

**Phylogenetic relationships of Abildgaardieae  
(Cyperaceae) inferred from chloroplast and  
nuclear DNA sequences and pollen data**

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This thesis is submitted for the degree of Doctor of Philosophy  
of the University of New England

**January 2004**

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**To:**

***MOJGAN***

***MARAL***

***&***

***NAHAL***

## **Declaration**

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.



Kioumars Ghamkhar

## Acknowledgments

There is no question in my mind that my gratitude for achieving this milestone should begin with my parents. Their tireless provision of the two foods (physical and mental) has been the foundation stone of my success. I have had parents of whom I am so proud. So much of who I am, I owe to them. In particular, thanks to my mum for her ongoing support.

I am deeply grateful to my supervisors Associate Professor Jeremy Bruhl, Adjunct Associate Professor Karen Wilson, and Dr Adam Marchant for being ideal supervisors. I am sincerely thankful for their wise counsel, invaluable advice, ongoing guidance, constructive criticism, encouragement, and patience throughout this study. I was lucky that they were not only my supervisors but also my best friends in Australia.

I extend my profound thanks to the Ministry of Science, Research, and Technology, the Government of Islamic Republic of Iran, for providing a scholarship during the period of my studies. I give thanks especially to Professor Javad Farhoodi for his enormous support.

I extend my sincere thanks to Professors Chris Quinn and John Thomson of Royal Botanic Gardens, Sydney, and Dr Helene Martin of the University of New South Wales for their kind advice during this study.

My sincere thanks are due to Ms Kerri Clarke of the University of New England (UNE), Associate Professor Jeremy Bruhl, and Mr Van Klaphake, for providing herbarium and silica gel plant materials, Adjunct Associate Professor Karen Wilson of Royal Botanic Gardens (RBG), Sydney, and Mr. George Orel of the University of Western Sydney for providing CTAB preserved plant material.

I have experienced a very positive working environment. Thanks to all my colleagues and officemates namely Dr Adam Marchant, Dr Andrew Perkins, Carolyn Porter, Nick Yee, and George Orel for their contributions. My special thanks go to

Dr Adam Marchant and Dr Andrew Perkins, who have been always the first point of help when I was in need. I would, also, like to thank my very special friend, Carolyn Porter, for helping me over the duration of my PhD. Her role in the earliest technical supervisions both in the molecular and SEM work is greatly acknowledged.

I appreciate supports from people at the Plant Sciences section at RBG, and Botany at UNE. My special thanks go to Dr Tim Entwisle, Dr Barry Conn, and Mr Anthony Martin at RBG and Mr Chris Cooper and Mr Douglas Clark at UNE.

I thank the molecular lab meeting group at RBG, Sydney, for the useful meetings and fruitful discussions and specifically Dr Peter Weston for his always useful comments.

I am grateful to my parents-in-law for their invaluable support and encouragement and for always being there for me. In addition, my brother and sisters and entire extended family are thanked for their support.

I have found that my very young and lovely daughter, Maral Ghamkhar, has been a great source of encouragement and motivation. I thank her and wish her the best for being reasonable and patient over the last four years.

Finally, I thank my lovely wife, Mojgan. Her incredible personal sacrifice and support are what has genuinely made all this possible. Without her kind and generous support, in particular, emotionally, I believe that this thesis would never have come into being. My gratitude to her is too deep for words to convey.

I hope that I will honour those individuals who have contributed to my success by carrying the torch high and passing it on with a light that burns brighter than when I received it.

This work received support from Botany, University of New England (N.C.W. Beadle Fund), the Plant Sciences section RBG, Sydney, and the Linnean Society of New South Wales (Joyce W. Vickery Scientific Research Fund).

Kioumars Ghamkhar

## Prologue

### Format

The format of this thesis follows wherever possible the format of *Australian Systematic Botany* except that:

- figures and plates are located throughout the text
- spaces have been placed between paragraphs
- section headings are numbered

### Thesis structure

Chapter one provides an introduction to the study, reviews previous approaches to taxonomic issues within and among Abildgaardieae and its allies, and outlines the relationships and taxonomic history of the study group. Aims and hypotheses to be tested are stated along with how and why these changed over the course of the study.

Chapter two reviews the process of selection of molecular methods. Theoretical considerations and selection of data sources are reviewed. Various molecular techniques and methods are described and compared and their phylogenetic value discussed.

The use and methodology of *trnL-trnF* non-coding region of chloroplast DNA is reviewed in chapter three. The methods and results of phylogenetic analyses for the study group taxa are described and discussed.

Chapter four deals with the use of the ITS region of the nuclear ribosomal DNA and phylogenetic analyses of separate ITS and combined ITS/*trnL-trnF* data. The results are discussed and compared with the previous studies.

Chapter five provides an overview and history of pollen morphology studies and presents and discusses characters of pollen morphology in the study group and phylogenetic analysis of such data. This chapter also deals with combined molecular (ITS/*trnL-trnF*) and morphological data using different approaches and analytical methods, and compares the results of separate analysis and combined molecular analysis. Comparison with previous studies is the final part of this chapter.

The last chapter, six, provides an overview of the phylogenetic relationships, implications and limitations of the present study. Future directions and priorities for systematic studies of Abildgaardieae and Arthrostylideae are presented.

**Data availability**

The datasets are available from the author on request.

## Abbreviations

AFLP	amplified fragment length polymorphism
AMB	ambitus
Bp	base pair(s)
CI	consistency index
cpDNA	chloroplast DNA
CTAB	hexadecyltrimethylammonium bromide (cetyl trimethylammonium bromide)
DELTA	description language for taxonomy
DNA	deoxyribonucleic acid
dNTP(s)	deoxyribonucleotide triphosphate(s)
EDTA	ethylenediamine tetra-acetic acid
g	gram(s)
GBSSI	granule-bound starch syntase
GHz	gigahertz
IGS	intergenic spacer
ILD	incongruence length difference
IR	inverted repeat
ITS	internal transcribed spacer
LM	light microscope/y
LSC	large single copy region
min	minute(s)
ML	maximum likelihood
ml	millilitre(s)
mM	millimolar
MP	maximum parsimony
NJ	neighbour-joining
NSW	New South Wales
NT	Northern Territory
NTS	non-transcribed spacer



PAUP	phylogenetic analysis using parsimony
PCR	polymerase chain reaction
pers. comm.	personal communication
pH	log of H <sup>+</sup> concentration
PHT	partition homogeneity test
QLD	Queensland
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
RI	rescaled consistency index
RNA	ribonucleic acid
rpm	revolutions per minute
SAW	successive approximation weighting
sec	second(s)
SEM	scanning electron microscope/y
SSM	slipped-strand mispairing
SSRs	simple sequence repeats
SSC	small single copy region
TAPS	tris-(hydroxymethyl)-methyl-amino-propanesulfonic acid sodium salt
Taq	thermus aquaticus
TBE	tris-borate/EDTA electrophoresis buffer
TBR	tree bisection reconnection
TEM	transmission electron microscope/y
Tris	tris (hydroxymethyl)-aminomethane
Tris HCl	tris (hydroxymethyl)-aminomethane hydrogen chloride
UV	ultraviolet
WA	Western Australia
μl	microliter(s)
μg	microgram(s)
°C	degrees Celsius

## Abstract

The need for a systematic study of the tribe Abildgaardieae became apparent when the boundaries between Abildgaardieae and Arthrostylideae in a previous study using morphological and physiological characters were unclear. Within Abildgaardieae, some species belonging to separate genera overlap in morphological and/or embryological characters (viz. *Fimbristylis hispidula*, *Abildgaardia ovata*, *Bulbostylis pilosa*) and their recognition in separate genera has been questioned.

The focus of this study was to estimate phylogeny for Abildgaardieae and evaluate the circumscription of six genera (49 species) within Abildgaardieae and Arthrostylideae. Parsimony and maximum likelihood analyses were conducted separately on three different datasets (ITS (nuclear ribosomal), *trnL-F* (plastid) DNA sequence, and pollen morphological data). The data sets were also combined and analysed using the same techniques.

The results showed that the three data sets produced different phylogenies. Separate data sets did not clearly resolve the relationships within Abildgaardieae or between Abildgaardieae and Arthrostylideae. By combining the three datasets, much higher resolution of the phylogenetic relationships was observed. ITS and *trnL-F* provided different insights into the relationships within Abildgaardieae and between the tribes disagreeing with the position of Arthrostylideae relative to Abildgaardieae and with the monophyly of *Abildgaardia*. Pollen morphology provided useful taxonomic characters for the Abildgaardieae–Arthrostylideae complex but it did not resolve the relationships further when analysed alone. When combined with ITS and *trnL-F*, pollen morphology further strengthened *trnL-F* results.

One of the genera of Abildgaardieae, *Bulbostylis*, formed a well-supported ‘basal’ clade distinct from related genera. Genera (two sampled) of Arthrostylideae were nested between *Bulbostylis* and the remaining genera (three sampled) of Abildgaardieae. Within these genera of Abildgaardieae a highly resolved clade composed of *Fimbristylis*, *Crosslandia* and *Abildgaardia vaginata* emerged indicating the need to redefine the limits of these genera. The data do not support recognition of the taxa within this last clade as distinct genera.

*Crosslandia*, *Abildgaardia*, and *Fimbristylis* formed a clade in all analyses. Total

evidence of molecular and pollen data indicate that Arthrostylideae are embedded in Abildgaardieae and *Bulbostylis* is monophyletic. *Fimbristylis* is monophyletic only with the inclusion of *Crosslandia* and *A. vaginata*. The rest of *Abildgaardia* is monophyletic and sister to this expanded *Fimbristylis*. Relationships within *Fimbristylis* largely do not conform to infrageneric classification.

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