

# UC Riverside

## UC Riverside Electronic Theses and Dissertations

### Title

Evolution and Origins of Polyploid Sonchus (Subgenus Sonchus) and the Woody Sonchus Alliance

### Permalink

<https://escholarship.org/uc/item/4f33v7qt>

### Author

Yao, Li

### Publication Date

2010

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA  
RIVERSIDE

Evolution and Origins of Polyploid *Sonchus* (Subgenus *Sonchus*) and  
the Woody *Sonchus* Alliance

A Dissertation submitted in partial satisfaction  
of the requirements for the degree of

Doctor of Philosophy

in

Plant Biology

by

Li Yao

December 2010

Dissertation Committee:

Dr. Norman Ellstrand, Co-Chairperson

Dr. Seung-Chul Kim, Co-Chairperson

Dr. Renyi Liu

Copyright by  
Li Yao  
2010

The Dissertation of Li Yao is approved:

---

---

Co-Chairperson

---

Co-Chairperson

University of California, Riverside

## ACKNOWLEDGEMENTS

A number of individuals and organization are deserving of thanks and acknowledgement for their contributions and generous help, both personal and professional, to complete this projects. Most of all I would like to thank my husband, Fei Liu. He gave up his career in China to move to America with me for my study in UC Riverside. His love and encouragement have sustained me throughout my years at Riverside. I love you, Fei. I am also extremely thankful for the precious son Stephen Liu, who was born during my studying in UCR. With his bright life, he has blessed our lives tremendously. Also, I especially want to thank my parents, Xueqin Yao and Xinlan Ren, for their constant love and their belief that I could accomplish my goals. I was deeply appreciated with their countless encouragement in my down times. Thank you, Dad and Mom.

A number of individuals have directly contributed to my dissertation. Firstly, I would like to thank my advisor and dissertation co-chairperson, Dr. Seung-Chul Kim for his guidance and advice throughout my research and my professional development. His expertise in molecular systematic and island evolution and ready help in the lab made this

project possible. Thanks are also for his constant support and encouragement. I also thank my co-major advisor, Dr. Norman Ellstrand for his tremendous input of the dissertation manuscripts. His passion for science is truly an inspiration and will be a lifetime gift for me in my future career. I am very proud to be one member of Ellstrand team. I thank Dr. Renyi Liu, the other member of my committee, for his keen suggestions as well as advisement on my research projects. Other faculty at UCR who at various junctures offered advice, ideas, and technical aid or sat on one or more of my committees include:

James Baldwin, Edith Allen, Christiane Weirauch, Shizhong Xu, Mikeal Roose.

I am grateful to the members of the Kim and Ellstrand Lab for their daily conviviality and helpfulness and to graduate students of Department of Botany and Plant Sciences who were both knowledgeable and personable. I would also like to acknowledge all my friends who have accompanied me throughout my graduate life.

Supporting organizations: Department of Botany and Plant Sciences, UCR; the UCR Graduate Division and Graduate Student Association of UCR provided funding for this project.

## DEDICATION

This work is dedicated to my parents.

ABSTRACT OF THE DISSERTATION

Evolution and Origins of Polyploid *Sonchus* (Subgenus *Sonchus*)  
and the Woody *Sonchus* Alliance

by

Li Yao

Doctor of Philosophy, Graduate Program in Plant Biology

University of California, Riverside, December 2010

Dr. Norman Ellstrand, Co-Chairperson

Dr. Seung-Chul Kim, Co-Chairperson

The Sonchinae is the most widely distributed subtribe in the tribe Cichorieae, with a discontinuous, almost cosmopolitan, and very peculiar phytogeographic distribution. In particular, genus *Sonchus* subgenus *Sonchus*, appears to be responsible for the origin of several island endemics both in the Pacific Ocean (*Dendroseris*, *Thamnosseris*, *Actites*, *Kirkianella* and *Embergeria* ) and the Atlantic Ocean (woody *Sonchus* alliance in the Macaronesian Islands). So polyploidy *Sonchus* and their relative Pacific polyploidy endemics are an ideal system to study the polyploidization for the island plants. Several hypotheses have been proposed based on the morphology and karyology. We use the cloning technique to retrieve the different ITS repeat types in order to test the origin of



several polyploidy *Sonchus* as well as polyploidy in the related Pacific islands. The Pacific endemics (*Dendroseris*, *Embergeria*, *Kirkianella*, and *Actites*) are all formed the monophyletic clade with all the their repeat types which support the single origin of these endemics. *S. arvensis* has been identified as a potential allopolyploid. The Stebbins hypothesis of allopolyploid origin for *S. oleraceus* is partially supported with confirmation of *S. asper* as one donor. The data also indicate a hybrid origin for *S. hydrophilus* which might have subsequently homogenized with ITS copies from the hybrid ancestor via concerned evolution.

The woody *Sonchus* alliance is one of the largest Macaronesia diverse endemic groups with 6 genera and approximately 31 species. Determining accurate phylogenetic relationships among the members of the woody *Sonchus* alliance presents challenges because of insufficient level of molecular variation and the convergent evolution of similar morphological traits in island settings. All taxa of woody *Sonchus* alliance were sampled to investigate the phylogenetic relationships as well as to test the potential role of hybridization and introgression using three independent low-copy nuclear genes: glyceraldehyde 3-phosphate dehydrogenase (G3pdh) , B12 and calmodulin (Cam) . B12

and Cam phylogeny is not well resolved due to the limited informative sites. The G3pdh data set was not significantly different from that of B12 and Cam, and subsequent combined analysis provided a better resolved and supported phylogeny within the alliance. In the MP combined tree, the basal lineages of monotypic genera were not identified. The all *Taekholmia* species except *T. arborea* formed a well-supported clade. It partly supports Boulos' classification to treat *Taekholmia* as a genus. But the *Dendrosonchus* is highly polyphyletic which does not support either Boulos' or Aldridge's classification of tree *Dendrosonchus*. Assessment of the role of hybridization and introgression was limited due to low sequence variability of B12 and Cam genes, however, potential hybridization has been recognized comparing unlinked gene regions.

The members of woody *Sonchus* alliance display extensive morphological, ecological, and anatomical diversity, but all taxa have a uniform chromosome number. Furthermore, all the members have no crossing barriers, there are extensive opportunities for hybridization between these species. We include two independent low-copy nuclear genes: glyceraldehyde 3-phosphate dehydrogenase (G3pdh) and Calmodulin (Cam) with the cloning with several key species to test the possible homoploid hybrid speciation.

There is no evidence in our data to support a hybrid origin for the monotypic species (*Babcockia platylepis*, *Sventenia bupleuroides* and *Lactucosonchus webbii*). The hybridization between *Dendrosonchus* and *Taeckholmia* is not supported in our data. *Sonchus tuberifer* might have hybrid origin between herbaceous and small woody species.

## TABLE OF CONTENTS

LIST OF TABLES .....	xi
LIST OF FIGURES .....	xii
Introduction .....	1
Chapter 1 ITS Evolution and the Origins of Polyploid <i>Sonchus</i> (Subgenus <i>Sonchus</i> ) and Related Pacific Endemic Genera in Sonchinae (Asteraceae: Cichorieae) .....	8
Chapter 2 Origin and Evolution of the Woody <i>Sonchus</i> alliance in the Canary Islands .....	41
Chapter 4 A Molecular Phylogenetic Study of Hybridization in Woody <i>Sonchus</i> Alliance (Asteraceae: Sonchinae) .....	74
Conclusions .....	99
Appendix 1 .....	102

## **LIST of TABLES**

1.2 List of investigated samples with sample number ( sample location, the number of clones sequenced, and the maximum number of sequence types found within each individual. .....	35
2.1 The species of the woody <i>Sonchus</i> alliance studied . Abbreviations of islands are as follows: LA Lanzarote, FU Fuerteventura, GC Gran Canaria, TE Tenerife, GO La Gomera, PA La Palma, HI E1 Hierro. Species with an asterisk represent the taxa included in the combined analysis. .....	68
2.2 The forward and reverse primers of G3pdh, Cam and B12 genes .....	69
2.3 Numbers of taxa and characters with tree statistics for maximum parsimony analyses of G3pdh, Cam, B12 and combined datasets. .....	70
3.1 List of investigated samples for Cam gene (species name, sample location, the number of clones sequenced) .....	93
3.2 List of investigated samples of G3pdh gene (species name, sample location, the number of clones sequenced) .....	94

**LIST of FIGURES**

1.1 Majority (50%) consensus tree based on Bayesian analysis (clade 1&2). The posterior probability values above are shown above branches .....36

1.2 Majority (50%) consensus tree based on Bayesian analysis (Clade 4). The posterior probability values above are shown above branches. ....37

1.3 Majority (50%) consensus tree based on Bayesian analysis (Clade 5). The posterior probability values above are shown above branches. ....38

1.4 Majority (50%) consensus tree based on Bayesian analysis( Clade 6-1). The posterior probability values above are shown above branches .....39

1.5 Majority (50%) consensus tree based on Bayesian analysis( Clade 6-2). The posterior probability values above are shown above branches .....40

2.1 Distribution of the woody *Sonchus* alliance in the Canary, Madeira, and Cape Verde archipelagos. The ages in millions of years of subaerial shields in the Canary Islands are indicated in parentheses. Taxa with an asterisk represent single island endemics. ....71

2.2 50% Majority rule consensus tree based on MP analysis of combined dataset. The MP bootstrap support values above 50% are shown above branches. ....72

2.3 50% Majority rule consensus tree based on MP analysis of G3pdh dataset. The MP bootstrap support values above 50% are shown above branches. .....	73
3.1 50% Majority rule consensus tree based on MP analysis of G3pdh dataset. The MP bootstrap support values above 50% are shown above branches (part 1) .....	95
3.2. 50% Majority rule consensus tree based on MP analysis of G3pdh dataset. The MP bootstrap support values above 50% are shown above branches (part 2) .....	96
3.3 50% Majority rule consensus tree based on MP analysis of Cam dataset. The MP bootstrap support values above 50% are shown above branches (part 1). .....	97
3.4 50% Majority rule consensus tree based on MP analysis of Cam dataset. The MP bootstrap support values above 50% are shown above branches (part 2). .....	98

## INTRODUCTION

Oceanic island archipelagos have been considered as among the ideal places on earth to seek an understanding of the origin and elaboration of biological diversity (Stuessy and Ono, 1998). Island floras typically display numerous endemic species, which often have evolved through adaptive radiation, as a set of closely related species each with strikingly different morphological and ecological features. Adaptive radiation refers to the diversification of a lineage into species that have adapted to a variety of different niches, and that differ in the morphological and physiological traits corresponding to those resources. The result of adaptive radiation is often characterized by extensive divergence in morphological traits and habit and, at the same time, by little divergence in molecular sequences or crossing ability.

The group of plants I am working on belongs to Asteraceae. Here, “*Sonchus*” does not just include the genus *Sonchus*, but refers to island plants and their relatives in the subtribe Sonchinae. The Sonchinae is the most widely distributed subtribe in the tribe Cichorieae. Genus *Sonchus* had been considered to related to *Aetheorhiza*, *Reichardia* and *Launaea* (Stebbins 1953, Boulos 1974). These four genera have been incorporated in the subtribe Sonchinae (Bremer 1994). Several molecular phylogenetic studies suggested that the subtribe Dendroseridinae (*Dendroseris* and *Thamnosseris*) is close to *Sonchus* (Jansen et al., 1991; Whitton et al., 1995; Lee and Baldwin, 2004). In particular,



*Dendroseris*, endemic to the Juan Fernandez Islands, was found to be deeply embedded within the Sonchinae (Kim et al., 1996a, b, 1999, 2007). The genus *Sonchus* is widely distributed worldwide except for Central and South America (Mejías and Andres, 2004). In particular, genus *Sonchus* subgenus *Sonchus*, appears to be responsible for the origin of several island endemics both in the Pacific Ocean (*Dendroseris* in the Juan Fernandez Islands, *Thamnosseris* in the San Ambrosio Islands, *Actites* in Australia, and *Kirkianella* and *Embergeria* in New Zealand) and the Atlantic Ocean (i.e., the woody *Sonchus* alliance in the Macaronesian Islands) (Kim et al., 2007).

The origins of a few polyploid *Sonchus* taxa have been previously discussed. Stebbins et al. (1953) proposed an allopolyploid origin for *S. oleraceus* ( $2n = 32$ ) through natural crossing of *S. tenerrimus* ( $2n = 14$ ) and *S. asper* ( $2n = 18$ ). *S. microcephalus* ( $2n = 30$ ) is proposed to have originated from *S. oleraceus* through a dysploid process (Mejías and Andres 2004). *S. arvensis* can also be considered an allotetraploid (Hsieh et al., 1972). Boulos (1973) also suggested that *S. hydrophlius* is probably an autoteraploid derived from *S. asper*.

Many Pacific island endemics are also polyploids. *Actites* ( $2n=36$ ) is a tetraploid endemic to Australia (Lander, 1976). Both *Embergeria* and *Kirkianella* are monotypic endemics to New Zealand. *Kirkianella* (a decaploid and decatetraploid) is morphologically very diverse (Garnock-Jones, 1988). *Embergeria*, a monotypic endemic to New Zealand, is tetraploid ( $2n = 36$ ). The eleven species of *Dendroseris*, endemics of

the Juan Fernandez Islands, are all tetraploid ( $2n = 36$ ). All these Pacific Island endemics in Sonchinae are indeed derived within the *Sonchus* sensu lato and that polyploidization played a fundamental role in their origins and evolutions on islands.

None of the above hypotheses has been systematically and rigorously tested. We use a phylogenetic framework to test above proposed hypotheses about the origins of several polyploid *Sonchus* species as well as the origins of the Pacific polyploid endemics. This is the focus of Chapter One.

Patterns of chromosomal evolution of *Sonchus* derivatives are apparently different between Pacific and Atlantic Ocean. Endemics from the Atlantic Oceanic Islands are diploids in case of the woody *Sonchus* alliance. All alliance members are diploids ( $2n = 18$ ), suggesting that adaptive radiation was not accompanied by polyploidization, and also that the ancestor of the alliance was a diploid. Chapter Two focuses on the woody *Sonchus* alliance. It is one of the most diverse groups of Macaronesia endemics (6 genera and approximately 31 species) representing an outstanding example of adaptive radiation (Aldridge 1975, 1979). The alliance is composed of 19 species of primarily woody members of *Sonchus* (subg. *Dendrosonchus*), seven species of *Taeckholmia*, one species of subg. *Sonchus* (*S. tuberifer*), and four monotypic genera *Babcockia*, *Lactucosonchus*, *Sventenia*, and *Chrysoprenanthes* (Kim et al. 1996a, b; Lee et al. 2005). Based on previous phylogenetic studies, resolution of phylogenetic relationships within the alliance has been a difficult challenge (Kim et al. 1996a; Lee et al. 2005). Additional molecular

characters are needed to resolve phylogenetic relationships within the alliance. More importantly, the independent markers will help to reveal the role of hybridization and introgression in the Macaronesian flora, which was rarely accessed (Francisco-Ortega et al. 1996; Brochmann et al. 2000). In Chapter Two, we used three low-copy nuclear genes as independent markers to attempt to further resolve phylogenetic relationships as well as to give insights into potential role of hybridization contributed to the radiation of woody *Sonchus* alliance.

Homoploid hybrid speciation is defined as the newly formed hybrid has the same chromosome number as the parental species. All members in woody *Sonchus* alliance are diploids ( $2n = 18$ ). Homoploid hybridization is possible. In Chapter Three, we include two independent low-copy nuclear genes with clones of several key species to test for possible homoploid hybrid speciation. We test the hybrid origin of *S. tuberifer* and the *S. gummifer* complex, the origins of three monotypic species, and the potential hybridization between *Dendrosonchus* and *Taeckholmia*.

## REFERENCE

Aldridge, A. 1975. Taxonomic and anatomical studies in *Sonchus* L. Subgenus *Dendrosonchus* Webb ex Schultz Bip. and related genera. Ph. D. Dissertation. University of Reading, England.

Aldridge, A. 1979. Evolution within a single genus: *Sonchus* in Macaronesia. Plants and islands, ed. Bramwell, D., Academic Press, New York : 279-290

Boulos, L. 1973. Révision systématique du genre *Sonchus* L. s.l. IV. Sous-genre 1. *Sonchus.*, *Bot. Notiser* 126 : 155–196.

Boulos, L., 1974. Révision systématique du genre *Sonchus* L. s.l. VI. Sousgenre3. *Origosonchus*. Genres *Embergeria*, *Babcockia* et *Taeckholmia*. *Bot. Notiser* 127: 402–451.

Bremer, K., 1994. Asteraceae: cladistics and classification. *Timber Press*, Portland.

Brochmann, C., Borgen, L. and Stabbetorp, O. E. 2000. Multiple diploid hybrid speciation of the Canary Island endemic *Argyranthemum sundingii* (Asteraceae). *Plant Syst. Evol.* 220:77-92.

Garnock-Jones, P. J. 1988. Asteraceae–Lactuceae. In: C.J. Webb, W.R. Sykes and P.J. Garnock-Jones, Editors, Flora of New Zealand vol. IV, Botany Division DSIR, Christchurch: 291–350.

Francisco-Ortega, J. F., Jansen, R. K. and Santos-Guerra, A. 1996. Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. *Proc. Natl. Acad. Sci. USA* 93: 4085-4090.

Hsieh T. S., Schooler A. B., Hell A. and Nalewaja J. A. 1972. Cytotaxonomic of three *Sonchus* species. *Amer. J. Bot.* 59: 789–796.

Jansen, R. K., Michaels, H. J., Palmer, J. D. 1991. Phylogeny and character evolution in the Asteraceae based on chloroplast DNA restriction site mapping. *Syst. Bot.* 16: 99–115.

Lander, N. S. 1976. *Actites*, a new genus of Compositae from Australia, *Telopea* 1: 129–135.

Lee C, Kim S.-C., Santos-Guerra A. and Lundy K. 2005. Chloroplast DNA phylogeny of the woody *Sonchus* alliance (Asteraceae) in the Macaronesian islands. *American Journal of Botany* 92: 2072-2085

Lee, J. and Baldwin, B.G., 2004. Subtribes of principally north American genera of Cichorieae (Compositae). *Novon* 14, 309–313.

Kim, S.-C., Crawford D. J. and Jansen R. K. 1996a. Phylogenetic relationships among the genera of the subtribe Sonchinae (Asteraceae): evidence from ITS sequences. *Syst. Bot.* 21: 417-432.

Kim, S.-C., Crawford D. J., Francisco-Ortega J. and Santos-Guerra A. 1996b. A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. *Proc. Natl. Acad. Sci. USA* 93: 7743-7748.

Kim, S.-C., Crawford D. J., Jansen R. K. and Santos-Guerra A. 1999. The use of a non-coding region of chloroplast DNA in phylogenetic studies of the subtribe Sonchinae (Asteraceae:Lactuceae). *Pl. Syst. Evol.* 215: 85-99.

Kim S-C, Lee C. and Mejías J. A. 2007. Phylogenetic analysis of chloroplast DNA *matK* gene and ITS of nrDNA sequences reveals polyphyly of the genus *Sonchus* and new relationships among the subtribe Sonchinae (Asteraceae: Cichorieae). *Mol Phylogenet Evol* 44:578–597

Mejías, J.A. and Andrés, C., 2004. Karyological studies in Iberian *Sonchus* (Asteraceae: Lactuceae): *S. oleraceus*, *S. microcephalus* and *S. asper* and a general discussion, *Folia Geobot.* 39: 275–291.

Soltis P. S. and Soltis D. E. 2009. The role of hybridization in plant speciation. *Annu Rev Plant Biol* 60: 561–588.

Stebbins, G.L., 1953. A new classification of the tribe Cichorieae, family Compositae. *Madrono* 12: 65–81.

Stuessy T.F. and Mikio O. (eds). 1998. *Evolution and Speciation of Island Plants*. Cambridge University Press, Cambridge.

Whitton, J., Wallace, R. S. and Jansen, R. K. 1995. Phylogenetic relationships and patterns of character change in the tribe Lactuceae (Asteraceae) based on chloroplast DNA restriction site variation. *Can. J. Bot.* 73: 1058–1073.

# CHAPTER 1

## **ITS Evolution and the Origins of Polyploid *Sonchus* (Subgenus *Sonchus*) and Related Pacific Endemic Genera in Sonchinae (Asteraceae: Cichorieae)**

### **Abstract:**

Polyploidy is a widespread, evolutionarily important process in plants. In the genus *Sonchus* subgenus *Sonchus*, polyploidy appears to be responsible for the origin of several island endemics both in the Pacific and the Atlantic Oceans. Therefore, polyploid *Sonchus* and their related Pacific polyploid endemics are ideal systems for studying the polyplodization of island plants. Several hypotheses regarding the origins of the polyploidy have been proposed based on the morphology and karyology. Here, we use the cloning technique to retrieve the different ITS repeat types in order to test the origin of several polyploid *Sonchus* as well as polyploidy in the related Pacific island endemics. The Pacific endemics (*Dendroseris*, *Embergeria*, *Kirkianella*, and *Actites*) all form a monophyletic clade containing all of their repeat types which support a single origin of

these endemics. *S. arvensis* has been identified as a potential allopolyploid. The Stebbins hypothesis of an allopolyploid origin for *S. oleraceus* is partially supported with confirmation of *S. asper* as one donor. The data also indicate a hybrid origin for *S. hydrophilus* whose ITS copies might have subsequently homogenized via concerted evolution from one hybrid ancestor.

Keywords: hybridization, island biogeography, phylogeny, polyploidy, *Sonchus*, ITS.



## INTRODUCTION

Polyploidization has long been considered as a major force in plant evolution and speciation (Stebbins, 1977; Husband, 2000; Otto and Whitton, 2000; Ramsey and Schemske, 2002; Soltis, 2009). Most plant lineages have been affected by polyploidization events during their evolutionary development (Soltis and Soltis, 1993, 2000). About 50% to 70% angiosperms have had at least one polyploidization event in history of their genome (Averett, 1980; Grant, 1981). Autopolyploids result via chromosome doubling in a single diploid species, whereas allopolyploidy involves a hybridization process between genetically distinct taxa, followed by genome doubling (Stebbins, 1947; Soltis and Soltis, 1993). In the recent years, much progress has been accomplished regarding the contribution of molecular data to the understanding of polyploid genome origin and its evolution (Soltis and Soltis, 1999; Soltis et al., 2004; Wendel and Doyle, 2004).

An ideal system to study evolution via polyploidization should be a group of closely related species included both diploid and polyploid. The Sonchinae is the most widely distributed subtribe in the tribe Cichorieae, with a discontinuous, almost cosmopolitan, and very peculiar phylogeographic distribution (Kim et al., 1996a). In particular, genus *Sonchus* subgenus *Sonchus*, appears to be responsible for the origin of

several island endemics both in the Pacific Ocean (*Dendroseris* in the Juan Fernandez Islands, *Thamnoseris* in the San Ambrosio Islands, *Actites* in Australia, and *Kirkianella* and *Embergeria* in New Zealand) and the Atlantic Ocean (i.e., the woody *Sonchus* alliance in the Macaronesian Islands) (Kim et al., 2007). The most common chromosome number of the subtribe Sonchinae is  $2n = 18$  ( $n = 9$ ) (Beuzenberg and Hair, 1984; Spooner et al., 1987; Mejías, 1993; Ardevol Gonzalez et al., 1993; Mejías and Andres, 2004). Polyploidy has been detected in subgenus *Sonchus* and in the genera *Embergeria*, *Kirkianella*, and *Dendroseris*, (Mejías and Andres 2004). Some of these polyploids are island endemics with restricted distribution (e.g., *S. kirkii* ( $2n = 36$ ), New Zealand endemic; *S. macrocarpus* ( $2n = 36$ ), Egyptian endemic; *S. malaianus* ( $2n = 54$ ), Indonesian endemic; *S. hydrophilus* (unknown, but autotetraploid was suggested by Boulos 1973, Australian endemic), while others are very widely distributed (e.g., *S. arvensis* ( $2n = 36, 54$ ), *S. oleraceus* ( $2n = 32$ ), and *S. gigas* ( $2n = 36$ )). Two related aneuploids in which have a chromosome number that is not a multiple of the haploid number ( $n = 9$ ) are *S. microcephalus* ( $2n = 30$ ) occurring on the Iberian Peninsula, *S. tenerrimus* ( $2n = 14$ ) on the Mediterranean, Macaronesia and the Middle East. Many related Pacific Islands endemics such as *Embergeria*, *Actites*, *Kirkianella*, and *Dendroseris* are polyploids. These chromosome numbers suggest an important role of polyploidization processes in the evolution of *Sonchus* as well as for some of its Pacific derivatives.

The origins of a few polyploidy *Sonchus* taxa have been previously discussed. Stebbins et al. (1953) proposed an allopolyploid origin for *S. oleraceus* ( $2n = 32$ ) through natural crossing of *S. tenerrimus* ( $2n = 14$ ) and *S. asper* ( $2n = 18$ ). The morphological characters of *S. oleraceus* are intermediate between the two putative parents. Prior phylogenetic molecular analysis indicates that *S. asper* and *S. oleraceus* are closely related taxa, but *S. tenerrimus* seems not be involved in the origin of *S. oleraceus* (Kim et al., 2007); The diploid chromosome number of *S. microcephalus* ( $2n = 30$ ) is proposed to have originated from *S. oleraceus* through a dysploid process (Mejías and Andres, 2004). The taxon shows very similar fruits and leaves to those of *S. tenerrimus*. In consequence, it can be proposed that the loss or silencing genetic information of *S. asper* is involved in the origin of *S. microcephalus*. *Sonchus arvensis* can also be considered an allotetraploid on the basis of regular bivalent associations during meiosis (Hsieh et al., 1972), but no hypothesis about its origin has been formulated. According to the observations of Boulos (1973), *S. macrocarpus* and *S. gigas* are close relatives of *S. asper*, suggesting they are derivatives of *S. asper*. Boulos (1973) also suggested that *S. hydrophlius* is probably an autoteraploid derived from *S. asper*. Palynological studies (Pons and Boulos, 1962) have shown that *S. hydrophlius* has a mixture of teracolporate and tricolporate pollen grains, suggesting a possible allopolyploidy origin. ITS sequencing data (Kim et al., 2004) suggested that *S. kirkii* share most recent common ancestor with *S. hydrophlius* in Australia.

Different patterns of chromosomal evolution of *Sonchus* derivatives are apparent between Pacific and Atlantic Oceans. Endemics from the Atlantic Oceanic Islands woody *Sonchus* alliance are diploids ( $2n = 18$ ). This suggests that adaptive radiation was not primarily accompanied by polyploidization, and also that the ancestor of the alliance was a diploid. In contrast, many Pacific Island endemics are polyploids. *Actites* ( $2n = 36$ ) is a tetraploid fleshy perennial herb descended from *Sonchus* that is endemic to coastal sand dunes and cliffs on the southern and eastern coasts of Australia (Lander, 1976). Several diploids (*S. maritimus*, *S. aquatilis*, *S. crassifolius*, *S. wightianus*, and *S. brachyotus*) and one tetraploid (*S. arvensis*) are possible parental ancestors (Kim et al., 2007). Both *Embergeria* and *Kirkianella* are monotypic endemics to New Zealand. *Kirkianella* (a decaploid and decatetraploid) is morphologically very diverse (Garnock-Jones, 1988). Phylogenetical study suggested that *Kirkianella* evolved within the *Sonchus* group via polyploidization (Kim et al., 2007). *Embergeria*, a monotypic endemic to the Chatham Islands of New Zealand, is a perennial and is somewhat succulent herb. This genus is tetraploid ( $2n = 36$ ) and has been considered to be closely related to section *Asperi* (*S. kirkii*; Webb et al., 1988). *Dendroseris*, which includes 11 species, is endemic to the Juan Fernandez Islands, off the coast of Chile and is also tetraploid ( $2n = 36$ ). The monophyletic clade *Dendroseris* is strongly suggested to be derived from one or more *Sonchus* taxa (Kim et al., 1996a, b, 1999, 2004, 2007). All these examples suggest that several Pacific Island endemics in Sonchinae are indeed derived from the *Sonchus* group and that polyploidization played a fundamental role in the origin and evolution of these

island endemic genera. We do not know, however, if the continental progenitors were diploids, whether they hybridized on the continent (becoming tetraploid), or whether polyploidization occurred after island colonization.

The internal transcribed spacer regions (ITS1 and ITS2) of the tandemly repeated nuclear ribosomal DNA clusters are frequently used as markers for phylogenetic analyses of diverse plants. ITS sequences are biparentally inherited in most angiosperms and are thus distinguished from maternally inherited chloroplast DNA (cpDNA) which is also in widespread use for detecting phylogenetic relationship (Baldwin, 1992; Alvarez and Wendel, 2003). ITS sequence data have provided extensive insights into phylogenetic history, polyploid ancestry and historical introgression (Alvarez and Wendel, 2003; Bailey et al., 2003). The ITS paralogues are generally homogenized by concerted evolution (Wendel et al., 1995; Alvarez and Wendel, 2003). Coexistence of multiple divergent ITS repeats in one genome is quite common as a consequence of hybridization, and by genomic processes like gene and chromosome segment duplication and various forms of homologous and nonhomologous recombination (Bailey et al., 2003). Detection of internal transcribed spacer (ITS) sequence polymorphisms has been reported from a comparison of direct sequences and combinations of direct sequences and clone sequences (O'Kane et al., 1996; Campbell et al., 1997; Widmer and Baltisberger, 1999). Because of its biparental inheritance, ITS sequence data may detect historic hybridization events and provide information on both the maternal and paternal progenitor lineages for

allopolyploids (Alvarez and Wendel, 2003; Mcfadden and Hutchinson, 2004). ITS sequences have been successfully used to document the origin of several species in the past decade; for example, in the following genera: *Paeonia* (Sang et al., 1995), *Amelanchier* (Campbell et al., 1997), *Oxalis* (Emshwiller and Doyle, 1998), *Cardamine* (Franzke and Mummenhoff, 1999), *Draba* (Widmer and Baltisberger, 1999), *Miscanthus* (Hodkinson et al., 2002), and *Eupatorium* (Siripun and Schilling, 2006).

In this study, we sampled extensively for the phylogenetic analysis of polyploid *Sonchus* species, examining Pacific island endemic polyploid species as well as their potential putative parental species based on the previous phylogenetic study of the subtribe Sonchinae (Kim et al., 2007). We first tested some of the proposed hypotheses about the origins of several *Sonchus* species. We also determined whether Pacific polyploidy endemics were monophyletic or polyphyletic. This represents the first study for systematic phylogenetic test of the origins of polyploids in Sonchinae.

## **MATERIALS AND METHODS**

### **Plant Materials and Sampling Strategy**

Currently, the genus *Sonchus* comprises 54 species and is divided into three subgenera (Boulos, 1972): The Subgenus *Dendrosonchus* (19 species) consists of woody

plants endemic to Macaronesia, the *subgenus* *Origosonchus* (14 species, herbaceous perennials) and the Subgenus *Sonchus* which comprises 21 species (annuals, biennials, and perennials) and includes several cosmopolitan weedy species. Polyploidy has been documented in subgenus *Sonchus*.

Several polyploid *Sonchus* were sampled extensively (Appendix 1), including seven species of subgenus *Sonchus*: *S. arvensis* ( $2n = 36, 54$ ), *S. oleraceus* ( $2n = 32$ ), *S. kirkii* ( $2n = 36$ ), *S. hydrophilus* ( $2n = \text{unknown}$ ), and two related aneuploid *S. microcephalus* ( $2n = 30$ ) and *S. tenerrimus* ( $2n = 14$ ). *S. asper* ( $2n = 18$ ) and *S. brachyotus* ( $2n = 18$ ), the proposed parents for *S. oleraceus*, were also included. Three Pacific Island monotypic endemic genera (*Embergeria*, *Actites*, and *Kirkianella*) were also included. *Embergeria*, a tetraploid ( $2n = 36$ ), is endemic to the Chatham Islands of New Zealand. Another monotypic New Zealand endemic genus, *Kirkianella*, shows mixed ploidy levels: decaploid ( $2n = 90$ ) and decatetraploid ( $2n = 126$ ). *Actites* is a monotypic endemic to Australia and is a tetraploid ( $2n = 36$ ). *Dendroseris* (Juan Fernandez Islands endemic) occurs off the coast of Chile. All chromosome counts indicate that they are tetraploids with  $2n = 36$ , and they show bivalent formation, so it may suggest allotetraploid (Sanders et al., 1983; Spooner et al., 1987). Six species were chosen to represent this genus; *D. berteroana*, *D. litoralis*, *D. micrantha*, *D. pinnata*, *D. pruinata* and *D. regia*. All taxa were sequenced for nrDNA ITS, and all those individuals was subsequently cloned.

We sampled multiple populations (Appendix 1) for all the taxa except *S. kirkii*, *S. microcephalus*, *S. hydrophilus*, and the species in *Dendroseris* due to restricted distribution of these taxa.

### **DNA Isolation, PCR, and Sequencing**

Methods of DNA isolation/sequencing followed those of Lee et al. (2005) and Kim et al. (2007). Total genomic DNA was isolated from fresh, dried, or herbarium leaf tissue using DNeasy Plant Mini kits (QIAGEN, Valencia, CA, USA). Polymerase chain reaction (PCR) products were purified using a QiaQuick PCR purification kit (QIAGEN). Primers were the same as those described by Lee et al. (2005) and Kim et al. (2007).

All of those individuals were cloned, and 10-25 clones were sequenced so that the different ITS repeat types could be sampled. All cloning was performed with the TOPO TA PCR cloning kit (Invitrogen, Carlsbad, CA, USA). The selected clones were sequenced with an ABI PRISM BigDye Terminator v3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems Foster City, CA, USA). Primers were used T7 and M13 reversed plasmid primers in both directions. Extension products were purified and separated on an ABI377 automated sequencing machine (Applied Biosystems). Base calling and sequence editing were performed with Sequencher 4.7 (Gene Codes, Ann



Arbor, MI, USA). We merged the identical sequence copies into one ribotype for each individual.

### **Phylogenetic Analysis: Parsimony and Bayesian**

*Hyoseris* was used as an outgroup for the ITS data based on the previous phylogenetic study (Kim et al. 2007). All 123 accessions (21 genera and 74 species) as well as newly sequenced additional cloning ITS copies were used for building the phylogenetic trees. Phylogenetic analyses using Fitch parsimony were performed with PAUP\* (version 4.0; Swofford 2001), using the heuristic search option with tree-bisection–reconnection (TBR) branch swapping and the multiple parsimony (MULPARS) options selected. Insertions and deletions (indels) were treated as missing data. Support for nodes was calculated by bootstrap analysis with 500 simple-addition bootstrap replicates. The data set was also analyzed using Bayesian methods. The GTR+G model of DNA substitutions for the maximum likelihood analysis was determined by the Akaike Information Criterion (AIC) in Mr. Modeltest 2.3 (Nylander, 2004). Bayesian inference was conducted using default parameters in MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with the selected model. The Bayesian Markov chain Monte Carlo (MCMC) algorithm was run for 10,000,000 generations with four simultaneously chains (three “cold” and one “heated”), starting from random trees and sampling every 100 generations. At the end of

the run, convergence was evaluated by visual inspection of a graph of likelihood as a function of generation. A conservative burn-in period was determined, and only postburn-in trees were saved. Then the trees were imported into PAUP\* and a majority rule consensus tree was produced in order to show posterior probabilities (PP).

## **RESULTS**

### **The ITS Repeat Types**

To recover low-copy ITS repeats that otherwise could not be obtained by the direct cloning method, ITS repeat types were exhaustively examined by sequencing 10-25 clones for each taxon. We obtained sequences for 17 taxa (26 individuals). In total, 393 full-length clones were sequenced. The ITS clone sequences obtained were consistent with previous reports and lengths of ITS1 and ITS2 are within the range of other *Sonchus* species previously reported (Kim et al. 1996a, b, 2004, 2007; Lee et al. 2005). Only the 182 different ITS repeat types were incorporated in this study (Table 1.1).

### **The ITS Phylogeny**

A total of 503 characters for 306 accessions were used for phylogenetic analyses. Of the 503 characters, 140 characters (27.83%) were constant, 65 variable characters (12.92%) were parsimony uninformative, and 298 characters (59.25%) were parsimony informative between outgroup and ingroup. The heuristic search found shortest trees with a tree length (TL) of 1051, a strict consensus tree with a consistency index (CI) of 0.544, and a retention index (RI) of 0.945. The 50% majority rule consensus tree recovered by the Bayesian analysis is presented in Figure 1.1 1.2, 1.3, 1.4 and 1.5 with the posterior probabilities in each clade. The topologies of the consensus trees derived from the two analyses were similar. The 50% bayesian majority tree was used to discuss the hypohese testing. The Bayesian tree presents five highly diverged clades supported strongly by posterior probabilities values (Figure 1.1 1.2, 1.3, 1.4 and 1.5). These clades are (1) Woody *Sonchus* alliance; (2) *Sonchus* subg *Sonchus* section *Pustulati*; (4) *Dendroseris*; (5) *Kirkianella*, *Embergeria*, *Actites* and *Sonchus* sections *Maritimi* and *Arvensese*; (6) *Sonchus* section *Sonchus* and *Asperi*.

In clades (1) and (2), all the members are diploid with a haploid chromosome number of nine. These two clades are derived off the basal part of the trees suggesting that nine is the basic and ancestral haploid chromosome number within the Sonchinae and that polyploidization and aneuploidization took place independently in several lineages after other lineages evolve from these basal lineages. The *Dendroseris* clade ( $2n = 36$ ) contains all the direct sequences as well as thirty-five ITS repeat types from cloned

sequences of six species (*D. berteroana*, *D. litoralis*, *D. micrantha*, *D. pinnata*, *D. pruinata* and *D. regia*) represented this genus. Clade 5 contains *Kirkianella*, *Embergeria*, *Actites*, and *S. brachyotus*. Clade 6 contains *S. tenerrimus*, *S. microcephalus*, *S. asper*, *S. kirkii* and *S. oleraceus*.

### **ITS Evolution of Pacific Endemics and subgenes *Sonchus* Polyploids**

In clade 5, *Dendroseris* is monophyletic with the posterior probability of 1.00. *Kirkianella* and *Embergeria* in New Zealand are both monophyletic and sister clades. Twenty-two cloned repeats of *Kirkianella* as well as 2 direct sequences all clustered as a monophyletic clade. The same was true for the *Embergeria* clade which included 14 cloned repeats. *Actites* in Australia also formed a monophyletic clade containing all the 8 cloned repeats and it is sister to the *S. arvensis* from Taiwan.

*Sonchus arvensis* from Taiwan, representing the Old World species, had its direct sequence and all the 4 repeat types clustered in the clade 5 with posterior probability (PP) of 1. Interestingly, the New World *S. arvensis* has the cloned repeat types (except one repeat type) clustered in the clade 6. The direct sequences for New World as well as one repeat type clustered with the Australian endemic *S. hydrophilus* with PP of 1 in clade 5. The Australian endemic *S. hydrophilus* has all 6 repeat types in the clade 5, but the previous direct sequences (Kim et al., 2007) are all in the clade 6 which includes all repeats types for *S. kirkii* ( $2n = 36$ ), the New Zealand endemic. Samples of *S. oleraceus*

( $2n = 32$ ) from India (8 cloned repeats), Egypt (2 cloned repeats) as well as Korea (direct sequence) all belong to the clade 6, but the taxon is apparently not monophyletic. Direct sequenced *S. oleraceus* from Korea, two repeat types from India and *S. asper* from Spain formed a small cluster within clade 5 with posterior PP of 0.98. *S. microcephalus* ( $2n = 30$ ), *S. bourgeaui* ( $2n = 16$ ) and *S. tenerrimus* ( $2n = 14$ ) from Australia are clustered with PP of 1. But all *S. tenerrimus* from Italy and Spain clustered into another cluster with PP of 1.

## DISCUSSION

### Evolution of Pacific island endemic polyploids

Species in *Dendroseris* are rosette trees and shrubs with extremely variable morphology. They have a very limited distribution and are exceedingly rare. All chromosome counts indicate that they are tetraploids with  $2n = 36$  (Sanders et al., 1983; Spooner et al., 1987). The origin of *Dendroseris* in the Juan Fernandez Islands has been elusive for several decades. This study confirms that *Dendroseris* is deeply embedded within the Sonchinae and strongly support it as a monophyletic group with all the thirty-five ITS repeat types included. According to the Bayesian 50% majority rule consensus

tree, *Dendroseris* is derived right after Woody *Sonchus* alliance and *Sonchus* section *Pustulati*. They share the most recent common ancestor with several other Pacific Island endemic genera as well as the species in subgenus *Sonchus*. The closest continental ancestor of *Dendroseris* remains undetermined. There is not enough evidence to determine whether its continental ancestor was diploid and became tetraploid subsequent to dispersal, or if polyploidization occurred prior to dispersal and radiation to the islands.

*Embergeria*, a tetraploid ( $2n = 36$ ), is endemic to the Chatham Islands of New Zealand. Subgenus *Sonchus*, especially section *Asperi* (*S. kirkii*; Webb et al., 1988), has been considered the most probable ancestor of *Embergeria* (Boulos, 1965; Pons and Boulos, 1972; Boulos, 1974). However, no repeats were clustered with section *Asperi* which indicate *Asperi* are not closely related to *Embergeria*. More likely, the *Arvenses* or *Maritimi* (excluding *S. palustris*) in clade 5 could be responsible for its origin. *Kirkianella*, Another monotypic New Zealand endemic genus, *Kirkianella* shows mixed ploidy levels: decaploid ( $2n = 90$ ) and decatetraploid ( $2n = 126$ ) (Beuzenberg and Hair, 1984). *Kirkianella* and *Embergeria* are sister clades with the posterior probability 0.92, which suggest that they might share the same ancestor. Since they have different ploidy levels, it is likely that they evolved through different polyploidization events. Since the putative parents are still unknown, we cannot conclude whether speciation was accompanied by polyploidization, because the polyploidization could have happened before dispersal to the island.

*Actites* is a fleshy perennial herb endemic to coastal sand dunes and cliffs on the Southern and eastern coasts of Australia and is tetraploid (Lander, 1976). Similar to *Embergeria*, it was suggested to have evolved from section *Asperi* (Wardle, 1963). This study does not support that hypothesis. The *Actites*, *S. arvensis* and *S. wightianus* formed a cluster with posterior probability 0.75 which suggests *Actites* probably has an Asian origin from diploid *S. arvensis* or *S. wightianus* via a polyploidization.

### **Evolution and Origins of Polyploid *Sonchus***

*S. arvensis* is both tetraploid (subsp. *uliginosus*;  $2n = 36$ ) and hexaploid (subsp. *arvensis*;  $2n = 54$ ), its two subspecies occur widely in non-Mediterranean Europe and North America. Previously Kim et al. (2007) showed *S. arvensis* shares its most recent common ancestor with *S. maritimus* and *S. crassifolius* in the ITS tree, while it is sister to *S. brachyotus* in matK phylogeny. In this study, both *S. arvensis* from Taiwan and *S. wightianus* from Bhutan might be involved in the origin of *Actites*. The direct sequenced *S. arvensis* from Texas and Wyoming as well as one cloned repeat types of *S. arvensis* from Wyoming formed a cluster which is sister to *S. maritimus* and *S. crassifolius*. The other 9 repeat types of *S. arvensis* from Wyoming clustered in clade 6 with *S. asper* and *S. oleraceus*. These data support a hybrid origin for *S. arvensis*. The parental repeats have been apparently homogenized by concerted evolution. In a polyploidy context, sequence heterogeneity is expected as a consequence of biparental inheritance in recently formed

species of hybrid origin (e.g., Ainouche et al., 2004; Soltis et al., 2004), with various degrees of repeat homogenization and concerted evolution (Wendel et al., 1995; Ainouche and Bayer, 1997; Rauscher et al., 2002; Kovarik et al., 2004). In the polyploids we have analyzed here, homogenization via concerted evolution seems to have occurred either toward maternal or paternal repeats for the *S. arvensis*, but the cloned ITS repeat types detected their rare copies for the New World *S. arvensis* which didn't show in the direct sequencing. But we are still not unsure about the parents for *S. arvensis*.

Stebbins hypothesized an allpolyploid origin for *S. oleraceus* ( $2n = 32$ ) through natural crossing of *S. tenerrimus* ( $2n = 14$ ) and *S. asper* ( $2n = 18$ ), because *S. oleraceus* is morphologically similar to those putative parents. Both *S. tenerrimus* and *S. oleraceus* have pinnatifidous leaves. These two taxa commonly co-exist in low-altitude areas of southern Spain, but karyological studies of *S. oleraceus* (Mejias and Andres 2004) show the regular presence of bivalents during meiosis, indicating no evidence of hybridization and therefore an effective reproductive isolation between these two species. In our study, *S. oleraceus* deeply clusters with *S. asper* in clade 6, but all the 20 repeat types from *S. tenerrimus* clustered in clade 5. So Stebbins' hypothesis is only partially supported by our data. It appears that *S. asper* is one donor for origin of the *S. oleraceus*, but it appears not the case for *S. tenerrimus*. But we can not rule out the possibility of ITS copies homogenized toward *S. asper*.



The proposed origin for *S. microcephalus* (n = 15) is through a process involving aneuploidy in *S. oleraceus* (n = 16) (Mejias and Andres 2004). *S. microcephalus* is very similar to *S. tenerrimus* in fruit and leaf morphology. Our data shows *S. bourgeaui* (n = 8), *S. tenerrimus* (n = 7) in Australia and all the 8 repeat types from *S. microcephalus* (Spain) clustered together with the PP of 1.00. The data support descending aneuploid series involving *S. bourgeaui* and *S. tenerrimus*. But all the repeat types we cloned from *S. tenerrimus* from Italy and Spain do not have the repeat types in this clade. A possible explanation for that could be *S. tenerrimus* in Italy and Spain might have homogenized with their ITS copies via concerted evolution. Moreover, by cloning the Australia species, we might find some repeat types filling the gaps between these two clades. There is no evidence to support that the proposed loss or silencing of some genetic information from *S. asper* is involved in the origin of *S. microcephalus*.

*S. kirkii* and *S. hydrophilus* are freshwater or littoral species with a deciduous pappus. *Sonchus kirkii* is tetraploid ( $2n = 36$ ) and had been considered closely related to section *Asperi*. Our results show that *S. kirkii* clustered with *S. hydrophilus* within clade 6, which suggests that *S. hydrophilus* might be closely related to *S. kirkii* and it belongs to the derived position on the phylogenetics tree. Therefore, both *S. kirkii* and *S. hydrophilus* may have originated from section *Asperi*. One interesting phenomenon is that the *S. hydrophilus* clones had all the 6 ITS repeat types in the clade 5. Collectively, these data might indicate a hybrid origin for *S. hydrophilus* which might have

subsequently homogenized its ITS copies from one hybrid ancestor via concerted evolution.

### **Conclusion Remarks:**

The role of polyploidization in the evolution of the genus *Sonchus* has been suggested to be important, based on the recurrence of polyploid species (ca. 30% of subgenus *Sonchus* are either tetraploids or hexaploids). This study represents the first comprehensive molecular documentation of origins of polyploids in the genus *Sonchus* as well as its several related Pacific island endemic relatives. Concerted evolution is often used to describe the unusual evolutionary behavior of multigene family members. The nrDNA tandem array such as ITS are subject to the process in which the individual repeats in a multigene family evolve in concert rather than independently. The concerted evolution leads to homogenization of all repeats in an array. Our cloning strategies successfully recovered the rare ITS copies which add new insights to the evolutionary stories of polyploidy *Sonchus*. We, however, could not strictly test hypotheses regarding the origins of the polyploidy *Sonchus* based on single marker. Future research needs to focus on finding several alternative low-copy nuclear intron regions to fully investigate the phylogeny of these polyploids and also to determine the role of polyploidization and hybridization in their evolution in the evolution of genus *Sonchus* and related taxa.

## REFERENCES

Aïnouche, M. L., and Bayer, R. J. 1997. On the origins of the tetraploid *Bromus* species (section *Bromus*, Poaceae): Insights from internal transcribed spacer sequences of nuclear ribosomal DNA. *Genome* 40: 730–743.

Aïnouche, M. L., Baumel, A. and Salmon, A. 2004. Biological relevance of polyploidy: Ecology to genomics. *Spartina anglica* C.E. Hubbard: A natural model system for analyzing early evolution changes that affect allopolyploid genomes. *Biological Journal of the Linnaean Society* 82: 475–484.

Alvarez, I. and Wendel, J. F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Mol. Phylogenet. Evol* 29: 417-434.

Ardévol González, J. F., Borgen, L. and Pérez de Paz, P. L., 1993. Checklist of chromosome numbers in Canarian vascular plants, *Sommerfeltia* 18: 1–59.

Averett, J. E. 1980. Polyploidy in plant taxa: summary. In: Polyploidy (Lewis WH, ed). New York: Plenum: 269-277.

Bailey, C. D., Carr, T. G., Harris, S. A. and Hughes C. E. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. *Mol Phylogenet Evol* 29:435–55s

Baldwin, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Mol. Phylogenet. Evol* 1, 3-16.

Beuzenberg, E. J. and Hair, J. B. 1984. Contributions to a chromosome atlas of the New Zealand flora-27: Compositae, *NZ. J. Bot.* 22: 353–356.

Boulos, L. 1965. *Sonchus* and *Embergeria*. In: Eichler, H.J. (Ed.), Supplement to Black's Flora of South Australia. Government Printer, *Adelaide* : 330–333.

Boulos, L. 1972. Révision systématique du genre *Sonchus* L. s.l. II. Étude caryologique. *Bot Not* 125:306–309

Boulos, L. 1973. Révision systématique du genre *Sonchus* L. s.l. IV. Sous-genre 1. *Sonchus.*, *Bot. Notiser* 126 : 155–196.

Boulos, L. 1974. Révision systématique du genre *Sonchus* L. s.l. VI. Sous-genre 3. *Origosonchus*. Genres *Embergeria*, *Babcockia* et *Taackholmia*, *Bot. Notiser* 127: 402–451.

Campbell, C. S., Wojciechowski, M. F., Baldwin, B. G., Alice, L. A., and Donoghue, M. J. 1997. Persistent nuclear ribosomal DNA sequence polymorphism in the *Amelanchier* agamic complex (Rosaceae). *Molecular Biology and Evolution* 14: 81–90.

Emshwiller, E., and Doyle, J. J. 1998. Origins of domestication and polyploidy in oca (*Oxalis tuberosa*: Oxalidaceae): nrDNA ITS data. *Am. J. Bot.* 85: 975–985.

Franzke, A. and Mummenhoff, K. 1999. Recent hybrid speciation in *Cardamine* (Brassicaceae) - conversion of nuclear ribosomal ITS sequences in *statu nascendi*. *Theor. Appl. Genet.* 98: 831–834.

Garnock-Jones, P. J. 1988. Asteraceae–Lactuceae. In: C.J. Webb, W.R. Sykes and P.J. Garnock-Jones, Editors, *Flora of New Zealand* vol. IV, Botany Division DSIR, Christchurch : 291–350

Grant V. 1981. *Plant speciation*. Columbia University Press, New York, New York, USA.

Hodkinson, T.R., Chase, M.W., Takahashi, C., Leitch, I.J., Bennett, M.D. and Renvoize, S.A. 2002. The use of DNA sequencing (ITS and trnL-F), AFLP, and fluorescent in situ hybridization to study allopolyploid *Miscanthus* (Poaceae). *Am. J. Bot.* 89: 279–286.

Hsieh T.S., Schooler, A.B., Hell, A. and Nalewaja, J.A. 1972. Cytotaxonomic of three *Sonchus* species. *Amer. J. Bot.* 59: 789–796.

Huelsenbeck, J. P. and Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17:754-755.

Husband, B.C. 2000. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proc. R. Soc. Lond. B.* 267: 217-223

Kim, S.-C., Crawford, D. J. and Jansen, R. K. 1996a. Phylogenetic relationships among the genera of the subtribe Sonchinae (Asteraceae): evidence from ITS sequences. *Syst. Bot.* 21: 417-432.

Kim, S.-C., Crawford, D. J., Francisco-Ortega, J. and Santos-Guerra, A. 1996b. A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. *Proc. Natl. Acad. Sci. USA* 93: 7743-7748.

Kim, S.-C., Crawford, D. J., Jansen, R. K. and Santos-Guerra, A. 1999. The use of a non-coding region of chloroplast DNA in phylogenetic studies of the subtribe Sonchinae (Asteraceae:Lactuceae). *Pl. Syst. Evol.* 215: 85-99.

Kim, S.-C., Lu, C. T. and Lepschi, B. J., 2004. Phylogenetic positions of *Actites megalocarpa* and *Sonchus hydrophilus* (Sonchinae: Asteraceae) based on ITS and chloroplast non-coding DNA sequences, *Aust. Syst. Bot.* 17: 73–81

Kim, S.-C., Lee, C. and Mejías, J. A. 2007. Phylogenetic analysis of chloroplast DNA *matK* gene and ITS of nrDNA sequences reveals polyphyly of the genus *Sonchus* and new relationships among the subtribe Sonchinae (Asteraceae: Cichorieae). *Mol Phylogenet Evol* 44:578–597

Kovarik, A., Matyasek, R., Lim, K. Y., Skalika, K., Koukalova, B., Knapp, S., Chase, M. and Leitch, A. 2004. Concerted evolution of allotetraploid 18-5.8-26S rDNA repeats in *Nicotiana* alloetretraploids. *Biological Journal of the Linnaean Society* 82: 615–625.

Lander, N.S. 1976. *Actites*, a new genus of Compositae from Australia, *Telopea* 1 : 129–135.

Lee, C., Kim, S.-C., Lundy, K. and Santos-Guerra, A. 2005. Chloroplast DNA phylogeny of the woody *Sonchus* alliance (Asteraceae: Sonchinae) in the Macaronesian Islands. *Am. J. Bot.* 92, 2072–2085.

Mcfadden, C.S., and Hutchinson, M.B. 2004. Molecular evidence for the hybrid origin of species in the soft coral genus *Alcyonium* (Cnidaria: Anthozoa: Octocorallia). *Mol. Ecol.* 13: 1495–1505.

Mejías J.A. 1993. Estudio cariológico del género *Launaea* Cass. en la Península Ibérica, *Lagascalia* 17 : 135–149.

Mejías, J.A. and Andrés, C. 2004. Karyological studies in Iberian *Sonchus* (Asteraceae: Lactuceae): *S. oleraceus*, *S. microcephalus* and *S. asper* and a general discussion, *Folia Geobot.* 39: 275–291.

- Nylander, J. A. A. 2004. Bayesian phylogenetics and the evolution of gall wasps. *Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology*, ISSN 1104-232X ; 937.
- O’Kane, S. L. Jr., Schaal, B. A. and Al-Shehbaz, I. A. 1996. The origins of *Arabidopsis suecica* (Brassicaceae) as indicated by nuclear rDNA sequences. *Systematic Botany* 21: 559–566.
- Otto, S. P. and Whitton J. 2000. Polyploid Incidence and Evolution. *Annual Review of Genetics* 34: 401-437.
- Pons, A. and Boulos, L. 1972. Révision systématique du genre *Sonchus* L. s.l. III. Étude palynologique, *Bot. Notiser* 125 : 310–319.
- Ramsey, J. and Schemske D. W. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology and Systematics* 33: 589-639.
- Rauscher, J. T., Doyle, J. J. and Brown, H. D. 2002. Internal transcribed spacer repeat-specific primers and the analysis of hybridization in the *Glycine tomentella* (Leguminosae) polyploid complex. *Molecular Ecology* 11: 2691–2702.
- Ronquist, F. and Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.
- Sanders, R.W., Stuessy, T. F. and Rodriguez, R. 1983. Chromosome numbers from the flora of the Juan Fernandez Islands. *Am. J. Bot.* 70: 799–810.
- Sang, T., Crawford, D. J. and Stuessy, T. F. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal

DNA: implications for biogeography and concerted evolution. *Proc. Natl. Acad. Sci. USA* 92: 6813–6817.

Siripun, K.C. and Schilling, E.E. 2006. Molecular confirmation of the hybrid origin of *Eupatorium godfreyanum* (Asteraceae). *Am. J. Bot.* 93: 319–325.

Soltis, D. E. and Soltis, P. S. 1993. Molecular data and the dynamic nature of polyploidy. *Critical Reviews in Plant Sciences* 12: 243-273.

Soltis, D. E. and Soltis, P. S. 1999. Polyploidy: recurrent formation and genome evolution. *Trends in Ecology and Evolution.* 14: 348-352

Soltis, P. S. and Soltis, D. E. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proc. Natl. Acad. Sci. USA* 97: 7051-7057

Soltis D. E., Soltis P. S., Pires J. C., Kovarik A. and Tate J. A. 2004. Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic, genomic and genetic comparisons. *Biological Journal of the Linnean Society* 82: 485–501.

Soltis P.S. and Soltis D.E. 2009. The role of hybridization in plant speciation. *Annu Rev Plant Biol* 60: 561–588.

Spooner, D. M., Stuessy, T. F., Crawford, D. J. and Silva, M. O. 1987. Silva, Chromosome numbers from the flora of the Juan Fernandez Islands. II, *Rhodora* 89: 351–356.

Stebbins, G. L. 1947. Types of polyploids: Their classification and significance. *Advanced Genetics* 1: 403–429.



Stebbins G.L. 1953. A new classification of the tribe Cichorieae, family Compositae. *Madroño* 12: 65–81.

Stebbins, G.L. 1971. *Chromosomal Evolution in Higher Plants*. Edward Publishers, Ltd: London.

Swofford, D.L. 2001. PAUP\*: phylogenetic analysis using parsimony (\*and other methods) version 4.0. Sinauer, Sunderland.

Wardle, P. 1963. Evolution and distribution of the New Zealand flora, as affected by Quaternary climates. *NZ J. Bot.* 1: 3–17.

Webb, C. J., Sykes, W. R. and Garnock-Jones, P. J. 1988. *Flora of New Zealand* vol. IV, Botany Division DSIR, Christchurch .

Wendel, J. F., Schnabel, A. and Seelanan, T. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proc. Natl. Acad. Sci. USA* 92: 280–284.

Wendel, J. F. and Doyle J. J. 2004. Polyploidy and evolution in plants. In R. Henry [ed.], *Diversity and evolution in plants*, CABI Publishing, Wallingford, UK: 97–117.

Widmer, A. and Baltisberrger, M. 1999. Molecular evidence for allopolyploid speciation and a single origin of the narrow endemic *Draba ladina* (Brassicaceae). *Am. J. Bot.* 86: 1282–1291.

Table 1.1 List of investigated samples with sample number ( sample location, the number of clones sequenced, and the maximum number of sequence types found within each individual.

<b>Species</b>	<b>Sample #</b>	<b>Sample location</b>	<b># of clone sequenced</b>	<b># repeat types</b>
<i>Actites</i>	3879-1	Australia	10	3
<i>Actites</i>	4657-1	Australia	10	5
<i>D. berteroana</i>	11321	Juan Fernandez	15	6
<i>D. litoralis</i>	6305	Juan Fernandez	15	8
<i>D. micrantha</i>	11582	Juan Fernandez	15	6
<i>D. pinnata</i>	12151	Juan Fernandez	15	5
<i>D. pruinata</i>	6445	Juan Fernandez	15	6
<i>D. regia</i>	9316	Juan Fernandez	15	4
<i>Embergeria</i>	149	New Zealand	15	8
<i>Embergeria</i>	17501	New Zealand	15	6
<i>Kirkianella</i>	150	New Zealand	20	12
<i>Kirkianella</i>	151	New Zealand	15	10
<i>S.arvensis</i>		Taiwan	15	4
<i>S. arvensis</i>		Wyoming (USA)	25	10
<i>S. brachyotus</i>		Korea	15	6
<i>S. asper</i>		Italy	15	12
<i>S. asper</i>		Korea	15	8
<i>S. asper</i>		Morocco	15	7
<i>S. asper</i>		Spain	15	6
<i>S. hydrophilus</i>		Australia	15	6
<i>S. kirkii</i>		New Zealand	15	6
<i>S. microcephalus</i>		Spain	25	8
<i>S. olearceus</i>		India	10	8
<i>S. oleraceus</i>		Egypt	10	2
<i>S. tenerrimus</i>	68	Italy	15	10
<i>S. tenerrimus</i>	38120	Spain	15	10

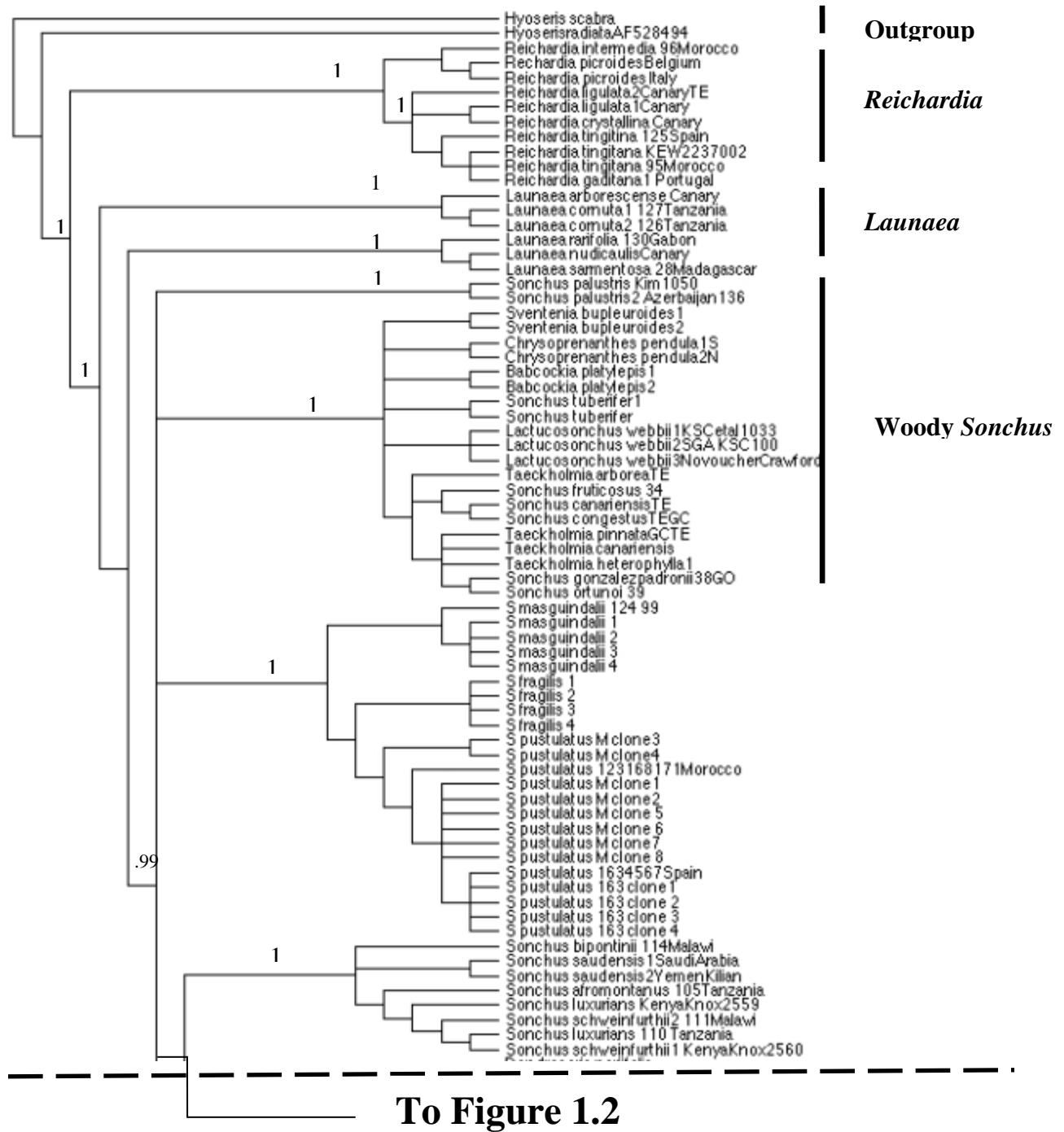


Figure 1.1 Majority (50%) consensus tree based on Bayesian analysis (clade 1&2). The posterior probability values above are shown above branches

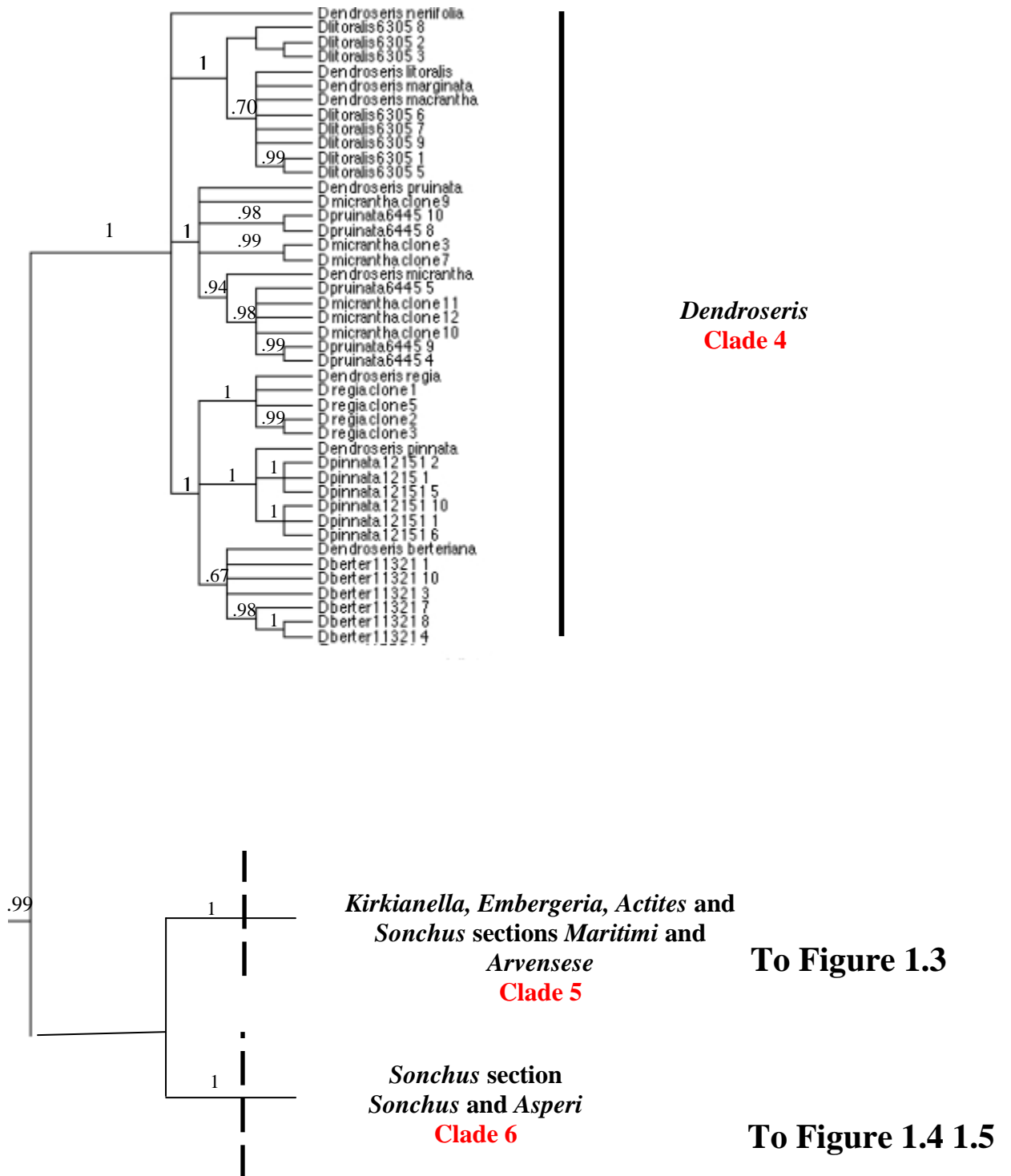


Figure 1.2 Majority (50%) consensus tree based on Bayesian analysis (Clade 4). The posterior probability values above are shown above branches.

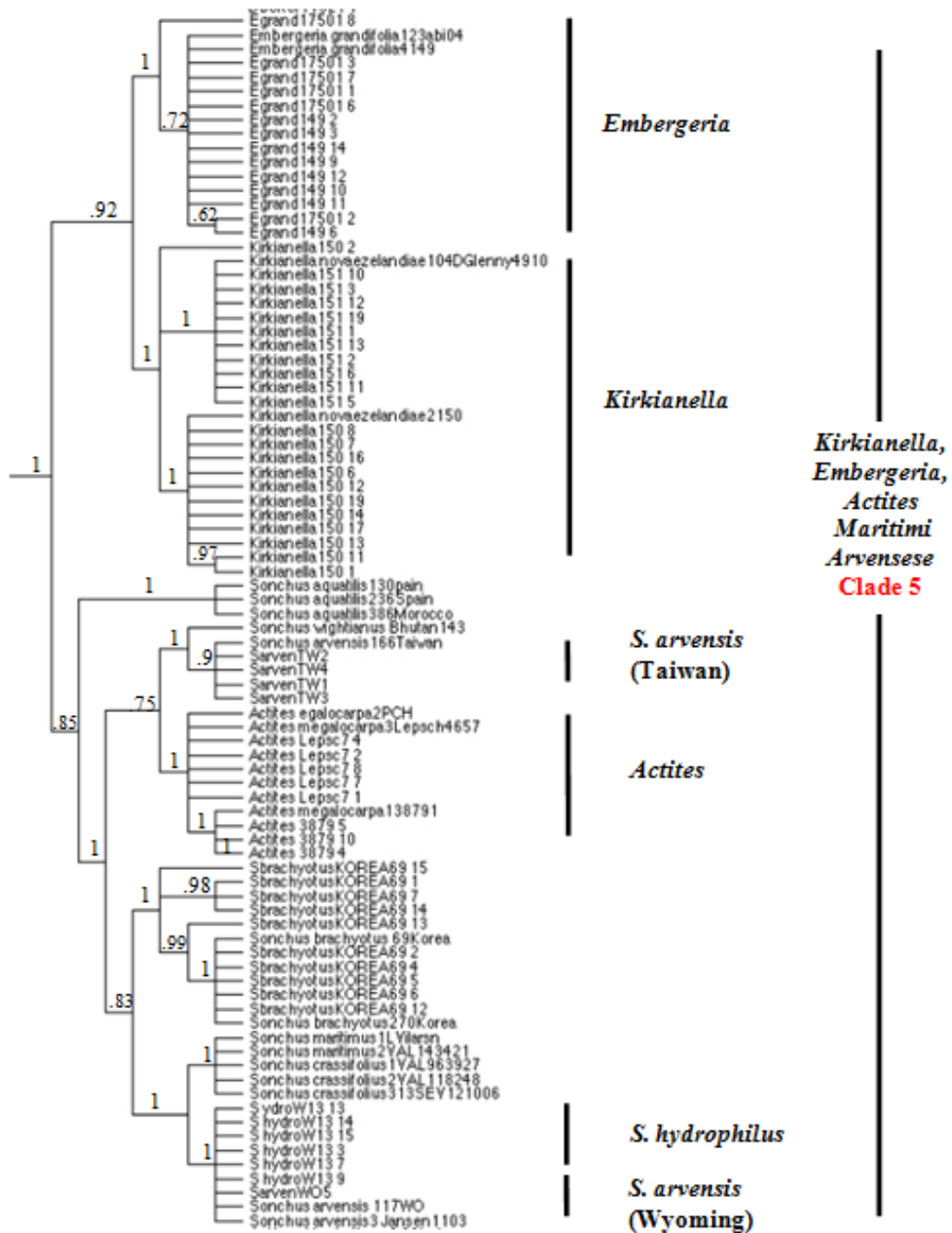


Figure 1.3 Majority (50%) consensus tree based on Bayesian analysis (Clade 5). The posterior probability values above are shown above branches

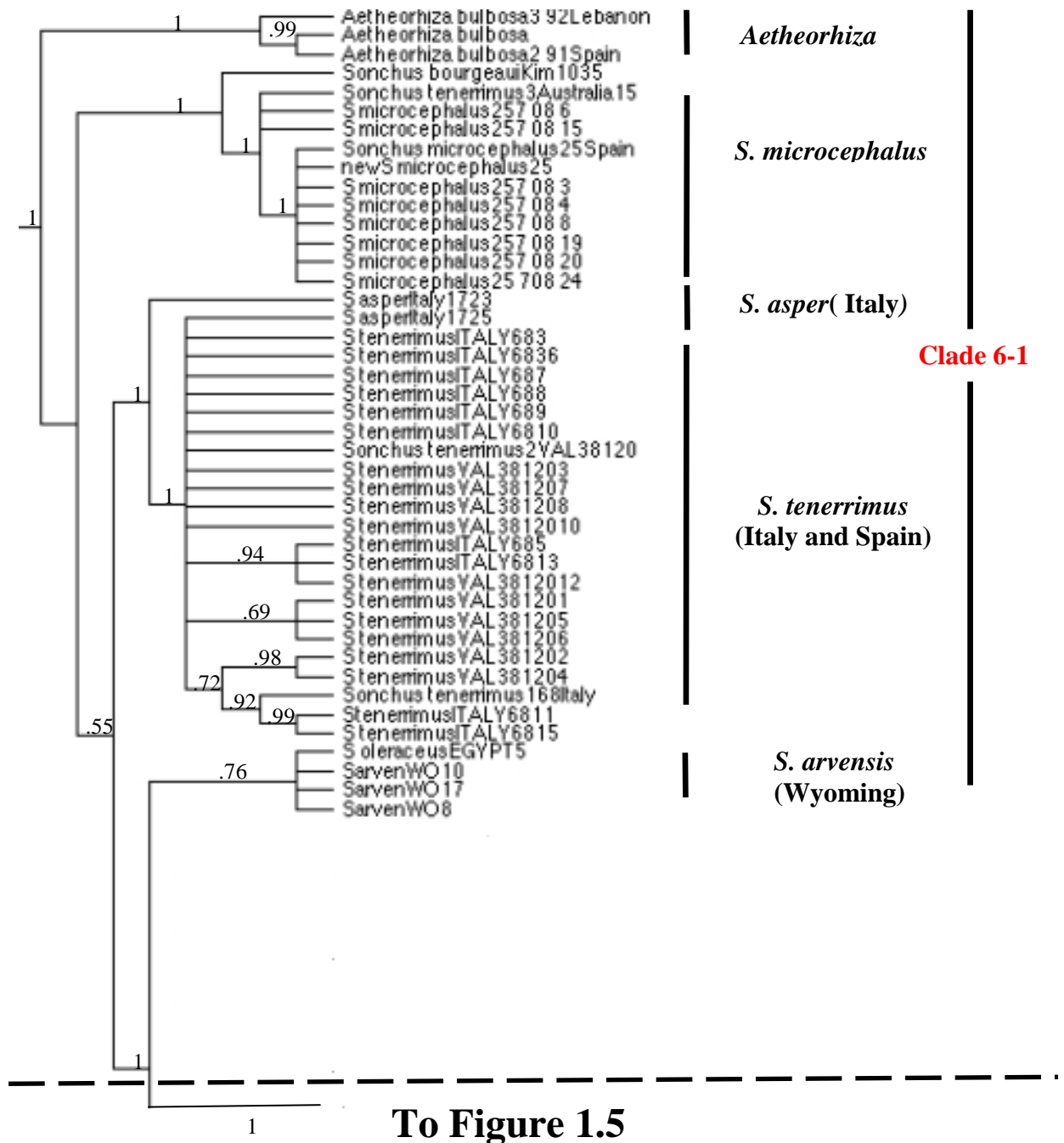


Figure 1.4 Majority (50%) consensus tree based on Bayesian analysis( Clade 6-1). The posterior probability values above are shown above branches

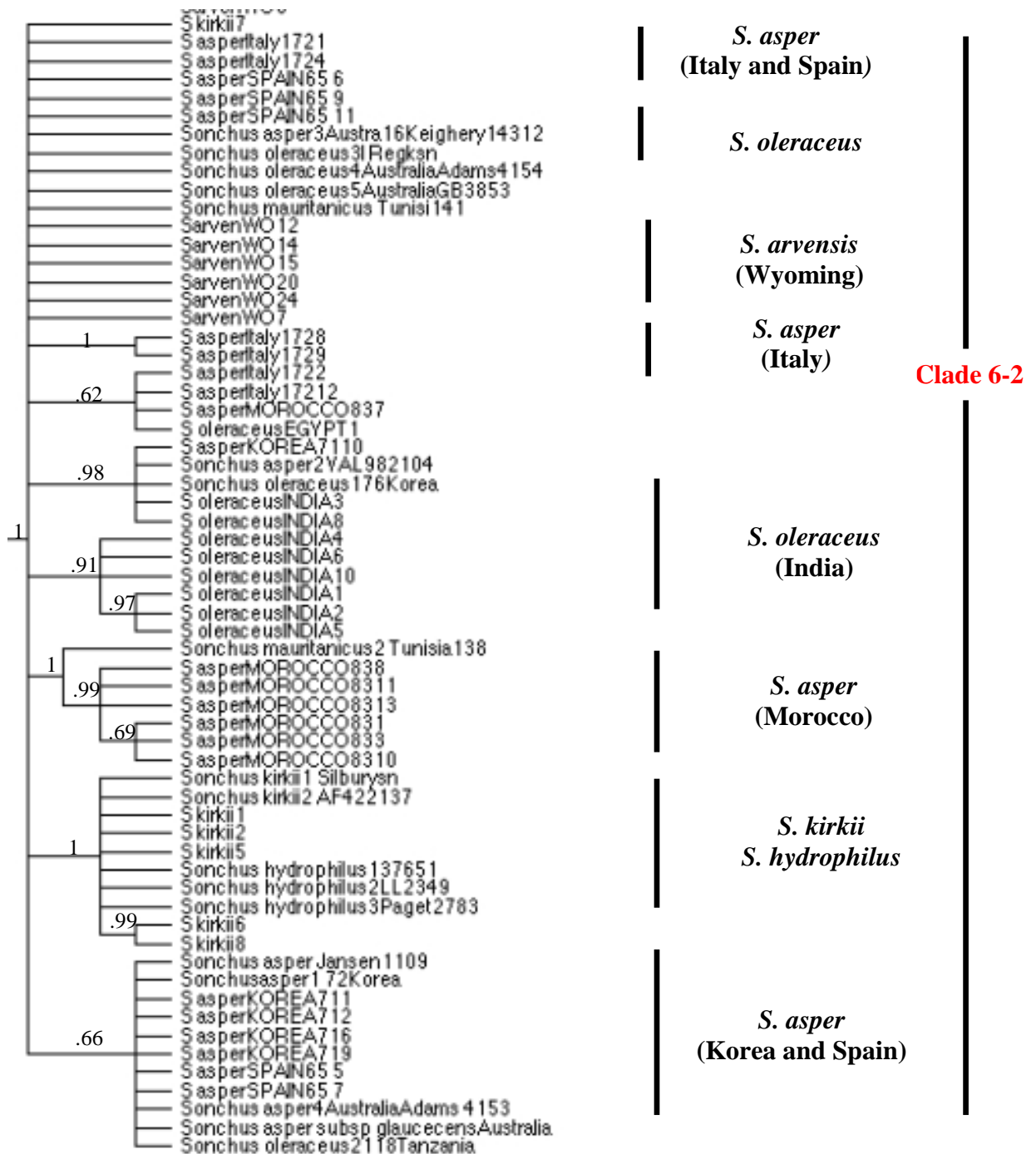


Figure 1.5 Majority (50%) consensus tree based on Bayesian analysis( Clade 6-2). The posterior probability values above are shown above branches

## CHAPTER 2

### **Origin and Evolution of the Woody *Sonchus* alliance in the Canary Islands**

#### **Abstract:**

The woody *Sonchus* alliance is one of the largest endemic groups of Macaronesia with 6 genera and approximately 31 species in Macaronesia, representing an example of adaptive radiation. Determining accurate phylogenetic relationships among the members of the woody *Sonchus* alliance presents challenges because of insufficient level of molecular variation and the convergent evolution of similar morphological traits in island settings. All taxa of the woody *Sonchus* alliance were sampled to investigate their phylogenetic relationships as well as to test the potential role of hybridization and introgression in their evolution using three independent low-copy nuclear genes: glyceraldehyde 3-phosphate dehydrogenase (G3pdh) , B12 and calmodulin (Cam). The B12 and Cam phylogenies were not well resolved due to the limited phylogenetically informative sites. The G3pdh phylogeny was not significantly different from those of B12 and Cam, and subsequent combined analysis provided a better resolved and supported phylogeny within the alliance. In the MP combined analysis, the basal lineages of monotypic genera were not identified. All *Taeckholmia* species except *T. arborea*



formed a well-supported clade. This result partly supports Boulos' classification that treats *Taeckholmia* as a distinct genus. But the subgenus *Dendrosonchus* is highly polyphyletic which does not support either Boulos' or Aldridge's classification of *Dendrosonchus*. Assessment of the role of hybridization and introgression in the alliance's evolution was limited due to low sequence variability of B12 and Cam genes. However, potential hybridization has been recognized comparing independent gene regions.

Keywords: Low-copy nuclear gene, adaptive radiation, woody *Sonchus* alliance, phylogeny, hybridization

## INTRODUCTION

Islands have held the fascination of biologists interested in natural history. Since Darwin's observations on the Galapagos, oceanic island archipelagos have provided clues about evolutionary patterns and processes and may rightly be considered as among the best places on earth to seek an understanding of the origin and elaboration of biological diversity. This idea has stimulated considerable modern work on the systematics, genetics, and ecology of island groups. Island floras are of great interest to evolutionary biologists (Stuessy and Ono, 1998), typically because they often contain many unique endemic species. The floras of some islands have an extremely high fraction of endemic species; for example, in Hawaii approximately 90% of the angiosperms are endemic (Groombridge, 1992). The endemics are morphologically divergent from continental relatives, presumably because they have evolved in environments that differ from those of the mainland. Often, gene flow from the mainland to an island is highly limited due to spatial isolation. This isolation can result in rapid fixation of mutations and subsequent speciation (Barton, 1998). In addition, a lack of competition with other species and the possibility for colonization of new habitats may promote speciation on islands (Crawford et al., 1987).

Most work on island plants in the last century has been systematic or biogeographic in focus addressing issues concerned with endemism, adaptive radiation, and the phylogenetic history of island taxa (Carlquist 1974; Bramwell 1976). Plants on islands often appear to have evolved through adaptive radiation, as a set of closely related species each with strikingly different morphological and ecological features but with low levels of genetic divergence. There are certain limitations using morphology to study both the origin and causes of species diversity both within an archipelago and on individual islands. Many island plants have diverged so dramatically from putative ancestral groups that it is difficult to ascertain their relationships and thus, to reconstruct the pattern of their evolution. Some morphological traits may be subject to high level of convergence as opposed to the characters undergoing radiation. On islands radiation can be concentrated in the relatively few characters that underline each radiation (Grant 1986, Baldwin and Robichaux 1995, Givnish 1995). Convergent evolution of the morphology can seriously skew the phylogeny when the characters converge independently as a result of selection imposed on several traits simultaneously by a shared environment. However, numerous molecular phylogenetic studies have subsequently shed improved light on the historical patterns of organismal evolution and their underlying mechanisms in islands (Baldwin et al., 1998). The ability to reconstruct the phylogenetic histories of taxa has been dramatically improved by the now fairly routine task of acquiring DNA sequence data from taxa. Within a phylogenetic framework one can answer fundamental questions such as whether ecologically and/or morphologically similar species on different islands

are the result of sharing a common ancestor or convergent evolution. Testing hypotheses about the ages of individual species groups or entire community assemblages is also possible within a phylogenetic framework (Kim et al., 2008). For these reasons, recent years have seen an increasing number of molecular phylogenetic analyses of island flora, primarily in the Canary Islands, Hawaiian Islands, Galapagos Islands, and the Caribbean Islands. In particular, the Macaronesian islands, especially the Canaries, have become a focus for study of the colonization and the diversification of different organisms (see review Juan et al. 2000).

The phytogeographic region of Macaronesia is comprised of five Atlantic archipelagos (Azores, Madeira, Selvagens, Canaries, and Cape Verde) off the western coasts of Europe and Africa, situated between latitudes 15 ° and 40° N. The region has 24 major islands that exhibit a broad range of variation both in their ecology and geology. Their geological ages vary from 21 million years (Myr) for Fuerteventura to 0.8 Myr for El Hierro (Rothe 1982; Mitchell-Thomé 1985; Galopim de Carvalho and Brandão 1991; Boekschoten and Manuputty 1993; Carracedo 1994). The combination of latitudinal gradients and northeastern trade winds has produced a number of distinct ecological zones (Bramwell 1972). The great habitat diversity and insular isolation are the main factors responsible for the rich flora of Macaronesia; at least 831 species and 40 genera are endemic to the region (Humphries 1979; Hansen and Sunding 1993; La Roche and Rodríguez-Piñero 1994).

The woody *Sonchus* alliance is one of the largest of the Macaronesian endemic groups (6 genera and approximately 31 species), representing a premier example of adaptive radiation (Aldridge 1975, 1979). The alliance is composed of 19 species of primarily woody members of *Sonchus* (subg. *Dendrosonchus*), seven species of *Taekholmia*, one species of subg. *Sonchus* (*S. tuberifer*), and four monotypic genera *Babcockia*, *Lactucosonchus*, *Sventenia*, and *Chrysoprenanthes* (Kim et al. 1996a, b; Lee et al. 2005). These taxa are all endemic to the Canary Islands (except three species of *Dendrosonchus* in Madeira and *S. daltonii* in the Cape Verde archipelago) (Figure 2.1) and display extensive morphological, ecological, and anatomical diversity (Aldridge 1977, 1978). Despite this diversity, all these taxa have a uniform chromosome number ( $n = 9$ ,  $2n = 18$ ; Ardévol Gonzalez et al. 1993), and the high fertility of frequent interspecific and intergeneric hybrids suggests genetic cohesiveness within the alliance (Aldridge 1976; Hansen and Sunding 1993).

The Canary archipelago, where most of the alliance occurs, is located in the Atlantic Ocean and consists of seven islands (Figure 2.1). These islands are of volcanic origin and form an approximately linear chain (McDougal and Schmincke 1976-77; Banda et al. 1981). In contrast to several remote archipelagos in the Pacific, such as the Hawaiian, Galapagos, and Juan Fernandez Islands, the Canaries have two unique biogeographical features. The proximity of the islands to the African continent (i.e., the eastern most island, Fuerteventura, is only about 100 km away from the west coast of

Morocco) suggests that colonizers could reach the islands relatively easily, and thus multiple colonization events may have occurred for some closely related taxonomic groups, like members of the woody *Sonchus* alliance. In addition, the oldest islands within the archipelago are 20 Myr which raises the possibility that some elements of the Canarian flora are much older in origin than others (Fernandez-Palacios and Anderson 1993; Fuster et al. 1993; Carracedo 1994). There also has been a long controversy over whether some of the woody Macaronesian endemics are relict elements of a flora that extended along the Mediterranean basin during the Tertiary period or are recent derivatives from continental ancestors (Carlquist 1962, 1974; Bramwell 1972, 1975, 1976; Sunding 1979; Böhle et al. 1994).

Phylogenetic analyses of plant taxa have been used to test for various evolutionary processes including reticulation. The examination of biparentally and uniparentally inherited markers in the same taxa is sensitive means for detecting the footprints of past reticulations. Previous investigations in the Hawaiian plants have concluded that hybridization has been of profound evolutionary significance in this flora (e.g., the silversword alliance; Baldwin et al. 1990). Single- or low-copy nuclear genes, especially their introns, can be very useful in phylogenetic reconstruction of closely related plant species (Sang 2002). Although there are many theoretical and practical questions concerning the phylogenetic utility of low-copy nuclear genes, recent studies have demonstrated that rapidly evolving introns of low-copy nuclear genes can provide

sufficient phylogenetic information to resolve interspecific relationships previously unresolved, or poorly resolved, by cpDNA or nrDNA (Doyle et al. 1996; Sang et al. 1997; Small et al. 1998; Emshwiller and Doyle 1999; Tank and Sang 2001).

Several phylogenetic analyses have already confirmed the monophyly of woody *Sonchus* despite their diversity in morphology (Kim et al., 1996a, Lee et al., 2005). This implies the woody *Sonchus* alliance was derived from a common ancestor. But resolution of phylogenetic relationships within the alliance has been a difficult challenge, in part because of a lack of genetic variation in ITS sequences. Moreover, the phylogeny remain highly unresolved by sequencing more than 4000bp chloroplast DNA (Lee et al., 2005). Therefore, additional molecular characters are needed to accurately estimate phylogenetic relationships within the alliance. More importantly, the independent markers will help to reveal the role if any of hybridization in the Macaronesian flora (Francisco-Ortega et al. 1996; Brochmann et al. 2000).

In this study, we used three low-copy nuclear genes as independent markers to the species of woody *Sonchus* alliance to investigate the phylogeny as well as to elucidate the extent to which hybridization has contributed to the radiation of woody *Sonchus* alliance.

## MATERIALS AND METHODS

### Taxon Sampling

In this study, we sampled all species of the alliance except *S. ortunoi* (Table 2.1). We included *Dendrosonchus* species and several species with restricted distributions in the Canaries (i.e., *Sonchus tuberifer*, *S. brachylobus*, *S. gandogeri*, *S. bornmuelleri*, *S. fauces-orci*, *T. capillaris*, *T. canariensis*, *T. arborea*, and *T. microcarpa*). We also sampled multiple populations of species that have wide geographic distributions on single or multiple islands: *Babcockia*, *S. congestus*, *S. hierrensis*, and *T. heterophylla*. We also included *S. bornmuelleri* and the *S. gummifer* complex sensu Aldridge (*S. gummifer*, *S. radicans*, and *S. tectifolius*). We adopted the classification of Boulos (1967, 1972, 1974a, b), which represents the first complete revision of the genus *Sonchus* and several other segregate genera.

### PCR Reaction and DNA Sequencing

Total genomic DNA was isolated from leaf tissue using the CTAB method of Doyle and Doyle (1987) and DNeasy plant mini kits (QIAGEN, Valencia, California, USA). Polymerase chain reaction (PCR) conditions were an initial 5 min at 95°C followed by 40 cycles of 1 min at 94°C, 1.5 min at 50°C, and 2 min at 72°C, with a final extension time of 10 min at 72°C. The PCR amplification primers of both nuclear genes



glyceraldehyde 3-phosphate dehydrogenase (G3pdh) and Calmodulin (Cam) were designed by Strand et al., 1997. The primer of the B12 is one of the universal primers designed for the phylogenetic analysis in the Asteraceae (Chapman et al., 2007). The primers sequences were listed in Table 2.2. PCR products of approximately 50  $\mu$ L of each sample were purified with the QiaQuick PCR Purification kit (QIAGEN) and direct sequencing of PCR products were carried out for the purified PCR products using Big Dye Terminator Cycle Sequencing reagents (Applied Biosystems, Foster City, California, USA). Sequencing primers used were identical to amplification primers.

Base calling and sequence editing were performed with Sequencher 4.1 (Gene Codes, Ann Arbor, Michigan, USA). The sequences were visually aligned using MacClade (version 4.06; Maddison and Maddison, 2003).

### **Phylogenetic Analysis**

Initially the G3pdh, Cam and B12 data sets were analyzed independently using an equally weighted, unordered maximum parsimony (MP) approach (Fitch, 1971) implemented in PAUP ver. 4.0 (Swofford, 2002). The MP analyses for each data set included a heuristic search for the most parsimonious trees; starting trees were obtained via stepwise addition. Sequences were added via simple addition with one tree held at each step. Branch swapping was performed via tree-bisection-recombination (TBR), and steepest descent and MulTrees options were in effect. Branches were collapsed if

maximum branch length was zero, and topological constraints were not enforced. Support for nodes was calculated by bootstrap analysis with 500 simple-addition bootstrap replicates, each with a maximum of 500 trees saved and TBR branch-swapping.

Pairwise congruence between three data sets were tested using the incongruence length difference (ILD) test (Farris et al., 1995) as implemented by the partition homogeneity test in PAUP\* for 50 replicates (heuristic search, simple addition, TBR branching swapping), each saving a maximum of 500 most parsimonious trees per replicate.

## **RESULTS**

### **Parsimony Analysis**

The B12 data set contained 34 taxa, including one outgroup taxon, and 405 total characters (total aligned sequence length) of which 384 were constant (94.81%). Of 21 variable characters (5.19%), 6 were parsimony un-informative (1.49%) and 15 were parsimony informative (3.70% of total). The Cam dataset contained 33 taxa, including two outgroup taxa, and 551 characters of which 482 were constant (87.48%). Of 69 variable characters (12.34%), 24 were parsimony informative (4.36% of total). The

G3pdh contained 42 taxa, including two outgroup taxa, and 825 characters of which 744 were constant (90.19%). Of 81 variable characters (9.89%), 40 were parsimony informative (4.85% of total). The combined dataset of 26 taxa (including one outgroup taxon) contained a total of 1779 characters of which 1661 were constant (93.37%), 129 were variable (7.25%), and 51 were parsimony informative (2.87% of total). (Table 2.3)

The shortest B12 trees were 45 steps long and had a consistency index (CI) of 0.778, homoplasy index (HI) of 0.222, retention index (RI) of 0.920, and a rescaled consistency index (RC) of 0.920. The shortest Cam trees were 103 steps long and had a consistency index (CI) of 0.825, homoplasy index (HI) of 0.175, retention index (RI) of 0.863, and a rescaled consistency index (RC) of 0.712. The shortest G3pdh trees were 108 steps long and had a consistency index (CI) of 0.824, homoplasy index (HI) of 0.176, retention index (RI) of 0.863, and a rescaled consistency index (RC) of 0.678. The shortest combined data trees were 162 steps long and had a CI of 0.735, HI of 0.175, RI of 0.791, and RC of 0.595.

### **Phylogenetic Relationships**

The B12 dataset had very low sequence variability. It only has 15 parsimony informative characters to resolve 34 taxa. Similarly, the Cam dataset had only 24 parsimony informative characters to resolve 33 taxa. As a result, the 50% majority tree from the MP analysis of both B12 and Cam data result in large numbers of unresolved

taxa and only few clades were identified. The G3pdh data have better resolution because of more parsimony informative characters (40) available to resolve 42 taxa.

The clades resolved by data from three independent regions that were present in the 50% majority tree were each bootstrap supported and were not in conflict with each other. The partition homogeneity test for pairwise comparison of the three datasets indicated that the partitions were not significantly different from random partitions. Therefore, we combined the three datasets to explore whether resolution and support would be improved by increasing the amount of sequencing data. The MP phylogeny based on the combined dataset provides better resolution than separate analysis. Therefore, the 50% majority rule consensus tree (Figure 2.2) is used as the working hypotheses for the woody *Sonchus* alliance. Unfortunately, only 26 accessions (representing 22 species), are completely overlapping between the three data sets. Particularly, two monotypic genera (*Chrysoprenanthes* and *Sventenia*) are not included in the combine datasets. So the 50% majority MP tree of G3pdh (Figure 2.3) is used to compensate for the limited plant representation of combined tree.

Both the combined and G3pdh tree do not identify the basal lineages such as *Babcockia*, *Sventenia*, *Chrysoprenanthes*, and *Lactucosonchus* suggested by previous ITS and cpDNA analysis (Lee et al., 2005). The four monotypic genera are not resolved in both trees. All five species of subg *Taeckholmia* formed a “core *Taeckholmia*” clade with 98% bootstrap support (BS) in combined datasets (Figure 2.2). In the G3pdh tree, “core

*Taeckholmia*” clade has 95% BS, and *T. capillaris* is sister to “core *Taeckholmia*” with the 61% BS. *Taeckholmia arboreus* from La Palma (PA) and Tenerife (TE) formed a small clade (62% BS) by itself (Figure. 2.3). *S. hienrensis* from La Palma (PA), El Hierro (HI) and La Gomera (GO) formed a clade with 86% BS with *S. daltonii* from Cape Verde Islands, which is 1600 km southwest of the Canaries. The Madeiran speices (*S. pinnatus* *S. fruticosus* and *S. ustulatus*) formed a clade with *S. gummifer* with 80% BS (Figure. 2.2). *S. palmensis* and *S. gandogeri* formed a clade with 95% BS. *Sonchus tuberifer* is sister to *S. acaulis* with 62% BS in the combined tree (Figure 2.2), but the other individual *S. tuberifer* from a clade with *Lactucosonchus* with 73% BS. Overall, the subgenus *Dendrosonchus* is polyphyletic in both trees.

## DISCUSSION

### Four Monotypic Genera

The previous work (Lee et al., 2005) suggested that the monotypic genus *Babcockia* diverged first. *Babcockia platylepis*, a small shrub with very large capitula is endemic to Gran Canaria (GC) with widely distributed in higher elevation. It has been considered to be one of subgenus *Dendrosonchus* species, *Sonchus platylepis*, by various

authors (Aldridge, 1975, 1976, 1979; Bramwell and Bramwell, 2001). Thus, we used *Babcockia* to root the trees. But none of our tree are supported with the basal position of *Babcockia* because the rest taxa are not monophyletic. Our analysis suggests that *Babcockia* might not be the oldest member of *Sonchus* alliance. But we cannot rule out the possibility of too few parsimony informative characters ( <5% ), which could not offer enough resolution to distinguish the basal linages. *Babcockia* should also be retained as a monotypic genus because it is not closely related to any members of subgenus *Dendrosonchus* and genus *Taekholmia* (Figure 2.2; Figure 2.3).

*Sventenia bupleuroides* is a small caudex perennial (up to 30 cm) which is endemic to Gran Canaria. It has entire leaves, beaked cypselas, and glandular hairs covering the inflorescence stalk, peduncles, and involucre bracts. *Chrysoprenanthes pendula* occurs in the mountain cliffs on the south and west sides of Gran Canaria. It is a small shrubby cliff plant and is unique in having heads with fewer florets. None of our trees suggested a basal position for either of these two species. Clearly, they are not clustered with any *Taekholmia* and *Dendrosonchus*. It might be reasonable to retain them as monotypic genera. But we lack enough parsimony informative characters to resolve the relationship clearly.

The monotypic genus *Lactucosonchus* is a herbaceous perennial with long tuberous roots (Bramwell and Bramwell, 2001) and it is sister to the *S. tuberifer*-1 from TE with 73% BS in the G3pdh tree (Figure 2.3), However in the combined data tree,

*Lactucosonchus* is not clustered with the other *S. tuberifer* from Teno, Tenerife (Figure 2.2). *S. tuberifer* from Teno clustered with several *Dendrosonchus* species without *Lactucosonchus* in the combined tree with 61% BS. *Lactucosonchus* occurs locally on a geologically young island, La Palma (2 Myr), *S. tuberifer* occurs on a geologically old part of western Tenerife (7.4 Myr) in the center of the diversity of *Dendrosonchus*. Because *Lactucosonchus* is from younger island, it is plausible that it is not the oldest member of the alliance. Since *S. tuberifer* is from the older island, it is possible that *Lactucosonchus* is the descendent from *S. tuberifer*, which explains the herbaceous habit with tuberous roots shared by *Lactucosonchus* and *S. tuberifer*. So it is very likely that *Lactucosonchus* is not the basal lineage of the woody *Sonchus* alliance.

### **Subgenus *Taeckholmia***

Boulos (1967) stated that some species which have narrow leaves and small capitula are different in many aspects from the rest of *Dendrosonchus* species which have broader leaves and large capitula and suggested placing them in a new genus *Taeckholmia*. However, Aldridge (1976) strongly argued that the genera *Babcockia* and *Taeckholmia* cannot be distinguished from the subgenus *Dendrosonchus* based on her extensive morphological investigation and suggested including them within *Dendrosonchus*.

Boulos' classification to separate *Taeckholmia* was supported by the current study. He recognized two subgenera within *Taeckholmia*, *Taeckholmia* (*T. pinnata*, *T. capillaris*, *T. canariensis*, and *T. microcarpa*) and *Pseudodendrosonchus* (*T. heterophylla*, *T. regis-jubae*, and *T. arborea*). In the combined tree (Figure 2.2), four *Taeckholmia* species (*T. heterophylla*, *T. microcarpa*, *T. pinnata* and *T. canariensis*) formed a “core *Taeckholmia*” with 98% BS. In the G3pdh tree (Figure 2.3), “core *Taeckholmia*” also strongly supported with 95% BS and *T. capillaries* is the sister to “core *Taeckholmia*” with 61% BS. The “core *Taeckholmia*” is also strongly supported by Cam tree (not shown) with 82% BS. *Taeckholmia arborea* is the only *Taeckholmia* species which does not cluster with “core *Taeckholmia*”. *Taeckholmia arborea* has broader leaf lobes (up to 5 mm) and somewhat more florets per head (15–20 florets). Thus, our molecular result is consistent with the morphology in *Taeckholmia*.

### **Subgenus *Dendrosonchus***

The molecular phylogenetic analysis presented here strongly suggests that subg. *Dendrosonchus* is not monophyletic. Based on our combined tree, it seems unreasonable to suggest any classification and species delimitations for *Dendrosonchus*. Aldridge (1977, 1978) treated *S. pinnatus*, *S. canariensis*, and *S. palmensis*, as three subspecies of *S. pinnata*. In our study shows *S. pinnatus* is close to those other two Maderia *Dendrosonchus* as well as to *S. gummifer*. Aldridge also recognized *S. gummifer*



complex containing three subspecies: *S. radicans*, subsp. *radicans* (north coast, Tenerife), subsp. *gummifer* (south coast, Tenerife), and subsp. *tectifolius* (south side of Anaga, east Tenerife). In the combined tree, *S. radicans* and *S. tectifolius* formed a small weakly supported cluster with 56% BS. *S. gummifer* clustered with three Madeira *Dendrosonchus*. Therefore, the *S. gummifer* complex is not fully supported by our data.

### ***Dendrosonchus* Species in Madeira and Cape Verde islands**

The Madeira archipelago which is approximately 400 km from the Canary Islands is very old (ca. 30 Myr). There are three *Dendrosonchus* species endemic to Madeira, *S. pinnatus*, *S. fruticosus* and *S. ustulatus* (subsp. *ustulatus* and subsp. *maderensis*). *S. ustulatus* occurs on dry rocky and sunny areas on the coast of Madeira. *Sonchus fruticosus* occurs in the Laurisilva and moist ravines in the interior of Madeira, and *S. pinnatus* occurs primarily on rocky slopes. Lee et al (2005) hypothesized Madeira species originated most likely from Tenerife. In our combined tree, three Madeira *Dendrosonchus* with *S. gummifer* from Tenerife formed a cluster with 70% BS which supported the hypothesis in previous study.

There is only one species of *Dendrosonchus*, *S. daltonii*, in the Cape Verde Islands, which are 1600 km southwest of the Canaries. *Sonchus daltonii* is a rosette shrub and is a critically endangered species, with only ca. 30 individuals remaining (Gomes et al., 1995). The combined tree shows that *S. daltonii* clustered with *S. hierrensis* from La

Palma (PA), El Hierro (HI) and La Gomera (GO) with 86% BS. The same cluster was supported by G3pdh tree with 63% BS. Therefore, the current study strongly suggests that *S. hierrensis* is very likely to be the parental donor for *S. daltonii*. This requires that the long-distance dispersal from the western Canary Islands. It is very likely it was a single event, because *S. daltonii* is clustered with only one *Dendrosonchus* species. Considering the 1600 km distance, it is plausible that the dispersal was likely accomplished by birds migrating between the two archipelagoes.

### **Insights Regarding Hybridization and Introgression**

Aldridge (1975) suggested a hybrid origin for several species in subgenus *Dendrosonchus*. For example, she hypothesized that *S. gondogeri* is the hybrid between *S. canariensis* and *S. hierrensis*. But in our combined tree, *S. gondogeri* is sister to *S. palmensis* with 95% BS. Neither *S. canariensis* nor *S. hierrensis* were close to *S. gondogeri*. Another hypothesis was that *T. heterophylla* was the hybrid derivative of *S. arboreus* and *S. leptcephalus*. In our tree, the *T. heterophylla* is in the “core *Taeckholmia*” clade. Neither putative parent is closely clustered with *T. heterophylla*.

The incongruence between G3pdh tree (Figure 2.3) and Cam tree (not shown) gives some insights into potential hybridization. For example, one *S. tuberifer* individual is sister to *Lactucosonchus* in the G3pdh tree, but in the combined tree, another individual

of *S. tuberifer* is sister to *S. acaulis*. These data might indicate a hybrid origin of *S. tuberifer*. The *S. gummifer* complex also shows incongruence.

The B12 marker has low variability, but Cam and G3pdh give moderate resolution. The future effort of cloning several critical species with both G3pdh and Cam genes will be useful to disentangle the potential role of hybridization within the woody *Sonchus* alliance. The result of that effort will be discussed in the next chapter.

## REFERENCES

- Aldridge, A. 1975. Taxonomic and anatomical studies in *Sonchus* L. Subgenus *Dendrosonchus* Webb ex Schultz Bip. and related genera. Ph. D. Dissertation. University of Reading, England.
- Aldridge, A. 1976. Macaronesian *Sonchus* subgenus *Dendrosonchus* s. l. (Compositae-Lactuceae), including a reappraisal of the species concept and new combinations. *Bot. Mac.* 2: 81-93.
- Aldridge, A. 1977. Anatomy and evolution in the Macaronesian *Sonchus* subgenus *Dendrosonchus* (Compositae-Lactuceae). Nodal and petiolar vascular patterns. *Bot. Mac.* 3: 41-59.
- Aldridge, A. 1978. Anatomy and evolution in the Macaronesian *Sonchus* subgenus *Dendrosonchus* (Compositae-Lactuceae). *Bot. J. Linn. Soci.* 76: 249-285.
- Aldridge, A. 1979. Evolution within a single genus: *Sonchus* in Macaronesia. *Plants and islands*, ed. Bramwell, D., Academic Press, New York : 279-290
- Baldwin, B. G., Kyhos, D. W., and Dvorak, J. 1990. Chloroplast DNA evolution and adaptive in the Hawaiian silversword alliance (Asteraceae-Madiinae). *Annals of the Missouri Botanical Garden* 77: 96-109.
- Baldwin, B. G., and Robichaux, R. H. 1995. Historical biogeography and ecology of the Hawaiian silversword alliance (Asteraceae): *new molecular phylogenetic perspectives*. *Hawaiian biogeography: evolution on a hot-spot archipelago*, ed. W. L. Wagner and V. A. Funk. Smithsonian Institution Press, Washington, D. C.: 259-287
- Baldwin, B. G., Crawford, D. J., Francisco-Ortega, F., Kim, S.-C., Sang, T. and Stuessy,

- T. F. 1998. Molecular phylogenetic insights on the origin and evolution of island plants. In: P.S.Soltis, D.E. Soltis, and J.J. Doyle. (eds.), *Molecular Systematics of Plants II*. Chapman and Hall : 410-441.
- Banda, E., Danobeitai, J. J., Surinach, E., and Ansorge, J. 1981. Features of crustal structure under the Canary Islands. *Earth Planet Sci. Let.* 55: 11-24.
- Barton, N.H. 1998. Natural selection and random genetic drift as causes of evolution on islands, *Evolution on Islands*; Oxford University Press: Oxford: 102–123.
- Boekschoten, G. J., and Manuputty, J. A. 1993. The age of the Cape Verde Isladns. *Courier des Forschungsinstitutes Senckenberg* 159: 3-5.
- Boulos, L. 1967. *Taeckholmia*, a new genus of Compositae from Canary Islands. *Botaniska Notiser* 120: 95–108.
- Boulos, L. 1972. Re´vision syste´matique du genre *Sonchus* L. s.l. I. Introduction et classification. *Botaniska Notiser* 125: 287–305.
- Boulos, L. 1974a. Re´vision syste´matique du genre *Sonchus* L. s.l. V. Sousgenre 2. *Dendrosonchus*. *Botaniska Notiser* 127: 7–37.
- Boulos, L. 1974b. Re´vision syste´matique du genre *Sonchus* L. s.l. VI. Sousgenre 3. *Origosonchus*. Genres *Embergeria*, *Babcockia* et *Taeckholmia*. *Botaniska Notiser* 127: 402–451.
- Bramwell, D. 1972. Endemism in the flora of the Canary Islands. Taxonomy, phytogeography, and evolution (ed. D. H. Valentine). Academic Press, New York. 141-159
- Bramwell, D. 1975. Some morphological aspects of the adaptive radiation of Canary Islands *Echium* species. *Anal. Inst. Bot. Cavanilles* 32: 241-254.
- Bramwell, D. 1976. The endemic flora of the Canary Islands; distribution, relationships

and phytogeography. in Biogeography and ecology in the Canary Islands, G. Kunkel, ed. The Hague, The Netherlands: Dr W. Junk: 207-240

Bramwell, D. and Bramwell, Z. 2001. Wild flowers of the Canary Islands, 2nd ed. Editorial Rueda, Madrid, Spain.

Brochmann, C., Borgen, L. and Stabbetorp, O. E. 2000. Multiple diploid hybrid speciation of the Canary Island endemic *Argyranthemum sundingii* (Asteraceae). *Plant Syst. Evol.* 220:77-92.

Böhle, U. -R., Hilger, H. and Martin, W. F. 1996. Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). *Proc. Natl. Acad. Sci. USA* 93: 11740-11745.

Carlquist, S. 1962. A theory of paedomorphosis in dicotyledonous wood. *Phytomorphology* 12: 30-45.

Carlquist, S. 1974. Island biology. New York. Columbia University Press.

Carracedo, J. C. 1994. The Canary Islands: an example of structural control on the growth of large oceanic-island volcanoes. *Journal of Volcanology and Geothermal Research* 60: 225-241.

Chapman M.A., Chang J.C., Weisman D., Kessii R.V., Burke J.M. 2007. Universal markers for the comparative mapping and phylogenetic analysis in the Asteraceae. *Theor Appl Genet* 115:747-755

Crawford D.J., Whitkus R. and Stuessy T. F. 1987. Plant evolution and speciation on oceanic islands. In: Urbanska KM (ed) *Differentiation and Diversification of Land Plants*, Springer-Verlag: Tokyo: 183-199.

Doyle, J. J. and Doyle J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochemical Bulletin* 19: 11-15.

Doyle, J. J., Kanazin, V. and Shoemaker, R. C. 1996. Phylogenetic utility of histone H3

intron sequences in the perennial relatives of soybean (*Glycine*: Leguminosae). *Mol. Phylogenet. Evol.* 6: 438-447.

Emshwiller, E. and Doyle, J. J. 1999. Chloroplast-expressed glutamine synthetase (ncpGS): potential utility for phylogenetic studies with an example from *Oxalis* (Oxalidaceae). *Mol. Phylogenet. Evol.* 12: 310-319.

Fernandez-Palacios, J. M. and Anderson, C. 1993. Species composition and within archipelago co-occurrence patterns in the Canary Islands. *Ecography* 16: 31-36.

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* 20: 406-416.

Francisco-Ortega, J. F., Jansen, R. K. and Santos-Guerra, A. 1996. Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. *Proc. Natl. Acad. Sci. USA* 93: 4085-4090.

Farris, J. S., Källersjö M., Kluge, A. G. and Bult, C. 1995. Constructing a significance test for incongruence. *Systematic Biology* 44: 570-572.

Fuster, J. M., Hernan, F., Cendero, A., Coello, J., Cabtagrel, J. M., Ancochea, E., and Ibarrola, E. 1993. Geochronology de la isla de El Hierro (Islas Canarias). *Bol. Soc. Esp. Hist. Nat.* 88: 1-4.

Galopim de Carvalho, A. M., and Brandão, J. E. 1991. Geologia do Archipelago da Madeira. Lisbon, Portugal: Museu Nacional de Historia Natural (Mineralogia e Geologia) da Universidade de Lisboa.

Givnish, T. J., Sytsma, K. J., Hahn, W. J. and Smith, J. F. 1995. Molecular evolution, adaptive radiation, and geographic speciation in *Cyanea* (Campanulaceae, Lobelioideae). in Hawaiian biogeography: evolution on a hot-spot archipelago, ed. W. L. Wagner and V. A. Funk. Smithsonian Institution Press, Washington, D. C. 299-337

Grant, P. R. 1986. Ecology and evolution of Darwin's finches. Princeton University Press.

- Groombridge B. (ed) .1992. Global Biodiversity: Status of the Earth's Living Resources. Chapman & Hall: London.
- Gomes, I., Gomes, S., Kilian N., Leyens T., Lobin W. and Veracruz M. T. 1995. Notes on the flora of the Cape Verde Islands, W Africa. *Willdenowia* 25: 177–196.
- Hansen, A., and Sunding, P. 1993. Flora of Macaronesia. Checklist of vascular plants, 4th revised ed. *Sommerfeltia* 17: 1-296.
- Humphries, C. J. 1979. Endemism and evolution in Macaronesia. in *Plants and islands*, D. Bramwell, ed. London, England: Academic Press: 171-199
- Juan, C., Emerson, B. C., Oromí, P. and Hewitt, G. M. 2000. Colonization and diversification towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology and Evolution* 15: 104-109.
- Kim, S.-C., Crawford, D. J., and Jansen, R. K. 1996a. Phylogenetic relationships among the genera of the subtribe Sonchinae (Asteraceae): evidence from ITS sequences. *Syst. Bot.* 21: 417-432.
- Kim, S.-C., Crawford, D. J., Francisco-Ortega, J., and A. Santos-Guerra. 1996b. A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. *Proc. Natl. Acad. Sci. USA* 93: 7743-7748.
- Kim, S.-C., Crawford, D. J., Jansen, R. K., and Santos-Guerra, A. 1999a. The use of a non-coding region of chloroplast DNA in phylogenetic studies of the subtribe Sonchinae (Asteraceae:Lactuceae). *Pl. Syst. Evol.* 215: 85-99.
- Kim, S.-C., Crawford, D. J., Francisco-Ortega, J., and Santos-Guerra, A. 1999b. Adaptive radiation and genetic differentiation in the woody *Sonchus* alliance (Asteraceae:Sonchinae) in the Canary Islands. *Pl. Syst. Evol.* 215: 101-118.
- Kim, S.-C., McGowen, M.R., Lubinsky, P., Barber, J.C., Mort, M.E. and Santos-Guerra, A. 2008. Timing and tempo of early and successive adaptive radiations in Macaronesia. *PLoS ONE* , 3(5) : e2139.



- Maddison, D.R., and Maddison, W.P. 2003. Sequence alignment program, release version 4.06. [www.macclade.org](http://www.macclade.org)
- McDougall, I. and Schmincke, H. U. 1976-1977. Geochronology of Gran Canaria, Canary Islands: age of shield building volcanism and other magmatic phases. *Bull. Volcanol.* 40: 57-77.
- Mitchell-Thomé, R. C. 1985. Radiometric studies in Macvaronesia. *Boletim do Museu Municipal do Funchal* 37: 52-85.
- La Roche, F. and Rodríguez-Piñero, J. C. 1994. Aproximación al número de táxones de la flora vascular silvestre de los archipiélagos macaronésicos. *Revista de la Academia Canaria de Ciencias* 6: 77-98.
- Lee C, Kim S.-C., Santos-Guerra A. and Lundy K. 2005. Chloroplast DNA phylogeny of the woody *Sonchus* alliance (Asteraceae) in the Macaronesian islands. *American Journal of Botany.* 92: 2072-2085
- Rothe, P. 1982. Zur geologie de Kapverdischen Inseln. *Courier des Forschungsinstitutes Senckenberg* 52: 1-9.
- Sang, T., Donoghue, M. J. and Zhang, D. 1997. Evolution of alcohol dehydrogenase genes in peonies (*Paeonia*): phylogenetic relationships of putative nonhybrid species. *Mol. Biol.Evol.* 14: 994-1007.
- Sang, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* 37: 121-147.
- Small, R. L., Ryburn, J. A., Cronn, R. C., Seelanan, T. and Wendel, J. F. 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear *Adh* sequences for phylogeny reconstruction in a recently diverged plant group. *Am. J. Bot.* 85: 1301-1315.
- Strand, A. E., Leebens-Mack, J. and Milligan. B. G. 1997. Nuclear DNA-based markers

for plant evolutionary biology. *Molecular Ecology* 6: 113–118

Stuessy T. F. and Mikio O. (eds). 1998. *Evolution and Speciation of Island Plants*. Cambridge University Press, Cambridge.

Sunding, P. 1979. Origins of the Macaronesian flora. In *Plants and islands* (ed. D. Bramwell), Academic Press, New York.

Swofford, D.L. 2002. PAUP\*. *Phylogenetic Analysis Using Parsimony (\*and Other Methods)*. Version 4. Sunderland: Sinauer Associates.

Tank, D. C. and Sang, T. 2001. Phylogenetic utility of the Glycerol-3-phosphate acyltransferase gene: evolution and implications in *Paeonia* (Paeoniaceae) *Mol. Phylogenet. Evol.* 19: 421–429

Table 2.1 The species of the woody *Sonchus* alliance studied . Abbreviations of islands are as follows: LA Lanzarote, FU Fuerteventura, GC Gran Canaria, TE Tenerife, GO La Gomera, PA La Palma, HI El Hierro. Species with an asterisk represent the taxa included in the combined analysis.

Species name	Location(s)	Habits
<i>Lactucosonchus</i> *	PA	Herbaceous perennial
<i>Babcockia</i> *	GC	Rosette subshrub
<i>Sventenia</i>	GC	Caudex perennial
<i>Chrysoprenanthes</i>	GC	Caudex perennial
<i>S. tuberifer</i> *	TE	Herbaceous perennial
<i>T. pinnata</i> *	TE/GC	Rosette subshrub
<i>T. microcarpa</i> *	TE	Rosette subshrub
<i>T. canariensis</i> *	GO	Rosette subshrub
<i>T. capillaris</i>	TE	Rosette subshrub
<i>T. heterophylla</i> *	GO	Rosette subshrub
<i>T. arborea</i>	PA/TE	Rosette subshrub
<i>S. bornmuelleri</i> *	PA	Rosette subshrub
<i>S. acaulis</i> *	TE/GC	Caudex perennial
<i>S. brachylobus</i>	GC	Rosette subshrub
<i>S. canariensis</i>	TE/GC	Rosette tree/shrub
<i>S. gandogeri</i> *	HI	Rosette tree/shrub
<i>S. congestus</i> *	TE/GC	Rosette tree/shrub
<i>S. fauces-orci</i> *	TE	Caudex perennial
<i>S. gonzalezpadronii</i>	GO	Caudex perennial
<i>S. hierrensis</i> *	PA/GO/HI	Rosette tree/shrub
<i>S. gummiifer</i> *	TE	Rosette tree/shrub
<i>S. radicans</i> *	TE	Rosette tree/shrub
<i>S. tectifolius</i> *	TE	Rosette tree/shrub
<i>S. palmensis</i> *	PA	Rosette tree/shrub
<i>S. pinnatifidus</i> *	LA/FU	Rosette tree/shrub
<i>S. pinnatus</i> *	Madeira	Rosette tree/shrub
<i>S. fruticosus</i> *	Madeira	Rosette tree/shrub
<i>S. ustulatus ssp ustulatus</i> *	Madeira	Rosette tree/shrub
<i>S. ustulatus ssp maderense</i>	Madeira	Rosette tree/shrub
<i>S. daltonii</i> *	Cape vadera	Rosette tree/shrub

Table 2.2 The forward and reverse primers of G3pdh, Cam and B12 genes

Locus Name	Fwd primer sequence (5' → 3')	Rev primer sequence (5' → 3')
G3pdh	GATAGATTTGGAATTGTTGAGG	AAGCAATTCCAGCCTTGG
Cam	AGCCTNTTCGACAAGGATGG	AGTGANCGCATCACAGTT
B12	CAAGTGGCTGCAGCCATGGG	ACATCRGGMACCATTCWCCGGTGT

Table 2.3. Numbers of taxa and characters with tree statistics for maximum parsimony analyses of G3pdh, Cam, B12 and combined datasets.

Data set	Number of Taxa		Maximum Parsimony				
			Sequence characters				
	Ingroup	Total	Total	Constant	Total Variable	Parsimony informative	
						number	% of total
G3pdh	39	42	825	744	81	40	4.85
Cam	32	33	551	482	69	24	4.35
B12	33	34	405	384	21	15	3.70
Combined	25	26	1779	1661	129	51	2.87

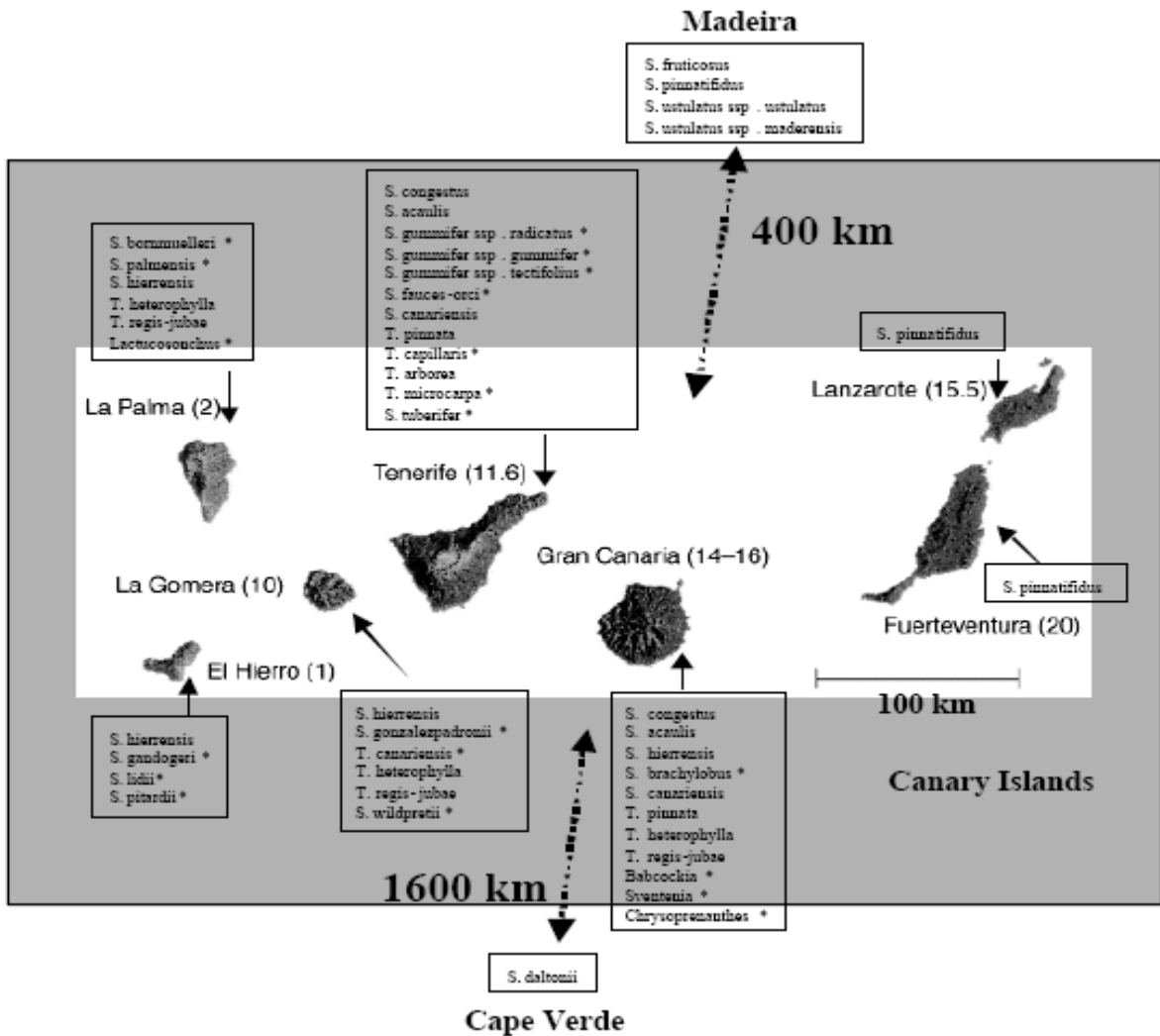


Figure 2.1 Distribution of the woody *Sonchus* alliance in the Canary, Madeira, and Cape Verde archipelagos. The ages in millions of years of subaerial shields in the Canary Islands are indicated in parentheses. Taxa with an asterisk represent single island endemics.

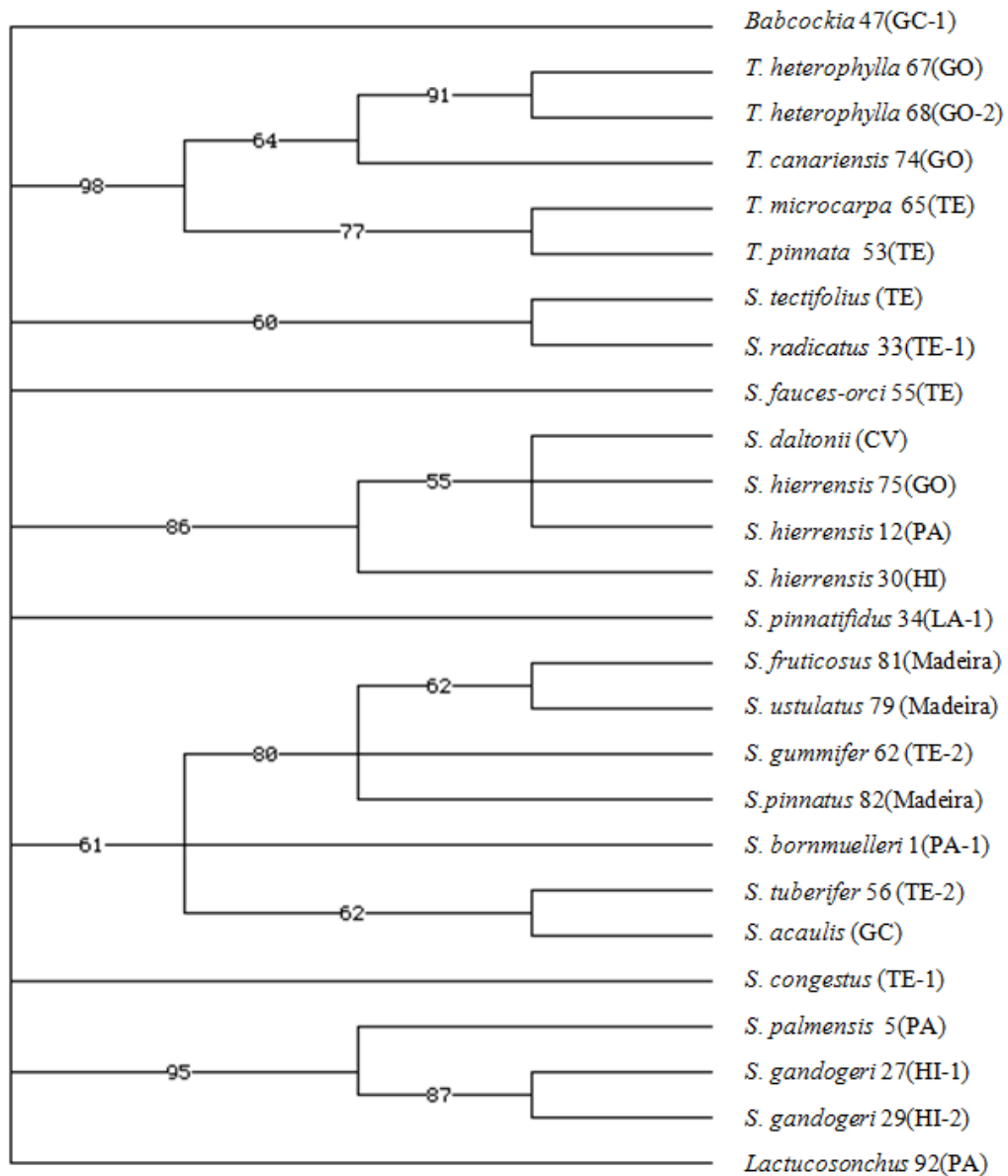


Figure 2. 2 50% Majority rule consensus tree based on MP analysis of combined dataset. The MP bootstrap support values above 50% are shown above branches.

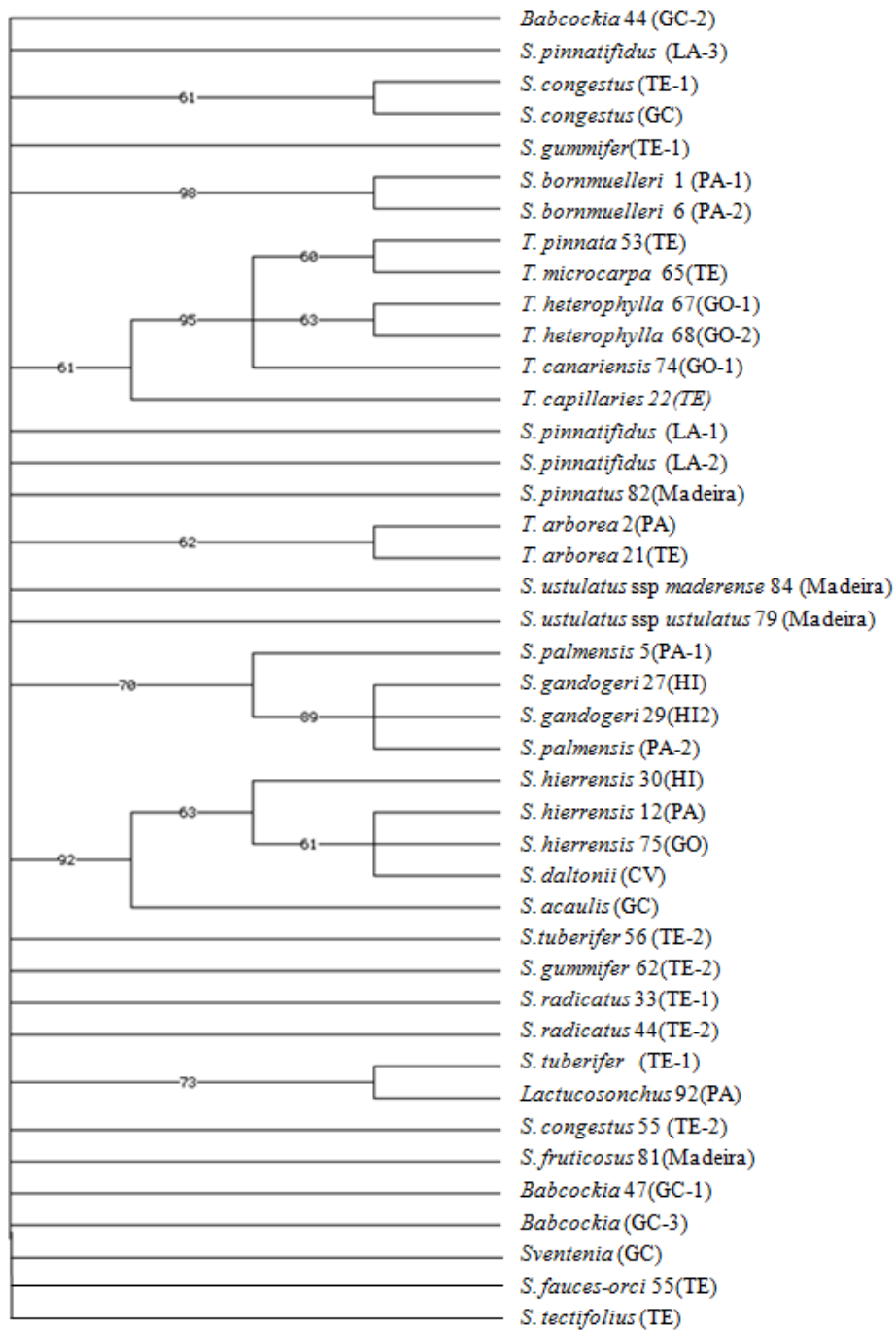


Figure 2.3 50% Majority rule consensus tree based on MP analysis of G3pdh dataset. The MP bootstrap support values above 50% are shown above branches.



## CHAPTER 3

### **A Molecular Phylogenetic Study of Hybridization in Woody *Sonchus* Alliance (Asteraceae: Sonchinae)**

#### **Abstract:**

Homoploid hybrid speciation is defined as the newly formed hybrid has the same chromosome number as the parental species. The members of the woody *Sonchus* alliance display extensive morphological, ecological, and anatomical diversity, but all taxa have a uniform chromosome number. Furthermore, all the members have no crossing barriers; there are extensive opportunities for hybridization between these species. We include two unlinked low-copy nuclear genes: glyceraldehyde 3-phosphate dehydrogenase (G3pdh) and Calmodulin (Cam) with the cloning with several key species to test the possible homoploid hybrid speciation. There is no evidence in our data to

support a hybrid origin for the monotypic genera (*Babcockia platylepis*, *Sventenia bupleuroides* and *Lactucosonchus webbii*). The hybridization between *Dendrosonchus* and *Taeckholmia* is not supported in our data. *S. tuberifer* might have a hybrid origin crossing a herbaceous and a small woody species.

Keywords: Homoploid hybrid speciation, low-copy nuclear gene, woody *Sonchus* alliance

## INTRODUCTION

Hybridization and introgression have been considered as important process in plant evolution and speciation (Grant 1981; Ellstrand et al. 1996, Rieseberg et al. 2003; Mallet 2007). Speciation by allopolyploidy involves hybridization and subsequent chromosome doubling. By contrast, with homoploid hybrid speciation the genome remains diploid. Whereas both homoploid hybridization and allopolyploidy can be potential sources of new species, allopolyploidy appears to be much more common than homoploid hybrid speciation, because homoploid hybrid species may be descended from early generation hybrids with reduced fitness, which is less likely to be the case in early generation allopolyploids (Grant 1981; Rieseberg and William 2007). The historic emphasis on allopolyploidy reflects the relative ease with which allopolyploid hybrid species can be identified based on chromosomal data. In contrast, homoploid hybrid species can not be easily detected and confirmed without detailed molecular analysis. Increasingly robust methods of phylogenetic reconstruction are helping overcome the challenges in detecting homoploid hybrid species. Several studies have shown the potential importance of the homoploid hybridization in plant evolution and speciation (Arnold et al. 2003; Rieseberg et al. 2003).

Island plants that are produced of the same adaptive radiation are particularly susceptible to hybridization, because of the general lack of reproductive barriers between closely related species. For example, previous investigations in the Hawaiian plants have concluded that hybridization has been of profound evolutionary significance in certain elements of this flora (e.g., the silversword alliance; Baldwin et al. 1990). Hybridization was also found on the California Islands, such as *Cercocarpus traskiae*, a rare tree endemic to Santa Catalina Island, which may be threatened by hybridization and introgression (Rieseberg et al 1989). Most evolutionary studies of oceanic islands have focused on the Pacific Ocean. But the role of hybridization in the Macaronesian flora of the Atlantic is certainly understudied (Francisco-Ortega et al. 1996; Brochmann et al. 2000).

The Macaronesian Islands in the Atlantic Ocean include 5 archipelagoes: Azores (9 islands), Salvagens (2 islands), Canaries (7 islands), Madeiras (3 islands), and Cape Verdes (10 islands). There are two unique features of these islands that contrast to the ones in the Pacific Ocean. First, macaronesian islands, especially the Canary archipelago, are relatively close to the continent. One of the closest islands of the Canaries, Fuerteventura, is only about 100 km from the west coast of Morocco. Second, the Macaronesian Islands are geologically much older than the archipelagoes in the Pacific, for example, Canaries (0.5–20.7 Myr), Madeiras (30 Myr), and Cape Verdes (6–20 Myr) (Francisco-Ortega et al. 2000).

The woody *Sonchus* alliance is one of the largest groups of macaronesian endemics (6 genera and approximately 31 species) and represents an example of adaptive radiation in Macaronesia (Aldridge 1975, 1979). Adaptive radiation refers to the diversification of a lineage into species that have adapted to a variety of different niches, and that differ in the morphological and physiological traits corresponding to those resources. The result of adaptive radiation is often characterized by extensive divergence in morphological traits and habit and, at the same time, by little divergence in molecular sequences or crossing ability. Despite the extensive diversity of the alliance, all members are diploids ( $2n = 18$ ) and have sporophytic self-incompatibility. Since selfing is not possible in this system and all the members have no crossing barriers, there are extensive opportunities for hybridization between these species.

One suspected case of homoploid hybridization in the woody *Sonchus* involves *S. tuberifer*. A phylogenetic analysis of woody *Sonchus* based on the three low copy nuclear genes has yielded topological incongruence between different genes with regard to this species. *S. tuberifer*, an endemic of Tenerife, is one of two members of the group that do not have a true woody habit. It is the only member in the woody *Sonchus* alliance which belongs to Subgenus *Sonchus*. *Sonchus tuberifer* and *Lactucosonchus* are both herbaceous perennials with tuberous roots. The inflorescences of *S. tuberifer* have 3-4 heads, and the leaves are toothed pinnate. In our result in previous chapter, *S. tuberifer* is sister to *Lactucosonchus* in the G3pdh tree, but in the combined tree, another individual

of *S. tuberifer* is sister to *S. acaulis*. This topological incongruence between independent inherited genes may indicate possible a hybrid origin for *S. tuberifer*, or alternatively, lineage sorting of ancient polymorphic sequences for rapidly radiated lineages in the alliance can also explain this pattern.

Aldridge (1976) recognized three subspecies in *S. radicans*: subsp. *radicans*, subsp. *gummifer* and subsp. *tectifolius*. For consistency, we use Boulos' (1967, 1972, 1974) classification. It recognized the species as the *S. gummifer* complex *sensu* Aldridge (*S. gummifer*, *S. radicans*, and *S. tectifolius*). All three members of this complex are endemic to Tenerife. *S. radicans* is distributed on the north coast and *S. gummifer* occurs on the south coast. *S. tectifolius* occurs in south side of Anaga in east Tenerife. *S. gummifer* is a small shrubby species with short woody stems and leaves arranged in basal rosettes. The leaves are pinnate with triangular leaf lobes that are not overlapping. *S. radicans* has very short woody stems and flat leaves arranged in rosettes. The heads are 1-2 cm in diameter and involucral bracts do not extend over the stem. *Sonchus tectifolius* is a short-woody-stemmed perennial with long, arching leaves with overlapping leaflets. In our result in previous chapter, *S. gummifer* is sister to the three Madeiran species (*S. ustulatus*, *S. fruticosus*, *S. pinnatus*), but *S. radicans* and *S. tectifolius* formed another clade, indicating the morphological similarity of the *S. gummifer* complex might not be monophyletic.

Besides the *S. tuberifer* and *S. gummifer* complex, we also want to investigate the origins of three monotypic genera (*Babcockia platylepis*, *Sventenia bupleuroides* and

*Lactucosonchus webbii*). *Babcockia platylepis* is endemic to the island of Gran Canaria. It is a shrub with rosette leaves and inflorescence with few heads consisting of very large and broadly ovate involucral bracts. *Sventenia bupleuroides* is about a 30 cm tall perennial with basal rosette leaves. It is endemic to Gran Canaria, more specifically from the cliffs of Tamadaba to the cliffs on Faneque Valley Agaete. *Lactucosonchus webbii*, a herbaceous perennial, is endemic to La Palma. There is no previous indication of any hybridization in the history of these three monotypic genera, nevertheless these need further investigation to test.

*Dendrosonchus* and *Taekholmia* species occur on the island of Tenerife and La Gomera. They are found in radically different environmental settings. Lee et al. (2005) stated the possibility of initial hybridization between *Dendrosonchus* and *Taekholmia*. We want to further test whether there is any evidence to indicate hybridization between these two major groups in the woody *Sonchus* alliance.

All members in the alliance are diploids ( $2n = 18$ ). Thus, homoploid hybridization is possible. Although some evidence indicates a hybrid origin of *S. tuberifer* as well as the *S. gummifer* complex. It is only preliminary, because it was based on individual direct sequence. The heterozygote sites are not fully recognized by the direct sequencing method. To recover heterozygote sites of G3pdh and Cam sequences that could not be obtained by the direct sequence, we cloned several species in this alliance. Our objectives are to test (1) the hybrid origin of *S. tuberifer* and the *S. gummifer* complex; (2) the

origins of three monotypic species; and (3) the potential hybridization between *Dendrosonchus* and *Taeckholmia*.

## MATERIALS AND METHODS

### Taxa Cloned

In this study, we cloned several species of the alliance. We included *Dendrosonchus* species (*S. brachylobus*, *S. fauces-orci*, *S. palmensis*, *S. tectifolius*, *S. congestus* and *S. gonzalezpadronii*), as well as two *Taeckholmia* (*T. pinnata* and *T. microcarpa*). We also cloned three monotypic genera (*Babcockia*, *Sventenia* and *Lactucosonchus*). We include two independent genes: glyceraldehyde 3-phosphate dehydrogenase (G3pdh) and Calmodulin (Cam) gene. We adopted the classification of Boulos (1967, 1972, 1974), which represents the first complete revision of the genus *Sonchus* and several other segregate genera. . For the Cam gene, the species examined have been listed in table 3.1, and the table 3.2 lists the species examined for G3pdh gene.



## **DNA Isolation, PCR, and Sequencing**

Total genomic DNA was isolated from leaf tissue using the CTAB method of Doyle and Doyle (1987) and DNeasy plant mini kits (QIAGEN, Valencia, California, USA). Polymerase chain reaction (PCR) conditions were an initial 5 min at 95<sup>0</sup>C followed by 40 cycles of 1 min at 94<sup>0</sup>C, 1.5 min at 50<sup>0</sup>C, and 2 min at 72<sup>0</sup>C, with a final extension time of 10 min at 72<sup>0</sup>C. The PCR amplification primers of both the nuclear gene glyceraldehyde 3-phosphate dehydrogenase (G3pdh) and the Calmodulin (Cam) gene were designed by Strand et al (1997).

All of those individuals in Table 3.1 and 3.2 were cloned, and between 2 and 11 clones per individual were sequenced so that the different repeat types could be identified. All cloning was performed with the TOPO TA PCR cloning kit (Invitrogen, Carlsbad, CA, USA). The selected clones were sequenced with an ABI PRISM BigDye Terminator v3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems Foster City, CA, USA). Primers were used T7 and M13 reversed plasmid primers in both directions. Extension products were purified and separated on an ABI377 automated sequencing machine (Applied Biosystems). Base calling and sequence editing were performed with Sequencher 4.1 (Gene Codes, Ann Arbor, Michigan, USA). The sequences were visually aligned using MacClade (version 4.06; Maddison and Maddison, 2003)

## **Phylogenetic Analysis**

The G3pdh and Cam datasets were analyzed independently using an equally weighted, unordered maximum parsimony (MP) approach (Fitch, 1971) implemented in PAUP ver. 4.0 (Swofford, 2002). The MP analyses for each dataset included a heuristic search for the most parsimonious trees; starting trees were obtained via stepwise addition. Sequences were added via simple addition with one tree held at each step. Branch swapping was performed via tree-bisection-reconnection (TBR), and steepest descent and MulTrees options were in effect. Branches were collapsed if maximum branch length was zero, and topological constraints were not enforced. Support for nodes was calculated by bootstrap analysis with 500 simple-addition bootstrap replicates, each with a maximum of 500 trees saved and TBR branch-swapping.

We performed another MP heuristic search of the two datasets independently, employing random addition instead of simple addition. The search was performed with 300 random addition sequences with TBR, MulTrees and Steepest descent in effect, while the maximum number of trees held was set to 20,000. Branch-swapping was via stepwise addition and 1 tree was held at each step. Branches were collapsed if their score was zero, gaps were coded as missing data, and characters were unordered. The bootstrap values were generated via an abbreviated analysis that included 100 bootstrap replicates, each with five random addition sequences. No more than 15,000 trees were saved per replicate, while all other parsimony parameters were constant with the heuristic searches.

## **RESULTS**

### **Parsimony Analysis**

The Cam dataset contained 59 taxa, including one outgroup taxon, and 551 characters of which 482 were constant (87.48%). Of 69 variable characters (12.34%), 25 were parsimony informative (4.54% of total). The G3pdh dataset contained 84 taxa, including one outgroup taxon, and 825 characters of which 696 were constant (84.36%). Of 129 variable characters (15.64%), 60 were parsimony informative (7.23% of total).

The shortest Cam trees were 103 steps long and had a consistency index (CI) of 0.825, homoplasy index (HI) of 0.175, retention index (RI) of 0.863, and a rescaled consistency index (RC) of 0.712. The shortest G3pdh trees were 160 steps long and had a consistency index (CI) of 0.863, homoplasy index (HI) of 0.137, retention index (RI) of 0.928, and a rescaled consistency index (RC) of 0.800.

### **Phylogenetic Relationships**

The Cam dataset had very low sequence variability. It only has 25 parsimony informative characters to resolve 59 taxa. As the result, the 50% majority tree from the MP analysis of Cam data resulted in large numbers of unresolved taxa and only few clades were identified. The G3pdh data had better resolution because of more parsimony

informative characters (60) to resolve 84 taxa. However, the clades resolved in two independent regions that were present in the 50% majority tree were each bootstrap supported and were not in conflict with each other. Since the MP phylogeny based on the Cam dataset is poorly resolved, and the G3pdh tree gives better resolution than separate analysis, the 50% majority MP tree of G3pdh (Figures, 3.1 and 3.2) is used as the working phylogenetic hypothesis for potential hybridization origin. The Cam tree (Figures, 3.3 and 3.4) is used for the additional information to confirm the G3pdh's hypothesis.

In the G3pdh tree, one individual of *S. tuberifer* clustered with all 4 clones of *Lactucosonchus* with 82 % bootstrap support (BS), but the other individual of *S. tuberifer* from Teno, near Masca, is sister to one clone of *S. tectifolius* (Figure 3.2). Unfortunately, we didn't obtain the Cam sequence from *S. tuberifer* from the Teno population. The *S. gummifer* complex (*S. gummifer*, *S. radicans*, and *S. tectifolius*) are in the unsolved group both the G3pdh and the Cam trees. The four *S. tectifolius* clones do not cluster with any of the other species in the *S. gummifer* complex. Four G3pdh clones of *Babcockia platylepis* clustered with its direct sequence with 65% BS. *Sventenia* clustered with its four clones with 100% BS in G3pdh tree (Figure 3.2). *Lactucosonchus webbii* clustered with its four clones with 98% BS and is sister to *S. tuberifer* with 79% BS in the G3pdh tree (Figure 3.2). The three clones of *T. pinnata* and *T. microcarpa* clustered with all *Taekholmia* species except *T. arboreus* with 83% BS in the G3pdh tree (Figure 3.1).

## DISCUSSION

### The Monotypic Genera

*Babcockia platylepis*, is widely distributed in the higher elevations (800–1600 m) of Gran Canaria. Aldridge (1976) based on her extensive morphological investigation, strongly argued that the genus *Babcockia* cannot be distinguished from *Sonchus* subg. *Dendrosonchus* and placed them within it. Our molecular data do not support Aldridge's argument, because *Babcockia* does not cluster with any *Dendrosonchus* species. All clones of *Babcockia* formed a clade with 65% BS. Even though we are not sure about whether *Babcockia* is derived earlier than other member of the alliance because of the unsolved basal lineages, it is likely *Babcockia* is monophyletic without any indication of a hybrid history.

*Lactucosonchus webbii* is a monotypic endemic for the island of La Palma in the Canaries. It is an herbaceous perennial with long tuberous roots and occurs in the north-facing under story of pine forest. It occurs in a relatively mesic habitat at high elevation. In the G3pdh tree, the direct sequence of *Lactucosonchus* as well as four cloned sequences clustered with *S. tuberifer* with 79% BS (Figure 3.2). It is likely that *Lactucosonchus* and *S. tuberifer* share the most recent common ancestor than other woody member in this alliance. If this is true, the herbaceous habit with tuberous roots

shared by them must have evolved in their shared ancestor. In the Cam tree, all the clones and direct sequences clustered by themselves with 83% BS (Figure 3.4). With the poorly resolved Cam tree, we cannot confirm the close relationship between the only two herbaceous members in this alliance, but none of the *Lactucosonchus* clones clustered in different lineages. So it is unlikely that *Lactucosonchus* has from a hybrid origin.

*Sventenia bupleuroides* is an extremely rare member that grows solely the high cliff-faces of Gran Canaria. In the G3pdh tree, *Sventenia* and its four clones were clustered with 100% BS (Figure 3.2). Like other monotypic species *Lactucosonchus* and *Babcockia*, there is no evidence in our molecular data to support a hybrid origin for this species.

### ***Dendrosonchus* and *Taeckholmia***

Previous study of the ITS phylogeny of the woody *Sonchus* suggested possible hybridization and introgression events between subg. *Dendrosonchus* and the genus *Taeckholmia*, because two species of subg. *Dendrosonchus*, *S. gonzalezpadronii* and *S. ortunoi*, were clustered with several species of *Taeckholmia* in a very strongly supported clade (Lee et al., 2005). Each of these *Dendrosonchus* and *Taeckholmia* species occur in radically different habitats. *Taeckholmia* species survive in a xeric environment with little available water or moisture. *Dendrosonchus* species are mostly robust shrubs in mesic habitats. In the G3pdh tree, all *Taeckholmia* species including the

clones of *T. pinnata* and *T. microcarpa* but not *T. arboreus* formed a cluster with 83% BS (Figure 3.1). None of the eleven clones of *S. gonzalezpadronii* cluster with any *Taeckholmia* species. In contrast to the ITS phylogeny, our data do not support hybridization between *Dendrosonchus* and *Taeckholmia*. Furthermore, it is very unlikely that *S. gonzalezpadronii* shares most recent common ancestor with any *Taeckholmia* species. Unfortunately, the Cam tree is highly unresolved with *Dendrosonchus* and *Taeckholmia* clades and we cannot give additional information about the relationship between them.

#### ***S. tuberifer* and the *S. gummifer* Complex**

*Sonchus tuberifer* is the only member of subg. *Sonchus* in the alliance. Our results show that *S. tuberifer* clustered with one clone of *S. tectifolius* with 81% BS, and the population in Teno clustered with all *Lactucosonchus* clones with 79% BS (Figure 3.2). It suggests that *S. tuberifer* might be closely related to *Lactucosonchus* which is also a herbaceous species and *S. tectifolius* which has very short woody stem. These results suggest that *S. tuberifer* might have hybrid origin between herbaceous and small woody species. *S. gummifer* complex (*S. gummifer*, *S. radicans*, and *S. tectifolius*) represents the highly unresolved part of the clade in both the G3pdh and Cam trees, suggesting that this complex is not monophyletic and does not support the Aldridge's view of the *S. gummifer* complex. *Sonchus tectifolius* showed diversified clone copies. One clone

clustered with *S. tuberosa* and the rest clustered with its direct sequence. These data might indicate a hybrid ancestry for *S. tuberosa*, but we cannot rule out the possibility of lineage sorting of ancient polymorphic G3pdh sequences for the rapidly radiated lineages.

**Closing Remarks:**

Based on our data, we find some support for homoploid hybrid speciation events for *S. tuberosa* and *S. tuberosa*. But the molecular sequence data here are not sufficient enough to confirm their parental taxa. Hypervariable nuclear markers (e.g. AFLPs), could be used to test hypotheses of diploid hybrid speciation at the population level. If the hybrid origins are supported based on phylogenetic approaches, extensive morphometric and genetic analysis of hybrid species including the parental species can be conducted not only to confirm diploid hybrid speciation but also to determine the frequency of hybridization events (e.g., Brochmann et al. 2000)



## REFERENCES

- Aldridge, A. 1975. Taxonomic and anatomical studies in *Sonchus* L. Subgenus *Dendrosonchus* Webb ex Schultz Bip. and related genera. Ph. D. Dissertation. University of Reading, England.
- Aldridge, A. 1976. Macaronesian *Sonchus* subgenus *Dendrosonchus* s. l. (Compositae-Lactuceae), including a reappraisal of the species concept and new combinations. *Bot.Mac.*, 2: 81-93.
- Aldridge, A. 1979. Evolution within a single genus: *Sonchus* in Macaronesia. Plants and islands, ed. Bramwell, D., Academic Press, New York : 279-290
- Arnold M. A., Bouck A. C. and Cornman R. S. 2003. Verne Grant and Louisiana irises: is there anything new under the sun? *New Phytol* 161:143–149
- Baldwin, B. G., Kyhos, D. W. and Dvorak, J. 1990. Chloroplast DNA evolution and adaptive in the Hawaiian silversword alliance (Asteraceae-Madiinae). *Annals of the Missouri Botanical Garden* 77: 96-109.
- Boulos, L. 1967. *Taeckholmia*, a new genus of Compositae from Canary Islands. *Botaniska Notiser* 120: 95–108.
- Boulos, L. 1972. Re´vision syste´matique du genre *Sonchus* L. s.l. I. Introduction et classification. *Botaniska Notiser* 125: 287–305.
- Boulos, L. 1974. Re´vision syste´matique du genre *Sonchus* L. s.l. V. Sousgenre 2. *Dendrosonchus*. *Botaniska Notiser* 127: 7–37.
- Brochmann, C., Borgen, L. and Stabbetorp, O. E. 2000. Multiple diploid hybrid speciation of the Canary Island endemic *Argyranthemum sundingii* (Asteraceae). *Plant Syst. Evol.* 220: 77-92.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochemical Bulletin* 19: 11–15.

Ellstrand, N.C., Whitikus, R. and Rieseberg, L.H. 1996 .Distribution of spontaneous plant hybrids. *Proc Natl Acad Sci USA* 93: 5090–5093

Francisco-Ortega, J. F., Jansen, R. K. and Santos-Guerra, A. 1996. Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. *Proc. Natl. Acad. Sci. USA* 93: 4085-4090.

Francisco-Ortega, J., Santos-Guerra, A., Kim, S.-C. and Crawford, D. J. 2000 . Plant genetic diversity in the Canary Islands: a conservation perspective. *American Journal of Botany* 87: 909-919

Grant, V. 1981. *Plant Speciation*, 2nd ed. New York: Columbia Univ. Press

Lee, C., Kim, S.-C., Santos-Guerra, A. and Lundy, K. 2005. Chloroplast DNA phylogeny of the woody *Sonchus* alliance (Asteraceae) in the Macaronesian islands. *American Journal of Botany*. 92: 2072-2085

Maddison, D. R. and Maddison, W.P. 2003. Sequence alignment program, release version 4.06. [www.macclade.org](http://www.macclade.org)

Mallet, J. 2007. Hybrid speciation. *Nature* 446:279–283

Rieseberg, L.H., Raymond, O., Rosenthal, D.M., Lai, Z., Livingstone, K., Nakazato, T., Durphy, J. L., Schwarzbach, A.E., Donovan, L.A. and Lexer, C. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301:1211–1216

Rieseberg, L.H. and Willis, J.H. 2007. Plant speciation. *Science* 317:910–14

Rieseberg, L. H., Zona, S., Aberbom, L. and Martin, T. D. 1989. *Conserv. Biol.* 3, 52.

Strand, A. E., Leebens-Mack, J. and Milligan, B. G. 1997. Nuclear DNA-based markers for plant evolutionary biology. *Molecular Ecology* 6: 113–118

Swofford, D.L. 2002. PAUP\*. Phylogenetic Analysis Using Parsimony (\*and Other Methods). Version 4. Sunderland: Sinauer Associates.

Table 3.1 List of investigated samples for Cam gene (species name, sample location, the number of clones sequenced)

<b>Species</b>	<b>Sample island</b>	<b># of clones</b>
<i>T. microcarpa</i>	TE	2
<i>T. pinnata</i>	GC	3
<i>S. fauces-orci</i>	TE	3
<i>S. congestus</i>	TE	4
<i>S. gonzalezpadronii</i>	GO	4
<i>Lactucosonchus</i>	PA	7
<i>Sventenia</i>	GC	4

Table 3.2 List of investigated samples of G3pdh gene (species name, sample location, the number of clones sequenced)

<b>Species</b>	<b>Sample island</b>	<b># of clones</b>
<i>T. microcarpa</i>	TE	3
<i>T. pinnata</i>	GC	3
<i>S.palmensis</i>	PA	1
<i>S.gonzalezpadronii</i>	GO	11
<i>S. tectifolius</i>	TE	4
<i>S. fauces-orci</i>	TE	4
<i>S.brachlobus</i>	GC	4
<i>Lactucosonchus</i>	PA	4
<i>Sventenia</i>	GC	4
<i>Babcockia</i>	GC	4



To Figure 3.1

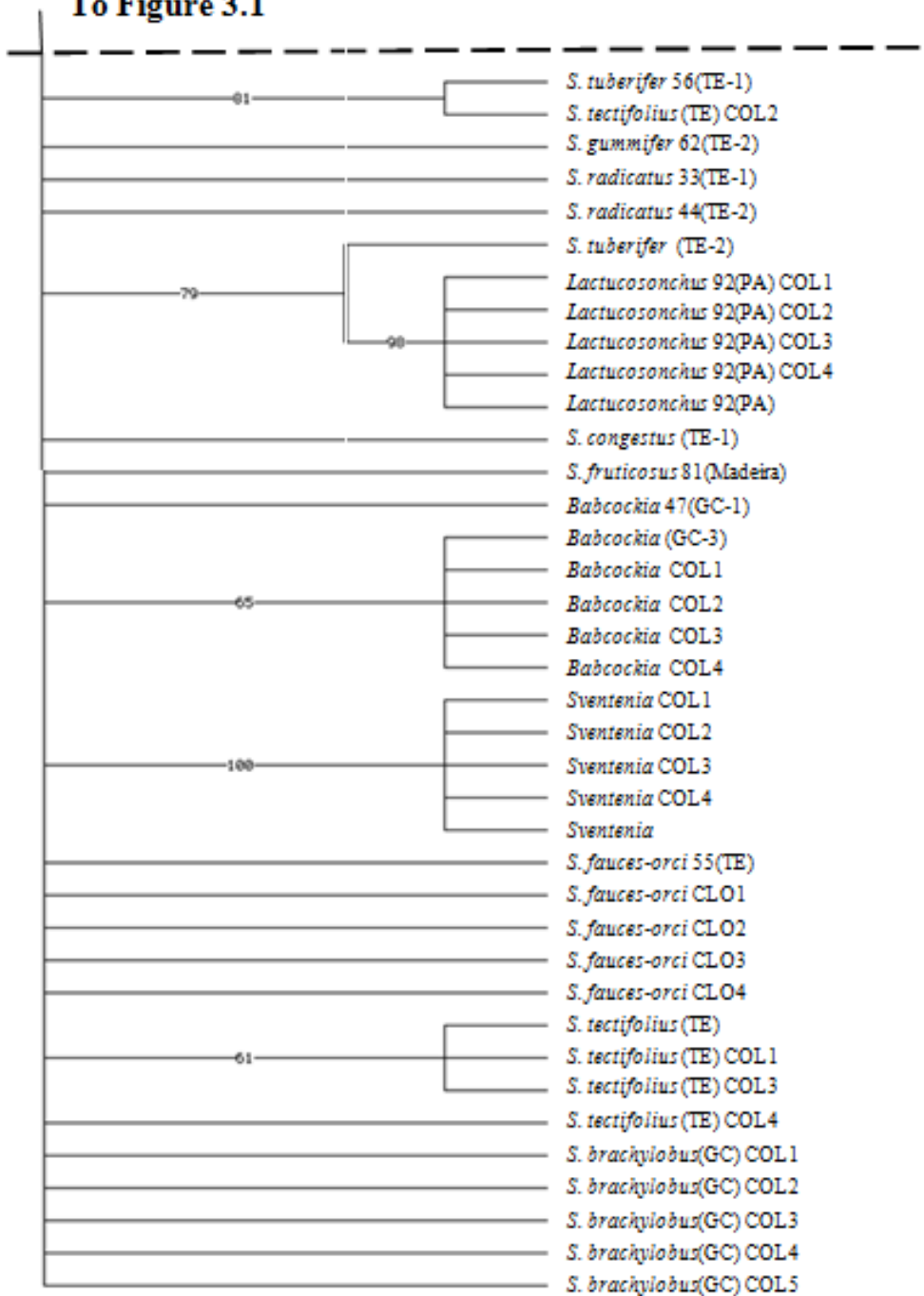
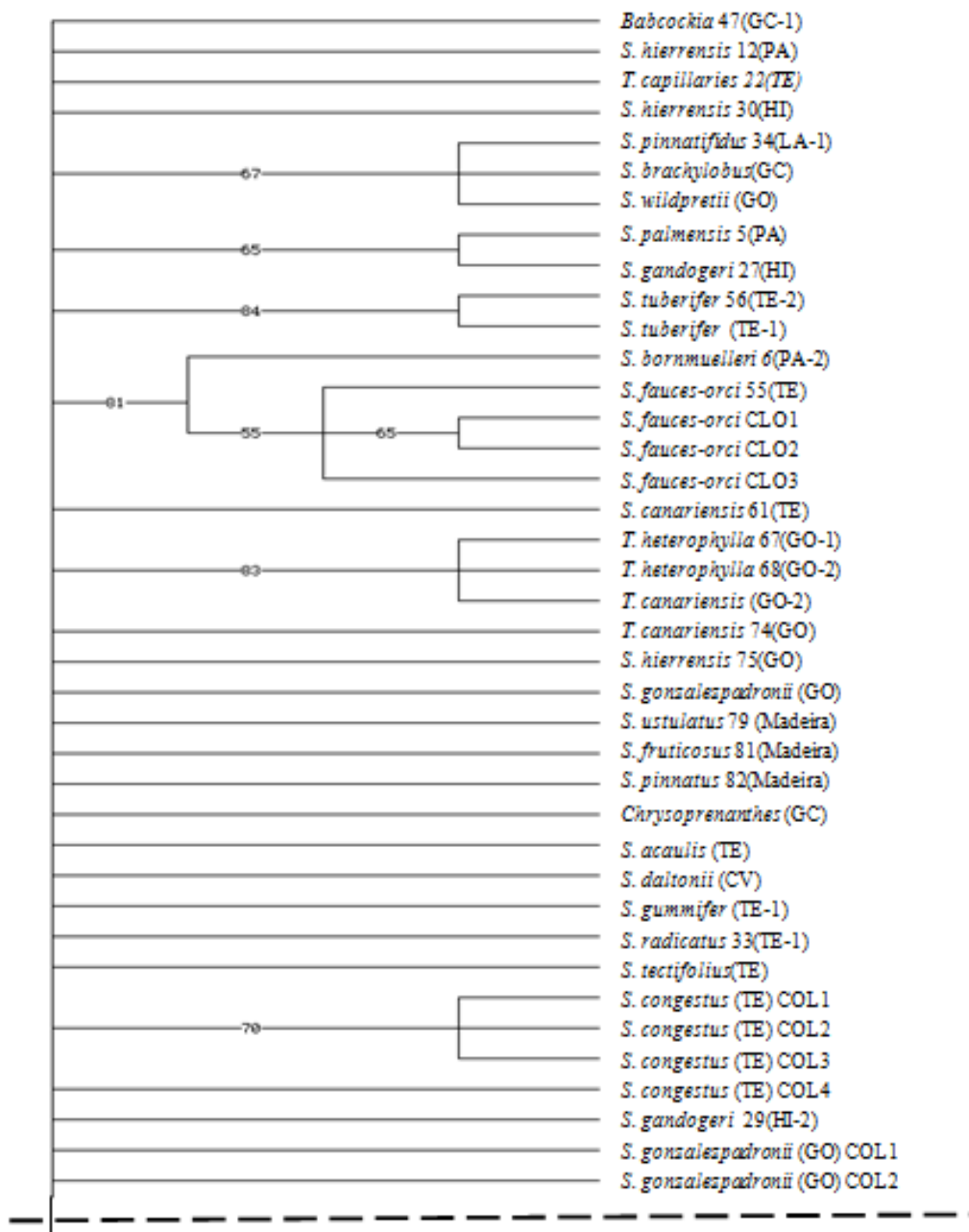


Figure 3.2 Majority (50%) consensus tree based on MP analysis of G3PDH dataset. The MP bootstrap support values above 50% are shown above branches.



### To Figure 3.4

Figure 3.3 Majority (50%) consensus tree based on MP analysis of CAM dataset. The MP bootstrap support values above 50% are shown above branches.



**To Figure 3.3**

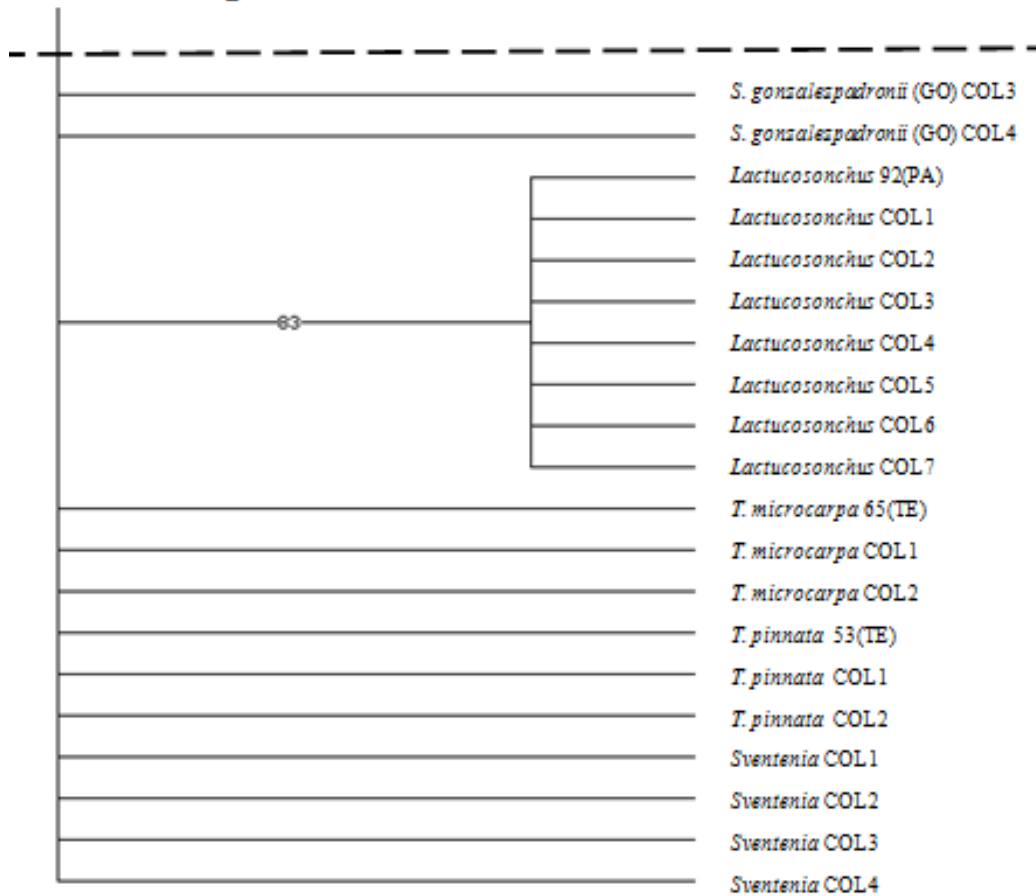


Figure 3.4 Majority (50%) consensus tree based on MP analysis of CAM dataset. The MP bootstrap support values above 50% are shown above branches.

## GENERAL CONCLUSIONS

This conclusion is written in order to summarize the most important findings of my Ph.D dissertation and perhaps more importantly to offer some insights of further investigation and topics for continued research. This dissertation takes multidisciplinary aspects to understand the origin and evolution of “*Sonchus*” as well as their island endemic derivatives. The effort of this dissertation work was to broaden our understanding of plant evolution and speciation on oceanic islands.

In the molecular phylogenetic analyses of polyploidy *Sonchus*, we interpreted the origins of Pacific Polyploid endemics. *Dendroseris* are probably autotetraploid. New Zealand endemics, *Kirkianella* and *Embergeria*, might share the same ancestor. Since they have different ploidy levels, it is likely that they evolved through different polyploidization events. *Actites* probably have an Asian origin from diploid *S. arvensis* or *S. wightianus*. Furthermore, several hypotheses of polyploidy *Sonchus* have been tested. Our data support a hybrid origin for *S. arvensis*. The ITS sequence has been homogenized via concerted evolution occurred either toward maternal or paternal repeats for *S. arvensis*. Stebbins’ hypothesis of *S. oleraceus* is only partially supported by our data. *S. asper* is one donor for *S. oleraceus*, but not *S. tenerrimus*. We confirmed the hybrid origin of *S. hydrophilus*. Our cloning strategies successfully recovered rare ITS copies

which add new insights of studying the evolutionary stories of polyploidy *Sonchus*. Our conclusions are preliminary regarding polyploid *Sonchus* because they are based on single marker. Future research needs to be focused on finding several alternative low-copy nuclear intron regions to fully investigate phylogeny of these polyploids and also to determine the role of polyplodization and hybridization.

The rest of the dissertation focuses on the woody *Sonchus* alliance, an outstanding example of adaptive radiation, in Canary Islands. We sequence three independent low-copy nuclear genes in order to investigate the phylogeny as well as to evaluate the role of hybridization contributed to the radiation of woody *Sonchus* alliance. Although all three genes have low sequence variability (<5%), the combined data gives moderate resolution. None of basal lineage was identified based on our data. The “core *Taeckholmia*” is also strongly supported containing all *Taeckholmia* species except *T. arborea*. *Dendrosonchus* is polyphyletic. With the poor resolution, it is not possible to suggest any classification and species delimitations for *Dendrosonchus*. We supported the previous hypothesis of Tenerife origin of Maderia species, most likely from *S.gummifer*. *S. hierrensis* is very likely to be the parental donor for *S. daltonii* in the Cape Verde Islands. We found some suspected hybrids, for example *S. tuberifer* and *S. gummifer* complex. The future effort of cloning several critical species with both G3pdh and Cam genes will be useful to disentangle the potential role of hybridization within woody *Sonchus* alliance presented in Chapter Three.

Research described in Chapter Three discovered *S. tuberifer* might have a hybrid origin between a herbaceous and a small woody species. Our data also indicate a hybrid ancestry for *S. tectifolius*. But the molecular sequence data here are not sufficient to confirm the parental taxa. Future study using hypervariable nuclear markers could test these hypotheses of diploid hybrid speciation at the population level. Once the diploid hybrid derivatives are supported based on phylogenetic approaches, extensive morphometric and genetic analysis of hybrid species including the putative parental species can be conducted not only to confirm diploid hybrid speciation but also to determine the frequency of hybridization events.

This project has several significant implications. It provides the first rigorous attempt to study the polyploid *Sonchus* and the related Pacific endemics. This study also elucidates phylogenetic relationships among the taxa within the woody *Sonchus* alliance based on unlinked molecular markers. It is the first attempt to test phylogenetic utility of low-copy nuclear genes in Macaronesian plant endemics. This study broadens our understanding of the role of hybridization and introgression in the evolution of Macaronesian endemics. Finally, phylogenetic data from DNA studies allow us to assess the process of diversification within the alliance which is valuable information to understand plant evolution and diversification on oceanic islands.

Appendix 1 List of samples of species cloned in the present study. The locality, voucher and herbarium numbers were reported previously in Kim et al. (2007)  
Taxon; Voucher (Location)

***Actites* Cass.**

*A. megalocarpa* (Hook. f.) Lander-1; Lepschi 3879-1 (Australia) ;  
*A. megalocarpa* (Hook. f.)Lander-3; Lepschi 4657-1 (Australia);

***Dendroseris* D. Don**

*D. berteroana* (Dcne) Hool. & Arn.-1;  
*D. litoralis* Skotts.-1;  
*D. micrantha* Hook. & Arn.-1;  
*D. pinnata* (Bert. & Dcne.) Hook. & Arn;  
*D. pruinata*(Johow) Skotts.-1;  
*D. regia* Skotts.; Landero & Ruiz 9361 (OS);

***Embergeria* Boulos**

*E. grandifolia* (T. Kirk) Boulos-1; Atkinson 118/85 (New Zealand);  
*E. grandifolia* (T. Kirk) Boulos-2; J. Santos ex cult(New Zealand);

***Kirkianella* Allan**

*K. novae-zelandiae* (Hook. f.) Allan-1; D. Glenny 4910 (New Zealand);  
*K.novae-zelandiae* (Hook. f.) Allan-2; D. Glenny 5036 (New Zealand);

**Subgenus *Sonchus* - Section *Arvenses* (Kirp.) Boulos**

*S.arvensis* L.-1; (Taiwan);  
*S. arvensis* L.-2; King & Garvey 11600 (USA);  
*S.brachyotus* DC.-2; (Korea);

**Section *Asperi* Boulos**

*S. asper* (L.) Hill-1; (Korea);  
*S. asper* (L.) Hill-2; VAL982104 (Spain) ;  
*S. asper* (L.) Hill-3; (Italy);  
*S. asper* (L.) Hill-4; (Morocoo);  
*S. hydrophilus* Boulos-1; Lepschi3765-1 (Australia);  
*S. kirkii* (T. Kirk) Allan-1; Silbury s.n. (New Zealand);

**Section *Sonchus***

*S. microcephalus* Mejias; SEV126508 (Spain);  
*S. olearceus* L.-2; Mlangwa 275 (Tanzania India);  
*S. oleraceus* L.-3; (Egypt); *S. tenerrimus* L.-1; -(Italy);  
*S. tenerrimus* L.-2; VAL38120(Spain) ;