

A new phylogeny of *Rumex* (Polygonaceae) adds evolutionary context to the diversity of reproductive systems present in the genus

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Abstract

Rumex is one of about 50 genera in the knotweed family, Polygonaceae. The genus comprises about 200 species with bisexual, or more commonly, unisexual flowers, with the species displaying monoecious, dioecious, synoecious (hermaphroditic) or polygamous reproductive systems. Some of the dioecious species have heteromorphic sex chromosomes, which is rare amongst angiosperms. We here present a plastid phylogeny of 67 species, representing all four subgenera. For this study, we used three chloroplast markers, *rbcL*, *trnH-psbA*, *trnL-F* and dense taxon sampling to reconstruct the most comprehensive molecular phylogeny of *Rumex* to date. The reconstructed phylogeny for this work resolves six major clades and one large grade in *Rumex* subg. *Rumex*. In addition, the species with known dioecious reproductive systems are resolved within a broader clade we term “the dioecious clade”. These results suggest that the species with divergent reproductive systems are more closely related to each other than to other species comprising the rest of the *Rumex* genus.

Keywords

Dioecious, *Emex*, heteromorphic sex chromosome systems, monoecious, synoecious

Introduction

Commonly known as docks and sorrels, *Rumex* L. (Polygonaceae) is a relatively large genus. *Rumex* encompasses four circumscribed subgenera, approximately 200 species and hundreds of described subspecies or varieties. Many species in *Rumex* are cosmopolitan in nature, spanning six continents of the world. However, many individual species are either regionally endemic, native or introduced on particular continents (Rechinger 1937).

The cosmopolitan distribution of *Rumex* species is indicative of their ability to thrive in a wide variety of environmental conditions. Described species are just as recurrent in dry and sandy soils as they are in marshes and cultivated fields, spanning the arctic, subarctic, boreal, temperate, tropical and subtropical localities (Löve and Kapoor 1967). Although several biological species demonstrate little to no niche preference (e.g. *Rumex crispus* L., *Rumex obtusifolius* L.), there are others that exhibit exceedingly precise ecological requirements (e.g. *Rumex bipinnatus* L.f., *Rumex pictus* Forrsk.). The large variation in the distribution of *Rumex* species might also account for the large deviation observed in the morphology of these species (Fig. 1), whereby some reach almost seven metres in height and others rarely exceed a few centimetres (Rechinger 1949; Löve and Kapoor 1967; Rechinger 1990).

In the 20th Century, progress in the taxonomic and cytological study of *Rumex* was largely accomplished by two researchers: Áskell Löve and Karl Heinz Rechinger (Rechinger 1937; Rechinger 1954a; Löve and Kapoor 1967). Löve extensively documented the cytological diversity of *Rumex* and he proposed a generic status for *Acetosa* and *Acetosella* (the groups with species bearing heteromorphic sex chromosomes) and subgeneric status for *Axillares* and *Platypodium*. Löve also considered *Rumex* to be composed of several smaller genera corresponding to a number of cytotypes (Löve 1957; Löve and Kapoor 1967; Mariotti et al. 2006, 2009).

Over the course of his career, Rechinger effectively monographed *Rumex*, using plant morphology and geographic distribution in his taxonomic treatments (Rechinger 1933, 1937, 1939, 1949, 1954a, b, 1984, 1990; Brandbyge and Rechinger 1989). By the mid-1900s, Rechinger had proposed a subgeneric status for *Platypodium* and maintained *Acetosa*, *Acetosella* and *Lapathum* as comparable subgenera (Rechinger 1954a; Table 1). Rechinger chose to retain *Rumex* as a single genus.

The reproductive systems of *Rumex* species vary extensively. Species of *Rumex* exhibit synoecious (hermaphroditic), monoecious, dioecious and polygamous

Table 1. Summary of the recognised subgenera in *Rumex*, with species diversity and reproductive systems present.

Subgenus	No. of species	Sexual system	Sex chromosomes
<i>Acetosa</i>	41	Dioecious, Gynodioecious, Polygamous	Yes (in part)- XX/XY ₁ Y ₂
<i>Acetosella</i>	5	Dioecious (rarely polygamous)	Yes- XX/XY
<i>Rumex</i> (= <i>Lapathum</i>)	126	Synoecious, Monoecious	No
<i>Platypodium</i>	1	Synoecious	No

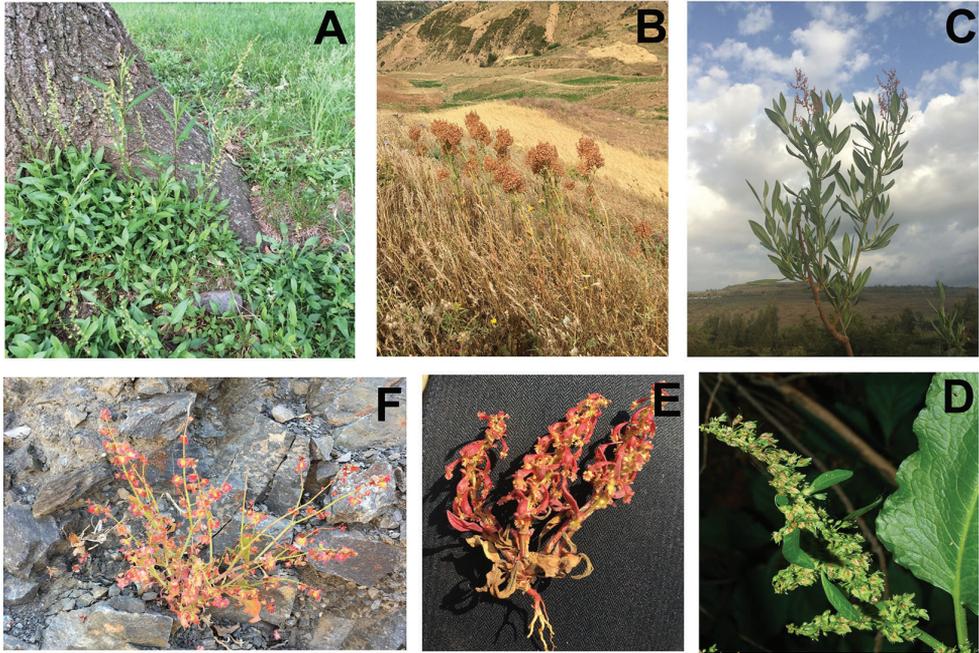


Figure 1. Depiction of morphological variation amongst the different subgenera of *Rumex*. **A** *Rumex acetosella* growing in Virginia, USA (subg. *Acetosella*) **B** *Rumex thyrsoides* growing in Morocco (subg. *Acetosa*) **C** *Rumex nervosus* growing in Ethiopia (subg. *Acetosa*) **D** *Rumex obtusifolius* growing in New York, USA (subg. *Rumex*) **E** *Rumex bucephalophorus* collected on the Moroccan coast (subg. *Platypodium*) **F** *Rumex papilio* growing in Morocco (subg. *Acetosa*). All photo credits J.M. Burke.

reproductive systems (Rechinger 1949; Rechinger 1954a; Löve and Kapoor 1967; Mosyakin 2005; Navajas-Pérez et al. 2005). Most of the reproductive system diversity has been described in subgenera *Acetosa* or *Acetosella*. In particular, most species in these subgenera are dioecious (Rechinger 1937, 1949, 1954a, 1984). A few species in subgenus *Rumex* have variable systems, especially between synoecy and monoecy (e.g. *Rumex crispus*, J. Burke, pers. obs.). There are also three species of *Rumex* endemic to the Hawaiian Islands (*Rumex albescens* Hillebr., *R. giganteus* Aiton and *R. skottsbergii* O. Deg. & I. Deg.), which are all monoecious (Wagner et al. 1999).

Rumex has two different sex chromosome systems exhibited in many of the dioecious species, classified in *Rumex* subg. *Acetosa* and *Rumex* subg. *Acetosella*. In *Rumex*, the documented sex chromosomes are heteromorphic. Two sex-determining chromosomal mechanisms are known: XX/XY and XX/XY₁Y₂ (Löve 1940, 1942, 1943, 1944; Löve and Löve 1948; Shibata et al. 1999, 2000; Navajas-Pérez et al. 2005; Cunado et al. 2007; Ming et al. 2011). The XX/XY₁Y₂ system is dosage-dependent and plant sex is based on the autosome to sex-chromosome ratio. In this system, female individuals have 14 chromosomes and male individuals have 15 chromosomes (Löve 1940, 1944; Löve and Kapoor 1967; Navajas-Pérez et al. 2005).

Recent molecular phylogenetic work has sought to resolve the placement of *Rumex* in the Polygonaceae more broadly (Sanchez and Kron 2008; Sanchez et al. 2009; Burke et al. 2010; Burke and Sanchez 2011; Sanchez et al. 2011; Schuster et al. 2011, 2013, 2015). These studies have placed *Rumex* alongside the other Rumices of Campderá (*Emex* and *Oxyria*), with the addition of *Rheum* as either sister to *Oxyria* (Burke et al. 2010; Schuster et al. 2011) or to *Rumex* + *Emex* (Schuster et al. 2013, 2015). One area that lacks clarity has been the placement of *Emex*, which sometimes appears to be nested within *Rumex* (e.g. Sanchez et al. 2011) and is sometimes placed as sister to *Rumex* (e.g. Burke et al. 2010). Moreover, the relationships of species within *Rumex*, including the relationship between *Rumex* and *Emex*, continue to be poorly understood due to insufficient sampling and paucity of data. To date, the relationships amongst species placed within Reehinger's subgenus *Rumex* are particularly obscure.

Here we present a new phylogeny of *Rumex*, constructed using three plastid gene regions (*trnH-psbA*, *rbcL* and *trnL-F*) and 67 *Rumex* species. We have used this phylogeny to test the placement and monophyly of its circumscribed subgenera, as well as discuss the broad patterns in the evolution of reproductive systems within *Rumex*.

Materials and methods

Taxon sampling and DNA Isolation

DNA was isolated from 109 accessions, representing 67 *Rumex* species. Of the 109 included accessions, a total of 99 *Rumex* accessions, six *Rheum* L. species, three *Emex* L. accessions and one species of *Persicaria* L. (Mill.) are represented. *Persicaria virginiana* (L.) Gaertn., *Rheum alexandrae* Batalin, *Rheum emodii* Wall., *Rheum nobile* Hook. f. & Thomson, *Rheum officinale* Baill., *Rheum palmatum* L. and *Rheum rhabarbarum* L. were included as outgroup species. Additional plant samples were obtained through the GenBank sequence database (Appendix A1). Samples were taken from a combination of herbarium specimens (K, NY, OSC, RAB, US), field collections and cultivated samples from collaborators. Herbarium acronyms follow the Index Herbariorum (Thiers 2019).

All fresh leaf samples were dried using silica gel. Plant tissue was homogenised using the FastPrep-24 5G Sample Preparation System (M. P. Biomedicals, LLC Santa Ana CA, USA). Total genomic DNA was extracted from herbarium specimen-sampled and silica-dried leaf tissues using a BIOLINE ISOLATE II Plant DNA Kit (Cat No. BIO-52070). Modification for herbarium material proceeded as follows: Cell lysis was carried out using 300 µl of buffer (PA1 or PA2) and 30 µl of proteinase K (20 µg/ml) and incubated for 18 hours at 65 °C on an orbital shaker.

Marker selection

For this first comprehensive phylogeny of the genus, we focused on plastid marker selection. Previous authors utilised nrITS as a nuclear marker (Schuster et al. 2011; Schuster et al. 2015). However, we did not utilise nrITS for this phylogeny due to

a number of issues that would interfere with accurate reconstruction of evolutionary relationships: 1) nrITS is extremely variable and difficult to align (66% of nrITS sequence data was excluded in Schuster et al. [2015] publication) and 2) Due to widespread polyploidy documented in multiple *Rumex* species, sequences of nrITS would not necessarily be low copy and there would be substantial issues with paralogy and orthology across multiple polyploidy events.

For plastid marker selection, we screened multiple markers that had previously been used in Polygonaceae reconstruction (Burke et al. 2010; Burke and Sanchez 2011; Koenemann and Burke 2020). We selected markers that both showed sufficient variation across the genus and were easily amplified for most taxa.

PCR amplification and sequencing

Amplification of DNA markers was completed for three plastid regions: *rbcL*, *trnH-psbA* and *trnL-F* (Table 2). *rbcL* was amplified using the following PCR conditions: 94 °C for 1 min, followed by 34 cycles of 94 °C/15 s, 54 °C/15 s and 72 °C/30 s and a final extension period of 5 min at 72 °C. *trnH-psbA* was amplified using the following PCR conditions: 94 °C for 2 min, followed by 34 cycles of 94 °C/30 s, 55 °C/30 s and 72 °C/30 s and a final extension period of 7 min at 72 °C. *trnL-F* was amplified using the following PCR conditions: 80 °C for 5 min, followed by 34 cycles of 94 °C/1 min, 55 °C/1 min and 72 °C/2 min and a final extension period of 5 min at 72 °C. PCR and gel electrophoresis were performed following standard protocols with no special conditions. PCR experiments were performed separately with only fresh or only herbarium material to help prevent cross-contamination.

Table 2. Gene regions used: name of primers, total length of region, % parsimony informative characters.

Gene region	Reference	Primer names	Total aligned length	PIC (%)
<i>rbcL</i>	Fazekas et al 2008	rbcLF, rbcLR	539	24 (4.5)
<i>trnH-psbA</i>	Shaw 2007	psbA, trnH	596	132 (22.1)
<i>3trnL-F</i>	Shaw 2005	3'trnL ^{UAAF} , trnF ^{GAA}	442	65 (14.7)
Combined			1577	221 (14.0)

PCR amplicons were sent to Eurofins Genomics (Louisville, KY) for Sanger sequencing. Sequences were edited using Geneious v. 10 (Biomatters Ltd.). Reviewed sequences were aligned with MUSCLE (Edgar 2004) and concatenated using MES-QUITE (Maddison 2005).

Phylogeny reconstruction

All phylogenetic analyses were completed using the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). Prior to the phylogenetic reconstructions, we performed ModelTest-NG (Darriba et al. 2020) for the concatenated matrix to determine the suggested model of evolution. ModelTest-NG indicated that the best fit was the General Time Reversible (GTR) model.

We performed Maximum Likelihood (ML) phylogeny reconstruction using GARLI v. 2.01.1067 (Zwickl 2006). We used the default GARLI parameters with the following exceptions: we performed 1000 search replications (10 iterations of 100 search replicates). In order to better search tree space, we increased the attachments per taxon setting to 150 and extended the generations without improvement parameter to 50000. To evaluate support for phylogenetic relationships, statistical bootstrapping was performed, specifying only one search replicate per bootstrap iteration for 100 iterations. All bootstrap trees were downloaded and used to generate a majority rule consensus tree in MESQUITE (Maddison 2005). The consensus tree was visualised in FigTree version 1.4.3 (Rambaut 2014).

We performed Bayesian Inference phylogeny reconstruction in MrBayes 3.2.7a (Ronquist et al. 2012). The priors were set to the defaults (Dirichlet). We set the seed number at 123. We conducted two independent Markov Chain Monte Carlo (MCMC) runs, each with four chains employing BEAGLE library acceleration (as recommended by CIPRES). Each MCMC run was set to complete 5 million generations, with trees sampled every 1,000 generations. The first 25% of trees in each run were discarded as burn-in. MrBayes then synthesised the two independent runs and extracted the majority rule consensus tree with posterior probabilities.

Posterior probability and bootstrap values were visualised using FigTree version 1.4.3 (Rambaut 2014) and MESQUITE (Maddison 2005). Posterior probabilities above 90% and bootstrap support values above 70% were considered significant and annotated in the final phylogeny.

Results

The most likely tree was generated using 109 specimen accessions. This included seven outgroup species, three accessions of *Emex* and 99 accessions of *Rumex*. The present phylogeny represents 67 *Rumex* species, more than twice the number of species of *Rumex* sampled in previous phylogenies (31 species in Navajas-Pérez et al. 2005; 13 species in Schuster et al. 2015). A total of 47 sequences were missing from the final matrix, yielding 14.4% missing data in the final analysis (Grant 2022). Table 2 summarises the variability of each of the gene regions. The most variable region was *trnH-psbA*, which consisted of 22.1% parsimony informative characters. The least variable region was *rbcL* which consisted of 4.5% parsimony informative characters.

The most likely tree recovered by GARLI received a likelihood score of $\ln = -5767.548440$.

The genus *Rumex* was recovered as monophyletic with strong support (100 Bayesian Posterior Probability/98 Maximum Likelihood Bootstrap) (Fig. 2). The analysis did not recover *Rumex* subgenus *Rumex*, the subgenus with the most species diversity, as monophyletic. In our phylogeny, species of subgenus *Rumex* form a grade at the base of the tree (“Basal Grade” – Fig. 2). *Emex* was recovered as monophyletic, just above the Basal Grade and sister to the dioecious clade. While the results indicate

Rumex. Furthermore, different gene regions reconstructed conflicting topologies for the placement of *Emex*. The *rbcL* phylogeny placed *Emex* within *Rumex* subgenus *Rumex* (50% bootstrap support). Both *trnh-psbA* and *trnL-F* placed *Emex* as sister to the *Rumex* genus (*trnh-psbA* < 50% bootstrap support and *trnL-F* 91% bootstrap support) (results not shown).

The remaining taxa, comprising the subgenera *Acetosa*, *Acetosella* and *Platypodium* form a highly supported (99/80) monophyletic group (Fig. 2). This group is denoted as “the dioecious clade” because it is here that we see the initial transition to dioecy of the known dioecious *Rumex* species resolved in this group. The relationships of the clades within this group are also well-supported. Our recovered phylogenetic tree did not recover subgenus *Acetosa* as monophyletic. Within the dioecious clade, subgenus *Acetosa* is comprised of three well-supported, monophyletic groups, Clade 2 (100/97), Clade 3 (100/78) and Clade 4 (100/97) and is nested below a pair of clades, represented by subgenus *Platypodium* (Clade 5) and subgenus *Acetosella* (Clade 6). The pair (*Platypodium* + *Acetosella*) is also well supported (100/81). Subgenus *Platypodium* was recovered as monophyletic with strong support (100/100) and consists of four accessions of its only circumscribed species: *Rumex bucephalophorus*. Species in subgenus *Acetosella* were recovered together with strong support (100/89), but the inclusion of *Rumex hastatulus* means the subgenus was not recovered as monophyletic.

In addition to corresponding largely to the established subgeneric system, the topology also largely corresponds to the diversity of the reproductive and sex chromosome systems present in *Rumex*. Species in subgenus *Rumex* (Basal Grade) are mostly hermaphroditic with no documented heteromorphic sex chromosomes. With no documented heteromorphic sex chromosomes, *Emex* is also represented as a clade and consists of purely monoecious species. Subgenus *Acetosa* consists entirely of dioecious species, with some members exhibiting the sex chromosome system XX/XY₁Y₂. Subgenus *Platypodium*, another hermaphroditic group with no reported sex chromosomes, is nested between subgenera *Acetosa* and *Acetosella*. Subgenus *Acetosella* consists of species that are both dioecious and have the sex chromosome system XX/XY.

Discussion

Our results produced a phylogeny of *Rumex*, with six major clades and one grade, largely congruent with Rechinger’s subgeneric classification. The placement of *Emex* conflicted, based on the molecular markers used. In our phylogeny, it is sister to the dioecious clade, but without strong support.

Within the phylogeny, the basal grade is mostly made up of species from *Rumex* subgenus *Rumex*. That subgenus *Rumex* was recovered as a grade rather than a clade is not surprising given the known extensive hybridisation amongst species of this subgenus. This phenomenon most certainly contributed to the lack of resolution in species-level relationships within subgenus *Rumex*. Additionally, although hybridisation between species in subgenus *Rumex* and species in the other subgenera are not

well documented, it is possible that such hybrids exist and serve to hinder our ability to distinguish subgenus *Rumex* as a clade. We suspect that increased taxon sampling and genetic data, especially from the nuclear genome, will help to resolve relationships amongst species in subgenus *Rumex*.

Although dioecious, the species included in Clade 2 and Clade 3 have no reported heteromorphic sex chromosome systems. The species included in Clade 4 exhibit a complex sex chromosome system (XX/XY_1Y_2). This placement suggests that this heteromorphic sex chromosome system was derived from dioecious ancestors. The genetic origin of heteromorphic sex chromosomes in *Rumex* is beyond the scope of this manuscript, but this result provides a framework to investigate potentially intermediary taxa that may contain homomorphic or transitional sex chromosome systems.

Subgenus *Platypodium* (Clade 5) was resolved as monophyletic and nested within “the dioecious clade”. Based on its plant and chromosome morphology, earlier studies concerning *Rumex bucephalophorus* have referred to it as the link between subgenus *Rumex*, which is predominantly synoecious and subgenus *Acetosella*, which is predominantly dioecious (Löve 1944). Although morphologically variable, *R. bucephalophorus* consistently exhibits a synoecious reproductive system. Its derivation from amongst the dioecious species in this phylogeny suggests a reversal from a dioecious condition.

Subgenus *Acetosella* (Clade 6) was not recovered as monophyletic. Known dioecious species, *R. hastatulus*, of subgenus *Acetosa* is nested within subgenus *Acetosella*. *Rumex hastatulus* is documented to exhibit two distinct karyotypes: a complex sex chromosome system (XX/XY_1Y_2 , North Carolina karyotype) which is characteristic of subgenus *Acetosa* and the simple sex chromosome system (XX/XY , Texas karyotype) which is characteristic of subgenus *Acetosella* (Navajas-Pérez et al. 2005; Mariotti et al. 2009; Hough et al. 2014). In addition, Rechinger’s 1937 treatment indicates a polygamous reproductive system for *R. hastatulus* (Rechinger 1937). Given the variability found within this species, *R. hastatulus* could have been placed in either subgenus (*Acetosa* or *Acetosella*), where species appear to have diversified according to the type of sex chromosome system they exhibit. This finding suggests the plasticity of reproductive and sex chromosome systems within *Rumex*, as a single species can exhibit two different karyotypes.

One of the striking features of the phylogeny recovered in this study is its congruence with the taxonomic system established by Rechinger (Rechinger 1933, 1937, 1939, 1949, 1954a, 1954b, 1984, 1990). Rechinger retained the diversity of species as a single genus, but divided them into four subgenera: *Rumex* (*Lapathum*), *Platypodium*, *Acetosa* and *Acetosella*. Each subgenus is prominently present in the topology. Subgenus *Platypodium* is monophyletic. Subgenus *Acetosella* is monophyletic even with the inclusion of *Rumex hastatulus*, whose placement has been ambiguous. The two largest subgenera, *Acetosa* and *Rumex*, were recovered as grades. The grade of subgenus *Acetosa* is well-resolved and well-supported. The grade of subgenus *Rumex* is both less well-resolved and less well-supported. The recovered topology, nevertheless, serves to confirm the major relationships amongst species in the genus, relationships for which Rechinger had proposed using only morphology.

In all, this work has provided a reconstructed phylogeny that differs from those currently published (Navajas-Pérez et al. 2005; Schuster et al. 2015) and has tested the placement and monophyly of its circumscribed subgenera. This work builds on those previous studies by providing an increased taxon sampling density, which has resulted in a more comprehensive reconstruction of the evolutionary history of *Rumex* and a more thorough examination of the stability of the subgeneric system. This work has provided an early outline of the evolution of reproductive systems in *Rumex*, suggesting an ordered plasticity and transitions from synoecy to dioecy to dioecy with heteromorphic sex chromosomes. Additionally, this work suggests a possible reversal from a dioecious condition. Future directions in *Rumex* research include the identification and application of nuclear markers that will allow for a more robust phylogeny, particularly with respect to the placement of *Emex*. Additionally, future genomic studies will serve to elucidate the evolution of the sex chromosomes and sex determining regions in *Rumex*.

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Appendix A1. List of taxa sampled and vouchers specimens

Abbreviations of herbaria where the voucher is housed are listed after the collection number. Sequences can be found on the Dryad database: <https://doi.org/10.5061/dryad.69p8cz8zs>

For sequences that we did not generate, accession information is given as found on GenBank.

GenBank sequences used for this study:

rbcL: *Rumex pamiricus* Rech. f. – JF944139.1, *Rumex sibiricus* Hulten-KC483892.1

trnH-psbA: *Rumex pamiricus*- JN047053.1

Table A1. DNA Sequences Generated for this Study.

Scientific name	Voucher
<i>Emex australis</i> Steinh.	P.C. Zietsma 4053, NY
<i>Emex spinosa</i> (L.) Campd.	Schuhwerk 90/328, NY
<i>Emex spinosa</i> (L.) Campd.	J.M. Burke 302, HUDC
<i>Persicaria virginiana</i> (L.) Gaertn.	J.M. Burke s.n., BH
<i>Rheum alexandrae</i> Batalin	Cultivated Material, HUDC
<i>Rheum emodii</i> Wall.	Cultivated Material, HUDC
<i>Rheum officinale</i> Baill.	Cultivated Material, HUDC
<i>Rheum palmatum</i> var. <i>taguticaum</i> L.	Cultivated Material, HUDC
<i>Rheum rhubarbarum</i> L.	Cultivated Material, HUDC
<i>Rheum nobile</i> Hook. f. & Thomson	Pradham 820581, BH
<i>Rumex abyssinicus</i> Jacq.	J.M. Burke 251, HUDC
<i>Rumex acetosa</i> L.	K.D. Grant s.n., HUDC
<i>Rumex acetosella</i> L.	R. Brand 1336, NY
<i>Rumex acetosella</i> L.	D.E. Atha 10521, NY
<i>Rumex acetosella</i> L.	K.D. Grant s.n., HUDC
<i>Rumex acetosella</i> L.	J.M. Burke 309, HUDC
<i>Rumex albescens</i> Hillebr.	Lorence 5224, K
<i>Rumex albescens</i> Hillebr.	Wood 14959, US
<i>Rumex alpinus</i> L.	Larsen 20708, US
<i>Rumex alpinus</i> L.	D.E. Atha 5114, NY
<i>Rumex altissimus</i> Alph, Wood	Shultz 8717, US
<i>Rumex altissimus</i> Alph, Wood	D.E. Atha 10857, NY
<i>Rumex alveolatus</i> Los.-Losinsk.	Rechinger 48318, US
<i>Rumex amurensis</i> F. Schmidt ex Maxim.	Barrett Lilan22p
<i>Rumex aquaticus</i> L.	Elias 7251, US
<i>Rumex arcticus</i> Trautv.	Shetler 4560, US
<i>Rumex arifolius</i> All.	K. Deguchi 4023, NY
<i>Rumex bequaertii</i> De Wild.	Germishuizen 3447, US
<i>Rumex berlandieri</i> Meisn.	Thieret 17178, US
<i>Rumex brachypodus</i> Rech. f.	J.M. Burke 312, HUDC
<i>Rumex brasiliensis</i> Link	R. Wasum 1655, NY
<i>Rumex brownii</i> Campd.	Wilson 10250, NY
<i>Rumex brownii</i> Campd.	Wilson 10250, US
<i>Rumex bucephalophorus</i> L.	Barrett 17RBTA5
<i>Rumex bucephalophorus</i> L.	J.M. Burke 293, HUDC
<i>Rumex bucephalophorus</i> L.	J.M. Burke 301, HUDC
<i>Rumex bucephalophorus</i> L.	J.M. Burke 304, HUDC
<i>Rumex chrysocarpus</i> Moris	D.E. Atha 13012, NY
<i>Rumex conglomeratus</i> Murray	D.E. Atha 10045, NY
<i>Rumex conglomeratus</i> Murray	J.M. Burke 271, HUDC
<i>Rumex conglomeratus</i> Murray	J.M. Burke 298, HUDC

Scientific name	Voucher
<i>Rumex conglomeratus</i> Murray	J.M. Burke 299, HUDC
<i>Rumex crispus</i> L.	J.M. Burke 268, HUDC
<i>Rumex cuneifolius</i> Campd.	J.C. Solomon 13044, US
<i>Rumex cyprius</i> Murb.	Kocher B-273, US
<i>Rumex densiflorus</i> Osterh.	Pinkava P12626, US
<i>Rumex dentatus</i> L.	D.G. Kelch 07.328, OSC
<i>Rumex giganteus</i> Aiton	K. Thorne 6736, NY
<i>Rumex giganteus</i> Aiton	Canfield 1304, US
<i>Rumex graminifolius</i> Gerogi ex Lamb.	Petrosky 1811, US
<i>Rumex hastatulus</i> Baldwin	D.E. Atha 10503, NY
<i>Rumex hastatus</i> D. Don	MacArthur 1291, US
<i>Rumex hastatus</i> D. Don	Barrett s.n.
<i>Rumex hymenosepalus</i> Torr.	Cultivated material, HUDC
<i>Rumex hymenosepalus</i> Torr.	A. Tiehm 15727, OSC
<i>Rumex induratus</i> Bioss. et Reut.	M.W. Chase 925, K
<i>Rumex induratus</i> Bioss. et Reut.	Barrett s.n.
<i>Rumex induratus</i> Bioss. et Reut.	J.M. Burke 310, HUDC
<i>Rumex intermedius</i> DC.	Rainha 5270, US
<i>Rumex japonicus</i> Houtt.	Bai-Zhang 4049, US
<i>Rumex kernerii</i> Borbás	Barta 2004-390, US
<i>Rumex lanceolatus</i> Thunb.	H.J. Venter 10295, NY
<i>Rumex longifolius</i> DC.	D. E. Atha 8858, NY
<i>Rumex lunaria</i> L.	NR. 8879, NY
<i>Rumex lunaria</i> L.	Barrett 17RLLM1
<i>Rumex lunaria</i> L.	Barrett 17RLTF1
<i>Rumex maritimus</i> L.	Shiu Ying Hu 13127, US
<i>Rumex mexicanus</i> Meisn.	D.E. Breedlove 13305, US
<i>Rumex microcarpus</i> Campd.	Barrett MJ-P40 (Seed)
<i>Rumex nepalensis</i> Spreng.	J.M. Burke 248, HUDC
<i>Rumex nervosus</i> Vahl	J.M. Burke 252, HUDC
<i>Rumex obtusifolius</i> L.	J.M. Burke s.n., BH
<i>Rumex obtusifolius</i> L.	J.M. Burke 270, HUDC
<i>Rumex orbiculatus</i> A. Gray	Ruee 43716, US
<i>Rumex orbiculatus</i> A. Gray	D.E. Atha et al. 8883/2010, NY
<i>Rumex pallidus</i> Bigelow	D.E. Atha 13922, NY
<i>Rumex palustris</i> Sm.	J.M. Burke 306, HUDC
<i>Rumex papilio</i> Coss. & Balansa,	S.L. Jury 13659, K
<i>Rumex papilio</i> Coss. & Balansa	J.M. Burke 303, HUDC
<i>Rumex patientia</i> L.	D.E. Atha 10674, NY
<i>Rumex paucifolius</i> Nutt.	Barrett 17RpCOT3.2
<i>Rumex paucifolius</i> Nutt.	Barrett 17RpCMC15.2
<i>Rumex peruanus</i> Rech. f.	V. Quipuscoa 1349, NY
<i>Rumex pictus</i> Forssk.	Barrett 17Rp.AR1
<i>Rumex pulcher</i> L.	J.M. Burke 294, HUDC
<i>Rumex pulcher</i> L.	J.M. Burke 295, HUDC
<i>Rumex pulcher</i> L.	J.M. Burke 296, HUDC
<i>Rumex rothschildianus</i> Aarons. ex Evenari	Barrett 17Rrs3.2
<i>Rumex sagittatus</i> Thunb.	Strobach B55575, US
<i>Rumex sagittatus</i> Thunb.	H.J. Venter 9995, NY
<i>Rumex salicifolius</i> Weinm.	W. Wood s.n., OSC

Scientific name	Voucher
<i>Rumex sanguineus</i> L.	J.M. Burke 316., HUDC
<i>Rumex scutatus</i> L.	Barrett s.n.
<i>Rumex skottsbergii</i> O.Deg. & I.Deg.	Degener 35050, US
<i>Rumex spiralis</i> Small.	D.E. Atha 9727, NY
<i>Rumex stenophyllus</i> Ledeb.	D.E. Atha 11389, NY
<i>Rumex stenophyllus</i> Ledeb.	R.L. McGregor 40643, OSC
<i>Rumex tianschanicus</i> Losinsk.	Barrett SH1-A-2007454
<i>Rumex thyrsoflorus</i> Fingerh.	Ollegard 261, US
<i>Rumex thyrsoflorus</i> Fingerh.	Elias 7282, US
<i>Rumex thyrsoides</i> Desf.	J.M. Burke 305, HUDC
<i>Rumex thyrsoides</i> Desf.	J.M. Burke 313, HUDC
<i>Rumex thyrsoides</i> Desf.	J.M. Burke 307, HUDC
<i>Rumex tuberosus</i> L.	S. Omar et al. 52591, K
<i>Rumex tuberosus</i> subsp. nov	J.M. Burke 308, HUDC
<i>Rumex usambarensis</i> (Dammer) Dammer	Ellemann 889, NY
<i>Rumex venosus</i> Pursh.	R.E. Brainerd 428, OSC
<i>Rumex vesicarius</i> L.	Brummit 15271, US

Supplementary material I

Aligned Data Matrix

Authors: Kirstie D. Grant

Data type: FASTA

Explanation note: Aligned sequence file.

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