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Phylogenetic analysis of chloroplast DNA *mat*K gene and ITS of nrDNA sequences reveals polyphyly of the genus *Sonchus* and new relationships among the subtribe Sonchinae (Asteraceae: Cichorieae)

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Abstract

Phylogenetic relationships among genera of the subtribes Sonchinae (11 genera and ca. 130 species) and Dendroseridinae (2 genera and 12 species) *sensu* Bremer were assessed with ITS and *mat*K gene sequences. The Sonchinae is strongly supported as paraphyletic, and subtribal rank of Dendroseridinae is poorly supported. This study re-delimits Sonchinae, which now includes 14 genera and ca. 146 species. Two genera, *Reichardia* and *Launaea*, diverge first, followed by a major radiation of the remaining species, including *Dendroseris* and *Thamnoseris*. The monotypic *Aetheorhiza* is more closely related to *Sonchus* than to *Launaea*. *Sonchus* is highly polyphyletic, occurring across nearly all clades of the trees. It is highly probable that several Pacific and Atlantic island endemics evolved within the *Sonchus* group. Polyploidization processes appear to have played an important role in evolutionary differentiation and speciation in *Sonchus*, especially in subgenus *Sonchus*. This study identifies 10 major clades within the Sonchinae, and emphasizes the need to re-delimit and re-circumscribe *Sonchus* and the Sonchinae.
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1. Introduction

The Cichorieae (formerly Lactuceae, Reveal, 1997; Lee et al., 2003) is perhaps the best-known and most easily recognized tribe of the Asteraceae, distinguished by the presence of milky latex and ligulate florets. It comprises 98 genera and more than 1550 species (excluding the numerous microspecies described in *Taraxacum* and *Hieracium*) and occurs predominantly in the Northern Hemisphere (Bremer, 1994). Previous classifications of the tribe into genera and subtribes have been proposed by numerous authors (e.g., Jussieu, 1789; Don, 1828; Lessing, 1832; Bentham, 1873; Hoffmann, 1891; Stebbins, 1953; Jeffrey, 1966; Sell, 1975). The most recent classification was given by Bremer whose proposals were based on morphological cladistic analysis (Bremer, 1993, 1994). Generally, subtribal classification has been upheld by subsequent molecular analyses (e.g., Jansen et al., 1991; Whitton et al., 1995; Kim et al., 1996a; Lee and Baldwin, 2004). The most salient changes in phylogenetic relationships in the Cichorieae as revealed by molecular phylogenetic studies concern the subtribe Dendroseridinae. The new results do not support its subtribal rank given by Stebbins (1953) and Bremer (1994), but support its placement within Jeffrey's *Sonchus* group, which includes *Sonchus*, *Dendroseris*,

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and *Thamnoseris*. Specifically, *Dendroseris*, endemic to the Juan Fernandez Islands, was found to be deeply embedded within the Sonchinae *sensu* Bremer (Kim et al., 1996a,b, 1999, 2004), and *Thamnoseris*, endemic to the San Ambrosio Islands, was found to be closely related to *Dendroseris* (B. Baldwin, personal communication). Another important deviation from Bremer's classification is the finding that *Prenanthes pendula*, endemic to the Canary Islands, is not closely related to any of its congeners, but that it represents one of several basal lineages of the recently radiated woody *Sonchus* alliance (Kim et al., 1996a,b). It has been subsequently raised to the rank of a monotypic genus, *Chrysoprenanthes* (Bramwell, 2003).

The Sonchinae (including Dendroseridinae) is the most widely distributed subtribe in the tribe Cichorieae, with a discontinuous, almost cosmopolitan, and very peculiar distribution phytogeographically (Kim et al., 1996a). In particular, genus *Sonchus* subgenus *Sonchus*, includes many widespread, successful, and noxious weeds and 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). The Sonchinae have been demonstrated repeatedly to be a well-defined subtribe of the Cichorieae (Kim et al., 1996a,b; Kilian, 1997; N. Kilian, personal communication). This allows us to assess phylogenetic relationships among the genera of the subtribe Sonchinae, which now include 14 genera and ca. 146 species.

Previous studies of Sonchinae (e.g., Kim et al., 1996a,b, 1999) have analyzed approximately 30% of the subtribe. Several widely distributed taxa, especially two subgenera of *Sonchus*, *Sonchus* and *Origosonchus*, were poorly represented in these surveys (33% and 15%, respectively), and *Launaea*, the second largest genus (ca. 54 species), was represented by only two species. Many of the subtribal relationships among the genera were either highly unresolved or weakly supported (Kim et al., 1996a,b, 1999, 2004). Several unexpected relationships, especially the position of *Dendroseris*, were suggested by this analysis, and should be independently verified by sampling other much longer regions of cpDNA.

In this study, we present a molecular phylogenetic assessment of relationships among all 11 genera of the Sonchinae (Bremer, 1993, 1994) plus *Chrysoprenanthes* (Bramwell, 2003), and the genus *Dendroseris* of the subtribe Dendroseridinae (Bremer, 1994) based on ITS of nrDNA and *mat*K chloroplast gene and flanking non-coding sequences. This represents the first comprehensive molecular phylogenetic study based on two independently inherited molecular markers with broad sampling, with the exception of the genus *Launaea* and subgenus *Origosonchus* from Africa. Our results identify several well-supported clades within the Sonchinae and suggest a need for reclassification.

2. Materials and methods

2.1. Taxon sampling

Several underrepresented taxa in the previous studies were sampled extensively (Appendix A). These included several taxa of subgenus *Sonchus* from Northern Africa and the Iberian Peninsula. Three species of section *Pustulati* from the Iberian Peninsula and North Africa: *S. pustulatus* (endemic to Spain, Morocco, and Algeria), *S. fragilis* (endemic to Morocco), and *S. masguindalii* (endemic to Morocco) were sampled. According to Boulos (1973), these taxa show several primitive morphological characteristics within subgenus *Sonchus*. We also sampled taxa of subg. *Sonchus* from the Iberian Peninsula and Mediterranean, representing several sections: *S. tenerrimus*, *S. oleraceus*, *S. microcephalus* (sect. *Sonchus*); *S. maritimus*, *S. aquatilis*, *S. crassifolius* (sect. *Maritimi*); *S. asper*, *S. mauritanicus* (sect. *Asperi*). Other species of subg. *Sonchus*, which occur widely in Europe, Asia and Australia, were also sampled: *S. brachyotus*, *S. wightianus* (sect. *Arvenses*), *S. palustris* (sect. *Maritimi*), and *S. hydrophilus* (sect. *Asperi*). The sampling of subg. *Sonchus* includes all but two species (*S. macrocarpus*, Egypt endemic, and *S. malaianus*, Indonesia endemic). For the subg. *Origosonchus*, only two species from Kenya, *S. luxurians* and *S. schweinfurthii*, were included in the previous phylogenetic analysis (Kim et al., 1996a). In this study, three additional species, *S. bipontini*, *S. afromontanus*, and *S. saudensis*, were sampled, which represents ca. 36% of the entire subgenus. In the case of the subg. *Dendrosonchus* and segregate genera in the Macaronesian Islands (i.e., the woody *Sonchus* alliance), all taxa were sequenced for nrDNA ITS and cpDNA *mat*K gene and *trn*T-L-F regions since the results strongly suggested monophyly of the entire alliance (Lee et al., 2005).

Two basal genera identified in the previous study, *Launaea* and *Reichardia*, were highly underrepresented and several additional species from each were included in this study: three taxa of *Reichardia* (*R. intermedia*, *R. gaditana*, and *R. crystallina*) and three species of *Launaea* (*L. cornuta*, *L. sarmentosa*, and *L. rarifolia*). A phylogenetic study of *Launaea* is currently underway by Norbert Kilian (personal communication).

We sampled multiple populations for many species of the two subgenera of *Sonchus*, *Sonchus* and *Origosonchus*, as well as several monotypic genera (i.e., *Actites*, *Embergeria*, *Kirkianella*, *Sventenia*, *Lactucosonchus*, *Babcockia*, and *Aetheorhiza*).

2.1.1. Outgroup selection

The subtribal relationships within the Cichorieae are still unknown and three subtribes of Bremer (1994) were used as outgroups: Hypochaeridinae (*Hyoseris*, *Hypochaeris*, *Picris*, *Helminthotheca*, *Leontodon*, and *Rhagadiolus*), Lactucinae (*Lactuca* and *Prenanthes*), and Crepidinae (*Taraxacum*). Selection of these genera is based on cpDNA restriction site analysis (Whitton et al., 1995) and representative

species with available ITS sequences in GenBank were included (Appendix A). An initial phylogenetic analysis suggests that *Hyoseris* is the closest outgroup genus to the Sonchinae, and so was used as an outgroup in subsequent ITS analyses. (This was also suggested as an outgroup by N. Kilian, personal communication.) For cpDNA analyses, *mat*K gene sequences of *Hoyseris* species were not available in GenBank, and so we sequenced two available DNA samples, *Prenanthes purpurea* and *Lactuca perennis*, and used them as outgroups.

2.2. DNA isolation, PCR, and sequencing

Total genomic DNA was isolated from fresh, dried, or herbarium leaf tissue using DNeasy plant mini kits (QIAGEN, Valencia, California, USA). PCR amplification of ITS of nrDNA and *mat*K gene was the same as described previously (Lee et al., 2005). PCR products were purified with the QiaQuick PCR Purification kit (QIAGEN). Direct sequencing of PCR products was performed with an ABI PRISM BigDye Terminator v3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems, Foster City, California, USA), and extension products were purified and subsequently separated on ABI377 automated sequencing machines (Applied Biosystems). We used the same sequencing primers for ITS and *mat*K gene that we used in the previous paper (Lee et al., 2005). Base calling and sequence editing were performed with Sequencher 4.1 (Gene Codes, Ann Arbor, Michigan, USA). Newly obtained ITS sequences were manually entered and visually aligned to the previously existing Sonchinae data matrix in MacClade (Madison and Madison, 2000). *mat*K gene and flanking region sequences were aligned manually due to low sequence variation. The two data matrices are available upon request from the first author.

2.3. Phylogenetic analyses

For the ITS data set, a total of 123 accessions was initially analyzed: 110 ingroup taxa (13 genera and 61 species) and 13 outgroup taxa (9 genera and 13 species). Later, *Hyoseris* was used as the sole outgroup. In the case of the *mat*K gene data set, outgroups were from the results of the ITS analysis (*Lactuca* and *Prenanthes*). Only a subset of ingroup taxa were analyzed (13 genera and 56 species). For each ITS and *mat*K data set, phylogenetic analyses using Fitch parsimony were performed with PAUP* 4.0 (Swofford, 2001) using the HEURISTIC search option with TBR branch swapping and MULPARS on. Gaps were treated as missing data and support for groups was examined by 1000 bootstrap replicates (Felsenstein, 1985) using the HEURISTIC search option from a simple addition sequence with TBR branch swapping. Pairwise sequence divergence was calculated using the Kimura 2-parameter method (Kimura, 1980) using PAUP*. Congruence between ITS and *mat*K data sets was 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 1000 most parsimonious trees per replicate. To explore character evolution, we used one of the most parsimonious trees based on the combined data set to map haploid chromosome numbers and morphological characters using the default parameters of MacClade version 4.0 (Madison and Madison, 2000).

3. Results

3.1. ITS phylogeny

Lengths of ITS 1 and ITS 2 in the newly sequenced taxa of the subtribe Sonchinae were within the size range reported previously (Kim et al., 1996a, 2004; Lee et al., 2005). Initial phylogenetic analysis of 13 outgroup genera supported the hypothesis that the genus *Hyoseris* is most closely related to the Sonchinae, and thus phylogenetic analyses with a total of 112 taxa, including 2 outgroup taxa and 110 ingroup taxa, were conducted. A total of 501 manually aligned characters were used for phylogenetic analyses. We found 194 constant characters (38.7%), 48 variable parsimony uninformative characters (9.6%), and 259 parsimony informative characters (51.7%) between ingroup and outgroup. The heuristic search resulted in more than 50,000 trees, with a tree length (TL) of 810, a consistency index excluding uninformative characters (CI) of 0.5548, and a retention index (RI) of 0.8931. The strict consensus tree is relatively well resolved within the subtribe Sonchinae (Fig. 1). Several different alignment parameters (i.e., gap opening and extension penalties) using the Clustal X program (Thompson et al., 1997) resulted in finding the same major lineages. Thus, description of major clades will be based on the manually aligned data set result. The major features of the strict consensus tree are the polyphyly of the genus *Sonchus*, basal position of *Reichardia* and *Launaea*, and derived nature of Pacific and Atlantic island endemics within the *Sonchus* group.

The several major clades found in this analysis are designated as follows: (1) the genus *Reichardia* clade (clade I), (2) the monotypic genus *Aetheorhiza* clade (clade II), (3) the genus *Sonchus* subgenus *Sonchus* sections *Sonchus* and *Asperi* clade (clade III), (4) the genus Sonchus subg. *Origosonchus* clade (clade IV), (5) the *Dendroseris* and *Thamnoseris* clade (clade V; see Section 4 about *Thamnoseris*), (6) the *Kirkianella*, *Actites*, *Embergeria*, and the genus *Sonchus* subg. *Sonchus* sections *Maritimi* and *Arvenses* clade (clade VI), (7) the genus *Sonchus* subg. *Sonchus* section *Pustulati* clade (clade VII), (8) the *Sonchus palustris* clade (clade VIII), and (9) the woody *Sonchus* alliance clade in the Macaronesian Islands (clade IX). The genus *Launaea* turned out not to be monophyletic in this study, but this is due to sampling error (independent molecular phylogenetic study of the genus *Launaea* with broader sampling by
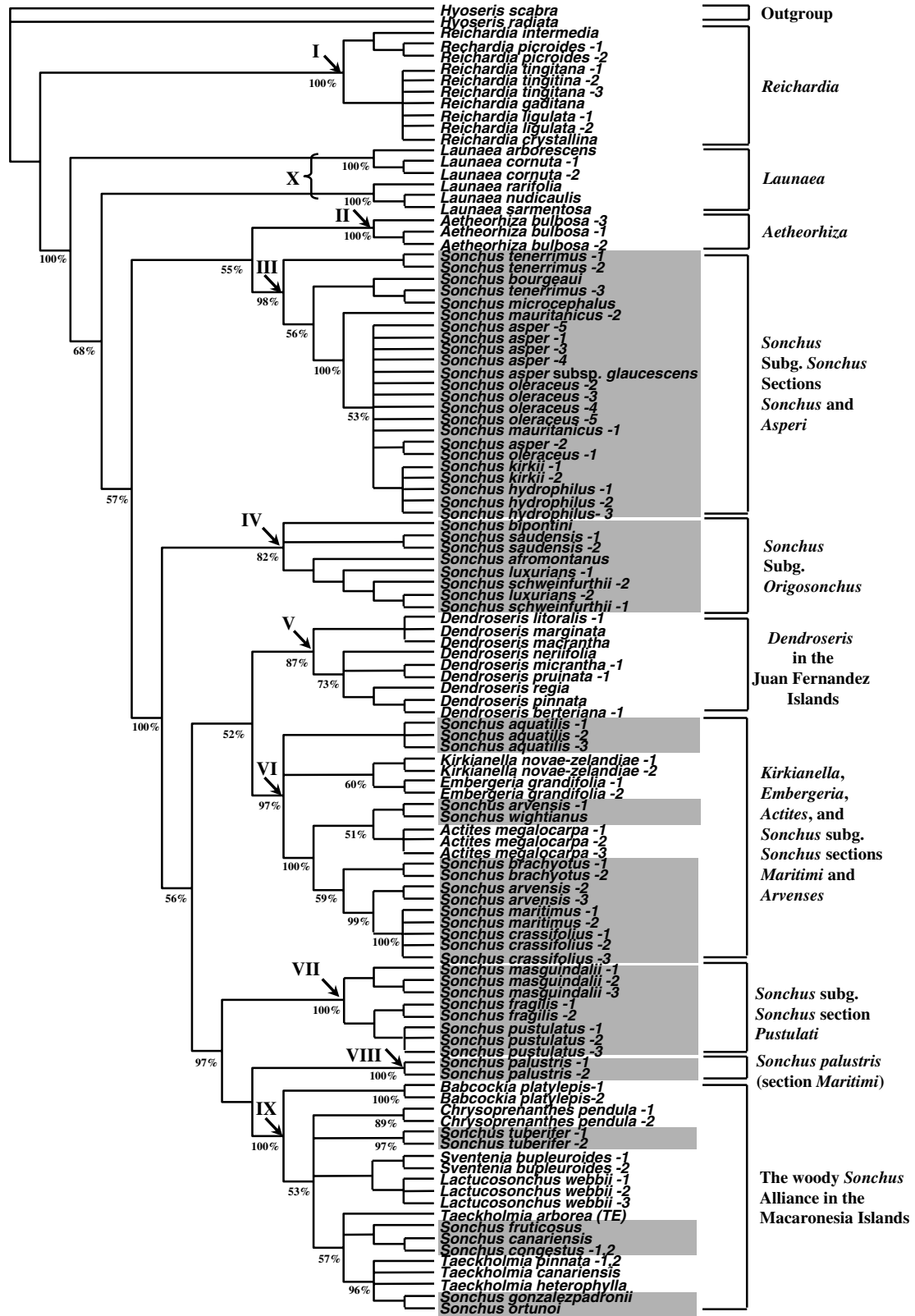
Fig. 1. Strict consensus tree of the subtribe Sonchinae based on ITS nrDNA sequences. The classification system of *Sonchus* and several segregate genera is based on Boulos (1972). The genus *Sonchus* is highlighted in gray box to show its polyphyly. Bootstrap supports are shown below branches.

Nobert Kilian strongly suggested that genus *Launaea* is monophyletic, personal communication). Therefore, we will recognize the *Launaea* clade in this study (clade X). Evidence of all major clades within the Sonchinae was strongly supported (above 82%) by bootstrap analysis (Fig. 1).

3.2. matK phylogeny

A total of 2990 aligned characters were used for phylogenetic analysis: partial *trn*K5′ and intergenic spacer 1–782 (782 bp), *mat*K gene 783–2318 (1536 bp), intergenic spacer 2319–2665 (347 bp), *trn*K3′ 2666–2700 (35 bp), intergenic spacer 2701–2965 (265 bp), and partial *psb*A gene 2966–2990 (25 bp). The length of *mat*K gene in all ingroup taxa is 1530 bp, with an exception of two taxa, *Sonchus maritimus*-2 (VAL14321, Spain) and *Sonchus aquatilis*-1 (Spain), which have 1536 bp. All but one insertions/deletions (indels) are found in intergenic spacers and are unique to one accession, homoplasious, or are synapomorphic to the clades recognized in the strict consensus tree (Fig. 2). Therefore, indels were treated as missing data in the phylogenetic analyses. Several different character weighing strategies of 1st, 2nd, 3rd position of codons and transition and transversion ratios found the same strict consensus trees (not shown) as the unweighted default search strict consensus tree (Fig. 2). Therefore, our discussion is based on the strict consensus tree. There are 2651 constant characters (88.6%), 151 variable but parsimony uninformative characters (5%), and 188 parsimony informative characters between ingroup and outgroup (6.3%) in the data matrix. The default heuristic search found more than 150,000 trees, with a TL of 425, a CI of 0.8565 (0.7741 excluding uninformative), and a RI of 0.9256. The strict consensus tree (Fig. 2) is somewhat less resolved compared to the ITS tree (Fig. 1), but it identifies several identical major clades found in the ITS tree. First, both *Reichardia* (clade I) and *Launaea* (clade X) are basal within the subtribe, sister to each other, and monophyletic (weak support for the monophyly of *Launaea*, <50% bootstrap value). The monophyletic clade of the woody *Sonchus* alliance (clade IX) branched off later, and the remainder of the subtribe remains highly unresolved. The *mat*K phylogeny identifies clade II (*Aetheorhiza*) and clade V (*Dendroseris*). The clade VII in the ITS phylogeny, i.e., the genus *Sonchus* subg. *Sonchus* section *Pustulati* clade, is no longer considered monophyletic: *S. fragilis* and *S. masguindalii* are part of a highly unresolved clade and *S. pustulatus* is deeply embedded within clade III (the genus *Sonchus* subg. *Sonchus* sections *Sonchus* and *Asperi*). The part of clade VI in the ITS phylogeny is sister to clade III in addition to *S. pustulatus*: two species of *Sonchus*, *S. brachyotus* and *S. arvensis* are sister to clade III, though weakly supported by 61% bootstrap value. *Sonchus palustris* (clade IV) is closely related to clade VIII (the genus *Sonchus* subg. *Origosonchus*): however, this is supported by a very low bootstrap value, <50%.

3.3. Congruence between ITS and matK phylogenies and combined data set analysis

There are several incongruences between ITS and *mat*K phylogeny. First, the woody *Sonchus* alliance branches off after the initial divergence of *Reichardia* and *Launaea* in the *mat*K gene tree, whereas it represents one of highly derived clades within the Sonchinae in the ITS phylogeny. Second, clade VII is monophyletic and sister to clades VIII (*S. palustris* clade) and IX (the woody *Sonchus* alliance clade), while it is not monophyletic and closely related to clade III in the *mat*K phylogeny. Clade VI is monophyletic and sister to the *Dendroseris* clade (clade V), while it is not monophyletic and part of clade VI, *Sonchus arvensis* and *S. tenerrimus*, is closely related to the clade III plus *S. pustulatus* in *mat*K phylogeny. Clade IV in the ITS phylogeny is sister to the clades V, VI, VII, VIII, and IX, whereas clade VIII is closely related to clade IV and represents one of several highly unresolved clades in the *mat*K phylogeny. The partition homogeneity test for the ITS and *mat*K indicated that the partitions were significantly different from random partitions ($P = 0.02$). Although the two phylogenies are incongruent, the ILD test is quite conservative and major clades with good bootstrap supports can be identified in both phylogenies. Furthermore, most conflicts occurred in somewhat weakly supported (less than 66%) clades with the exception of *Sonchus pustulatus*. Therefore, we decided to combine the two data sets to improve resolution and support. The same heuristic search with 89 taxa sequenced both ITS and *mat*K gene found more than 50,000 equally parsimonious trees with a TL of 1280, a CI of 0.6594 (0.5856 excluding parsimony uninformative characters), and a RI of 0.8587. The strict consensus tree is shown in Fig. 3. The tree representing a combined data set identified the same 10 major clades identified in separate analysis, but relationships among them differed slightly. One major difference between combined and independent data set analysis is the position of the woody *Sonchus* alliance: the combined data analysis, like cpDNA tree, suggested that it represents a primitive group after initial divergence of *Reichardia* and *Launaea*. However, basal position of this group is very weakly supported and when weakly supported (<52%) branches are collapsed, the combined data set tree is almost identical to the two independent data set trees: 10 major strongly supported clades are still recognized, but relationships among them need to be further resolved.

3.4. Chromosomal and morphological traits evolution

Since the combined data set analysis identified 10 major clades within the subtribe Sonchinae, we decided to investigate the evolution of chromosomal and three morphological traits using one of the most parsimonious trees (Figs. 4 and 5). It became quite clear that the haploid chromosome number of 9 is basic and ancestral within the Sonchinae and that polyploidization and aneuploidization took place
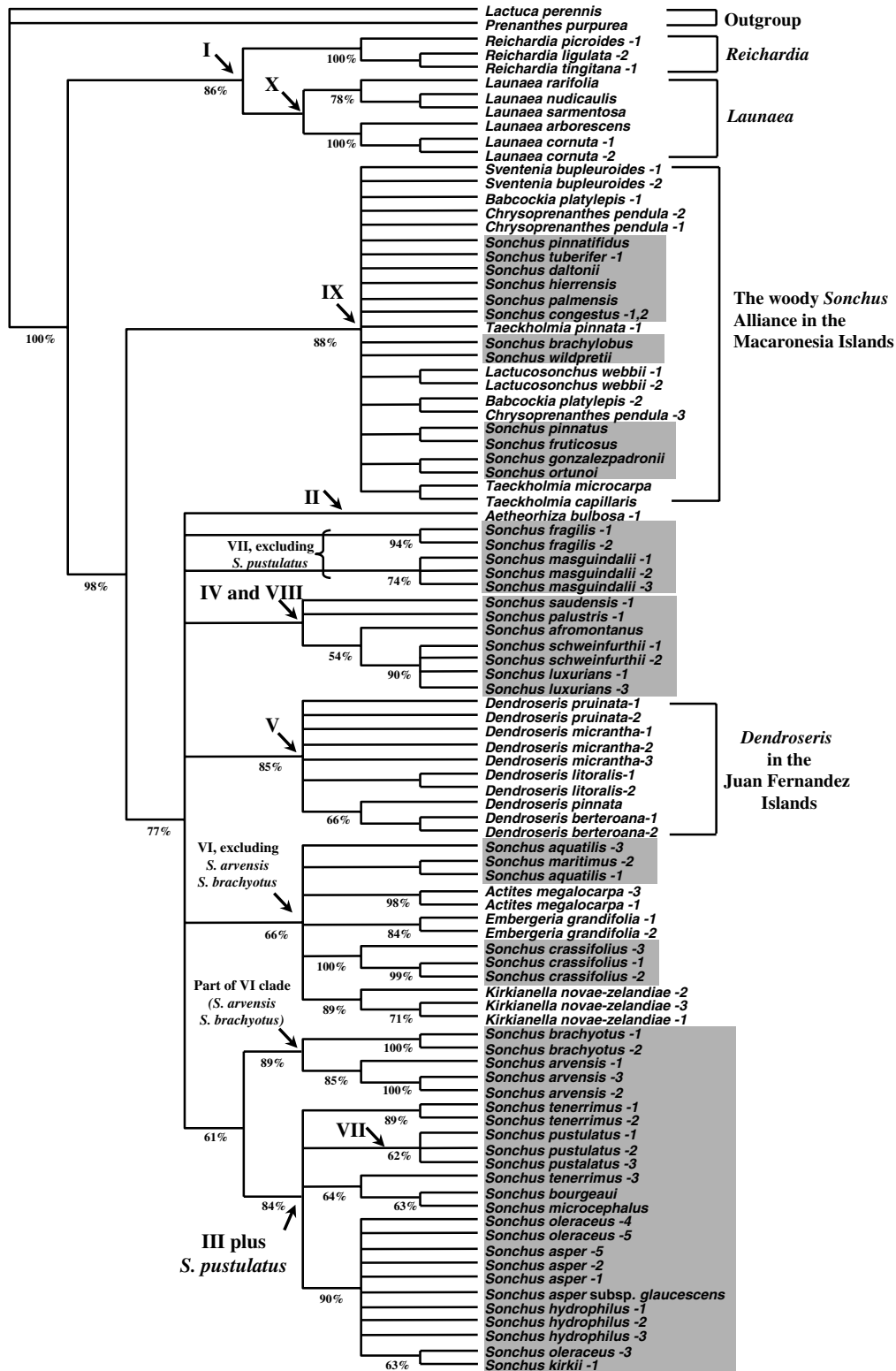
Fig. 2. Strict consensus tree of the Sonchinae based on cpDNA matK gene and flanking sequences. The clade numbers are same as in Fig. 1. Bootstrap supports are shown below branches.

independently in several lineages (Fig. 4). Habit mapping on the tree (Fig. 5) also strongly suggested that the herbaceous (annual/biennial/perennial) habit is plesiomorphic within the subtribe and the suffrutescent and shrub/tree habits evolved independently several times from herbaceous ancestors. Lastly, the presence of a deciduous and monomorphic pappus seemed to be ancestral within the Sonchinae.
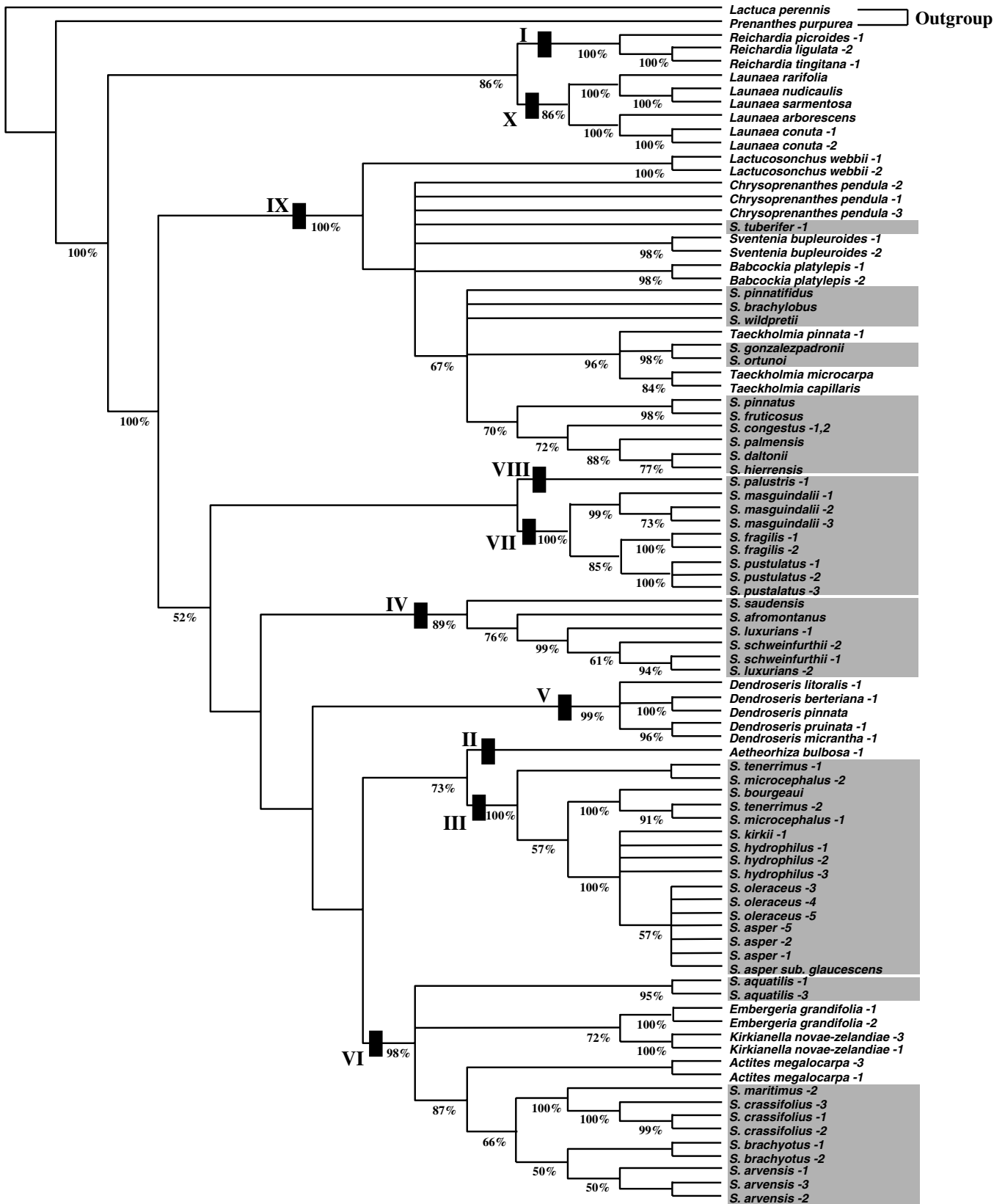
Fig. 3. Strict consensus tree of the Sonchinae based on combined data set. Bootstrap supports are shown below branches. (*S.* = *Sonchus*). The clade numbers are same as in Fig. 1.

Fig. 4. Mapping of chromosome numbers on one of the most parsimonious trees based on combined data set. Haploid numbers were obtained from the Missouri Botanical Garden W³TROPICOS data base (Index to Plant Chromosome Numbers Data Base), Kilian (1997), Mejías and Andrés (2004), Roux and Boulos (1972), Gallego (1980), Sanders et al. (1983), and Spooner et al. (1987).
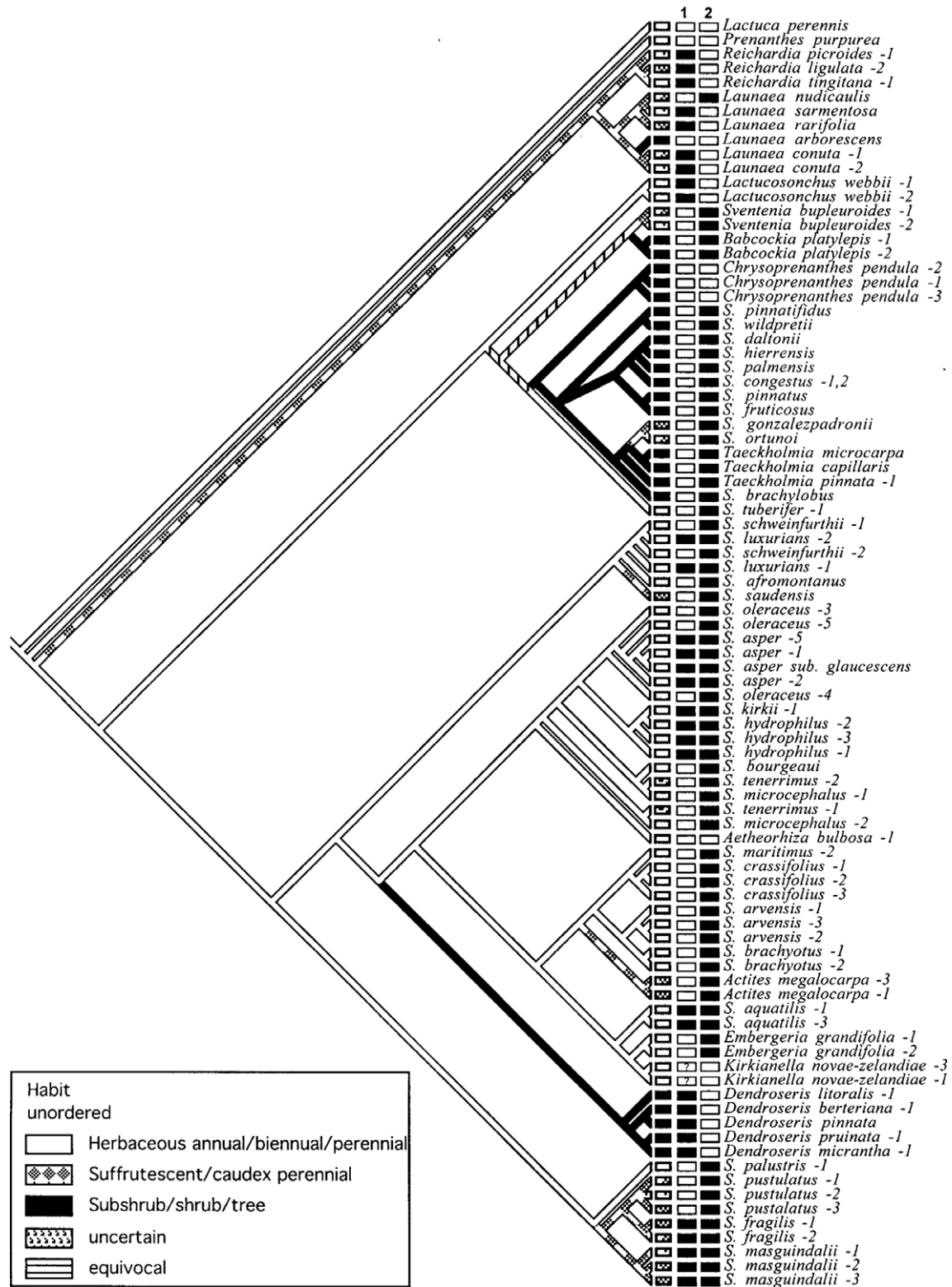
Fig. 5. Mapping of habit, pappus deciduousness, and pappus dimorphism on one of the most parsimonious trees based on combined data set. Habit is mapped on the tree, with numbered columns of boxes: (1) pappus deciduousness: persistent (white); deciduous (black); deciduous and persistent (gray) and (2) pappus dimorphism: monomorphic (white); dimorphic (black). ? = unknown.

4. Discussion

4.1. Major lineages within the subtribe

Several major lineages within the subtribe Sonchinae are strongly supported (Figs. 1–3). This study confirms the same major clades found earlier (Kim et al., 1996a) and delineates several other major clades as well.

4.1.1. Reichardia and Launaea clade (I and X, respectively)

These two genera, one of the two major alliances recognized Bremer within the Sonchinae, represent the most primitive members within the subtribe. *Reichardia* is a strongly supported monophyletic group based on both ITS and *mat*K phylogenies (100% bootstrap supports). *Launaea*, on the other hand, appears to be paraphyletic in the ITS phylogeny (Fig. 1), but monophyletic (very weak bootstrap support, >50%) in the *mat*K phylogeny (Fig. 2). The combined data set tree shows that both genera are reciprocally monophyletic (Fig. 3) and represent the first split within the subtribe. An independent molecular phylogenetic study of *Launaea* with almost complete sampling strongly suggested its monophyly and basal position within the Sonchinae (N. Kilian, personal communication). This current study was based on only five species, representing five of eight sections (ca. 10% of the whole genus; Kilian, 1997), and thus it is highly likely that *Launaea* is monophyletic. It has been previously suggested that *Launaea* may be the oldest stock in the Sonchinae (Stebbins, 1953; Kilian, 1997). The ITS phylogeny suggests that the first split is between *Reichardia* and the remainder of the ingroup, and *Launaea* shares a more recent common ancestor with *Sonchus* and related genera than with *Reichardia*. However, the *mat*K and combined data set phylogeny suggests that *Reichardia* and *Launaea* share a most recent common ancestor and can be separated from *Sonchus* and related genera within the subtribe (this is also supported by several taxonomically relevant features; Kilian, 1997).

We estimated the divergence time of *Reichardia* and *Launaea* within the Sonchinae based on ITS sequences. Several different ITS substitution rates have been estimated: $3.94 \pm 0.10 \times 10^{-9}$/yr (*Dendroseris*, Sang et al., 1994); 3.62×10^{-9}/yr (Curcubitaceae, Jobst et al., 1998); 35×10^{-10}/yr (herbaceous perennial *Astragalus*, Wojciechowski et al., 1999); and 30×10^{-10}/yr (the Hawaiian silversword alliance, Baldwin and Sanderson, 1998). On average, the ITS substitution rate is 0.725% per million years. Obviously, this rate will only give us a very rough estimate. Nevertheless, based on this rate, the average pairwise sequence divergence between the most basal lineage (*Launaea* and *Reichardia*) and the rest of Sonchinae is $20.364\% \pm 0.0169$ (standard deviation). This suggests that the common ancestor of the two major lineages within the subtribe evolved approximately 28 million years ago (Mya), in the mid-Oligocene period. The age of the Asteraceae has been estimated to be at least 38 Mya, near the Eocene–Oligocene boundary (Bremer and Gustafsson, 1997), and this divergence time is within the estimated time of origin of the family. This suggests that the ancestral lineages of the Sonchinae radiated in the mid-Oligocene of the Tertiary period. These two basal genera occur throughout Africa, the Mediterranean Basin, Southern Europe, Southwest and Central Asia, Middle East, and the Macaronesian Islands. Since all the Macaronesian Island species are derived from a single continental ancestor, it is highly likely that the Sonchinae originated somewhere in Africa, the Mediterranean Basin, or Eurasia.

4.1.2. Monotypic genus Aetheorhiza clade (II)

This monotypic genus shares several morphological characteristics with *Launaea* and *Sonchus* (Kim et al., 1996a), and is designated as a distinctive genus based solely on its habit [i.e., (sub)scapose and stoloniferous]. In the strict consensus tree based on ITS sequences (Fig. 1), *Aetheorhiza* is sister to the clade containing several cosmopolitan species of *Sonchus* sections *Sonchus* and *Asperi* (though very weakly supported, 55%). In the *mat*K phylogeny, it represents one of the highly unresolved members in the Sonchinae (Fig. 2). The combined analysis, like the ITS phylogeny, suggests a close relationship to *Sonchus* sections *Sonchus* and *Asperi* (Fig. 3). Therefore, it is clear that *Aetheorhiza* shares a most recent common ancestor with the *Sonchus* group and diverged as a part of the radiation of the *Sonchus* group. Like other *Sonchus*-related genera, this occurred after *Reichardia* and *Launaea* had already diverged. Based on the average ITS substitution rate, it appears that *Aetheorhiza* diverged from the rest of the *Sonchus* group approximately 14.5 Mya ($10.52\% \pm 0.005$ sequence divergence), in the late Miocene period.

4.1.3. Sonchus subg. Sonchus, sections Sonchus and Asperi clade (III)

This clade consists primarily of two sections of *Sonchus*: subgenus *Sonchus*, *Sonchus* and *Asperi*. It has strong bootstrap support (98%) in the ITS phylogeny. Both sections turned out not to be monophyletic in ITS, *mat*K, and the combined phylogeny. Section *Asperi* was created by Boulos (1972), but it shares many overlapping morphological characters with section *Sonchus* (except in achene morphology). Section *Sonchus* displays variability in the basic chromosome number ($x = 7, 8, 9$), while section *Asperi* has a consistent basic chromosome number (x) of 9. This study strongly indicates that the two sections are not natural groups, and are probably best represented as a single section.

4.1.4. Sonchus subg. Origosonchus clade (IV)

Five species of this subgenus were sampled (out of 15 species, 30% sampling), but it appears nonetheless that it is a good monophyletic group based on the ITS phylogeny (82% bootstrap support). In the case of *mat*K phylogeny, *Sonchus palustris* is closely related to the subg. *Origosonchus* clade (Fig. 2). This relationship is, however, very weakly supported (>50%) by only one substitution and

perhaps due to low sequence variation in the *mat*K gene and flanking regions. The combined phylogeny suggests its monophyly with strong bootstrap support (89%) (Fig. 3). Therefore, it seems likely that *Origosonchus*, consisting of generally herbaceous perennials with generally persistent pappus and endemic to Africa, sub-Sahara, Madagascar, and Comores, is the only natural subgenus within the genus *Sonchus*.

4.1.5. Dendroseris clade (V)

This Juan Fernandez Islands endemic, occurring off the coast of Chile, is monophyletic and well supported by all phylogenies (87%, 85%, and 99% in ITS, *mat*K, and combined phylogeny, respectively). Species of *Dendroseris* are exceedingly rare and consist of rosette trees and shrubs with extremely variable morphology. All chromosome counts indicate that they are tetraploids with $n = 18$ ($2n = 36$) (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 that it most likely derived from the *Sonchus* group. The *mat*K phylogeny (Fig. 2) is highly unresolved in regards to recently radiated Sonchinae, and their origins could not therefore be tested. The combined tree (Fig. 3) also suggests uncertainty about the origin of *Dendroseris*. However, the ITS tree (Fig. 1) suggests that *Dendroseris* shares a most recent common ancestor with several other Pacific Island endemic genera (i.e., *Kirkianella*, *Embergeria*, *Actites*), and with subgenus *Sonchus* sections *Maritimi* (except *S. palustris*) and *Arvenses*. The support for this, however, was weak by bootstrap value (52%). The subg. *Sonchus* sections *Maritimi* and *Arvenses* occur very widely, and are mostly diploids, with an exception in *S. arvensis* ($2n = 36, 54$). The Pacific Island monotypic endemic genera, *Kirkianella*, *Embergeria*, and *Actites*, are all polyploids. The closest continental ancestor of *Dendroseris* remains undetermined. It is also unclear whether the continental ancestor was diploid and became tetraploid subsequent to dispersal, or if polyploidization occurred prior to dispersal and radiation to the islands. The monotypic *Thamnoseris* is a San Ambrosio Islands endemic, off the coast of Chile. It is a stout shrub or small tree with leaf rosettes at the summit of the stems, a similar habit to *Dendroseris*. It was placed in the subtribe Dendroseridinae (Stebbins, 1953; Bremer, 1994). Based on ITS sequences, this genus, along with *Dendroseris*, constitutes a unique clade (B. Baldwin, personal communication). Therefore, clade V includes *Dendroseris* and *Thamnoseris* and will be referred to as the *Dendroseris–Thamnoseris* clade.

4.1.6. The Pacific Island monotypic endemic genera, Kirkianella, Actites, Embergeria, and the genus Sonchus subg. Sonchus, sections Maritimi and Arvenses clade (VI)

This clade includes three Pacific Island endemic genera, *Kirkianella*, *Embergeria*, and *Actites*. *Embergeria*, a tetraploid ($2n = 36$), is endemic to the Chatham Islands of New Zealand. It has been viewed as a survivor of Pleistocene glaciation and was hypothesized to have originated during the late Pliocene period when subg. *Sonchus* migrated to New Zealand (Wardle, 1963; Lander, 1976). Subgenus *Sonchus*, especially section *Asperi*, was considered the most probable ancestor of *Embergeria* (Boulos, 1965a; Pons and Boulos, 1972; Boulos, 1974). However, this study strongly suggests that the species of section *Asperi* are not closely related to *Embergeria* at all. Rather, it appears more likely that sections *Arvenses* or *Maritimi* (excluding *S. palustris*) are responsible for its origin. Another monotypic New Zealand endemic genus, *Kirkianella*, is morphologically variable, and shows mixed ploidy levels: decaploid ($2n = 90$) and decatetraploid ($2n = 126$) (Beuzenberg and Hair, 1984). The ITS and combined phylogenies suggest that it is sister to *Embergeria* (Fig. 1), while relationship is unresolved in the *mat*K tree. The origin of these two New Zealand endemics remains uncertain because of low resolutions in ITS and *mat*K phylogenies. It seems likely, however, that sections *Arvenses* or *Maritimi* (excluding *S. palustris*) are involved in their origins.

Actites is a monotypic endemic to Australia and is a tetraploid. Like *Embergeria*, it was suggested to have evolved from section *Asperi* during the late Pliocene in Australia (Wardle, 1963). This study does not support that hypothesis. Rather, the ITS and combined data suggest that *Sonchus* section *Arvenses*, especially *S. arvensis* and *S. wightianus* from Asia, is involved in the origin of *Actites*. However, the *mat*K phylogeny suggested that phylogenetic relationships of *Actites* relative to other species in this clade is unresolved and that two species of *Sonchus* section *Arvense*, *S. brachyotus* and *S. arvensis*, are more closely related to the clade III including *S. pustulatus*.

Both phylogenies suggest that the two sections of subg. *Sonchus*, *Arvenses* and *Maritimi*, are not monophyletic. One species of section *Maritimi*, *S. palustris*, is not closely related to any other species. Instead, it is closely related to the woody *Sonchus* alliance in the Macaronesian Islands. In addition, *S. aquatilis* is not closely related to *S. maritimus*, which confirms the specific level status of the taxa, usually subordinated as subspecies of *S. maritimus* (Mejías and Valdés, 1988). Both sections are morphologically quite similar, but can be distinguished by somewhat overlapping floret number per capitulum and number of involucre scales, as well as more or less longer pappus in section *Arvenses*. It seems likely that section *Arvenses* evolved from section *Maritimi* based on their geographic distribution and the presence of polyploids in section *Arvenses* (all species of section *Maritimi* are diploids).

4.1.7. Sonchus subg. Sonchus, section Pustulati clade (VII)

This is the first molecular phylogenetic study to include three species of *Sonchus* section *Pustulati*. Boulos recognized four species in this section: *S. pustulatus*, *S. fragilis*, *S. masguindalii*, and *S. briquetianus*. However, we believe that *S. briquetianus* is a variety of *S. tenerrimus* based on morphology (J. Mejías, unpublished data). This section

shows highly restricted disjunct distribution accomplishing ca. 15 known small populations in all: *S. pustulatus* in Spain, Morocco, and Algeria; *S. fragilis* in Morocco; and *S. masguindalii* in Morocco. They are morphologically similar to the highly polymorphic *S. tenerrimus* and are often considered not "true species" because they were not well known. They are small suffrutescent perennials (10–30 cm height) and because of having primitive characteristics within *Sonchus*, they were considered as potential ancestors of the subg. *Dendrosonchus* in the Macaronesian Islands (Boulos, 1973).

This study provides several interesting aspects of the evolutionary history of this section. The ITS phylogeny suggests that section *Pustulati* is monophyletic (100% support) and distantly related to *S. tenerrimus* species (Fig. 1). Within section *Pustulati*, *S. pustulatus* is sister to *S. fragilis* and *S. masguindalii* is in turn sister to the clade of *S. pustulatus*–*S. fragilis*. This clade, along with *S. palustris*, are the closest continental ancestors of the woody *Sonchus* alliance (97% bootstrap support). In contrast, the *mat*K phylogeny suggests that section *Pustulati* is not monophyletic and that *S. pustulatus* is most closely related to the species of the clade III (*Sonchus* sections *Asperi* and *Sonchus*). Nonmonophyly in the *mat*K phylogeny is due to low sequence variation (i.e., lack of synapomorphies between *S. fragilis* and *S. masguindalii*). However, the same cannot be said for the unexpected phylogenetic position of *S. pustulatus*, which has a very divergent chloroplast haplotype from two species in section *Pustulati*. Fifteen changes are needed to force the monophyly of the section. The unexpected position of *S. pustulatus* is perhaps due to introgressive hybridization (see later Section 4.2).

Phylogenetic relationships among three species are congruent with crossing experiments. *Sonchus fragilis* and *S. pustulatus* from Morocco seem to be completely interfertile (J. Mejías, unpublished data), whereas *S. masguindalii* is not cross compatible with other two species. Also, *S. fragilis* is morphologically similar to *S. pustulatus*, but can be distinguished based on vegetative characters. Therefore, crossing ability and morphological similarities agree with phylogenetic relationships.

4.1.8. Sonchus palustris clade (VIII)

Sonchus palustris, an herbaceous perennial with wide distribution in non-Mediterranean Europe, is the only a highly divergent species in section *Maritimi*. In the ITS phylogeny, *S. palustris* is sister to the woody *Sonchus* alliance (clade IX) and clade VII (sect. *Pustulati*) is in turn sister to the clades VIII and IX. This relationship is very strongly supported by bootstrap values (97%), and is the first time that the closest continental ancestor of the woody *Sonchus* alliance in the Macaronesian has strong support. Thus, the ITS phylogeny suggests that the common ancestor of the alliance was somewhat like the continental species, such as species in sect. *Pustulati* or *Sonchus palustris*. In the case of the *mat*K phylogeny, *S. palustris* was placed in the clade IV (subg. *Origosonchus*), but this is tentative

designation because of the low variation in the *mat*K gene sequences (by only 1 bp change). The combined tree (Fig. 3) shows sister relationship between *S. palustris* and section *Pustulati* with very weak support (<50%), indicating uncertain position of this taxon.

4.1.9. The woody Sonchus alliance (clade IX)

The monophyly of the entire alliance was strongly supported by ITS, *mat*K gene, and combined phylogenies (100%, 88%, and 100%, respectively). One striking difference between ITS and cpDNA phylogeny is the position of the alliance: the ITS tree suggests a more derived nature of the alliance relative to other *Sonchus* species, while *mat*K phylogeny suggests its early divergence within the radiated *Sonchus* group. Based on the ITS tree, the alliance derived from a continental ancestor somewhat like, *S. palustris* and the species in sect. *Pustulati*. *Sonchus palustris*, an herbaceous perennial, is widely distributed in non-Mediterranean Europe and it was previously suggested as the closest continental ancestor (Kim et al., 1996b). The species of sect. *Pustulati* are restricted to southwestern Spain, Morocco, and Algeria. They are small herbaceous suffrutescent perennials (up to 30 cm height) and have been suggested to be an ancestor of the subg. *Dendrosonchus* (Boulos, 1973). The ITS tree suggests an herbaceous/suffrutescent habit for the common ancestor of the alliance and a geographic origin in western Europe and/or the Iberian Peninsula and Morocco.

The *mat*K phylogeny placed the woody *Sonchus* alliance clade in basal position within the *Sonchus* group, and herbaceous *Sonchus* species and other Pacific island endemics arose subsequently. Topologically, the woody *Sonchus* alliance shares a most recent common ancestor with the rest of the *Sonchus* group, but this basal position does not necessarily indicate that all herbaceous *Sonchus* species evolved from island woody ancestor, as suggested by Aldridge (1975, 1979). We cannot explain the topological incongruence between ITS and matK tree (but see later Section 4.2). If the *mat*K phylogeny reflects true species relationships, then it may suggest that the common ancestor of the alliance was herbaceous or weakly suffrutescent. It subsequently dispersed to the Canary Islands, mostly likely Gran Canaria (Lee et al., 2005), and underwent radiation on the islands.

We estimate the dispersal of the continental ancestors to the islands, as well as radiation on the islands, based on ITS sequences (on average 0.725% per Myr). It appears that the continental ancestor was dispersed to the islands between 10.8 and 13.5 Myr (7.832% ± 0.004 between *S. palustris* and the alliance and 9.786% ± 0.009 between sect. *Pustulati* and the alliance, respectively). This timing correlates with the origin of Gran Canaria (approximately 13.9 Myr; Carracedo, 1984). The average sequence divergence within the entire alliance is 2.313% ± 0.009 and suggests that initial divergence of the alliance took place around 3.2 Myr (mid Pliocene) and following radiation of the subg. *Dendrosonchus* and *Taeckholmia* took place

about 2.3 Myr ($1.636\% \pm 0.008$). It seems likely that the common cpDNA haplotype on the continent went extinct during the first glaciation in the Northern Hemisphere (2.8 Mya; Flint, 1971) and/or at the beginning of Sahara desertification (2.5 Mya; Williams, 1982). Since there is a significant time gap between the initial dispersal of the putative ancestor to the islands and the radiation of the alliance, as well as phenotypic differentiation among the basal lineage, we cannot rule out the possibility of mass extinction in early stage of evolution. It also suggests that the radiation of the core alliance members is quite recent, mid- to late-Pliocene.

4.2. Hybridization and introgression

We detected several incongruences between the ITS and matK phylogenies. It appears that hybridization and introgression could explain several of the incongruences between them. In the case of *Sonchus pustulatus*, it was embedded within the clade III in matK phylogeny, while it formed a monophyletic group with other species in sect. *Pustulati* in the ITS phylogeny. This raises the possibility that hybridization has taken place between *S. pustulatus* and other species of *Sonchus* in the clade III, most likely *S. tenerrimus* or a closely related sympatric species, and subsequently backcrossed toward *S. pustulatus*. This caused the capture of foreign chloroplast haplotype in *S. pustulatus* and fixation of *S. pustulatus* ITS type.

Another example of incongruence resulting from possible hybridization is *S. arvensis*. *Sonchus arvensis* shares its most recent common ancestor with *S. maritimus* and *S. crassifolius* in the ITS tree, while it is sister to *S. brachyotus* and closely related to the species in clade III in matK phylogeny. *Sonchus maritimus* (a diploid; $2n = 18$) is mainly distributed throughout the Mediterranean region, the Middle East, and Southern Africa. *Sonchus crassifolius* is endemic to the central Iberian Peninsula. *Sonchus arvensis* is a tetraploid (subsp. *uliginosus*; $2n = 36$) and hexaploid (subsp. *arvensis*; $2n = 54$) and two subspecies occur widely in non-Mediterranean Europe and North America. *Sonchus brachyotus* is diploid and occurs in Eastern and Central Asia. Geographic distributions and chromosome number of these species suggest that three species, *S. maritimus*, *S. crassifolius*, and *S. brachyotus*, are possibly involved in the origin of *S. arvensis*. Based on two phylogenies, it is highly likely that *S. brachyotus* is the maternal contributor, while *S. maritimus* and/or *S. crassifolius* is the paternal parent.

In addition to above examples where those species were placed within different clades in ITS and matK phylogenies, one striking topological incongruence is noticeable and this cannot be explained by hybridization and introgression. The matK gene tree (Fig. 2) suggested basal position of the woody *Sonchus* alliance relative to other *Sonchus* and the Pacific Islands endemics. In contrast, it was placed in highly derived position within the Sonchinae based on ITS phylogeny: the derived nature of the alliance

is very strongly supported by several branches with high bootstrap values. However, the sister relationship between the alliance and the rest of Sonchinae, excluding *Reichardia* and *Launaea*, is strongly supported (98%) in the matK phylogeny, while the branch toward the rest of Sonchinae clade is moderately supported (77%). Eighteen substitutions (seven of which are homoplasious) support the sister relationship between the alliance and the rest of clade, while the alliance is supported by only four substitutions (three of which are homoplasious) and the rest of clade has three substitutions (only one of which is homoplasious). Therefore, this incongruence might be due to multiple hits at nucleotide sites or nucleotide bias shared convergently among lineages. In fact, three homoplasious substitutions in the alliance also occurred in African *Sonchus* and *Launaea* species, *S. schweinfurthii* and *L. sarmentosa*.

4.3. Chromosomal evolution within the Sonchinae

The most common chromosome number of the subtribe Sonchinae is $2n = 18$ ($n = 9$) (Roux and Boulos, 1972; Beuzenberg and Hair, 1984; Spooner et al., 1987; Mejías, 1993; Ardévol González et al., 1993; Mejías and Andrés, 2004). Since this number is most common and the basic one for most polyploids in the subtribe, the ancestral chromosome number (base number) is considered 9 ($x = 9$): this is also supported by chromosome number mapping in this study (Fig. 4). Several lines of chromosomal evolution within the Sonchinae have occurred. First, although aneuploidy is not common, several examples can be found in *Sonchus*, *Reichardia*, and *Launaea* (Mejías and Andrés, 2004). *Reichardia* is morphologically the most homogenous genus in the subtribe and the majority of taxa have chromosome number of $n = 8$. Two species, *R. macrophylla* and *R. dichotoma*, have $n = 9$, while the circum-Mediterranean *R. picroides* has $n = 7$ (Gallego, 1980; Siljak-Yakovlev, 1981). We were not able to resolve phylogenetic relationships within the genus well, and *R. macrophylla* and *R. dichotoma* were not included in this study. Therefore, we cannot confirm whether the ancestral basic number of $n = 9$ was subsequently reduced in the evolutionary process in the genus *Reichardia*. It seems, however, likely that the reduction of chromosome number from the ancestral basic number of 9 has taken place. *Launaea* is another basal genus within the subtribe and it has a wide range of chromosome numbers, $n = 5$, 6, 7, 8, and 9 (Kilian, 1997). It was suggested that reduction from an ancestral basic number of $x = 9$ occurred in several sections in *Launaea* (Kilian, 1997). We were not able to include many species of *Launaea*, but both ITS and matK phylogenies seem to suggest reduction of chromosome numbers in the evolutionary process in *Launaea*. Kilian (1997) provided two lines of evidence to support this reduction hypothesis from an ancestral basic number of $x = 9$ and this needs to be further tested based on a robust phylogenetic framework. Within the *Sonchus* group, aneuploidy seems to be restricted to subgenus *Sonchus* section *Sonchus* (clade

III). Our molecular data support a descending aneuploid series involving *S. bourgeaui* ($n = 8$) and *S. tenerrimus* ($n = 7$) from the ancestral basic number of $x = 9$, but they are not in accordance with the proposed origin of *S. microcephalus* ($n = 15$) through a process involving chromosome number reduction in *S. oleraceus* ($n = 16$) (Mejías and Andrés, 2004).

A very different pattern of chromosomal evolution between the Pacific and the Atlantic Ocean can be seen. Endemics from the Atlantic Oceanic Islands are diploids (e.g., the woody *Sonchus* alliance, clade IX). In contrast, many Pacific Islands endemics are polyploids (e.g., *Embergeria*, *Actites*, *Kirkianella*, and *Dendroseris*). The woody *Sonchus* alliance is composed of 6 genera and about 31 species and occurs in the Macaronesian Islands, i.e., the Canary Islands, Madeira, and Cape Verde. The majority of these islands are quite old and the woody *Sonchus* alliance represents one of the spectacular examples of adaptive radiation among angiosperms in Macaronesia (Kim et al., 1996b; Lee et al., 2005). Since all alliance members are diploids, this suggests that adaptive radiation was not accompanied by a polyploidization process on the islands and also that the ancestor of the alliance was a diploid. Pacific Island endemics, on the other hand, are quite different from the ones in the Atlantic. *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). Our study suggested that several diploids (*S. maritimus*, *S. aquatilis*, *S. crassifolius*, *S. wightianus*, and *S. brachyotus*) and one tetraploid (*S. arvensis*) are possibly involved in its origin. Both *Embergeria* and *Kirkianella* are monotypic endemics to New Zealand. *Kirkianella* (a decaploid and decatetraploid) is morphologically very diverse and three forms are recognized (Garnock-Jones, 1988). This genus has been considered closely related to *Sonchus*, *Launaea*, or *Crepis* (Allan, 1961). Our study clearly suggested that *Kirkianella* evolved within the *Sonchus* group, most likely from subg. *Sonchus* sections *Arvenses* and *Maritimi* (excluding *S. palustris*) through a polyploidization process. *Embergeria*, a monotypic endemic to the Chatham Islands of New Zealand, is a perennial and somewhat succulent herb. This genus is tetraploid ($2n = 36$) and had been considered closely related to section *Asperi* (*S. kirkii*; Webb et al., 1988). Like *Kirkianella*, our results show that it is closely related to other *Sonchus* species in the clade VI. Lastly, *Dendroseris*, which includes 11 species, is endemic to the Juan Fernandez Islands, off the coast of Chile and is also tetraploid ($2n = 36$). The ITS data suggest that it is closely related to the clade VI with very weak bootstrap support (52%) and the *mat*K tree (Fig. 2) shows unresolved relationships within the Sonchinae. Both data strongly suggest that genus *Dendroseris* was derived within the *Sonchus* group. All these examples suggest that several Pacific Islands endemics in Sonchinae are indeed derived within the *Sonchus* group, probably from sections *Arvenses* and/or *Maritimi*, and that polyploidization played a fundamental role in origin and evolution

of these genera on islands. We do not know, however, whether the continental progenitors were diploids, hybridized on the continent (becoming tetraploid), and subsequently dispersed and rapidly radiated on the islands.

Several examples (ca. 30%) of polyploids (i.e., tetraploids and hexaploids) within genus *Sonchus* subgenus *Sonchus* are observable, suggesting the important role of polyploidization processes in the evolution of *Sonchus* (two subgenera, *Dendrosonchus* and *Origosonchus*, are all diploids). Some of these species are highly restricted (e.g., *S. kirkii*, New Zealand endemic; *S. macrocarpus*, Egyptian endemic; *S. malaianus*, Indonesia endemic; *S. hydrophilus*, Australia endemic), while others are very widely distributed (e.g., *S. oleraceus*, *S. gigas* and *S. arvensis*). Three species included in this study, i.e., *S. hydrophilus*, *S. kirkii*, and *S. arvensis*, were all in derived positions within the clades (III and VI), showing the polyploidization process. Another large genus in the Sonchinae, *Launaea*, on the other hand, has about 16% of species (5 out of 30 counted species; Kilian, 1997) that are polyploids, while about 40% of species (13 out of 30 counted species) demonstrate descending aneuploidy. This suggests that in genus *Launaea* descending aneuploidy processes played a much more important role than polyploidization.

4.4. Evolution of morphological traits

Several morphological traits such as habit, achenes, and pappus, have been used to delimit genera in the subtribe Sonchinae as well as in infrageneric classification of genus *Sonchus*. We investigated the evolutionary trends of some important traits based on our phylogenetic framework. First, we mapped habit on one of the most parsimonious trees based on combined data set (Fig. 5). Due to very limited sampling, we were not able to confidently identify trends in two basal genera, especially in the very diverse genus *Launaea*. Kilian (1997, p. 26) pointed out that both annual and frutescent growth form in *Launaea* have evolved from more or less herbaceous ancestors. However, it seems very clear that the herbaceous habit is plesiomorphic in *Sonchus* and the genera sharing a common ancestor with *Sonchus*, with eight independent origins of the suffrutescent and shrub/tree habit (Fig. 5). Whether the herbaceous habit is plesiomorphic or apomorhic has been quite controversial, but the trends in Sonchinae agreed with other Macaronesian woody endemics (Mes, 1995; Böhle et al., 1996). Both the woody *Sonchus* alliance in Macaronesia Islands and *Dendroseris* in the Juan Fernandez Islands independently evolved shrub and tree like habits from herbaceous ancestors, supporting Carlquist's hypothesis (1974). These examples illustrate the derived nature of woody species in otherwise predominantly herbaceous taxa in adaptation to certain environments, e.g., insular habitats. Not all island endemics evolved suffrutescent or shrub habit. For example, *Actites* and *Embergeria*, are succulent and herbaceous perennials endemic to Australia and New Zealand, respectively. *Kirkianella*, a New Zealand endemic,

is also an herbaceous perennial. The habit mapping also strongly suggests that suffrutescent/caudex perennial habit has independently evolved at least four times with one reversal from shrub habit. Most species with a suffrutescent/caudex habit are confined to arid environments, especially section *Pustulati* and *S. saudensis* (Fig. 5). *Actites* occurs in coastal sand dunes, representing a special adaptation to arid and saline environments.

The same trend can be seen in genus *Launaea*, which includes species with a variety of habits, ranging from perennial and annual herbs to scoparious or spinescent subshrubs, cushion-forming rosette shrubs and spinescent shrubs. Kilian (1997) claimed that woody form evolved independently and is correlated to similar environments in different species groups of *Launaea* as well as in different genera of the Lactuceae. Therefore, this study provides strong evidence that the woody growth form has evolved independently from herbaceous ancestors in different lineages of Sonchinae and this appears to be the rule rather than the exception in Lactuceae.

The function of pappus in Compositae is generally considered to relate to fruit dispersal (Zohary, 1950; Shmida, 1985). The deciduous pappus can hinder long distance dispersal and variation in pappus persistence results in variation in potential dispersal distances. Progressive loss of dispersal ability on oceanic islands is noticeable in many members of the sunflower family (Carlquist, 1965). It seems that a deciduous pappus is plesiomorphic within the subtribe Sonchinae and that a persistent pappus evolved at least four times (Fig. 5). No significant associations were found between pappus deciduousness/dimorphisms and growth- or life-form. Also, pappus deciduousness appears not to be correlated to pappus dimorphism (but see Shmida, 1985). For example, subgenus *Dendrosonchus* species in the Macaronesian Islands have dimorphic pappus: deciduous bristles and persistent or slightly deciduous downy hairs. Several species occur widely on single or multiple islands and soft persistent downy hairs are likely responsible for dispersal of these species within and between archipelagoes. In contrast, the Juan Fernandez tree lettuces, genus *Dendroseris*, have monomorphic and early deciduous pappus and most species are exceedingly rare. This genus clearly illustrates the progressive loss of dispersal ability and fruit gigantism upon arrival on islands. Specifically, *D. litoralis* has variously shaped and widened fruits, topped with only vestigial threads. The fruits of *D. litoralis* are now suited to little more than dropping to the ground beneath the tree that bears them (Carlquist, 1965). Two other Pacific island endemics, *Actites* and *Embergeria*, have a dimorphic and persistent pappus, maintaining the dispersal ability of fruits. The Chatham Islands endemic *Embergeria*, giant sowthistle, is a large (up to 1.5 m tall), succulent perennial and occurs sporadically on coastal dunes, in coastal forest and scrub on sandy soils. The fruits are generally larger than those in other *Sonchus* species, but since they possess a persistent pappus and

broadly winged and flattened they can easily be dispersed by wind. *Actites* has a dimorphic pappus with soft, persistent, downy hairs that are probably responsible for dispersal of fruits.

The subgenus *Sonchus* species have typical dimorphic pappus, but the nature of pappus is exceedingly variable. Three independent reversals to a deciduous pappus, after evolving a persistent one, are noticeable (i.e., high evolutionary flexibility; Shmida, 1985): *S. asper*, *S. kirkii*, *S. hydrophilus*, and *S. aquatilis* (Fig. 5). All three, *S. kirkii*, *S. hydrophilus*, and *S. aquatilis*, are freshwater or littoral species and have a deciduous pappus. Therefore, it is plausible that hydrochory can permit long distance dispersal of these taxa. Unlike several invasive and cosmopolitan weedy species with dimorphic and persistent pappus, e.g., *S. oleraceus*, *S. arvensis*, and *S. tenerrimus*, *S. asper* has an unusual dimorphic pappus, which can easily be removed. We believe that wind dispersal mediated by the pappus is common in this taxon, which is consistent with its invasive weedy behavior. In general, widely distributed species tend to have a dimorphic and persistent pappus, but the exceedingly variable nature of pappus persistence combined with pappus dimorphism within the Sonchinae suggest that these traits have evolutionary flexibility and various fruit dispersal mechanisms have been evolved over ecological time.

4.5. The genus concept of Sonchus

Generic delimitation of *Sonchus* has been controversial, especially with regard to the island endemics. In many instances, the morphology of the endemics differs dramatically from their closest continental relatives (ancestors-sister groups). In fact, it is only with the use of molecular data, that the closest relatives of the island groups have been elucidated (see examples in Baldwin et al., 1998). Second, the restriction of the endemics to small, distant islands has been used as further evidence for their interpretation as distinct, very isolated lineages. Molecular phylogenetic studies on different Asteraceae from a variety of archipelagos have shown island groups nested within large continental genera and the situation for Sonchinae appears to be the rule rather than the exception, which has resulted in several cases of synonym complexity and nomenclatural instability. For example, the genera *Embergeria* and *Actites* from the Pacific were once considered as *Sonchus*, *S. grandifolius* and *S. asper* var. *megalocarpa* (Kim et al., 2004). *Sonchus asper* var. *megalocarpa* was afforded specific status by Black (1929) as *S. megalocarpus* (Hook. f.) J.M. Black. Boulos (1965a) later transferred it into the genus *Embergeria*, as *E. megalocarpa* (Hook. f.) Boulos. Most recently, Lander (1976) erected the monotypic genus *Actites* to accommodate *A. megalocarpa*, separating his new genus from *Sonchus* and *Embergeria*. *Embergeria grandifolia* (Kirk) Boulos was segregated from *Sonchus* in 1965. In the Canary Islands, two genera, *Babcockia* and *Taeckholmia*, were first described by Boulos (1965b, 1967), respectively, but were later merged into *Sonchus*

(Aldridge, 1975, 1976). These two genera were originally described as *Sonchus*, elevated as distinct genera, and then merged again based on the absence of phenotypic gaps and unique characteristics between *Sonchus* and two genera. All these genera share very similar morphological characteristics with *Sonchus* and differ only by a few characters. The validity of *Actites* as a new genus was challenged (Cooke, 1986; Sennikov and Illarionova, 2001) and *Embergeria* was not treated as a distinct genus from *Sonchus* in the recent treatment of New Zealand flora (Garnock-Jones, 1988). It was suggested that *E. grandifolia* may be closely related to *S. kirkii*, in which case it should be included in *Sonchus*.

There are two other examples in which other genera were placed in *Sonchus* and subsequently raised to generic rank in the Macaronesian Islands. First, *Chrysoprenanthes pendula*, originally placed in *Prenanthes*, was re-placed in *Sonchus* based on morphology and anatomy of its fruits (Sennikov and Illarionova, 1999). Later, Bramwell (2003) did not find their claims convincing and created a new monotypic genus, *Chrysoprenanthes*. This genus is morphologically somewhat different from subg. *Dendrosonchus* species, especially in having only few (5 or 6) florets per capitulum. The genus *Lactucosonchus* was originally treated as *Sonchus* subgenus *Lactucosonchus* (Sventenius, 1968). Like *Sonchus tuberifer*, it is an herbaceous perennial with tuberous root. It is endemic to La Palma, one of youngest islands in the Canary Archipelago. The present study strongly suggests that they represent basal lineages within the Macaronesian *Sonchus* Clade IX (Figs. 1 and 2), indicating an origin within the *Sonchus* group. It is also true that the other monotypic genus in the Macaronesian *Sonchus* clade, *Sventenia*, is clearly derived from the *Sonchus* group. Based on morphological and anatomical structure of the achenes, Sennikov and Illarionova (1999, 2001) suggested merging several segregates (i.e., *Actites*, *Babcockia*, *Embergeria*, *Kirkianella*, *Lactucosonchus*, *Sventenia*, and *Taeckholmia*) into a single genus *Sonchus*. Our study strongly demonstrates that the genus *Sonchus* as currently treated does not include all the descendents of the common ancestor. Further, it suggests that *Dendroseris*, *Thamnoseris*, and *Aetheorhiza* represent integral parts of the main radiation of the *Sonchus* group.

The main results of this study suggest that several clades of *Sonchus* (i.e., III, IV, VI, VII, VIII and part of IX in Fig. 1) should be recognized as distinct genera, or that all segregate and closely related genera, including *Dendroseris* and *Thamnoseris*, should be amalgamated as one genus *Sonchus*. Traditionally, several characters, primarily habit, geographic distribution, and achene/pappus morphology, have been used to distinguish genera in Sonchinae. These characters tend to be continuous across several genera and show typical convergent features, especially habit and pappus morphology among insular endemics. Also, despite taxonomic importance, some floral and fruit characters show a higher degree of evolutionary lability, which

may in part be driven by their simple genetic basis, as in other groups of Asteraceae (e.g., Smith and Parker, 1971; Smith, 1974, 1976; Jansen et al., 1987; Vlot et al., 1992; Battjes et al., 1994; Van Houten et al., 1994; Ganders et al., 2000; Kimball and Crawford, 2004). Habit and life cycle have also been thought to be unstable characters and liable to reversions (Funk, 1982; Barret et al., 1996). Therefore, the potential evolutionary lability of floral and fruit characters and highly polyphyletic nature of the genus *Sonchus* lead us to support the option of one large genus *Sonchus*, and eight identified clades of subgeneric rank. Although paraphyly is not precluded under the ICBN (Greuter et al., 2000), monophyly has been sometimes claimed to promote nomenclatural stability (Pfeil and Crispy, 2005). The number of substitutions leading toward those segregate and closely related genera also does not warrant their generic recognition since they are within the range of the *Sonchus* group substitution variation. In the ITS tree, on average nine substitutions occurred in segregate/closely related genera, while on average, 12 substitutions were found in the major lineages of *Sonchus* group. Also, in the cpDNA tree, we detected far more substitutions within the *Sonchus* group compared to the ones in segregate/closely related genera. A revised classification of the subtribe Sonchinae is necessary and it will be presented separately.

Appendix A

Appendix. Taxa, voucher information, and GenBank accession numbers for samples examined in the study. Accession numbers with asterisk were reported in various

previous studies (Kim et al., 1996b, 2004; Lee et al., 2005). -; no voucher specimen or DNA sequences. SGA; Santos-Guerra, Arnoldo. KSC; Kim, Seung-Chul; KN, Kilian, Norbert. SEV; University of Sevilla Herbarium, Spain. VAL; University of Valencia Herbarium, Spain. MO; Missouri Botanical Garden Herbarium. RNG; University of Reading Herbarium. OS; Ohio State University Herbarium. E; Royal Botanical Garden Edinburgh Herbarium. TEX; University of Texas Herbarium. CANB; Australian National Herbarium. We followed the classification of Boulos (1972) for genus *Sonchus*.

Taxon; Voucher; Accession Nos.: ITS 1, ITS 2 (only one accession number includes both ITS 1, 5.8S, and ITS 2 sequences); *mat*K.

Outgroup taxa:

Helminthotheca Vaill. Ex Zinn - *H. echioides* (L.) Holub; Gutermann 23831; AF528491*; -. *Hyoseris* L. - *H. scabra* L.; -; Z93831*, Z93847*; -. *H. radiata* L.; Gutermann 23604; AF528494*; -. *Hypochaeris* L. - *H. uniflora* Hoffm.; -; Z93829*, Z93845*; -. *H. maculata* L.; -; Z93822*, Z93838*; -. *Lactuca* L. -; *L. perennis* L.; -; L48143*, L48144*; -; *L. sativa* L.; -; L13957*; -. *Leontodon* L. - *L. autumnalis* L.; Stuessy 15541; AF528486*; -. *Picris* L. - *P. hieracioides* L.; Stuessy 15409*; AF528490*; -. *Prenanthes* L. - *P. purpurea* L.; -; L48151*, L48152*; -. *P. altissima* L.; -; L48149*, L48150*; -. *Rhagadiolus* Scop. - *R. edulis* Gaertn.; Gutermann 31695; AF528495*; -. *Taraxacum* Weber - *T. officinale* Weber; -; L48337*, L48338*; -.

Ingroup taxa:

Actites Cass. - *A. megalocarpa* (Hook. f.) Lander-1; Lepschi 3879-1 (Australia) (CANB); AY458011*; -. *A. megalocarpa* (Hook. f.) Lander-2; Heyligers 98005-1 (Australia) (CANB); AY458012*; -. *A. megalocarpa* (Hook. f.) Lander-3; Lepschi 4657-1 (Australia) (CANB); AY458013*; -.

Aetheorhiza Cass. - *A. bulbosa* (L.) Cass.-1; Jansen 1105 (TEX); L48135*, L48136*; -. *A. bulbosa* (L.) Cass.-2; OPTIMA ITER VI107 (Spain) (RNG); DQ507888, DQ507939; -. *A. bulbosa* (L.) Cass.-3; Pagher 1021 (Lebanon) (RNG); DQ507889, DQ507940; -.

Babcockia Boulos - *B. platylepis* (Webb) Boulos-1; SGA & KSC 111; DQ072470-1*; DQ022965*. *B. platylepis* (Webb) Boulos-2; SGA & KSC 112; DQ072472-3*; DQ022966*.

Chrysoprenanthes (Sch. Bip.) Bramwell - *C. pendula* (Sch. Bip.) Bramwell-1; SGA & KSC 116; L48155*, L48156*; DQ022995*. *C. pendula* (Sch. Bip.) Bramwell-2; KSC 1051 (OS); L48157*, L48158*; DQ022993*. *C. pendula* (Sch. Bip.) Bramwell-3; SGA & KSC 117; -; DQ022996.

Dendroseris D. Don - *D. berteroana* (Dcne) Hool. & Arn.-1; Stuessy et al. 11589 (OS); L49522*, L49523*; -. *D. berteroana* (Dcne) Hool. & Arn.-2; -; -; DQ508015. *D. litoralis* Skottsb.-1; Stuessy et al., 11973 (OS); L49508*, L49509*; -. *D. litoralis* Skottsb.-2; -; -; DQ508014. *D. marginata* (Bert. & Dcne.) Hook. & Arn.; Stuessy et al. 11999 (OS); L49510*, L49511*; -. *D. macrantha* (Bert. & Dcne.) Skottsb.; Stuessy et al. 5149 (OS); L49512*, L49513*; -.

D. micrantha Hook. & Arn.-1; Stuessy et al. 11582(OS); L49514*, L49515*; -. *D. micrantha* Hook. & Arn.-2; -; -; DQ508017. *D. micrantha* Hook. & Arn.-3; -; -; DQ508018. *D. neriifolia* Hook. & . Arn.; Stuessy et al. 11534 (OS); L48518*, L48519*; -. *D. pinnata* (Bert. & Dcne.) Hook. & Arn.; Stuessy et al. 11334 (OS); L49520*, L49521*; -. *D. pruinata* (Johow) Skottsb.-1; Stuessy et al. 11349 (OS); L49516*, L49517*; -. *D. pruinata* (Johow) Skottsb.-2; -; -; DQ508016. *D. regia* Skottsb.; Landero & Ruiz 9361 (OS); L49524*, L49525*; -.

Embergeria Boulos - *E. grandifolia* (T. Kirk) Boulos-1; Atkinson 118/85 (New Zealand) (OS); L48139*, L48140*; -. *E. grandifolia* (T. Kirk) Boulos-2; J. Santos ex cult. (New Zealand); DQ507912, DQ507962; DQ508011.

Kirkianella Allan - *K. novae-zelandiae* (Hook. f.) Allan-1; D. Glenny 4910 (New Zealand); L48141*, L48142*; - *K. novae-zelandiae* (Hook. f.) Allan-2; D. Glenny 5036 (New Zealand); DQ507911, DQ507961; DQ508012. *K. novae-zelandiae* (Hook. f.) Allan-3; J. Marris s.n. (New Zealand); -; DQ508013.

Lactucosonchus (Sch. Bip.) Svent. - *L. webbii* (Sch. Bip.) Svent.-1; KSC et al. 1033; -; DQ022986*. *L. webbii* (Sch. Bip.) Svent.-2; SGA & KSC 100; DQ072460-1*; DQ022987*. *L. webbii* (Sch. Bip.) Svent.-3; -; L48161*, L48162*; DQ022988*. *L. webbii* (Sch. Bip.) Svent.; -; DQ507928, DQ507978; -.

Launaea Cass. - *L. arborescens* (Batt.) Murb.; Kim S.-C. et al. 1040 (OS); L48145*, L48146*; -. *L. cornuta* (Hochst. ex Oliv. & Hiern) C. Jeffrey-1; Gereau et al. 6169 (Tanzania) (MO); DQ507885, DQ507936; DQ507981. *L. cornuta* (Hochst. ex Oliv. & Hiern) C. Jeffrey-2; Massawe 17 (Tanzania) (MO); DQ507886, DQ507937; DQ507980. *L. nudicaulis* (L.) Hook. f.; Kim S.-C. et al. 1053 (OS); L48147*, L48148*; -. *L. rarifolia* (Oliv. & Hiern) Boulos; Lee White 0857 (Gabon) (MO); DQ507887, DQ507938; DQ507982. *L. sarmentosa* (Willd.) Kuntze; McPherson et al. 14857(Madagascar) (MO); DQ507884, DQ507935; DQ507983.

Reichardia Roth - *R. crystallina* (Schultz Bip.) Bramwell; -; DQ507883, DQ507934; -. *R. gaditana* (Willk.) Coutinho; -(Portugal); -; -. *R. intermedia* (Schultz Bip.) Coutinho; Jury 15830 (Morocco) (RNG); -; DQ207879, DQ5507930; -. *R. ligulata* (Vent.) Kunkel & Sunding-1; -; DQ507882, DQ507933; -. *R. ligulata* (Vent.) Kunkel & Sunding-2; Kim S.-C. et al. 1044 (OS); L48165*, L48166*; -. *R. picroides* (L.) Roth-1; Belgium Bot. Gard. 2871; L48153*, L48154*; -. *R. picroides* (L.) Roth-2; OPTIMA ITER VIII1175 (Italy) (RNG); -; DQ207878, DQ507929; -. *R. tingitana* (L.) Roth-1; KEW 223-70-02 090; DQ507880, DQ507931; DQ507979. *R. tingitana* (L.) Roth-2; Jongkind & Nieuwenhuis 806 (Spain) (MO); -; -. *R. tingitana* (L.) Roth-3; Jury 19511(Morocco) (RNG); DQ507881, DQ507932; -.

Sonchus L.-Subgenus *Dendrosonchus* Sch. Bip. ex Boulos - *S. brachylobus* Webb & Berth.; SGA & KSC 228; -; DQ022977*. *S. canariensis* (Sch. Bip.) Boulos; SGA & KSC 149; L48291*, L48292*; -. *S. congestus* Willd.-1;

KSC et al. 1000; L48173*, L48174*; DQ023030*. *S. congestus* Willd.-2; SGA & KSC 154; L48175*, L48176*; -. *S. daltonii* Webb; -; -; DQ023015*. *S. fruticosus* L. fil; -; L48125*, L48126*; DQ023001*. *S. gonzalezpadronii* Svent.; SGA & KSC 166; DQ072492-3*; DQ023031*. *S. hierrensis* (Pitard) Boulos; SGA & KSC 133; -; DQ023018*. *S. ortunoi* Svent.; -; L48129*, L48130*; DQ023032*. *S. palmensis* (Sch. Bip.) Boulos; SGA & KSC 145; -; DQ023026*. *S. pinnatifidus* Cav.; SGA & KSC 125; -; DQ022971*. *S. pinnatus* Ait.; KSC 1996; -; DQ022998*. *S. wildpretii* U. & A. Reifenberger; SGA & KSC 180; -; DQ022978*.

Subgenus *Origosonchus* Boulos - *S. afromontanus* R. E. Fries; Gereau 1739 (Tanzania) (MO); DQ507915, DQ507965; DQ507985. *S. bipontini* Aschers.; LaCroix 4681 (Malawi) (MO); DQ507918, DQ507968; -. *S. luxurians* (R. E. Fries) C. Jeffrey-1; Knox 2559 (Kenya); L48297*, L48298*; -. *S. luxurians* (R. E. Fries) C. Jeffrey-2; Gereau 1732 (Tanzania) (MO); DQ507916, DQ507966; -. *S. luxurians* (R. E. Fries) C. Jeffrey-3; Knox 2561 (Kenya); -; DQ507986. *S. schweinfurthii* Oliv. et Hiern-1; Knox 2560 (Kenya); L48295*, L48296*; -. *S. schweinfurthii* Oliv. et Hiern-2; Phillips 2319 (Malawi) (MO); DQ507917, DQ507967; DQ507984. *S. saudensis* Boulos-1; ASG s.n. (Saudi Arabia); DQ507913, DQ507963; DQ507987. *S. saudensis* Boulos-2; KN s.n. (Yemen); DQ507914, DQ507964; -.

Subgenus *Sonchus* - Section *Arvenses* (Kirp.) Boulos - *S. arvensis* L.-1; -(Taiwan); DQ507906, DQ507957; DQ508001. *S. arvensis* L.-2; King & Garvey 11600 (USA) (MO); DQ507907, DQ507958; DQ508002. *S. arvensis* L.-3; Jansen 1103 (TEX); L48307*, L48308*; -. *S. brachyotus* DC.-1; -(Korea); DQ507909, DQ507959; DQ508005. *S. brachyotus* DC.-2; -(Korea); DQ507910, DQ507960; DQ508006. *S. wightianus* DC.; Sinclair & Long 5599 (Bhutan) (E); DQ507908, -; -.

Section *Asperi* Boulos - *S. asper* (L.) Hill-1; -(Korea); DQ507890, DQ507941; DQ508004. *S. asper* (L.) Hill-2; VAL982104 (Spain) (VAL); DQ507891, DQ507942; DQ508003. *S. asper* (L.) Hill-3; Keighery 14312 (Australia) (CANB); AY458003*; -. *S. asper* (L.) Hill-4; Adams 4153 (Australia) (CANB); AY458004*; -. *S. asper* (L.) Hill-5; Jansen 1109 (TEX); L48301*, L48302*; DQ022963. *S. asper* subsp. *glaucescens* (Gordan) Ball; Lepschi 4663-2 (Australia) (CANB); AY458005*; -. *S. hydrophilus* Boulos-1; Lepschi 3765-1 (Australia) (CANB); AY458006*; -. *S. hydrophilus* Boulos-2; Lepschi & Lally 2349 (Australia) (CANB); AY458008*; -. *S. hydrophilus* Boulos-3; Paget 2783 (Australia) (CANB); AY458009*; -. *S. kirkii* (T. Kirk) Allan-1; Silbury s.n. (New Zealand); L48299*, L48300*; -. *S. kirkii* (T. Kirk) Allan-2; - (New Zealand); AF422137*; -. *S. mauritanicus* Boiss. & Reut.-1; Davis & Lamond D57029 (Tunisia) (E); DQ507895, DQ507946; -. *S. mauritanicus* Boiss. & Reut.-2; Davis & Lamond D57016 (Tunisia) (E); DQ507894, DQ507945; -.

Section *Maritimi* (Kirp.) Boulos - *S. aquatilis* Pourr.-1; SEV121001 (Spain) (SEV); DQ507900, DQ507951; DQ507996. *S. aquatilis* Pourr.-2; SEV215735 (Spain) (SEV); DQ507901, DQ507952; -. *S. aquatilis* Pourr.-3; Jury14558 (Morocco) (RNG); DQ507902, DQ507953; DQ507997. *S. crassifolius* Pourr.-1; VAL963927 (Spain) (VAL); DQ507903, DQ507954; DQ508008. *S. crassifolius* Pourr.-2; VAL118248 (Spain) (VAL); DQ507904, DQ507955; DQ508009. *S. crassifolius* Pourr.-3; SEV121006 (Spain) (SEV); DQ507905, DQ507956; DQ508010. *S. maritimus* L.-1; L. Vilar s.n. (OS); L48309*, L48310*; -. *S. maritimus* L.-2; VAL143421 (Spain) (VAL); DQ507899, DQ507950; DQ507995. *S. palustris* L.-1; Kim S.-C. 1050 (OS); L48311*, L48312*; -. *S. palustris* L.-2; Rechinger 49632 (Azerbaijan) (E); DQ507919, DQ507969; -.

Section *Pustulati* Boulos - *S. fragilis* Ball-1; SEV215728 (Morocco) (SEV); DQ507926, DQ507976; DQ507991. *S. fragilis* Ball-2; SEV215729 (Morocco) (SEV); DQ507927, DQ507977; DQ507992. *S. masguindalii* Pau & Font Quer -1; ASG s.n. (Morocco); DQ507923, DQ507973; DQ507998. *S. masguindalii* Pau & Font Quer-2; SEV215732 (Morocco) (SEV); DQ507924, DQ507974; DQ507999. *S. masguindalii* Pau & Font Quer-3; SEV215733 (Morocco) (SEV); DQ507925, DQ507975; DQ508000. *S. pustulatus* Willk.-1; SEV215730 (Morocco) (SEV); DQ507920, DQ507970; DQ507988. *S. pustulatus* Willk.-2; SEV215731 (Morocco) (SEV); DQ507921, DQ507971; DQ507989. *S. pustulatus* Willk.-3; Ait Laflcih et al. P0003353 (Morocco) (RNG); DQ507922, DQ507972; DQ507990.

Section *Sonchus* - *S. bourgeaui* Sch. Bip.; Kim S.-C. 1035 (OS); L48305*, L48306*; -. *S. microcephalus* Mejías; SEV126508 (Spain) (SEV); DQ507898, DQ507949; DQ508007. *S. olearceus* L.-1; -(Korea); DQ507892, DQ507943; -. *S. olearceus* L.-2; Mlangwa 275 (Tanzania) (MO); DQ507893, DQ507944; -. *S. olearceus* L.-3; I. Regk s.n. (OS); L48303*, L48304*; -. *S. olearceus* L.-4; Adams 4154 (Australia) (CANB); AY457987*; -. *S. olearceus* L.-5; Gibson and Brown 3853 (Australia) (CANB); AY458002*; -. *S. tenerrimus* L.-1; -(Italy); DQ507896, DQ507947; DQ507993. *S. tenerrimus* L.-2; VAL38120 (Spain) (VAL); DQ507897, DQ507948; DQ507994. *S. tenerrimus* L.-3; Lepschi 3070 (Australia) (CANB); AY458000*; -.

Section *Tuberiferi* Boulos - *S. tuberifer* Svent.-1; KSC et al. 1045 (OS); L48313*, L48314*; DQ022967*. *S. tuberifer* Svent.-2; SGA & KSC 120; DQ072468-9*; -.

***Sventenia* Font Quer** - *S. bupleuroides* Font Quer-1; KSC et al. 1041; L48315*, L48316*; DQ022989*. *S. bupleuroides* Font Quer-2; KSC 1067; DQ072462-3*; DQ022990*.

***Taeckholmia* Boulos** - *T. arborea* (DC.) Boulos; SGA & KSC 225; L43825*, L48326*; -. *T. canariensis* Boulos; KSC et al. 1043; L48323*, L48324*; -. *T. capillaris* (Svent.) Boulos; SGA & KSC 209; -; DQ023013*. *T. heterophylla* Boulos; KSC et al. 1037; L48333*, L48334*; -. *T. microcarpa* Boulos; SGA & KSC 205; -; DQ023012*. *T. pinnata* (L. f.) Boulos-1; SGA & KSC 201; L48319*, L48320*; DQ023010*. *T. pinnata* (L. f.) Boulos-2; SGA & KSC 202; L48317*, L48318*; -.

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