



**Radiation of the Endemic Genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands: Evidence from Sequences of the ITS Regions of Nuclear Ribosomal DNA**

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## RADIATION OF THE ENDEMIC GENUS *DENDROSERIS* (ASTERACEAE) ON THE JUAN FERNANDEZ ISLANDS: EVIDENCE FROM SEQUENCES OF THE ITS REGIONS OF NUCLEAR RIBOSOMAL DNA<sup>1</sup>

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Phylogenetic relationships among nine of the 11 species of the endemic genus *Dendroseris* on the Juan Fernandez Islands were inferred from nucleotide sequences of the internal transcribed spacer regions (ITS) of the 18-26S nuclear ribosomal DNA. Sequences were determined for 15 populations of *Dendroseris* and one population for each of two outgroups from the genera *Sonchus* and *Sventenia*. Little length variation was detected in the ITS regions of *Dendroseris*, with ITS 1 253 or 254 bp long and ITS 2 224 or 225 bp. The sequence data provide strong support for the holophyly of *Dendroseris* despite the distinct morphological differences among the three subgenera. The molecular data also indicate that subg. *Dendroseris* and *Phoenicoseris* are holophyletic, but do not support holophyly of subg. *Rea*. The ITS sequences did not resolve relationships among subgenera, supporting the hypothesis of rapid adaptive radiation of *Dendroseris* on the islands. Relative rate tests indicate that rates of nucleotide substitutions in the ITS regions are not significantly different among the different lineages of *Dendroseris* following adaptive radiation. Comparisons of average pairwise sequence divergence of *Dendroseris* species in the ITS regions and chloroplast genome indicated that ITS sequences have evolved about 38 times faster than cpDNA in the genus. Rates of ITS sequence divergence of *Dendroseris* were estimated to be faster than  $(3.94 \pm 0.10) \times 10^{-9}$  per site per year, and likely  $(6.06 \pm 0.15) \times 10^{-9}$  per site per year.

The Juan Fernandez Islands lie 580 km west of mainland Chile at 33° S latitude. The archipelago consists of two major islands—Masatierra and Masafuera—which are approximately 4 million and 1–2 million years old, respectively (Stuessy et al., 1984). The islands, with an unusually high level of endemic flora (60% at the specific level), offer an excellent model system for studying plant phylogeny (Stuessy, Crawford, and Marticorena, 1990). Further, these islands with known ages and rich endemism should serve as a simplified system for studying molecular evolution.

The endemic genus *Dendroseris* D. Don. (Asteraceae: Lactuceae) is the most speciose genus on the Juan Fernandez Islands and consists of 11 species in three subgenera: subg. *Dendroseris* Skottsb. (*D. litoralis* Skottsb., *D. macrantha* [Bertero & Dcne.] Skottsb., *D. macrophylla* D. Don, and *D. marginata* [Bertero & Dcne.] Hook. & Arn.); subg. *Phoenicoseris* Skottsb. (*D. berteroaana* [Dcne.] Hook. & Arn., *D. pinnata* [Bertero ex Dcne.] Hook. & Arn., and *D. regia* Skottsb.); and subg. *Rea* (Bertero ex Dcne.) Skottsb. (*D. gigantea* Johow, *D. micrantha* [Bertero ex Dcne.] Hook. & Arn., *D. neriifolia* [Dcne.] Hook. & Arn., and *D. pruinata* [Johow] Skottsb.). The three subgenera are quite distinct morphologically, ranging from palmiform and rosette trees to rosette shrubs and sparsely branched trees, which represent the typical habits often encountered in plants on oceanic islands (Carlquist, 1974). Eight of the species are endemic to the older island of Masatierra, and one species in each subgenus (*D. macro-*

*phylla*, *D. regia*, and *D. gigantea*) occurs on the younger island of Masafuera.

The species of *Dendroseris* are exceedingly rare, and all chromosome counts indicate that they are tetraploid with  $n = 18$  (Sanders, Stuessy, and Rodriguez, 1983; Spooner et al., 1987). Previous studies of *Dendroseris* have included anatomy (Carlquist, 1967), morphology (Sanders et al., 1987), allozymes (Crawford, Stuessy, and Silva O., 1987; Brauner, Crawford, and Stuessy, unpublished data), and restriction site mutations of cpDNA and nrDNA (Crawford et al., 1992). The phylogenies based on morphology and restriction site mutations both indicated that subg. *Dendroseris* and subg. *Phoenicoseris* are holophyletic, but that subg. *Rea* is paraphyletic (Sanders et al., 1987; Crawford et al., 1992). The phylogeny produced by morphology was rooted between subg. *Phoenicoseris* and the other two subgenera. However, relationships among the subgenera were not resolved by restriction site data, and an unresolved tetrachotomy formed at the base of the cladogram, consisting of subg. *Dendroseris*, subg. *Phoenicoseris*, *D. micrantha* and *D. pruinata* of subg. *Rea*, and *D. neriifolia* of subg. *Rea* (Fig. 2). The lack of resolution at the subgeneric level was attributed to the rapid radiation of *Dendroseris* relative to the slow mutation rate in the chloroplast genome. Another question needing to be addressed is the holophyly of the genus. Since the three subgenera are morphologically so distinct and only one cpDNA restriction site mutation has been found that is shared by all the *Dendroseris* species (Fig. 2, R. K. Jansen, University of Texas, Austin, TX, personal communication), further data are needed to support holophyly of the genus.

Sequences of the internal transcribed spacers of nrDNA (ITS) have recently been shown to be useful for resolving phylogenetic relationships within genera and among closely

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TABLE 1. Populations of *Dendroseris* and outgroups used as sources of DNA.

Subgenus and species	Collection number <sup>a</sup>	Locality <sup>b</sup>
Subg. <i>Dendroseris</i>		
<i>D. littoralis</i>	6305A	Cultivated in CONAF garden, San Juan Bautista
	6435	Cultivated in yard on La Polvora Street, San Juan Bautista
	11,973	Puerto Francés, Quebrada La Piña, in tree fern forest
<i>D. macrantha</i>	5149	Cultivated in yard in San Juan Bautista
<i>D. marginata</i>	11,999	Puerto Francés, on steep sea cliffs
Subg. <i>Rea</i>		
<i>D. micrantha</i>	11,399	Cerro Alto, on S side toward Valle Inglesa
	11,582	Puerto Francés, down ridge from La Pascua into Valle Francés
<i>D. neriifolia</i>	11,534	Quebrada Lapiz, in ravine
<i>D. pruinata</i>	11,349	Morro Spartán, E side of Santa Clara
	12,085	El Camote, near top of ridge
Subg. <i>Phoenicoseris</i>		
<i>D. berteriana</i>	11,589	Puerto Francés, Quebrada La Piña
	11,958	Puerto Francés, across from Quebrada La Piña
<i>D. pinnata</i>	11,334	Top of ridge overlooking Corrales de Molina
	11,741	Cordón Salsipuedes
<i>D. regia</i>	Landero & Ruiz, 9316	Quebrada Guatón, west side of Masafuera
<i>Sonchus fruticosus</i>		Grown from seeds collected from the Jardín Botánico, Madeira
<i>Sventenia bupleuroides</i>		DNA provided by R. K. Jansen

<sup>a</sup> All collection numbers are those of Stuessy et al., 1984 except as given for *D. regia*.

<sup>b</sup> All collections of *Dendroseris* were made on Masatierra except *D. regia* from Masafuera, and one collection (11,349) of *D. pruinata* from Santa Clara.

related plant genera (Baldwin, 1992, 1993; Ritland, Ritland, and Straus, 1993; Savard, Michaud, and Bousquet, 1993; Wojciechowski et al., 1993; Kim and Jansen, in press). Further, divergence rates of the ITS regions in certain genera of the *Madiinae* (Asteraceae), including genera of the silverword alliance endemic to Hawaii, were suggested to be about an order of magnitude faster than cpDNA (Baldwin, 1992). Thus, we hoped to resolve phylogenetic relationships among the subgenera of *Dendroseris* using these faster evolving regions of DNA. However, in the Winteraceae, divergence rates of the ITS regions have been shown to be very slow (Suh et al., 1993). Therefore, studying ITS sequences of *Dendroseris* may also provide an opportunity to compare mode and tempo of ITS and cpDNA evolution.

The primary purposes of the present study were to use ITS sequences to: 1) examine the holophyly of *Dendroseris*; 2) attempt to resolve relationships among the subgenera; and 3) compare evolutionary patterns and rates between ITS regions and cpDNA in *Dendroseris*.

## MATERIALS AND METHODS

For this study, 15 accessions representing 15 populations of nine *Dendroseris* species and two accessions of two outgroup species were sequenced (Table 1). When materials were available, more than one population of each *Dendroseris* species was sequenced, but only one accession was available for *D. macrantha*, *D. neriifolia*, and *D. regia*. Leaves used for DNA were from three sources: 1) collected in the field and then either placed in sealable plastic bags with silica gel or put on wet ice until returned to the lab; 2) taken from plants grown from seed in the greenhouse; or 3) taken from herbarium specimens at the Herbarium of The Ohio State University (OS). Voucher specimens are deposited in OS.

Total DNA was isolated from leaf tissue using the CTAB method of Doyle and Doyle (1987), and purified in CsCl/ethidium bromide gradients. Double-stranded DNA of the complete ITS regions in each genomic DNA (5'18S–3'26S) were amplified by 30 cycles of symmetric PCR using the primers ITS 4 and ITS 5 of Baldwin (1992). The first cycle consisted of 3 minutes at 95 C to denature double-stranded template DNA, 1 minute at 50 C to anneal primers to single-stranded template DNA, and 1 minute at 72 C to extend primers. Denaturation time at 95 C was reduced to 1 minute, and primer extension time was gradually increased from 45 seconds by the addition of 4 seconds to each 72 C period in the next 29 cycles. A final extension period of 5 minutes at 72 C terminated the PCR reaction. The amplification products were purified by agarose gel (1 × TAE buffer) electrophoresis, and the concentrated DNAs were recovered using glass powder (U.S. Bioclean, U.S. Biochemical, Cleveland, OH).

Sequencing was done using the dideoxy chain termination reaction employing Sequenase (version 2.0, U.S. Biochemical). Modification to the Sequenase protocol included denaturation of the double-stranded DNA by boiling the DNA/primer mix for 3 minutes, following by snap-chilling the annealing mixture for 7 minutes in an ice water bath. In addition, 1 μl DMSO was added to both the labeling and termination reactions to reduce the effects of DNA secondary structure (Cosner, Jansen, and Lambers, in press). Both strands were sequenced using two forward primers, ITS 3 and ITS 5, and two reverse primers, ITS 2 and ITS 4 (Baldwin, 1992).

Separation of the sequences was done in 6% acrylamide gels using wedge spacers. Both short (bromphenol blue migrated to end of gel) and long (8 hours at 1,500 volts) gels were run so that both strands of the whole ITS region could be read easily. Gels were fixed for 30 minutes in 10% acetic acid, transferred to 3-MM Whatmann paper, dried under vacuum for 2 hours at 80 C, and exposed to Kodak XAR x-ray film for 24–72 hours.

Sequence limits of the two ITS regions were determined by comparison with known sequences for the coding regions 18S, 5.8S, and 25S of nrDNA (Yokota et al., 1989; Baldwin, 1992; Kim and Jansen, in press). All sequences

TABLE 2. Sequence divergences of ITS regions (ITS 1 and ITS 2) for species of *Dendroseris* and the two outgroup species. Actual numbers of unambiguous divergent sites above the diagonal and calculated sequence divergence values below diagonal. Taxa designations same as in Fig. 1.

	SVEN	SONC	LIT	MAR	MAC	MIC	PRU	NER	PIN	BER	REG
SVEN	—	14	37	37	37	35	35	37	39	37	41
SONC	0.0324	—	37	37	37	35	35	37	41	39	43
LIT	0.0818	0.0827	—	0	0	11	11	12	19	17	19
MAR	0.0819	0.0828	0.0000	—	0	11	11	12	19	17	19
MAC	0.0818	0.0827	0.0000	0.0000	—	11	11	12	19	17	19
MIC	0.0771	0.0803	0.0234	0.0234	0.0234	—	0	9	15	13	18
PRU	0.0770	0.0802	0.0234	0.0234	0.0234	0.0000	—	9	15	13	18
NER	0.0817	0.0850	0.0256	0.0256	0.0256	0.0191	0.0191	—	15	15	19
PIN	0.0867	0.0947	0.0410	0.0410	0.0410	0.0322	0.0321	0.0322	—	4	10
BER	0.0864	0.0897	0.0365	0.0365	0.0365	0.0278	0.0277	0.0321	0.0084	—	8
REG	0.0935	0.0995	0.0410	0.0410	0.0410	0.0388	0.0387	0.0410	0.0212	0.0169	—

could be aligned manually because indels (insertions and deletions) were very infrequent and each consisted of one or two bases.

The outgroups were selected because an extensive survey of the Lactuceae for cpDNA restriction site mutations (R. K. Jansen, personal communication) provided compelling evidence that *Sonchus* and *Sventenia* are closely related to *Dendroseris*. From among the several species of *Sonchus* for which we have sequence data (S. C. Kim, unpublished data), we selected the one most similar to *Dendroseris* (i.e., *S. fruticosus*).

Variable sites (65 sites) of the ITS sequences among species of ingroups and outgroups were analyzed by Wagner parsimony using PAUP (version 3.1.1, Swofford, 1993). Indels were not included in the analyses. The shortest trees were found using the Branch-and-Bound method. Bootstrap analyses (Felsenstein, 1985) were carried out with 1,000 replicates using the Branch-and-Bound algorithm. The DNADIST program of PHYLIP (version 3.4, Felsenstein, 1991) was employed to calculate sequence divergence values between species based on the entire ITS 1 and ITS 2 regions (481 sites) using the Kimura two-parameter method (Kimura, 1980) with the transition-transversion ratio setting at 0.9474 from the parsimony tree (Fig. 3). The DNAML program of PHYLIP 3.4 was used to construct a maximum likelihood tree for the sequences of the entire ITS 1 and ITS 2 regions (481 sites).

To examine whether rates of sequence divergence are significantly different in the different lineages of *Dendroseris*, the relative rate tests (Wu and Li, 1985; Li and Tanimura, 1987) were employed. *Sonchus* was used as the reference taxon for all tests because these lineages form an unresolved tetrachotomy at the base of the cladogram (Fig. 3). Sequence divergence values for the tests were calculated using Kimura two-parameter method. Standard errors for estimated relative rate differences between the lineages were calculated according to Li and Tanimura (1987) and Kimura (1980), and were used for significance tests.

## RESULTS

Complete sequences for ITS 1 and ITS 2 regions were determined for 15 accessions representing nine of the 11 described species of *Dendroseris* and one accession of each of the two outgroup taxa (Fig. 1). There are minimal length

differences between species of *Dendroseris*. For ITS 1, three species (*D. litoralis*, *D. macrantha*, and *D. marginata*, all in subg. *Dendroseris*) have 253 bp and all other species have 254 bp. The length of ITS 2 is 224 bp in *Dendroseris pinnata* and it is 225 bp long in all other species. Thus, in *Dendroseris*, there is a total of only two 1-bp indels, one in ITS 1 and another in ITS 2. With regard to the two outgroups, four independent 1-bp indels in ITS 1 and four indels in ITS 2, one of 2-bp and three of 1-bp, are needed to align them with *Dendroseris* sequences.

No unambiguously different sites were found between populations of the same species. The only differences detected between different populations of the same species were: at site 206 of ITS 1 in *D. litoralis*, populations 6305A and 6435 have C, but population 11,973 has both C and T; at site 197 of ITS 2 in *D. micrantha*, population 11,399 has T, but 11,582 has both C and T (Fig. 1). Since these differences did not affect divergence values for populations of the same species, only species abbreviations are shown in Table 2 and Figs. 3, 4. The number of ambiguous sites detected is very low, and they are found at only eight sites (Fig. 1).

A total of 65 unambiguous variable sites (33 in ITS 1, 32 in ITS 2) was found in the outgroups and ingroup, with 41 phylogenetically informative sites. There are 33 variable sites in *Dendroseris*, 15 in ITS 1, and 18 in ITS 2. Of these, eight are cladistically informative in ITS 1 and ten are informative in ITS 2. Four equally most parsimonious trees were obtained with a consistency index of 0.945 including autapomorphies and 0.918 excluding them, and a retention index of 0.938. The strict consensus tree generated from them and bootstrap values are shown in Fig. 3. The same consensus tree was obtained when transversion to transition was weighted 10 to 1 in the parsimony analyses. Sequence divergence values calculated by the two-parameter method are shown in Table 2. The tree constructed by maximum likelihood is shown in Fig. 4. All branch lengths are significantly positive except the one containing subg. *Rea* and subg. *Phoenicoseris*.

## DISCUSSION

One of the purposes of this study was to reexamine the holophyly of *Dendroseris*. Sanders et al. (1987) agreed

with Carlquist (1967) and suggested that *Dendroseris* represents a holophyletic group despite the considerable morphological differences among the three subgenera. Sanders et al. (1987) indicated that because all species of *Dendroseris* are widely divergent from any known continental taxa they likely resulted from a single introduction. However, the subgenera in turn are so distinct from each other morphologically that it would be useful to have other data to test the holophyletic hypothesis. Although all *Dendroseris* species that have been counted have a chromosome number of  $n = 18$  (Sanders, Stuessy, and Rodriguez, 1983; Spooner et al., 1987), this is a common base number ( $n = 9$ ) in the Lactuceae (Tomb, 1977). The rather high genetic identities (0.77–0.99) between *Dendroseris* species (Crawford et al., 1987; Brauner, Crawford, and Stuessy, unpublished data) argue for a close relationship and origin from one introduction. Chloroplast DNA restriction site mutations, however, provided rather weak support for holophyly with three mutations recently reported as occurring relative to the three species of *Sonchus* used as outgroups (Crawford et al., 1992). Further, additional analyses of Lactuceae data have revealed only one unique mutation for *Dendroseris* (Fig. 2; R. K. Jansen, personal communication). By contrast, ITS data provide strong support for holophyly of *Dendroseris*, with 25 mutations relative to outgroups, and the *Dendroseris* clade occurred in all 1,000 of bootstrap replicates (Fig. 3). Also, the branch length containing *Dendroseris* in the maximum likelihood tree is significantly positive ( $P < 0.01$ ) (Fig. 4). In another island group, Baldwin (1992) also found strong support for holophyly of the Hawaiian silverword alliance using ITS sequences.

Another purpose of this study of *Dendroseris* was to use ITS sequences to provide some resolution of relationships among the subgenera of *Dendroseris* because the restriction site data were inconclusive (Fig. 2). Some success was anticipated because the rate of sequence divergence appears to be higher in the ITS regions than in the chloroplast genome (Baldwin, 1992), but see also Suh et al. (1993). If this were the case then one might expect that mutations could have accumulated in the common ancestor of the different subgenera. Indeed, higher sequence divergence rates in ITS regions than in cpDNA were found in the genus (to be discussed later), and there were many more characters from the ITS sequences (65) than from the cpDNA restriction site data (13) for constructing cladograms. However, the strict consensus tree from parsimony analysis of the 65 variable sites in the ITS regions (Fig. 3) shows the same unresolved tetrachotomy at the base of the cladogram as was seen with the restriction site data (Fig. 2). The maximum likelihood tree generated from sequences of the entire ITS regions does not show any significant support for relationship among the subgenera (Fig. 4). These results further demonstrate that the subgenera must have radiated very rapidly after the ancestor of *Dendroseris* dispersed to the island, and there was insufficient time for the accumulation of mutations in either the chloroplast genome or the ITS regions. Thus, the results suggest that the early evolution of *Dendroseris* fits the model of Carlquist (1974) for rapid adaptive radiation in an island archipelago. It would be of interest to seek a more accurate picture of such a rapid radiation using faster evolving DNA sequences.

Regarding relationships within the subgenera, three species from each of three subgenera were examined using ITS sequences. Among the nine species, *D. macrantha* and *D. regia* were not included in the previous restriction site study because of unavailability of sufficient plant material. This is, therefore, the first molecular phylogenetic study of congeners from both islands of Masatierra and Masafuera (*D. regia* is from Masafuera). In both the parsimony and maximum likelihood trees constructed from ITS sequences, the holophyly of subgenera *Dendroseris* and *Phoenicoseris* is supported (Figs. 3, 4), as both morphology (Sanders et al., 1987) and restriction site data suggested (Fig. 2, Crawford et al., 1992). ITS sequence data provided additional insights into relationships within subg. *Phoenicoseris*. Morphologically, *D. regia* was considered as the most derived species in the subgenus (Sanders et al., 1987). The ITS phylogeny, however, suggests that *D. regia* may have diverged from the common ancestor of *D. berteriana* and *D. pinnata* prior to divergence of the latter two species (Fig. 3).

Nucleotide substitutions in the ITS regions show an apparently unequal distribution pattern among the subgenera and species (Fig. 3). The species of subg. *Phoenicoseris* possess the largest number of substitutions, whereas species of subg. *Rea* have the fewest substitutions within the genus. In the extreme case, *D. regia* has 14 substitutions while *D. micrantha* and *D. pruinata* have accumulated only four substitutions after the radiation of the subgenera. Thus, the hypothesis that the rates of ITS sequence divergence are different among the lineages needed to be tested. Since there is no sequence divergence within subg. *Dendroseris* or between *D. micrantha* and *D. pruinata*, the relative rate tests were performed among four lineages while using one sequence representing subg. *Dendroseris* and one sequence representing *D. micrantha* and *D. pruinata*. The largest test value, 0.73 (standard error of 0.0262 for the relative rate difference value of 0.0192), was found between *D. regia* and *D. micrantha* and *D. pruinata*. This value indicates that the rates are not significantly different between them, and thus the molecular clock cannot be rejected for ITS sequence evolution in *Dendroseris*.

No unambiguous nucleotide substitutions were found between populations of the same *Dendroseris* species, which is concordant with the very high genetic identities at allozyme loci and the lack of cpDNA mutations within and among populations of each species (Crawford et al., 1987; Crawford et al., 1992; Brauner, Crawford, and Stuessy, unpublished data). Likewise, Soltis and Kuzoff (1993) detected little or no variation in ITS 1 sequences in two species of *Lomatium* (Umbelliferae). Also, very few ambiguous sites were found in the ITS regions of *Dendroseris* species, suggesting that copies are highly homogeneous, despite the chromosome number of  $n = 18$  (Sanders, Stuessy, and Rodriguez, 1983) and the duplicated isozyme loci (Crawford et al., 1987) suggesting that the species are tetraploid. Hybridization may cause additivity of different parental nucleotides at the same site in the ITS regions of the hybrids (Kim and Jansen, in press), but speciation by hybridization has been viewed as highly unlikely for *Dendroseris* (Sanders et al., 1987), and the ITS data are concordant with this.

Rates of sequence divergence in the ITS regions remain

## ITS 1

SVEN	TCGAACCCTG	CAAAGCAGAA	CGCCCTGTGA	ACATGTAAT	ACAACTCGGT	GTTGTTGAGA	(60)
SONC	.....	.....	..A..C....	.....	.....	.....	
LIT1	.....	.....	..A..C....	.....	.....	..C.....	
LIT2	.....	.....	..A..C....	.....	.....	..C.....	
MAR	.....	.....	..A..C....	.....	.....	..C.....	
MAC	.....	.....	..A..C....	.....	.....	..C.....	
MIC1	.....	.....	..A..C....	.....	.....	..C.....	
MIC2	.....	.....	..A..C....	.....	.....	..C.....	
PRU	.....	.....	..A..C....	.....	.....	..C.....	
NER	.....	.....	..A..C....	.....	..Y.....	..C.....	
PIN	.....	.....	..A..C....	.....	.....	..C.....	
BER	.....	.....	..A..C....	.....	.....	..C.....	
REG	.....	.....	..A..C....	.....	.....	..C.....	
SVEN	CTGGGCCTTA	GGTTTTGATC	AGCAATACCA	CCCGGTTTGT	TTCCATGGTA	TCTTCTTTTA	(120)
SONC	-.....G...	.....C....	.....C....	T.....G...	.....	.....	
LIT1	-.....G...	.....C.T...	.....C....	T.....G...	.....	...-.G..G	
LIT2	-.....G...	.....C.T...	.....C....	T.....G...	.....	...-.G..G	
MAR	-.....G...	.....C.T...	.....C....	T.....G...	.....	...-.G..G	
MAC	-.....G...	.....C.T...	.....C....	T.....G...	.....	...-.G..G	
MIC1	-.....G...	.....C....	.....C....	T.....G...	.....	...-.G..G	
MIC2	-.....G...	.....C....	.....C....	T.....G...	.....	...-.G..G	
PRU	-.....G...	.....C....	.....C....	T.....G...	.....	...-.G..G	
NER	-.....G...	.....C....	.....C....	T.T...G...	.....	...-.G..G	
PIN	-.....G...	.....C....	..T..A....	T...A.G...	.....	...-.G..G	
BER	-.....G...	.....C....	..T..A....	T...A.G...	.....	...-.G..G	
REG	-.....G...	.....C....	..T..A....	T...A.G...	.....	...-.G..G	
SVEN	TGGTACCATG	GATGTCACAT	CGGATTATAA	CAAACCCCGG	CACGGCATGT	GCCAAGGAAA	(180)
SONC	.....	.....A..	.....	.....	.....	.....	
LIT1	.....	.....C..	.....C....	.....	.....	.....	
LIT2	.....	.....C..	.....C....	.....	.....	.....	
MAR	.....	.....C..	.....C....	.....	.....	.....	
MAC	.....	.....C..	.....C....	.....	.....	.....	
MIC1	.....	.....C..	.....C....	.....	.....	.....	
MIC2	.....	.....C..	.....C....	.....	.....	.....	
PRU	.....	.....C..	.....C....	.....	.....	.....	
NER	.....	.....C..	.....C....	.....	.....	.....	
PIN	..C.....	.....C..	.....C....	.....	.....	.....	
BER	..CA.....	.....C..	.....C....	.....	.....	.....	
REG	..C.....	.....C..	.....C....	.....	.....	.....	
SVEN	ACGAAATATA	AGAAGGTATC	TACTTGATTT	GCCCCGTTTT	GCGGTGTGCA	TGCAGGTGGT	(240)
SONC	.....	.....	.....	...Y....	A.....	.....	
LIT1	.....AG	.....	.....C....	.....	-.....	.....	
LIT2	.....AG	.....	.....Y....	.....	-.....	.....	
MAR	.....AG	.....	.....C....	.....	-.....	.....	
MAC	.....AG	.....	.....C....	.....	-.....	.....	
MIC1	.....AG	.....	.....C....	.....	.....	.....T...	
MIC2	.....AG	.....Y...	.....C....	.....	.....	.....T...	
PRU	.....AG	.....	.....C....	.....	.....	.....T...	
NER	..T.....AG	.....	.....C....	.....	.....	.....	
PIN	..W.....AG	.....	..T..C....	.....	.....	..A..A...	
BER	.....AG	.....	.....C....	.....	.....	..A..A...	
REG	.....AG	.....	.....C....	..T.....	.....	..A.....	
SVEN	AGCATTCTTT	AAAAATA	(256)				
SONC	.....	...-..					
LIT1	...C.C....	...C..					
LIT2	...C.C....	...C..					
MAR	...C.C....	...C..					
MAC	...C.C....	...C..					
MIC1	...C.M....	...C..					
MIC2	...C.M....	...C..					
PRU	...C.C....	...C..					
NER	...C.C....	...C..					
PIN	T..C.C....	...C..					
BER	T..C.C....	...C..					
REG	T..C.C....	...C..					

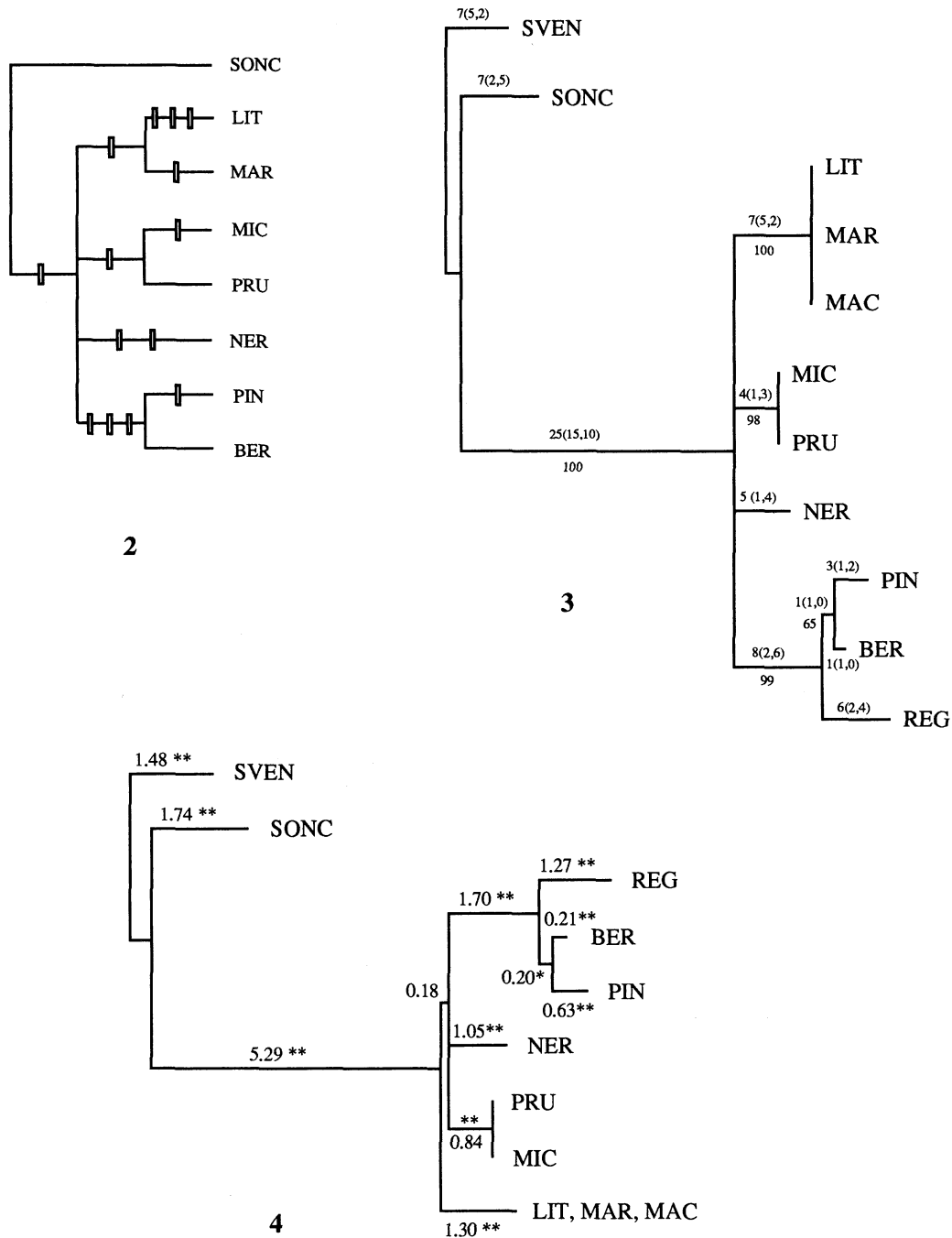
## ITS 2

SVEN	ATCGTGTGCG	CCCCCGCCAA	ACATCCCCTA	A-GGTAATCA	TGGTGATGGG	GGCGGAAATT	(60)
SONC	.....G..	.....A....	.....A....	.....A....	.....A....	...C.....	
LIT1	....C.....	....T.....	.....TGA.	.G.....	.....	.....	
LIT2	....C.....	....T.....	.....TGA.	.G.....	.....	.....	
MAR	....C..R.	....T.....	.....TGA.	.G.....	.....	.....	
MAC	....C.....	....T.....	.....TGA.	.G.....	.....	.....	
MIC1	....C.....	....T.....	.....TGA.	.G.....	.....	.....	
MIC2	....C.....	....T.....	.....TGA.	.G.....	.....	.....	
PRU	....C.....	....T.....	.....TGA.	.G.....	.....	.....	
NER	....C.....	....T.....	.....TGA.	.G.....	.....	.....	
PIN	....C.....	....T.....	-...TGAT	.G.....	.....	.....	
BER	....C.....	....T.....	.....TGAT	.G.....	.....	.....	
REG	....C.....	....T.....	.....TGCT	.G.....	.....	.....	
SVEN	GGCCTCCCGT	TCTTGTGTCT	GGTTGGCCCA	AAGATGAGTC	CCC-TACGGC	GGATGCACAA	(120)
SONC	..--.....	.....C	.....T	.....	.....	.....	
LIT1	.....W	.....TC	.....T	.....C	...C...T	.....	
LIT2	.....W	.....TC	.....T	.....C	...C...T	.....	
MAR	.....W	.....TC	.....T	.....C	...C...T	.....	
MAC	.....W	.....TC	.....T	.....C	...C...T	.....	
MIC1	.....	.....C	.....T	.....	...C.....	.....	
MIC2	.....	.....C	.....T	.....	...C.....	.....	
PRU	.....	.....C	.....T	.....	...C.....	.....	
NER	.....	.....C	.....T	.....	...C.....	.....	
PIN	.....	.....C	.....T	.....	...C.....	.....	
BER	.....	.....C	.....T	.....	...C.....	.....	
REG	.....	.....C	.....T	.....	...CA.....	.....	
SVEN	CTAGTGGTGG	TTGAACAGAC	CCTCGTCTTG	TGTTGTGTGT	CGTGAGCTGT	GAAGGAAGTT	(180)
SONC	.....	.....	.....	.....	.....	.....	
LIT1	.....	....T....	.....C	.....	.....G	..G...C.	
LIT2	.....	....T....	.....C	.....	.....G	..G...C.	
MAR	.....	....T....	.....C	.....	.....G	..G...C.	
MAC	.....	....T....	.....C	.....	.....G	..G...C.	
MIC1	.....	....T....	.....	.....	.....A	..G.T...C.	
MIC2	.....	....T....	.....	.....	.....A	..G.T...C.	
PRU	.....	....T....	.....	.....	.....A	..G.T...C.	
NER	.....	....T....	....A..	.....	.....G	..CG...C.	
PIN	.....	....T....	.....	.....	.....G	..G...C.	
BER	.....	....T....	.....	.....	.....G	..G...C.	
REG	.....	....T....	.....	.....	.....G	..T.G.....	
SVEN	CTCATTTCAG	ACCCTACTGT	ATCGTTAAAA	GACGATATAT	CGACC	(225)	
SONC	.....	.....	.....	.....	.....	.....	
LIT1	....CT..	.....	.....	A.T.....	.....	.....	
LIT2	....CT..	.....	.....	A.T.....	.....	.....	
MAR	....CT..	.....	.....	A.T.....	.....	.....	
MAC	....CT..	.....	.....	A.T.....	.....	.....	
MIC1	..A...T..	....C....	.....	A.T.....	.....	.....	
MIC2	..A...T..	....C....	.....	A.T.....	.....	.....	
PRU	..A...T..	....C....	.....	A.T.....	.....	.....	
NER	....T...A..	..C....	.....	A.T.....	.....	.....	
PIN	....T...C..	....	.....	A.T.....	.....	.....	
BER	....T...C..	....	.....	A.T.....	.....	.....	
REG	....CT..C..	....	.....T..	A.T.....	.....	.....	

Fig. 1. Aligned ITS sequences from *Dendroseris* species and the two species used as outgroups, *Sonchus fruticosus* (SONC) and *Sventenia bupleuroides* (SVEN). Abbreviations of *Dendroseris* species are: *D. litoralis*, 6305A and 6435 (LIT1), 11,973 (LIT2); *D. marginata* (MAR); *D. macrantha* (MAC); *D. micrantha*, 11,399 (MIC1), 11,582 (MIC2); *D. pruinata* (PRU); *D. nerifolia* (NER); *D. pinnata* (PIN); *D. berteriana* (BER); *D. regia* (REG). Dots (.) indicate matched sequences to the first taxon, and bars (-) indicate the gaps. Polymorphic sites are represented by the following symbols: M = A or C; R = A or G; Y = C or T; W = A or T.

unclear, and the only available estimates in two plant groups vary considerably. Baldwin (1992) suggested that sequence divergence of ITS regions was about an order of magnitude faster than that of cpDNA in certain genera of *Madiinae*. Suh et al. (1993), however, found very slow

rates of ITS sequence divergence in Winteraceae ( $3.2\text{--}5.2 \times 10^{-10}$  substitutions per site per year for ITS 1 and  $3.6\text{--}5.7 \times 10^{-10}$  for ITS 2). In *Dendroseris*, the average pairwise ITS sequence divergence between species is  $2.67\% \pm 1.27\%$ , which is about 38 times higher than that esti-



Figs. 2-4. 2. Cladogram for species of *Dendroseris* based on cpDNA restriction site mutations. An open bar represents a cpDNA mutation. Redrawn and modified from Crawford et al. (1992). 3. The strict consensus tree of the four most parsimonious trees generated from ITS sequences. Numbers above lines are the number of nucleotide substitutions for a branch followed by the number of transitions and transversions, respectively, in parentheses. Numbers below branches give the percentage that a group occurred in 1,000 bootstrap replications. Abbreviations for taxa same as in Fig. 1. 4. Maximum likelihood tree constructed from ITS sequences. Branch lengths are given above the branches. Those values with one or two asterisks are significantly positive ( $P < 0.05$ ,  $P < 0.01$ , respectively). The one branch of length 0.18 is not significantly positive. Designations of taxa same as in Fig. 1.

mated from cpDNA restriction site mutations (0.07%) (Crawford et al., 1992). To estimate rates of ITS sequence divergence in *Dendroseris*, the average of pairwise sequence divergence between species in the four different lineages (subg. *Dendroseris*; *D. micrantha* and *D. pruinata*; *D. nerifolia*; and subg. *Phoenicoseris*) was calculated to

be  $3.15\% \pm 0.77\%$ . As indicated earlier, the lack of informative substitutions for resolving relationships among the subgenera suggests a rapid radiation of these lineages from the common ancestor. Thus, the sequence divergence values between species in different lineages could be viewed as the sequence divergence within the genus

since the ancestor dispersed to the island. Further, we may assume for the sake of argument that the ancestor of *Dendroseris* arrived on Masatierra soon after its formation about 4 million years ago (Stuessy et al., 1984). Under these assumptions and using the average sequence divergence value between species from different lineages (i.e.,  $3.15\% \pm 0.77\%$ ), the average rate of nucleotide substitutions per site per year within the genus would be  $(3.94 \pm 0.10) \times 10^{-9}$  per site per year. However, since this is the earliest time when the ancestor may have arrived on the island, this estimate represents the slowest rate of divergence. Estimates from cpDNA restriction site mutations suggested that the two most divergent species (*D. litoralis* and *D. pinnata*) may have diverged some 2.6 million years ago. If we assume this later time of arrival of the ancestor of *Dendroseris*, then the average rate of ITS sequence divergence in the genus would be  $(6.06 \pm 0.15) \times 10^{-9}$  per site per year.

The distribution of substitutions of ITS sequences can also be compared to the distribution of restriction site mutations in cpDNA on phylogenetic trees. One similarity in the two trees is the relatively high number of synapomorphies supporting subg. *Phoenicoseris* (Figs. 2, 3). One evident difference between the data sets involves the number of unique changes detected within species of subg. *Dendroseris* and *Phoenicoseris*. No ITS sequence differences are present in species of subg. *Dendroseris*, whereas three cpDNA restriction site mutations were found in *D. litoralis*, and one cpDNA mutation was found in *D. marginata* (Figs. 2, 3). By contrast, only one cpDNA mutation distinguishes *D. berteriana* and *D. pinnata*, whereas four substitutions in the ITS regions were found (Figs. 2, 3). Whether these different mutation patterns represent different evolutionary tempo between the ITS regions and chloroplast genome needs further investigation.

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