American Journal of Botany 80(2): 222–238. 1993.

MOLECULAR PHYLOGENETICS OF CALYCADENIA (COMPOSITAE) BASED ON ITS SEQUENCES OF NUCLEAR RIBOSOMAL DNA: CHROMOSOMAL AND MORPHOLOGICAL EVOLUTION REEXAMINED[1]

BRUCE G. BALDWIN[2]

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721

Phylogenetic patterns within *Calycadenia* were estimated from 18-26S nuclear ribosomal DNA sequences of the internal transcribed spacer (ITS) region in 19 representatives of all species in *Calycadenia*, including *Osmadenia* (*C.*) *tenella*, and in two outgroup species. In pairwise comparisons among the *Calycadenia* and *Osmadenia* sequences, divergence ranged from 0 to 11.2% of nucleotides in ITS 1 and from 0 to 8.6% in ITS 2. Of 62 nucleotide sites with potential phylogenetic information, 51.6% were in ITS 1, 46.8% were in ITS 2, and 1.6% were in the 5.8S subunit. A highly resolved, strict consensus tree from Wagner parsimony analysis of these data agrees well with morphological and cytological evidence. This tree suggests that: 1) the monotypic *Osmadenia tenella* is the sister-group to *Calycadenia*; 2) the base chromosome number in *Calycadenia* is $n = 7$, from which other numbers were derived; 3) species with multiple T-glands on cylindrical bracts and chromosome numbers of $n = 5$ or 6 (or 7 in *C. oppositifolia*) form a monophyletic group derived from an $n = 7$ species similar or identical in genomic structure to *C. hooveri* or *C. villosa*; 4) *C. spicata* ($n = 4$) is the product of an independent dysploid reduction from $n = 7$; 5) *C. multiglandulosa* and *C. pauciflora*, sensu Keck, are not monophyletic taxa; and 6) loss of chromosomal homology between *Calycadenia* species, as reflected by meiotic chromosomal association in hybrids, is positively correlated with time since evolutionary divergence. These results offer little evidence of homoplasy in chromosomal and phenotypic characters in *Calycadenia* and provide further support for the phylogenetic utility of plant ITS sequences.

[redacted]

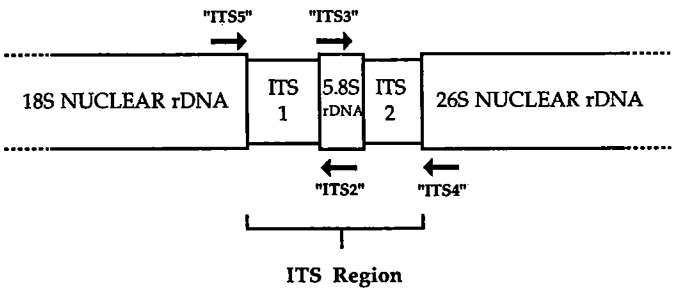

Fig. 1. Organization of the internal transcribed spacer (ITS) region of 18-26S nuclear ribosomal DNA. Approximate positions of primers used for DNA amplification and sequencing are indicated by arrows. Primer names follow White et al., 1990. Primer sequences (5′ to 3′): "ITS2" = GCTGCGTTCTTCATCGATGC; "ITS3" = GCATCGATGAAGAACGCAGC; "ITS4" = TCCTCCGCTTATTGATATGC; "ITS5" = GGAAGTAAAAGTCGTAACAAGG. Modified from Fig. 1 in Baldwin (1992).

diversified, annual tarweeds. Sequence data for this purpose were obtained from the internal transcribed spacer (ITS) region of 18-26S nuclear ribosomal DNA (nrDNA) (Fig. 1). Among nuclear gene regions, 18-26S nrDNA is attractive for phylogeny reconstruction because of its high copy number (Rogers and Bendich, 1987), rapid concerted evolution (cf. Arnheim et al., 1980; Zimmer et al., 1980; Arnheim, 1983), and diverse rates of evolution within and among component subunits and spacers (Appels and Dvorak, 1982; reviewed in Jorgansen and Cluster, 1988). Phylogenetic analysis of ITS region sequences from representatives of several genera in the subtribe Madiinae of Compositae (Baldwin, 1992) yielded results highly concordant with a phylogeny of these same species based on chloroplast DNA (cpDNA) restriction site mutations (Baldwin, 1989; Baldwin et al., 1991). In each pairwise comparison of the Madiinae DNAs, nucleotide sequence divergence was an order of magnitude higher in the ITS region than that estimated from cpDNA restriction sites. Based on these findings, the ITS region appeared to hold great promise for resolving evolutionary patterns among more closely related species in Madiinae, e.g., within *Calycadenia.*

MATERIALS AND METHODS

Plant samples—Total DNAs from 21 populations in *Arnica, Calycadenia, Hemizonia,* and *Osmadenia* were isolated from fresh leaves of pooled individuals (see Table 1) and purified on cesium chloride gradients, using the methods described by Palmer (1986) without separation of organelles or the methods of Doyle and Doyle (1987).

Sequencing strategy—Single-stranded DNAs from the ITS region of each sample were generated by asymmetric polymerase chain reaction (PCR) using the primers "ITS5" and "ITS4" (yielding a 5′26S-3′18S strand) or "ITS3" and "ITS4" (yielding a 5′26S-3′5.8S strand) (White et al., 1990; Fig. 1) in a 1:20 ratio. In several cases, the primers "ITS5" and "ITS2" (White et al., 1990; Fig. 1) were used as above to amplify single-stranded DNA of the ITS 1 region (5′5.8S-3′18S) for resequencing. PCR reactions, purification of PCR products, and direct dideoxy sequencing of resultant single-stranded DNAs were conducted by the methods detailed in Baldwin (1992).

TABLE 1. *Collections examined for ITS nucleotide sequence variation*[a]

Arnica mollis Hook.[b]—*BGB*[c] *680,* 0.25 mile SW of Winnemucca Lake, Alpine County, California.

Calycadenia ciliosa E. Greene (chromosome race "Corning"[d])—*RLC*[e] *2131,* 2 miles NE of Calahan on Gazelle-Calahan Road, Siskiyou County, California.

Calycadenia ciliosa E. Greene (chromosome race "Lewiston"[d])—*RLC 2157,* 4 miles SW of Lewiston on Lewiston Road, Trinity County, California.

Calycadenia fremontii A. Gray—*BGB 710,* ca. 1 mile SW of junction of Broyles Road and State Highway 99, Butte County, California.

Calycadenia hispida (E. Greene) E. Greene—*GDC*[f] *1161,* ca. 3 miles NE of Honcut, Butte County, California.

Calycadenia hooveri G. D. Carr—*BGB 682,* 4.8 miles SE of junction with State Highway 120 on Willms Road, Stanislaus County, California.

Calycadenia multiglandulosa DC. subsp. *bicolor* (E. Greene) Keck—*BGB 490,* 2 miles S of junction with McCourtney Road on Auburn Road, Nevada County, California.

Calycadenia multiglandulosa DC. subsp. *cephalotes* (DC.) Keck—*RLC 2138,* Bootjack Camp area, Mount Tamalpais, Marin County, California.

Calycadenia mollis A. Gray—*RLC 2213,* ca. 2.3 miles W of State Highway 41 on State Highway 49, Madera County, California; *RLC 2215,* 0.3 mile S of State Highway 168 on Tollhouse Road, Fresno County, California.

Calycadenia oppositifolia (E. Greene) E. Greene—*RLC 2026,* 0.5 mile S of Chico Airport on Cohasset Road, Butte County, California; *RLC 2032,* 4.6 miles from Quincy–Oroville Highway on Bloomer Lookout Road, Butte County, California.

Calycadenia pauciflora A. Gray (chromosome race "Elegans"[g])—*RLC 2120,* ca. 5 miles NW of Middletown on State Highway 175, Lake County, California.

Calycadenia pauciflora A. Gray (chromosome race "Pauciflora"[g])—*RLC 2153,* 3.7 miles W of Colusa County line on State Highway 20, Lake County, California.

Calycadenia spicata (E. Greene) E. Greene—*RLC 2217,* ca. 5 miles S of Knights Ferry on Knights Ferry–La Grange Road, Stanislaus County, California.

Calycadenia truncata DC. subsp. *scabrella* (E. Drew) Keck—*BGB 605,* 1.5 miles E of junction with Plum Creek Road on Paynes Creek Loop, Tehama County, California.

Calycadenia truncata DC. subsp. *truncata*—*BGB 619,* ca. 0.1 mile SE of junction with Comings Camp Trail on Devils Peak Trail, Santa Lucia Mountains, Monterey County, California.

Calycadenia villosa DC. ("Erecta"[h])—*GDC 1163,* 1.4 miles SE of Jolon, Monterey County, California; ("Depressa"[h]) *GDC 1164,* 0.1 mile S of State Highway 58 on road to Navajo Campground, San Luis Obispo County, California.

[b]*Hemizonia perennis* Keck—*D. W. Kyhos s.n.,* midway between Colonet and San Antonio del Mar, Baja California, Mexico.

Osmadenia tenella Nutt.—*GDC 1365,* 2.1 miles S of junction with State Highway 76 on Pala to Lilac Road, San Diego County, California.

[a] DNA analyzed from each population was from two to 15 pooled individuals. Vouchers are at DAV (B. G. Baldwin, G. D. Carr, and D. W. Kyhos collections) or the Eastern Washington University herbarium (R. L. Carr collections).

[b] Outgroup species.

[c] The author.

[d] See Carr and Carr, 1983.

[e] Robert L. Carr.

[f] Gerald D. Carr.

[g] See Carr, 1975b.

[h] See Carr, 1977.

Sequence analysis—DNA sequences were aligned manually by sequential pairwise comparisons (cf. Swofford and Olsen, 1990). Subunit and spacer boundaries of these DNA sequences were determined by comparison to the corresponding boundaries in *Daucus carota* and *Vicia* *faba,* which have been defined by S1 nuclease mapping (Yokota et al., 1989).

Numbers and proportions of nucleotide sites with different nucleotide states were calculated for all possible pairwise comparisons of combined ITS 1 and ITS 2 sequences from the study species. Only those sites with fixed nucleotide character states in all sequences were compared, i.e., those sites without gaps or polymorphisms in any of the aligned sequences.

Phylogenetic analysis — Aligned nucleotide sites with potential phylogenetic information, i.e., with each of at least two nucleotide states in two or more sequences, were included in a data matrix. Sequences that were identical at all potentially informative sites were merged for the phylogenetic analyses. Gaps were treated as missing data. The matrix included sequence data from representatives of 19 populations of all *Calycadenia* species, including the monotypic *Osmadenia* (*C.*) *tenella.* ITS sequences from the extrasubtribal species *Arnica mollis* and the tarweed *Hemizonia perennis* served as outgroups. *Arnica mollis* was chosen for this purpose because of its high ITS sequence similarity to members of Madiinae (Baldwin, 1992). *Hemizonia perennis* was chosen as an outgroup because of the high alignability of its ITS sequences with those of *Calycadenia* and *Osmadenia* species and the close ITS relationship between *Hemizonia* and *Calycadenia*/*Osmadenia* (Baldwin, unpublished data).

The data matrix was analyzed by Wagner parsimony using the "branch-and-bound" option of PAUP (version 3.0L, D. L. Swofford, Illinois Natural History Survey), with collapse of zero-length branches, to find the maximally parsimonious trees.

The decay index for individual clades, i.e., the number of additional evolutionary steps required before at least one of the possible trees fails to resolve a particular sister-group relationship, was calculated by examining the strict consensus of all equal-length trees up to six steps longer than the shortest trees (cf. Donoghue et al., 1992). Bootstrap values for particular clades were calculated from 100 replicate Wagner parsimony analyses using the PAUP "heuristics" option and "closest" addition sequence of the taxa.

Additional Wagner parsimony analyses of potentially informative sites from separate ITS 1 and ITS 2 data were conducted to assess the relative contribution of each spacer to phylogenetic resolution in *Calycadenia.* The "branch-and-bound" option of PAUP was used for the ITS 1 analysis. The "heuristics" option of PAUP with the "closest" addition sequence of the taxa was used for the ITS 2 analysis because of limitations imposed by the data set.

RESULTS

ITS length variation — The entire ITS region varied in

sequences required gaps at 1.2% of nucleotide sites, none of which were adjacent positions. Alignment of *Calycadenia* and *Osmadenia* ITS 2 sequences necessitated gaps at 2.7% of nucleotide sites. There was a need for gaps at adjacent ITS 2 sites in two instances: 1) an identical deletion of three base pairs in both *C. mollis* accessions, 21–23 positions from the 5.8S/ITS 2 boundary and 2) a deletion of two base pairs in *Osmadenia tenella,* 31–32 positions from the 5.8S/ITS 2 boundary. No gaps were needed to align the *Calycadenia* 5.8S sequences (Table 2).

Repeat-unit variation — No evidence of ITS length variants or major sequence variants within DNA accessions was found. PCR products were resolved in every case as single, sharp, double-stranded and single-stranded DNA bands on 3% agarose gels. In addition, individual DNA sequences exhibited low levels of potential polymorphism at nucleotide sites, i.e., two bands at a single position that could indicate multiple nrDNA repeat types.

ITS sequence divergence — Among *Calycadenia* and *Osmadenia* accessions, pairwise sequence comparisons indicated ITS 1 sequence divergence ranging from 0 to 11.2%. Infraspecific ITS 1 sequence divergence ranging from 0 in *C. mollis* to 4.3% in *C. truncata* (Table 3). ITS 2 sequence divergence from pairwise comparisons of *Calycadenia* and *Osmadenia* DNAs ranged from 0 to 8.6%. Within-species ITS 2 sequence divergence ranged from 0 in *C. mollis* to 3.0% in *C. truncata* (Table 3). Complete identity existed among all *Calycadenia* and *Osmadenia* 5.8S sequences except for a single mutation at site 397 (Tables 2, 4) that differentiated *C. hooveri* from the other species.

ITS nucleotide site variation — A character matrix of 647 characters was necessary to align *Calycadenia, Osmadenia,* and outgroup DNAs (Table 2). No alignment ambiguities were encountered. Of these 647 characters, 162 (25.0%) were variable. ITS 1 accounted for most (58.0%) of this variation compared to 39.5% in ITS 2 and 2.5% in the 5.8S subunit.

Potentially informative characters accounted for 9.6% of all ITS region sites and 38.3% of variable sites, excluding sites where mutations were shared only between sequences that were identical at all potentially informative positions (see Table 4). Similar numbers and proportions of ITS 1 sites (32; 12.3%) and ITS 2 sites (29; 13.0%) were potentially informative. Among variable positions, however, the percentage of ITS 2 sites that were potentially informative (45.3%) was higher than in ITS 1 (34.0%). One potentially informative character, separating the outgroup from the ingroup, occurred in the 5.8S subunit.

Phylogenetic analyses — Eleven minimum-length trees were generated from Wagner parsimony analysis of po-

TABLE 2. *Aligned DNA sequences of the ITS region in 18-26S nuclear ribosomal DNA from 19 representatives of* Calycadenia *and* Osmadenia *and from two outgroup species (see Table 1)*

Taxa[a]	Nucleotide sites[b]
	ITS 1 →
	10 20 30 40 50 60
1[c]	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTAAAGAGGAT
2[c]	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATAAGGAC
3	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGACTCATGAGGAC
4	TCGAATCCTGCATAGCAGAATGACCCGTGAACATGTACAACAACATGGTCTCATAAGGAC
5	TCGAATCCTGCATAGCAGAATGACCCGTGAACATGTACAACAACATGGTCTCATAAGGAC
6	TCGAATCCTGCATAGCAGAAYGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAC
7	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGTCTCATGAGGAC
8	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAC
9	TCGAATCCTGCGTAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAT
10	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAT
11	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAT
12	TCGAATCCTGCACAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAC
13	TCGAATCCTGCACAGCAGAACGACCCGTGAACACGTAAAACAACATGGCCTCATGAGGAC
14	TCGAATCCTGCACAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAC
15	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCACGAGGAC
16	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCACGAGGAC
17	TCGAAYCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAC
18	TCGAATCCTGCATAGCAGMACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAC
19	TCGAACCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAC
20	TCGAAYCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAC
21	TCGAATCCTGCATAGCAGAACGACCCGTGAACACGTACAACAACATGGCCTCATGAGGAC
	70 80 90 100 110 120
1	CGGATCA--TCTGTTTCGGTCCTYGTTAAGCCACGTCGACATTGTGTTTATGATCTCCTT
2	CG-ATCA-TTCTTCTTTGGTCCTTGTATGGCCACGTCGAC-CCGTGTTAATGATCTCCTT
3	CG-ATTG-CTTTGCTTCGGGCCTTRTGTGGCCACGTCGAC-CGGCGTTGATGGTCTCCTT
4	AG-TTCA-CTTTGCTTCGGTCCTTGTGTGGCTACGTCGAC-CTGTGTTGATGATCTCCTT
5	AG-TTCA-CTTTGCTTCGGTCCTTGTGTGGCTACGTCGAC-CTGTGTTGATGATCTCCTT
6	TG-ATCA-TTTTGCTTCTGTCCTTGTGTGGCCACGTCGAC-CTCTGTTGATGATCTCCTT
7	CG-ATCA-TTTTGCTTCTGTCCTTGTGTGGCCACGTCGAC-CTGTGCTGATGATCTCCTT
8	CG-ATCA-TTTTGCTTCGGTCCTTGTGCGGCCACGTCGRC-CTGTGTCGATGATCTCCTT
9	CG-ATCCATTTTGCTTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTCGATGATCTCCTT
10	CG-ATCAATTTTGCTTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTCGATGATCTCCTT
11	CG-ATCAATTTTGCTTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTCGATGATCTCCTC
12	CA-ATCAATTTTGGTTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTCGATGATCTCCTT
13	CA-ATCAATTTTGGTTCGGTCCTTGTGCGGTCACGTCAAC-CTGTGTCGATGATCTCCTT
14	CA-ATCAATTTTGGTTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTCGATGATCTCCTT
15	CG-ATCAATTTTGATTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTCGATGATCTCCTT
16	CG-ATCAATTTTGATTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTCGATGATCTCCTT
17	CG-ATCAATTTTGATTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTTKATGATCTCCTT
18	CG-ATCAATTTTGATTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTTKATGATMTCCTT
19	CG-ATCAATTTTGATTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTTGATGATCTCCTT
20	CG-ATCAATTTTGATTCGGTCCTTGTGYGGTCACGTCGAC-CTGTGTYGATGATYTCCTT
21	TG-ATCAATTTTGATTCGGTCCTTGTGCGGTCACGTCGAC-CTGTGTCGATGACCTCCTT

TABLE 2. *Continued*

Taxa[a]	Nucleotide sites[b]
	130 . 140 . 150 . 160 . 170 . 180 .
1	TTTAGGACTCATGGACATCATGCTGGCACAACAACAACCCCC-GGCACAACATGTGCCAA
2	TTCGGGAATCATTAACATCGTGTCGGC---ATAACAACCCCCCGGCACGGCATGTGCCAA
3	TGCGGGAGTCATGGACATCGTGTCGGCACAATAACAACCCCC-GGCACGGCACGTGCCAA
4	GGTGGGACTCATTGACATTGTGTTGGCACAATAACAACCCCCCGGCACGGCATGTGCCAA
5	[illegible]
6	-GCGGGACTCATCGACATCGCGTTGGCACAATAACAACCCCC-GGCACGGCACGTGCCAA
7	-GCGGGACTCATCGACATTGTGTTGGCACATTAACAACCCCC-GGCACGGCACGTGCCAA
8	[illegible]
9	[illegible]

TABLE 2. *Continued*

Taxa[a]	Nucleotide sites[b]
	5.8S →
	250 . 260 . 270 . 280 . 290 . 300 .
1	GCGTGGCTTCTTTCAAATCTTAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
2	TCGTGGCTTCTTTCTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
3	CCGTGACTTCTTTCTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
4	TCATGGCTTCTTTTTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
5	TCATGGCTTCTTTTTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
6	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
7	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
8	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
9	[illegible]
10	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
11	ACGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
12	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
13	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
14	[illegible]
15	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
16	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
17	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
18	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
19	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
20	TCGTGGCTTCTTTGTAATCATAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCG
21	TCGTGGCTTCTTTGTAATCATAAACGACTCTYGGCAACGGATATCTCGGCTCACGNATCG
	310 . 320 . 330 . 340 . 350 . 360 .

TABLE 2. *Continued*

Taxa[a]	Nucleotide sites[b]
	370 . 380 . 390 . 400 . 410 . 420 .
1	GTTTTTGAACGCAAGTTGCGCCTGAAGCCTTCTGGTTGAGGGCACGTCTGCCTGGGCGTC
[redacted]	
3	GTTTTTGAACGCAAGTTGCGCCCGAAGCCTTCTGGCTGAGGGCACGTCTGCCTGGGCGTC
4	GTTTTTGAACGCAAGTTGCGCCCGAAGCCTTCTGGCTGAGGGCACGTCTGCCTGGGCGTC
[redacted]	
6	GTTTTTGAACGCAAGTTGCGCCCGAAGCCTTCTGGCTGAGGGCACGTCTGCCTGGGCGTC
7	GTTTTTGAACGCAAGTTGCGCCCGAAGCCTTCTGGCTGAGGGCACGTCTGCCTGGGCGTC
8	GTTTTTGAACGCAAGTTGCGCCCGAAGCCTTCTGGCCGAGGGCACGTCTGCCTGGGCGTC
[redacted]	

TABLE 2. *Continued*

Taxa[a]	Nucleotide sites[b]
	490 . 500 . 510 . 520 . 530 . 540 .
1	TGGTCTCCCGTGGTAATGACGCGGTTGGCCTAAATATGAGTCCCATCAAGAGGGACGCAC
2	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAACACGAGTCCTGTCGAGATGGACGCAC
3	TGGTCTCCCGTGTCCATGACGCGGTTGGCCTAAATATGAGTCCCGTCAAGATGGACGCAT
4	TGGTCTCCCGTGTCCATGATGTGGTTGGCCTAAATATGAGTTCCATTGAGATGGACGCAC
5	TGGTCTCCCGTGTCCATGATGTGGTTGGCCTAAATATGAGTTCCATTGAGATGGACGCAC
6	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATGCGAGTCCCGTCGAGATGGACGCAC
7	TGGTCTCCCGTGTCTATGATGCGGTTGGCCTAAATACGAGTCCCATCGAGATGGACGCAC
8	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATACGGGTCCCGTCGAGATGGACGCAC
9	TGGTCTCCYGTGTCYATGATGCGGTTGGCCTAAATACGAGTCCCGTCGAGATGGACGCAC
10	TGGTCTCCCGTGTCCATGATGTGGTTGGCCTAAATACGAGTCCCRTCGAGATGGACGCAC
11	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATACGAGTCCTGTCGAGATGGACGCAC
12	TGGTCTCCCGTGTCCATGATGCGGCTGGCCTAAATGCGAGTCCCGTCGAGATGGACGCAC
13	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATGCGAGTCCCGTCGAGATGGACGCAC
14	TGGTCTCCCGTGTCCATGATGCGGCTGGCCTAAATGCGAGTCCCGTCGAGATGGACGCAC
15	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATACGAGTCCCGTCGAGATGGACGCAC
16	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATACGAGTCCCGTCGAGATGGACGCAC
17	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATACGAGTCCCGTCGAGATGGACGCAC
18	[illegible]
19	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATACGAGTCCCGTCGAGATGGACGCAC
20	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATACGAGTCCCGTCGAGATGGACGCAC
21	TGGTCTCCCGTGTCCATGATGCGGTTGGCCTAAATACGAGTCCTGTCGAGATGGACGCAC
	550 . 560 . 570 . 580 . 590 . 600 .
1	GACTARTGGTGGTTGATTACACAGTCGTCTCGTGTCGTGCGTCTTGATTCTTGAGGSGTA
2	GACTAGTGGTGGTTGATTACACAGTCGTCTCGTGTCGTGCGTTTTGATTCTTGAATTGGA
3	GACTAGTGGTGGTTGATAACACAGTCGTCTCGTGTTGTGCGTTTTGATTCTTGAAGGGRA
4	GATTAGTGGTGGTTGATTACACAGTCGTCTCGTGTCGTGCGTTTTGATTCTTGAAGGGGG
5	GATTAGTGGTGGTTGATTACACAGTCGTCTCGTGTCGTGCGTTTTGATTCTTGAAGGGGG
6	GACTAGTGGTGGTTGATTACACAGTCGTCTCGTGTCGTGCGTTTTGATTCTTGAAGGGTG
7	GACTAGTGGTGGTTGATTACACAGTCGTCTCGTGTCGTGCGTTTTTATTCTTGAAGGGTG
8	GACTAGTGGTGGTTGATTACACAGTCGTCTCGTGCCGTGCGTTTTGATTCTTGAAGGGTG
9	[illegible]

TABLE 2. *Continued*

Taxa[a]	Nucleotide sites[b]
	610 . 620 . 630 . 640 .
1	AGGCTCTTAGAATA-CCCTGATGTGTTGTCTTCTGATGGCGCTTCGA
2	ATACTCTTAAAATA-CCCTGATGTGTTGTCTTCTGATGGCGCTTCGA
3	AGACTCTTAAAATAACCCCAATGTGTTGTCTTTTGATGGCGCTTCGA
4	AGACTCTTATAATAACCCTGATGTGTTGTCTTCTGAYGGCGCTTCGA
5	AGACTCTTATAATAACCCTGATGTGTTGTCTTCTGAYGGCGCTTCGA
6	AGACTCCTAAAATAACCCTAACGTGTTGTCTTCTGATGGCTCTTCGA
7	AGACTCTTAAAATAACCCTAACGTGTTGTCTTTTGATGGCGCTTCGA
8	AGACTCTTAAAATAACCCCGACGTGTTGTCTTCTGATGGCGCTTCGA
9	AGACTCTTAAAATAACCCYGACGTGTTGTCTTTTGATGGCGCYTCGA
10	AGACTCTTAAAATAACCCCGACGTGTTGTCTTTTGATGGCGCTTCGA
11	AGACTCTTAAAATAACCCCGACGTGTTGTCTTCTGATGGCGCTTCGA
12	AGACTCTTAAAATAACCCTGACGTGTTGTCTTCTGATGGCGCTTCGA
13	AGACTCTTAAAATAACCCTGACGTGTTGTCTTCTGATGGCGCTTCGA
14	AGACTCTTAAAATAACCCTGACGTGTTGTCTTCTGATGGCGCTTCGA
15	AGACTCTTAAAATAACCCTGACGTGTTGTCTTCTGATTGCGCTTCGA
16	AGACTCTTAAAATAACCCTGACGTGTTGTCTTCTGATTGCGCTTCGA
17	AGACTCTTAAAATAACCCCGACGTGTTGTCTTCTGATTGCGCTTCGA
18	AGACTCTTAAAATAACCCCGACGTGTTGTCTTCTGATTGCGCTTCGA
19	[illegible]
20	AGACTCTTAAAATAACCCCGACGTGTTGTCTTCTGATTGCGCTTCGA
21	AGACTGTTAAAATAACCCTGATGTGTTGTCTTCTGATTGCGCTTCGA

[a] 1, *Arnica mollis*; 2, *Hemizonia perennis*; 3, *Osmadenia tenella*; 4, *Calycadenia mollis* (Fresno Co.); 5, *C. mollis* (Madera Co.); 6, *C. truncata* subsp. *truncata*; 7, *C. t.* subsp. *scabrella*; 8, *C. hooveri*; 9, *C. villosa* "Depressa"; 10, *C. v.* "Erecta"; 11, *C. spicata*; 12, *C. multiglandulosa* subsp. *bicolor*; 13, *C. multiglandulosa* subsp. *cephalotes*; 14, *C. hispida*; 15, *C. oppositifolia* (Cohasset Road); 16, *C. o.* (Bloomer Lookout Road); 17, *C. ciliosa* "Lewiston"; 18, *C. c.* "Corning"; 19, *C. fremontii*; 20, *C. pauciflora* "Pauciflora"; 21, *C. p.* "Elegans".

[b] Numbered 5′ to 3′ from the 18S subunit/ITS 1 border to the ITS 2/26S subunit border. A, C, G, T = dATP, dCTP, dGTP, dTTP. K = G/T; M = A/C; N = A/C/G/T or uncertain nucleotide state; R = A/G; S = C/G; W = A/T; Y = C/T. Hyphens = gaps or missing nucleotides.

[c] Outgroup species.

strict consensus of these 11 trees is presented in Fig. 2. One of these 11 trees is shown in Fig. 3 to indicate types and numbers of point mutations supporting each clade, as optimized by ACCTRAN in PAUP, and phylogenetic information from length mutations.

Collapse of phylogeny branches in the strict consensus tree required one to seven or more additional evolutionary steps (Fig. 2). Bootstrap values for the consensus clades ranged from 58% to 100% (Fig. 2). The g_1 statistic for 10,000 random trees from these data was −0.7 (cf. Huelsenbeck, 1991).

Wagner parsimony analysis of the data matrix (Table 4) after removal of all sites with any polymorphisms or gaps yielded one minimum-length tree. This single tree was identical to the strict consensus tree (Fig. 2) from analysis of the entire data matrix.

ITS 2 tree (Fig. 5) did, however, resolve *Calycadenia* as a monophyletic genus, unlike the otherwise better-resolved ITS 1 tree (Fig. 4).

DISCUSSION

ITS sequence comparisons—The ITS region of the study species is similar to other Compositae (Baldwin, 1992), *Vicia faba* (Yokota et al., 1989), and *Sinapis alba* (Rathgeber and Capesius, 1989) in having an ITS 1 spacer that is larger than ITS 2 (see Fig. 1). In contrast, ITS 2 is larger than ITS 1 in other reported angiosperms (*Oryza sativa*, Takaiwa, Oono, and Sugiura, 1985; *Cucumis melo*, Kavanagh and Timmis, 1988; *Lycopersicon esculentum*, Kiss et al., 1988; *Vigna radiata*, Schiebel and Hemleben, 1989;

species (164 bp) is one of two reported sizes of this highly conserved region in angiosperms (163 to 164 bp).

ITS evolution—The ITS region in *Calycadenia* and *Osmadenia* has evolved primarily by point mutations, judging from the moderately high levels of ITS sequence divergence between and even within species (Table 3), the minor proportion of sites that required gaps for sequence alignment (Table 2), and the absence of evident ITS length variants within DNA accessions. Such structural conservatism of ITS sequences has been attributed to their role in the production of mature rRNAs from primary transcripts, i.e., in forming secondary RNA structures that bring the ends of the 18S, 5.8S, and 26S regions into close proximity for processing (Gonzalez et al., 1990; Thweatt and Lee, 1990; Venkateswarlu and Nazar, 1991).

ITS phylogenetic resolutions—*Cytology*—Cytological investigation has provided abundant data on chromosomal relationships at both the infraspecific and interspecific level within *Calycadenia* (Carr, 1975a, b, c, 1977; Carr and Carr, 1983). Meiotic analyses of hybrids and chromosome number distribution in Madiinae led Carr (1975b) to conclude that $n = 7$ was the base chromosome number in *Calycadenia* from which other numbers (n = 4, 5, and 6) were derived by dysploidy. In addition, Carr (1975b) concluded that lack of meiotic chromosomal association in F_1 hybrids between *Osmadenia* (*C.*) *tenella* and *Calycadenia* species gave new justification for resurrection of *Osmadenia* for *C. tenella*. Both of these conclusions from cytology are supported by the ITS region consensus tree (Fig. 2), notwithstanding a possible secondary origin of $n = 7$ in *C. oppositifolia* and an unknown origin of $n = 9$ in *O. tenella*.

The ITS region tree (Fig. 2) further suggests that the n = 5 and 6 lineage (including *C. oppositifolia*, $n = 7$) is monophyletic and originated from a common ancestor with a $n = 7$ genome very similar or identical in structure to that of *C. hooveri* or *C. villosa*. These last two species are visibly differentiated chromosomally by only one reciprocal translocation and one or more paracentric inversions (Carr, 1975a, c).

The ITS region tree (Fig. 2) also suggests that the genome with the lowest chromosome number in *Calycadenia*, that of *C. spicata* ($n = 4$), also arose from a $n = 7$ genome similar or identical in structure to that of either *C. hooveri* ($n = 7$) or *C. villosa* ($n = 7$), but independent of the n = 5 and 6 lineage. This interpretation is congruent with T-gland distribution data (see "Morphology"). The absence of genomic entities bridging this chromosome number gap between $n = 7$ and $n = 4$ suggests considerable extinction or another example of "saltational reorganization of chromosomes" (Carr, 1975b, c, 1980) in *Calycadenia*, as G. D. Carr previously hypothesized for the origin of a chromosome race in *C. pauciflora*. These examples and a recent report of an amazing shift between $n = 8$ and $n = 3$ in *Hymenoxys texana* (J. Coulter & Rose) Cockerell (Strother and Brown, 1988) underscore the need for caution in interpreting chromosome number relationships in Compositae.

Morphology—No explicit phylogenetic treatment of *Calycadenia* morphological data has been attempted. The

TABLE 3. *Pairwise divergence between combined ITS 1 and ITS 2 nucleotide sequences from 21* Calycadenia, Osmadenia, *and outgroup DNAs*[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	—	0.116	0.123	0.128	0.128	0.123	0.123	0.109	0.121	0.114	0.114	0.123	0.133	0.123	0.119	0.119	0.121	0.121	0.121	0.121	0.130
2	50	—	0.093	0.086	0.086	0.088	0.093	0.074	0.095	0.091	0.086	0.098	0.100	0.098	0.093	0.093	0.093	0.093	0.093	0.093	0.098
3	53	40	—	0.093	0.093	0.086	0.079	0.070	0.084	0.086	0.088	0.088	0.093	0.088	0.088	0.088	0.088	0.088	0.088	0.088	0.098
4	55	37	40	—	0.000	0.072	0.065	0.060	0.072	0.067	0.072	0.079	0.086	0.079	0.074	0.074	0.074	0.074	0.074	0.074	0.077
5	55	37	40	0	—	0.072	0.065	0.060	0.072	0.067	0.072	0.079	0.086	0.079	0.074	0.074	0.074	0.074	0.074	0.074	0.077
6	53	38	37	31	31	—	0.037	0.035	0.051	0.051	0.047	0.049	0.056	0.049	0.049	0.049	0.049	0.049	0.049	0.049	0.058
7	53	40	34	28	28	16	—	0.028	0.035	0.040	0.044	0.049	0.056	0.049	0.042	0.042	0.042	0.042	0.042	0.042	0.051
8	47	32	30	26	26	15	12	—	0.026	0.026	0.026	0.033	0.040	0.033	0.023	0.023	0.023	0.023	0.023	0.023	0.037
9	52	41	36	31	31	22	15	11	—	0.014	0.023	0.035	0.042	0.035	0.026	0.026	0.026	0.026	0.026	0.026	0.035
10	49	39	37	29	29	22	17	11	6	—	0.023	0.035	0.042	0.035	0.026	0.026	0.026	0.026	0.026	0.026	0.040
11	49	37	38	31	31	20	19	11	10	10	—	0.035	0.042	0.035	0.026	0.026	0.026	0.026	0.026	0.026	0.035
12	53	42	38	34	34	21	21	14	15	15	15	—	0.016	0.000	0.026	0.026	0.026	0.026	0.026	0.026	0.040
13	57	43	40	37	37	24	24	17	18	18	18	7	—	0.016	0.033	0.033	0.033	0.033	0.033	0.033	0.047
14	53	42	38	34	34	21	21	14	15	15	15	0	7	—	0.026	0.026	0.026	0.026	0.026	0.026	0.040
15	51	40	38	32	32	21	18	10	11	11	11	11	14	11	—	0.000	0.005	0.005	0.005	0.005	0.019
16	51	40	38	32	32	21	18	10	11	11	11	11	14	11	0	—	0.005	0.005	0.005	0.005	0.019
17	52	40	38	32	32	21	18	10	11	11	11	11	14	11	2	2	—	0.000	0.000	0.000	0.019
18	52	40	38	32	32	21	18	10	11	11	11	11	14	11	2	2	0	—	0.000	0.000	0.019
19	52	40	38	32	32	21	18	10	11	11	11	11	14	11	2	2	0	0	—	0.000	0.019
20	52	40	38	32	32	21	18	10	11	11	11	11	14	11	2	2	0	0	0	—	0.019
21	56	42	42	33	33	25	22	16	15	17	15	17	20	17	8	8	8	8	8	8	—

[a] Numbers of divergent sites from pairwise sequence comparisons appear in the lower left half of matrix. Proportions of divergent sites to total sites appear in the upper right half of matrix. Comparisons were limited to nucleotide positions without gaps or polymorphic states (428 of 483 positions).

TABLE 4. *Matrix of ITS nucleotide positions with potential phylogenetic information from 21* Calycadenia, Osmadenia, *and outgroup species*

Taxa[a]	Nucleotide sites[b]
	1111111111122222222234444444444455555555555555666666
	14566667778902233345679001222235944555555566001122277999012233
	39501291488182335949039470134834646023457917026745856249090238
1[c]	TCGTCG-CTGACTTTGACTCCTAYGATTCTCCTTATGCCAGTCACCATCAATCTATATGTCG
2[c]	TCACCGTCCGTCTTCTACCCCTACGTTTCTTCTCACTAGTGTCATCACTGGTCTAGATGTCG
3	TAGCCGCTCGTCTGCGACCCCCACTTTTCCTCCCTTTAT-GGCACCATCGATTTARACATTG
4,5	TTACAGCTCGTCTGTTATTCCTTTGTTTCTTTCC-TCATTGGTATTATCAGTCTAGGTGTCG
[illegible]	[illegible]
7	TTGCCGTTCTTCTGCCATTCCCTCGATTCCCGCATTCACTAGTATCACCAGTCTATGTACTG
8	TCGCCGTTCGCCCGCGATCCCCTCGCTTCCCGCATTYACCAGCATCACCGGCCTATGCGCCG
9	TCGTCGTTCGCTCGCGGTTCCCTCGCTTTCCGCATTCAYTAGTGTCACCGGTCCAGGYGCTG
10	TCGTCGTTCGCTCGCGGTTCCTTCGCTTTCCGCATYCACTAGCGTTACCRGTCYAGGCGCTG
11	TCGTCGTTCGCTCGCGATTCCCTCGCTTTTCGCATTCACTAGCGTCACTGGTCCATGCGCCG
12,14	CCGCCATTGGCTCGCGATTACCTCGTCTTCCGCATTCCATAGCGTCGCCGGTTCATGTGCCG
13	CCGCCATTGGCTCACGATTACCTTGTCTTCCGCATTCACTAGCGTCGCCGGTTCATGTGCCG
15,16	TCGCCGTTAGCTCGCGATTAACTCGCCTTCCGCATCCATCAGCGTCACCGGTCCATGTGCCT
17	TCGCCGTTAGCTTGCGATTAACTCCCCATCCGCATTCAGCAGCGTCACCGGYCCGTGCGCCT
18	TCGCCGTTAGCTTGYGATTAACTCCCCATCCGCATTCAGCAGCGTCACCGGTCCGTGCGCCT
[illegible]	[illegible]
20	TCGCCGTTAGYTYGCGATTAACTCSCCATCCGCATTCASCAGCGTCACCGGYCCRNGCGCCT
21	TCGCTGTTAGCTCGCGATTAACTCGC-TTCCGCATTCACTAGTGTCACTGGTCYATGTGTCT

[a] Sample numbers assigned in Table 2. Two-number designations indicate merged sequences that are identical at all potentially informative sites.
[b] Nucleotide sites designations and sequence symbols defined in Table 2.
[c] Outgroup species.

phylogenetic distribution of discrete morphological character-states in the *Calycadenia* ITS region tree (Fig. 2) can, however, be assessed by parsimony.

Calycadenia was named for the unique vascularized, tack-shaped, or T-shaped, glandular appendages that occur on the leaves surrounding the capitulae in all of the species (Carlquist, 1959). *Osmadenia* is unique from *Calycadenia* in lacking these glands and for possessing beaked achenes, a feature found in most other *Hemizonia*-like tarweeds (three of four *Hemizonia* sections and *Holocarpha*). The ITS region tree (Fig. 2) suggests retention of beaked achenes in *Osmadenia* and a single origin of T-glands in *Calycadenia* following divergence from a common ancestor with *Osmadenia*.

Within *Calycadenia*, the distribution of T-glands on

by a morphological character. The species of this lineage have smooth ray-achene surfaces, unlike the irregular, wrinkled ray-achene surfaces of *C. mollis* and *C. truncata*.

Biogeography—Two tentative conclusions about the biogeographic history of *Calycadenia* are possible based on the ITS region phylogeny (Fig. 2), despite extensive and overlapping distributions of several species. *Calycadenia* and *Osmadenia* are both endemic to the California Floristic Province (Raven and Axelrod, 1978). Unlike the species of *Calycadenia, O. tenella* occurs in the southwestern California Floristic Province, from Los Angeles County to northern Baja California. *Calycadenia* species occur from central western California and the Tehachapi Mountains, i.e., San Luis Obispo and Kern

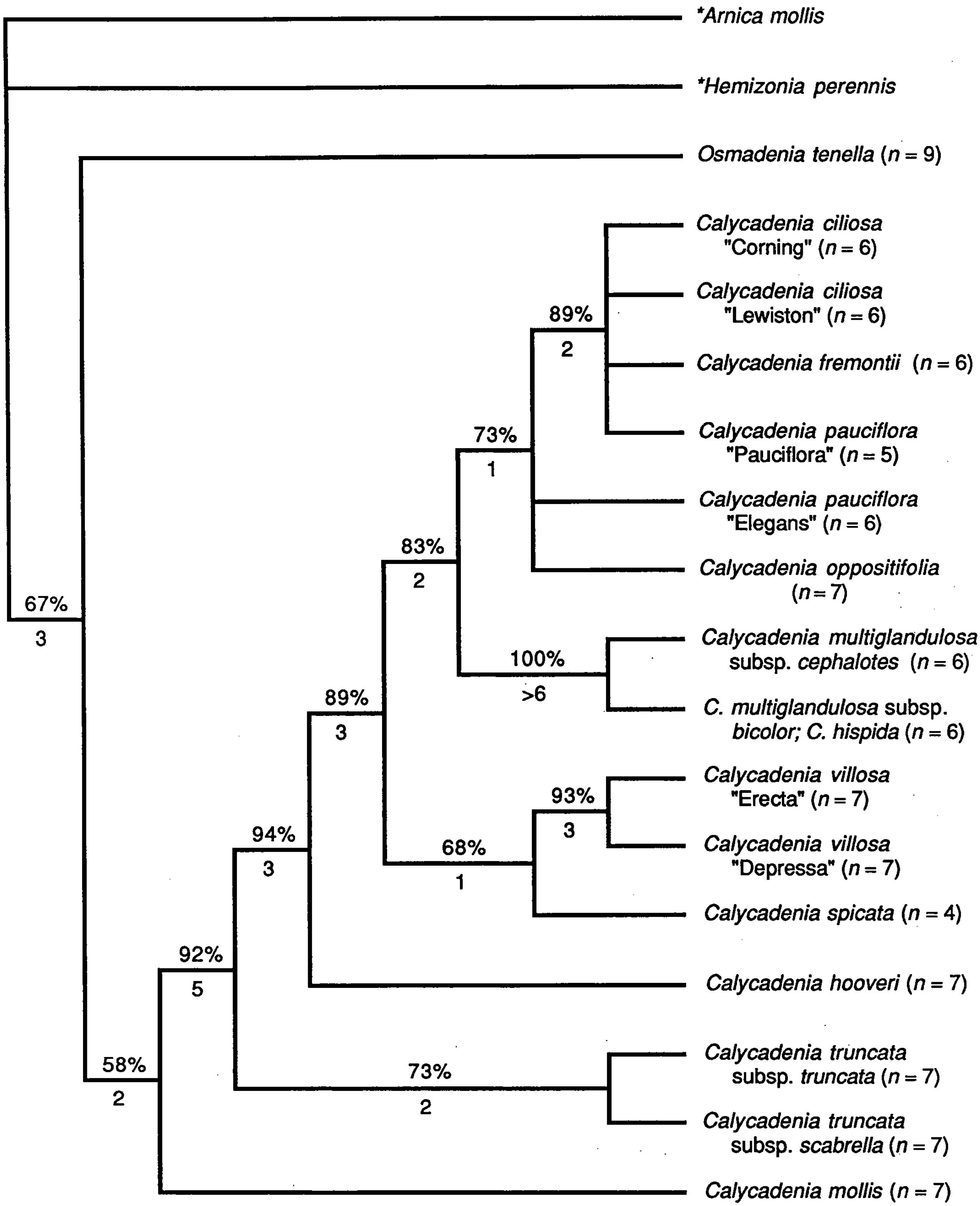

Fig. 2. Strict consensus of 11 maximally parsimonious Wagner trees from analysis of potentially informative ITS region sequence variation among *Calycadenia, Osmadenia,* and outgroup species (Table 4). Percentages above branches are bootstrap values. Numbers below branches are decay index values, i.e., numbers of additional evolutionary steps required to break the corresponding sister-group relationship in at least one of the maximally parsimonious trees. n = haploid chromosome number. For each tree: consistency index = 0.61; retention index = 0.71; tree length = 127 steps.

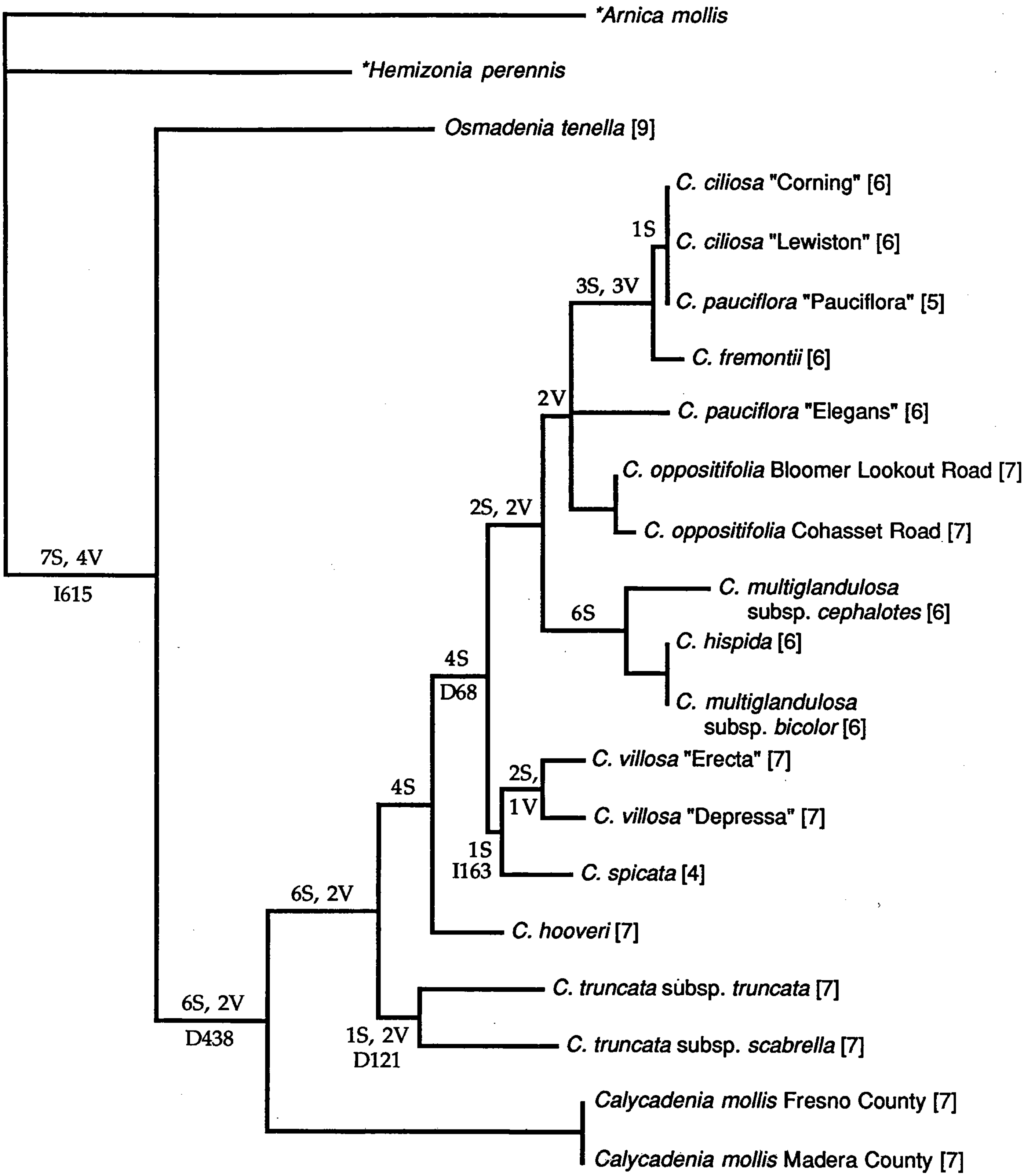

Fig. 3. One of the 11 maximally parsimonious Wagner trees from analysis of ITS region sequence variation among the *Calycadenia, Osmadenia,* and outgroup species. Branch lengths correspond to numbers of mutations, with uninformative variation reincluded, as optimized by ACCTRAN (PAUP). Numbers of transition (S) and transversion (V) mutations that define branch-points are indicated. Phylogenetically informative deletion (D) and insertion (I) mutations, not included in the analysis, are indicated by sequence position in Table 2. Only those length mutations whose branch assignment is unambiguous are shown. Numbers in brackets are haploid chromosome numbers.

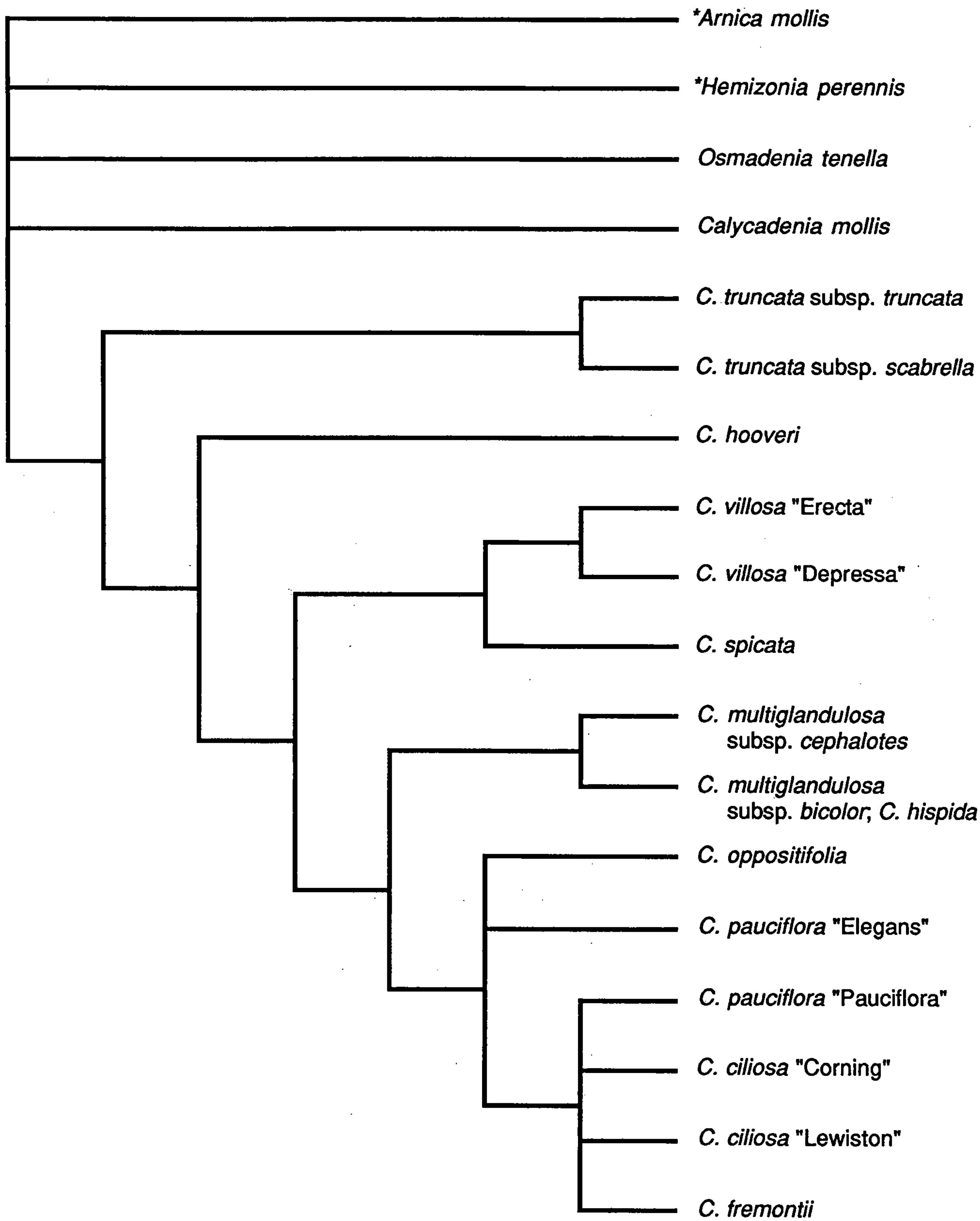

Fig. 4. Strict consensus of six maximally parsimonious trees from analysis of ITS 1 sequence variation among *Calycadenia, Osmadenia,* and outgroup species. For each tree: consistency index = 0.68; retention index = 0.79; tree length = 62.

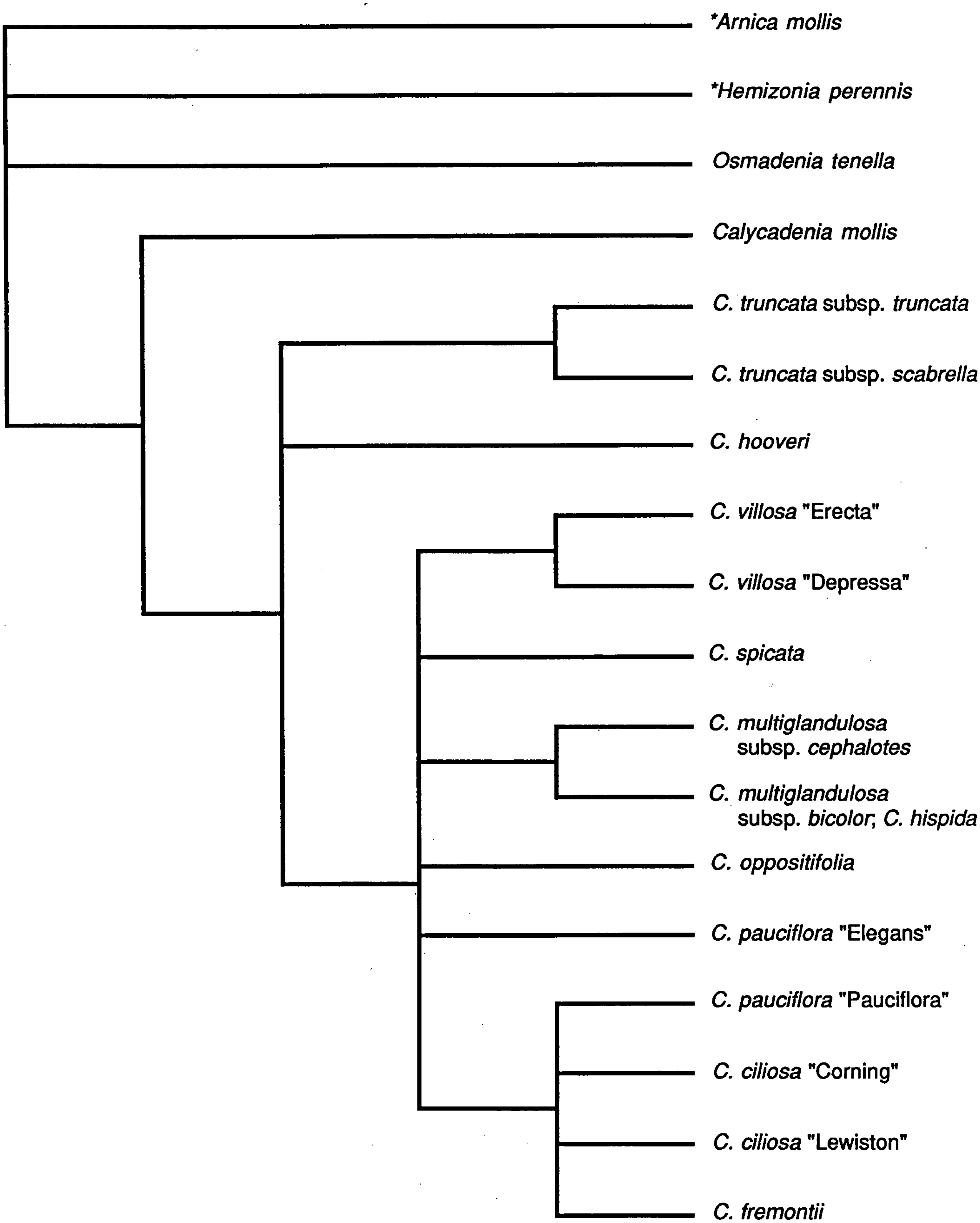

Fig. 5. Strict consensus of 90 maximally parsimonious trees from analysis of ITS 2 sequence variation among *Calycadenia, Osmadenia,* and outgroup species. For each tree: consistency index = 0.53; retention index = 0.61; tree length = 64.

divergence was found between populations of a species. Of the seven species represented by DNAs from more than one population, four were monophyletic and one was potentially monophyletic in the ITS region consensus tree (Fig. 2). The remaining two species, *C. multiglandulosa* and *C. pauciflora,* sensu Keck (1959), were paraphyletic.

Unlike any other pair of *Calycadenia* species, *C. multiglandulosa* and *C. hispida* intergrade morphologically and have structurally identical nuclear genomes (Carr, 1977). Based on these criteria, submergence of *C. hispida* within *C. multiglandulosa,* the species with priority, has been proposed (G. D. Carr and R. L. Carr, personal communication) and is in accord with the ITS region consensus tree (Fig. 2) resolution.

Calycadenia multiglandulosa subsp. *bicolor,* unlike the coastal *C. m.* subsp. *cephalotes,* has a Sierra Nevada foothill distribution that strongly overlaps that of *C. hispida.* The close ITS relationship of *C. m.* subsp. *bicolor* and *C. hispida* (Figs. 2, 3) suggests 1) a common Sierran foothill origin with subsequent ecological isolation and differentiation, 2) hybridization between these interfertile entities, or 3) lineage sorting. These plants are somewhat isolated ecologically. *Calycadenia multiglandulosa* is generally found on serpentine-derived soils, whereas *C. hispida* is not. A hybrid swarm between these taxa was, however, recorded by G. D. Carr in Tuolumne County, California near Rawhide (*G. D. Carr 599,* DAV). Examination of ITS sequences from additional populations of *C. multiglandulosa* and *C. hispida* on both sides of the Central Valley in California is necessary to confidently assess the biogeographic significance of these findings.

The ITS region phylogeny (Fig. 2) and morphological and cytological investigations (Carr, 1975a, b, c; Carr and Carr, 1983) strongly indicate that *C. pauciflora* sensu Keck is not monophyletic. As presently recognized, *C. pauciflora* encompasses several chromosomal and morphological "races." The two entities examined in this study, known informally as "Pauciflora" ($n = 5$) and "Elegans"

headed but self-incompatible *C. pauciflora* entities. The ITS region tree (Fig. 2) corroborates G. D. Carr's hypothesis (Carr, 1975b, c) that *C. hooveri* is not part of the *C. pauciflora* species complex. The position of *C. hooveri* on the ITS tree indicates that it is, in fact, a distinctive element of the basal $n = 7$ grade with which *C. villosa* is associated.

Evolutionary divergence and chromosomal homology—One impetus for assessing molecular phylogeny of *Calycadenia* was to examine the hypothesis that extent of meiotic chromosomal association in hybrids between diploid species is negatively correlated with time since their divergence from a common ancestor. This hypothesis has formed an important basis for taxonomic decisions (e.g., generic delimitation) by some cytotaxonomists.

In *Calycadenia,* the species can be divided into two groups on the basis of meiotic chromosomal pairing in hybrids (Carr, 1975b, 1977). Hybrids between species of the first group are characterized by a high degree of meiotic chromosomal association in multivalents or bivalents. These species are *C. villosa, C. hooveri, C. spicata, C. multiglandulosa, C. hispida, C. oppositifolia, C. ciliosa, C. pauciflora,* and *C. fremontii.* In contrast, hybrids between species of the second group display primarily univalents at meiosis. This second group comprises *Osmadenia tenella, C. mollis, C. truncata,* and *C. villosa.*

Based on the ITS region tree (Fig. 2), the species whose hybrids display considerable meiotic association of chromosomes indeed share a more recent common ancestry than the species whose hybrids show low meiotic pairing. The ITS region phylogeny does not, therefore, allow rejection of the hypothesis that breakdown of chromosomal homology, as reflected by meiotic association, is an indicator of evolutionary divergence in diploid plants.

ITS and plant phylogeny—Close agreement between the ITS consensus tree (Fig. 2) and parsimony-based interpretations of cytological and morphological data from *Calycadenia* suggests that the ITS region can prove useful

a repeated gene family. *Theoretical and Applied Genetics* 63: 361–365.

Arnheim, N. 1983. Concerted evolution of multigene families. *In* M. Nei and R. Koehn [eds.], Evolution of genes and proteins, 38–61. Sinauer, Sunderland, MA.

———, M. Krystal, R. Schmickel, G. Wilson, O. Ryder, and E. Zimmer. 1980. Molecular evidence for genetic exchanges among ribosomal genes on non-homologous chromosomes in man and apes. *Proceedings of the National Academy of Sciences, USA* 77: 7323–7327.

Baldwin, B. G. 1989. Chloroplast DNA phylogenetics and biosystematic studies in Madiinae (Asteraceae). Ph.D. dissertation, University of California. Davis, CA.

———. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.

———, D. W. Kyhos, J. Dvorak, and G. D. Carr. 1991. Chloroplast DNA evidence for a North American origin of the Hawaiian silversword alliance (Asteraceae). *Proceedings of the National Academy of Sciences, USA* 88: 1840–1843.

Carlquist, S. 1959. The leaf of *Calycadenia* and its glandular appendages. *American Journal of Botany* 46: 70–80.

Carr, G. D. 1975a. *Calycadenia hooveri* (Asteraceae), a new tarweed from California. *Brittonia* 27: 136–141.

———. 1975b. Chromosome evolution and aneuploid reduction in *Calycadenia pauciflora* (Asteraceae). *Evolution* 29: 681–699.

———. 1975c. Chromosome evolution and aneuploid reduction in *Calycadenia pauciflora* (Asteraceae). Ph.D. dissertation, University of California. Davis, CA.

———. 1977. A cytological conspectus of the genus *Calycadenia* (Asteraceae): an example of contrasting modes of evolution. *American Journal of Botany* 64: 694–703.

———. 1980. Experimental evidence for saltational chromosome evolution in *Calycadenia pauciflora* Gray (Asteraceae). *Heredity* 45: 107–112.

Carr, R. L., and G. D. Carr. 1983. Chromosome races and structural heterozygosity in *Calycadenia ciliosa* Greene (Asteraceae). *American Journal of Botany* 70: 744–755.

Donoghue, M. J., R. G. Olmstead, J. F. Smith, and J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. *Annals of the Missouri Botanical Garden* 79: 333–345.

Doyle, J. J. 1992. Gene trees and species trees: molecular systematics

of the 17S-25S spacer region from tomato rDNA. *Nucleic Acids Research* 16: 7179.

Kyhos, D. W., G. D. Carr, and B. G. Baldwin. 1990. Biodiversity and cytogenetics of the tarweeds (Asteraceae: Heliantheae-Madiinae). *Annals of the Missouri Botanical Garden* 77: 84–95.

Palmer, J. D. 1986. Isolation and structural analysis of chloroplast DNA. *Methods in Enzymology* 118: 167–186.

———, C. R. Shields, D. B. Cohen, and T. J. Orton. 1983. Chloroplast DNA evolution and the origin of amphidiploid *Brassica* species. *Theoretical and Applied Genetics* 65: 181–189.

Rathgeber, J., and I. Capesius. 1989. Nucleotide sequence of the 18S-25S spacer region from mustard DNA. *Nucleic Acids Research* 17: 7522.

Raven, P. H., and D. I. Axelrod. 1978. Origin and relationships of the California flora. *University of California Publications in Botany* 72: 1–134.

Rieseberg, L. H., and D. E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* 5: 65–84.

Rogers, S. O., and A. J. Bendich. 1987. Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. *Plant Molecular Biology* 9: 509–520.

Schiebel, K., and V. Hemleben. 1989. Nucleotide sequence of the 18S-25S spacer region from rDNA of mung bean. *Nucleic Acids Research* 17: 2852.

Soltis, D. E., P. S. Soltis, and B. D. Ness. 1989. Chloroplast-DNA variation and multiple origins of autopolyploidy in *Heuchera micrantha* (Saxifragaceae). *Evolution* 43: 650–656.

Soltis, P. S., and D. E. Soltis. 1991. Multiple origins of the allotetraploid *Tragopogon mirus* (Compositae): rDNA evidence. *Systematic Botany* 16: 407–413.

Stebbins, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York, NY.

Strother, J. L., and L. E. Brown. 1988. Dysploidy in *Hymenoxys texana* (Compositae). *American Journal of Botany* 75: 1097–1098.

Swofford, D. L., and G. J. Olsen. 1990. Phylogeny reconstruction. *In* D. Hillis and C. Mortiz [eds.], Molecular systematics, 411–501. Sinauer, Sunderland, MA.

Takaiwa, F., K. Oono, and M. Sugiura. 1985. Nucleotide sequence of the 17S-25S spacer region from rice rDNA. *Plant Molecular Biology* 4: 355–364.

Thweatt, R., and J. C. Lee. 1990. Yeast precursor ribosomal RNA: