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STATUS OF *DESCURAINIA TORULOSA* (BRASSICACEAE)

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ABSTRACT.—*Descurainia torulosa* was established in 1983 based on material collected in northwestern Wyoming. The species is now known from 2 disjunct populations in southwestern Wyoming, and recent collections have expanded the known distribution within the northern portion of its range. The north-south disjunct distribution and apparent habitat differences have stimulated questions concerning the degree of relatedness between the disjunct elements to the point that the southern populations have been suggested to be a distinct taxon. Recognition of *D. torulosa* as a distinct species has also been questioned. This paper is based on field examination of *D. torulosa* populations, study of all known *D. torulosa* herbarium specimens, and analysis of nucleotide sequence from the internal transcribed spacer regions 1 and 2 of nuclear ribosomal DNA (ITS-1 & 2) from population exemplars. Cladistic analysis of the sequence data support our conclusion, based on morphological analysis. Northern and southern populations of *D. torulosa* are conspecific. Western North American *Descurainia* is in need of modern, critical taxonomic revision.

Key words: Brassicaceae, Descurainia, *taxonomic status, DNA, ITS-1 & 2, rare plant.*

Descurainia torulosa was described by Reed Rollins (1983) from material collected near Brooks Lake, Fremont County, northwestern Wyoming. Rollins (1983) considered *D. torulosa* distinctive because of the branching habit, short stature, closely appressed but flaring torulose siliques, and extremely short pedicels. The taxon is endemic to Wyoming, with a disjunct distribution (Fig. 1), and had been designated a category 1 (threatened) candidate for federal listing as threatened or endangered (U.S. Fish and Wildlife Service 1990). A reevaluation of the situation (Marriott 1991) resulted in the recommendation that *D. torulosa* be reassigned to category 2 (additional information needed) status.

In 1988 several specimens of *Descurainia* collected in Wyoming were sent by the Rocky Mountain Herbarium (RM) to Brassicaceae expert Reed Rollins (Harvard University) for his determination. Three collections, 2 from Park County in northwestern Wyoming (R. Kirkpatrick 5191a; E. Evert 10062) and 1 from Pine Butte in Sweetwater County approximately 240 km to the south (Dueholm 10779), were given provisional determinations as "*D. torulosa* (?)" (Rollins 1988). Rollins indicated that more material was needed to clarify the situation for *D. torulosa.*

The apparent rarity of *Descurainia torulosa,* as well as the curious disjunct distribution (Fig. 1) and differing ecological settings for the northern and southern populations, stimulated field research by pertinent federal land management agencies. These studies (Dorn 1989, Marriott 1991, 1992) produced much new information on the distribution, ecology, and morphological aspects of *D. torulosa.* They also drew attention to 2 taxonomic questions. Does *D. torulosa* merit recognition as a distinct species? If so, do the northern and southern population systems represent the same taxon? Dorn (1989) first suggested that *D. torulosa* might be just a variant form of *D. incana* (= *D. richardsonii*), a common, widespread species in the Rocky Mountain region.

The objective of this study was to evaluate the taxonomic status of *Descurainia torulosa* Rollins and, using morphological and DNA sequence data, to assess the degree of divergence between the northern and southern population systems.

MATERIALS AND METHODS

Between 18 and 20 July 1997, we surveyed documented (Marriott 1992) *Descurainia torulosa* populations in Fremont, Park, Sweetwater,

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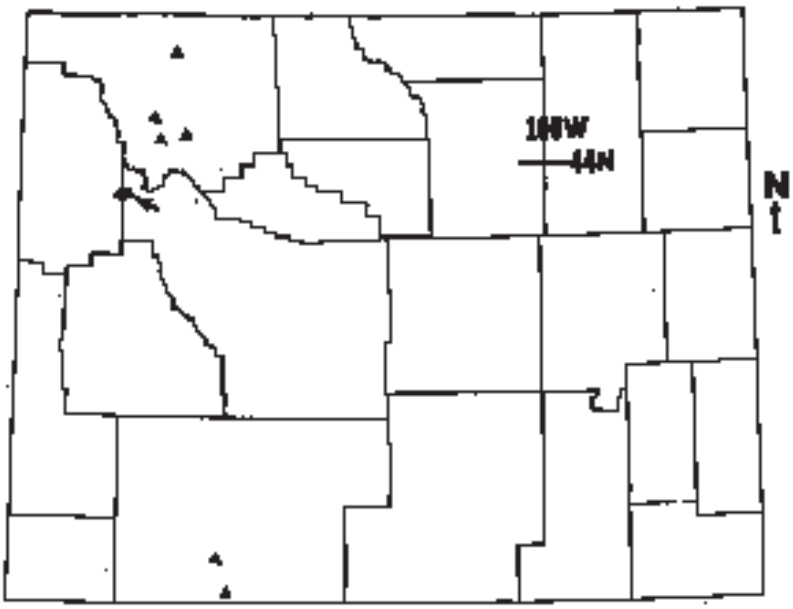

Fig. 1. Map of the state of Wyoming showing the distribution of *Descurainia torulosa* populations. County lines are shown, and the arrow indicates the location for the type collection.

and Teton counties of western Wyoming. At each site 4–10 individuals were collected for DNA extraction, and a like number selected as herbarium voucher specimens. We recorded observations of plant habit, reproductive status, and habitat for each specimen. Latitude and longitude were determined using the Global Positioning System. For comparison purposes, DNA samples and voucher specimens were also secured for *D. incana* var. *viscosa* and *D. sophia* from locations in and around Laramie, Wyoming (Table 1). Additional specimens of varieties of *D. incana* and *D. pinnata* (see Table 1) were obtained from dry herbarium specimens on file at the Rocky Mountain Herbarium (RM). All vouchers have been filed at RM.

Young leaf tissues of mature *Descurainia torulosa* plants were excised, immediately frozen in liquid nitrogen, and stored on dry ice for transport to the laboratory (Millan et al. 1996). Upon return to the lab, DNA was extracted following the protocol of Torres et al. (1993). We extracted DNA from dry herbarium leaf material using the same protocol and determined DNA concentrations by UV spectroscopy.

Symmetric polymerase chain reaction (PCR) amplification of ITS-1 and ITS-2 regions was conducted using the forward and reverse strand primers, ITS5 and ITS4 respectively, from White et al. (1990). Each 50-µl PCR mixture included 50 ng of DNA extract, 0.5 µM of each primer, 100 µM each of 4 dNTPs, 5 µl of PCR buffer (100 mM Tris-Cl [pH 8.3], 500 mM KCL, 15 mM $MgCl_2$, and 0.1% [w/v] gelatin), and 1 unit of *Taq* polymerase (Stratagene, La Jolla, CA). The reaction mixture was overlain with 60 µl of sterile mineral oil. Thermal cycling was performed using an MJ Programmable Thermal Cycler (MJ Research, Inc.) programmed for 2 min initial denaturation at 94°C, 25 PCR cycles of 1 min denaturation at 94°C, 2 min of annealing at 54°C, and 2 min of extension at 72°C. The final extension time of the last cycle was increased to 4 min. Reactions lacking template DNA were included as negative controls.

PCR product yield and quality were initially evaluated via electrophoresis of a 10-µl aliquot in 1.0% agarose using a TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). Successful amplifications were then purified using Centricon 100 columns (Amicon, Inc.) following the manufacturer's directions, and then lyophilized. The lyophilized double-stranded products were sent to Macromolecular Resources, Colorado State University, for automated sequencing.

The ITS-1 & 2 region DNA sequences (5′ to 3′) obtained from the automated sequencer

TABLE 1. DNA sources[a].

Taxon	Locality	Collection	GenBank Number
Descurania incana			
var. *incana*	WY, Carbon Co., Coal Creek Canyon	Redder 56	AF205582
var. *incana*	WY, Natrona Co., Pathfinder Dam	Roderick 4822	AF205583
var. *viscosa*	WY, Albany Co., Eagle Rock	Bricker 531	AF118859
D. pinnata			
var. *brachycarpa*	WY, Carbon Co., Third Sand Creek	Roderick 1124	AF205584
var. *filipes*	WY, Carbon Co., Rawlins	Roderick 697	AF205585
var. *nelsonii*	WY, Carbon Co., N. Shirley Basin	Roderick 6116	AF205586
D. sophia	WY, Albany Co., Laramie	Bricker 548	AF118860
	WY, Carbon Co., Medicine Bow River	Roderick 3565	AF205587
D. torulosa	WY, Sweetwater Co., Pine Butte	Bricker 550	AF118861
	WY, Sweetwater Co., Lion Bluffs	Bricker 551	AF118862
	WY, Teton Co., Breccia Cliffs	Bricker 552	AF118863
	WY, Fremont Co., Brooks Lake	Fertig 16913	AF118865

[a]All vouchers are on file, Rocky Mountain Herbarium (RM), University of Wyoming.

output were double-checked against their respective chromatograms and then manually aligned. Position number 1 in the aligned sequence corresponded to the 1st position where sequence was available for all samples. Sequence alignment on the 3′ end was terminated at position 624 because alignments beyond that position became dubious. Ambiguous base determinations within the 624 base pair span were coded in the matrix as "N." The complete data matrix of aligned sequence was subjected to parsimony analysis using the program PAUP 3.1.1 (Swofford 1991).

There is no known explicit phylogenetic hypothesis for the member species of *Descurainia.* As a consequence, relationships amongst the taxa included in this study (Table 1) are unknown. *Descurainia sophia* was originally selected as the potential outgroup since it is not native to North America and is presumably distinct from the *Descurainia* species native to the Rocky Mountain region. We conducted a preliminary cladistic analysis to explore the relationship between *D. sophia* and the remaining taxa included in this study. This preliminary analysis utilized the HEURISTIC search option with the TREE BISECTION-RECONNECTION (TBR) branch swapping option, and the MULPARS, COLLAPSE, and ACCTRAN optimization options, with sequence from *Sinapis alba* (Rathgeber and Capesius 1989) designated as the outgroup. A more intensive phylogenetic analysis for those taxa listed in Table 1 was executed using the EXHAUSTIVE search option, with *D. sophia* designated as the outgroup, saving all most parsimonious trees. To evaluate relative branch support, we conducted 500 replicates of bootstrap analysis and decay analysis.

DNA sequences generated in this study have been submitted to GenBank (Table 1). The matrix of aligned ITS-1 & 2 sequences is available from the authors.

RESULTS

The preliminary analysis, rooted with *Sinapis alba,* resulted in 3 equally most parsimonious trees of 164 steps (not shown). The strict consensus of these 164 step trees had a topology exactly like that illustrated in Figure 2, with the addition of *Sinapis alba* as the rooting outgroup. This analysis confirmed an outgroup position for *Descurainia sophia* relative to the *torulosa-incana-pinnata* ingroup, at least until a more appropriate outgroup is identified.

The exhaustive search, rooted with *Descurainia sophia*, resulted in 3 equally most parsimonious trees of 86 steps with the consistency index of 0.826 and retention index of 0.795. The g1 value for this analysis was –1.7068, indicating significant phylogenetic signal within the sequence data. Sixty-eight of 624 characters are phylogenetically informative, while 459 are invariant. Pairwise mean and absolute distances are provided in Table 2.

Taxa in the *torulosa-incana-pinnata* ingroup occupy 2 clades (Figure 2). Population exemplars for *Descurainia torulosa* are all located in the same clade, along with 1 variety each from *D. pinnata* and *D. incana.*

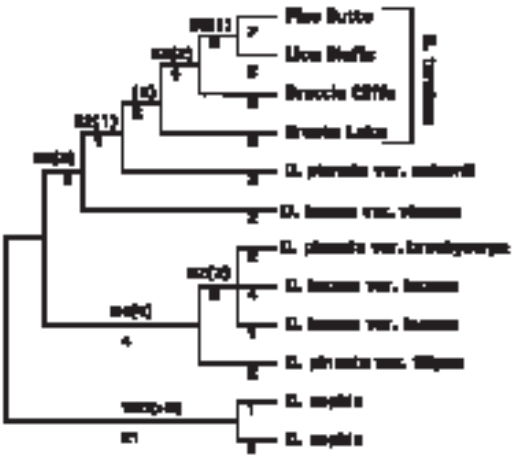

Fig. 2. Strict consensus of the 3 equally most parsimonious trees (86 steps) obtained in an exhaustive search rooted with *Descurainia sophia.* Bootstrap values >50% and decay values (in parentheses) are located above the branches. Numbers below each branch indicate the number of base substitutions.

DISCUSSION

Cladistic analysis of the molecular data suggests that *Descurainia torulosa* is part of a complex that includes both *D. incana* and *D. pinnata,* and that each of these taxa is paraphyletic. Furthermore, the present sample set reveals 2 clades within this complex, with the population exemplars for *D. torulosa* comprising the terminal cluster in 1 of these clades. Finally, the 2 known southern populations (Pine Butte and Lion Bluffs) are more similar to each other than either is to the sampled northern populations (Breccia Cliffs and Brooks Lake). However, the sampled northern populations do not form a similar clade.

A thorough examination of all known *Descurainia torulosa* specimens demonstrates that the diagnostic morphological characters used by Rollins (1983) to distinguish this species are variable and unreliable in terms of species determination. Rollins (1983) noted that the procumbent, probably perennial, habit of *D. torulosa* was unique for *Descurainia* in North America. Some details concerning the growth habit remain unresolved. Plants from the Sweetwater County populations appear to be annuals (also see Marriott 1992), although vegetative rosettes, suggesting a biennial or perennial habit, were encountered as well. Plants from northern populations appear to be biennial, although some flowering/fruiting individuals appeared to be in the 1st season of growth. The long-lived perennial habit does not appear to exist within *D. torulosa.* Contrary to the type description, the growth form for all small, flowering individuals examined ranges from erect to decumbent, but definitely not procumbent. Larger, presumably older, flowering specimens tend to be more decumbent in form.

Rollins (1983) placed diagnostic significance in a suite of fruit characters, namely the closely appressed but flaring (curving outwards) torulose siliques and the extremely short pedicels (≤ 2.5 mm). In Dorn's (1992) treatment of *Descurainia,* no mention of growth habit or form is made in the diagnostic key for species. Fruit characters, however, are relied upon heavily, and Dorn (1992) adds fruit pubescence as the primary character to distinguish *D. torulosa* (hairy) from *D. incana* (usually glabrous). In comparing the spectrum of fruit characters putatively diagnostic for *D. torulosa* against those from scores of *Descurainia* specimens from throughout the Rocky Mountain region on file at RM (e.g., *D. californica* [Gray] Schulz, *D. incana* [Bernh. ex Fisch. & Meyer] Dorn, *D. pinnata* [Walt.] Britt.), we have concluded that there is no unequivocal character, nor suite of characters, that can be used to distinguish *D. torulosa* with absolute reliance. Plants determined as *D. torulosa* have siliques ranging from appressed to weakly divergent, and the degree to which siliques are torulose ranges from mild to obvious, but is not qualitatively different from torulose fruits seen in a wide range of *D. incana* specimens. Fruit pubescence, which Rollins (1983) did not specify, but which Dorn (1992) emphasized as diagnostic for *D. torulosa,* is also polymorphic. Except for plants from the Pine Butte population in Sweetwater County, all other known populations of *D. torulosa* have plants with conspicuously pubescent ovary walls and fruits. Furthermore, plants from the Lion Bluff population, also Sweetwater County and ca 55 km northwest of the Pine Butte site, are otherwise indistinguishable from plants in the Pine Butte population, except for having hairy ovary and fruit walls. Although Dorn was aware of these southern populations (Dorn 1989), it appears that he did not include them within his circumscription of *D. torulosa,* as he listed the *D. torulosa* distribution ("nw" and "c") to include only the northern populations. Finally,

TABLE 2. Matrix of pairwise distances between taxa/populations included in this analysis. Values above the diagonal are mean distances. Values below the diagonal are absolute distances measured in number of character-state changes.

	Sample[a]	1	2	3	4	5	6	7	8	9	10	11	12
1	531	–	0.040	0.020	0.013	0.015	0.013	0.020	0.025	0.017	0.010	0.023	0.056
2	548	24	–	0.051	0.053	0.050	0.048	0.043	0.049	0.043	0.045	0.045	0.015
3	550	12	31	–	0.015	0.023	0.026	0.036	0.041	0.033	0.022	0.038	0.066
4	551	8	32	9	–	0.015	0.013	0.028	0.028	0.022	0.012	0.030	0.064
5	552	9	30	14	9	–	0.018	0.026	0.031	0.023	0.017	0.030	0.060
6	fer	8	29	16	8	11	–	0.023	0.028	0.022	0.013	0.026	0.064
7	697	12	26	22	17	16	14	–	0.012	0.007	0.022	0.008	0.055
8	1124	15	30	25	17	19	17	7	–	0.005	0.023	0.010	0.061
9	4822	10	26	20	13	14	13	4	3	–	0.020	0.008	0.057
10	6116	6	27	13	7	10	8	13	14	12	–	0.023	0.055
11	Desc	14	27	23	18	18	16	5	6	5	14	–	0.051
12	3565	34	9	40	39	36	39	33	37	34	33	31	–

[a]531 = *Descurainia incana* var. *viscosa*; 548 = *D. sophia*; 550 = Pine Butte population of *D. torulosa*; 551 = Lion Bluffs population of *D. torulosa*; 552 = Breccia Cliffs population of *D. torulosa*; fer = Brooks Lake population of *D. torulosa*; 696 = *D. pinnata* var. *filipes*; 1124 = *D. pinnata* var. *brachycarpa;* 4822 = *D. incana* var. *incana*; 6116 = *D. pinnata* var. *nelsonii*; Desc = *D. incana* var. *incana*.

the short pedicel is very nearly diagnostic for *D. torulosa.* However, individual specimens of *D. incana* have been encountered that have pedicels as short as 3 mm.

Descurainia torulosa exhibits an interesting disjunct distribution. The north and south populations appear to occupy distinctly different habitats. The southern populations are located in the Green Basin, the northern populations associated with the Absaroka Mountains (Knight 1994), and the major vegetation types in these regions are quite different. If, however, the physical aspects of populational microhabitats are considered, these disjunct populations are not so different. The north-south disjuncts occupy a similar elevational range, but more specifically, the microhabitats are very similar. These populations are found at the base of cliffs in a narrow zone where the cliff face above provides a somewhat sheltering alcove. The rooting substrates for these populations, consisting of exfoliated, coarsely textured rock materials from the cliff, are also physically similar. Overall, the observable microhabitat similarities for the north and south disjunct populations of *D. torulosa* are striking.

In conclusion, morphological evidence does not support recognizing *D. torulosa* as a species distinct from *D. incana.* There is no single unequivocal diagnostic character or suite of characters that serve to distinguish *D. torulosa* from *D. incana.* Furthermore, molecular data presented here indicate that *D. torulosa* is part of a complex that includes morphological entities currently placed in both *D. incana* and *D. pinnata*. The taxonomic status of *D. torulosa* is not resolved, and modern revisionary study of the North American elements of *Descurainia* is needed before resolution of the issue can be clarified. The increased morphological sample evaluated in this study, in concert with molecular data, suggest that *D. torulosa* is more appropriately treated at the infraspecific level. However, until species concepts and relationships within *Descurainia* are better clarified, the question of how to best treat *D. torulosa* (e.g., place in synonymy, treat as an infraspecific taxon, or as distinct species) must wait.

SPECIMENS EXAMINED

Fremont Co.: Wind River Range, near Brooks Lake, 2 mi NW of lake, 10,000 ft, 8 July 1966, *R.W. Scott 761*, (Holotype: GH, Isotype: RM); southern Absaroka Mts, Continental Divide ridge ca 1.3–2 mi NW of Brooks Lake and 0.4 mi W of Upper Jade Lake, ca 2–2.5 air mi NE of Togwotee Pass, 10,080–10,280 ft, 23 July 1990, *H. Marriott 11282* (RM); southern Absaroka Range, E side of Continental Divide ridge, ca 0.4 mi W of Upper Jade Lake, ca 1.3 air mi NW of Brooks Lake, ca 2 air mi NE of Togwotee Pass, 26 July 1996, *Fertig 16913* (RM).

Park Co.: Absaroka Range, North Fork Shoshone River drainage, ridge E of Sweetwater Creek, ca 4–5 mi N of US Hwy 14, 16, & 20, 7500–8000 ft, 16 June 1986, *Evert 10062* (RM); Absaroka Range, North Fork Shoshone River drainage, many pinnacled ridges E of Clearwater Creek, ca 3–4 miles N of US Hwy 14, 16, & 20, 7200–8000 ft, 19 June 1986, *Evert 10143* (RM); Absaroka Mt, along Hunter Creek Trail, ca 3 mi E of South Fork Ranger Station, ca 43 mi SW of Cody, ca 8800 ft, 29 July 1989, *Evert 18092* (RM); Absaroka Mts, ca 22 air mi SW of Meeteetse in the vicinity and W of the jct of Middle Fork of Wood River and Beaver Creek, 7500–8300 ft, 26 July 1984, *Kirkpatrick 5049* (RM); Absaroka Range, ca 21 air mi W of Meeteetse, along ridge between the North Fork Pickett Creek and Little Rose Creek up to "peak" 11448, 10,000–11,400 ft, 29 July 1984, *Kirkpatrick 5191a* (RM); Absaroka Range, E of Wapiti Ridge, on ridge between Houlihan and Bobcat Creeks ca 2 air mi SE of Citadel Mt, 8000–9000 ft, 12 July 1996, *R. Hartman 55177* (RM).

Sweetwater Co.: N side of Pine Butte, below top of rim, ca 8300 ft, 20 July 1980, *K. Dueholm 10779* (RM); Washakie Basin, N and NW side of Pine Butte, ca 33 air miles SE of Rock Springs, 8500 ft, 21 July 1987, *H. Marriott 10635* (RM); Lion Bluffs at NE end of summit of Quaking Asp Mt, ca 12 air mi SE of Rock Springs, 8300–8400 ft, 22 July 1991, *A. Flinck & H. Marriott 1* (RM); Pine Butte, W exposure along base of cliffs, 7600 ft, 18 July 1997, *J.S. Bricker 549 & G.K. Brown* (RM); Pine Butte, W exposure along base of cliffs, 7600 ft, 18 July 1997, *J.S. Bricker 550 & G.K. Brown* (RM); E terminus of Asp Mt at Lion Bluffs, 18 July 1997, *J.S. Bricker 551 & G.K. Brown* (RM).

Teton Co.: Southern Absaroka Mts, base of cliffs at E end of Breccia Cliffs, ca 1.9 air mi NNW of Togwotee Pass, 10,100 ft, 23 July

1990, *H. Marriott 11293*, (RM); southern Absaroka Mts, base of cliffs near W end of Breccia Cliffs, ca 3.6 air mi NNW of Togwotee Pass (N of Lost Lake), 10,500 ft, 27 July 1990, *A. Flinck #1* (RM); southern Absaroka Mts, base of cliffs at W end of Sublette Ridge, ca 0.6 mi W of Togwetee Pass, 20 July 1997, *J.S. Bricker 552 & G.K. Brown* (RM); southern Absaroka Mts, base of cliffs at W end of Sublette Ridge, ca 0.6 mi W of Togwetee Pass, 20 July 1997, *J.S. Bricker 553 & G.K. Brown* (RM).

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