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Is the serpentine aster, *Symphyotrichum depauperatum* (Fern.) Nesom, a valid species and actually endemic to eastern serpentine barrens?

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Abstract. Serpentine aster, *Symphyotrichum depauperatum* (Fern.) Nesom, is the 'flagship' species of the eastern serpentine barrens, inhabiting 20 of the 26 remaining occurrences of significant size of this globally rare community type and long recognized as its only known endemic species. Previous studies have called into question both the validity of the taxon and its status as a true endemic of the serpentine barrens. We used amplified fragment length polymorphism (AFLP) analysis to compare seven serpentine barrens populations, one alleged diabase glade population, and two populations each of the two species with which *S. depauperatum* is lumped by some authors. Our analysis supports the validity of *S. depauperatum* as a distinct species, which grows almost entirely on shallow soils overlying serpentinite bedrock in Pennsylvania and Maryland, but it confirms an earlier hypothesis that *S. depauperatum* also includes small, disjunct populations on diabase glades in North Carolina.

Introduction

Strategies for establishing key areas for conserving biodiversity fall into several general categories: focusing on threatened or endangered species and their habitat, centers of endemism, biodiversity hotspots, and flagship or keystone species (World Conservation Union 1999; Harrison and Inouye 2002; Pavlik 2003; Root et al. 2003). In all of these cases, however, the lowest taxonomic unit of interest is typically the species. Populations at the periphery of a species' range may harbor unique characteristics, which could be related to different selection pressures than in the center of its range (Linhart and Grant 1996). Even though edge-of-range populations, ecotypes, varieties, and subspecies are important reservoirs of genetic diversity, rarely are conservation projects aimed at taxa below the species level given priority, despite acknowledgment by state, federal, and international agencies that the term endangered species applies to taxonomic units at or below the species level (World Conservation Union 2001; U.S. Fish and Wildlife Service 2003). Six of the nine plant species delisted (Clayton 1993; Jordan 1993; Rutman 1993; Smith 1996; Kennedy 1999) or proposed for delisting (Tarp 2002) from the U.S. federal endangered and threatened species list were lowered in conservation status because of taxonomic revision below the species level. For taxa that occur in restricted ecological communities and that historically have been classified as full species, revising the taxonomic status to an infraspecific level can potentially have severe consequences, for example, by deflecting attention and funding away from habitat protection and other conservation efforts. *Symphyotrichum* (*Aster* sensu lato) *depauperatum* (Fern.) Nesom, is potentially one such taxon. It has long been recognized as the only known endemic species of the eastern North American serpentine barrens, but its taxonomic status is unresolved.

The taxonomic status of *Symphyotrichum depauperatum* as a good species is unresolved. Jones (1984) placed *S. parviceps* (Burgess) Nesom as a subspecies of *S. pilosum* (Willd.) Nesom and *S. depauperatum* as an ecological variant of *S. pilosum* var. *parviceps*. Based on morphological measurements of herbarium and fresh specimens, Hart (1990) suggested that *S. depauperatum* was a group of serpentine-tolerant populations of *S. parviceps*. Currently, *S. depauperatum* is globally rare (ranked G2, meaning the species is imperiled because there are only 6–20 occurrences in total) and listed as a state endangered species in Maryland (Maryland Wildlife and Heritage Division 2001) and Pennsylvania (Rhoads and Block 2000; Pennsylvania Natural Diversity Inventory 2003), where more than 90% of eastern serpentine barrens occur (Latham 1993; Tyndall and Hull 1999). If *S. depauperatum* were assigned an infraspecific designation, as suggested by Jones (1984) and Hart (1990), then protections afforded or encouraged by endangered species status may no longer accrue.

The designation of *S. depauperatum* as an eastern serpentine barren endemic has also been questioned, with the identification as *S. depauperatum* from three diabase glade populations in Granville County, North Carolina (Levy and Wilbur 1990). In contrast, the North Carolina Natural Heritage Program database lists *S. parviceps* as the state-endangered glade aster and does not list *S. depauperatum* as present in North Carolina (North Carolina Natural Heritage Program 2003). If the three diabase glade populations are truly *S. parviceps*, then *S. depauperatum* should continue to be recognized as an eastern serpentine barren endemic.

In this study, we used amplified fragment length polymorphism (AFLP) analysis to assess the genetic relationships among seven populations of *S. depauperatum* from Maryland and Pennsylvania, one alleged population of *S. depauperatum* or *S. parviceps* collected at a diabase glade in North Carolina, and two populations each of two closely related species (*S. pilosum* and *S. parviceps*) collected in the Midwest. We tested two specific hypotheses: (1) *S. depauperatum* is not a subspecies of *S. pilosum* or *S. parviceps*, and (2) *S. depauperatum* is endemic to the eastern serpentine barrens. A working assumption of the first hypothesis is that genetic similarity reflects phylogeny, which has been established by an extensive treatment of *Aster* sensu lato by Nesom (1994).

Materials and methods

Tissue samples from *S. depauperatum*, *S. parviceps*, and *S. pilosum* were collected from 11 native populations and 1 seed lot purchased from a native plant seed

Table 1. Collection information on two *S. pilosum*, two *S. parviceps*, and eight *S. depauperatum* populations. Taxonomy based on Nesom (1994). Collections by authors except where noted.

Species	Site	Code	Collected	County	State[1]
Symphyotrichum pilosum	Ford's Crossing	pil-ILf	12 Sept. 2001[2]	McLean	IL
S. pilosum	Minnesota	pil-MN	–[3]	–	MN
S. parviceps	Ford's Crossing	par-ILf	12 Sept. 2001[2]	McLean	IL
S. parviceps	Riverside Park	par-ILr	12 Sept. 2001[2]	Sangamon	IL
S. depauperatum	Cedar Barrens	dep-PAc	10 Oct. 2001	Chester	PA
S. depauperatum	Brinton's Quarry	dep-PAb	10 Oct. 2001	Chester	PA
S. depauperatum	Fern Hill	dep-PAf	10 Oct. 2001	Chester	PA
S. depauperatum	Goshenville	dep-PAg	10 Oct. 2001	Chester	PA
S. depauperatum	Soldiers Delight Natural Environmental Area	dep-MDs	11 Sept. 2001	Baltimore	MD
S. depauperatum	Robert E. Lee Park	dep-MDr	11 Sept. 2001	Baltimore	MD
S. depauperatum	Pilot Barrens	dep-MDp	11 Sept. 2001	Cecil	MD
S. depauperatum	North Carolina	dep-NC	Oct. 2001[4]	Granville	NC

[1]IL = Illinois, MN = Minnesota, PA = Pennsylvania, MD = Maryland, NC = North Carolina.
[2]Collected by Vern LeGesse.
[3]Prairie Moon Nursery (purchased seed).
[4]Collected by Dr. Mark Basinger and Dr. Robert L. Wilbur.

vendor (Table 1). Plants collected in the field were placed in silica gel and stored at 4 °C until needed. The purchased seeds of *S. pilosum* were germinated under greenhouse conditions and DNA was extracted from randomly selected individuals. Total genomic DNA was extracted from approximately 0.1 g of silica-dried leaves from field-collected and 0.5 g fresh leaf material using an E.Z.N.A.® plant DNA miniprep kit (Omega Bio-Tek, Doraville, Georgia).

AFLP fingerprints were generated following the AFLP System II (Gibco BRL, Life Technologies, Inc., Gaithersburg, Maryland) protocol. Two individuals of each of the three species were used to survey all combinations of three forward and five reverse primers. Two primer combinations using a fluorescently labeled forward primer (5′-FAM-GACTGCGTACCAATTCAG-3′) and two reverse primers (5′-GATGAGTCCTGAGTAACAC-3′ and 5′-GATGAGTCCTGAGTAACAG-3′) were selected for this study, with 100 and 85 bands, respectively. Approximately 120 ng of total genomic DNA was digested with the *EcoRI*/*MseI*(2 U) restriction enzyme combination in a 20 µl reaction vessel and incubated at 22 °C overnight. Incubating at 70 °C for 20 min and then placing the tube on ice halted restriction enzyme activity. Adapter ligation solution (19.2 µl) and DNA ligase (0.8 µl) were added, incubated at 22 °C for 2 h, and the product was diluted 1:50 with TE buffer for preselective amplification. Preselective and selective amplifications followed the AFLP System II protocol. Electrophoresis was performed on the final PCR products in 5% denaturing acrylamide on an ABI Prism 377 sequencer, where profiles were recorded digitally.

Relative mobility of fragments was determined with the inclusion of an internal size standard with each sample. ABI Genescan software was used to visualize and score profiles. Binary profiles from the absence or presence of fragments were constructed for each taxon. Relationships among taxa were investigated using principal components analysis (PCA) and parallel analysis (PA) to establish which PCA axes were appropriate for interpretation (SAS Institute 1989). Parallel analysis was used to derive the 95th percentile eigenvalues for each successive PCA axis, based on a Monte Carlo analysis of Longman et al.'s (1989) regression equations. Only axes with eigenvalues greater than the PA eigenvalues were retained for interpretation (Franklin et al. 1995). The multi-response permutation procedure (MRPP) was used to determine if the three species were significantly different from one another, based on AFLP frequency data (PC-Ord, MjM Software Design, Gleneden Beach, Oregon).

Results

The analysis yielded 185 bands, of which six were unique to *S. pilosum*, six were unique to *S. parviceps*, and 83 unique to *S. depauperatum*. Among the 8 *S. depauperatum* populations in this study, 42 of the 83 bands were represented in at least 2 populations; the Maryland, Pennsylvania, and North Carolina populations possessed 27, 13, and 1 population-specific band, respectively. These data were considered to be purely descriptive, because sample sizes ($N = 2$–5) were insufficient for calculating population-level genetic diversity measures.

The first three axes of the PCA accounted for 24.9% (axis 1 eigenvalue = 23.86), 16.5% (axis 2 eigenvalue = 15.83), and 12.9% (axis 3 eigenvalue = 12.39) of the variance (Figure 1). Parallel analysis indicated that the first three axes were statistically significant and appropriate for interpretation. PCA analysis revealed predictable associations, with *S. pilosum* and *S. parviceps* separating from *S. depauperatum* on the first axis and from each other on the third axis (Figure 1). MRPP results indicated significant differences among the three species ($T = -4.35$, $A = 0.08$, $P < 0.01$).

Genetic relationships among *S. depauperatum* populations revealed three associations corresponding to the states in which they occur (Figure 1). The separation of the North Carolina population from the Maryland and Pennsylvania populations along the second axis (Figure 1) indicates that this population is genetically different from the other seven *S. depauperatum* populations in this study.

Discussion

The *S. depauperatum* populations in this study were genetically different, based on AFLP analysis, from the phylogenetically closely related *S. pilosum* and *S. parviceps*. In an earlier comparative morphological study, Hart (1990) suggested that *S. depauperatum* was a disjunct serpentine-tolerant variant of the more widely distributed *S. parviceps*. However, the results of our taxonomic study, which included the two *S. depauperatum* populations investigated by Hart (1990), indicate that *S.*

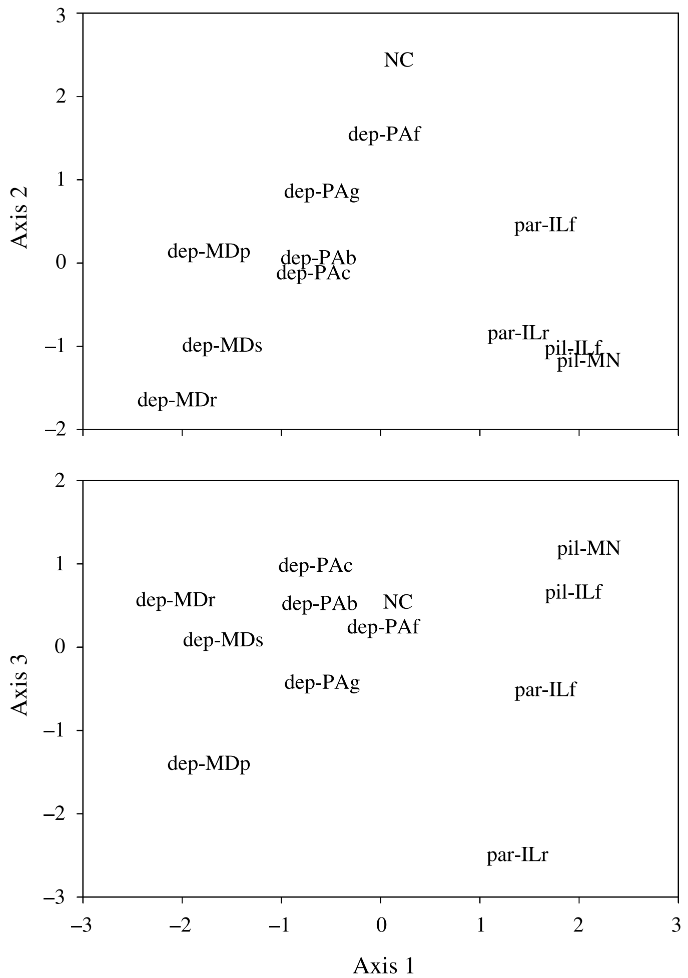

Figure 1. Principal components analysis depicting the relationships among eight *S. depauperatum*, two *S. pilosum*, and two *S. parviceps* populations, based on AFLP analysis of 185 bands. Symbols: dep = *S. depauperatum*; par = *S. parviceps*; pil = *S. pilosum*; IL = Illinois; MD = Maryland; MN = Minnesota; NC = North Carolina; PA = Pennsylvania; lowercase letters following state abbreviations refer to specific collection sites.

depauperatum is as genetically dissimilar to *S. parviceps* as *S. pilosum*, a close relative, is to *S. parviceps*. The species groupings supported in this study are also consistent with previous cytogenetic studies of North American *Symphyotrichum* species (Semple et al. 1983; Semple and Chmielewski 1985; Chmielewski and Semple 1989; Jones 1989; Levy and Wilbur 1990).

Symphyotrichum depauperatum is not, however, strictly endemic to eastern North American serpentine barrens, as is *Cerastium velutinum* var. *villosissimum*

(Gustafson et al. 2003), based on the inclusion of the North Carolina populations. The North Carolina plants analyzed in this study were indeed *S. depauperatum*. There are some similarities in the chemical composition of the mafic diabase and ultramafic serpentinite, such as high levels of Fe and Mg and low P (Brooks 1987: p.14; Froelich and Gottfried 1999: p.205); however, the diabase soils have much lower Mg, Cr, and Ni concentrations and much higher Ca concentrations than the serpentine soils (Brooks 1987; T. Clark, North Carolina Geological Survey, personal communication). It is possible that these populations of *S. depauperatum* in North Carolina are relicts from a grassland expansion across eastern North America around 8000–4500 years ago (Deevey and Flint 1957; Wright 1976; Haas and McAndrews 2000). There is evidence that serpentine grasslands in Maryland and Pennsylvania were maintained by burning and used as hunting and foraging grounds by indigenous peoples as late as 1731 (Marye 1955; Tyndall and Hull 1999), but we are unaware of any evidence for an anthropogenic origin of the North Carolina diabase glades. Subtle similarities of soils weathered from serpentinite and diabase, eastward expansion of the North American grasslands, and maintenance by activities of indigenous peoples are a few of the possible explanations for the extreme disjunct occurrence of *S. depauperatum* in North Carolina.

Our AFLP genetic analysis supports traditional systematic morphological and cytogenetic studies that indicate that *S. depauperatum* is a species closely related to the more widespread *S. pilosum* and *S. pariveps*. *S. depauperatum* occurs on 20 of the 26 eastern North American serpentine barrens of greater than 2.0 ha area known to exist (Latham 1993) and three small populations on diabase glades in north-central North Carolina. With only three diabase populations in the entire large area of diabase soils extending from Georgia north along eastern North America to Nova Scotia, we recommend retaining the description of *S. depauperatum* as an eastern serpentine barrens endemic species, while acknowledging the unique, highly disjunct populations in North Carolina.

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