BIOSYSTEMATIC ANALYSIS OF THE *THELESPERMA SUBNUDUM* COMPLEX (ASTERACEAE)

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ABSTRACT

Chromosome studies and phylogenetic analyses of morphology, allozymes, and nrDNA-*ITS* sequence data were used to resolve taxonomic relationships in the *Thelesperma subnudum* complex. Of special interest is the placement of three globally rare members of the complex: *Thelesperma caespitosum*, *T. pubescens*, and *T. subnudum* var. *alpinum*. The first two rare taxa yield only diploid (*n*=12) chromosome counts; *T. subnudum* var. *alpinum* is consistently tetraploid (*n*=24). *Thelesperma subnudum* var. *subnudum* exhibits both diploid (*n*=12) and tetraploid (*n*=24) populations. Phylogenetic analyses of individual data sets strongly support (bootstrap 88%–99%) *T. subnudum* var. *alpinum* as being more closely related to *T. pubescens* and *T. caespitosum* than to *T. subnudum* var. *subnudum*. Combined analyses give the strongest support (bootstrap 100%) for the clade of *T. pubescens*, *T. caespitosum*, and *T. subnudum* var. *alpinum*. Preliminary evidence suggests that *T. subnudum* var. *alpinum* may be an allotetraploid resulting from hybridization between *T. pubescens* and *T. subnudum*. Based on the available evidence, we propose the following nomenclatural changes: ***Thelesperma pubescens*** Dorn var. ***caespitosum*** (Dorn) C.J. Hansen, stat. nov., and ***Thelesperma windhamii*** C.J. Hansen, nom. et stat. nov.

KEY WORDS: *Thelesperma*, Asteraceae, nrDNA-*ITS* sequences, phylogenetics, and systematics.

RESUMEN

Se han usado estudios cromosómicos y análisis filogenéticos de datos morfológicos, alozimas y de secuencias de nrDNA-ITS para tratar de resolver las relaciones taxonómicas dentro del complejo *Thelesperma subnudum*. De especial interés es la posición taxonómica de los tres miembros del complejo: *Thelesperma caespitosum*, *T. pubescens* y *T. subnudum* var. *alpinum*, considerados globalmente raros. Los dos primeros taxa mostraron solamente poblaciones diploides (*n* = 12), mientras que *T. subnudum* var. *alpinum* se mostró constantemente como tetraploide (*n* = 24). *Thelesperma subnudum* var *subnudum* mostró tanto poblaciones diploides (*n* = 12) como tetraploides (*n* = 24). Los resultados del análisis filogenético de diferentes tipos de datos soportan más fuertemente (bootstrap 88%–99%) el agrupamiento de *T. subnudum* var. *alpinum* con *T. caespitosum* y *T. pubescens*, que su agrupamiento con *T. subnudum* var. *subnudum*. Los análisis combinados mostraron el máximo soporte (bootstrap 100%) para el clado compuesto por *T. subnudum* var. *alpinum*, *T. caespitosum* y *T. pubescens*. Evidencias preliminares sugieren que *T. subnudum* var. *alpinum* podría ser un alotetraploide resultante de la hibridación entre *T. subnudum* y *T. pubescens*. De acuerdo con la evidencia disponible proponemos los siguientes cambios nomenclaturales: ***Thelesperma pubescens*** Dorn var. ***caespitosum*** (Dorn) C.J. Hansen, stat. nov. y ***Thelesperma windhamii*** C.J. Hansen, nom. et stat. nov.

PALABRAS CLAVE: *Thelesperma*, Asteraceae, nrDNA-*ITS* secuenciación, filogenética y sistemática.

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Thelesperma Lessing (Asteraceae: Heliantheae, Coreopsidinae) comprises about 15 species, mostly herbaceous perennials (annuals and subshrubs rare), native to south-central and western North America and warm-temperate South America (Melchert 1963; Bremer 1994a). The genus is thought to be monophyletic based on shared characters such as strongly dimorphic involucral bracts, scarious-margined inner involucral bracts that are connate from 1/5 to 1/2 their lengths, opposite leaves, and pappi that are absent or composed of hispid or serrulate awns (Bremer 1994a). Previous taxonomic studies of *Thelesperma* have focused on morphology (Shinners 1950a, b; Alexander 1955) and cytology (Melchert 1963; Greer 1997; Greer & Powell 1999) and were not explicitly phylogenetic. Nevertheless, past work on the genus has identified some species groups that may be monophyletic. One of these is the *Thelesperma subnudum* complex, which is restricted to the Colorado Plateau and areas adjacent to the Rocky Mountains in western North America (Fig. 1). This group includes five commonly accepted taxa: *T. subnudum* A. Gray var. *subnudum*, *T. subnudum* A. Gray var. *alpinum* S.L. Welsh, *T. pubescens* Dorn, *T. caespitosum* Dorn and *T. marginatum* Rydb. Features used to distinguish members of the *T. subnudum* complex are given in Table 1.

Delimitation of taxa within the *Thelesperma subnudum* complex has varied, and at least three classifications have appeared (Table 2). Dorn (1990), who discovered and named *T. pubescens* and *T. caespitosum*, recognized each as distinct species. He treated *T. marginatum* as a separate species and accepted *alpinum* as a variety of *T. subnudum*. In the 2nd edition of *A Utah Flora*, Welsh et al. (1993) classified both *T. caespitosum* and *T. subnudum* var. *alpinum* as varieties of *T. subnudum*; *T. marginatum* and *T. pubescens* were not treated because the former is not known from Utah and discovery of the latter in Utah postdates publication of the flora. Cronquist et al. (1994) recognized only *T. subnudum* and *T. pubescens* at the species level; *T. caespitosum* and *T. subnudum* var. *alpinum* were included within *T. pubescens*, and *T. marginatum* was treated as a variety of *T. subnudum*.

These divergent classifications result from differing interpretations of the available morphological and cytological information. Additional genetic data (e.g., allozyme and DNA) are needed to allow an informed choice between competing taxonomies. The need to pursue these studies has been heightened recently by conservation concerns. *Thelesperma pubescens*, *T. caespitosum*, and *T. subnudum* var. *alpinum* are rare taxa restricted to a few localized populations (Fig. 1). All three have been listed as potentially endangered (category 2) by the U.S. Fish and Wildlife Service (U.S. Dept. of Interior 1985, 1993). The accumulation of genetic data is an important step in determining their eligibility for federal protection under the Endangered Species Act.

Our objectives in this study are threefold: 1) to develop baseline genetic data for members of the *Thelesperma subnudum* complex, 2) to analyze the data in

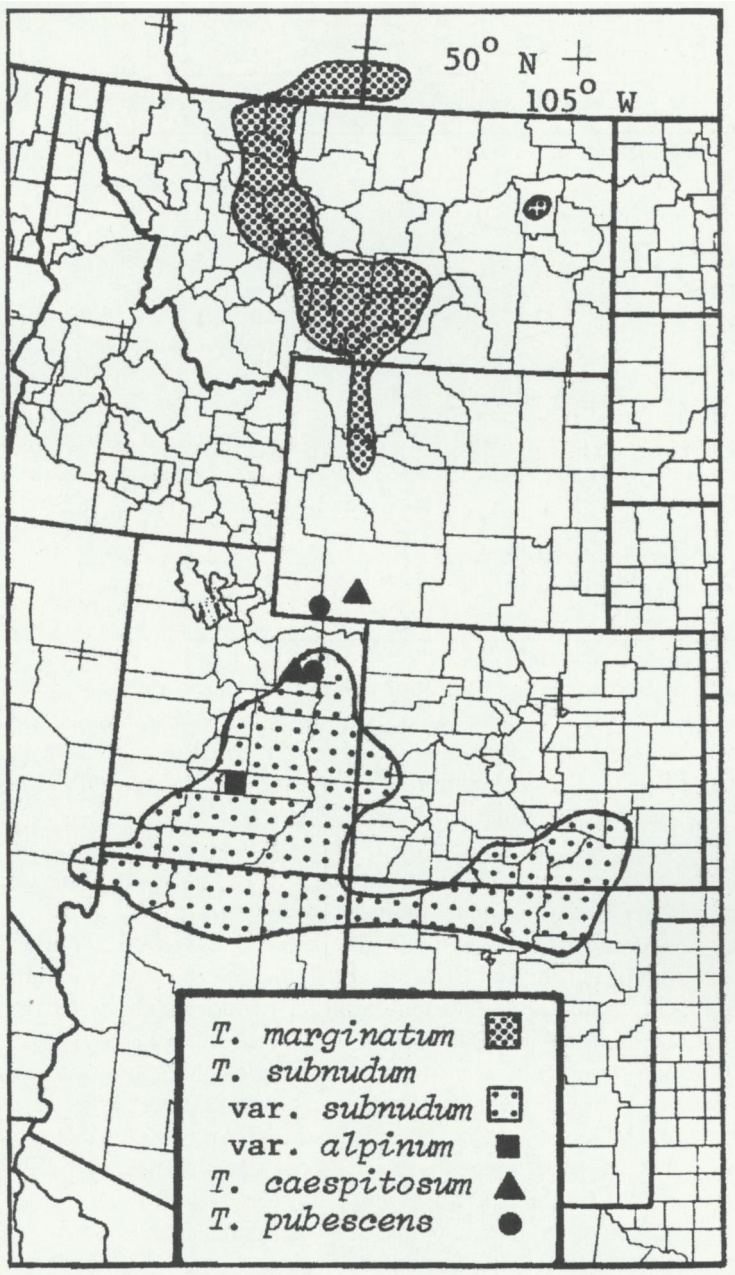

FIG. 1. Distribution of taxa in the *Thelesperma subnudum* complex. (Modified from Dorn 1990).

TABLE 1. Selected morphological, ecological, and distributional features distinguishing taxa of the *Thelesperma subnudum* complex. Modified from Dorn 1990.

Taxa	Leaf segments	Pubescence	Heads	Rootstock	Habitat	Elevation (m)
T. subnudum	long, broad	none	1-several, radiate (rarely) discoid	somewhat creeping	common, sandy soils	1050–2310
T. subnudum var. *alpinum*	short, narrow	lower stems & leaves	1(2), discoid	somewhat creeping, branched caudex	specialized, Carmel Form. Navajo Sandstone	1830–2680
T. marginatum	long, broad	none	1-several, discoid	somewhat creeping	common, sandy out wash	2130–2835
T. pubescens	short, narrow	leaves	1(2), discoid	branched caudex	specialized, weathered Bishop conglomerate	2430–2715
T. caespitosum	short, narrow	petioles (lower blade)	1(2), discoid	branched caudex	specialized, whitish shale	1220–2680

concert with morphology to produce an explicit phylogenetic hypothesis for these taxa, and 3) to compare that hypothesis to existing classifications and propose any desirable taxonomic changes. Through this process, we hope to shed light on the relationships and possible origin of *T. subnudum* var. *alpinum*, the rarest and most enigmatic member of the group.

MATERIAL AND METHODS

Determination of Chromosome Numbers/Ploidy Level

Ploidy levels were determined for all taxa in the *T. subnudum* complex using a combination of chromosome counts and analyses of allozyme banding patterns. For chromosomal observations, capitula (in bud) were obtained from field populations and fixed in Farmer's solution (3 parts ethanol:1 part glacial acetic acid). Chromosome counts were made from meiotic figures obtained using standard squash techniques and acetocarmine staining (Turner & Johnston 1961; Strother 1972). Preparations were examined under phase contrast on a Zeiss Axioplan2 Microscope. Images of chromosome squashes were saved electronically using Zeiss Image® software from Carl Zeiss, Inc.

In populations for which flower buds were unavailable, ploidy level was determined by careful examination of allozyme banding intensities across a variety of enzymes (Danzmann & Bogart 1982a; Dessauer & Cole 1984; Pryer & Haufler 1993). Because allozyme markers are additive, codominant, and

TABLE 2. Previous taxonomic treatments of *Thelesperma subnudum* and allied taxa.

Dorn (1990)	Welsh et al. (1993)	Cronquist et al. (1994)
T. subnudum var. *subnudum*	*T. subnudum* var. *subnudum*	*T. subnudum* var. *subnudum*
T. subnudum var. *alpinum*	*T. subnudum* var. *alpinum*	*T. pubescens*
T. pubescens	Not treated	*T. pubescens*
T. caespitosum	*T. subnudum* var. *caespitosum*	*T. pubescens*
T. marginatum	Not treated	*T. subnudum* var. *marginatum*

inherited in a Mendelian fashion, the expressions of alleles at various ploidy levels are expected to be proportional to their gene dosages. In our study, chromosomally documented diploids in *Thelesperma* always showed balanced band patterns at heterozygous loci. If a heterozygote showed unequal band intensities of 3:1 (in a monomeric enzyme) or 9:4:1 (in a dimeric enzyme), the particular individual always proved to be tetraploid (the only type of polyploid encountered during our study). Thus, chromosomally unknown populations could be assigned to a specific ploidy level based on the presence or absence of unbalanced heterozygous allozyme patterns.

Morphological Data

Our coding of morphological character states is based on observation of approximately 300 herbarium sheets from ASC, BRY, GH, MONTU, NMU, NY, RM, UT, and UTC (herbarium designators follow Holmgren et al. 1990). These data were supplemented by information obtained from Melchert (1963), Welsh (1983), Dorn (1983, 1990), Jansen et al. (1991), Ryding & Bremer (1992), Welsh et al. (1993), Cronquist et al. (1994), and Karis & Ryding (1994). Ingroup taxa included *T. subnudum* var. *subnudum*, *T. subnudum* var. *alpinum*, *T. pubescens*, *T. caespitosum*, and *T. marginatum*, plus the related species *T. filifolium* (Hook.) A. Gray, *T. longipes* A. Gray, and *T. megapotamicum* (Spreng.) Kuntze. *Bidens* has been identified as a possible sister genus to *Thelesperma* (Ryding & Bremer 1992) and two species of that diverse genus, *B. cernua* L. and *B. frondosa* L., were chosen as outgroup taxa. A total of 16 characters (14 binary and 2 multi-state) was included in the morphological analysis (Tables 3, 4).

nr DNA-ITS Sequence Data

Samples used in the DNA study are identified by their GenBank accession numbers in Table 5. *ITS* sequences for the outgroups *Bidens cernua* and *B. frondosa* were obtained from GenBank; voucher data for these collections can be found in Ganders et al. (2000). The *ITS* sequences for all *Thelesperma* taxa were obtained directly by extracting total DNA from the leaf tissue of dried specimens using a basic CTAB extraction protocol (Hillis et al. 1996). Two different individuals from each taxon were sampled in order to check for intraspecific differences. The *ITS*-1 & 2 and 5.8S regions were amplified using the polymerase chain

TABLE 3. Morphological characters and character states used in phylogenetic analysis. All characters treated as unordered.

1. Herbs (0); subshrubs (1).
2. Perennial (0); annual (1).
3. Plants with taproots, sometimes creeping (0); fibrous roots (1); rhizomes (2).
4. Plants without a branching caudex (0); plants with a branching caudex (1).
5. Plants not marcescent basally (0); plants marcescent basally (1).
6. Leaves cauline (0); leaves basal, much reduced distally (1).
7. Leaves simple, entire or dentate only (0); leaves pinnately or ternately lobed (1).
8. Leaves glabrous (0); only leaf petioles or leaf margins pubescent (1); leaves pubescent throughout (2).
9. Inner involucral bracts essentially free (0); inner involucral bracts connate 1/5 or more (1).
10. Inner involucral bracts without scarious margins (0); inner involucral bracts with scarious margins (1).
11. Capitula with ray flowers (0); capitula without ray flowers (1).
12. Disc florets equally lobed (0); disc florets unequally lobed (1).
13. Cypselae straight (0); cypselae incurved (1).
14. Cypselae pubescent throughout (0); cypselae glabrous or apically pubescent only (1).
15. Pappus present (0); pappus absent (1).
16. Stems pubescent (0); stems glabrous (1).

TABLE 4. Morphological data matrix for taxa in *Bidens* and *Thelesperma*. Characters as listed in Table 3. Variable characters are coded as 'v'.

Taxa	Character Number 12345 67891 11111 1 0 12345 6
Bidens cernua	01100 00000 v0000 0
Bidens frondosa	01100 01000 10000 0
Thelesperma caespitosum	00011 11111 10111 1
Thelesperma filifolium	01000 01011 01110 1
Thelesperma longipes	10000 11011 10110 1
Thelesperma marginatum	00000 11011 10110 1
Thelesperma megapotamicum	00200 01011 11110 1
Thelesperma pubescens	00011 11211 10111 1
Thelesperma subnudum	00000 11011 v0110 1
Thelesperma subnudum var. *alpinum*	00011 11011 10110 1

reaction (PCR) and two primers in a 1:1 ratio. Primers used in PCR amplification and DNA sequencing were *ITS*-4 (White et al. 1990) and a modified sequence of *ITS*-5 (White et al. 1990). The latter, designated *ITS*-I, has the sequence: GTC CAC TGA ACC TTA TCA TTT AG (L. Urbatsch, pers. comm.). The following PCR cycling protocol was used in amplification: a 95°C hot start for 12 min.; 40 cycles of: 95°C for 20 sec., 54°C for 30 sec., and 73°C for 1 min.; a 4 min. final extension at

73°C; and ending with a hold at 4°C. To confirm base positions, the *ITS*-1 & 2 and intervening 5.8S regions of *Thelesperma* were sequenced in both directions. The contigs, or unidirectional sequences, were assembled using Sequencher (Gene-Codes, Ann Arbor, MI) and visually checked. Base positions that gave equally strong, contrasting signals on both strands were coded as ambiguous according to the IUPAC-IUB ambiguity code set included in the program Sequencher. All characters were coded as unordered, and gap characters ("-") were treated as missing data rather than a fifth character state (Baldwin 1993).

Allozyme Data

An electrophoretic survey was performed to analyze allozyme variation at a variety of enzyme loci. A total of 765 plants were sampled from 25 natural populations (about 30 plants/population) representing all taxa in the *Thelesperma subnudum* complex, *T. longipes* A. Gray, and one outgroup population of *Bidens cernua* L. (Table 5). Fresh leaf material was collected in the field and kept on ice until returning to the laboratory (2-3 days maximum). Tissue was then ground using a mortar and pestle in the phosphate-PVP extraction buffer of Soltis et al. (1983). Extracts were absorbed onto wicks cut from Whatman 3MM filter paper and stored at -80°C until electrophoresis.

A total of 19 enzymes was surveyed electrophoretically for variability. Six enzymes, representing 11 putative loci, provided consistent, interpretable results. Two buffer systems were used to resolve these enzymes in 11.5% (w/v) starch gels. A tris-citrate/borate buffer system (System 6 of Soltis et al. 1983) provided good resolution for leucine aminopeptidase (LAP), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), and triosephosphate isomerase (TPI). A pH 7.5 modification of the morpholine-citrate buffer system of Odrzykoski and Gottlieb (1984) was used to resolve malate dehydrogenase (MDH) and shikimate dehydrogenase (SkDH). Staining schedules and protocols followed Soltis et al. (1983) and Murphy et al. (1996).

Genotypes were inferred directly from electromorphs observed on the stained gels, based on the assumption that enzyme substructure and compartmentalization parallel those observed in other flowering plants (Gottlieb 1981). When enzymes showed more than one allozyme locus, the most anodal (fastest migrating) locus was designated number 1, the next fastest number 2, etc. If more than one allele was present at a locus, the most anodal allele was designated *a*, the next fastest *b*, etc.

In a departure from common practice, the allozyme alleles detected at each locus were coded as present or absent for each taxon and included in a phylogenetic analysis. Although advocated by some (Mickevich & Johnson 1976; Mickevich & Mitter 1983; Buth 1984), this approach has been criticized by Swofford et al. (1996) because it violates the assumption of character independence. Similar treatment of allozyme data from *Mimulus* sect. *Erythranthe*

TABLE 5. Populations used in morphology, allozyme, and *nrDNA-ITS* studies of *Thelesperma*. Letters before collection numbers identify the following collectors: H = C.J. Hansen; S = C.J. Stubben; W = M. D. Windham; Wo = L. Allphin Woolstenhulme. All vouchers are deposited at the University of Utah herbarium (UT) unless otherwise indicated by upper case herbarium designators (based on Holmgren et al. 1990) following the collection numbers. x = no allozyme samples.

Taxa	(Code)	Geographic origin & voucher	Allozyme sample size	Sources for DNA (GenBank #)
T. caespitosum	(Thca1)	Utah: Duchesne Co.: *H and S 97-80*	30	#AY017361
	(Thca2)	Wyo: Sweetwater Co.: *H and S 97-91*	30	#AY017360
T. filifolium				
var. *filifolium*	(Thfi2)	Tex: Potter Co.: *Higgins 16391* (BRY)	x	#AY017365
var. *intermedium*	(Thfi1)	Wyo: Goshen Co.: *Nelson 25622* (RM)	x	#AY017364
T. longipes	(Thlo2)	NewM: Doña Ana Co.: *S 385*	29	#AY017355
	(Thlo1)	Tex: Culberson Co.: *Higgins 17688* (BRY)	x	#AY017354
T. marginatum	(Thma2)	Wyo: Fremont Co.: *H 97-134*	30	#AY017357
	(Thma1)	Wyo: Natrona Co.: *Hartman 38509* (RM)	x	#AY017356
T. megapotamicum	(Thme1)	NewM: Sandoval Co.: *Atwood et al. 24016* (BRY)	x	#AY017366
	(Thme2)	Utah: San Juan Co.: *Atwood 22534* (BRY)	x	#AY017367
T. pubescens		Wyo: Sweetwater Co.: *H and S 97-101*	29	
	(Thpu1)	Wyo: Uinta Co.: *H and S 97-111*	30	#AY017358
	(Thpu2)	Wyo: Uinta Co.: *H and S 97-117*	30	#AY017359
T. subnudum		Ariz: Coconino Co.: *Wo and H 97-141*	31	
		Ariz: Coconino Co.: *Wo and H 97-142*	33	
		Ariz: Coconino Co.: *Wo and H 97-143*	35	
		Ariz: Mohave Co.: *W 97-330*	30	
	(Thsu2)	Ariz: Mohave Co.: *H et al. 97-43*	39	#AY017351
		Nev: Clark Co.: *W 98-247*	27	
		Utah: Carbon Co.: *W and Heckel 2417*	30	
		Utah: Duchesne Co.: *H and Nielsen 97-71*	30	
		Utah: Emery Co.: *H and Wi 97-66*	30	
		Utah: Garfield Co.: *H 97-79*	30	

continued

Taxa	(Code)	Geographic origin & voucher	Allozyme sample size	Sources for DNA (GenBank #)
		Utah: Grand Co.: *H and W 97-69*	30	
		Utah: Uintah Co.: *H and Nielsen 97-72*	30	
	(Thsu4)	Utah: Washington Co.: *H et al. 97-45*	36	#AY017352
		Utah: Wayne Co.: *H and W 97-57*	30	
		Utah: Wayne Co.: *H and W 97-68*	23	
var. *alpinum*	(Thwi1)	Utah: Wayne Co.: *H 97-73*	31	#AY017362
		Utah: Wayne Co.: *H 97-74*	30	
	(Thwi2)	Utah: Wayne Co.: *Anderson 922* (BRY)	x	#AY017363
Bidens cernua	(Bice)	Utah: Utah Co.: *H 97-144*	30	
		Ganders et al. 2000	x	#U67098
Bidens frondosa	(Bifr)	Utah: Utah Co.: *Welsh 608* (BRY)	x	
		Ganders et al. 2000	x	#U67094

(Windham unpubl.) produced a phylogenetic tree highly concordant with information from other sources, suggesting that these data may contain a strong phylogenetic signal in spite of their perceived limitations. The allozyme analysis undertaken here is presented as an experiment to further assess the value of enzyme data in phylogenetic reconstruction.

Phylogenetic Analyses

All phylogenetic analyses were performed using the computer program PAUP (*Phylogenetic Analysis Using Parsimony, version 3.1*; Swofford 1991) utilizing random stepwise addition. Only the shortest trees were retained in each search. All characters were considered unordered and given equal weights, with multistate characters treated as 'uncertain.' Characters and character states were tracked, organized, and manipulated using the computer program *MacClade 3.0* (Maddison & Maddison 1992). Nodal support in each topology was determined by100 bootstrap replicates (BS; Felsenstein 1985), as well as by calculating Bremer support values (BV; Bremer 1988, 1994b).

Separate and combined phylogenetic analyses were conducted on three data sets: morphology, *nrDNA-ITS* sequences, and allozymes. Examining all relevant data in a combined fashion can produce a more robust estimate of phylogeny than separate analyses by maximizing congruence among different sources of data in phylogenetic inference (Hillis 1987; Kluge 1989; de Queiroz et al. 1995; Nixon & Carpenter 1996). To estimate levels of congruence among data sets, incongruence length differences (ILDs) were calculated (Mason-Gamer &

Kellogg 1996; Johnson & Soltis 1998). This index measures the amount of extra homoplasy that results from the combination of two or more data sets, as described by Mickevich & Farris (1981) and Farris et al. (1994, 1995). The ARNIE program in the Random Cladistics software package (Siddall 1995) was used to determine the significance of ILDs. α-values less than 0.05 were considered sufficient evidence to reject the null hypothesis of data set homogeneity. Combined analyses included only those taxa common to all phylogenetic data sets.

RESULTS

Chromosome Data

All individuals sampled from populations of *T. pubescens* and *T. caespitosum* proved to be diploid with chromosome counts of $n = 12$ (Table 6; Figs. 2A–B). Individuals sampled from populations of *T. subnudum* var. *alpinum* consistently were tetraploid with $n = 24$ (Fig. 2C). Our analyses revealed that some Colorado Plateau populations of *T. subnudum* var. *subnudum* are diploid (n=12; Fig. 2D) whereas others are exclusively tetraploid (n=24; Fig. 2E). Although the geographic ranges of these cytotypes overlap and both appear to be common (Windham, Hansen, & Allphin unpubl. data), we have yet to identify a locality where they occur together. Both ploidy levels of *T. subnudum* var. *subnudum* are represented in the morphological, DNA, and allozyme analyses that follow.

A chromosome number was not obtained directly for *T. marginatum*, and no previously published counts were found in the *Index to Plant Chromosome Numbers*. However, an analysis of allozyme banding intensities in our collection from Fremont Co., Wyoming, provided strong evidence that the plants at this locality are diploid. This sample of *T. marginatum* showed only balanced heterozygote banding patterns, as is expected in diploid organisms (Danzmann & Bogart 1982b; Wendel & Weeden 1989) and observed in all chromosomally verified diploid populations of *Thelesperma*.

Morphological Data

Parsimony analysis of 16 morphological characters yielded two most-parsimonious trees, the strict consensus of which is shown in Figure 3. Relative to the outgroup species chosen, *Thelesperma* was strongly supported as a monophyletic group (BV=6, BS=100%). The first branch within the *Thelesperma* clade produces a trichotomy that separates *T. megapotamicum* and *T. filifolium* (both with unequally lobed disc florets and a well developed pappus) from the other species. The clade encompassing the remaining taxa is weakly supported (BV=1, BS=70%), branching to form a polytomy in which each taxon (with the exception noted below) occupies its own unresolved branch. The only deviation from this pattern is the grouping of *T. caespitosum*, *T. pubescens*, and *T. subnudum* var. *alpinum* in a single clade with strong support (BV=2, BS=88%). Within this clade, the last taxon is moderately supported as sister to *T. caespitosum* and *T. pubescens*.

TABLE 6. Chromosome counts on taxa belonging to the *Thelesperma subnudum* complex. Apparent first counts for a taxon are marked by a double asterisk following the relevant name. Letters before collection numbers identify the following collectors: H = C.J. Hansen; S = C.J. Stubben; W = M. D. Windham; Wo = L. Allphin Woolstenhulme.

Thelesperma caespitosum Dorn (= *T. pubescens* Dorn var. *caespitosum* (Dorn) C.J. Hansen)**			
n = 12	Utah	Duchesne Co.	1.2 mi up jeep road to Anthro Mtn. from Chokecherry Canyon (T7S, R4W, S18); *H and S 97-80* (UT)
n = 12	Wyo	Sweetwater Co.	ca. 3.3 mi past bridge on dirt road to Scott's Bottom SE of Green River (T18N, R106W, S31); *H and S 97-91* (UT)
Thelesperma pubescens Dorn (= *T. pubescens* Dorn var. *pubescens*)**			
n = 12	Wyo	Sweetwater Co.	ca. 0.3 mi S of switchback on dirt road leading up to Cedar Mtn. Summit (T13N, R112W, S10) *H and S 97-101* (UT)
n = 12	Wyo	Uinta Co.	ca. 0.6 mi past left fork to Sage Creek Mtn. (T13N, R113W, S3); *H and S 97-111* (UT)
n = 12	Wyo	Uinta Co.	NE summit of Hickey Mtn. at radio tower (T13N, R114W, S13); *H and S 97-117* (UT)
Thelesperma subnudum Gray			
n = 12	Ariz.	Mohave Co.	W of Wolf Hole Valley ca. 3.85 km ENE of Mustang Knoll (T39N, R12W, S31); *Ha, S, W and Wo 97-43* (UT)
n = 24	Utah	Washington Co.	W base of Smithsonian Butte (T42S, R11W, S21); *H, S, W and Wo 97-45* (UT)
Thelesperma subnudum Gray var. ***alpinum*** Welsh (= *T. windhamii* C.J. Hansen)**			
n = 24	Utah	Wayne Co.	WSW of Teasdale near the base of Boulder Mtn. (T29S, R4E, S20); *W 93-144* (UT)
n = 24	Utah	Wayne Co.	ca. 0.6 mi W of State St. in Teasdale on road to Bullberry Creek (T29S, R4E, S21); *H 97-73* (UT)
n = 24	Utah	Wayne Co.	ca. 0.8 mi S of SR 24 on dirt road to Government Creek (T29S, R4E, S18); *H 97-74* (UT)

ITS Sequence Data

Of 663 characters (aligned length), 61 were variable, and 6 were phylogenetically informative. The sequence of *T. caespitosum* was incomplete with approximately 87 bp missing compared to the other sequences. Possible non-specific amplification of the *ITS* region in *T. caespitosum* resulted in a double signal on the chromatogram. Multiple attempts to re-extract DNA and obtain a clearer signal failed. To determine if there was a loss of phylogenetically informative characters in the missing region of *T. caespitosum*, two different sequence length scenarios were analyzed. The first scenario involved aligning all sequence lengths equal to that of *T. caespitosum* (i.e., no gaps in *T. caespitosum* but with an 87 bp truncation in all other taxa). The second scenario was to align full sequence lengths in all taxa except *T. caespitosum* (i.e., 87 bp gap in *T. caespitosum* only). In both analyses, the same single most-parsimonious tree was obtained

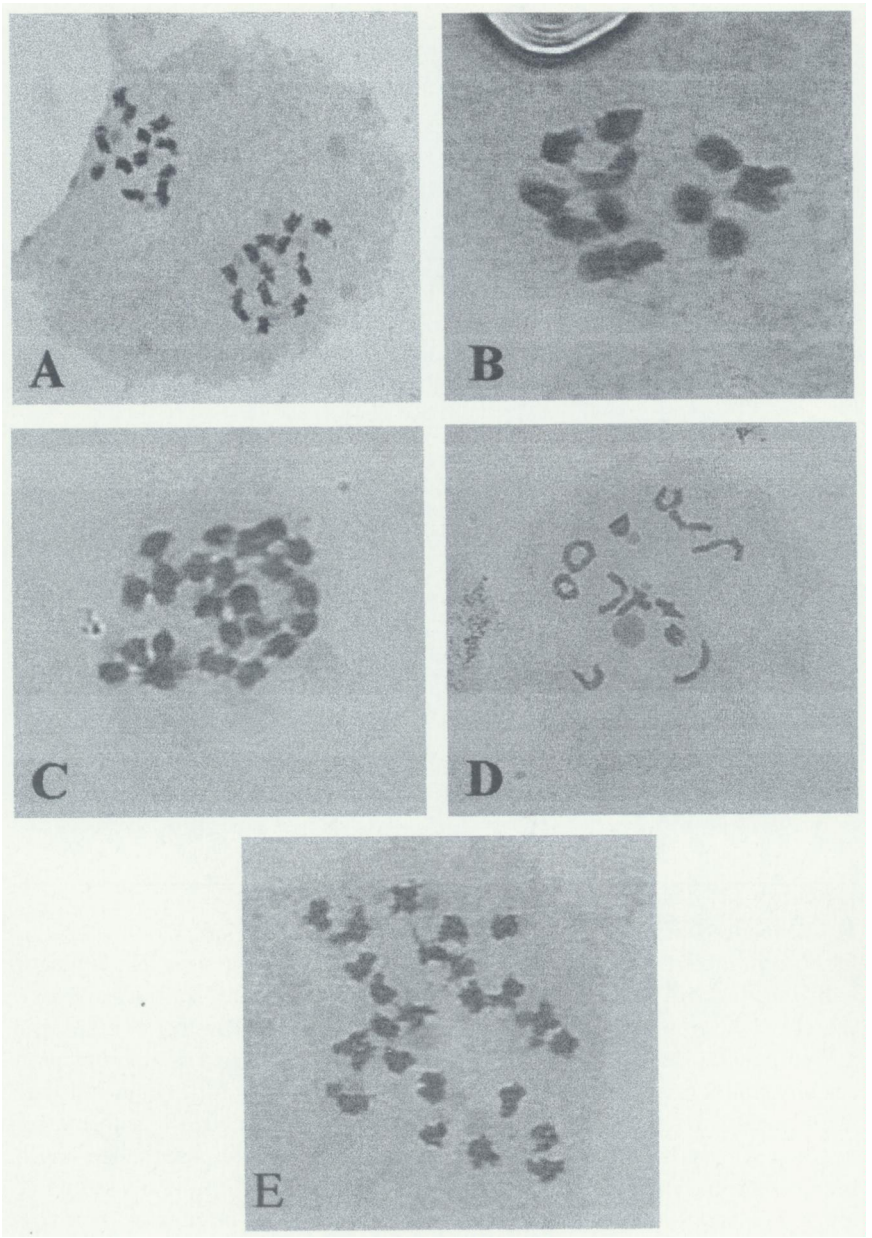

Fig. 2. Photomicrographs of chromosomes from four taxa in the *Thelesperma subnudum* complex. A) *T. pubescens*, n=12 (= var. *pubescens*, B) *T. caespitosum*, n=12 (= *T. pubescens* var. *caespitosum*), C) *T. subnudum* var. *alpinum*, n=24 (= *T. windhamii*), D) *T. subnudum*, n=12, E) *T. subnudum*, n=24.

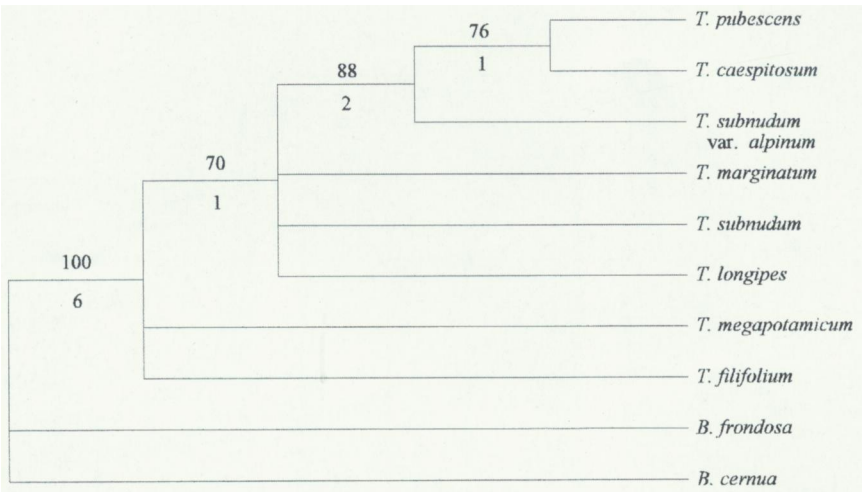

FIG. 3. Strict consensus of 2 most-parsimonious trees based on morophological data. Bootstrap values are above the line, Bremer support values below. Length= 19, CI= 0.947, RI= 0.941.

(Fig. 4). Furthermore, bootstrap support only differed by 1–2 percent, being slightly higher in the second scenario. These results suggest that very few phylogenetically important characters are located in the 87 bp gap of *T. caespitosum* and subsequent combined analyses utilized the second scenario alignment.

The topology of the single most-parsimonious tree from the *ITS* sequence analysis was similar to that derived from morphology (Figs. 3, 4). The monophyly of *Thelesperma* is again supported by a 100% bootstrap value; Bremer support in the ITS data is significantly higher (BV=16). The only topological difference between the morphological and ITS trees involves the placement of *T. filifolium*. In the *ITS* analysis, this species forms a clade with all *Thelesperma* other than *T. megapotamicum* on a relatively well supported branch (BV=2; BS=86%). As in the morphological tree, all taxa on this branch form an unresolved polytomy with the exception of *T. pubescens*, *T. caespitosum*, and *T. subnudum* var. *alpinum*. Support for this clade is significantly higher in the *ITS* tree (BV=5; BS=99) and, once again, *T. subnudum* var. *alpinum* receives moderate support as the sister taxon of *T. caespitosum* and *T. pubescens*. A comparison of pair-wise distances based on the aligned *ITS* sequences shows very little divergence among these rare taxa (Table 7).

Allozyme Data

Missing allozyme data made up 5.3% of the total data matrix due to poor staining resolution of SKDH and PGI in populations of *T. marginatum* and *T. longipes*. The results of a phylogenetic analysis based on presence/absence data yielded

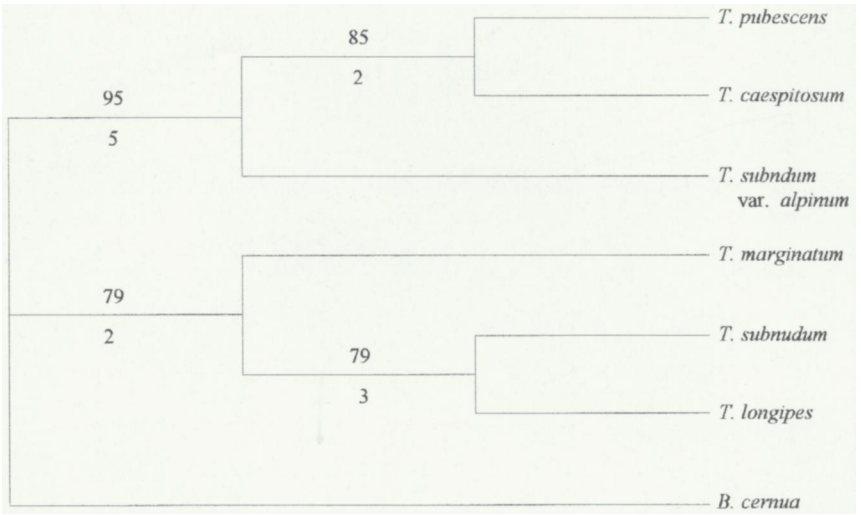

FIG. 4. Single most-parsimonious tree from an exhaustive search based on ITS sequence data only. Bootstrap values are above the line, Bremer support values are below. Length= 70, CI= 0.986, RI= 0.968.

a single most-parsimonious tree (Fig. 5). Relative to the outgroup species *Bidens cernua*, the subset of *Thelesperma* taxa included in the allozyme analysis form two distinct clades. One consists of the three rare endemics, which show a strong association (BV= 5, BS=95%) comparable to that observed in the morphological and *ITS* trees (Figs. 3, 4). Once again, *T. subnudum* var. *alpinum* is sister to *T. caespitosum* and *T. pubescens*, and there is increased support for this topology (BV=2; BS=85%). The other *Thelesperma* clade recovered in the allozyme analysis (Fig. 5) consists of *T. marginatum*, *T. subnudum*, and *T. longipes*, which group together with moderate support (BV=2; BS= 79%). Nearly identical support (BV=3; BS=79%) exists for the placement of *T. marginatum* as the sister taxon to *T. subnudum* and *T. longipes*.

Combined Data Set Analysis

Statistical comparisons of trees resulting from the individual data sets revealed that they were highly congruent and amenable to being combined in a single analysis. This combined analysis of morphology, allozymes, and nrDNA-*ITS* sequences resulted in a single most-parsimonious tree, the topology of which was identical to that of the analysis based on allozymes alone (Fig. 6). Bootstrap and Bremer support values for the *(marginatum (subnudum/longipes))* clade are not significantly different from those derived from the allozyme analysis. Support for the *(alpinum (pubescens/caespitosum))* clade is increased by combining data sets. The association of these three taxa is supported by a 100% bootstrap estimate, and the Bremer support value increases to 12. Statistical

TABLE 7. Pair-wise divergence in *nrDNA-ITS* sequences for *Thelesperma* and *Bidens*. Percent divergence above diagonal; total number of nucleotide differences below diagonal (generated in PAUP 3.1). Codes for taxa provided in TABLE 6.

	Thsu2	Thsu4	Thlo1	Thlo2	Thma1	Thma2	Thpu1	Thpu2	Thca1	Thca2	Thwi1	Thwi2	Thfi1	Thfi2	Thme1	Thme2	Bice	Bifr
Thsu2		0	0.40	0.60	0.80	0.80	1.4	1.0	1.2	1.0	0.80	0.80	0.40	0.40	1.0	1.0	6.3	5.7
Thsu4	0		0.40	0.60	0.80	0.80	1.4	1.0	1.2	1.0	0.80	0.80	0.40	0.40	1.0	1.0	6.3	5.7
Thlo1	2	2		0.20	0.80	0.80	1.4	1.0	1.2	1.0	0.80	0.80	0.40	0.40	1.0	1.0	6.3	5.9
Thlo2	3	3	1		1.0	1.0	1.6	1.2	1.5	1.2	1.0	1.0	0.60	0.60	1.2	1.2	6.5	6.1
Thma1	4	4	4	5		0	1.8	1.4	1.5	1.4	1.2	1.2	0.80	0.80	1.4	1.4	6.7	6.1
Thma2	4	4	4	5	0		1.8	1.4	1.5	1.4	1.2	1.2	0.80	0.80	1.4	1.4	6.7	6.1
Thpu1	7	7	7	8	9	9		0.40	0.50	0.40	0.60	0.60	1.6	1.4	2.0	2.0	7.1	6.7
Thpu2	5	5	5	6	7	7	2		0	0	0.20	0.20	1.2	1.0	1.6	1.6	6.9	6.5
Thca1	5	5	5	6	6	6	2	0		0	0.20	0.20	1.2	1.0	2.0	2.0	6.9	6.4
Thca2	5	5	5	6	7	7	2	0	0		0.20	0.20	1.2	1.0	1.6	1.6	6.9	6.5
Thwi1	4	4	4	5	6	6	3	1	1	1		0	1.0	0.80	1.4	1.4	6.7	6.3
Thwi2	4	4	4	5	6	6	3	1	1	1	0		1.0	0.80	1.4	1.4	6.7	6.3
Thfi1	2	2	2	3	4	4	8	6	5	6	5	5		0.20	1.0	1.0	6.3	5.9
Thfi2	2	2	2	3	4	4	7	5	4	5	4	4	1		1.0	1.0	6.3	5.9
Thme1	5	5	5	6	7	7	10	8	8	8	7	7	5	5		0	6.1	5.7
Thme2	5	5	5	6	7	7	10	8	8	8	7	7	5	5	0		6.1	5.7
Bice	31	31	31	32	33	33	35	34	28	34	33	33	31	31	30	30		5.5
Bifr	28	28	29	30	30	30	33	32	26	32	31	31	29	29	28	28	27	

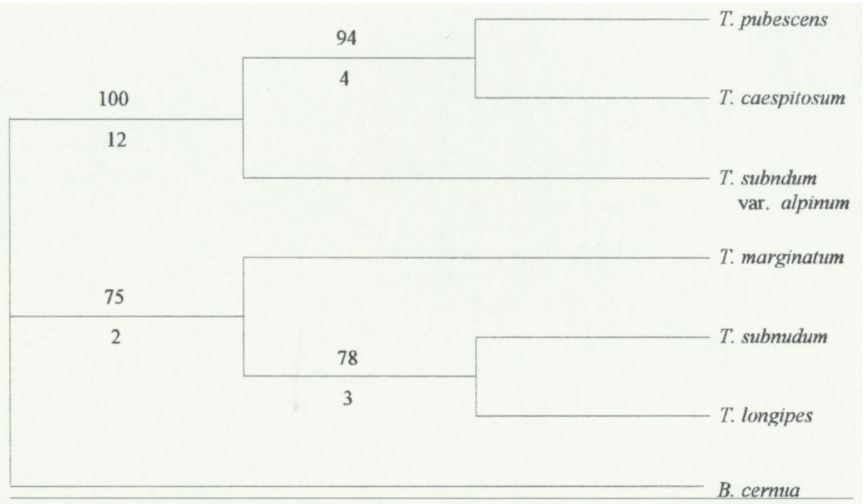

Fig. 5. Single most-parsimonious tree from an exhaustive search based on allozyme data only. Bootstrap values are above the line, Bremer support values are below. Length= 75, CI= 0.827, RI= 0.690.

support for the placement of *T. subnudum* var. *alpinum* as sister to the other two taxa increases as well (BV=4; BS=94%).

DISCUSSION

We gathered molecular and morphological data, which were analyzed separately and in combination to obtain the best estimate of the phylogeny of the *T. subnudum* complex. Tree topologies were highly concordant, and the single most-parsimonious tree from the combined analysis was more resolved and exhibited improved nodal support over any of the individual analyses.

The level of congruence among data sets in this study indicates that allozymes contain valuable phylogenetic information that can be recovered through parsimony analysis. This suggests that concerns regarding the independence of characters (Swofford et al. 1996) should not disqualify allozymes from playing at least a limited role in phylogenetic studies. Because this approach is relatively untested, however, we will refrain from placing undue emphasis on the allozyme tree in the following taxonomic discussion. This applies to the combined analysis as well, because the topology of that tree may be unduly influenced by the relative abundance of informative allozyme characters. The taxonomy outlined below addresses only those patterns independently observed in all data sets.

The relationships portrayed in our phylogenetic trees can be summarized as follows. *Thelesperma pubescens* and *T. caespitosum* are closest relatives, forming a clade in all analyses and showing very little divergence from one another

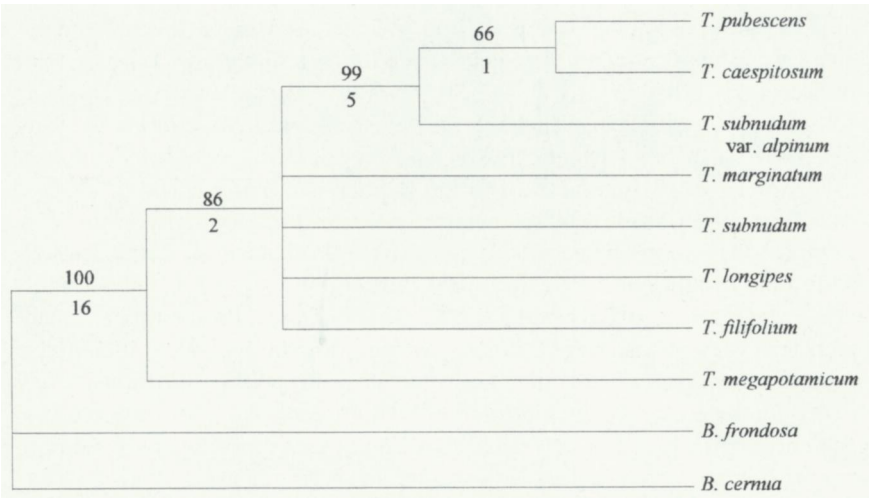

FIG. 6. Single most-parsimonious tree derived from combined analysis of allozyme, morphology, and *ITS*-DNA data sets. Bootstrap values are above the line, Bremer support values are below. Length= 137, CI= 0.905, RI= 0.772.

(Tables 4 & 7). Another relationship consistent across all analyses is the placement of *T. subnudum* var. *alpinum* as the sister taxon to the *T. pubescens/caespitosum* clade. Bootstrap support for this topology ranges from 88% in the morphological analysis to 100% in the combined analysis. Relationships among other taxa of the *Thelesperma subnudum* complex are poorly resolved, with the aforementioned clade forming a polytomy with *T. subnudum*, *T. marginatum*, and *T. longipes* in the morphology and *ITS* analyses (Fig. 3). In the allozyme and combined analyses (Fig. 4), those three taxa form a separate, moderately supported clade with *T. marginatum* basal to *T. subnudum* and *T. longipes*. These results are not wholly concordant with any of the proposed classifications of the *T. subnudum* complex summarized in Table 2, suggesting that nomenclatural changes are warranted.

The *Thelesperma pubescens/T. caespitosum* clade

Dorn (1990) recognized both taxa in this clade at the species level; Cronquist et al. (1994) combined them (and *T. subnudum* var. *alpinum*) under the name *T. pubescens*. Welsh et al. (1993) were mute on the matter of *T. pubescens* (which was not known to occur in Utah at the time the flora was published), but probably would consider it a variety of *T. subnudum*, the treatment accorded to *T. caespitosum*. The last classification is the least practicable in light of the data presented in this study. To treat *T. caespitosum* as a variety of *T. subnudum* and maintain monophyly, our data (Figs. 3, 4) indicate that other species would have to be subsumed within *T. subnudum* as well. These include *T. marginatum*, *T.*

longipes, and possibly even *T. filifolium*. We consider such a species concept, encompassing taxa not previously included in the *T. subnudum* complex, to be unacceptably broad.

Our data indicate that *Thelesperma pubescens* and *T. caespitosum* are sister taxa showing minimal genetic divergence. They differ by a single morphological character involving the distribution of pubescence on the leaves (Tables 3 & 4; Dorn 1990). Recent collections from near Anthro Mountain on the West Tavaputs Plateau in Duchesne Co., Utah (BRY- *Goodrich et al. 25159*) provide additional insight on their relationship. In that population, individual plants range from having strictly petiolar pubescence (*T. caespitosum* type) to complete leaf blade pubescence (*T. pubescens* type). Whether a result of incomplete primary divergence or secondary convergence resulting from hybridization or selection, there is complete intergradation. It appears that distribution of pubescence, in the absence of correlated characteristics, is probably a tenuous foundation for recognizing species in the *T. subnudum* complex.

Under the Biological Species Concept (Mayr 1942), *T. caespitosum* and *T. pubescens* are "groups of actually or potentially interbreeding populations" that probably should be recognized as conspecific. Invoking the Cohesion Species Concept (Templeton 1989), *T. pubescens* and *T. caespitosum* also qualify as conspecific based on phenotypic similarities (cohesion) due to gene flow by means of presumed interbreeding. Because these two taxa lack unique, diagnosable sets of morphological and molecular characteristics and don't appear to have separate evolutionary trajectories, they also would be considered a single species under the Phylogenetic Species Concept (Cracraft 1983; Davis & Nixon 1992).

With regard to the *caespitosum/pubescens* clade, our data are most congruent with the classification presented by Cronquist et al. (1994). In that treatment, *T. pubescens* is considered specifically distinct from *T. subnudum*, with *T. caespitosum* included within *T. pubescens*. We propose two modifications to this classification. First, we exclude *T. subnudum* var. *alpinum* from synonymy with *T. pubescens* for reasons that will be discussed below. Second, we propose to recognize *T. caespitosum* as a variety of *T. pubescens*. Excluding the intergradient population on Anthro Mountain, Utah, there is a definite correlation among pubescence, substrate, and geography (Table 1; Fig. 1). We feel that the incipient divergence in this clade is best recognized at the varietal level.

Origin and relationships of *Thelesperma subnudum* var. *alpinum*

This taxon appears to be exclusively tetraploid (Table 6), a point that is central to hypotheses regarding its evolutionary origin and to its classification. Tetraploids typically are derived from diploids through the incorporation of additional sets of chromosomes (see Harlan & DeWet 1975), and they are informally grouped according to the similarity of their constituent genomes (Crawford 1989). Polyploids containing genomes that are very similar chromosomally and genetically

(usually derived from within a single species) are considered autopolyploids. Because of genetic similarity to their progenitor diploids, autopolyploids are rarely given species status (e.g., Mosquin 1966). Polyploids that contain well-differentiated genomes (often obtained through hybridization between different species) are considered allopolyploids and usually treated as distinct species. The proper classification of *T. subnudum* var. *alpinum* thus hinges on whether it proves to be an autopolyploid or an allopolyploid.

Classifications of this taxon by Dorn (1990) and Welsh et al. (1993) imply that its closest relative is *Thelesperma subnudum*, and suggest that it may be an autopolyploid derived from within that species. Two lines of evidence refute that putative relationship. Although the taxon shows some morphological similarities to *T. subnudum* (Dorn 1990), the *ITS* sequence data clearly indicate a closer relationship to *T. pubescens* and *T. caespitosum*. In fact, all data sets developed during this study support the placement of *T. subnudum* var. *alpinum* as sister to these taxa, not *T. subnudum* var. *subnudum* (Figs. 3 & 4). Further evidence that var. *alpinum* is not an autopolyploid derivative of *T. subnudum* comes from the fact that known autopolyploids abound in this species and do not resemble var. *alpinum*. These undisputed autopolyploids in *T. subnudum* appeared identical to diploid *T. subnudum* in morphology and *ITS* sequences (Table 7). In our analyses, they were recognizable only through chromosome studies or the detection of unbalanced heterozygosity in allozyme markers shared exclusively with diploid *T. subnudum*.

Despite significant genetic similarity, it also seems unlikely that *Thelesperma subnudum* var. *alpinum* is an autopolyploid derivative of either *T. pubescens* or *T. caespitosum*. It varies toward *T. subnudum* in some morphological characters, most notably the somewhat creeping rootstock. The two taxa are similar enough to convince Dorn (1990) to maintain them as varieties of a single species. The presence, in most individuals, of a PGI-1 allele otherwise found only in *T. subnudum* and *T. marginatum* provides further evidence of genetic links to taxa outside the *T. pubescens/caespitosum* clade. Variety *alpinum* also shows several autapomorphic traits that distinguish it from *T. pubescens* and *T. caespitosum*, including pubescent flowering stems and unique allozyme variants.

We suspect that *T. subnudum* var. *alpinum* may be an allotetraploid resulting from hybridization between diploid *T. subnudum* and either *T. caespitosum* or *T. pubescens*. This would explain the pattern of shared characters and apparent morphological intermediacy that has led to such divergent classifications (Dorn 1990 vs. Cronquist et al. 1994). It also would explain allozyme banding patterns at the PGI-1 locus, where most individuals of var. *alpinum* are heterozygous for alleles derived from the *pubescens/caespitosum* and *subnudum/marginatum* clades respectively. At this one locus, var. *alpinum* approaches fixed heterozygosity, one of the genetic hallmarks of allopolyploidy. The absence of fixed heterozygosity at other allozyme loci may be due to homoeologous chro-

mosome pairing or extensive gene silencing (Windham 1988). Over time, allopolyploids lose expression of duplicated parental genes through various genetic processes, especially null mutations (Roose & Gottlieb 1976; Werth & Windham 1991). Given enough time, the polyploid taxon becomes genetically "diploidized" (Grant 1981).

A parallel process may explain the lack of diagnostic *T. subnudum ITS* sequences in var. *alpinum*. Recently formed allopolyploids would be expected to show the *ITS* sequences of both diploid parents (Soltis et al. 1995; Cook & Soltis 1999; 2000; Gernandt & Liston 1999). With time, however, *ITS* loci often experience concerted evolution, which randomly eliminates one of the parental sequences (Sang et al. 1995; Wendel et al. 1995; Polanco et al. 1999). Under this scenario, var. *alpinum* still could be an allopolyploid hybrid in which the original *T. subnudum* sequence has been lost to concerted evolution.

Each of the possible evolutionary origins (two autopolyploid and one allopolyploid) of *Thelesperma subnudum* var. *alpinum* discussed above would support a different classification for this taxon. The hypothesis that var. *alpinum* is an autopolyploid derived from *T. subnudum*, the only scenario congruent with the classifications of Dorn (1990) and Welsh et al. (1993), can be rejected. To uphold *alpinum* as a variety under *T. subnudum* and still maintain monophyly, we would have to expand the species definition of *T. subnudum* to include the entire complex plus *T. longipes* and, possibly, *T. filifolium* (Figs. 3 & 4). In our opinion, lumping half of the species in the genus *Thelesperma* into one species is not a desirable solution.

Our data are not sufficiently robust to distinguish between an autopolyploid origin of var. *alpinum* from within the *T. pubescens/caespitosum* clade or an allopolyploid origin through hybridization between members of that clade and *T. subnudum*. Nevertheless, we can propose a classification that would be phylogenetically congruent regardless of which scenario proves more plausible. To include var. *alpinum* within *T. pubescens* (as done by Cronquist et al. 1994) would be cladistically indefensible if *alpinum* subsequently is shown to be an allopolyploid. However, if we anticipate that the latter hypothesis is more plausible and recognize var. *alpinum* as a distinct species, such a treatment remains valid in the event that *alpinum* maintains its current position as the basal branch of the *T. pubescens/caespitosum* clade. Variety *alpinum* is morphologically distinct from the other members of this clade and, because of its polyploid chromosome number, is probably genetically isolated from the strictly diploid taxa. Thus, it can be recognized as a species under the Biological Species Concept (Mayr 1942) as well as the Phylogenetic Species Concept (Cracraft 1983; Davis & Nixon 1992; Davis 1996).

Other taxa in the *Thelesperma subnudum* complex

The two remaining taxa typically assigned to this complex are *T. subnudum*

and *T. marginatum*. Dorn (1990) treated them as distinct species; Cronquist et al. (1994) considered the latter a variety of *T. subnudum*. These taxa are part of an unresolved polytomy in the morphological and *ITS* data sets (Fig. 3), but form a moderately supported clade with *T. longipes* in the allozyme and combined trees (Fig. 4). The placement of *T. subnudum* as sister to *T. longipes* instead of *T. marginatum* in the latter trees suggests a relationship at odds with previous classifications, which exclude *T. longipes* from the *T. subnudum* complex. This result should be confirmed by additional studies before taxonomic revisions are proposed. Even if the allozyme data are discounted, there still is no support for expanding *T. subnudum* to include *T. marginatum* as proposed by Cronquist et al. (1994). Recognition of *T. marginatum* as a variety of *T. subnudum* would require its placement as sister to *T. subnudum* in a phylogenetic analysis. That these two taxa do not form a clade in any of our analyses suggests that they should continue to be treated as separate species. Each has a unique, diagnosable set of morphological and molecular characteristics indicative of a distinct evolutionary trajectory, thus satisfying the definition of a phylogenetic species (Davis & Nixon 1992).

In order to implement the classification outlined above, two nomenclatural innovations are necessary:

Thelesperma pubescens Dorn var. **caespitosum** (Dorn) C.J. Hansen, stat. nov. BASIONYM: *Thelesperma caespitosum* Dorn, Madroño 37: 293. 1990. TYPE: U.S.A. WYOMING. Sweetwater Co.: T18N, R106W, SE1/4 of SE1/4 of Sect. 31 and SW1/4 of SW1/4 of Sect. 32, 5 km SE of Green River, barren white shale ridge, 1890 m, 22 Jun 1988, *Dorn 4948* (HOLOTYPE: RM!).

Thelesperma windhamii C.J. Hansen, nom. et stat. nov. BASIONYM: *Thelesperma subnudum* A. Gray var. *alpinum* Welsh, Great Basin Naturalist 43: 369. 1983. TYPE: U.S.A. UTAH. Wayne Co.: T28S, R4E, S13 (NE1/4), 3 mi due N of Bicknell, bristlecone pine forest on multicolored clay hills, 2745 m, 20 Jul 1980, *Atwood and Thompson 7646a* (HOLOTYPE: BRY!).

This new name for *T. subnudum* var. *alpinum* honors the junior author, M.D. Windham, who proposed and co-directed the study. We chose not to raise the epithet *alpinum* to species rank because the taxon never occupies truly alpine habitats and most populations occur at moderate elevations (ca. 2200 m) in semi-desert regions.

KEY TO THE TAXA IN THE *THELESPERMA SUBNUDUM* COMPLEX (ASTERACEAE)

1. Leaves and stems glabrous or essentially so; leaves mostly 3–9 cm long; stems mainly 9–35(–50) cm tall, scattered along a somewhat creeping rhizomatous rootstock; rays present or absent; plants widely distributed.
   2. Ray florets present (rarely absent), scarious margins of inner involucral bracts mostly 1–1.5 mm or more wide; Nevada, Utah, Arizona, New Mexico, and Colorado _____ **T. subnudum**
   2. Ray florets absent, scarious margins of inner involucral bracts mostly 0.5–1 mm wide; Wyoming, Montana, Alberta, Saskatchewan _____ **T. marginatum**

1. Leaves or lower stems pubescent; leaves 1.5–4 cm long; stems mainly 3–19 cm tall, clustered on a thick, branching caudex with old, persistent leaf bases; ray florets absent; plant distribution restricted.
   3. Pappus a toothed crown; lower portion of flowering stems pubescent; plants from Wayne Co., Utah _____ **T. windhamii**
   3. Pappus absent; lower portion of flowering stems glabrous; plants from NE Utah and SW Wyoming.
      4. Leaves pubescent throughout; plants appearing gray-green _____ **T. pubescens** var. **pubescens**
      4. Leaves pubescent only on petioles or (rarely) on the proximal portion of the blade; plants appearing green _____ **T. pubescens** var. **caespitosum**

It is hoped that this revision of the *Thelesperma subnudum* complex will resolve some of the taxonomic confusion in the group. Although the treatment of *T. pubescens* and *T. caespitosum* as conspecific makes the aggregate taxon less rare, long-term monitoring and land management still will be necessary. All three rare taxa are restricted to specific substrates, and populations of *T. pubescens* near Green River and Hickey Mountain in Wyoming are endangered due to off-road vehicle use and oil and gas development. Populations of *T. windhamii* located west of Teasdale, Utah, are similarly threatened by off-road vehicle use.

To improve phylogenetic resolution, future studies should sequence more rapidly evolving regions of the *Thelesperma* genome, such as the external transcribed spacer regions of ribosomal DNA (Baldwin & Markos 1998). Artificial hybridization studies also might prove useful for revealing reproductive barriers and species boundaries. Ultimately, research should be expanded to include all taxa of *Thelesperma*. Deciphering relationships within *Thelesperma* will aid in identifying possible sister genera and thereby contribute to our knowledge of higher level relationships in the Coreopsidinae and Heliantheae.

ACKNOWLEDGMENTS

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