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EVIDENCE FOR A CLOSE RELATIONSHIP BETWEEN IOSTEPHANE AND VIGUIERA (ASTERACEAE: HELIANTHEAE)[1]

EDWARD E. SCHILLING[2] AND JOSE L. PANERO

Department of Botany, University of Tennessee, Knoxville, Tennessee 37996-1100; and
Grinnell College, Grinnell, Iowa 50112-0806

The phylogenetic relationship of *Iostephane* is assessed using data from morphology, flavonoid chemistry, and chloroplast DNA and nuclear ribosomal DNA restriction fragment analysis. Morphological evidence supports placement of *Iostephane* in subtribe Helianthinae, but fails to clarify the placement of the genus within this assemblage. Further evidence for the placement of *Iostephane* in subtribe Helianthinae is provided by the presence in all species of the genus of floral flavonoids of the chalcone/aurone type, which provides a distinctive trait for the subtribe within the tribe Heliantheae. Analysis of chloroplast DNA from two species of *Iostephane*, *I. heterophylla* and *I. madrensis*, in comparison to *Viguiera* and related genera indicates that the restriction site patterns with 16 enzymes for the *Iostephane* species are virtually identical to one another as well as to those of *Viguiera* sect. *Maculatae*. Data from restriction fragment patterns of nuclear rDNA are concordant with the results from chloroplast DNA in suggesting a direct relationship between the two groups. The close phylogenetic relationship between *Iostephane* and *Viguiera* sect. *Maculatae* suggested by the DNA restriction fragment data was not suggested by any other set of data.

Iostephane Benth. is a small genus of about four species endemic to Mexico. The genus forms a readily recognizable assemblage based on its subscapiform, heavy-rooted perennial herb habit and preference for pine, oak, or pine-oak forests between 1,500 and 3,000 m (Strother, 1983). Because there is variability between species for the presence or absence of a pappus, a character often considered to be important at the generic level in Asteraceae, the genus has only recently been circumscribed in its current constitution (Strother, 1983). The relationships of the genus have been suggested to lie with subtribe Helianthinae (Robinson, 1981; Strother, 1983) based on reproductive characters, including the presence of pales, hermaphroditic disk flowers, striate and carbonized achenes, sterile rays, a single stigmatic surface, and style ducts outside the veins in the shaft (Robinson, 1981).

Robinson (1981) suggests that the subtribe Helianthinae is one of the most concisely characterized subtribes of the tribe Heliantheae. The bulk of subtribe Helianthinae is formed by a group of species currently classified as

Viguiera and its related genera, including *Helianthus* L., *Heliomeris* Nutt., *Pappobolus* S. F. Blake, *Simsia* Pers., and *Tithonia* Desf. ex Gmelin. In addition to morphological characters, *Viguiera* and related genera have been shown to be characterized by the shared presence of floral chalcone/aurone pigments (Harborne and Smith, 1978; Schilling, 1983; Rieseberg and Schilling, 1985; Schilling and Panero, 1988; Schilling, Panero, and Bohm, 1988; Schilling and Spooner, 1988), a type of flavonoid that is otherwise uncommon in tribe Heliantheae except in subtribe Coreopsidinae (Crawford and Stuessy, 1981). Data from chloroplast DNA (cpDNA) restriction fragment variation provides further evidence for the relatedness of this group, and suggests that *Viguiera* as currently constituted forms a paraphyletic assemblage relative to the other genera (Schilling and Jansen, 1989).

The current study is an attempt to determine the phylogenetic relationships of *Iostephane*. Initially, a single sample of the genus was analyzed as a possible outgroup to polarize chloroplast DNA restriction site changes within *Viguiera* and related genera. Additional samples were then analyzed to verify the somewhat surprising result that its chloroplast DNA restriction site pattern is very similar to that of *Viguiera* sect. *Maculatae* (S. F. Blake) Panero & E. Schilling, a group that is phenetically very different. Additional data from morphology, chemistry, and restriction fragment analysis of a second molecule, nuclear ribosomal DNA

(rDNA), were then sought to provide further perspective on these results.

MATERIALS AND METHODS

The following samples of *Iostephane* were analyzed for DNA restriction site variation, using living plants grown at the University of Tennessee greenhouses (all originating from Mexico; vouchers at TENN): *I. heterophylla*: Durango, *Panero and Schilling 1549* (I1); *Breedlove 44199* (I2; grown from seed provided by Strybing Arboretum); Guerrero, *Schilling and Panero 86-A* (I3); Jalisco, *Schilling and Panero 88-53* (I4); and *I. madrensis*: Guanajuato, *Schilling and Panero 88-22* (IM). Analysis of morphology and floral flavonoids utilized these samples, as well as the following, using material collected from natural populations: *I. papposa,* Oaxaca, *Panero et al. 617*; *I. trilobata,* Oaxaca, *Schilling and Panero 88-34.*

For morphological studies, the 33 samples of *Viguiera, Helianthus, Heliomeris, Simsia,* and *Tithonia* (species names in Fig. 1; sources in Table 1 of Schilling and Jansen, 1989) analyzed previously for cpDNA variation were considered to be exemplars of their respective taxa, and were analyzed together with samples of *Iostephane* species. A total of 27 characters (Table 1) was scored for these samples, including ones used traditionally for taxonomic delimitation as well as novel characters from leaf venation and floral micromorphology (the latter discussed fully in Panero, in press). Deletion of samples with identical scores for these characters resulted in a data set of 25 samples used for cladistic analysis (deleted samples included *Helianthus giganteus,* two samples of *Viguiera dentata, V. deltoidea, V. incana, V. puruana, V. tomentosa,* and *V. triangularis*). The genus *Flourensia* was utilized as an outgroup to polarize character changes. Wagner parsimony was performed utilizing the PAUP program (version 2.4, D. Swofford, Illinois Natural History Survey) implemented on an IBM-PC. Most parsimonious trees were sought by implementing the multiple parsimony (MULPARS) with global branch swapping (SWAP = GLOBAL) options. A strict consensus tree was generated using the CONTREE program.

Methods for flavonoid analysis generally followed Mabry, Markham, and Thomas (1970) and Markham (1982). Dried flowering heads (ca. 1 g) were ground and extracted overnight in methanol. The extract was filtered, concentrated, and chromatographed on Whatmann 3MM paper two-dimensionally using t-buta-

TABLE 1. *Characters scored for cladistic analysis of* Viguiera, Iostephane, Helianthus, Tithonia *and* Simsia

1. Habit (0^a = woody; 1 = herbaceous)
2. Habit (0^a = not scapose; 1 = scapose)
3. Chromosome number (0^a = 18; 1 = 17; 9 = 8)
4. Leaves lobed (0^a = absent; 1 = present)
5. Leaf vein course (0 = straight/sinuous; 1 = zigzag)
6. Leaf vein areolation (0 = well developed; 1 = imperfect)
7. Synflorescence structure (0 = dichasial; 1 = monochasial)
8. Head shape (0 = hemispherical; 1 = campanulate/cylindrical)
9. Phyllary shape (0^a = narrowed gradually; 1 = narrowed abruptly)
10. Pale apex shape (0 = entire; 1 = trifid)
11. Ray corolla UV nectar guides (0 = absent; 1 = present)
12. Disk corolla shape (0^a = straight; 1 = bulbous at base of throat)
13. Disk corolla lobe color (0^a = yellow; 1 = black)
14. Disk corolla lobe sclerified cells (0 = absent; 1 = present)
15. Disk corolla elongate moniliform glands (0 = rare; 1 = present)
16. Disk corolla tube glands (0 = present; 1 = absent)
17. Stamen connective pubescence (0 = none; 1 = present)
18. Crystals in staminal filaments (0 = few; 1 = abundant)
19. Anther color (0 = black; 1 = orange)
20. Anther collar cells (0 = thickened; 1 = heavily thickened)
21. Anther endothecial cells (0 = polarized; 1 = partially polarized)
22. Style appendage (0 = minute; 1 = well developed)
23. Style branch shape (0 = deltoid; 1 = tapering)
24. Ray ovary (0 = short; 1 = long, narrow)
25. Disk achene pappus (0 = persistent; 1 = deciduous; 9 = absent)
26. Disk achene pappus (0 = present; 1 = absent)
27. Disk achene type (0^a = biconvex; 1 = very flattened)

[a] Plesiomorphic character state, polarized relative to *Flourensia*.

nol : acetic acid : water (3:1:1) and 15% acetic acid. Flavonoid spots, visualized with UV light, were cut from chromatograms and extracted in MeOH, followed by Sephadex LH-20 column chromatography. Compound identification was based on color under UV light, UV spectral data, and cochromatography with authentic compounds identified from previous studies of *Helianthus* and *Viguiera*. Localization of compounds of the chalcone and aurone types was done by visual inspection of dried, intact flowering heads that were flooded with ammonia vapor, which produces a red color reaction with these compounds.

Methods for DNA restriction fragment analysis generally followed Jansen and Palmer (1988). Preparations of total DNA were made from 1.5 g of fresh leaves by the procedure of Doyle and Doyle (1987). Restriction endonuclease digestions (utilizing the following enzymes: *Ava*I, *Bam*HI, *Ban*I, *Ban*II, *Bcl*I, *Bgl*II,
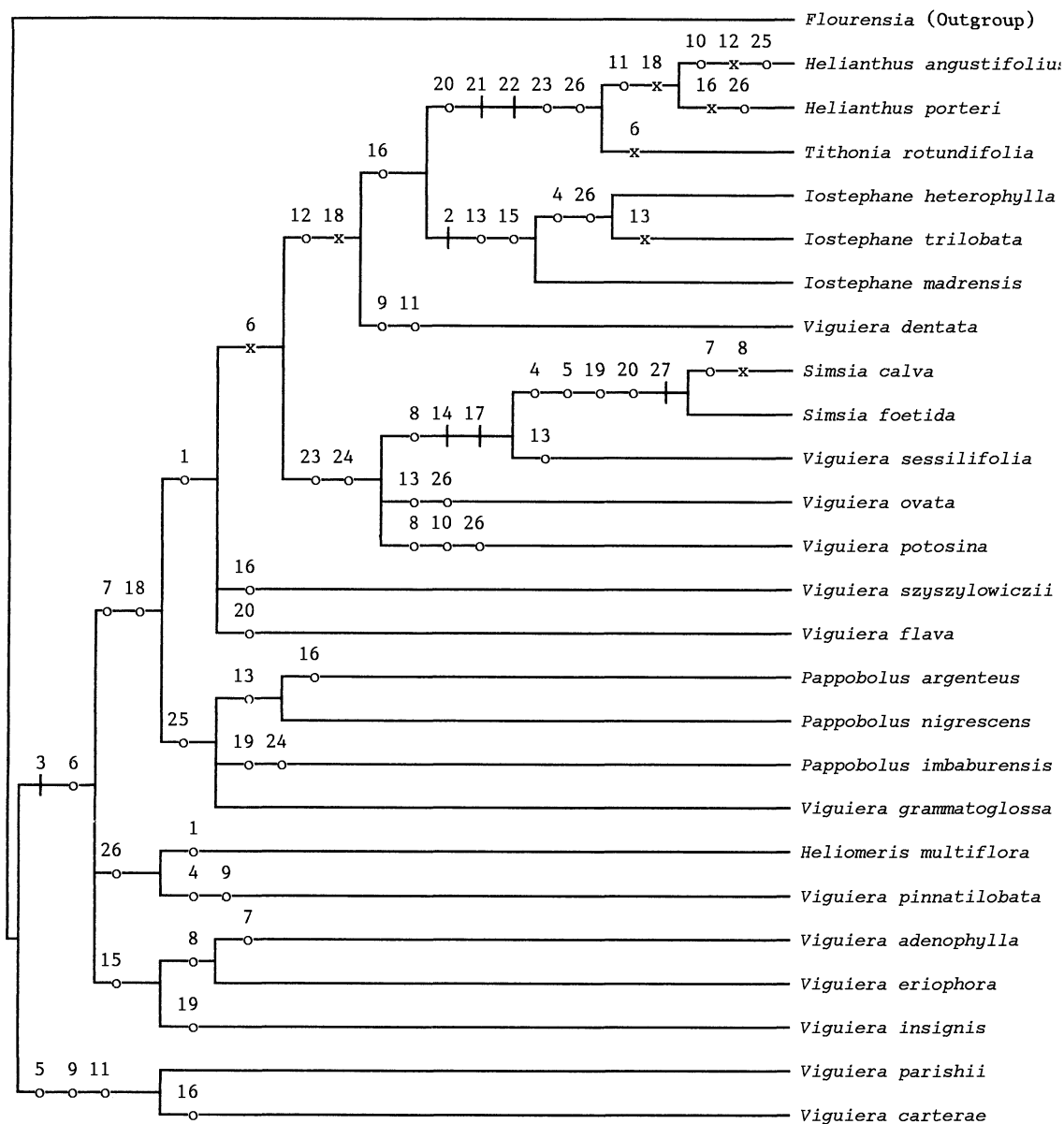
Fig. 1. Strict consensus tree generated from parsimony analysis using morphological data, showing relationships of *Iostephane* and related genera. Characters numbered as in Table 1. |, apomorphy; O, parallelism; ×, reversal.

*Bst*NI, *Bst*XI, *Cla*I, *Eco*RI, *Eco*RV, *Hae*II, *Hinc*II, *Hind*III, *Nco*I, *Nsi*I), agarose gel electrophoresis, and bidirectional transfer of DNA fragments from agarose gels to Zetabind (AMF CUNO) nylon filters were performed as described in Palmer (1986) and Jansen and Palmer (1987). Preparation of digoxigen-labeled probes and filter hybridizations followed the manufacturer's instruction ("Genius Kit," Boehringer Mannheim Biochemicals, Indianapolis). The 22 cloned restriction fragments of lettuce cpDNA were combined into batches

for filter hybridization experiments. Mapping of fragments was done relative to data of Jansen and Palmer (1987, 1988) and Schilling and Jansen (1989). Because the cpDNA restriction fragment site pattern for *Iostephane* samples was almost identical to that of samples of *Viguiera* sect. *Maculatae*, it was possible to place these unequivocally on the cladogram from restriction site mutation data generated by Schilling and Jansen (1989). Ribosomal DNA variation was detected by hybridization to plasmids containing a single 18S-28S rDNA

TABLE 2.   *Chemistry and distribution of* Iostephane *floral flavonoids*[a]

Species	Quercetin 3-glucoside	Quercetin 3-glucuronide	Coreopsin/ Sulfurein[b]	Ligules	Disk
I. heterophylla	+	+	+	−	+
I. madrensis	+	+	+	−	+
I. papposa	+	+	+	+	+
I. trilobata	+	+	+	+	+

[a] +, compound present; −, compound not detectable.
[b] Coreopsin spontaneously isomerizes to form sulfurein, so the two compounds occur together in plant extracts.

repeat unit from *Helianthus argophyllus* (originally isolated by Mike Arnold), and mapped relative to data from *Helianthus* of Rieseberg (unpublished data).

RESULTS

Morphological analysis—Parsimony analysis of morphological data using PAUP produced a total of 54 equally parsimonious minimum length trees. A strict consensus tree produced from these is shown in Fig. 1. This tree has a length of 67 steps and a consistency index of 0.40. An estimate of the homoplasy excess ratio (HER) for this tree was calculated using equations (4) and (5) from Archie (1990), and this produced a value of HER = 0.43. This value suggests that there is a high proportion of phylogenetically random variation in the data set. In agreement with cpDNA data, the woody habit appears to be plesiomorphic within this group, with the basal clades including primarily woody taxa. One of these, *V.* subg. *Bahiopsis,* is placed as the sister group to the remaining taxa. Although this is also in agreement with cpDNA data, it should be noted that this position is influenced by the choice of an outgroup with a chromosome base number of $x = 18$. Another basal clade is formed by the samples of *V.* sect. *Maculatae. Iostephane* is placed with other herbaceous taxa in a large terminal clade as the sister group to *Helianthus* and *Tithonia.* These taxa are not, however, well resolved, and most of the defining characters are parallelisms or reversals. This situation can be illustrated by consideration of the effect of adding an additional character (the presence or absence of foliar subsessile glands) that was removed because of its high level of homoplasy, and because chemical studies of individual taxa have suggested that the loss of such glands has occurred repeatedly in different groups (Schilling, 1983; Schilling and Panero, 1988; Schilling, Panero, and Bohm, 1988). After addition of this character, the herbaceous clade in the consensus tree becomes altered

TABLE 3.   *Chloroplast DNA restriction site mutations differentiating samples of* Iostephane *and* Viguiera *sect.* Maculatae *from other members of* Viguiera *and related genera, numbered following Schilling and Jansen (1989)*

Probe[a]		Enzyme	Mutation
13.	18.8 Kb	*Hinc*II	4.6 = 4.3 [+0.3]
19.	14.7 Kb	*Bcl*I	3.8 + 2.7 = 6.5
26.	14.7 Kb	*Eco*RV	1.4 + 2.1 = 3.5
47.	10.6 Kb	—	Deletion
61.	10.6 Kb	*Nsi*I	0.5 + 0.5 = 1.0
72.	7.7 Kb	*Hinc*II	7.2 = 4.6 + 2.6
75.	7.0 Kb	*Bam*HI	3.7 + 3.7 = 7.4
105.	6.7 Kb	*Nsi*I	16.7 = 12.2 + 4.5
109.	6.3 Kb	*Ban*II	1.2 + 2.5 = 3.7
113.	6.3 Kb	*Eco*RI	0.9 [+0.3] = 1.2
128.	4.6 Kb	*Eco*RI	1.8 [+0.1] = 1.9
144.	9.0 Kb[b]	*Bcl*I	4.0 + 2.6 = 6.6
148.[c]	3.8 Kb	*Ava*I	3.2 = 2.2 + 1.0

[a] From lettuce *Sac*I cpDNA clone bank (Jansen and Palmer, 1987), listed by fragment size.
[b] Fragment from petunia 15.4 Kb region not cloned from lettuce.
[c] Mutation newly reported in this paper.

that most of its internal nodes are unresolved; only *Simsia* and *Iostephane* are retained as defined groups (not shown).

Floral flavonoid chemistry—Samples of all species of *Iostephane* produced identical profiles for floral nonanthocyanic flavonoids, which included two quercetin glycosides and the isomeric chalcone/aurone pair coreopsin/ sulfuerin (Table 2). This is notable given the differences between species in ray flower coloration (*I. heterophylla* has purple rays whereas the other species have yellow ones). There were, however, significant differences in localization of the chalcone/aurone pigments. These compounds were produced throughout the ligule of *I. trilobata* and *I. papposa,* whereas they were restricted to the disk corolla lobes in *I. madrensis* and *I. heterophylla* (Table 2). Thus it is expected that the coloration of the ligule detected by pollinators sensitive to UV light is different for *I. madrensis* compared to the other two species with yellow ligules.

Chloroplast DNA analysis—All of the *Iostephane* samples shared the same ten restriction site mutations (Table 3) that distinguish *Viguiera* sect. *Maculatae* relative to *V.* subg. *Bahiopsis* (the "Baja California Group"), and lacked any additional site mutations characteristic of other taxa of *Viguiera* and related genera. No apomorphic mutations were detected that would distinguish *Iostephane* from *V.* sect. *Maculatae,* although some samples of both groups have individual autapomorphies.
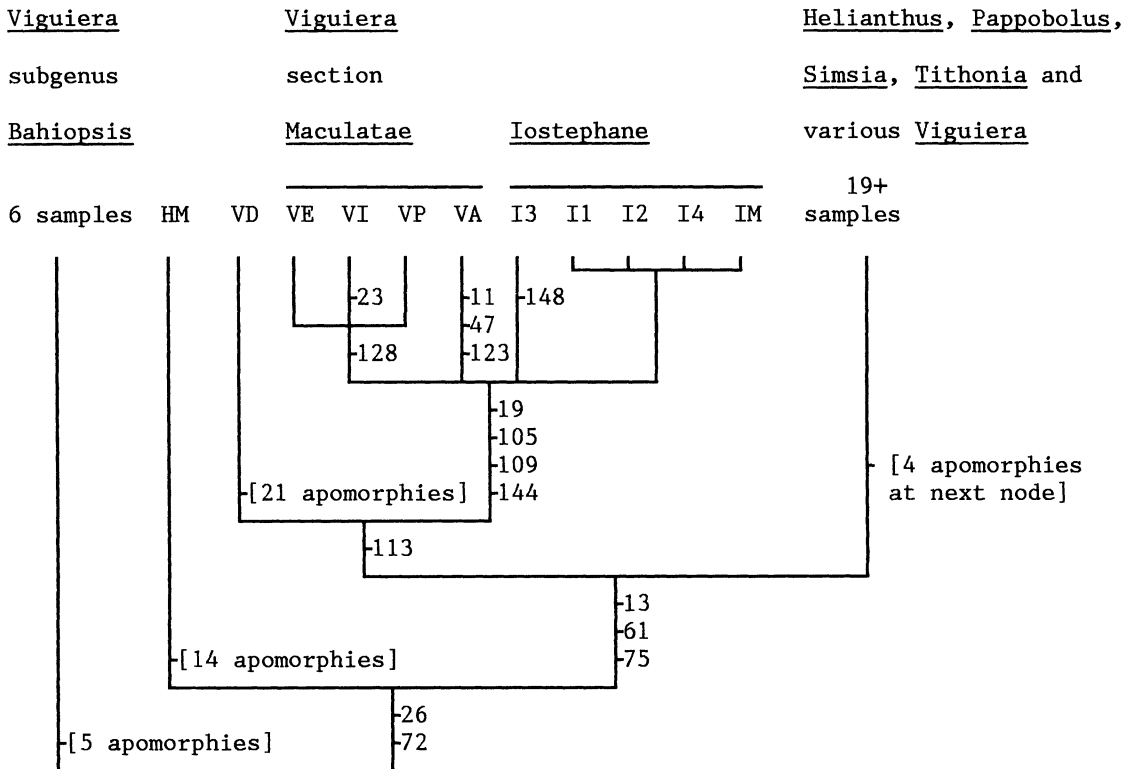
Fig. 2. Single most parsimonious Wagner tree of *Viguiera* and related species based on cpDNA restriction site mutation data, from Schilling and Jansen (1989), showing placement of samples of *Iostephane* (numbered as in text). Mutations (numbered as in Table 3) or numbers of mutations shown along branches. HM, *Heliomeris mutiflora*; VA, *V. adenophylla*; VD, *V. dentata*; VE, *V. eriophora*; VI, *V. insignis*; VP, *V. puruana*.

Hence, the *Iostephane* samples can be placed (Fig. 2) to form an unresolved polychotomy, together with *V. adenophylla* and the other samples of *V.* sect. *Maculatae,* in the most parsimonious Wagner tree produced by Schilling and Jansen (1989). No additional homoplasy is introduced into this tree by inclusion of the samples of *Iostephane.*

Nuclear rDNA analysis —Variation in restriction sites readily interpreted relative to data from *Helianthus* (Rieseberg, unpublished data) was available for the following enzymes: *Bcl*I, *Bgl*II, *Eco*RI, *Eco*RV, *Hind*III, *Nsi*I. Other enzymes either produced no variability among samples or produced band patterns that were not easily interpreted without further experimentation. In addition to restriction site variation, there were also differences in the length of the basic repeat unit. The postulated rDNA restriction site mutations are shown relative to the most parsimonious cpDNA tree (Fig. 3). Except for some size variation, the restriction fragment patterns of the samples of *Iostephane* were the same as those of samples of *V.* sect. *Maculatae* (Fig. 3). There were no additional

bands in the *Iostephane* samples that would provide possible evidence of polymorphism for rDNA molecules.

DISCUSSION

Comparison of morphological and DNA restriction site data suggests that *Iostephane* is phyletically close to a taxon, *Viguiera* sect. *Maculatae,* from which it is phenetically quite distinct. Like many genera of Asteraceae, *Iostephane* is recognized by the presence of discrete autapomorphies. There are, however, relatively few characters that are potential synapomorphies to represent its relationships to other taxa. This problem is exacerbated in Asteraceae because of the high degree of homoplasy exhibited by other characters.

Cladistic analysis of morphology, including both traditional taxonomic characters as well as novel ones from leaf venation and floral micromorphology, fails to clarify the relationships of *Iostephane* (Fig. 1). In the consensus tree, *Iostephane* is placed in a clade formed by the herbaceous members of *Viguiera* and related genera. Although *Iostephane* is further
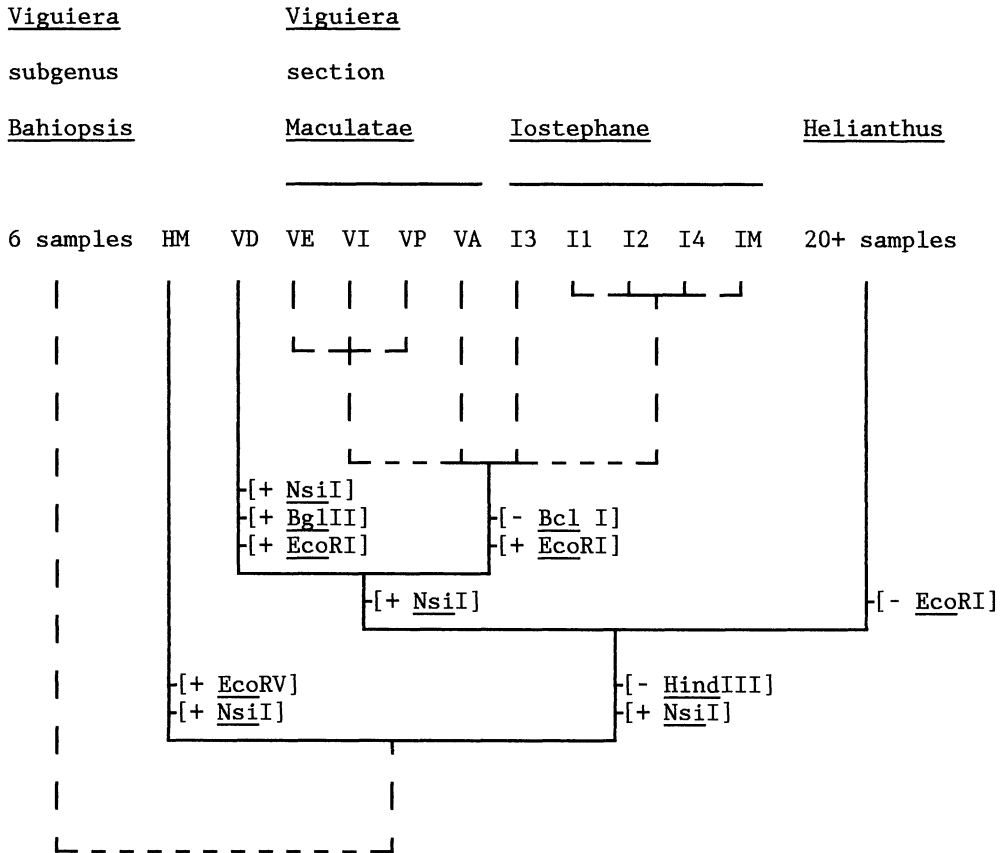
Fig. 3.   Postulated restriction site mutations in nuclear ribosomal DNA, showing site gains (+) and losses (−), plotted on most parsimonious Wagner tree based on chloroplast DNA data (Fig. 2). Dashed lines show parts of tree that are unsupported by nuclear ribosomal DNA information. Abbreviations as in Fig. 2.

placed as a sister group to *Helianthus* and *Tithonia,* all of the characters that support this placement exhibit a high degree of homoplasy in the analysis. The overall high level of homoplasy and lack of resolution provided by the cladistic analysis, however, suggest that morphological data provide little information regarding the relationships of taxa of *Viguiera* and related genera. The estimates of the excess homoplasy ratio (Archie, 1990) indicate that the data set includes significant amounts of phylogenetically random variation. This is notable in that exemplars of taxa were used; inclusion of all members of each taxon could be expected to raise levels of homoplasy and lower overall resolution even further. The lack of resolution of relationships between taxa provides confirmation that the current taxonomy of this group is essentially a phenetic one. Individual taxa are recognized primarily on the basis of autapomorphies, and there are few morphological synapomorphies to indicate phylogenetic relationships between such taxa. This is particularly notable for *Iostephane,*

which is clearly distinguished by autapomorphies in growth habit but lacks any of the distinctive apomorphies that characterize other named taxa of this assemblage.

Variability and homoplasy in morphological features complicate recognition of many of the named taxa of *Viguiera* and related genera. For instance, the taxa of *Pappobolus* do not form a monophyletic group in the cladistic analysis of morphology (Fig. 1), even though considerations of biogeography and overall morphology (Panero, in press) suggest that *Pappobolus* is monophyletic. In several other groups, including *Helianthus, Simsia, Tithonia, Viguiera* sect. *Maculatae,* and *V.* subg. *Bahiopsis,* there is similar variation in defining features, although it is not reflected in the cladogram because only a subset of taxa was analyzed.

The presence in *Iostephane* heads of compounds of the chalcone/aurone type (Table 2) provides supporting evidence that *Iostephane* should be placed taxonomically in subtribe Helianthinae, near *Viguiera* and related genera.

Compounds of the chalcone/aurone type have a limited distribution in Asteraceae, but are characteristic of *Viguiera* and related genera (Crawford and Stuessy, 1981). Floral flavonoid chemistry does not, however, provide evidence to indicate more specifically the relationships of *Iostephane* within this assemblage. Although Schilling and Spooner (1988) reported the presence of quercetin 3-glucuronide to be characteristic of *Simsia,* this glycoside has now been found in various species of *Viguiera* as well (unpublished data). There were no differences in compound profiles between species of *Iostephane* (Table 2), but the distribution of compounds within the head provides another character to distinguish among the yellow-rayed species, with *I. madrensis* lacking ligule compounds whereas *I. trilobata* and *I. papposa* produce them.

Data from cpDNA restriction fragment analysis also support the relationship of *Iostephane* to *Viguiera* and related genera, and provide evidence of a direct relationship to *Viguiera* sect. *Maculatae* (Fig. 2). *Iostephane* and *V.* sect. *Maculatae* share four unique restriction mutations (three losses and one gain) relative to all other taxa of *Viguiera* and related genera that have been examined to date (Fig. 2; Table 3), generating a confidence level of 100% when assessed by the bootstrap method (Felsenstein, 1985; Schilling and Jansen, 1989). The two groups also share six further restriction sites relative to *V.* subg. *Bahiopsis,* the apparent sister group to other members of *Viguiera* and related genera, and lack any of the additional mutations that characterize various other members of this assemblage (Schilling and Jansen, 1989).

That there should be a direct phylogenetic relationship between *Iostephane* and *V.* sect. *Maculatae* is surprising, given the great differences in morphology that occur between the two groups. Members of *Iostephane* are all subscapose perennial herbs, whereas those of *V.* sect. *Maculatae* are all large shrubs or small trees (Figs. 4, 5). Although both groups are endemic to Mexico, there are also differences in habitat preference between them, with *Iostephane* occurring in pine and pine/oak forests (Strother, 1983), while members of *V.* sect. *Maculatae* occur primarily in tropical deciduous forests and extend only rarely into oak or oak/pine forests (Panero and Schilling, 1988). Previous taxonomic treatments of *Iostephane* have also failed to suggest a direct relationship with any member of *Viguiera* (e.g., Strother, 1983). For example, taxa of *Iostephane* have been at times variously placed in a total of eight other genera (*Coreopsis, Simsia, Echina-*

cea, Ximenesia, Helianthella, Pionocarpus, Rudbeckia, Gymnolomia), but never in *Viguiera* (Strother, 1983). Among the morphological characters utilized in the current study (Table 1), only one, presence of distinctive moniliform trichomes on the corolla, links *Iostephane* with *V.* sect. *Maculatae,* and the cladistic analysis of morphological data would suggest this to be a parallelism (Fig. 1).

Two alternative hypotheses that might be considered to explain the similarity in cpDNA restriction fragment patterns between *Iostephane* and *V.* sect. *Maculatae* are wide hybridization and lineage sorting, but neither appear to be very likely. It might be hypothesized that the habit of *Iostephane* originated from a paternal parent in an initial hybridization event, whereas the maternally inherited cpDNA genome is retained from a member of *V.* sect. *Maculatae.* An initial problem with this explanation is that the subscapose growth habit of *Iostephane* is observed in only a few, relatively specialized members of related taxa, notably members of *Helianthus* ser. *Angustifolii* such as *H. carnosus* and *H. radula* (Heiser et al., 1969), and that the few crosses that have been attempted between *Helianthus* and either *V.* sect. *Maculatae* or *Iostephane* have failed (unpublished data). Further, data from rDNA restriction site variation are completely consistent with cpDNA data in indicating a direct relationship between *Iostephane* and *V.* sect. *Maculatae* (Fig. 3); there is no evidence from this biparentally inherited molecule, such as polymorphism for or recombination of restriction site patterns, to indicate that hybridization between widely different parents has been involved in the origin of *Iostephane.* It might also be proposed that lineage sorting from a polymorphic ancestral pool of cpDNA genomes has resulted in retention of the same genomes in phyletically different groups. Additional survey (Schilling, unpublished data) of cpDNA restriction site patterns in over 70 other samples of *Helianthus, Pappobolus, Simsia, Tithonia,* and *Viguiera* species, all of which would presumably be descended from this same ancestral gene pool, has failed to indicate any evidence for the retention of such a cpDNA genome elsewhere.

There are some notable parallels between the situation of *Iostephane* and *Viguiera* and that of another group of Asteraceae, the Hawaiian silversword alliance. Data from cpDNA restriction fragment analysis suggest that the most speciose genus of the silversword alliance, *Dubautia,* may be paraphyletic relative to the other two genera, *Argyroxiphium* and *Wilkesia* (Baldwin, Kyhos, and Dvorák, 1990). In par-
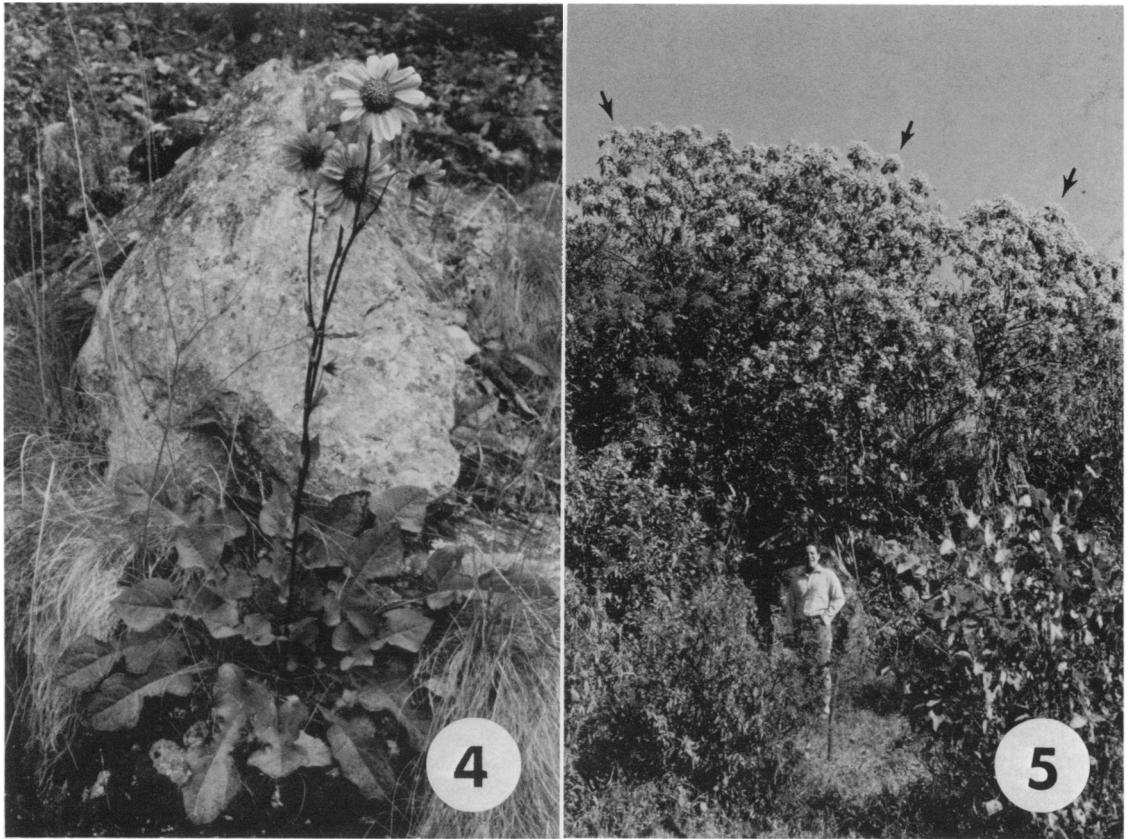
Figs. 4, 5.   Habit of *Iostephane* and *Viguiera* sect. *Maculatae*. **4.** *Iostephane heterophylla* in Jalisco, Mexico. Plant is ca. 6 dm tall. **5.** *Viguiera puruana* (arrows) in Michoacán, Mexico. Human in picture is ca. 1.8 m tall.

ticular, cpDNA data (Baldwin, Kyhos, and Dvorák, 1990) suggest that *Wilkesia,* which occurs in dry scrub and woodland habitats and produces striking cauline, yuccalike rosettes of monocotlike leaves, has as its immediate sister group a lineage of *Dubautia* species that are rain forest trees and shrubs with typical dicot habit and leaf venation (Carr, 1985). Divergence in the Hawaiian silversword alliance as a whole has produced a variety of growth forms that inhabit a range of habitats and exhibit a broad range of physiological diversity (Carr, 1985; Robichaux et al., 1990). Because the montane topography of Mexico may result in the occurrence of high altitude habitat "islands," particularly during periods of extreme climate, it might be hypothesized that the same types of forces that produced the radiation in the Hawaiian silversword alliance may also be responsible for the divergence of *Iostephane* and *V.* sect. *Maculatae.*

The case of *Iostephane* provides another of a growing list of examples where the phylogeny inferrable from cpDNA data is not in agreement with the current classification scheme.

For example, it appears that *Solanum* is paraphyletic relative to *Lycopersicon* (tomatoes), with the potatoes (*S.* sect. *Petota*) being the immediate sister group to tomatoes (Spooner, Anderson, and Jansen, 1990). Similarly, the monotypic *Heterogaura* is the sister species to a member of *Clarkia* (Sytsma and Gottlieb, 1986a, b). Soltis, Soltis, and Bothel (1990) have suggested that in herbaceous Saxifragaceae most monotypic, phenetically distinct taxa are likely to be phyletic ingroups to larger, diverse genera. There has not yet appeared any consensus in how to resolve such conflicts between phenetic and phylogenetic groups in plants. Schilling and Jansen (1989) note that other genera related to *Viguiera,* such as *Helianthus, Pappobolus* (= *Helianthopsis*), *Tithonia,* and *Simsia,* are recognized primarily by distinctive morphological features (autapomorphies), leaving a paraphyletic assemblage not characterized by any apomorphies (but mostly sharing one or more sympleisomorphies) as *Viguiera.* In the current situation, *Iostephane* differs from *Viguiera* by autapomorphies in features of the achene (shape and type of pap-

pus) and the involucre that have traditionally been considered to be important generic level characters in tribe Heliantheae. Similar types of variation in pappus type have been noted in *Pappobolus* (Panero, in press), and there is considerable variability in the involucre within *Viguiera*. A classification in agreement with the cpDNA phylogeny would be achieved by submerging *Iostephane* within *Viguiera*, but it appears advisable to await resolution of the broader problems of generic delimitation involving *Viguiera* and related genera before making a formal taxonomic proposition.

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