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Rhodiola integrifolia: hybrid origin and Asian relatives

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Abstract: We investigated the relationship among North American *Rhodiola integrifolia* Raf., *Rhodiola rosea* L., and *Rhodiola rhodantha* (A. Gray) H. Jacobsen by sequencing a fragment of the nuclear-encoded chloroplast-expressed glutamine synthetase (ncpGS) gene from these three species and from selected Asian representatives of the genus. We found that *R. integrifolia* is a hybrid descendant of *R. rosea* and *R. rhodantha* lineages. We also found that the ncpGS gene and the internal transcribed spacer regions of the nuclear ribosomal DNA of Asian *Rhodiola algida* Fisch. & Mey. are very similar to those of *R. rhodantha*. In accordance with known chromosome numbers and species morphology, we therefore propose to move *R. algida* into the subgenus *Clementsia* of the genus *Rhodiola*, which so far contains only two species, *R. rhodantha* and Asian *Rhodiola semenovii* (Regel & Herder) Boriss.

Key words: Rhodiola integrifolia, *Rhodiola rhodantha*, *Rhodiola rosea*, *Rhodiola algida*, allopolyploidy, glutamine synthetase.

Résumé : Les auteurs ont étudié les relations des espèces nord-américaines *Rhodiola integrifolia* Raf., *Rhodiola rosea* L., et *Rhodiola rhodantha* (A. Gray) H. Jacobsen, en séquençant un fragment du gène de la glutamine synthétase du noyau exprimé dans le chloroplaste (GSncl), chez ces trois espèces et chez représentants asiatiques de ce genre. Ils ont constaté que le *R. integrifolia* constitue un hybride provenant des lignées *R. rosea* et *R. rhodanta*. Ayant également constaté que le gène GSncl et les régions de l'EIT (espaceur interne transcrit) de l'ADN chloroplastique du *Rhodiola algida* Fisch. & Mey. sont très semblables à ceux du *R. rhodantha* et correspondant aux nombres chromosomiques connus ainsi qu'à la morphologie de l'espèce, il proposent donc de placer le *R. algida* dans le sous-genre *Clementsia* du genre *Rhodiola*, lequel jusqu'à maintenant ne contenait que deux espèces, le *R. rhodanta* et le *Rhodiola semenovii* (Regel & Herder) Boriss asiatique.

Mots-clés : Rhodiola integrifolia, *Rhodiola rhodantha*, *Rhodiola rosea*, *Rhodiola algida*, allopolyploïdie, glutamine synthétase.

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Introduction

The genus *Rhodiola* L. (Sedoideae, Crassulaceae), which comprises up to 90 species (Zhengyi and Raven 2001), has its origin and largest diversity in the mountains of central Asia (Mayuzumi and Ohba 2004). *Rhodiola* is usually considered a highly derived group within the Crassulaceae, but its exact position within the family has not been fully resolved (Mayuzumi and Ohba 2004; Gontcharova and Gontcharov 2009). Recent molecular analysis of Asian Crassulaceae revealed that the genus *Rhodiola* is well separated from the *Sedum* species, while it forms a clade together with the small genus *Pseudosedum*, which may have to be reduced to a synonym of *Rhodiola* (Mayuzumi and Ohba 2004; Gontcharova et al. 2006).

The following three *Rhodiola* species occur in North America: *Rhodiola rosea* L., *Rhodiola integrifolia* Raf., and *Rhodiola rhodantha* (A. Gray) H. Jacobsen. *Rhodiola rosea* is the most widespread species of the genus. It grows along the northern coastlines and in alpine habitats of Asia, Europe, and eastern North America from Nunavut to North Carolina. *Rhodiola integrifolia* is found in Siberia and western North America from Alaska to New Mexico with disjunct occurrence in Minnesota and New York state. *Rhodiola rhodantha* is endemic to the Rocky Mountains of the United States (U.S. Department of Agriculture 2011).

Rhodiola integrifolia, first described in 1832 by Rafinesque, is morphologically very similar to *R. rosea* and has repeatedly been treated as a subspecies or variety of *R. rosea* by different authors of the 20th century (The International Plant Name

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Index 2011). *Rhodiola integrifolia* differs from *R. rosea* in usually having dark red petals whereas *R. rosea* usually has yellowish petals. Clausen (1975) also found the petal width of staminate flowers and median leaf size to be significantly larger in *R. integrifolia*. *Rhodiola rhodantha* is clearly different from *R. rosea* and *R. integrifolia* and many other *Rhodiola* species, and had once even been moved into a separate genus *Clementsia* (Rose) (Britton and Rose 1903), now recognized as *Rhodiola* subgen. *Clementsia* (Rose) H. Ohba.

Cytogenetic studies (for example: Uhl 1952) of the North American *Rhodiola* species revealed haploid chromosome numbers of $n = 7$ for *R. rhodantha*, $n = 11$ for *R. rosea*, and $n = 18$ for *R. integrifolia* (referred to as the 11-chromosome strain and the 18-chromosome strain of *Sedum rosea* (L.) Scop. by Uhl). Uhl (1952) proposed the following two hypotheses for the origin of the 18-chromosome strain: (1) as a hexaploid of an ancestral *Rhodiola* species with a haploid chromosome number of $n = 6$; or (2) as an allopolyploid of the 11-chromosome strain and some other parent lineage with a haploid chromosome number of $n = 7$, like *R. rhodantha*.

We tested Uhl's hypotheses by sequencing a fragment of the nuclear-encoded chloroplast-expressed glutamine synthetase (ncpGS) gene from the 3 North American *Rhodiola* species and 50 Asian relatives of the genus (unpublished data), including *Rhodiola algida* Fisch. & Mey. and *Rhodiola semenovii* (Regel & Herder) Boriss. The ncpGS gene is a single-copy gene in most diploids and has been successfully used to identify the parent lineages of allotetraploid species in several studies (e.g., Emshwiller and Doyle 1999; Clarkson et al. 2010).

Several species of *Rhodiola* have been used in traditional medicine. *Rhodiola rosea* has, by far, the most extensive ethnobotanical record, and its rhizomes are currently marketed as adaptogenic supplements to strengthen mental and physical performance in stress situations (Brown et al. 2002; Schittko 2004; Vastag 2007). Within North America, usage of *Rhodiola* species has been reported mainly from the Bering Sea coastal areas. Literature records exist for both *R. rosea* and *R. integrifolia* (Moerman 2003). It is not clear whether this is due to the historical uncertainty of *R. integrifolia*'s taxonomic independence or if *R. rosea* did extend from Asia into the American continent. One yellow-flowered *R. rosea* like specimen with narrow petals is preserved from Teller, Port Clarence (E. Scammon 5529, GH) (Moran 2000) and was sequenced in this study among other specimens from the Bering Sea area to get a better idea of the extent of distribution of these two *Rhodiola* species in the Bering Sea area.

Materials and methods

DNA was extracted from individual plants using the Nucleospin Plant Kit (Macherey-Nagel, Düren, Germany) followed by chloroform extraction. To amplify a stretch of 372–382 bp of the ncpGS gene (glutamate-ammonia ligase, E.C. 6.3.1.2) using a polymerase chain reaction (PCR), we designed the following primer pair: GGTGATTGGAATGGTGC (GSF) and GCCTTGTTTCTCAGTATCG (GSR). The amplicon corresponds to a region extending from exon 8 to exon 9 of the *Arabidopsis thaliana* ncpGS gene, but contains an additional intron in *Rhodiola* interrupting exon 8 of *A. thaliana*. The internal transcribed spacer (ITS) region (599 bp) was amplified with ITS4 and ITS5 (White et al. 1990). PCR reactions were kept at 95 °C for 4 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 51 °C, and 30 s at 72 °C, followed by 7 min at 72 °C. PCR products were either directly submitted for commercial sequencing (Iowa State University, DNA Facility, Ames, Iowa, USA) or cloned using the TOPO TA cloning kit (Life Technologies, Grand Island, N.Y., USA) with subsequent sequencing of the plasmid DNA from isolated colonies. Cloning was necessary for the *R. integrifolia* ncpGS sequence only, but it was also used to replicate the ncpGS data obtained for the parental species and whenever better quality ITS sequences were needed to identify different herbarium specimens from the Bering Sea area. Sequences were edited and aligned in BioEdit (Hall 1999). Table 1 lists all the samples included in the study. Following the current, although controversial (Moran 2000), infraspecific classification of *R. integrifolia*, all samples from the Bering Sea area belong to *R. integrifolia* subspecies *integrifolia*, whereas the samples from the Rocky Mountains were *R. integrifolia* subspecies *procera*.

Results

Glutamine synthetase clones of *R. integrifolia* were found to be either identical to clones of *R. rosea* or *R. rhodantha* or recombinant sequences of these two parent lineages (Fig. 1) from different subgenera of *Rhodiola* (3.7% ITS and 5.3% ncpGS sequence divergence between *R. rosea* and *R. rhodantha*, counting indels as one position). Some clones also showed individual nucleotides unique to *R. integrifolia*. All other sequences of the genus *Rhodiola* (unpublished data) were different from the three American species.

Although subgeneric treatments of the genus have only placed *R. semenovii* together with *R. rhodantha*, we discovered that the sequences of *R. rhodantha* and *R. algida* share 99.0% (ITS) and 98.4% (ncpGS) identical positions, whereas each of these species has less sequence similarity when compared with *R. semenovii* (*R. rhodantha*: 97.4% ITS and 97.8% ncpGS sequence identity; *R. algida*: 96.8% ITS and 97.8% ncpGS sequence identity, counting indels as one position).

Herbarium specimens from Alaska and the Bering Sea were all found to be *R. integrifolia*, including the yellow-flowered specimen collected by Scamman in 1949. One of the Chukotsk specimens (ALAAC V115350) was found to be *R. rosea*.

Discussion

Our data are in agreement with the hypothesis by Uhl (1952) that *R. integrifolia* is an allopolyploid species derived from hybridization of *R. rhodantha* and *R. rosea* lineages. Both parental alleles of the ncpGS gene are maintained in *R. integrifolia*. In addition, sequencing revealed a number of recombinant ncpGS clones. Multiple sequences from individual plants have been recovered in some previous studies using the ncpGS gene (Emshwiller and Doyle 1999; Ionta et al. 2007), but not in others (Clarkson et al. 2010), and it is not known to what extent these recombinants represent artifacts caused by PCR recombination (Cronn et al. 2002). It is also possible that some variation in our template DNA is due to endopolyploidy, which is common in succulent plants, including the Crassulaceae (De Rocher et al. 1990; Barow 2006).

Whereas both parent alleles of the ncpGS gene are maintained in *R. integrifolia*, concerted evolution seems to have

Table 1. Sample information.

Rhodiola species	Material and voucher	Locality	GenBank accession numbers	Sequence obtained from
R. algida	Fresh leaves from plant in cultivation JE Hermsmeier 1	Seeds collected by W. Peschel in Altai, Russia (49°31′N, 88°01′E)	ITS: JQ228604 ncpGS: JQ228607	PCR product PCR product
R. integrifolia	Herbarium sample ALAAC 81656	Seward Peninsula, Alaska	ITS: JQ228592	1 colony
R. integrifolia	Herbarium sample ALAAC V125521	Seward Peninsula, Alaska	ITS: JQ228594	2 colonies
R. integrifolia	Herbarium sample ALAAC V135051	Seward Peninsula, Alaska	ITS: JQ228595	2 colonies
R. integrifolia	Herbarium sample ALAAC 92435	Seward Peninsula, Alaska	ITS: JQ228596	PCR product
R. integrifolia	Herbarium sample ALAAC 6623	St. Matthew Island, Alaska	ITS: JQ228598	2 colonies
R. integrifolia	Herbarium sample ALAAC 92615	St. Lawrence Island, Alaska	ITS: JQ228599	PCR product
R. integrifolia	Herbarium sample ALAAC V113690	Chukotka, Russia	ITS: JQ228600	PCR product
R. integrifolia	Herbarium sample GH E. Scamman 5529	Seward Peninsula, Alaska	ITS: JQ228601	4 colonies
R. integrifolia	Herbarium sample UBC V175863	Richards Island, Northwest Territories	ITS: JQ228602	1 colony
R. integrifolia	Fresh leaves from plant in cultivation JE Hermsmeier 3	Seeds collected by U. Hermsmeier at Williams Lake, New Mexico	ITS: JX102569 ncpGS clones 1a–f: JQ228610- JQ228615	PCR product 6 colonies
R. integrifolia	Fresh leaves from plant in cultivation	Seeds collected by R. Day-Skowron, Rocky Mountain Rare Plants, in Clear Creek County, Colorado	ITS: JX102568 ncpGS clones 2a–l: JQ228616- JQ228627	PCR product 12 colonies
*R. rhodantha**	Fresh leaves from plant in cultivation JE Hermsmeier 2	Seeds obtained from Jelitto Perennial Seeds (Schwarmstedt, Germany)	ITS: JQ228605 ncpGS: JQ228608	PCR product PCR product, 2 colonies
R. rosea†	Fresh leaves from plant in cultivation	Seeds collected by W. Peschel in Altai, Russia (51°03′N, 85°41′E)	ITS: JX102567 ncpGS: JQ228609	PCR product PCR product
R. rosea	Herbarium sample ALAAC V115350	Chukotka, Russia	ITS: JQ228597	PCR product
R. semenovii	Herbarium sample JE Janßen and Martins 2205	Talas-Ala-Too mountains, Kyrgystan	ITS: JQ228603 ncpGS: JQ228606	PCR product PCR product

Note: ITS, internal transcribed spacer; PCR, polymerase chain reaction.

*Three more nuclear-encoded chloroplast-expressed glutamine synthetase (ncpGS) colonies obtained from DNA of a plant from Island Lake, Wyoming, were sequenced to replicate the analysis. All three sequences were identical to JQ228608.

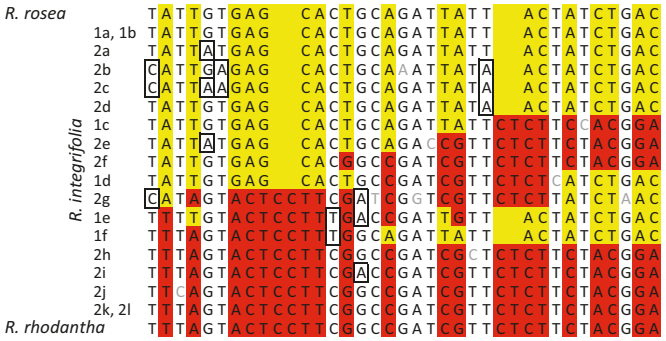
†Identical ncpGS sequences were obtained from replicate samples from France, the Faroe Islands, Norway (all PCR products), and Newfoundland (one colony sequenced).

‡Voucher is from the parent plant from Williams Lake, New Mexico.

operated to maintain only one ITS parental lineage. Only 5–7 of 599 bp were different between *R. integrifolia* and *R. rhodantha*, whereas 25–27 of 599 bp differed between *R. integrifolia* and *R. rosea*. Phylogenetic trees based on ITS sequences support *R. integrifolia* to be a sister taxon of *R. rhodantha*, but not of *R. rosea* (Guest 2009). On the other hand, morphological studies have always placed *R. integrifolia* close to *R. rosea*, or combined them in one taxon (The International Plant Name Index 2011). A hybrid origin of *R. integrifolia* helps to understand these controversies between morphological and molecular data.

Based on extensive morphological studies, the genus *Rhodiola* L. is currently divided into four subgenera (Ohba 1981*a*, 1981*b*, 1982, 1987). *Rhodiola integrifolia*, *Rhodiola rosea*, and *Rhodiola algida* are in subgenus *Rhodiola* (L.) H. Ohba, which is by far the largest subgenus of the genus. *Rhodiola rhodantha* is in the small subgenus *Clementsia* (Rose) H. Ohba, which otherwise only contains *Rhodiola semenovii* (Regel & Herder) Boriss. from the Tien-Shan and Pamiro-Alai mountains (Komarov and Yuzepchuk 1939). We propose to add *Rhodiola algida* (Ledeb.) Fisch. & Mey., native to the Altai mountains (Komarov and Yuzepchuk 1939), to this subgenus. All three species, *R. rhodantha*, *R. algida*, and *R. semenovii*, have dense inflorescenses with 5-merous perfect flowers with relatively large (8–10 mm) white to pink petals (Komarov and Yuzepchuk 1939; Clausen 1975). A haploid chromosome number of seven has been reported for *R. algida* (Amano et al. 1995), *Rhodiola dumulosa*, and *R. rhodantha*

Fig. 1. Alignment of glutamine synthetase sequences. Only variable positions (37 of 387 sequenced) are shown. Nucleotides specific to *Rhodiola rhodantha* (GenBank accession JQ228608) are shown on a dark grey background; those specific to *Rhodiola rosea* (GenBank accession number JQ228609) are shown on a light grey background. Nucleotides shared by *Rhodiola integrifolia* clones (GenBank accession numbers JQ228610 to JQ228627) but not found in any of the parents are boxed. Nucleotide deviations unique to a single sequence are shown in a grey font.



(Uhl 1952), while all other *Rhodiola* species investigated so far had larger chromosome numbers. *Rhodiola semenovii* has not been analyzed yet. It is interesting that *R. algida* from Asia, like its close relative *R. rhodantha* from North America, may form hybrids with *R. rosea*. Intermediate forms have been observed in the Altai Mountains whenever the two species overlapped (Peschel 2002). Our results concur with Mayuzumi and Ohba (2004), who sequenced 17 *Rhodiola* species (ITS and trnL-F) as part of a study on Asian Sedoideae and found that a revision of the current infrageneric classification of the genus *Rhodiola* is required.

The current distribution of *R. rosea* and *R. rhodantha* does not overlap. One or both parental lineages must have been more widespread at some point in history for hybridization to have taken place. The lineage that led to the American endemic species *R. rhodantha* must have separated from closely related lineages that led to Asian *R. semenovii* and *R. algida* first and, while spreading onto the American continent, must have occurred north of its current species boundaries. Easternmost species boundaries of *R. rosea* in Siberia have been delimited by different authors in different ways. According to Ohba (1981*b*), *R. rosea* does not reach the coastlines but has its easternmost species boundaries in the Kolyma River area in Siberia. According to the Flora Arctica URSS (Busunova et al. 1984), *R. rosea* reaches all the way to the Bering Sea. Hultén (1945) had mapped its eastward extension onto the American continent based on yellow-flowered specimens rarely found in westernmost Alaska. Yellow flowers are typical for *R. rosea* but not *R. integrifolia*, except for a population in New Mexico, which has yellow petals with red only at the apex (Clausen 1975). Our results are in agreement with the Flora Arctica URSS (Busunova et al. 1984) but do not provide evidence for *R. rosea* in Alaska. All specimens sequenced from Alaska were found to be *R. integrifolia*, including the yellow-flowered specimen from Port Clarence. It remains unknown if hybridization has occurred in North America and (or) Siberia.

A hybrid origin of *R. integrifolia* raises questions about its potential as a medicinal plant. We do not know how much of the pharmacological and phytochemical properties of *R. rosea* are maintained in the hybrid offspring. It is clear that native people have used *Rhodiola* plants on both sides of the Bering Sea (Brown et al. 2002; Moerman 2003) even if species were not always consistently identified in the ethnobotanical literature due to the uncertain taxonomic history of *R. integrifolia*. In particular, some records from Alaska (Moerman 2003) may mistakenly refer to *R. rosea* meaning *R. integrifolia*.

Rhodiola integrifolia is described to be a variable species by Hultén (1945) and Clausen (1975) and is considered a collective species in the Flora USSR (Komarov and Yuzepchuk 1939). Most recently, Olfelt and Handzic (2011) proposed species status for one of the four recognized subspecies of *R. integrifolia* on the American continent. Hybridization between *R. integrifolia* and *R. rhodantha* has been documented by Guest (2009) in the Rocky Mountains of Colorado. Intermediate forms between *R. rosea* and *R. integrifolia* (Hultén 1945), treated as *Rhodiola borealis* by Komarov and Yuzepchuk (1939), have been reported from Siberia where these two species overlap (Busunova et al. 1984). Clearly, more research is needed on the taxonomy and ethnobotany of *R. integrifolia*.

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References

Amano, M., Wakabayashi, M., and Ohba, H. 1995. Cytotaxonomical studies of Siberian Sedoideae (Crassulaceae) I. Chromosomes of *Rhodiola* in the Altai Mountains. J. Jpn. Bot. **70**: 334–338.

Barow, M. 2006. Endopolyploidy in seed plants. Bioessays, **28**(3): 271–281. doi:10.1002/bies.20371. PMID:16479577.

Britton, N.L., and Rose J.N. 1903. New or noteworthy North American Crassulaceae. Bull. New York Bot. Gard. **3**(9): 1–45.

Brown, R.P., Gerbard, P.L., and Ramazanov, Z. 2002. *Rhodiola rosea*: a phytomedicinal overview. Herbalgram, **56**: 40–52. Available from http://rhodiola.ph/pressrelease/pdf/HerbGram.pdf [accessed 2 September 2011].

Busunova, I.O., Gladkova, V.N., Saikonnikova, T.I., Petrovsky, V.V., Rebristaja, O.V., Taraskina, N.N., Tichomirov, V.N., and Jurtzev, B.A. 1984. Droseraceae-Rosaceae. *In* Flora Arctica URSS, **9**(1). *Edited by* B.A. Jurtzev. Izdat. Akad. Nauk, Leningrad, Russia. pp. 10–15.

Clarkson, J.J., Kelly, L.J., Leitch, A.R., Knapp, S., and Chase, M.W. 2010. Nuclear glutamine synthetase evolution in *Nicotiana*: phylogenetics and the origins of allotetraploid and homoploid (diploid) hybrids. Mol. Phylogenet. Evol. **55**(1): 99–112. doi:10.1016/j.ympev.2009.10.003. PMID:19818862.

Clausen, R.T. 1975. *Sedum* of North America north of the Mexican Plateau. Cornell University Press, Ithaca, New York.

Cronn, R., Cedroni, M., Haselkorn, T., Grover, C., and Wendel, J.F. 2002. PCR-mediated recombination in amplification products derived from polyploidy cotton. Theor. Appl. Genet. **104**: 482–489. Available from http://www.eeob.iastate.edu/faculty/WendelJ/pdfs/TAG2002-PCRrecombination.pdf [accessed 2 September 2011].

De Rocher, E.J., Harkins, K.R., Galbraith, D.W., and Bohnert, H.J. 1990. Developmentally regulated systemic endopolyploidy in suc-

culents with small genomes. Science, **250**(4977): 99–101. doi: 10.1126/science.250.4977.99. PMID:17808240.

Emshwiller, E., and Doyle, J.J. 1999. Chloroplast-expressed glutamine synthetase (ncpGS): Potential utility for phylogenetic studies with an example from *Oxalis* (Oxalidaceae). Mol. Phylogenet. Evol. **12**(3): 310–319. doi:10.1006/mpev.1999.0613. PMID: 10413625

Gontcharova, S.B., and Gontcharov, A.A. 2009. Molecular phylogeny and systematic of flowering plants of the family Crassulaceae DC. Mol. Biol. **43**(5): 794–803. doi:10.1134/S0026893309050112.

Gontcharova, S.B., Artyukova, E.V., and Gontcharov, A.A. 2006. Phylogenetic relationships among members of the subfamily Sedoideae (Crassulaceae) inferred from the ITS region sequences of nuclear rDNA. Russ. J. Genet. **42**(6): 654–661. doi:10.1134/S102279540606010X.

Guest, H. 2009. Systematic and phylogeographic implications of molecular variation in the western North American roseroot, *Rhodiola integrifolia* (Crassulaceae). [Online.] M.Sc. thesis, Department of Biology, University of Victoria, Victoria, B.C. Available from https://dspace.library.uvic.ca:8443//handle/1828/2812 [accessed 2 September 2011].

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. **41**: 95–98.

Hultén, E. 1945. Flora of Alaska and Yukon. V. Gleerup, Lund, Sweden. pp. 895–898.

Ionta, G.M., Judd, W.S., Williams, N.H., and Whitten, W.M. 2007. Phylogenetic relationships in *Rhexia* (Melastomataceae): evidence from DNA sequence data and morphology. Int. J. Plant Sci. **168**(7): 1055–1066. doi:10.1086/518837.

Komarov, V.L., and Yuzepchuk, S.V. 1939. Flora of the U.S.S.R. Vol. 9. Rosales and Sarraceniales. Izdatel'stvo Akademii Nauk SSSR, Moskau-Leningrad. pp. 20–36.

Mayuzumi, S., and Ohba, H. 2004. The phylogenetic position of eastern Asian Sedoideae (Crassulaceae) inferred from chloroplast and nuclear DNA sequences. Syst. Bot. **29**(3): 587–598. doi: 10.1600/0363644041744329.

Moerman, D. 2003. Native American ethnobotany. A database of foods, drugs, dyes and fibers of Native American peoples, derived from plants. Available from http://herb.umd.umich.edu [accessed 18 November 2011].

Moran, R. 2000. *Rhodiola integrifolia* – a rambling account, with two new combinations. Cactus Succul. J. **72**: 137–139.

Ohba, H. 1981*a*. A revision of the Asiatic species of Sedoideae (Crassulaceae). Part 1. *Rosularia* and *Rhodiola* (subgen. *Primuloides* and *Crassipedes*). J. Fac. Sci. Univ. Tokyo, Sect. III, **12**: 337–405.

Ohba, H. 1981*b*. A revision of the Asiatic species of Sedoideae (Crassulaceae). Part 2. *Rhodiola* (subgen. *Rhodiola* sect. Rhodiola). J. Fac. Sci. Univ. Tokyo, Sect. III, **13**: 65–119.

Ohba, H. 1982. A revision of the Asiatic species of Sedoideae (Crassulaceae). Part 3. *Rhodiola* (subgen. *Rhodiola* sect. Pseudorhodiola, Prainia & Chamaerhodiola). J. Fac. Sci. Univ. Tokyo, Sect. III, **13**: 121–174.

Ohba, H. 1987. Biogeography of the genus *Rhodiola*. Acta Phytotax. Geobot. **38**: 211–223.

Olfelt, J., and Handzic, N. 2011. A new species of North American *Rhodiola* (Crassulaceae). [Online.] Botany, **2011**. Available from http://www.botanyconference.org/engine/search/index.php?func=detail&aid=522 [accessed 18 November 2011].

Peschel, W. 2002. Prüfung einer Kollektion verschiedener Herkünfte von *Rhodiola rosea* L. aus einer weltweiten Sammlung stammend bezüglich der Eignung für eine spätere Arzneimittelentwicklung. FNR (Agency for Renewable Resources), Gülzow-Prüzen, Germany.

Schittko, U. 2004. Plant portrait: Roseroot (*Rhodiola rosea*). Journal of Medicinal and Spice Plants, **9**(1): 42–43.

The International Plant Name Index. 2011. [Online.] Available from http://www.ipni.org [accessed 2 September 2011].

Uhl, C.H. 1952. Heteroploidy in *Sedum rosea* (L.) Scop. Evolution, **6**(1): 81–86. doi:10.2307/2405505.

U.S. Department of Agriculture. 2011. The PLANTS database. [Online.] U.S. Department of Agriculture, Natural Resources Conservation Service, National Plant Data Team, Greensboro, NC 27401-4901, USA. Available from http://plants.usda.gov/java/ [accessed 2 September 2011].

Vastag, B. 2007. Warming to a Cold War herb: Soviet secret finds its way west. Sci. News, **172**(12): 184–189. doi:10.1002/scin.2007.5591721209.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols: a guide to methods and applications. *Edited by* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White. Academic Press, San Diego, California. pp. 315–322.

Zhengyi, W., and Raven, P.H. 2001. Flora of China. Vol. 8. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis. pp. 251–268.