
A MORPHOMETRIC EVALUATION OF
CORYDALIS CASEANA AND ITS SUBSPECIES WITH
SPECIAL ATTENTION TO *C. AQUAE-GELIDAE*

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PREFACE

This report is the result of a cooperative Challenge Cost Share project between the Institute for Applied Ecology (IAE) and a federal agency. IAE is a non-profit organization dedicated to natural resource conservation, research, and education. Our aim is to provide a service to public and private agencies and individuals by developing and communicating information on ecosystems, species, and effective management strategies and by conducting research, monitoring, and experiments. IAE offers educational opportunities through 3-4 month internships. Our current activities are concentrated on rare and endangered plants and invasive species.

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INTRODUCTION

Cold-water corydalis (*Corydalis aquae-gelidae*) (Figure 1) is listed as a USFS Survey and Manage species and is a candidate for endangered species listing with the Oregon Department of Agriculture. The species is restricted to western Oregon and Washington forests, primarily in riparian areas adjacent to rivers and streams. It is closely related to *C. caseana* (fitweed), including Sierra corydalis (*C. caseana* ssp. *caseana*) and Cusick's corydalis (*C. caseana* ssp. *cusickii*). Cold-water corydalis is known to occur from southern Washington to Linn County, Oregon. Sierra corydalis is distributed from northern to central California in the Sierra Mountains and Cusick's corydalis ranges from northeast Oregon to adjacent Idaho.

A recently discovered population of *Corydalis* (Traverse Creek, aka Saddleblanket Mtn.) on the Willamette National Forest occurs in Lane County, south of the known range of cold-water corydalis and north of the known range of Sierra corydalis. Individuals in this population resemble cold-water corydalis but also appear intermediate between the two species in some characteristics. Because Sierra corydalis is currently not considered a species of management concern but cold-water corydalis is, it is crucial for proper management to determine which species this population belongs to.

Taxonomy of Corydalis caseana and subspecies

There are about 300 species of *Corydalis*, a north-temperate genus of Europe, Asia, North America, and tropical African mountains (Mabberley 1993). Within *Corydalis caseana*, a strictly North American species, there are currently five subspecies recognized (Stern 1997). These taxa are widely distributed in the west and tend to be geographically isolated from one another (Figure 2). These taxa include the following: *brachycarpa*, *brandegei*, *caseana*, *cusickii*, and *hastata*. In addition, *C. aquae-gelidae*, which was originally described in the 1950's (Peck 1957), has been recognized as a subspecies of *C. caseana* (Lidén 1996), but this treatment was not followed in the Flora of North America (Stern 1997).

Objectives

The goal of this study is to clarify the taxonomy of *Corydalis aquae-gelidae*, with special emphasis on the Traverse Creek population on the Willamette National Forest. The specific objectives are:

- To determine if the Traverse Creek population is morphologically aligned with populations of *aquae-gelidae* to the north or some other subspecies of *C. caseana* such as *cusickii* to the east or *caseana* to the south.
- To determine if the subspecific taxonomy of *C. caseana* and *C. aquae-gelidae* is statistically verifiable when samples from throughout the geographic range of the species are included, and, if so, identify morphological traits that can consistently distinguish the taxa. In so doing, material from Oregon, California, Idaho, and Colorado will be involved in the study, including specimens of *C. aquae-gelidae*, *C. caseana* ssp. *brandegei*, *C. caseana* ssp. *caseana*, and *C. caseana* ssp. *cusickii*.

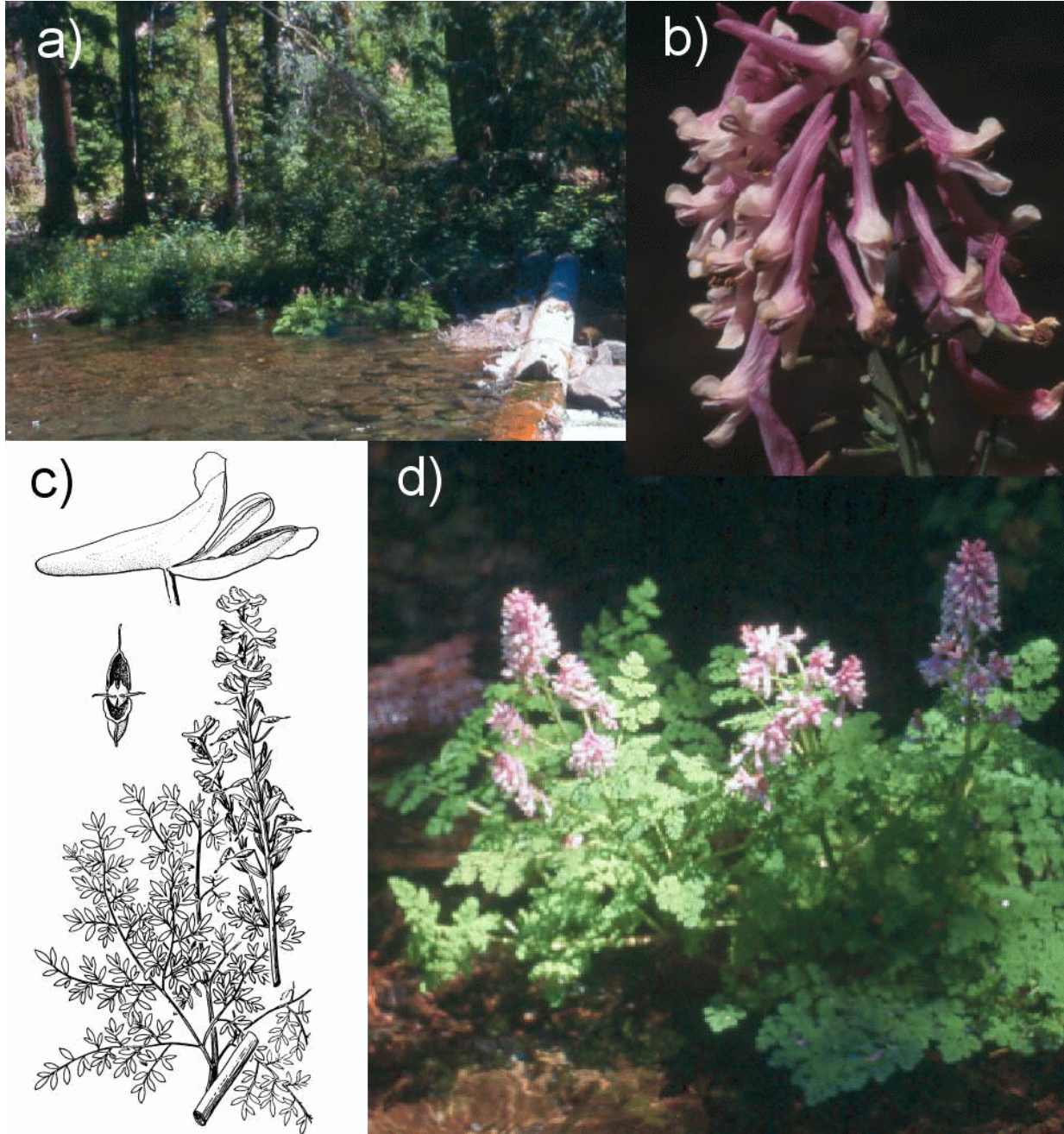


Figure 1. *Corydalis aquae-gelidae* along the Clackamas River in Oregon. A) habitat of plants along the river bank, b) inflorescence showing individual flowers, note the crests on the upper and lower petals, c) line drawing of the species from Hitchcock and Cronquist (1965), d) habit of the plant.

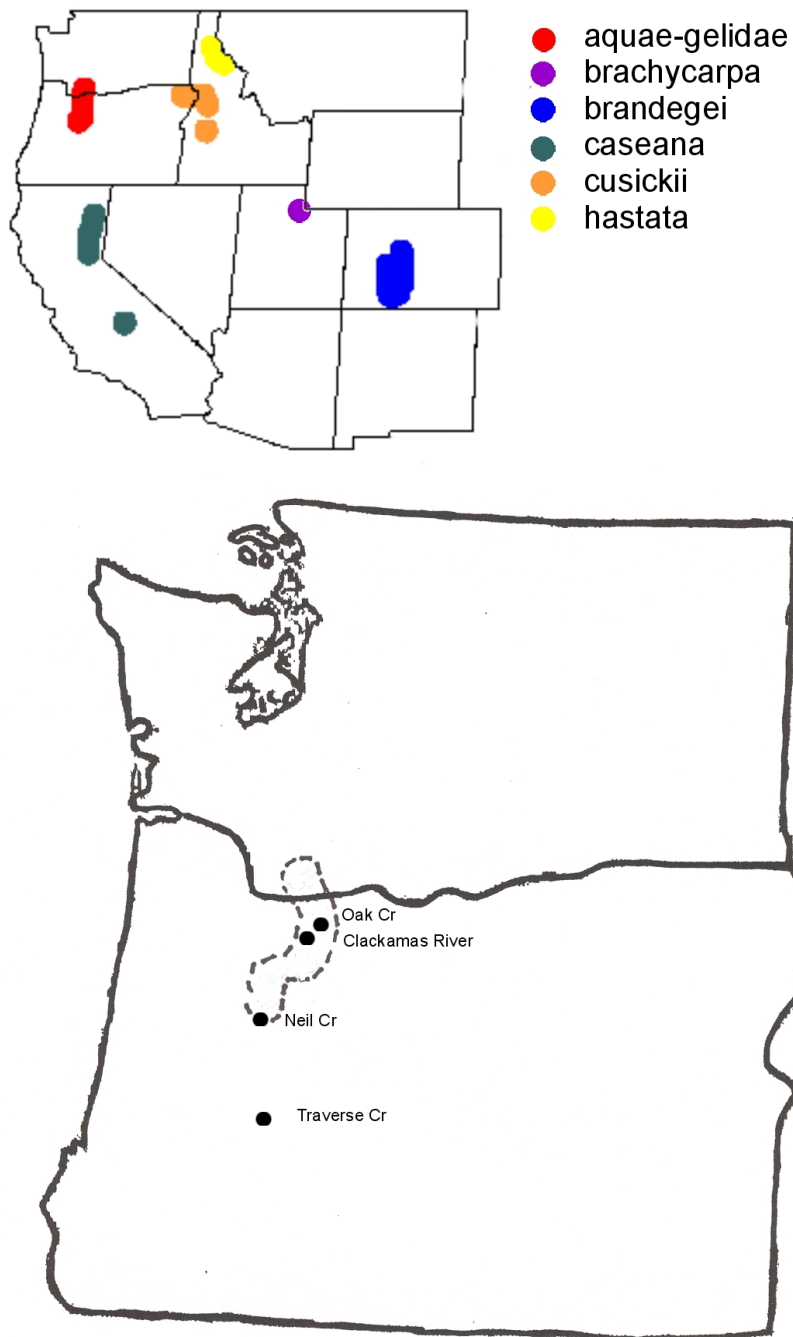


Figure 2. **Top:** Distribution of *Corydalis aquae-gelidae* and five subspecies of *C. caseana* in western North America (after Stern 1997). **Bottom:** Distribution of *C. aquae-gelidae* in Oregon and Washington and locations of the four populations used to make measurements of fresh plants used in this study.

METHODS

Study materials

Two sources of study material were included in this study: fresh plants collected in Oregon and Colorado in 2000 and herbarium specimens from three herbaria housed at Oregon State University: Oregon State University (OSC), University of Oregon (ORE), and Morton Peck/Willamette University (WILLU). These samples are listed in Table 1.

Table 1. Samples of each taxon included in morphometric analyses.

taxon	location	material	no. of samples
<i>aquae-gelidae</i>	Traverse Creek, Willamette NF	fresh	20
	Niel Creek, Salem District, BLM	fresh	18
	Clackamas River, Mt. Hood NF	fresh	16
	Oak Grove Fork of Clackamas River	fresh	20
	Clackamas Co, OR	herbarium	3
	Marion Co., OR	herbarium	2
	Linn Co., OR	herbarium	1
	<i>brandegei</i>	Ohio Pass, CO (N38 50' 14.5", W107 065' 34.9")	fresh
Kebler Pass, CO (N38 51' 10.2" W107 06' 27.2")		fresh	6
Irwin Pass, CO		fresh	4
<i>caseana</i>		Tehama Co., CA	herbarium
	Butte Co., CA	herbarium	4
	Prattville Co., CA	herbarium	1
	Grizzly Range, CA	herbarium	1
	<i>cusickii</i>	Wallowa Co., OR	herbarium
Baker Co., OR		herbarium	4

Morphological traits

Twenty-three morphological traits were included in our examination of variation *aquae-gelidae* using fresh material (Table 2). These included both vegetative and reproductive features, such as various flower characteristics, inflorescence sizes, and leaf and leaflet sizes. Measurements were made in millimeters with a ruler (e.g., leaflet length and width). Fewer traits (19) were measured from herbarium specimens because it was difficult to measure some characteristics on dried herbarium specimens and some collections did not have complete material, such as fruits for counting ovules or lower leaves. One characteristic, the number of times lower leaves were divided, was omitted from analyses of *aquae-gelidae* populations because this trait did not vary at this level.

Analytical approach

The morphological data were analyzed with Principal Components Analysis (PCA) to identify any morphological discontinuities or overlap between populations of *aquae-gelidae* (including Traverse Cr.) and between subspecies of *Corydalis caseana*. In addition, Discriminant Analysis was used to compare groups of specimens of from the various *aquae gelidae* populations and among the subspecies.

Discriminant analysis--Discriminant analysis (DA) tests for a significant difference between samples with multivariate data assigned to groups *a priori*, and can identify which samples in the data set were assigned to the “wrong groups” (Mardia *et al.*, 1979; STATGRAFICS reference manual, 1993). We used DA to determine if the populations of *aquae-gelidae* (including Traverse Creek) were significantly different from one another and if *aquae-gelidae* and three subspecies of *C. caseana* differed. This technique also was useful for identifying which morphological traits could be useful for distinguishing the populations or taxa.

Principal components analysis--We used principal components analysis (PCA) to evaluate patterns of morphological variation in *Corydalis*, such as morphological discontinuities or overlap between the populations or taxa. PCA is an ordination technique that reduces the dimensionality of the data, extracting new axes with maximum variance. Instead of having one axis (or dimension) for each morphological character, PCA can reduce the number of variables by finding linear combinations of the variables that explain most of the variability (Gauch, 1982; STATGRAFICS reference manual, 1993). The axes extracted by PCA decrease in importance from the first to the second, and so on. PCA performs an eigenanalysis to determine the *eigenvalue* of each axis (the percentage of variation accounted for by the axis, i.e., its importance), and an *eigenvector* of ordination scores for each sample. In addition to PCA, we performed Factor Analysis (FA) to determine the factor weights of each variable on each component axis (after discarding all axes after the first six). The factor weights indicate the importance and correlation of each morphological character to the axes. We used FA because it yields factor weights that are related to the total variance explained by each component (factor weights are scaled so that their sum of squares is equal to the associated eigenvalue).

We plotted the ordination scores of the first three PCA axes against one another to display

patterns of variation in the morphological data. Because our morphological characters differed in their units of measure, we standardized all values prior to analysis. We conducted these analyses first on all morphological data from four populations of *aquae-gelidae* (including Traverse Creek) and again including *aquae-gelidae* and three subspecies of *C. caseana*: *brandegei*, *caseana*, and *cusickii*.

Table 2. Twenty three morphological traits quantified for a numerical analysis of *Corydalis aquae-gelidae* and subspecies of *C. caseana*.

<i>Morphological trait</i>	<i>notes</i>
Floral Measurements	
spur length	measured from distal to proximal ends
spur width at middle	between stipe and distal end
spur width at base	Proximal end, adjacent to stipe connection
flower length	from spur tip to petal tips
lower petal length	inflection point to distal end
upper petal length	inflection point to distal end
petal separation	distance separating the two outer petals
throat opening	Lower (non-spurred) outer petal
wing width	Lower (non-spurred) outer petal, outside of throat
upper crest width	size of the crest (a membranous protrusion or extension) on the spurred petal
lower crest width	size of the crest (a membranous protrusion or extension) on the non-spurred, outer petal
bract length	length of floral bract at anthesis
bract width	
stipe length	
style length	As measured on developing fruit
Inflorescence Measurements	
number of flowers	flowers on primary inflorescence
inflorescence length	distance from tip of axil flower to tip of lowest flower
Fruit Measurements	
number of ovules	count of ovules (fertilized and unfertilize) in developing ovaries
Leaf Measurements	
number of times divided	
leaf length	length of longest leaf from base of petiole to tip of leaf
leaf width	width of same leaf
leaflet length	measured second leaflet from tip(ante-penultimate position) of major (first order) leaf division; repeated five times.
leaflet width	As above.

RESULTS AND DISCUSSION

Discriminant analysis

***Aquae-gelidae* populations**--Discriminant Analysis of the *aquae-gelidae* populations indicated that the populations were significantly different from one another ($P < 0.0001$) in several discriminant functions (Table 4). All samples of each population were correctly classified in DA. The first discriminant function placed Traverse Creek and Oak Grove at opposite ends of morphological variation, while the second function distinguished Niel Creek from the other three sources (Figure 4). Flower bract width, crest width of the upper petal, and inflorescence length were ranked highest in the first discriminant function, and bract width, lower petal wing width, and spur width (measured at base of flower) were highly ranked in the second discriminant function. These morphological traits appear to vary substantially among populations. For example, Traverse Creek plants had the longest upper petal crests (1.41 mm) and widest flower bracts (2.71 mm) while plants from Oak Grove had the shortest crests (0.6 mm) and plants from Niel Creek had the narrowest bracts (1.68 mm) (Table 3). Although Traverse creek plants appeared to be significantly different from the other *aquae-gelidae* populations, they were fairly closely placed along the Discriminant functions, suggesting some separation between the populations but also some close affinity.

***Aquae-gelidae* and *caseana* subspecies**--The three subspecies of *C. caseana* included in this analysis and *C. aquae-gelidae* were generally strongly distinguishable in DA ($P < 0.00001$) (Table 4, Figure 4). All samples of each subspecies were correctly classified. The most important traits for the first discriminant function were number of leaf divisions, leaflet width, and spur width (measured at the middle). For the second function, leaflet length and width as well as flower length were ranked highest. In terms of distinguishing the taxa from one another, *aquae-gelidae* was strongly isolated by discriminant function 1 (Figure 4), and this appears to be due to the small leaflets of this taxon (mean 8.19 mm long by 3.51 mm wide) and 4-times divided leaves, in contrast to the larger leaflets (15.18-29.36 mm long by 5.25-11.19 mm wide) and 3-times divided leaves of the other subspecies. *Brandegei* was segregated by the second function primarily on the basis of its very large leaflets (29.36 mm long by 11.19 mm wide) and wide spurs (4.14 mm at mid-point). *Caseana* and *cusickii* were marginally separable from one another by the second function based on wider petal separation in *cusickii* (10.02 mm) and slightly larger leaflets than *caseana* (Table 3).

Table 3. Summary of plant traits measured for *Corydalis* samples and the average measurement for each group evaluated. Mean values for each *aquae-gelidae* population are reported on the left while those for each taxon are listed on the right. All measurements (aside from counts) are in mm.

	<i>aquae-gelidae</i> populations					<i>aquae-gelidae</i> and <i>caseana</i> subspecies			
	Clackamas	Oak Grove	Niel Cr	Traverse Cr.	Mean	<i>aquae-gelidae</i>	<i>brandegei</i>	<i>caseana</i>	<i>cusickii</i>
N:	16	20	18	20	74	79	14	6	9
Floral measurements									
spur length	9.53	11.18	11.23	9.46	10.37	10.27	12.21	13.19	11.48
spur width at middle	2.56	2.86	3.36	3.18	3.00	2.98	2.83	3.21	3.04
spur width at base	3.11	3.45	4.03	3.93	3.65	3.64	4.14	3.75	3.78
flower length	21.60	23.28	22.29	20.11	21.82	21.56	22.14	22.11	22.61
lower petal length	4.92	5.43	5.11	4.89	5.10	5.11	5.64	5.78	5.72
upper petal length	4.20	4.45	4.29	5.14	4.54	4.58	4.40	4.86	5.25
petal separation	6.71	6.68	7.11	6.11	6.64	6.61	6.44	6.28	10.02
flower throat opening	0.91	1.14	0.83	1.74	1.18				
wing width	1.77	1.35	0.89	0.66	1.15	1.10	1.04	0.63	0.69
upper crest width	0.74	0.60	0.83	1.41	0.90	0.90	0.46	0.48	0.86
lower crest width	0.61	0.48	0.74	1.33	0.80	0.79	0.23	0.50	0.85
bract length (at anthesis)	8.33	8.60	6.21	8.62	7.96	7.98	6.55	7.07	9.96
bract width	2.21	1.95	1.68	2.71	2.15	2.13	1.02	1.01	2.33
stipe length	9.75	8.91	7.54	7.62	8.41	8.32	5.51	4.58	6.96
style length	3.63	4.05	4.01	3.79	3.88	3.91	4.08	4.31	5.63
Inflorescence measurements									
no. flowers	34.13	55.25	28.33	34.55	38.54	37.91	25.50	39.00	36.78
inflor. length	128.81	86.55	88.19	119.49	104.99	105.18	103.71	106.13	117.78
Fruit measurements									
no. ovules	5.75	7.32	4.30	6.28	5.96				
Leaf measurements, lowest leaf									
no. times divided	4.00	4.00	4.00	4.00	4.00	4.00	3.00	3.00	3.00
leaf length	40.88	43.10	39.64	46.35	42.66				
leaf width	25.06	25.10	24.97	29.60	26.28				
leaflet length	6.71	6.19	11.02	9.06	8.25	8.19	29.36	15.18	16.38
leaflet width	2.77	4.19	4.35	3.66	3.78	3.51	11.19	6.06	5.23

Notes: leaflet size: antepenultimate leaflet; lower wing width = (width at base of throat - inside throat width)/2;
bract width = width at widest point.

Table 4. Discriminant function coefficients (standardized) for each morphological character from discriminant analyses of four *aquae-gelidae* populations and also for three *caseana* subspecies and *aquae-gelidae*. Only the first two functions are shown. Large individual coefficients are in bold type.

Morphological characters	Discriminant functions			
	<i>aquae-gelidae</i> populations		<i>aquae-gelidae</i> and <i>caseana</i> subspecies	
	function 1	function 2	function 1	function 2
spur length	-0.12436	0.221608	-0.0741	-0.13822
spur width, middle	0.157298	0.197366	0.217677	-0.46064
spur width, base	-0.22873	0.70706	-0.05583	0.243255
flower length	-0.5005	-0.07353	-0.10009	0.421411
lower petal length	-0.05865	0.05199	-0.01762	-0.00314
upper petal length	0.084638	-0.15356	-0.00766	-0.25748
petal separation	-0.01679	0.022714	-0.00602	-0.50631
flower throat opening	0.47927	-0.56331		
wing width	0.136056	-0.80804	0.008392	0.160067
upper crest width	0.741134	-0.0828	0.05717	0.175069
lower crest width	0.074887	-0.17491	0.122058	-0.27743
bract length (at anthesis)	-0.64443	0.517293	-0.1271	-0.27961
bract width	0.811133	-0.96203	0.095892	0.304256
stipe length	0.121995	-0.49336	0.100503	0.131645
style length	0.01011	0.089254	-0.06715	-0.37929
no. flowers	-0.54625	0.311811	0.094076	-0.14332
inflor. length	0.577994	-0.44587	-0.14398	-0.11632
no. ovules	-0.20932	-0.64502		
No. times divided			0.9000	0
leaf length	-0.40869	0.012915		
leaf width	0.194148	0.183182		
leaflet length	0.456588	0.301706	-0.39858	0.514888
leaflet width	-0.12532	0.173605	-0.12732	0.487352
eigenvalue	6.7	5.1	27.7	2.9
df	66	42	57	36
chi-square	324.3	202.1	486.7	165.6
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001

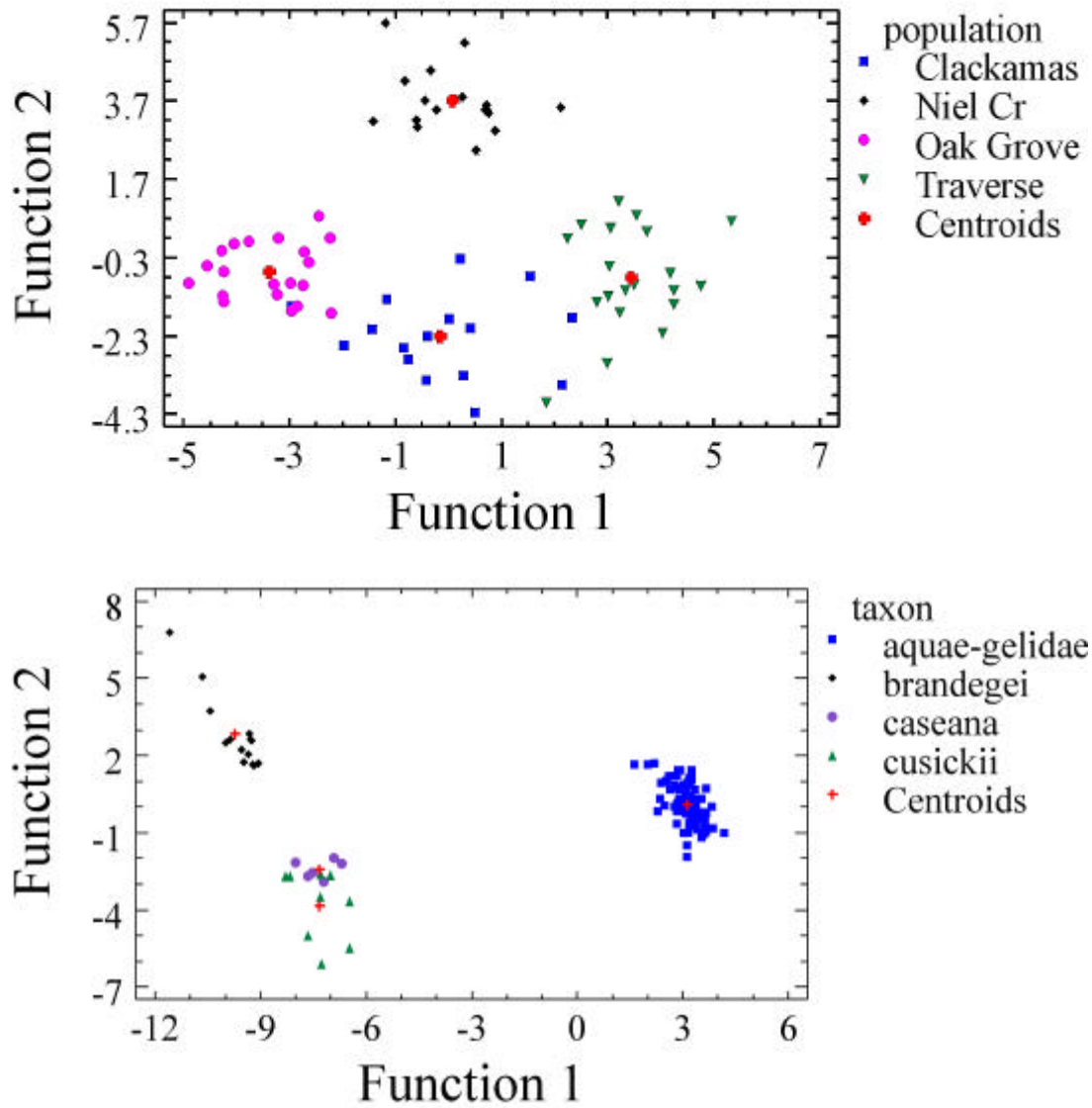


Figure 3. Discriminant Analysis of *Corydalis*. **Top:** four populations of *C. caseana* spp. *aquae-gelidae*. **Bottom:** *C. aquae-gelidae* and three subspecies of *C. caseana*.

Principal components analysis

***Aquae-gelidae* populations**--The first three components from PCA accounted for nearly 46% of the variation in the morphological data (Table 5); the remaining axes did not appear to explain interpretable variation, and were discarded. Crest size of the upper and lower petals as well as flower throat opening were heavily weighted on the first component, the second was strongly loaded by flower length, lower petal length, and petal separation, and the third represented bract length and width and flower number (Table 5). When the first two components were plotted against one another (Figure 5), there was no clear separation between the populations, reflecting the clinal nature of the morphological variation within the subspecies. However, there was also little overlap between Traverse Creek and the other populations, suggesting that this population is closely related to them but isolated both morphologically and geographically. The factor weights for component 1 suggest that petal crest size was the most important trait that separated Traverse Creek from the other populations (see Table 3). PCA components 2 did not appear to separate the populations well, but component 3 tended to isolate Niel Creek from the others, primarily because of the large bracts subtending the flowers at the population.

***Aquae-gelidae* and *caseana* subspecies**--With four subspecies included in the analysis, the first three Principal components accounted for 50.5% of the variation. Component 1 was very strongly weighted by leaflet size and number of leaf divisions, component 2 was correlated with petal crest sizes and spur width, while the third component reflected flower length and wing width, and number of flowers on the central inflorescence. As with populations of *aquae-gelidae*, the subspecies of *C. caseana* were not markedly isolated by PCA, reflecting their close affinity for one another (Figure 6). The first component served to place *aquae-gelidae* (including the Traverse Creek population) at one end of the spectrum and *brandegei* at the other – a clear reflection of the differences in leaflet size and leaf division that these taxa showed (Table 3). Subspecies *caseana* and *cusickii* were placed intermediately on component 1, but were partially separated by axis 2, apparently on the basis of the wider spurs and smaller petal crests of *caseana*.

Table 5. Factor weights and analytical results for the first three principal components resulting from analyses of four *aquae-gelidae* populations and also *aquae-gelidae* and three *caseana* subspecies. The three largest individual coefficients are in bold type. Factor weights are the relative importance (or correlation) of each morphological character with each principal component.

Morphological characters	<i>aquae-gelidae</i> populations			<i>aquae-gelidae</i> and <i>caseana</i> subspecies		
	component 1	component 2	component 3	component 1	component 2	component 3
spur length	0.1077	0.5821	-0.3560	0.6729	-0.1136	0.3421
spur width, middle	-0.5128	0.3596	-0.3981	0.1043	-0.6435	0.1070
spur width, base	-0.5509	0.2714	-0.4150	0.3498	-0.5521	-0.0663
flower length	0.3113	0.7326	-0.0412	0.3638	-0.1921	0.7514
lower petal length	-0.0001	0.6697	0.1209	0.3545	-0.3309	0.1796
upper petal length	-0.4465	0.0218	-0.0060	-0.0213	-0.4320	-0.3144
petal separation	0.0635	0.5980	0.1157	0.1324	-0.4742	0.5023
flower throat opening	-0.5882	-0.2872	0.1251			
wing width	0.3819	0.3710	0.2951	-0.1114	0.2120	0.5503
upper crest width	-0.8625	-0.1213	0.0326	-0.5084	-0.6514	-0.3329
lower crest width	-0.8324	-0.1676	-0.0342	-0.5191	-0.6351	-0.3161
bract length (at anthesis)	-0.2600	0.2353	0.7013	-0.3269	-0.4655	0.2722
bract width	-0.4653	0.1787	0.5818	-0.5445	-0.5201	0.1390
stipe length	0.4165	0.1451	0.1593	-0.5043	0.0442	0.4550
style length	-0.1315	0.5598	-0.2545	0.3388	-0.4797	0.1470
no. flowers	0.1838	0.2032	0.5936	-0.2362	0.0620	0.5611
inflor. length	0.0372	-0.4105	0.3595	-0.2043	0.0631	0.0067
no. ovules	0.0379	0.0365	0.5177			
no. times divided				-0.8323	0.1208	0.0784
leaf length	-0.5280	0.2206	0.4537			
leaf width	-0.4280	0.1856	0.4272			
leaflet length	-0.5232	0.1214	-0.3796	0.8365	-0.1132	-0.2354
leaflet width	-0.2783	0.5252	-0.0135	0.8144	-0.1076	-0.1965
eigenvalue*	4.13	3.15	2.79	4.33	2.9	2.3
percentage variance (cumulative)	18.8	14.1 (33.1)	12.7 (45.8)	22.8	15.4 (38.2)	12.3 (50.5)

*Derived from Factor Analysis

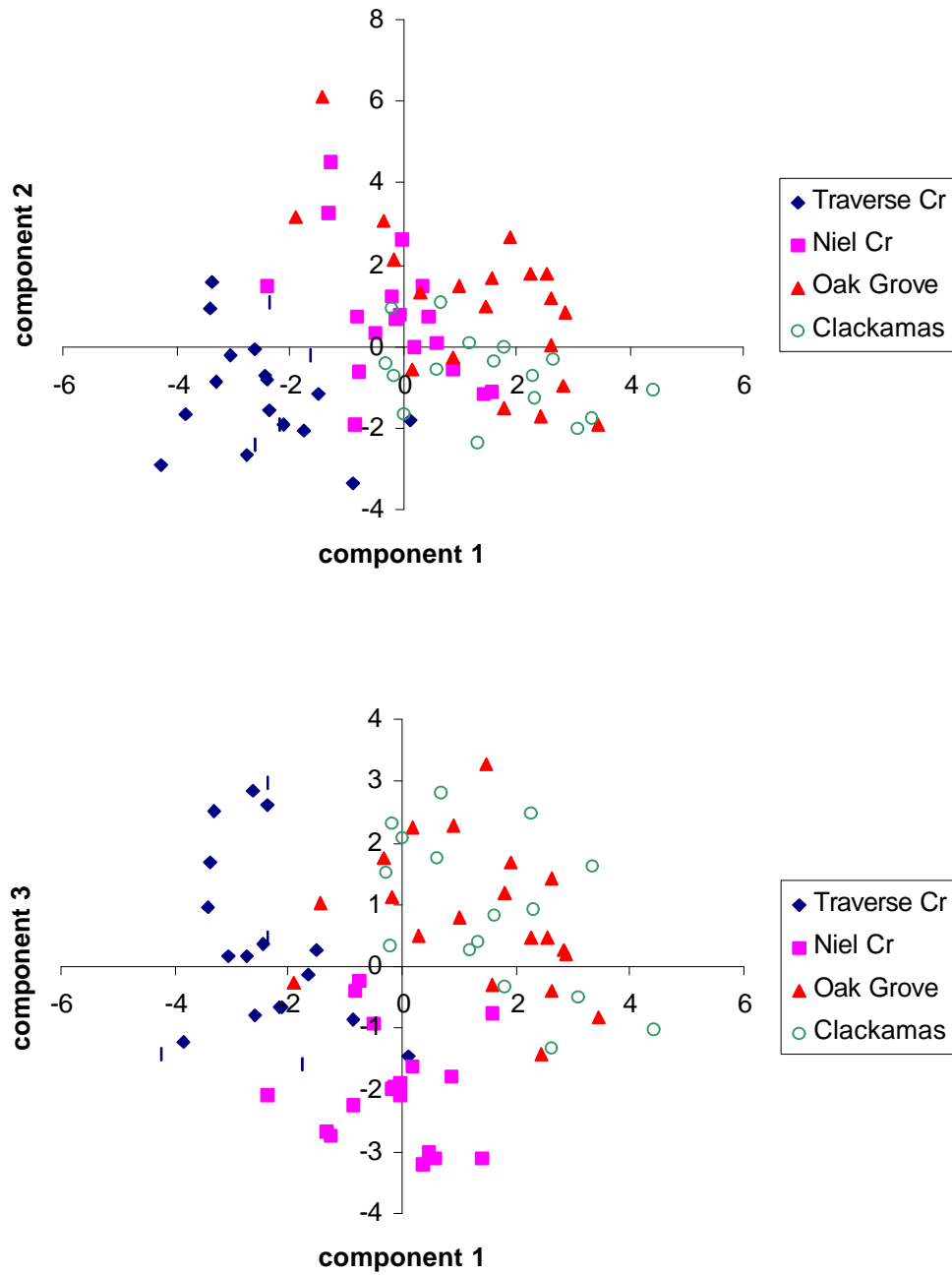


Figure 5. Principal components analysis of four populations of *Corydalis aquae-gelidae*. **Top:** components 1 and 2. **Bottom:** components 1 and 3.

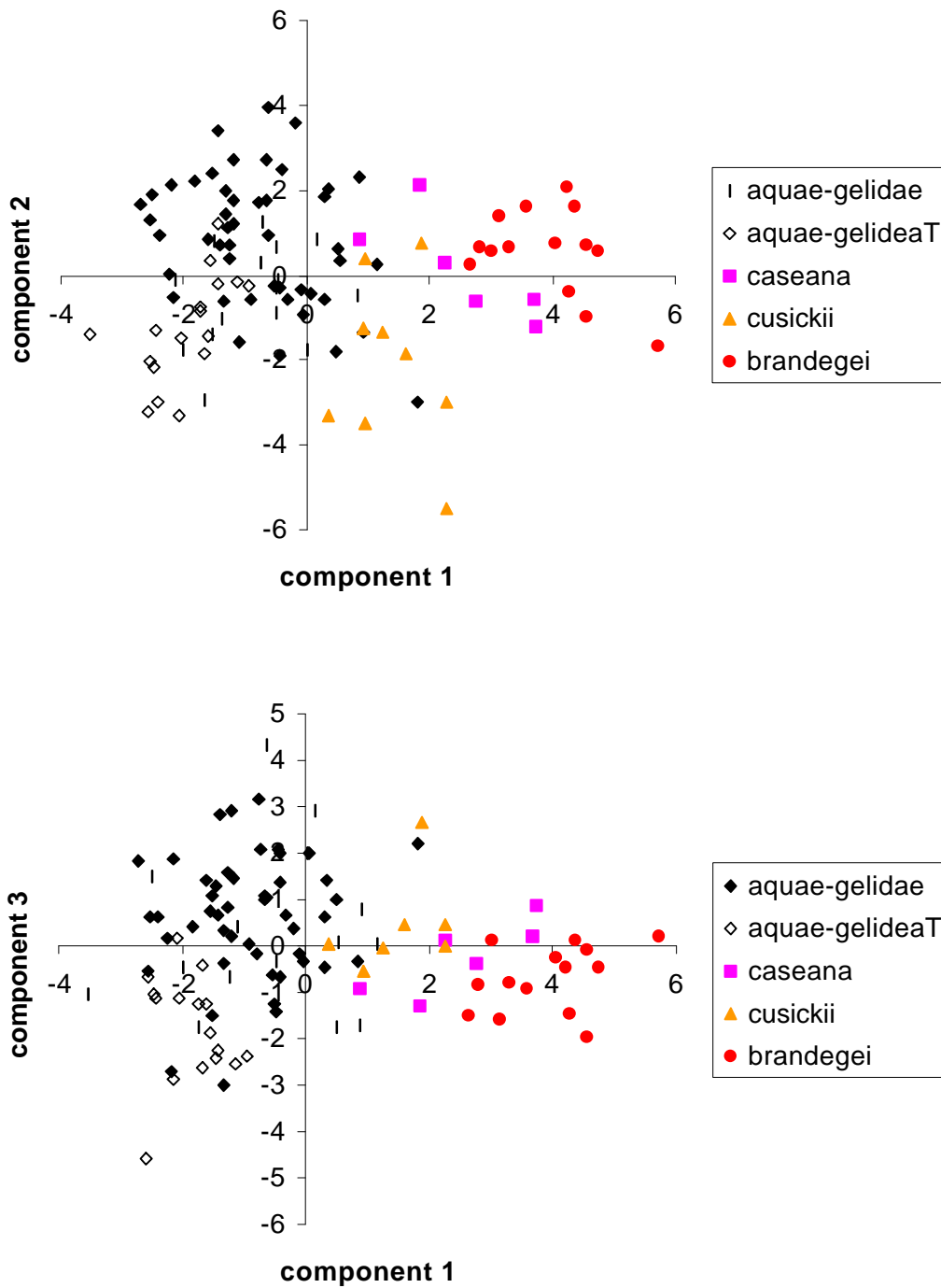


Figure 6. Principal components analysis of *C. aquae-gelidae* and three subspecies of *Corydalis caseana*. **Top:** components 1 and 2. **Bottom:** components 1 and 3. Open diamonds (*aquae-gelidae*T) represent Traverse Creek plants, a population that appears more closely related to *aquae-gelidae* than to any other subspecies of *C. caseana*.

Taxonomic status of the Traverse Creek population

The population of *Corydalis* at Traverse Creek appears to be most closely related to *C. aquae-gelidae*. It is somewhat separated from the other populations of *C. aquae-gelidae* included in these analyses (both PCA and DA), but all of the populations appear to have some distinguishing features. This suggests only that there may be widespread variability in morphometric traits in this species. Also, the pattern observed here corroborates genetic patterns described by Liston and Thorsen (2000) for *C. aquae-gelidae*. For example, they found that the Oak Grove population was genetically isolated, despite its proximity to other populations. We also found Oak Grove to be separate based on morphological information from the nearby population at Clackamas River. Finally, although the Traverse Creek population shares some traits with *C. cusickii*, such as large crests on the outer petals (Table 3), it is clearly aligned with *C. aquae-gelidae* in PCA (Figure 6).

Taxonomic status of Corydalis aquae-gelidae

Our results, though preliminary, are in agreement with Lidén's (1996) interpretation of *C. caseana* in which *C. aquae-gelidae* is considered a subspecies of this more widespread taxon. We recommend that this treatment be followed by future treatments. The four taxa included in PCA were separated spatially but without any notable discontinuities. In Discriminant Analysis, the taxa were generally isolated from one another, and *C. aquae-gelidae* was strongly isolated. However, the separation of *C. aquae-gelidae* was heavily weighted by leaf dissection and leaflet size, characters that can vary in each of the taxa. When leaf traits were removed from the analyses, the separation of *C. aquae-gelidae* was much reduced (not shown). *C. aquae-gelidae* is clearly a close relative of the *C. caseana* complex, and recognizing it as a subspecies of *C. caseana* would be consistent with other treatments that place the various geographic segments of this group under *C. caseana* (e.g., Ownbey 1947, Stern 1997). Lidén and Zetterlund (1997) have provided dichotomous keys to all of the non-tuberous species of *Corydalis*, including *C. caseana* and all six subspecies (including *aquae-gelidae*). This key notes that the leaves of *aquae-gelidae* are more divided and the spurs of the flowers are shorter than in *caseana* and most other subspecies, characteristics that are consistent with the results of the current study (see Table 3).

Additional taxonomic work

If additional taxonomic work is deemed necessary to delineate further the subspecific taxa of *C. caseana* and explore the differences between the Traverse Creek population from the rest of *C. aquae-gelidae*, it is recommended that the other two subspecies *hastata* and *brachycarpa*, be included. Also, it is recommended that a molecular genetic approach be pursued, possibly utilizing micro-satellites or internal simple sequence repeats (ISSRs). The morphometric approach used in the study reported here could be confounded if the observed morphological patterns are environmentally controlled. A genetic method would not be vulnerable to this problem, but combining both approaches would allow genetic differences to be juxtaposed with physical traits observable in the field and herbarium.

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