SYSTEMATIC IMPLICATIONS OF DNA RESTRICTION SITE VARIATION IN *Hymenoxys* AND *Tetraneuris* (ASTERACEAE, HELENIEAE, GAILLARDIINAE)

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Abstract: Generic delimitation in the *Hymenoxys* complex has long been problematic. One taxonomic extreme would recognize only *Hymenoxys,* whereas the other would split the obviously related taxa into as many as eight genera. This study examined restriction site variation in both cpDNA and nrDNA in the *Hymenoxys* complex. Fifty-six populations representing 21 species (four with two varieties each) and six of the eight possible genera were analyzed using 21 enzymes, which resulted in the detection of 358 restriction site changes of which 171 were potentially phylogenetically informative. Wagner parsimony using synapomorphic characters generated 26,600 equally parsimonious trees of 223 steps with a consistency index of 0.76 and a retention index of 0.98. Bootstrap analysis indicated that the major clades were strongly supported. The DNA tree supports the recognition of *Tetraneuris* as a genus separate from *Hymenoxys,* and the inclusion in *Hymenoxys* of taxa that at times have been split into the genera *Dugaldia, Macdougalia, Phileozera, Picradenia,* and *Plummera.*

Resumen: Delimitaciones genéricas en el complejo *Hymenoxys* han sido siempre problemáticas. Unos preferirían ver a *Hymenoxys* como un solo género y otros lo separarían en tantos como ocho géneros. Este estudio examina la variación en sitios reconocidos en el cpDNA y en el nr DNA por enzimas de restricción en el complejo *Hymenoxys.* Cincuenta y seis poblaciones representando 21 especies (cuatro con dos variedades cada una) y seis de los ocho posibles géneros fueron analizadas usando 21 enzimas, resultando en la detección de 358 sitios de los cuales 171 tuvieron el potencial de ser filogenéticamente informativos. Parsimonia de Wagner usando caracteres sinapomórficos generaron 26,600 árboles de equivalente parsimoniedad contando todos con 233 pasos con un índice de consistencia de 0.76 y un índice de retención de 0.98. El analisis bootstrap indicó que la mayoría de los clados están fuertemente sostenidos. El árbol de DNA sostiene el mantener a *Tetraneuris* como un género separado de *Hymenoxys* y la inclusión en *Hymenoxys* de taxones que en algun momento fueron separados a nivel de género como *Dugaldia, Macdougalia, Phileozera, Picradenia* y *Plummera.*

Keywords: *Asteraceae, Hymenoxys, Tetraneuris,* DNA, phylogeny, systematics

Hymenoxys sensu lato includes taxa that are referable to *Dugaldia* Cass., *Hymenoxys* Cass., *Macdougalia* A. Heller, *Phileozera* Buckley, *Picradenia* Hook., *Plummera* A. Gray, *Rydbergia* Greene, and *Tetraneuris* Greene.

A reasonable case can be made on morphologic, cytologic (chromosome number), and chemical grounds for combining all of the taxa discussed here into one genus *(Hymenoxys)* or splitting them into at least the eight genera listed above (Bierner [1994] provides a comparison of these taxa with regard to morphology, cytology, flavonoid chemistry, monoterpene chemistry, and sesquiterpene lactone chemistry).

Difficulties with generic delimitation in this group are illustrated by differences in the treatments presented by various workers during this century. Rydberg (1915) recog-

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nized *Dugaldia, Hymenoxys, Macdougalia, Plummera, Rydbergia,* and *Tetraneuris,* and placed the taxa referable to *Phileozera* and *Picradenia* in *Hymenoxys.* Turner and Powell (1977) recognized only *Dugaldia* and *Hymenoxys,* and submerged *Macdougalia, Phileozera, Picradenia, Plummera, Rydbergia,* and *Tetraneuris* in *Hymenoxys.* Robinson (1981) recognized *Hymenoxys, Macdougalia, Plummera,* and *Tetraneuris,* and submerged *Phileozera, Picradenia* and *Rydbergia* in *Hymenoxys,* and *Dugaldia* in *Helenium* L. Bierner (1994) recognized only *Hymenoxys* and *Tetraneuris,* and submerged *Dugaldia, Macdougalia, Phileozera, Picradenia, Plummera,* and *Rydbergia* in *Hymenoxys* as subgenera.

As major evidence for this latter treatment, Bierner (1994) cited preliminary unpublished analyses of DNA restriction site data (the information now being published in this paper) that clearly separated the taxa of *Tetraneuris* into one branch of the DNA phylogenetic tree and grouped the other taxa into another branch. He also cited the presence of 6-methoxy flavone aglycones, flavonol 3-O-acetyl glycosides, and seco-pseudoguaianolides in all of the groups except for *Tetraneuris,* and the presence of 6,8-dimethoxy flavone aglycones and monoterpene glycosides only in *Tetraneuris.* The one exception was *Hymenoxys texana* (tentatively placed by Bierner [1994] and Spring et al. [1994] in *Hymenoxys* subgenus *Phileozera*), which contains monoterpene glycosides and lacks seco-pseudoguaianolides (Spring et al., 1994). In fact, the phenogram produced by Spring et al. (1994) based on presence or absence of chemical components isolated from glandular trichomes placed *Hymenoxys texana* with *Tetraneuris.*

This paper presents a phylogenetic analysis of DNA restriction site data from taxa in this complex. The major questions focus on the status of *Dugaldia, Macdougalia, Plummera,* and *Tetraneuris.* The working hypothesis at the beginning of this project (based on all evidence available when the study began in 1992) was to recognize *Dugaldia, Hymenoxys, Macdougalia, Plummera,* and *Tetraneuris* as distinct genera, and to recognize *Phileozera, Picradenia,* and *Rydbergia* as subgenera of *Hymenoxys.* Hence, the generic names *Dugaldia, Macdougalia,* and *Plummera* are used throughout this article even though the results of this study indicate that they are in fact congeneric with *Hymenoxys.*

Representatives of *Hymenoxys* subgenera *Hymenoxys* and *Rydbergia* were not available for this study. We feel confident, however, from evidence presented by Bierner (1994) and Spring et al. (1994) that the taxa comprising these subgenera are clearly associated with those referable to *Dugaldia, Macdougalia, Phileozera, Picradenia,* and *Plummera.*

Materials and Methods

DNA restriction site variation was examined in samples from 56 populations representing 25 taxa of *Hymenoxys* sensu lato (Table 1): one of the three taxa of *Dugaldia,* two of the three taxa of *Hymenoxys* subgenus *Phileozera,* 10 of the 11 taxa of *Hymenoxys* subgenus *Picradenia,* the one taxon of *Macdougalia,* both taxa of *Plummera,* and nine of the 11 taxa of *Tetraneuris.* DNA restriction site variation was compared to two taxa included as outgroups (Table 1): *Helenium drummondii* and *Psilostrophe villosa.*

Total DNAs were isolated from leaf material, all of which was field collected except for that of *Hymenoxys texana* (greenhouse-grown at the University of Texas at Austin). DNAs from the Turner collection and the Bierner 1988 collections were extracted from fresh material by Ki-Joong Kim, those from the Bierner 1989 and 1991 collections were extracted by the first author from material stored at –70°C, and those from the Bierner 1992 collections were extracted by the first author from fresh material. In all cases, the CTAB method of Doyle and Doyle (1987) was used, and the

TABLE 1. Sources of DNA for taxa of *Hymenoxys* sensu lato, *Helenium drummondii*, and *Psilostrophe villosa*. Multiple samples of a taxon are given unique numbers within the taxon that follow the scientific name. All vouchers are deposited at TEX.

Taxon	Voucher	Location
Dugaldia Cass.		
Dugaldia hoopesii (A. Gray) Rydb. 1	*Bierner 92-25*	Grant Co., New Mexico
Dugaldia hoopesii (A. Gray) Rydb. 2	*Bierner 92-39*	Garfield Co., Utah
Helenium L.		
H. drummondii Rock	*Bierner 89-1*	Hardin Co., Texas
Hymenoxys Cass. subgenus *Phileozera* (Buckl.) Cockerell		
H. odorata DC. 1	*Bierner 89-11*	Val Verde Co., Texas
H. odorata DC. 2	*Turner 15805*	Tom Green Co., Texas
H. texana (J. Coulter & Rose) Cockerell	*Bierner 92-46*	Harris Co., Texas*
Hymenoxys Cass. subgenus *Picradenia* (Hook.) Cockerell		
H. brachyactis Wooten & Standley	*Bierner 88-77*	Torrance Co., New Mexico
H. cooperi (A. Gray) Cockerell 1	*Bierner 89-17*	San Bernardino Co., California
H. cooperi (A. Gray) Cockerell 2	*Bierner 89-19*	Clark Co., Nevada
H. cooperi (A. Gray) Cockerell 3	*Bierner 89-21*	Garfield Co., Utah
H. cooperi (A. Gray) Cockerell 4	*Bierner 89-23*	Coconino Co., Arizona
H. cooperi (A. Gray) Cockerell 5	*Bierner 89-24*	Coconino Co., Arizona
H. helenioides (Rydb.) Cockerell 1	*Bierner 92-40A*	Garfield Co., Utah
H. helenioides (Rydb.) Cockerell 2	*Bierner 92-40B*	Garfield Co., Utah
H. jamesii Bierner	*Bierner 89-26*	Coconino Co., Arizona
H. lemmonii (Greene) Cockerell	*Bierner 88-69*	Lander Co., Nevada
H. quinquesquamata Rydb.	*Bierner 88-73*	Cochise Co., Arizona
H. richardsonii (Hook.) Cockerell var. *floribunda* (A. Gray) K. L. Parker 1	*Bierner 92-38*	Garfield Co., Utah
H. richardsonii (Hook.) Cockerell var. *floribunda* (A. Gray) K. L. Parker 2	*Bierner 92-33*	Coconino Co., Arizona
H. richardsonii (Hook.) Cockerell var. *floribunda* (A. Gray) K. L. Parker 3	*Bierner 92-45*	Bernalillo Co., New Mexico
H. richardsonii (Hook.) Cockerell var. *floribunda* (A. Gray) K. L. Parker 4	*Bierner 92-28*	Grant Co., New Mexico
H. richardsonii (Hook.) Cockerell var. *floribunda* (A. Gray) K. L. Parker 5	*Bierner 92-26*	Grant Co., New Mexico
H. richardsonii (Hook.) Cockerell var. *richardsonii*	*Bierner 92-42*	Daggett Co., Utah
H. rusbyi (A. Gray) Cockerell	*Bierner 88-75*	Grant Co., New Mexico
H. subintegra Cockerell	*Bierner 92-34*	Coconino Co., Arizona
Macdougalia A. Heller		
M. bigelovii (A. Gray) A. Heller	*Bierner 89-25*	Coconino Co., Arizona
Plummera A. Gray		
P. ambigens S. F. Blake	*Bierner 92-29*	Graham Co., Arizona
P. floribunda A. Gray	*Bierner 92-31*	Cochise Co., Arizona
Psilostrophe DC.		
P. villosa Rydb.	*Bierner 89-2*	Live Oak Co., Texas
Tetraneuris Greene		
T. acaulis (Pursh) Greene var. *acaulis* 1	*Bierner 89-29*	Torrance Co., New Mexico
T. acaulis (Pursh) Greene var. *acaulis* 2	*Bierner 88-63*	San Miguel Co., New Mexico
T. acaulis (Pursh) Greene var. *arizonica* (Greene) K. L. Parker 1	*Bierner 92-35*	Coconino Co., Arizona

T. acaulis (Pursh) Greene var. *arizonica* (Greene) K. L. Parker 2	*Bierner 92-41*	Uintah Co., Utah
T. acaulis (Pursh) Greene var. *arizonica* (Greene) K. L. Parker 3	*Bierner 88-59*	Emery Co., Utah
T. argentea (A. Gray) Greene 1	*Bierner 92-44*	Bernalillo Co., New Mexico
T. argentea (A. Gray) Greene 2	*Bierner 88-62*	Los Alamos Co., New Mexico
T. ivesiana Greene 1	*Bierner 92-37*	Kane Co., Utah
T. ivesiana Greene 2	*Bierner 89-22*	Garfield Co., Utah
T. linearifolia (Hook.) Greene var. *arenicola* Bierner 1	*Bierner 89-4*	Brooks Co., Texas
T. linearifolia (Hook.) Greene var. *arenicola* Bierner 2	*Bierner 91-12*	Brooks Co., Texas
T. linearifolia (Hook.) Greene var. *arenicola* Bierner 3	*Bierner 91-13*	Brooks Co., Texas
T. linearifolia (Hook.) Greene var. *arenicola* Bierner 4	*Bierner 91-14*	Hidalgo Co., Texas
T. linearifolia (Hook.) Greene var. *linearifolia* 1	*Bierner 91-18*	Atascosa Co., Texas
T. linearifolia (Hook.) Greene var. *linearifolia* 2	*Bierner 91-4*	Live Oak Co., Texas
T. linearifolia (Hook.) Greene var. *linearifolia* 3	*Bierner 91-5*	Live Oak Co., Texas
T. linearifolia (Hook.) Greene var. *linearifolia* 4	*Bierner 91-8*	Live Oak Co., Texas
T. scaposa (DC.) Greene var. *argyrocaulon* (K. L. Parker) K. L. Parker 1	*Bierner 89-5*	Jim Hogg Co., Texas
T. scaposa (DC.) Greene var. *argyrocaulon* (K. L. Parker) K. L. Parker 2	*Bierner 91-15*	Jim Hogg Co., Texas
T. scaposa (DC.) Greene var. *argyrocaulon* (K. L. Parker) K. L. Parker 3	*Bierner 88-36*	Jim Hogg Co., Texas
T. scaposa (DC.) Greene var. *scaposa* 1	*Bierner 89-28*	Torrance Co., New Mexico
T. scaposa (DC.) Greene var. *scaposa* 2	*Bierner 88-52*	Jeff Davis Co., Texas
T. scaposa (DC.) Greene var. *scaposa* 3	*Bierner 89-13*	Brewster Co., Texas
T. scaposa (DC.) Greene var. *scaposa* 4	*Bierner 89-10*	Blanco Co., Texas
T. turneri (K. L. Parker) K. L. Parker 1	*Bierner 91-2*	Goliad Co., Texas
T. turneri (K. L. Parker) K. L. Parker 2	*Bierner 91-3*	Bee Co., Texas
T. turneri (K. L. Parker) K. L. Parker 3	*Bierner 91-6*	Live Oak Co., Texas
T. turneri (K. L. Parker) K. L. Parker 4	*Bierner 91-1*	Goliad Co., Texas
T. turneri (K. L. Parker) K. L. Parker 5	*Bierner 91-9*	Live Oak Co., Texas

*Plant material of *Hymenoxys texana* used in this study was grown in the Welch greenhouse at the University of Texas at Austin from seed collected in Harris Co., Texas.

DNAs were then further purified in cesium chloride/ethidium bromide gradients as described by Sambrook et al. (1989). Restriction enzyme digestions, agarose gel electrophoresis, bi-directional transfer of DNA fragments from gels to Zetabind (CUNO Inc., Meriden, Connecticut) nylon filters, labeling of recombinant plasmids by nick-translation, filter hybridizations, and autoradiography were performed as described in Palmer (1986), Jansen and Palmer (1987), and Palmer et al. (1988). Enzyme digestions were done using 21 six-base pair restriction endonucleases: *Ava*I, *Ava*II, *BamH*I, *Ban*I, *Ban*II, *Bcl*I, *Bgl*II, *BstN*I, *BstX*I, *Cla*I, *Dra*I, *Eco*0109, *EcoR*I, *EcoR*V, *Hae*II, *Hinc*II, *Hind*III, *Nci*I, *Nsi*I, *Ssp*I, and *Xmn*I. Restriction fragment data were obtained from hybridizations done with 15 cloned SacI restriction fragments of lettuce cpDNA (Jansen and Palmer, 1987) and three subclones of the *Helianthus argophyllus* Torrey & A. Gray ribosomal repeat (M. Arnold, unpublished). We did not map

sites for either cpDNA or nrDNA; however, this was unnecessary because levels of variation were low enough to allow us to reliably interpret restriction site changes (Jansen et al., in press). We were also able to distinguish between restriction site and length changes, because the latter were evident with most of the 21 enzymes examined.

Restriction site data were subjected to Wagner parsimony (Farris, 1970) analyses using a Macintosh Quadra 700 microcomputer and PAUP version 3.1.1 (Swofford, 1993). Tree Bisection Reconnection (TBR) branch-swapping and Mulpars options were used to search for the most parsimonious Wagner trees, and 100 random additions were performed to search for islands of equally parsimonious trees (Maddison, 1991). *Helenium drummondii* and *Psilostrophe villosa* were selected as outgroups, but it became obvious after a first computer run with PAUP that *Psilostrophe villosa* belonged in the ingroup. *Helenium drummondii*, therefore, served as the outgroup for subsequent analyses. The PAUP CONTREE option was used to construct a strict consensus tree. Bootstrap (Felsenstein, 1985) analyses (1000 replicates) in PAUP were used to derive confidence intervals using the same options as the parsimony analyses except that only one random addition was performed without Mulpars.

Results

We were able to detect 358 restriction site changes, of which 187 were autapomorphic and 171 were potentially synapomorphic. The list of all character changes and the data matrix for the 171 synapomorphic characters have been deposited under *Hymenoxys* at TEX and are also available from the first author.

Of the 187 autapomorphic characters, 154 (82%) were confined to three taxa: 73 (39%) in *Helenium drummondii*, 39 (21%) in *Psilostrophe villosa*, and 42 (22%) in *Hymenoxys texana*. The other 33 autapomorphic characters were distributed among 19 populations representing 15 taxa; eight populations had one autapomorphy, nine populations had two, one population had three, and one population had four.

Wagner analyses of the 171 synapomorphic characters, of which 144 (84%) were in the chloroplast genome, produced 26,600 equally most parsimonious trees of 223 steps with a consistency index (excluding autapomorphic characters) of 0.76, and a retention index of 0.98. Because of limitations of computer memory, it is likely that not all of the equally parsimonious trees were detected. Topology of the consensus tree using all 171 synapomorphic characters is congruent with that of the tree produced without using nrDNA characters. The consensus tree along with character support and bootstrap values, and with autapomorphic characters added to it, is shown in Figure 1.

The ingroup taxa are separated into two clades. One includes taxa referable to *Hymenoxys* subgenus *Picradenia*, *H.* subgenus *Phileozera*, *Dugaldia*, *Macdougalia*, and *Plummera*. The monophyly of this clade is strongly supported by the presence of 33 shared characters and a bootstrap value of 100%. The other clade contains taxa referable to *Tetraneuris* and *Psilostrophe*. It too is strongly supported as monophyletic by 12 shared characters and a bootstrap value of 88%.

Within the first clade, the separation of *Hymenoxys texana* from the other taxa is very strongly supported by a bootstrap value of 100%, and the separation of *H. odorata* is supported by a bootstrap value of 77%. In addition, separation of *H. brachyactis* is weakly supported by a bootstrap value of 62%, the two *Plummera* taxa are associated by a bootstrap value of 64%, and *Dugaldia hoopesii* 2 and *Hymenoxys helenioides* 2 are strongly united with a bootstrap value of 99%. There is also a weak subgroup of *Hymenoxys rusbyi*, *H. subintegra*, and *H. cooperi* 3, 4, and 5 supported by a bootstrap value of 49%.

In the second clade, 12 characters and a

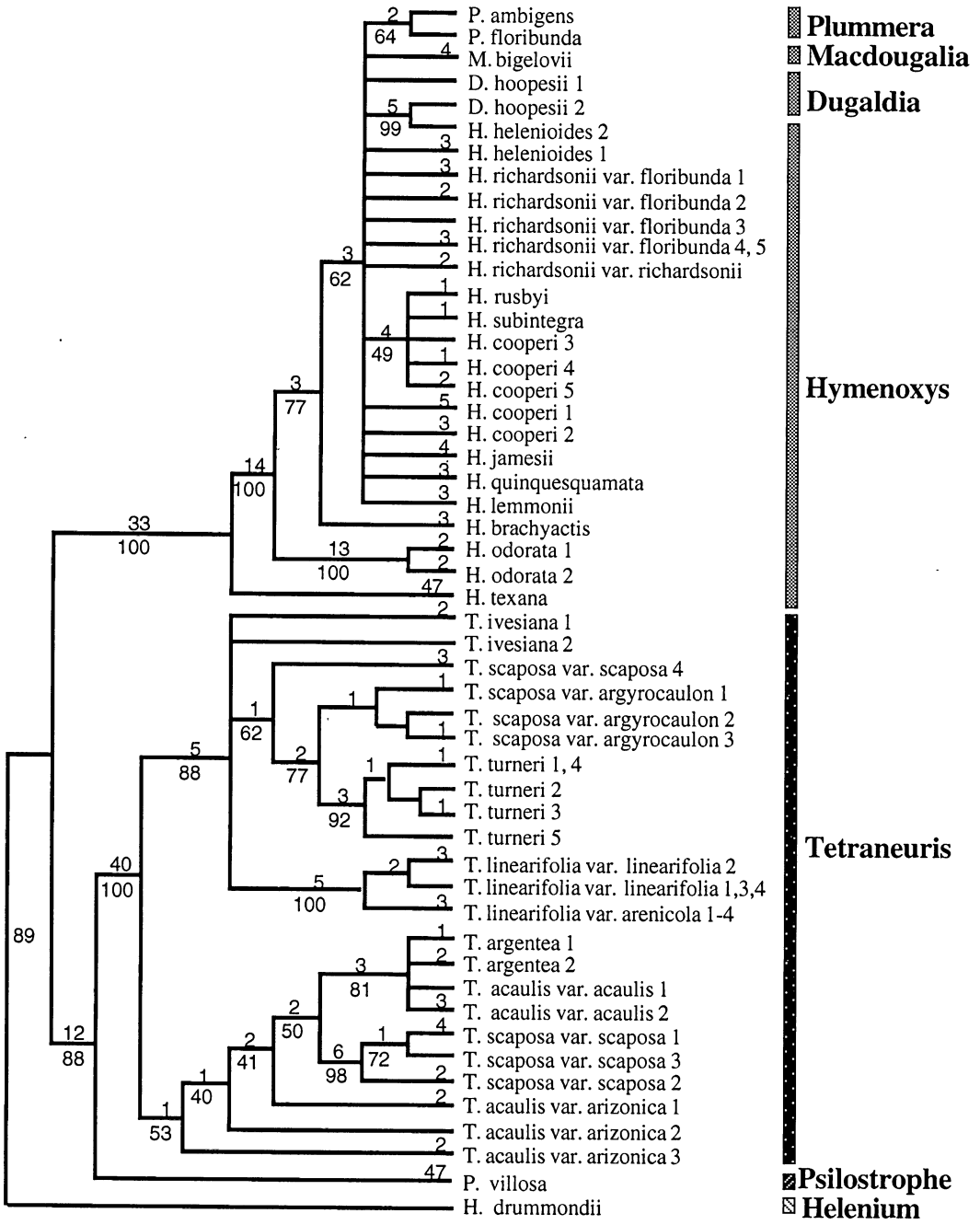

FIG. 1. Strict consensus tree of *Hymenoxys* sensu lato based on 171 phylogenetically informative DNA restriction site changes. Numbers of site changes shown above each branch; bootstrap values shown below branches. The tree has 223 steps, a consistency index of 0.76 and a retention index of 0.98.

bootstrap value of 88% unite *Psilostrophe* and *Tetraneuris*. It should be noted, however, that *Psilostrophe* is separated from *Tetraneuris* by 87 characters, two more than separate the *Tetraneuris* clade and the clade containing *Hymenoxys*, *Dugaldia*, *Macdougalia*, and *Plummera*.

The monophyletic nature of *Tetraneuris* by itself is very strongly supported by the presence of 40 synapomorphies and a bootstrap value of 100%. Within *Tetraneuris*, five synapomorphies and a bootstrap value of 88% unite *Tetraneuris ivesiana*, *T. scaposa* var. *scaposa* 4, *T. scaposa* var. *argyrocaulon*, *T. turneri*, *T. linearifolia* var. *linearifolia*, and *T. linearifolia* var. *arenicola*. The *T. linearifolia* varieties are strongly associated by five synapomorphies and a bootstrap value of 100%, and *T. scaposa* var. *argyrocaulon* is associated with *T. turneri* by two synapomorphies and a bootstrap value of 77%. Among the other *Tetraneuris* taxa, there is a strong association of *T. argentea* with *T. acaulis* var. *acaulis* supported by a bootstrap value of 81%, and a strong association of *T. scaposa* var. *scaposa* 1, 2, and 3 (note that *T. scaposa* var. *scaposa* 4 is well separated) supported by a bootstrap value of 98%.

In several cases, multiple populations of the same taxon were identical with regard to DNA restriction site changes. *Hymenoxys richardsonii* var. *floribunda* 4 and 5 were identical to one another, as were *T. turneri* 1 and 4, *T. linearifolia* var. *linearifolia* 1, 3, and 4, and all four populations of *T. linearifolia* var. *arenicola*. More often, multiple populations of the same taxon exhibited differences in restriction sites. *Dugaldia hoopesii* 1 and 2 were different from one another, as were *Hymenoxys helenioides* 1 and 2, *H. richardsonii* var. *floribunda* 1, 2, 3, and 4/5, *H. cooperi* 1, 2, 3, 4, and 5, *H. odorata* 1 and 2, *Tetraneuris ivesiana* 1 and 2, *T. scaposa* var. *scaposa* 1, 2, 3, and 4, *T. scaposa* var. *argyrocaulon* 1, 2, and 3, *T. turneri* 1/4, 2, 3, and 5, *T. linearifolia* var. *linearifolia* 1/3/4 and 2, *T. argentea* 1 and 2, *T. acaulis* var. *acaulis* 1 and 2, and *T. acaulis* var. *arizonica* 1, 2, and 3.

Discussion

DNA restriction site variation strongly supports the notion that *Dugaldia*, *Macdougalia*, and *Plummera* are congeneric with *Hymenoxys*. In fact, these taxa align much more closely with the taxa of *H.* subgenus *Picradenia* than do *H. odorata* and *H. texana*. As a matter of consistency, if *Dugaldia*, *Macdougalia*, and *Plummera* were to be recognized as separate genera, it would be necessary to recognize *Hymenoxys odorata* and *H. texana* at the generic level. Taxonomically, *Macdougalia* has been included in *Hymenoxys* by most recent workers (e.g., Turner and Powell, 1977; Bierner, 1994), but submersion of *Plummera* in *Hymenoxys* has been suggested only by Turner et al. (1973), Turner and Powell (1977), and Bierner (1994), and only Bierner (1994) has submerged *Dugaldia* in *Hymenoxys*.

Conversely, the taxa of *Tetraneuris* are clearly separated from the other taxa, forming a monophyletic clade supported by the presence of 40 shared characters and a bootstrap value of 100%. In fact, *Tetraneuris* is separated from *Hymenoxys*, *Dugaldia*, *Macdougalia*, and *Plummera* by a total of 85 character changes and is separated from *Psilostrophe* by 87 character changes, degrees of separation that we believe merit recognition at the generic level.

Psilostrophe was chosen to serve as an outgroup and was not a focus of this study. However, its association with *Tetraneuris* in the DNA phylogenetic tree is noteworthy, because many workers, including Bentham (1873), Rydberg (1914), and Turner and Powell (1977) have placed *Psilostrophe* in a different subtribe from *Hymenoxys* sensu lato as discussed here. It is apparent from Fig. 1 that *Psilostrophe* is distinctly closer to *Tetraneuris*, with which it shares 12 synapomorphies, than to *Hymenoxys*; however, 87 characters separate *Psilostrophe* and *Tetraneuris*. Keeping in mind that this study was intended to examine relationships among *Hymenoxys* and its putative closest

relatives, many taxa in subtribe Gaillardiinae were not included. The position of *Psilostrophe* will be much clearer, therefore, when we are able to examine other genera of subtribe Gaillardiinae such as *Amblyolepis* DC., *Baileya* Harv. & A. Gray, *Balduina* Nutt. (including *Actinospermum* Elliott), *Gaillardia* Foug. (including *Agassizia* A. Gray & Engelm. and *Guentheria* Spreng.), *Helenium* (including *Actinea* A. L. Juss., *Cephalophora* Cav., *Hecubaea* DC., *Leptopoda* Nutt., and *Tetrodus* Cass.), *Marshallia* Schreb., and *Plateilema* (A. Gray) Cockerell.

On morphologic grounds, *Hymenoxys texana* seems to belong within the genus *Hymenoxys*, and DNA restriction site data clearly associate it with *Hymenoxys* (33 synapomorphies with a bootstrap value of 100%). But, it is separated from the other taxa by 61 character changes (bootstrap value of 100%), and its relationship to any other taxon in this clade is unclear. Furthermore, different from *Hymenoxys* and similar to *Tetraneuris*, *H. texana* possesses monoterpene glycosides and lacks seco-pseudoguaianolides (Spring et al., 1994), and its very unusual chromosome numbers of n = 8 and 3 (Strother and Brown 1988) are unlike any reported from other taxa in either *Hymenoxys* or *Tetraneuris* (mainly n = 15 with some dysploidy and polyploidy; Bierner, 1994). Despite the conflicting data, it is our opinion that the 33 synapomorphies that *H. texana* shares with the *Hymenoxys* taxa is compelling evidence for maintaining it in *Hymenoxys*, but the 61 character changes by which it differs from the other taxa is compelling evidence for separating it into a different subgenus, *Picradeniella* Cockerell, as suggested by Cockerell (1904).

Recent work by Anderson et al. (1996) provides convincing evidence that *Hymenoxys helenioides* is a hybrid between *Dugaldia hoopesii* and *H. richardsonii* var. *floribunda*. In our study, *D. hoopesii* 2, *H. helenioides* 1 and 2, and *H. richardsonii* var. *floribunda* 1 were all collected at the same locality. *Dugaldia hoopesii* 2 from Utah differs from *D. hoopesii* 1 from New Mexico by four character changes, and yet *H. helenioides* 2 (unlike *H. helenioides* 1) is identical to *D. hoopesii* 2 with regard to cpDNA restriction sites. This suggests to us that an individual of *D. hoopesii* in the Utah population was likely the female parent of *H. helenioides* 2.

Another finding is the separation of *Hymenoxys cooperi* 3, 4, and 5 from *H. cooperi* 1 and 2. Populations 1 and 2 are from California and Nevada in the general vicinity of the type locality of *H. cooperi* (California, San Bernardino County, Providence Mountains). Populations 3, 4, and 5 are from Utah and Arizona in the general vicinity of the type locality of *H. biennis* (A. Gray) H. M. Hall (Arizona, Mohave County, Mokiak Pass). On morphologic grounds, the first author cannot find characters that consistently separate these populations and, therefore, has treated *H. biennis* as conspecific with *H. cooperi* (Bierner, unpublished). DNA restriction site data suggest that this decision should be reexamined.

Likewise, as noted in the results section, there is considerable restriction site variation among multiple populations of several other taxa. All of these taxa exhibit morphologic variation among their populations, often to the extent that the variants have been described as species or varieties. For example, *Hymenoxys richardsonii* var. *floribunda* has eight taxonomic synonyms (sensu Bierner, unpublished). Population 1 from this study is from Garfield County, Utah, and is perhaps referable to *H. richardsonii* subsp. *macrantha* (Nelson) Cockerell var. *utahensis* Cockerell, population 2 is from Coconino County, Arizona, and is perhaps referable to *H. floribunda* (A. Gray) Cockerell var. *arizonica* Cockerell or *H. floribunda* var. *intermedia* Cockerell, population 3 is from Sandia Crest in Bernalillo County, New Mexico, and is morphologically somewhat distinct from other populations (not described in the literature; Bierner, pers. obs.), and populations 4 and 5 (which were identical to one another with

regard to DNA restriction site changes) are from Grant County, New Mexico, and are probably referable to *H. metcalfei* Cockerell. As with *H. cooperi*, therefore, DNA restriction site data suggest that populations of several taxa in this complex have diverged from one another to some extent and should be examined more closely to determine whether taxonomic recognition of any of these populations is warranted.

Within *Tetraneuris*, *T. ivesiana*, *T. scaposa* var. *scaposa* 4, *T. scaposa* var. *argyrocaulon*, *T. turneri*, *T. linearifolia* var. *linearifolia*, and *T. linearifolia* var. *arenicola* are strongly associated by DNA restriction site data. *Tetraneuris ivesiana* is found in the four-corners area of northeast Arizona, southeast Utah, southwest Colorado, and northwest New Mexico (the populations in this study were collected in Utah), *T. scaposa* var. *scaposa* ranges from southern Nebraska south to Kansas, Colorado, Oklahoma, New Mexico, Texas, and northern Mexico, making a close approach to the range of *T. ivesiana* in northwest New Mexico (population 4 was collected in southcentral Texas west of Austin), *T. linearifolia* var. *linearifolia* ranges from northcentral Oklahoma south to south Texas and northern Mexico and west to west Texas and southeastern New Mexico (the populations in this study were collected in south Texas), and *T. scaposa* var. *argyrocaulon*, *T. turneri*, and *T. linearifolia* var. *arenicola* are all south Texas endemics. At first glance, therefore, it would seem unusual to relate *T. ivesiana* from the four-corners area with taxa restricted to south Texas; however, both *T. scaposa* var. *scaposa* and *T. linearifolia* var. *linearifolia* bridge the geographic gap. DNA restriction site data further group *T. scaposa* var. *scaposa* 4, *T. scaposa* var. *argyrocaulon*, and *T. turneri*, with *T. scaposa* var. *argyrocaulon* and *T. turneri* being strongly associated with one another. Also, the *T. linearifolia* varieties, consistent with their taxonomic treatment, form a very strongly supported clade.

Although *Tetraneuris scaposa* var. *scaposa* 4 is clearly related to *T. ivesiana*, *T. scaposa* var. *argyrocaulon*, *T. turneri*, *T. linearifolia* var. *linearifolia*, and *T. linearifolia* var. *arenicola* as described above, the *T. scaposa* var. *scaposa* 1, 2, and 3 populations are separated into a different clade and associated with one another with a bootstrap value of 98%. As mentioned above, population 4 was collected in southcentral Texas, but population 1 was collected in northcentral New Mexico (Torrence County), and populations 2 and 3 were collected in west Texas (Jeff Davis and Brewster counties). DNA restriction site data, therefore, indicate that considerable divergence has occurred among populations that we are recognizing in this study as *T. scaposa* var. *scaposa*, and a thorough examination of the *T. scaposa* complex should be undertaken.

Finally, *Tetraneuris argentea* and *T. acaulis* var. *acaulis* are monophyletic with a bootstrap value of 81%. The ranges of these taxa overlap in northern New Mexico, and although the former has stem leaves and the latter is scapose, both are characterized by dense appressed silky pubescence, which is not seen in the other taxa of *Tetraneuris*.

Conclusions

In summary, our DNA restriction site studies support the following conclusions:

1. *Dugaldia*, *Macdougalia*, and *Plummera* are congeneric with *Hymenoxys*.
2. Tetraneuris is a genus distinct from but related to *Hymenoxys*.
3. *Psilostrophe* belongs within subtribe Gaillardiinae and is more closely related to *Tetraneuris* than to *Hymenoxys*.
4. *Hymenoxys texana* resides within *Hymenoxys*, but it should be placed in its own subgenus, *Picradeniella*.
5. *Hymenoxys helenioides* appears to be a hybrid between *Hymenoxys richardsonii* var. *floribunda* and *Dugaldia hoopesii* (= *Hymenoxys hoopesii* [A. Gray] Bierner).
6. Populations referable to *Hymenoxys biennis* may be distinct from *H. cooperi*.
7. Populations of several taxa in this complex (e.g., *Hymenoxys richardsonii* var. *flori-*

bunda sensu Bierner) have diverged from one another to some extent and may warrant taxonomic recognition.

8. *Tetraneuris ivesiana*, *T. scaposa* var. *scaposa* (from central Texas), *T. scaposa* var. *argyrocaulon*, *T. turneri*, *T. linearifolia* var. *linearifolia*, and *T. linearifolia* var. *arenicola* form a phylogenetically related subgroup, within which *T. scaposa* var. *scaposa* (from central Texas), *T. scaposa* var. *argyrocaulon*, and *T. turneri* are further associated.

9. *Tetraneuris linearifolia* var. *linearifolia* and *T. linearifolia* var. *arenicola* are a closely related varietal pair.

10. Populations recognized in this study as *Hymenoxys scaposa* var. *scaposa* have diverged from one another.

11. *Tetraneuris argentea* and *T. acaulis* var. *acaulis* are phylogenetically closely related.

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