

# Molecular systematics and taxonomy of lake cress (*Neobeckia aquatica*; Brassicaceae), an imperiled aquatic mustard

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## Abstract

The North American lake cress, *Neobeckia aquatica* (Eaton) Greene, is a rare, aquatic member of the mustard family, Brassicaceae. The phylogenetic position and taxonomic status of this species have been uncertain due to the considerable overlap of morphological characters that exists among the five genera of aquatic cresses: *Armoracia*, *Cardamine*, *Nasturtium*, *Neobeckia*, and *Rorippa*. In this study, DNA sequence data from the chloroplast gene *rbcL* were compared for these aquatic cress genera and were used to construct a phylogenetic tree depicting their evolutionary interrelationships. Molecular data support a natural relationship of the aquatic cresses, but specifically indicate that *Neobeckia* is more closely related to *Rorippa* than it is to *Armoracia*. This analysis indicates that the incomplete fruit septa of *Neobeckia* and *Armoracia* are parallelisms which should not be used to justify the merger of these genera. DNA sequence data also indicate a close relationship between *Cardamine* and *Nasturtium*, and their distinctness from the other aquatic cress genera. A relatively high level of DNA sequence divergence separates *Neobeckia* from all other aquatic cress genera. *Neobeckia* and *Rorippa* share similar vegetative and seed features, but differ by habit, petal color, and fruit characteristics. The taxonomic decision is made to retain *Neobeckia* as a monotypic genus rather than to merge it with *Rorippa*.

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## 1. Introduction

Taxonomists recognize the mustards (Brassicaceae) as a distinctive, natural angiosperm family, yet the precise boundaries of many genera have proven to be notoriously difficult to delimit (Rollins, 1962; Jones, 1976; Cronquist, 1988). The family is relatively large (350 genera) but has few aquatic (or helophytic)

species, confined to the genera *Armoracia* (horseradish), *Cardamine* (bitter cress), *Nasturtium* (watercress), *Neobeckia* (lake cress), *Rorippa* (yellow cress), and *Subularia* (awlwort) (Cronquist, 1981; Cook, 1990). This group includes the economically important horseradish (*Armoracia rusticana* Gaertn., Mey., & Scherb.) and watercress (*Nasturtium officinale* R. Br.) as well as lake cress (*Neobeckia aquatica* (Eaton) Greene), a rare species with minor commercial value as a specimen aquarium plant (Rataj and Horeman, 1977).

Perhaps the most unusual of the aquatic cresses is the lake cress, *Neobeckia aquatica*. This species grows as an emergent or can thrive as a submersed plant in water depths exceeding 4.5 m (La Rue, 1943; Patman and Iltis, 1961). *Neobeckia* is extremely polymorphic with considerable variation in the morphology of its submersed and emergent leaves. Cauline leaves display heterophylly, with highly dissected submersed leaves and divided to entire emergent leaves (Mac Dougal, 1914). In addition, the plants grow from a basal rosette with leaves that can vary from nearly entire to highly pinnately dissected (La Rue, 1943).

An exceptional feature of *Neobeckia* is its prodigious capacity for vegetative reproduction. Pringle (1879) and later Foerste (1881, 1889) noted that cauline leaves shed from flowering plants produced adventitious plantlets from petiolar bases that became lodged upon mud. Foerste (1881) also reported vegetative layering of *Neobeckia* stems which rooted and produced branches along the bottom. La Rue (1943) observed that most reproduction in the species is asexual via vegetative reproduction and regeneration. The early formation of an abscission layer causes leaves to drop from plants even when slightly touched (Muenscher, 1930; La Rue, 1943; Voss, 1985). Bud regeneration in *Neobeckia* has been observed from mutilated leaves, pieces of leaf divisions less than 5 mm long, stem pieces, roots, and detached ovaries; normal plants have been grown from small masses of terminal bud meristematic tissue less than 0.5 mm in length (La Rue, 1943).

La Rue (1943) remarked that once established, *Neobeckia* plants can become extremely abundant due to their effective means of asexual reproduction. Nevertheless, the species has not flourished. *Neobeckia* inhabits the coastal plain and Mississippi embayment region (Stuckey, 1993), but has always been "... rare south of Missouri and New York" (Hotchkiss, 1967). The historical range of *Neobeckia aquatica* includes 26 states in the United States and two Canadian provinces (Table 1). Although herbarium records indicate a fairly extensive past distribution, the number of extant populations has declined in recent years. The present rarity of *Neobeckia* has prompted concern for its protection in several regions (e.g. Stuckey and Roberts, 1977; Roosa and Eilers, 1978; Crow et al., 1981). Stuckey (1987) emphasized that disappearance of the species has been most pronounced in the central portion of its range, the region where it was once most abundant. Throughout North America, the scarcity of records since 1950 indicates that up to 84% of all *Neobeckia* populations may now be lost (Table 1).

Although tribal limits in the Brassicaceae remain somewhat tentative, the aquatic cresses (*Armoracia*, *Cardamine*, *Nasturtium*, *Neobeckia*, and *Rorippa*) appear to comprise a natural group. Hayek (1911) included the genera within

Table 1

Reported historical distribution of lake cress (*Neobeckia aquatica*). The number of extant sites (by county) is uncertain for most states; sites with collections since 1950, however, provide a crude estimate of extant populations

State/province	No. of known sites		References
	Pre 1950	1950 and after	
<i>United States</i>			
Alabama	2	0	Al-Shehbaz and Bates, 1987; Stuckey, 1987
Arkansas	8	0	Al-Shehbaz and Bates, 1987; Stuckey, 1987
Florida	3	1	Al-Shehbaz and Bates, 1987; Stuckey, 1987
Georgia	2	0	Al-Shehbaz and Bates, 1987; Stuckey, 1987
Illinois	41	2	Mohlenbrock and Ladd, 1978; Stuckey, 1987; Al-Shehbaz and Bates, 1987
Indiana	14	0	Deam, 1940; Stuckey, 1987
Iowa	1	0	Al-Shehbaz and Bates, 1987; Stuckey, 1987; Roosa and Eilers, 1978
Kentucky	5	1	Beal and Thieret, 1986; Stuckey, 1987
Louisiana	6	3	Al-Shehbaz and Bates, 1987; Stuckey, 1987
Massachusetts	1	0	Eaton, 1822; Stuckey, 1987
Maryland	1	0	Stuckey, 1987
Michigan	13	5	Voss, 1985; Stuckey, 1987
Mississippi	3	1	Al-Shehbaz and Bates, 1987; <i>Bryson 8865</i> (KNK)
Missouri	12	0	Steyermark, 1963; Stuckey, 1987
New Jersey	4	0	Stuckey, 1987
New York	28	3	Clausen, 1940; Ogden, 1974; Stuckey, 1987; House, 1924; Muenscher, 1930
North Carolina	?	0	Muenscher, 1944; Beal, 1977; Stuckey, 1987
Ohio	11	3	McCormac, 1992
Oklahoma	3	1	Nelson and Couch, 1985; Stuckey, 1987
Pennsylvania	1	0	Muenscher, 1944; Stuckey, 1987
South Carolina	?	0	Muenscher, 1944; Stuckey, 1987
Tennessee	5	2	Al-Shehbaz and Bates, 1987; Stuckey, 1987
Texas	2	1	Correll and Correll, 1975; Stuckey, 1987
Vermont	8	4	Muenscher, 1930; <i>Grout s.n.</i> (VT); <i>Griffin s.n.</i> (VT); <i>Pringle s.n.</i> (VT); <i>Countryman 796</i> (VT); <i>Cushman 6016</i> (CONN); <i>Hellquist s.n.</i> (NASC); <i>Thompson s.n.</i> (VT)
Virginia	1	0	Al-Shehbaz and Bates, 1987; Stuckey, 1987
Wisconsin	4	1	Al-Shehbaz and Bates, 1987; Krueger, 1993
<i>Canada</i>			
Ontario	9	3	Stuckey, 1987
Quebec	1	0	Stuckey, 1987
Totals	189	31	

the single tribe *Arabideae*; Schulz (1936) differed only by placing *Armoracia* within tribe *Drabeae*. Both tribes are viewed as closely related and highly advanced in the family (Schulz, 1936). The aquatic genus *Subularia* is unrelated to

these cress genera and has been assigned to the phylogenetically intermediate tribe *Lepidieae* (Hayek, 1911; Schulz, 1936).

The question of generic limits has been a long standing problem among putatively related aquatic cresses. Stuckey (1972; p. 286) suggested that *Armoracia* (including *Neobeckia*), *Cardamine*, *Nasturtium*, and *Rorippa* were closely related but advocated that "A study of the affinities of these and other Cruciferae is needed". Rollins (1962) reasoned that the weak differentiation among all mustard genera was due to the family's recent evolutionary history. It is not surprising that generic boundaries have been particularly difficult to reconcile among members of phylogenetically advanced tribes such as *Arabideae* and *Drabeae*. This problem is exemplified by *Neobeckia* which, in addition to being recognized as a monotypic genus, has been assigned variously to *Armoracia*, *Nasturtium*, and *Rorippa* (Al-Shehbaz and Bates, 1987). Bateman (1955; p. 65) maintained that attempts at phylogenetic classifications of the Brassicaceae have been complicated because "... all characters of possible systematic importance vary independently ...". Indeed, differences among the aquatic cress genera are relatively minor and exhibit considerable overlap (Table 2).

A better understanding of the relationships of *Neobeckia* should provide important life history insights that may benefit programs directed at its conservation. For example, fungal and viral pathogens are known to seriously impact populations of horseradish and watercress (Crisp, 1976). Knowing whether *Neobeckia*

Table 2  
Features compared among the aquatic cresses

	<i>Armoracia</i>	<i>Cardamine</i>	<i>Nasturtium</i>	<i>Neobeckia</i>	<i>Rorippa</i>
Wet habitats	yes	yes	yes	yes	yes
Chromosome series	$x=8$	$x=8$	$x=8$	$x=8$	$x=8$
Petals	white	white/purple	white	white	white <sup>1</sup> / yellow
Style beak	<0.5 mm	0.3–15 mm	0.1–2 mm	2–4 mm	0.1–3 mm
Replum partition	incomplete	complete	complete	vestigial	complete
Heterophylly	yes	no	yes <sup>2</sup>	yes	yes <sup>3</sup>
Gemmpary	no	yes <sup>4</sup>	no	yes	yes <sup>3</sup>
Fruit	silicle	silique	silique	silicle	silicle/silique
Valve shape	convex	flat	convex	convex	convex
Elastic valves	no	yes	no	no	no
Seeds	2 rows	1 row	1 or 2 rows	2 rows	1 <sup>1</sup> or 2 rows
Compatibility	SI	SI, SC	SC	?	SI, SC

Compiled from Arber, 1920; Fernald, 1950; Rollins, 1962, 1993; Ball, 1964; Valentine, 1964; Stodola, 1967; Crisp, 1976; Voss, 1985; Al-Shehbaz and Bates, 1987; Gleason and Cronquist, 1991.

SI, self-incompatible; SC, self-compatible.

<sup>1</sup> *Rorippa crystallina* Rollins.

<sup>2</sup> Submersed leaves simple, emergent leaves divided.

<sup>3</sup> *Rorippa amphibia*.

<sup>4</sup> *Cardamine pratensis* L.

is closely or distantly related to either of these species could help assess its susceptibility to similar infectious agents.

This study reexamines relationships among the aquatic cresses to clarify the phylogenetic position of *Neobeckia*. Because affinities of these species have not been resolved satisfactorily by comparative morphology, DNA sequence data have been used to perform a comparative phylogenetic analysis for this group of interesting aquatic mustards.

## 2. Methods

Total genomic DNA was isolated from green leaves of *Armoracia rusticana*, *Cardamine pensylvanica* Willd., *Nasturtium officinale*, *Neobeckia aquatica*, *Rorippa amphibia* (L.) Besser, and *Rorippa sylvestris* (L.) Besser using a modified CTAB procedure (Doyle and Doyle, 1987). Voucher specimens for these species have been deposited at the University of Connecticut herbarium (CONN). A 1,347 bp portion of the chloroplast gene *rbcL* (approximately 94% of the coding region) was amplified enzymatically using a thermostable polymerase (see Les et al., 1993) and synthetic oligonucleotide primers:

5'-ATGTCACCACAAACAGAACTAAAGC-3' (forward) and

5'-AATTTGATCTCCTTCCATATTTTCGCA-3' (reverse).

The double-stranded amplification products were purified and sequenced directly following the methods outlined in Les et al. (1993) using the universal sequencing primers kindly provided by G. Zurawski (DNAX, Palo Alto, CA). Additional *rbcL* sequences of *Capparis hastata* Jacq. and *Brassica oleracea* L. were obtained for comparison from GenBank (accession numbers: M95754, M88342); the sequence of *Brassica campestris* L. was obtained from the *rbcL* database (Chase et al., 1993). The sequences of the two *Brassica* species differed by only one substitution (a transition at position 66). Consequently, only the sequence of *B. campestris* was used in the analyses.

Alignment of the 1,347 bp sequences for all species was straightforward and required no gaps. The phylogenetic significance of the sequence data was assessed using unweighted maximum parsimony methods. Parsimony analyses employed the computer program PAUP, v. 3.1.1 (Swofford, 1993) to perform an exhaustive search using the *Capparis* sequence as an outgroup for rooting. A bootstrap analysis (500 replicates) provided estimates of confidence intervals for branch lengths in the resulting phylogeny. Pairwise sequence divergence and transition:transversion ratios were calculated using a computer program written by S. Duncan, Indiana University.

3. Results

Eighty-four nucleotide substitutions were detected at 80 sites (5.9% of total) among the eight taxa compared in this study (Fig. 1). The majority of these (62/74%) represented synonymous first and third codon position substitutions; 22 nucleotide substitutions (26%) were involved in 19 amino acid substitutions (Fig. 1). Among the mustards (Capparis excluded), only eight amino acid substitutions were detected, indicating that the protein was approximately 98.2% conserved at the amino acid level.

The level of rbcL sequence divergence was low among the aquatic cress genera (0.5-1.3%), higher between the aquatic cresses and Brassica (2.1-2.9%) and highest between the mustard genera and Capparis (2.3-3.8%); the sequences of the two Rorippa species were identical (Fig. 1). Transition to transversion ratios varied from 1 to 3.7, but showed no correlation with percent sequence divergence (Table 3). A single minimal length (most parsimonious) tree (87 steps) was

Table with multiple rows of nucleotide and amino acid sequences for various taxa (C.h., B.c., C.p., N.a., N.o., R.s.). Nucleotide positions are numbered 1-74. Amino acid sequences are shown in bold type. Substitutions are indicated by bolding the nucleotide and the corresponding amino acid.

Fig. 1. Nucleotide and amino acid substitutions (bold type) for variable codons within a 1,347 bp portion of rbcL compared for Capparaceae (C.h., Capparis hastata) and seven Brassicaceae: B.c., Brassica campestris (tribe Brassiceae); C.p., Cardamine pensylvanica; A.r., Armoracia rusticana; and: A.r., Armoracia rusticana; C.p., Cardamine pensylvanica; N.a., Neobeckia aquatica; N.o., Nasturtium officinale; R.a., Rorippa amphibia; R.s., Rorippa sylvestris (tribe Arabideae). Positions of all variable nucleotides observed are numbered consecutively from the ATG (methionine) transcription initiating codon of rbcL (codons with invariant sites have been omitted). The amino acid sequence of this proportion of rbcL (approximately 94% of the total sequence) is 99.1% conserved among the six species of aquatic Arabideae.

Table 3

Matrix of percent sequence divergence (upper) and transition: transversion ratios (lower) for *rbcl* in pairwise comparisons of taxa studied

	1	2	3	4	5	6	7	8
1 <i>Armoracia rusticana</i>	—	2.3	3.0	0.7	0.5	1.2	0.7	0.7
2 <i>Brassica campestris</i>	2.44	—	3.8	2.2	2.1	2.9	2.6	2.6
3 <i>Capparis hastata</i>	1.28	1.43	—	2.9	2.7	3.4	3.3	3.3
4 <i>Cardamine pensylvanica</i>	2.00	2.00	1.05	—	0.5	1.3	1.0	1.0
5 <i>Nasturtium officinale</i>	1.33	1.80	1.12	1.00	—	1.2	0.9	0.9
6 <i>Neobeckia aquatica</i>	2.20	2.25	1.19	1.83	1.67	—	1.0	1.0
7 <i>Rorippa amphibia</i>	1.50	2.18	1.25	1.60	1.40	3.67	—	0.0
8 <i>Rorippa sylvestris</i>	1.50	2.18	1.25	1.60	1.40	3.67	—	—

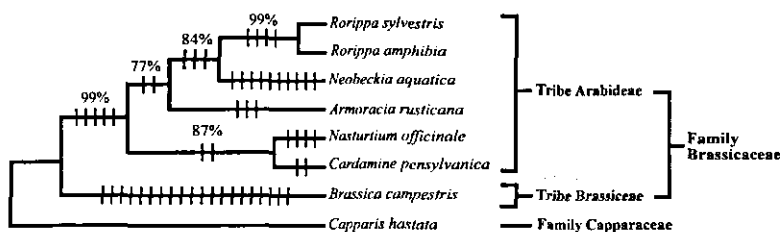


Fig. 2. Phylogeny for aquatic members of tribe *Arabideae* derived from *rbcl* sequence data. The number of substitutions supporting each branch are indicated by vertical bars; percentages above branches provide estimates of confidence intervals from 500 bootstrap replications. This is the single shortest tree resulting from unweighted maximum parsimony analysis of the data; the consistency index for the cladogram is 0.97. The *rbcl* data depict lake cress (*Neobeckia*) as a genetically distinct sister group to the genus *Rorippa*.

obtained with a consistency index (CI) of 97% (Fig. 2); a CI of 86% was obtained when phylogenetically uninformative characters were excluded. The retention index for this cladogram is 88%. Bootstrap values indicated moderate support for the association of *Neobeckia* with *Rorippa* species (84%) and for the association of *Cardamine* and *Nasturtium* (87%). Support for the association of all six aquatic mustard taxa was high (99%).

#### 4. Discussion

The *rbcl* gene has been used extensively in studies of plant phylogenetic relationships (e.g. Chase et al., 1993) including several investigations of aquatic angiosperms (Les et al., 1991, 1993). Owing to the high degree of conservation in the coding region, the greatest utility of *rbcl* sequence comparisons has been to assess phylogenetic relationships among distant taxonomic groups such as families (e.g. Rodman et al., 1993). The suitability of *rbcl* sequence data for use in infrafamilial phylogenetic studies has not been explored adequately, presumably

because such comparisons are likely to yield an insufficient number of nucleotide substitutions to thoroughly resolve phylogenetic relationships.

A major problem with DNA sequence data, however, is associated with homoplasy resulting from multiple nucleotide substitutions ('multiple hits') that can occur repeatedly at the same site over time. Ritland and Eckenwalder (1992) concluded that the 'optimal' sequence divergence for molecular studies is approximately 10%, a level where the number of 'single hit' sites is expected to be at a maximum and the number of multiple-hit sites at a minimum. However, even at 10% sequence divergence, a significant level of homoplasy will occur in trees generated from sequence data (Ritland and Eckenwalder, 1992).

A lower level of sequence divergence among taxa (i.e. < 10%) has the advantage of identifying substitutions that mostly represent single-hit sites, thereby reducing a major contributing factor to homoplasy in such analyses. The limiting factor when comparing taxa of recent origin (where DNA sequence divergence is minimal) is simply locating enough phylogenetically informative substitutions to produce an adequately resolved phylogeny. Whether a group of closely related taxa will yield a set of DNA sequences that is both of low divergence yet contains relatively large numbers of informative characters can only be evaluated empirically by trial and error.

The *rbcl* sequences of the aquatic cresses comprise a fortuitous data set where the overall level of nucleotide divergence between species is low (0–3.5%), yet the number of informative sites is sufficient to completely resolve phylogenetic relationships. The consistency index of 97% reflects the low level of homoplasy associated with the low level of sequence divergence among taxa. Accordingly, this comparison should represent a relatively reliable assessment of phylogenetic interrelationships for the aquatic cress taxa.

The *rbcl* phylogeny is consistent with the hypothesis that all five genera of aquatic cresses are monophyletic and closely related. This conclusion remains tentative, however, given that a relatively small number of mustard genera have been surveyed. Nevertheless, the *rbcl* data better support the tribal classification of Hayek (1911) who placed all of the general within the single tribe *Arabideae* rather than the classification of Schulz (1936) who placed *Armoracia* within tribe *Drabeae*. The *rbcl* sequence data indicate that all of the aquatic cress genera are relatively differentiated at the molecular level from *Brassica campestris*, a species of the putatively primitive tribe *Brassicaceae* (Schulz, 1936).

Below, the taxonomy of aquatic cresses is reviewed and discussed in context of affinities suggested by the present molecular analysis.

#### 4.1. Affinities of *Neobeckia* and *Armoracia*

The superficial similarity of *Neobeckia* and horseradish (*Armoracia*) led Eaton (1822) to describe the former as a variety of horseradish. Wiegand (1925) also included *Neobeckia* within *Armoracia* noting its resemblance in stature, flower size, fruit shape, and dissected leaves to horseradish. Several recent taxonomic treatments (e.g. Voss, 1985; Al-Shehbaz and Bates, 1987; Gleason and Cronqu-



ist, 1991) have adopted the placement of *Neobeckia* in the genus *Armoracia* on the basis of their apparent similarity.

Both genera have similar features (Table 2). Cultivated horseradish is understandably not perceived as 'aquatic', yet all species of *Armoracia* are typically marsh plants (Ball, 1964; Voss, 1985). Although generally recognized by the large, simple, sorrel-like leaves of garden-grown plants, horseradish is heterophyllous and will produce pinnately dissected lower leaves if grown under submersed conditions (Jennings and Avinoff, 1953).

Like horseradish, *Neobeckia* is a deeply rooted perennial (Muenscher, 1944; Fernald, 1950; Patman and Iltis, 1961; Al-Shehbaz and Bates, 1987) but overwinters by dormant stem bases and dormant vegetative plantlets (La Rue, 1943). When grown under terrestrial conditions, lake cress plants form 'radish-like underground members' that are capable of overwintering (Mac Dougal, 1914). Mac Dougal (1914) also observed that terrestrial grown lake cress plants produce thicker roots (up to 1 cm) than plants grown as aquatic (roots < 2 mm thick). The thickened roots are comparable to radishes in texture and taste (Mac Dougal, 1914). Horseradish is easily propagated by root cuttings (Schery, 1972; Al-Shehbaz and Bates, 1987), but root propagation of *Neobeckia* is difficult (La Rue, 1943).

Imprecise descriptions of fruit structure have confused efforts to assess the relationship of lake cress to horseradish. In mustards, an encircling, thick, frame-like rib known as the replum forms in the suture of the two carpel margins (Fahn, 1982). Typically, the fusion of two membranes that originate from the replum creates a 'false' septum (not of carpellary origin) and results in a bilocular fruit (Fahn, 1982). The fruits of most mustards (including species of *Cardamine*, *Nasturtium*, and *Rorippa*) possess both a replum and a complete septum.

Wood (1889) described the fruits of both horseradish and lake cress as "1-celled from the incomplete partition." Because most mustard fruits are bilocular, this feature has been accorded considerable taxonomic importance. Wiegand (1925), however, implied that a septum was present in horseradish but was only partially complete or entirely absent in lake cress. Al-Shehbaz and Bates (1987) indicated that an incomplete septum occurred in both species, yet Fernald (1950), Jennings and Avinoff (1953), and Gleason and Cronquist (1991) distinguished lake cress as possessing a unilocular fruit and horseradish a bilocular fruit.

For clarification, fruits of horseradish (*Armoracia rusticana*, Eames 2000 CONN) and lake cress (*Neobeckia aquatica*, Bryson 8865 KNK) from herbarium material were examined. The horseradish fruits possessed a septum that was approximately 60-90% complete. In lake cress, the septum was essentially absent. In several specimens of lake cress examined at the Gray Herbarium (GH), the septum was approximately 10% complete. The taxonomic significance of the incomplete septum of horseradish and the virtual absence of a septum in lake cress is questionable. Although uncommon, unilocular fruits or incomplete to fenestrate septa occur in other mustard genera such as *Aphragmus*, *Eutrema*, *Lesquerella*, *Scolioxon* (Rollins, 1993), *Neslia*, *Raphanus*, and *Cardaria* (Fernald,

1950). The incomplete septum of *Lesquerella perforata* Rollins (Rollins, 1993) is nearly identical to the type found in *Neobeckia*.

Phylogenetically (Fig. 2), the abnormal septa of lake cress and horseradish represent parallelisms (a complete septum exists in *Rorippa*). Owing to their nearly exclusive vegetative reproduction, the independent loss of septa in *Armoracia* and *Neobeckia* is conceivable given that selection to retain features associated with sexual reproduction or seed dispersal is likely to be relaxed.

Seed failure is commonly associated with both lake cress (Foerste, 1881; La Rue, 1943; Muenscher, 1944; Godfrey and Wooten, 1981; Al-Shehbaz and Bates, 1987; Gleason and Cronquist, 1991) and horseradish (Weber, 1949; Stokes, 1955; Gleason and Cronquist, 1991). Although horseradish is typically highly sterile, viable seed has been obtained on occasion (Courter and Rhodes, 1969). Foerste (1881) observed only a few seeds to ripen in a population of lake cress in Illinois, but doubted their viability. La Rue (1943) reported that no ripe seeds were produced in a flowering population studied from Cheboygan, Michigan. Pringle (1879) remarked that vegetative plantlets "... have one year's start of those resulting from seeds of the same year ..." but did not elaborate on whether he actually observed reproduction from seed to take place. In a survey of the Lake Champlain basin, Muenscher (1930) reported flowering plants to be rare and fruiting individuals to be 'very scarce'. Mac Dougal (1914) followed transplanted lake cress populations for 10 years and observed flowering in only one plant and never observed reproduction from seed. The vigorous fruiting but poor seed maturation of lake cress plants in Ohio, led McCormac (1992) to conclude that reproduction was probably entirely vegetative. It remains to be demonstrated whether viable seed is ever produced in lake cress populations.

As with septum features, it is apparent that the sterility of horseradish and lake cress has arisen independently in these species and probably results from different causes. Horseradish (*Armoracia rusticana*) is tetraploid (Easterly, 1963), however, *Neobeckia aquatica* is triploid (D.H. Les, G.J. Anderson and M.A. Cleland, unpublished data, 1993). Triploidy in lake cress is likely to represent the major cause of its sterility and is probably a significant factor contributing to its present rarity (D.H. Les, G.J. Anderson and M.A. Cleland, unpublished data, 1993). Brezezinski (1909) suggested that horseradish was an interspecific hybrid, a hypothesis supported by Weber (1949) who described abortive anthers, high pollen sterility, irregular meiosis, and a low proportion of ovules containing gametophytes in the species. Stokes (1955) alternately suggested that deleterious mutations thwarting seed production may have arisen during the long period of clonal propagation in horseradish and also that the species was self-incompatible.

In *Neobeckia*, the shedding of leaves during the flowering period may limit resources available for seed maturation. Pringle (1879) commented that abscised leaves were neither withered nor senescent, but were "... green and gorged with elaborated material for growth ...". It is evident that these lost resources (and the loss of future photosynthetic contributions from shed leaves) are not available to developing fruits. However, leaf loss is not always associated with reduced seed production. Snoad (1981) demonstrated that in peas, leafless plants

have higher seed output than leafy plants when grown at high densities. Although rosette leaves of lake cress are usually retained throughout the growing season (La Rue, 1943), the relationships of cauline leaf loss, resource limitation, and seed maturation deserve further investigation.

Sporophytic homomorphic self-incompatibility occurs in many Brassicaceae and has been documented from at least 11 tribes and 80 species (Bateman, 1955). Self-incompatibility is known in *Armoracia* (Stokes, 1955; Bateman, 1955; Fryxell, 1957), *Cardamine* (Correns, 1913; Bateman, 1955; East, 1940; Fryxell, 1957), and *Rorippa* (Stace, 1975). Self-fertile species, however, have evolved repeatedly throughout the mustard family and occur in *Cardamine*, *Rorippa*, and *Nasturtium* (East, 1940, Bateman, 1955). In the absence of controlled crosses it is not presently possible to determine whether *Neobeckia* is self-incompatible. Reduced seed set is predicted in clonal self-incompatible species that encounter bottlenecks because of the widespread loss of incompatibility alleles (Reinartz and Les, 1994).

#### 4.2. Affinities of *Neobeckia*, *Cardamine*, and *Nasturtium*

The former placement of lake cress (*Neobeckia*) in *Nasturtium* must be evaluated in the proper context. Asa Gray, who first assigned lake cress to *Nasturtium* in 1835, adopted a broad concept of the genus that included species of *Nasturtium*, *Rorippa*, and *Armoracia*. It is interesting that Gray (1889) indicated lake cress to be “near *N. amphibium*”, i.e. the species now recognized as *Rorippa amphibia*. Schulz (1936) also retained lake cress in *Nasturtium*, but within section *Rorippa*. Therefore, the previous associations of lake cress and *Nasturtium* actually represented proposed affinities to the genus *Rorippa* and not to *Nasturtium sensu stricto* (i.e. watercress species). Stuckey (1972) remarked that *Nasturtium* was not closely related to the North American species of *Rorippa* and Rollins (1978) agreed that watercresses (*Nasturtium*) were “only marginally related” to species of *Rorippa*. A distant relationship of *Nasturtium* and *Rorippa*, as hypothesized by Stuckey (1972) and Rollins (1978), is supported by the *rbcl* phylogeny.

Lake cress has not been assigned to *Cardamine*, probably on the basis of morphological inconsistencies such as the single row of seeds in the latter (Table 2). Both *Neobeckia* and *Cardamine* exhibit gemmipary, which is relatively common among the aquatic cresses. Like *Neobeckia*, adventitious buds can arise at the base of any detached simple leaf or any isolated leaflet of a compound leaf in *Cardamine*, *Dentaria*, and *Rorippa*; in some taxa even small fragments of a leaflet will eventually produce young plants (Sculthorpe, 1967). Arber (1920) illustrated adventitious plantlets of *Cardamine pratensis* arising from the base of an apical leaflet and from detached pinnules.

The close (i.e. sister-group) relationship of *Cardamine* and *Nasturtium* indicated by *rbcl* sequence data is not surprising given the similarity of certain species in these genera. Rollins (1978) remarked that assignment of taxa to *Cardamine* or *Nasturtium* was “obscure” and often extremely difficult to assess.

*Cardamine pensylvanica* is frequently misidentified as *Nasturtium officinale*, particularly when in vegetative condition (Voss, 1985).

Although *Nasturtium* differs from *Cardamine* by its compound leaves, submersed plants of *Nasturtium microphylla* produce entire leaves that resemble those of *Cardamine* species (Rollins, 1978). *Cardamine* species also differ from *Nasturtium* by having seeds in one rather than two rows, however, seeds of the tetraploid *Nasturtium microphylla* Reichb. are also uniseriate (Green, 1962). Crossing studies have shown that *N. microphylla* is an allotetraploid hybrid with the diploid *N. officinale* as one parent; the other unknown parent is suspected to be a species of *Cardamine* (Howard and Manton, 1946).

#### 4.3. Affinities of *Neobeckia* and *Rorippa*

The similarity of *Neobeckia* to species of *Rorippa* is striking (Table 2) and provides a rationale for merging these genera (e.g. Muenscher, 1944; and others). Jonsell (1968), however, regarded the placement of lake cress in *Rorippa* as "questionable". *Neobeckia* differs from *Rorippa* mainly by its white petals and vestigial septum (Fassett, 1957; Jonsell, 1968; Stuckey, 1972). The pinnate underwater leaves of *Neobeckia* differ from some *Rorippa* species, but similar dissected submersed leaves occur in *Rorippa amphibia* (Jonsell, 1968). Specimens of *Neobeckia aquatica* have been mistaken for *Rorippa amphibia* on several occasions (Marie-Victorin, 1930).

A well-developed system of vegetative reproduction occurs in *Rorippa* and *Neobeckia*. *Rorippa amphibia* produces adventitious plants on dislodged leaves, particularly following water level drops (Stodola, 1967). Norton (1933) also observed the production of plantlets from submersed stems and branches of *Rorippa amphibia*. Allogamous, perennial *Rorippa* species have a highly developed capacity for vegetative reproduction and can regenerate from fragments of stems and roots (Jonsell, 1968). Highly developed vegetative reproduction, including the exogenous formation of adventitious shoots from roots, has also been reported in *Rorippa austriaca* (Crantz) Besser (Wilson, 1927).

Schulz (1936) regarded petal color as the major distinction between *Rorippa amphibia* and *Neobeckia*. These vegetatively similar species are difficult to distinguish in the absence of fruit and flowers. The label notation: "flowers yellow, fading to white", on a specimen of *Rorippa amphibia* that was originally misidentified as lake cress (Zika 9175 VT), indicates the underlying white pigmentation of *Rorippa* flowers. In his generic description of the genus, Stuckey (1972) also commented that the yellow petals of *Rorippa* become white when dry. The enigmatic species *Rorippa crystallina* Rollins shares several features with lake cress including similar leaves and whitish rather than yellow petals (Rollins, 1962, 1993; Stuckey, 1972). White petals also characterize *Rorippa floridana* Al-Shehbaz & Rollins (Rollins, 1993) and the New Zealand species *Rorippa gigantea* (Hook. f.) Garnock-Jones (Garnock-Jones, 1978). The petals of *Rorippa alpina* (S. Wats.) Rydb. are described as "whitish-yellow" (Rønning, 1972). These exam-

ples emphasize that white petal color is yet another mustard characteristic that conforms to imprecise generic boundaries.

Further evidence of relatedness between *Neobeckia* and *Rorippa* is found in their seed morphology (see figs. 9 and 23 in Murley, 1951). In a key to the seeds of northeastern North American Cruciferae (Murley, 1951), similar features of *Neobeckia* seeds key it among *Rorippa* species.

In the *rbcL* phylogeny (Fig. 2), the association of *Neobeckia* with *Rorippa* has moderate bootstrap values (84%), and is supported by three substitutions (Fig. 1: positions 207, 513, and 684) that occur nowhere else among the taxa compared. A recent common ancestry of *Rorippa* and *Neobeckia* is indicated by these shared mutations. Similarly, the absence of these substitutions in *Armoracia*, *Nasturtium*, and *Cardamine* provides compelling evidence against the transfer of *Neobeckia* to any of these genera.

There are eight nucleotide substitutions (six transitions and two transversions at positions 148, 447, 954, 993, 1020, 1107, 1212, 1341 that are unique to lake cress in this comparison; an additional two transitions (at positions 976, 133) are reversals to the outgroup state (Fig. 1). These mutations indicate a high degree of divergence for lake cress relative not only to *Rorippa*, but other aquatic cresses as well (Fig. 2). It is true that not all *Rorippa* species were included in this study, however, the sampled species are among the putatively closest related group to *Neobeckia*. This observation, together with the fact that a combination of taxonomic features (white petals, submersed habit, vestigial fruit septum) can be used to separate *Neobeckia* and *Rorippa*, is the basis of the decision to retain the monotypic genus *Neobeckia* rather than to merge lake cress with the genus *Rorippa*.

The identical *rbcL* sequences for *Rorippa amphibia* and *R. sylvestris* are not surprising given that a close relationship of these species and their recent divergence is evidenced by their successful ability to hybridize (Jonsell, 1968). The single substitution differing between the two sequences of *Brassica* (*B. campestris*, *B. oleracea*) also indicates a low level of intrageneric divergence for *rbcL* in the mustard family.

## 5. Conclusions

A phylogenetic analysis of DNA sequence data supports the close evolutionary relationship of the aquatic cresses and indicates the phylogenetic position of *Neobeckia* (lake cress) as a genetically distinct, sister genus to *Rorippa*. This relationship is consistent with a re-examination of other evidence that establishes a high degree of similarity between these genera. Taxonomically, it is difficult to argue against the inclusion of lake cress within *Rorippa*, given that the major distinguishing feature is the vestigial septum, and even this may simply reflect a prolonged history of vegetative reproduction.

However, *Neobeckia* possesses a suite of characteristics that may not be unique singly, but together set it apart from *Rorippa*. *Neobeckia* is truly aquatic, whereas, *Rorippa* species are essentially helophytic. The combination of heterophylly, highly

dissected leaves, white petals, vestigial replum, and triploid chromosome number, would be cause enough to place lake cress in a distinct section even if it were included in *Rorippa*. Although many recent authors have favored the transfer of the lake cress to the horseradish genus *A Armoracia*, and the merger of the genera *Rorippa* and *Nasturtium*, these taxonomic practices are not recommended given that doing so would be inconsistent with the relationships indicated by the molecular based phylogeny. Conservationists should consider the unique phylogenetic status of lake cress in the mustard family when establishing priorities for protection.

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