside *Cyperus* s.l. The second part briefly focuses on three articles outside Cyperaceae. A few illustrations were already included in the previous chapters and are not repeated here (Figs. 2.2, 2.13 & 8.11).

For these articles my contribution was focused on drawing the botanical illustrations and maps and giving their correct interpretations. Good illustrations are most valuable for taxonomic works certainly in large and taxonomically difficult taxa as for example *Cyperus* s.l. (Cyperaceae), *Peperomia* (Piperaceae) and *Paspalum* (Poaceae) since they allow for correct interpretation in addition to the descriptions and keys. Detailed line drawings have the advantage over pictures that they not only show the different important structures but also show the interpretations of the authors.

A.5.2 Illustrating novelties in Cyperaceae

Publications of new species, names and combinations is still one of the most important bases of taxonomy and working on such a large and diverse group as Cyperaceae gives many opportunities to provide science with stunning novelties.

Figures A.5.2-A.5.11 were produced for several published papers with the following references:

1) Reynders M., Dhooge S. & Goetghebeur P. 2006 A new Central African species, *Cyperus vandervekenii* (Cyperaceae) from the sources of the Nile in Rwanda. Novon 16: 512–515.

Abstract:

Cyperus vandervekenii Reynders, Dhooge & Goetghebeur, from Rwanda, is fully described and illustrated. It can easily be distinguished from the related *Cyperus graciliculmis* Lye by its slender subquadrangular culms, single pseudolateral spikelets, and by the associated bract shorter than the spikelet.

2) Samain M.-S., Reynders M. & Goetghebeur P. 2006 *Kyllinga beninensis* (Cyperaceae), a new species from Bénin. Novon 16: 516–519.

Abstract:

Kyllinga beninensis Samain, Reynders & Goetghebeur, a new species of Cyperaceae from the Borgou-Sud region of Benin, is fully described and illustrated. This species can be recognized by the slender habit with swollen stem base, the tiny white head consisting of a single spike, and the spikelets with two glumes and one flower. Morphological differences with the species *K. microbulbosa* Lye from East Africa, to which *K. beninensis* bears a superficial resemblance, are discussed.

3) Prata A.P., Camelbeke K., Reynders M., Goetghebeur P., Fedon I. & Huber O. 2007 *Bulbostylis medusae* (Cyperaceae), a new species from Venezuela. Novon 17: 67-71.

Abstract:

Bulbostylis medusae Prata, Reynders & Goetghebeur from Venezuela is fully described and illustrated. This species differs from all other South American *Bulbostylis* Kunth species by the combination of long-ciliated leaf sheaths, leaf apices, bracts, and spikelet axes. The new species resembles *B. sellowiana* (Kunth) Palla, and a comparison of the two species is made.

4) Larridon I., Reynders M. & Goetghebeur P. 2008 *Cyperus limiticola*, a New Name for a Madagascan *Cyperus* (Cyperaceae). Novon 18(2): 187-188.

Abstract:

There is a need to propose a new name in *Cyperus* L. for *C. paucispiculatus* Chermezon because the name currently used is n illegitimate later homonym of *C. paucispiculatus* Böckeler. A new name, *C. limiticola* Larridon & Reynders, is provided here.

5) Larridon I., Reynders M. & Goetghebeur P. 2009 Novelties in *Nemum* (Cyperaceae). Belg. J. Bot. 141 '2008': 157-177.

Abstract:

Five new taxa of the African genus *Nemum* (Cyperaceae) are recognised. The new combination *Nemum angolense* is made; three new species (*Nemum atracuminatum*, *N. capitatum* and *N. raynalii*) and a new subspecies (*Nemum spadiceum* subsp. *spadolense*) are described. The taxa are described and illustrated, and differences from their closest relatives are discussed. An identification key and commentaries on the interspecific affinities are included.

Fig. A.5.2 Habit drawings of Andean scirpoid species for the PhD dissertation of Sandra Dhooghe (2004). A. *Phylloscirpus boliviensis,* drawn from Beck 22360 (GENT). B .*Phylloscipus acaulis, drawn from* Laegard 101436 (GENT). C. *Zamaioscirpus muticus*, drawn from Renvoize & Laegard 5144 (Gent).
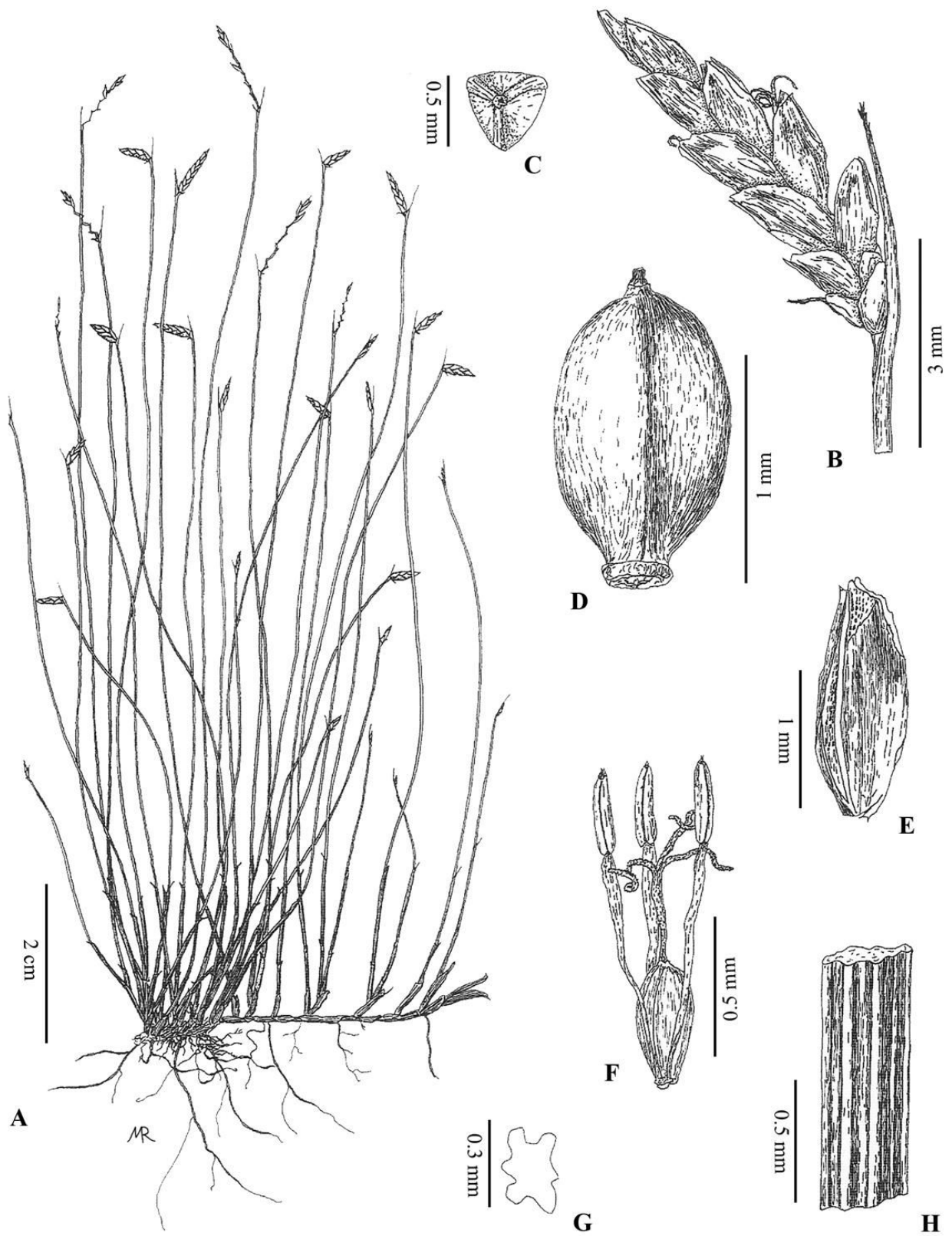
Fig. A.5.3 *Cyperus vandervekenii* Reynders, Dhooge & Goetghebeur: A. habit; B. inflorescence; C. fruit upper view; D. fruit lateral view; E. glume; F. flower; G. stem section; H. stem detail (drawn from the holo-type: *Van der Veken* 10116, GENT). (Reynders *et al.,* 2006).
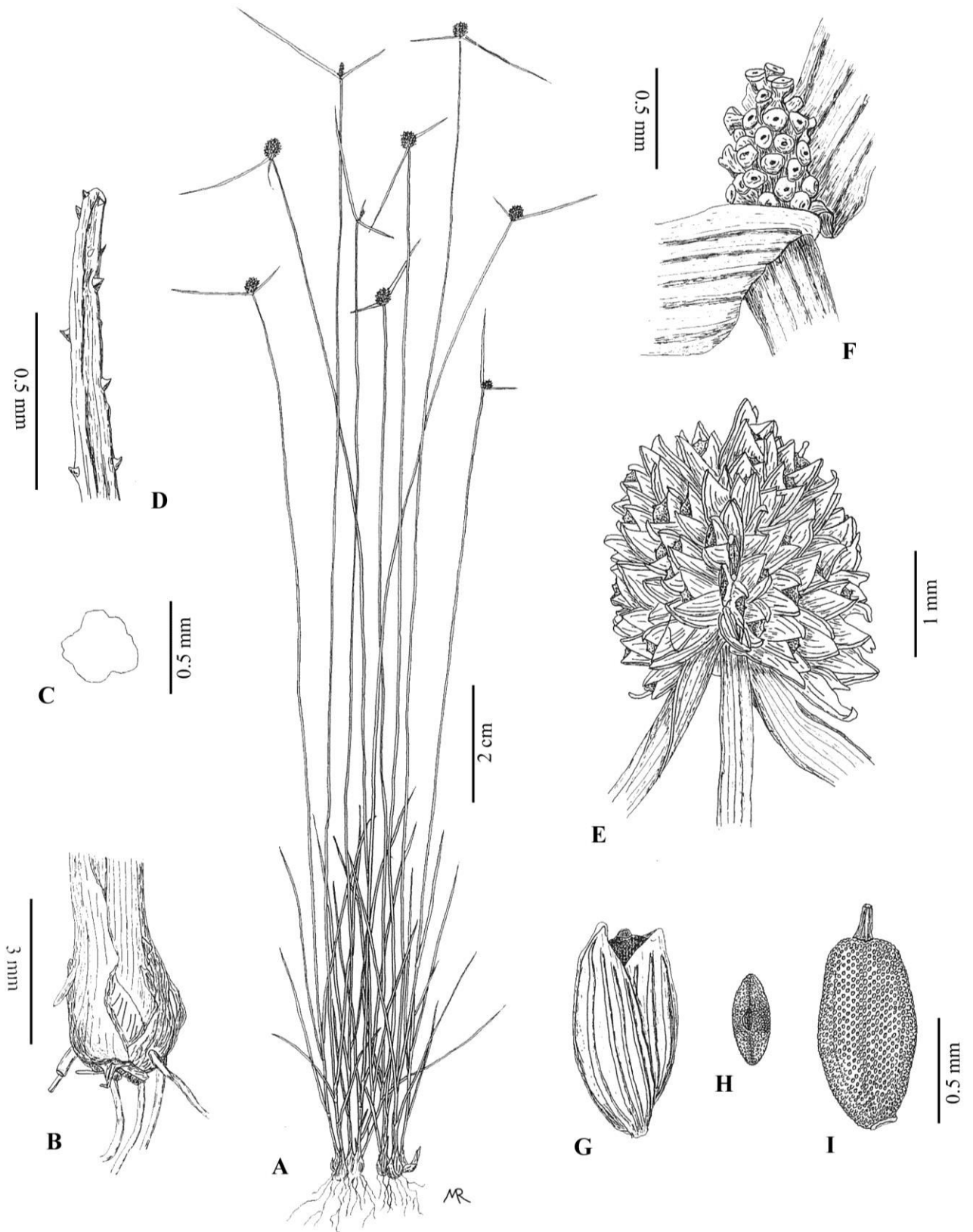
Fig. A.5.4 *Kyllinga beninensis* Samain, Reynders & Goetghebeur: A. habitus; B. swollen stem base; C. culm section; D. leaf tip; E. inflorescence; F. receptaculum; G. spikelet; H. fruit upper view; I. Fruit lateral view (Drawn from the holotype: *Sinsin* 3038, WAG). (Samain *et al.,* 2006)
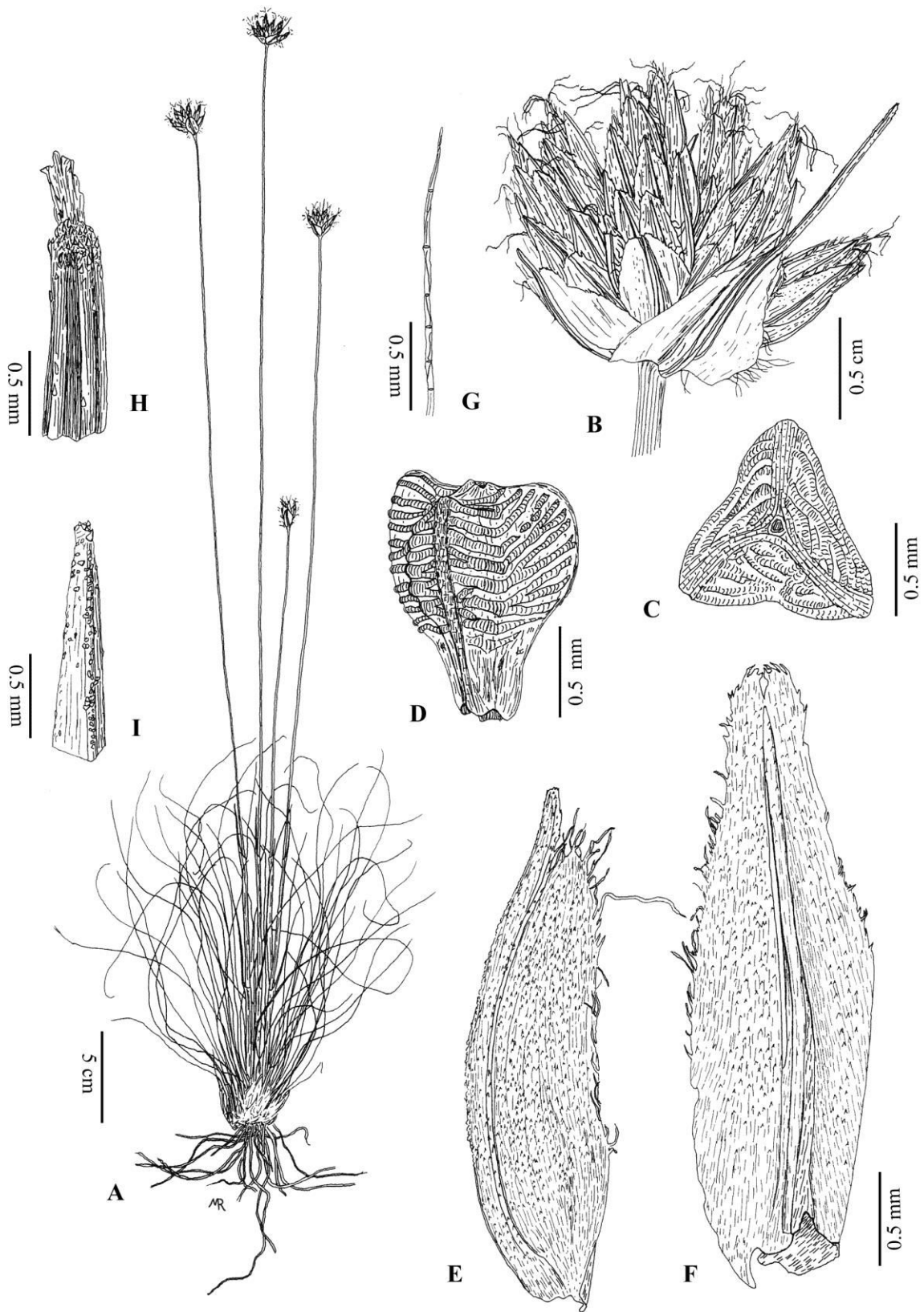
Fig. A.5.5 *Bulbostylis medusae* Prata, Reynders & Goetghebeur: A. habit; B. inflorescence; C. fruit upper view; D. fruit; E. lower glume; F. upper glume; G. hair from base of glumes; H. leaftip with bunch of hairs (*Gröger 913*, holo GENT); I, leaf tip of *Bulbostylis sellowiana* Palla (*Furlan, Guilietti, Harley, Wanderley & Varanda 4569*, GENT). (Prata *et al.,* 2007)

Fig. A.5.6 *Nemum angolense (C.B. Clarke) Larridon & Goetgh.* Habit. A. Slender annual of which at least one inflorescence bears more than one spikelet (based on *Hess 52/1370*). B. Robust perennial habit with a clearly anthelate inflorescence (based on *Hess 52/2100*). C. Intermediate form with nearly capitate inflorescences (based on *Robinson 2323*). (Larridon *et al.,* 2009)
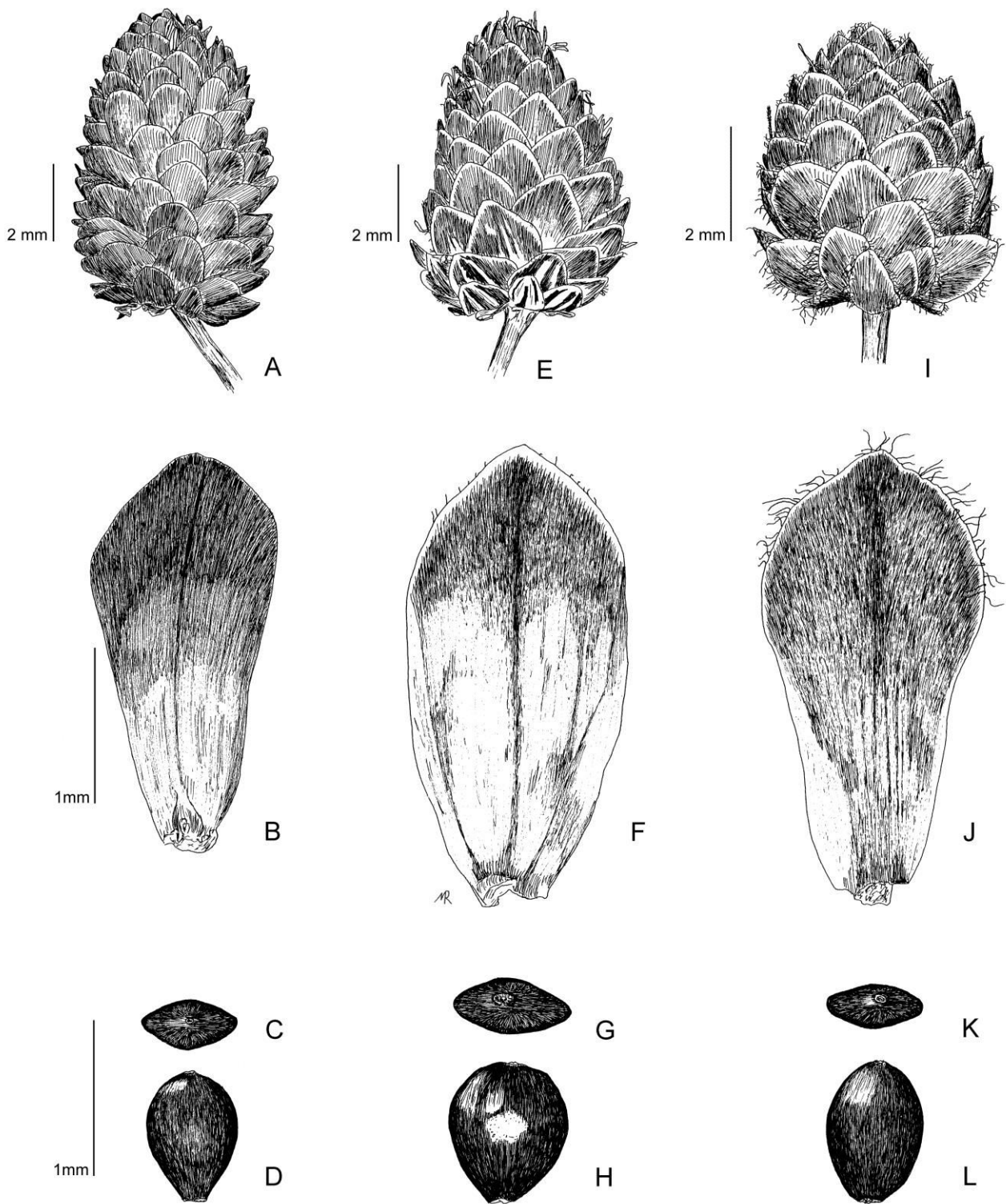
Fig. A.5.7 *Nemum angolense*. (C.B. Clarke) Larridon & Goetgh. Details. A-D. Based on *Robinson 2323*. A. Spikelet. B. Glume. C. Nutlet (apical view). D. Nutlet (lateral view). E-H. Based on *Le Testu 4113*. E. Spikelet. F. Glume. G. Nutlet (apical view). H. Nutlet (lateral view). I-L Based on *Hess 52/1370*. I. Spikelet. J. Glume. K. Nutlet (apical view). L. Nutlet (lateral view). (Larridon *et al.,* 2009)
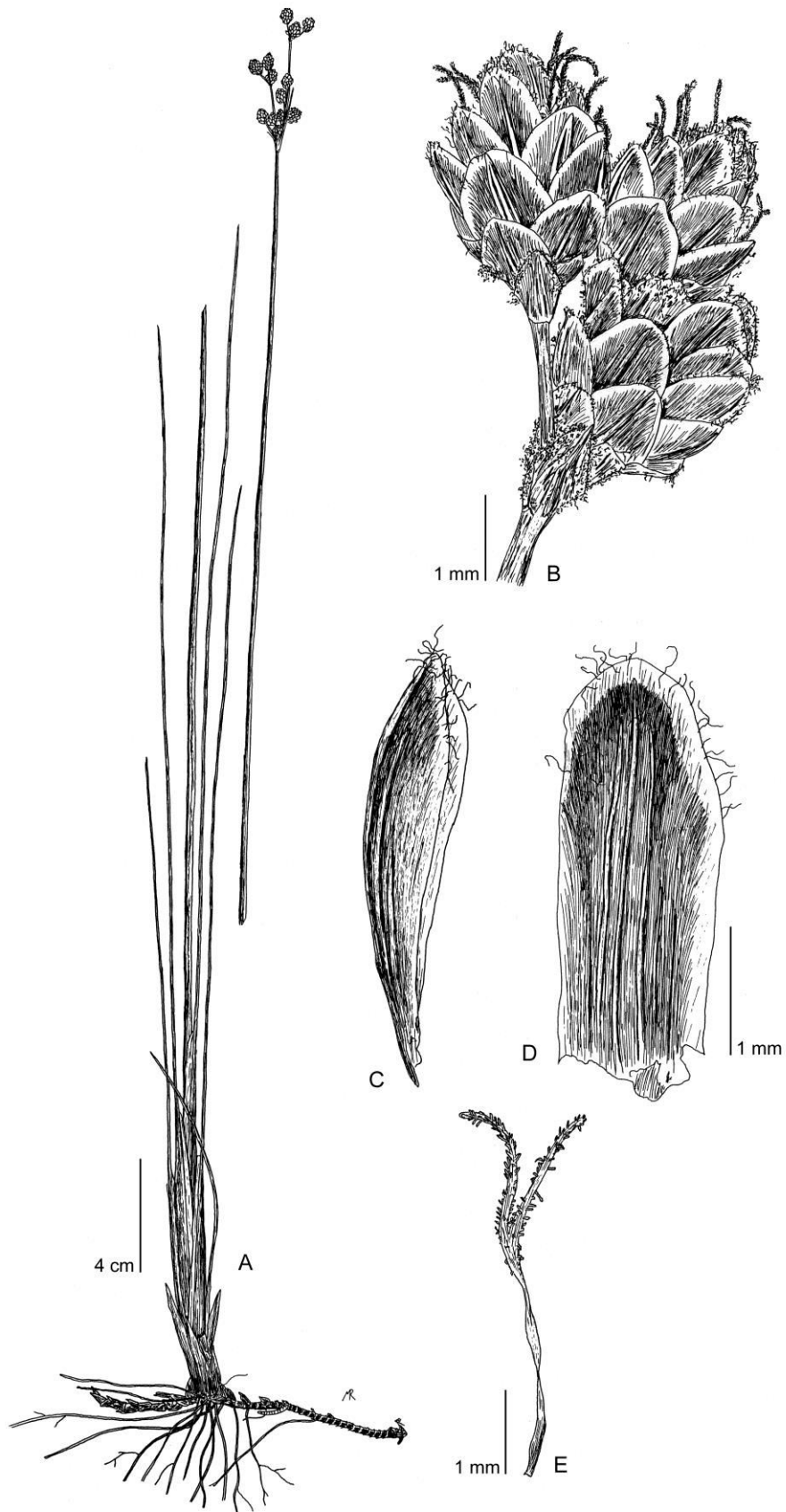
Fig. A.5.8 *Nemum raynalii* S.S. Hooper ex Larridon & Goetgh. (based on *Milne-Redhead* 4290). A. Habit. B. Anthela of spikelets. C. Glume (lateral view). D. Glume (abaxial view) E. Bifid style. (Larridon *et al.,* 2009)
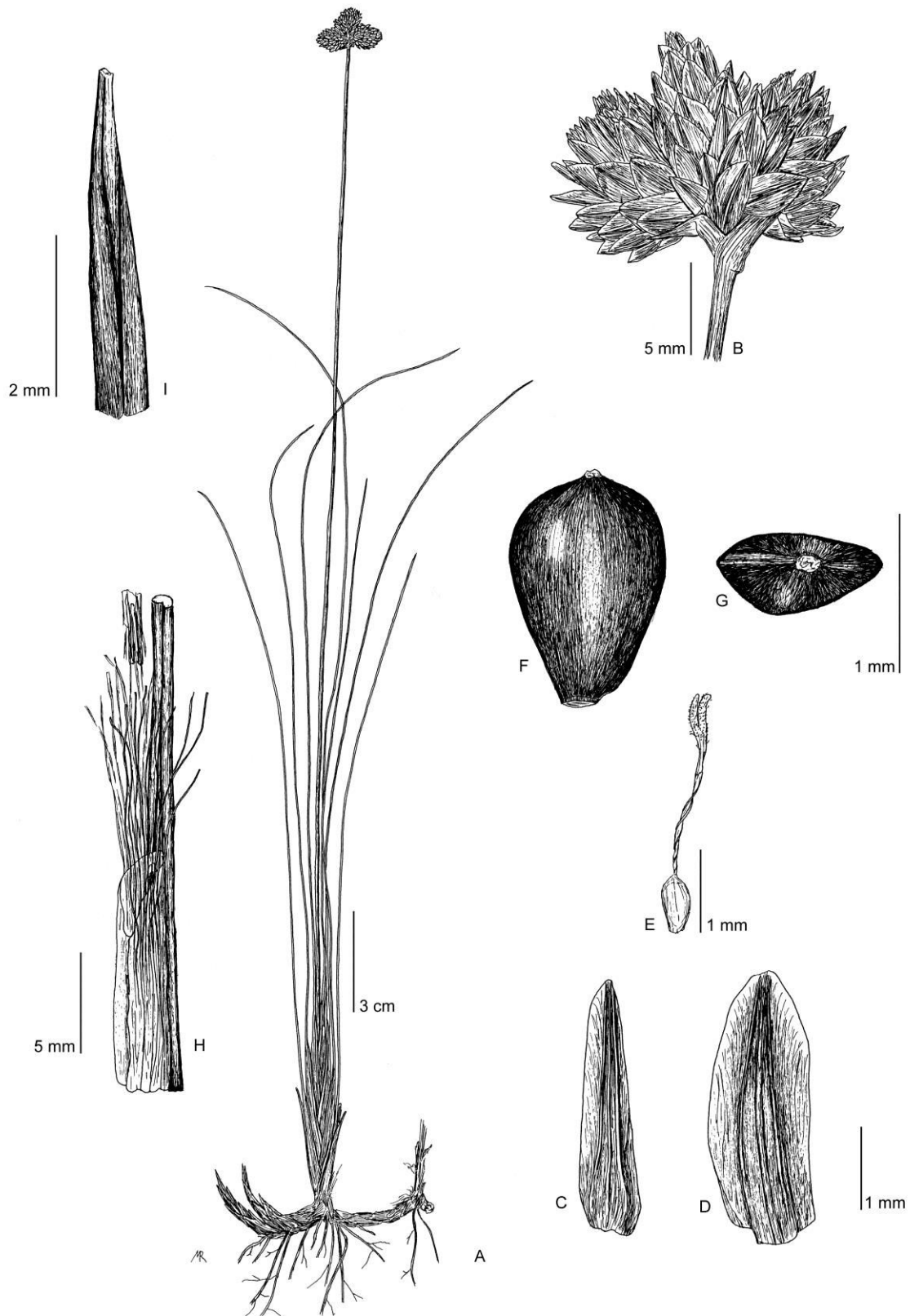
Fig. A.5.9 *Nemum capitatum* S.S. Hooper ex Larridon & Goetgh. (based on *Adam 22456bis*). A. Habit. B. Head of spikelets. C. Glume (lateral view). D. Glume (abaxial view) E. Bifid style. F. Nutlet (lateral view). G. Nutlet (apical view). H. Transition leaf sheath to leaf blade. I. Almost ensiform tip of leaf. (Larridon *et al.,* 2009)
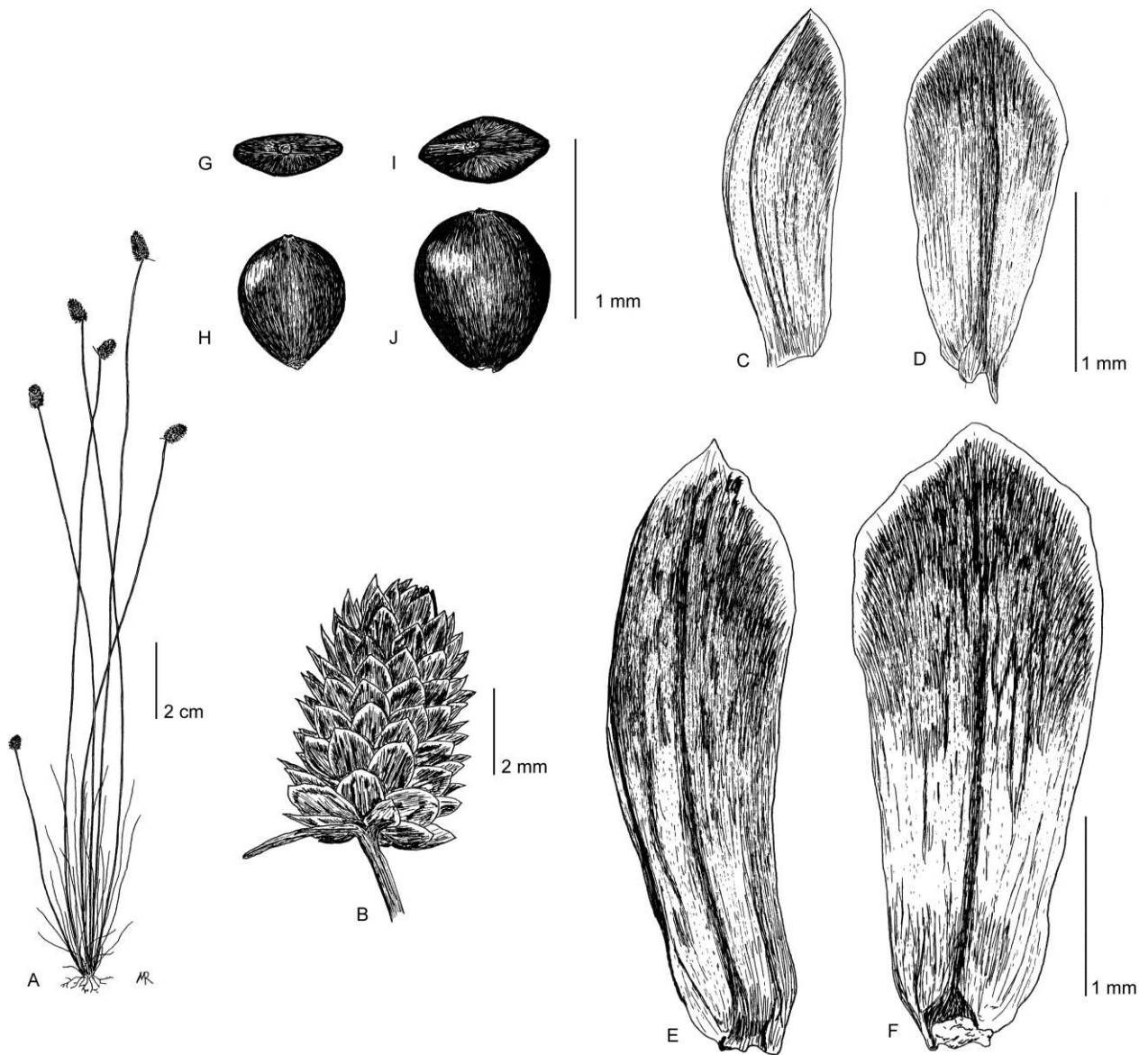
Fig. A.5.10 *Nemum spadiceum* (Lam.) Desv. ex Ham. A-D. & G-H. *Nemum spadiceum* subsp. *spadiceum* (based on *Laegaard* 21213). A. Habit. B. Spikelet. C. Glume (lateral view). D. Glume (abaxial view). G. Nutlet (abaxial view). H. Nutlet (lateral view). E-F. & I-J. *Nemum spadiceum* subsp. *spadolense* Larridon & Goetgh. (based on Gerard 3949). E. Glume (lateral view). F. Glume (abaxial view*). I. Nutlet (apical view). J. Nutlet (lateral view).* (Larridon *et al.,* 2009)

Fig. A.5.11 *Cyperus limiticola* Larridon & Reynders: A, habit; B, inflorescence; C, detail of spikelets (Viguier & Humbert 1256, P). (Larridon *et al*., 2008)

A.5.3 Leaving the Cyperpath, just for a little while…

A.5.3.1 Illustrative contributions to the taxonomy of *Paspalum* (Poaceae)

Figures A.5.12-A.5.13 were produced for two published papers with the following references:

1) Verloove F. & Reynders M. 2007. Studies within the genus *Paspalum* (Paniceae, Poaceae) in Europe: 1. *Paspalum distichum* subsp. *paucispicatum*, an overlooked taxon in France. *Willdenowia* 37: 199-204.

Abstract:

The present paper reports about the naturalization of the Mexican grass species *Paspalum paucispicatum* in the Loire valley in France. The species has long been confused with and erroneously referred to as *P. dilatatum, P. distichum, P. longipilum* or *P. pubiflorum*. A revision of several relevant herbaria enabled us to reconstruct the species' invasion history in France. Main diagnostic features are given and an identification key for *Paspalum* in France is presented. *P. paucispicatum*, of a debated taxonomic rank in its area of origin but clearly delimited in France, is probably better treated as a subspecies of *P. distichum;* hence the new combination at subspecific level is validated.

2) Verloove F. & Reynders M. 2007. Studies within the genus *Paspalum* (Paniceae, Poaceae) in Europe – 2. The *Quadrifaria* group. *Willdenowia* 37: 423-430.

Abstract:

The South American *Paspalum quadrifarium* has been reported as a naturalized xenophyte in Tuscany and Liguria, Italy, since at least the 1960s. In the present contribution the discovery of the closely related *P. exaltatum,* also of South American origin, in Liguria, Italy, is reported. These are the only known occurrences in Europe of both species, which are very similar to each other and likely to be confused. In the present paper their diagnostic features are discussed and original line drawings and SEM photographs for both taxa are presented. Their current distribution in Italy is shown and some ecological remarks are added.
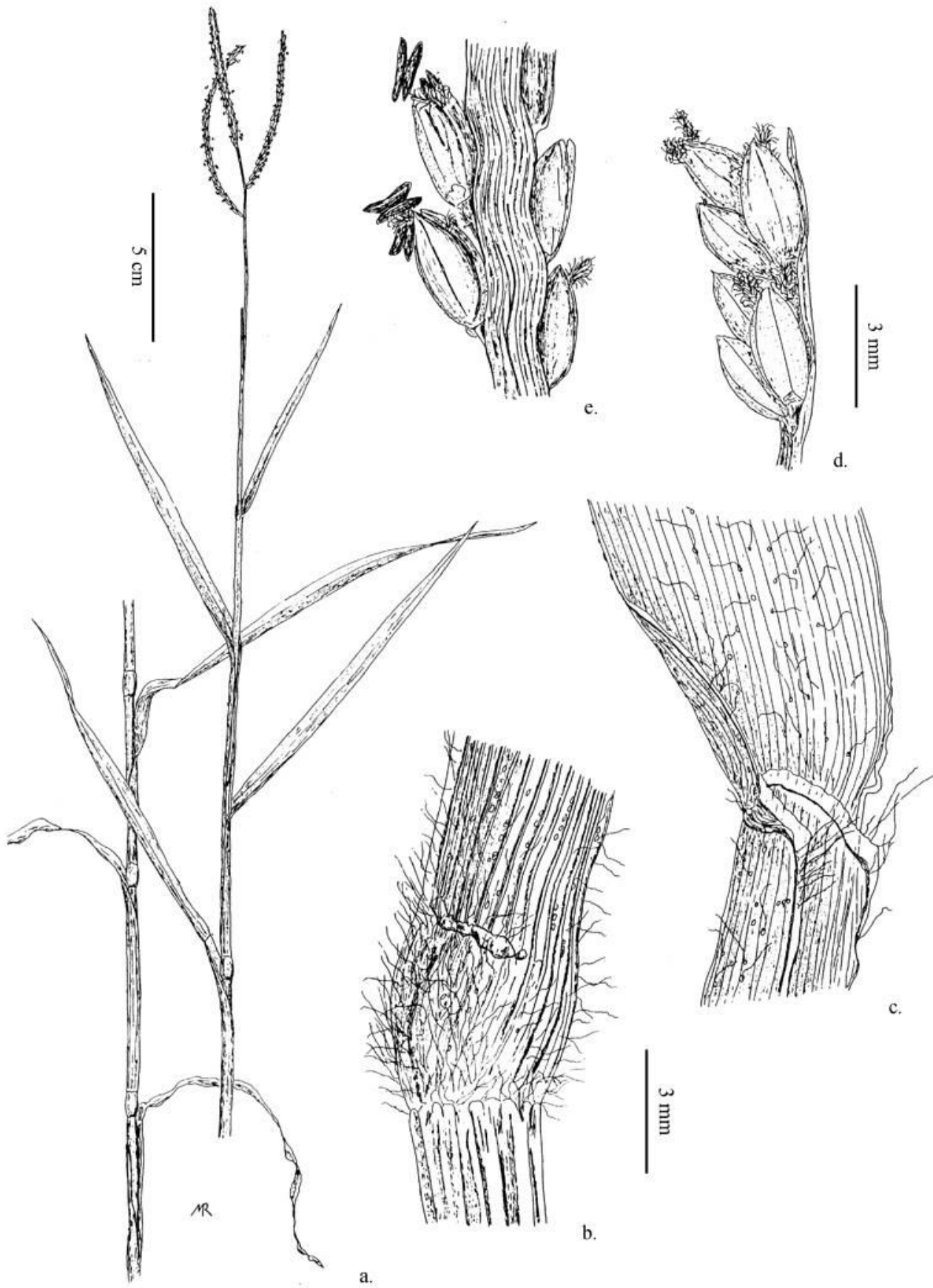
Fig. A.5.12 *Paspalum distichum L. subsp. paucispicatum* (Vasey) F. Verloove & M. Reynders comb. nov. – a: habit; b: node; c: leaf sheath with ligula; d,e: spikelets grouped two by two. Drawn after Verloove 4489. (Verloove & Reynders, 2007a)
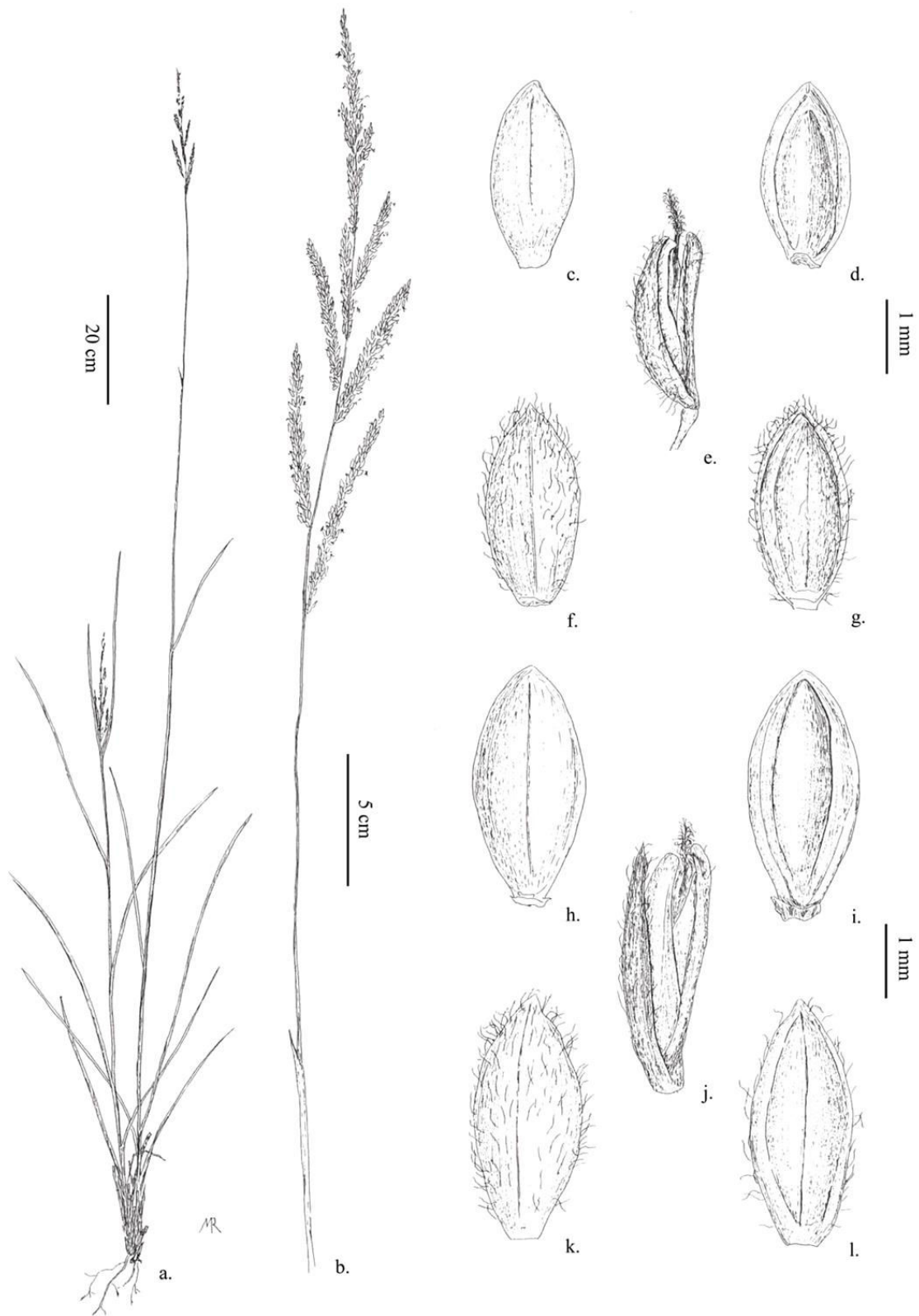
Fig. A.5.13 *Comparison of Paspalum quadrifarium* Lam. and *Paspalum exaltatum* J. Presl & C. Presl in *Italy – a: habit; b: inflorescence; P. quadrifarium:* c: upper floret in dorsal view showing lemma; d: upper floret in ventral view showing palea and margins of lemma; e: spikelet in lateral view; f: spikelet in dorsal view showing upper glume; g. spikelet in ventral view showing sterile lemma; *P. exaltatum:* h: upper floret in dorsal view; i: upper floret in ventral view; j: spikelet in lateral view; k: spikelet in dorsal view; l. spikelet in ventral view. Drawn after F. Verloove 6004 and F. Garbari s.n. (Verloove & Reynders, 2007b)

A.5.3.2 Contributions to the taxonomy of *Peperomia* (Piperaceae)

Figures A.5.14-A.5.20 were produced for a published paper with the following reference:

1) Mathieu G., Samain M.S., Reynders M. & Goetghebeur P. 2007. Taxonomy of the *Peperomia* species (Piperaceae) with pseudo-epiphyllous inflorescences, including four new species. *Bot. J. Linn. Soc.* 157: 177-196.

Abstract:

Twelve of the estimated 1500–1700 taxa in Peperomia show a particular feature: their terminal inflorescences seem to originate from the base of a leaf blade as a result of the presence of a sessile leaf at the base of these inflorescences. Three of these 12 taxa occur in Ecuador and four species are reported from Colombia. from Peru, two species are known from historical herbarium collections, and hree other species are new to science. One new species is described from Bolivia. Collections of all 12 species are quite rare and, in the past, their identification seems to have been rather problematic. Previous authors have referred to these inflorescences as epiphyllous but, as shown here, this is not a correct description. To make a clear distinction from genuine epiphyllous inflorescences, the term 'pseudo-epiphyllous' is proposed. In addition to the publication of four new species of Peperomia, emended descriptions are provided for the other species, synonymy is treated, and lectotypes are designated. The architecture of the plants is discussed and a key to the 12 species is provided.
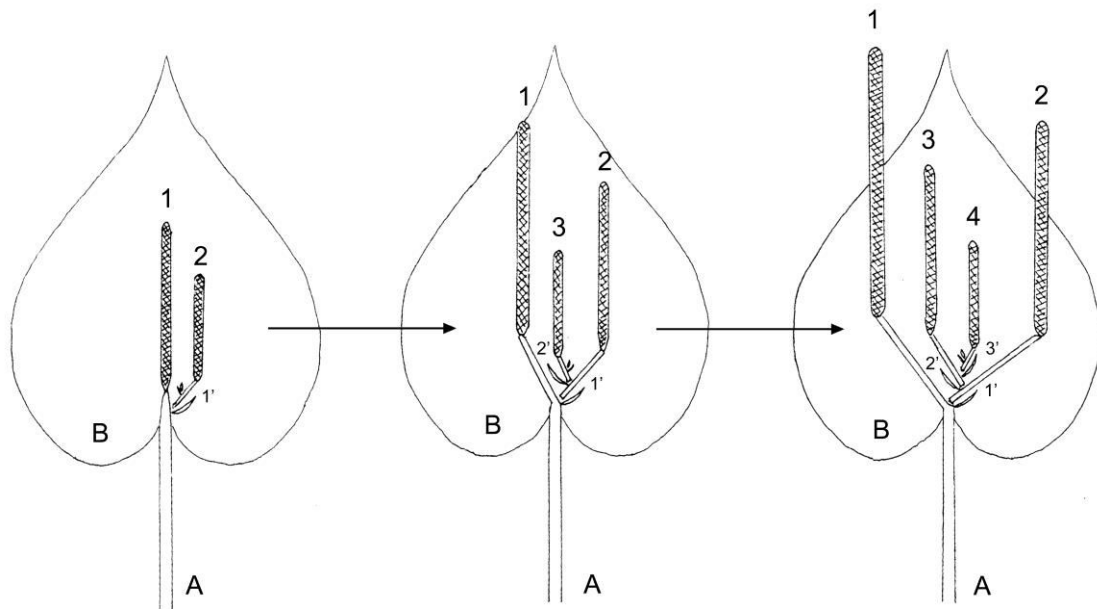
Fig. A.5.14 Developing order in a compound inflorescence of pseudo-epiphyllous *Peperomia* species: A, 'fertile' branch; B,'fertile' leaf; 1–4, successively developing spadices; 1'-3', successive bracts subtending the axillary bud from which the next spadix develops. (Mathieu *et al.,* 2007)
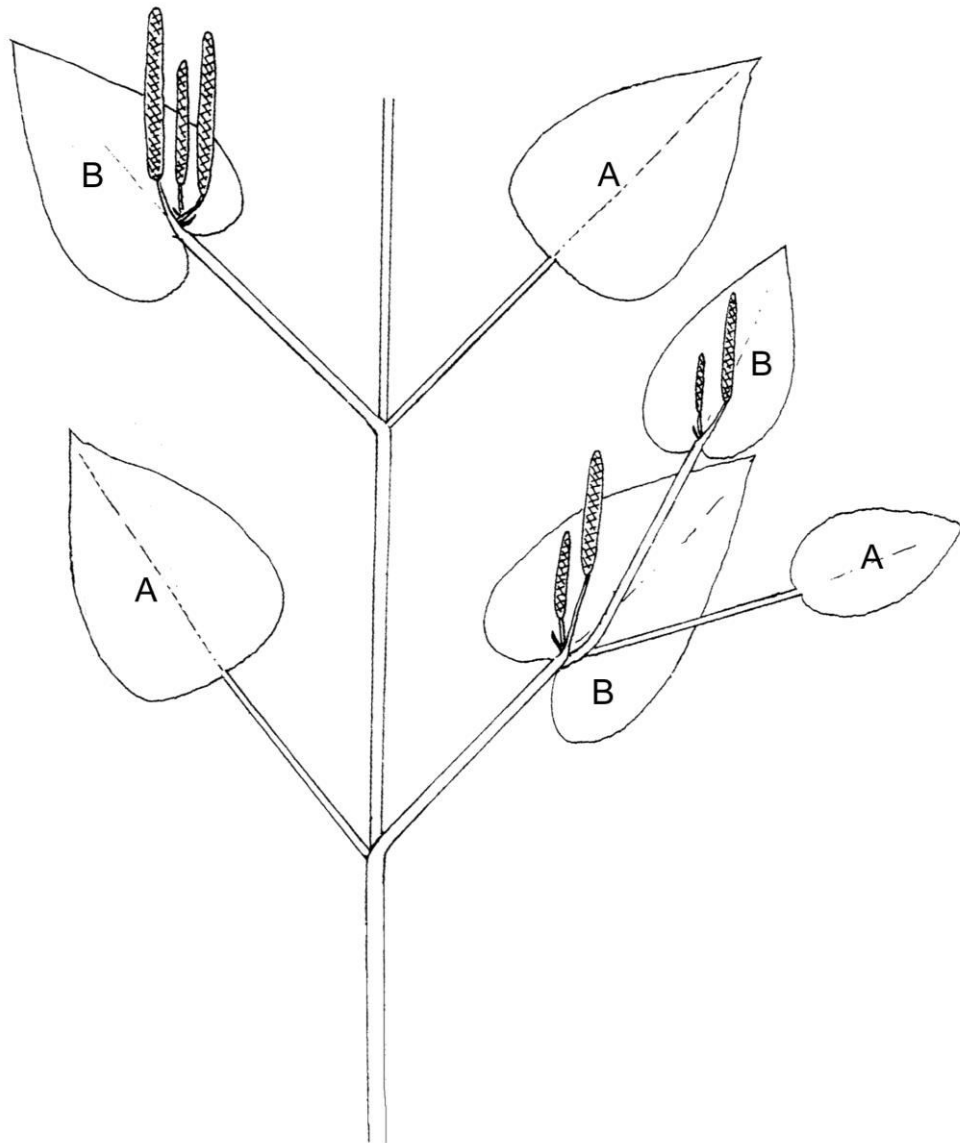
Fig. A.5.15 Architecture of the holotype of *Peperomia palmiriensis*: A, 'vegetative' (petiolate) leaf; B, 'fertile'(sessile) leaf. (Mathieu *et al.,* 2007)
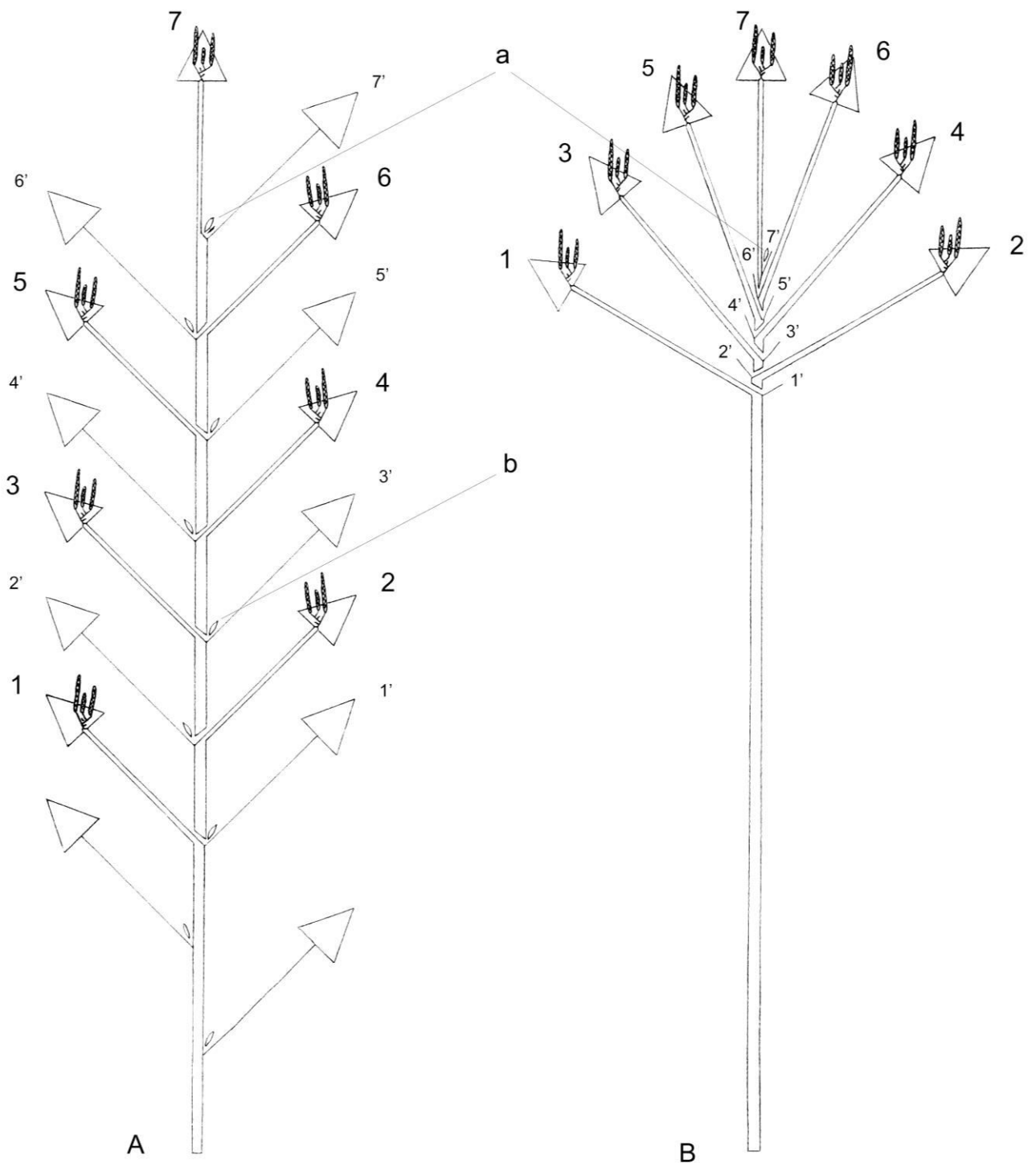
Fig. A.5.16 Architecture of pseudo-epiphyllous *Peperomia* species: A, with sympodial branching; B, with condensed lateral branching; 1–7, successive 'fertile' leaves; 1'-7', successive 'vegetative' leaves; a, axillary bud that develops into the next'fertile' branch; b, bract being part of a serial axillary bud. (Mathieu *et al.,* 2007)
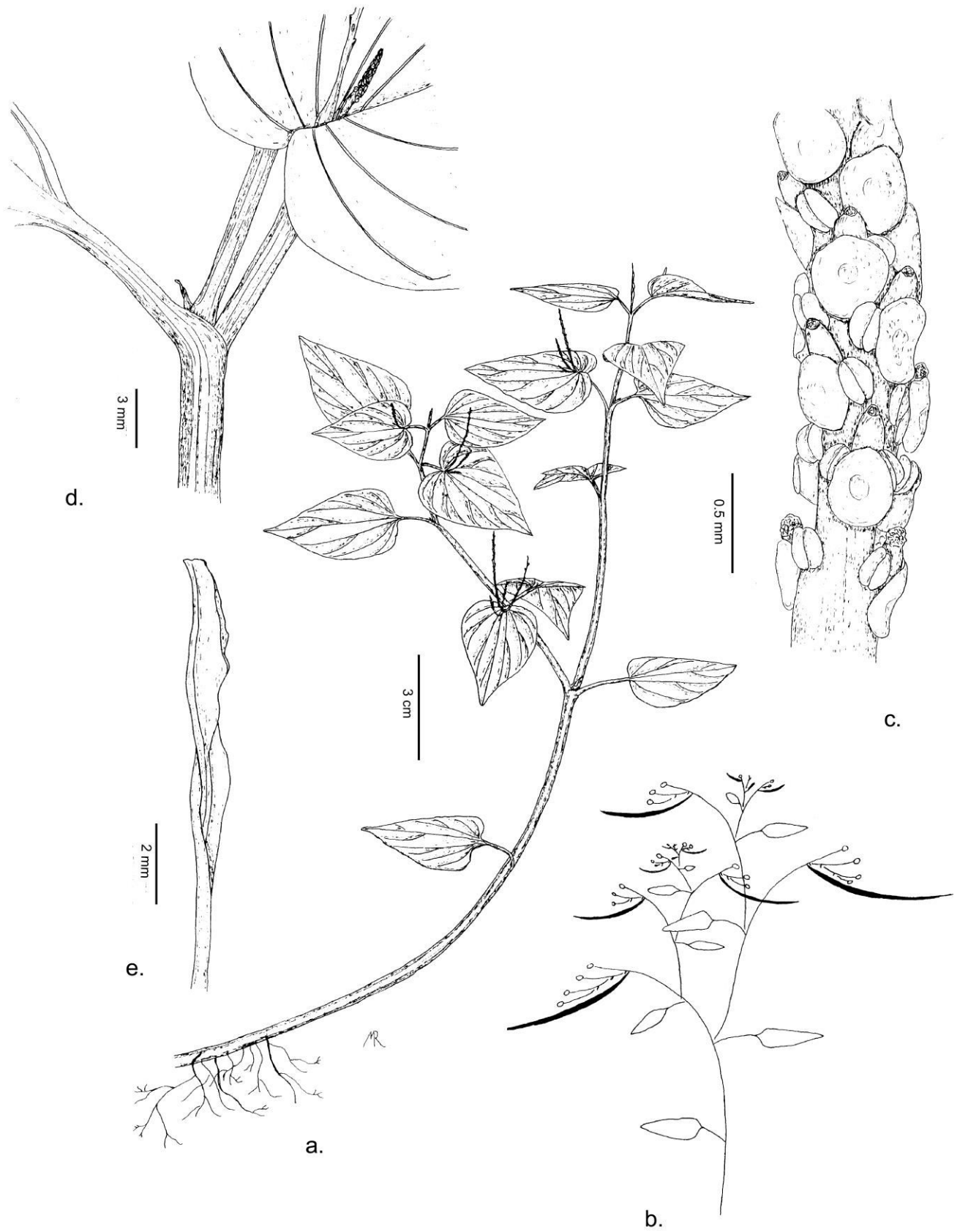
Fig. A.5.17 *Peperomia kjellii* G.Mathieu: A, general habit; B, architecture; C, part of the rachis; D, branching detail; E, young terminal sympodial shoot; left, petiolate 'vegetative' leaf; right, branch with a sessile 'fertile' leaf; middle, sympodial shoot; holotype. (Mathieu *et al.,* 2007)
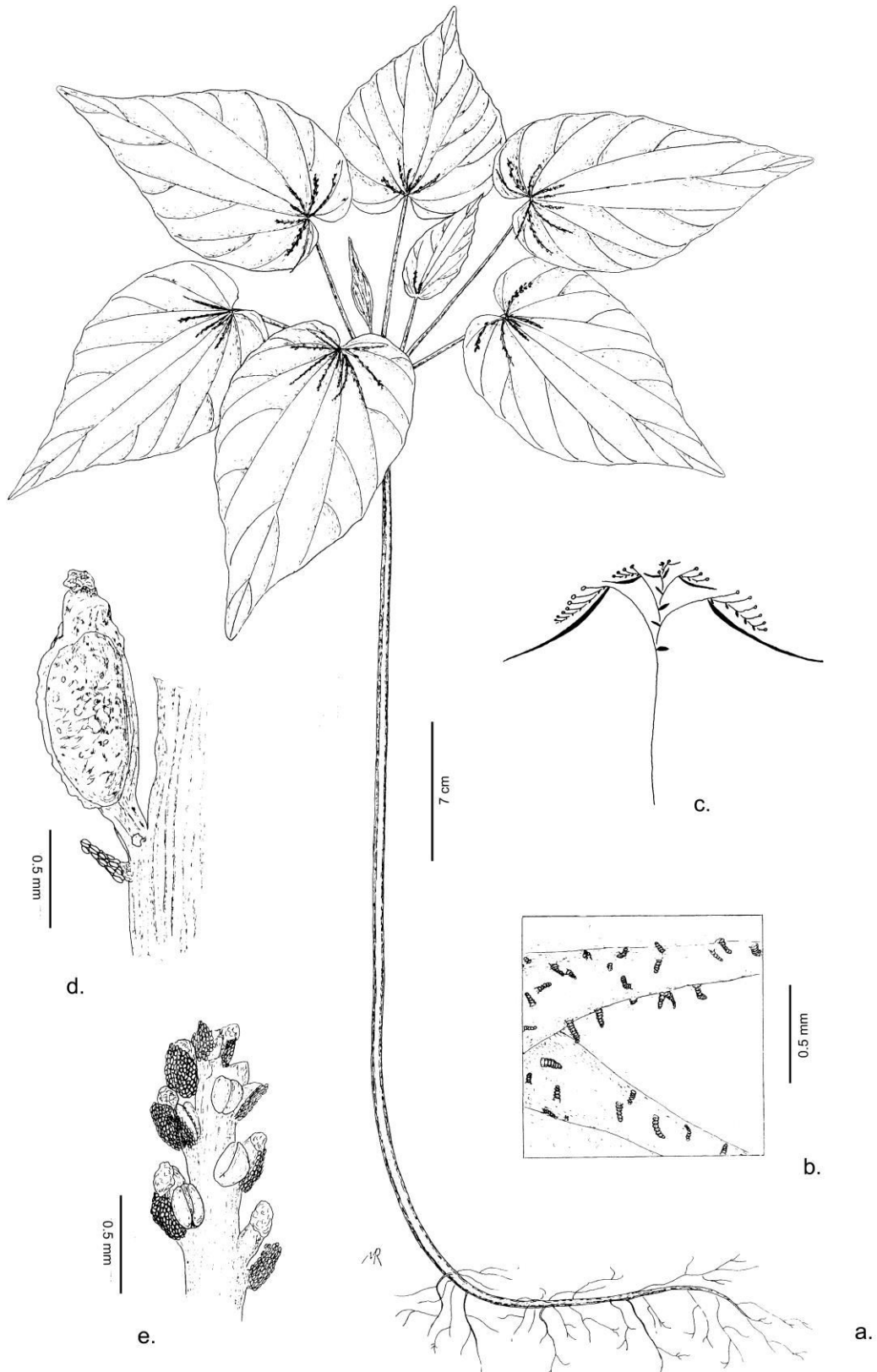
Fig. A.5.18 *Peperomia magnifoliiflora* G.Mathieu: A, general habit; B, basal part of the main nerves abaxially; C, architecture; D,fruit; E, distal part of the rachis; holotype. (Mathieu *et al.,* 2007)

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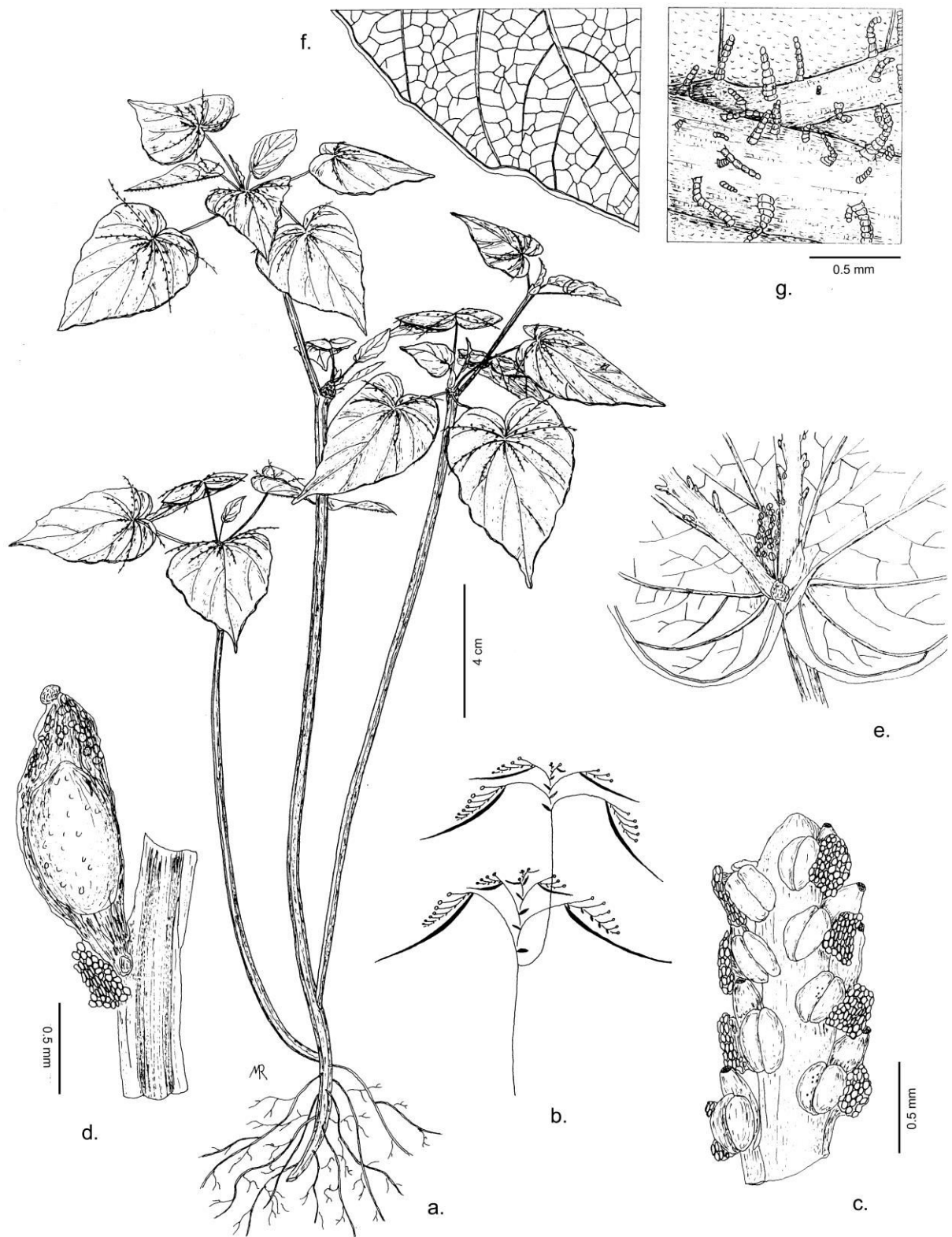
Fig. A.5.19 *Peperomia pseudophyllantha* Samain; A, general habit; B, architecture; C, apical part of rachis; D, fruit; E, basal part of 'fertile' leaf;F, leaf margin adaxially; G, middle part of the main nerves abaxially; holotype. (Mathieu *et al.,* 2007)
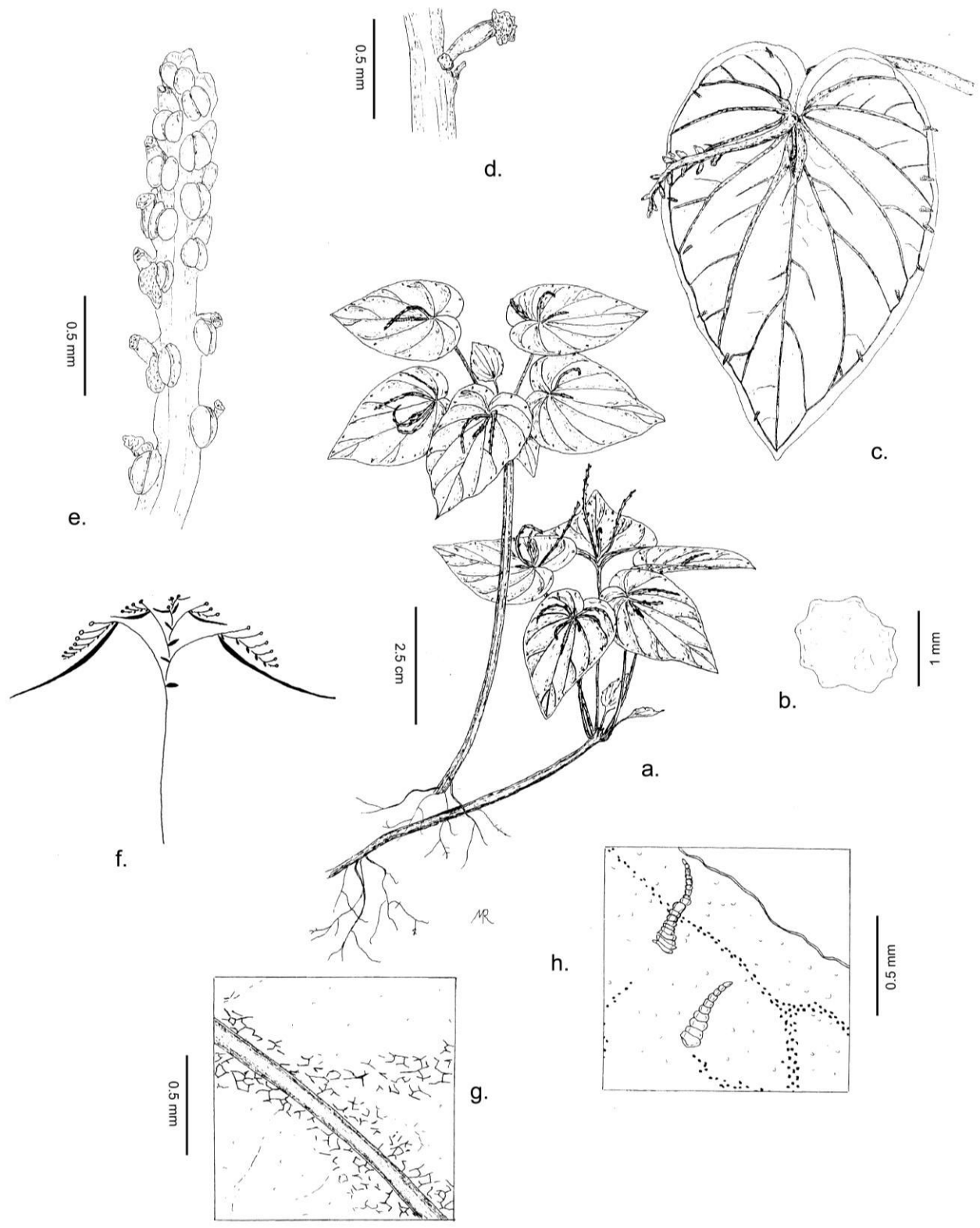
Fig. A.5.20 *Peperomia pinoi* G. Mathieu*:* A, general habit; B, cross-section of stem; C, 'fertile' leaf; D, single flower; E, apical part of rachis; F, architecture; G, main nerve abaxially; H, perimarginal zone adaxially; holotype. (Mathieu *et al.,* 2007)

Appendix VI Poster presentations

An print is included of the posters presented on diverse symposia during this PhD study:

Poster 1: Presented on the PhD symposium Faculty of Sciences, Ghent University. ICC International Convention Center, Ghent. 3rd May 2005

Poster 2: Presented on the Africa's Great Rift: Diversity and Unity symposium. Royal Academy for Overseas Sciences, Brussels. 29th and 30th September 2005

Poster 3: Presented on *the* XVIIIth AETFAT Congress, Yaoundé, Cameroon. 26 February – 2nd March 2007. And also on the PhD symposium Faculty of Sciences, Ghent University. ICC International Convention Center, Ghent. 24th April 2007

Poster 4: Presented on the XIXth AETFAT Congress, Antanarivo, Madagascar. 25th April - 1st May 2010

Poster 5: Presented on: Botanical Diversity: exploration, understanding and use, National Botanic Garden of Belgium, Meise. 16th to 18th September 2010

The nutlet, a key character in the taxonomy of *Pycreus* (Cyperaceae)

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Dorsal vascular bundles
Ventral vascular bundles
Central vascular bundles
Lateral vascular bundles

A. Basal type: lateral bundles are present, central bundles going to the ovulum
B. Cyperus type: lateral bundles are absent, dorsal bundles going to the style
C. Dorsoventrally compressed type: reduction of the abaxial carpel
D. Laterally compressed type: reduction of the abaxial carpel and formation of lateral bundles that go to the stylebranches
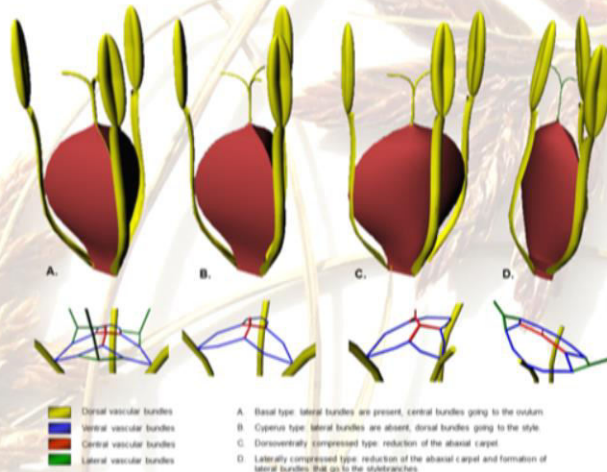
The laterally compressed pistil

Pycreus is a mainly African genus with about 120 species. It is especially represented by annuals from habitats with a strong seasonality.

Many authors were not convinced about the generic status of *Pycreus* and kept the species under *Cyperus* sensu lato[1].

The laterally compressed pistil, with two style branches, is however an important apomorphy for *Pycreus*. The origin of this pistil type demanded a completely new architecture of the gynoecium, a difficult evolutionary step that occurred probably only once (Fig. 1).

But there is more than *Pycreus*. *Kyllinga* and *Queenslandiella*, also related to *Cyperus*, show a similar pistil, but the anatomical structure is not yet sufficiently known.

← **Fig. 1.** 3D reconstruction (Rhino 3D) of Cyperaceae flowers and pistil vascular bundles, based on Blaser (1941)

From isodiametric to zonate

Adaptation to similar habitats resulted in similarities in the overall morphology of *Pycreus* species, being the source for many misclassifications that were often based on non homologous characters[1].

Variation in small structures as glumes and nutlets seems to be very large (Fig. 2). Especially the nutlet epidermal cells and their silica bodies will be important in discovering relationships between the several species and sections. Due to the lack of technology, these characters haven't been studied in detail.

By a SEM study of the nutlet surface of many *Pycreus*, *Cyperus* and *Kyllinga* specimens, with removing the outer cell wall by acid treatment, it became apparent that the isodiametric type with a large central silica body is the primitive state in *Pycreus* (Fig. 3).
The zonate type is unique for *Pycreus* in the Cyperoid clade and originated from the isodiametric type. Many species seem to have an intermediate or mixed cell type. Elongation of the cells resulted in transversal costae and other typical decorations of the fruit wall.

References
[1]. Kükenthal, G. (1936) Pflanzenreich 101 : 390.
[2]. Blaser, H.W. (1941) Amer. J. Bot. 28(7) : 542-551, 1-86 fig.

← **Fig. 2.** Variation in nutlet shapes in the genus *Pycreus*

From top to bottom: *Pycreus intactus*; *Pycreus hildebrandtii*; *Pycreus divulsus* subsp. *africanus*; *Pycreus vicinus*; *Pycreus muricatus*.

→ **Fig. 3.** Variation in nutlet epidermal cells. The isodiametric type is the plesiomorphic state in the genus, it is also present in related genera as *Cyperus*, *Kyllinga* and *Queenslandiella*.
From left to right and from top to bottom: *Kyllinga polyphylla*: plesiomorphic state; *Pycreus melas*, silica bodies with sattelites on the top; *Pycreus micromelas*; *Pycreus melanacme*; *Pycreus rhizomatosus* with sattelites around the central silica body; *Pycreus acuticarinatus* with slightly elongated cells; *Pycreus muricatus* with zonate cells; *Pycreus rehmanianus*, the transverse walls are pushed upward due to the extreme elongation of the cells.
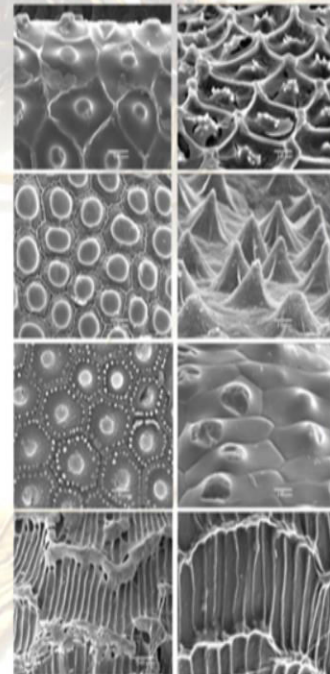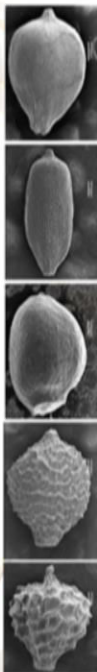
A key to the Cyperaceae of Rwanda and Burundi.

Reynders, Marc

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With 22 genera and approximately 200 species the Cyperaceae form the third largest flowering plant family of Rwanda and Burundi. These species can be found in almost all habitats, from Lake Tanganyika to the tops of the Virunga Mountains, often with a strong ecological importance. In Rwanda and Burundi two endemic species occur in the Albertine Rift region[2].

For Rwanda a Cyperaceae key was already available[1] but needed to be updated. There is no flora for Burundi yet. Because Rwanda and Burundi are biogeographically very similar the species of the two countries are combined in one key.

Differences between the two countries can be found in the Virunga region (Rwanda) and the Mosso (Burundi).

This study is based on 2.456 specimens from the herbaria of Ghent University (GENT) and the National Botanical Garden of Belgium (BR). The keys were tested by students of Ghent University.

These keys form an important working tool for future floristic, fytosociological and ecological research, especially of the wetlands in these countries. A working version of this key can be requested at the authors address. In the future an interactive version will become available on the internet.

REFERENCES

[1] Maquet P. (1987) in Troupin G. et al., Flore du Rwanda : Spermatophytes : 4, Musee Royal de l'Afrique Centrale Tervuren – Annales – serie IN-8° - sciences economiques – n°16.

[2] Reynders M. (2004), De Cyperaceae van Rwanda en Burundi, bijdrage tot een nieuwe flora. Scriptie ingediend tot het behalen van de graad van Licentiaat in de Biologie. Promotor Dr. Goetghebeur P. Universiteit Gent.
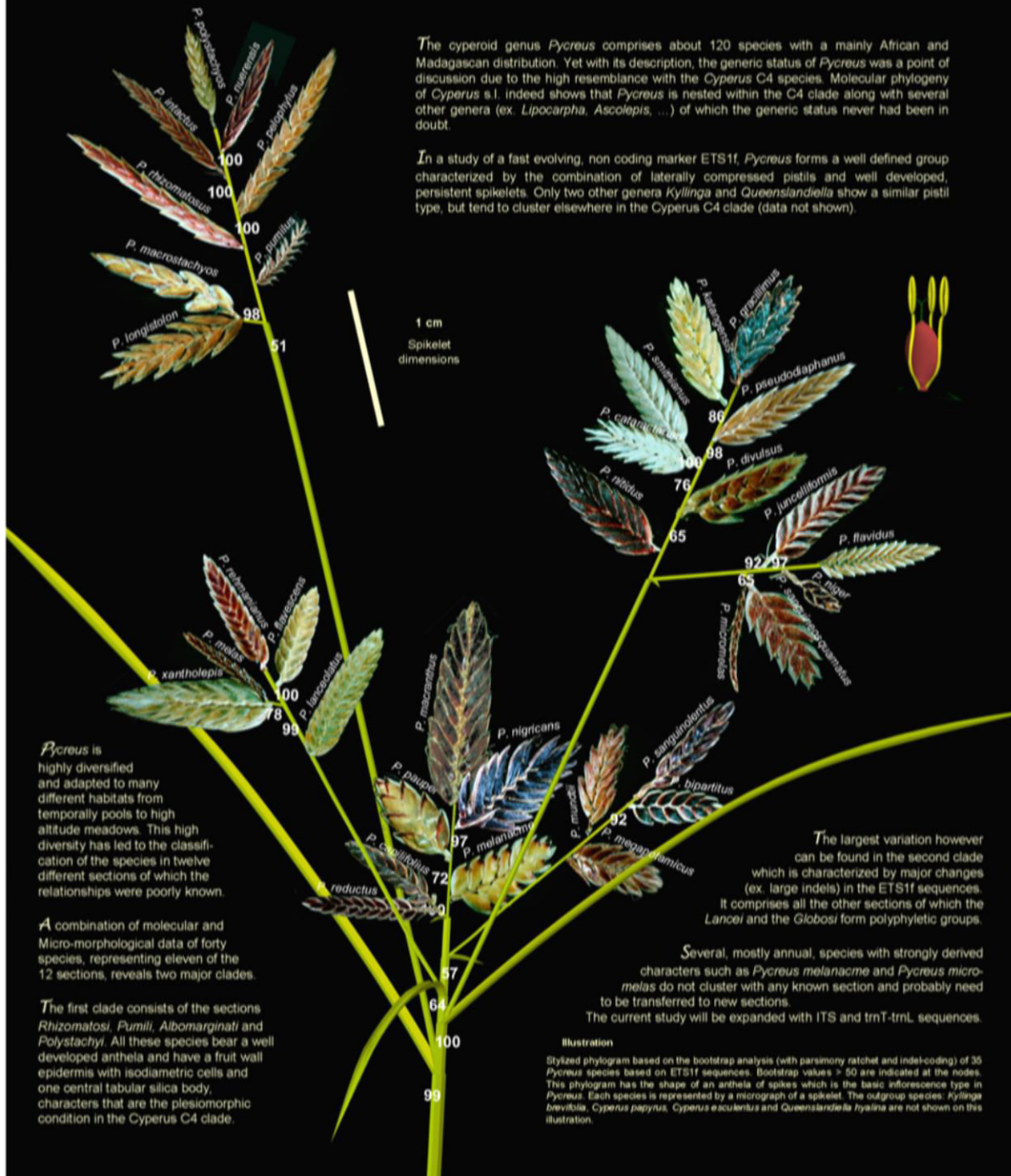
Phylogeny and evolution of the mainly African genus *Pycreus* (Cyperaceae), based on molecular and morphological data.

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*T*he cyperoid genus *Pycreus* comprises about 120 species with a mainly African and Madagascan distribution. Yet with its description, the generic status of *Pycreus* was a point of discussion due to the high resemblance with the *Cyperus* C4 species. Molecular phylogeny of *Cyperus* s.l. indeed shows that *Pycreus* is nested within the C4 clade along with several other genera (ex. *Lipocarpha*, *Ascolepis*, ...) of which the generic status never had been in doubt.

*I*n a study of a fast evolving, non coding marker ETS1f, *Pycreus* forms a well defined group characterized by the combination of laterally compressed pistils and well developed, persistent spikelets. Only two other genera *Kyllinga* and *Queenslandiella* show a similar pistil type, but tend to cluster elsewhere in the Cyperus C4 clade (data not shown).

1 cm
Spikelet dimensions

*P*ycreus is highly diversified and adapted to many different habitats from temporally pools to high altitude meadows. This high diversity has led to the classification of the species in twelve different sections of which the relationships were poorly known.

A combination of molecular and Micro-morphological data of forty species, representing eleven of the 12 sections, reveals two major clades.

*T*he first clade consists of the sections *Rhizomatosi*, *Pumili*, *Albomarginati* and *Polystachyi*. All these species bear a well developed anthela and have a fruit wall epidermis with isodiametric cells and one central tabular silica body, characters that are the plesiomorphic condition in the Cyperus C4 clade.

*T*he largest variation however can be found in the second clade which is characterized by major changes (ex. large indels) in the ETS1f sequences. It comprises all the other sections of which the *Lancei* and the *Globosi* form polyphyletic groups.

*S*everal, mostly annual, species with strongly derived characters such as *Pycreus melanacme* and *Pycreus micromelas* do not cluster with any known section and probably need to be transferred to new sections. The current study will be expanded with ITS and trnT-trnL sequences.

Illustration

Stylized phylogram based on the bootstrap analysis (with parsimony ratchet and indel-coding) of 35 *Pycreus* species based on ETS1f sequences. Bootstrap values > 50 are indicated at the nodes. This phylogram has the shape of an anthela of spikes which is the basic inflorescence type in *Pycreus*. Each species is represented by a micrograph of a spikelet. The outgroup species *Kyllinga brevifolia*, *Cyperus papyrus*, *Cyperus esculentus* and *Queenslandiella hyalina* are not shown on this illustration.

Do you understand the fibrillous *Pycrei* (Cyperaceae)?

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The fibrillous habit.

From Central Africa, several *Pycreus* species are known of which the old leaf sheaths break up into fibres and have a strongly reduced inflorescence.

This habit is encountered in several other Cyperaceae genera reflecting convergence in their adaptation to environments prone to high levels of disturbance. In *Pycreus*, taxonomy of these species is poorly understood which is mainly due to misinterpretation and synonymisation of different species based on their eye catching common characteristics.

A combination of molecular and morphological research reveals the true relationships of the different taxa.

← **Fig. 1.** Stylised phylogram based on a maximum parsimony analysis of the ETS 1 nuclear marker. Bootstrap values are indikated on each node. *Pycreus africanus* (*P.* Sect. *Tuberculati* Cherm.) forms the outgroup of the clade which contains the most important fibrous *Pycreus* species.

Cleaning up the taxonomic mess.

The current circumsciption[1&2] of *P. fibrillosus* is incorrect and in fact corresponds to *P. scaettae*, a separate species, which should be removed from synonymy with *P. fibrillosus*. However, the immature UPS type of *P. fibrillosus* does correspond to *P. scaettae* var *katangensis* Chermezon[3], which was also synonymised under *P. fibrillosus* by Kükenthal[2] but has different spikelets than *P. scaettae*.

ETS1 molecular study shows *P. fibrillosus* is most related to *P. gracillimus*, a poorly known afroalpine species with blackish glumes, while *P. scaetae* is more related to *P. smithianus* and *P. cataractarum*, all characterized by white spikelets with a strait rachilla.

All species surrounding the fibrillous *Pycrei* schow already a tendency for leafsheaths, breaking up in firbres suggesting a multiple origin of the fibrillous habit within two different but related clades.

References
1. Haines & Lye (1981) Sedges & Rushes East Afr.: 278.
2. Kükenthal, G. (1936) Pflanzenreich 101 : 390.
3. Chermezon (1935) Bull. Jard. Bot. Brux. 13: 278.

← **Fig. 2.** The characteristic habit with old leaf sheaths splitting into fibres. Inflorescences are strongly recuded to a single spike of spikelets. Here shown for *Pycreus gracilimus* Chiovenda, a rare afroalpine species.

↑ **Fig. 3.** Comparision of spikelets of different fibrous *Pycreus* species clearly showing the difference between *P. fibrillosus* (b & f) and *P. scaettae* (c & g). *P. fibrillosus* has a flexuous rachilla which is hardly visible between the glumes. Lower glumes mostly have 1-2 additional nerves on their wings. These characteristics are in common with *P. gracilimus* (a & e). *P. scaettae* has larger spikelets with strongly imbricate glumes and a strait rachilla which is visible between the glumes, which are mutual characterisites with *P. smithianus* (d & h).