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A Phylogenetic Analysis of Western North American *Draba* (Brassicaceae) Based on Nuclear Ribosomal DNA Sequences from the ITS Region

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ABSTRACT. To clarify relationships among western North American members of the genus *Draba*, we produced a molecular phylogeny using nucleotide sequences from both internal transcribed spacers of nuclear ribosomal DNA and the 5.8S rRNA gene (collectively, ITS). Sequence data from 17 *Draba* taxa and two outgroups were subjected to both parsimony and maximum likelihood analyses. The phylogenetic results support previously proposed informal groupings for the sampled species. Western North American *Draba* are divided into two well-supported clades: 1) all taxa with a chromosome number based on $x = 8$, and 2) taxa whose chromosome base numbers appear to be aneuploid, deviating from x = 8. The resulting phylogenetic framework also reveals the taxonomic limitations of flower color, chromosome base number, and growth habit for predicting relationships within the genus.

Species of *Draba* L., the largest genus of the Brassicaceae with roughly 350 described taxa worldwide (Al-Shehbaz 1987), occupy a variety of habitats and occur on every continent except Australia and Antarctica. They are morphologically diverse, and the genus shares numerous physical features with other genera in the family. Despite the elusive nature of characters unique to *Draba*, the majority of species have relatively short fruits (silicles) that are compressed parallel to the septum; are low-growing perennial herbs; have leaves arranged in a basal rosette, with flowering stems that are scapose or leafy; are either white or yellow flowered (Rollins 1993). The plants exhibit a diversity of trichomes, including simple, bifurcate, cruciform, stellate, dendritic, and pectinate. These may occur singly or in combination, often with different trichome types on different parts of the plant, allowing the recognition of many taxa based on trichome features alone (Mulligan 1976). The hypothesized base chromosome number for the genus is $x = 8$ (Koch and Al-Shehbaz 2002).

Nearly one third of all drabas occur on the North American continent, where Rollins (1993) recognized 104 species. Most of these occupy mountainous regions of the western United States, which rivals any area of the world with respect to diversity and endemism in *Draba* (Windham 2000). This diversity makes the region an excellent candidate for studies of speciation and character evolution in the genus. A complete phylogeny is not yet available for *Draba*, hindering attempts to understand the evolution of the genus. In the absence of such information, existing sectional classifications of the genus provide a tentative hypothesis of species relationships.

The most comprehensive infrageneric classification of *Draba* worldwide is that of Schulz (1927), who divided it into 17 sections. Attempts to apply Schulz's sectional treatment to the New World drabas have been fraught with difficulty, and North American authors have characterized his sections as ''poorly drawn,'' ''ill defined,'' and ''confusing'' (Fernald 1934; Hitchcock 1941; Mulligan 1976; Al-Shehbaz 1987; Koch and Al-Shehbaz 2002). As such, Schulz's treatment is of little use in determining the relationships of North American species.

A more recent classification with greater applicability to North American *Draba* was proposed by Mulligan (1976), based on his extensive studies of Alaskan and Canadian species. Using a combination of chromosome number, flower color, breeding system, and hybridization studies, Mulligan (1976) split the high-latitude native North American drabas into three groups. One of these comprised all white-flowered taxa with euploid ($x = 8$) chromosome numbers and partial interfertility. These were clearly related to Eurasian boreal species assigned by Schulz (1927) to section *Leucodraba* DC., and Mulligan (1976) tentatively placed them in that section. In addition, he proposed two informal species assemblages. All yellow-flowered species with euploid ($x = 8$) chromosome numbers were assigned to one group (hereafter designated MG1) and all yellow-flowered aneuploids ($x = 9$, 10, 12, 14, 15, etc.) were placed in a second group (MG2). Sterile artificial hybrids sometimes were produced within these groups but not between them, suggesting that they might constitute phylogenetic units. Mulligan's (1976) informal classification of Alaskan and Canadian *Draba* may be an improvement over the ill-defined sections of Schulz (1927), but its general application is restricted by a paucity of chromosome counts.

Koch and Al-Shehbaz (2002) found little support for the sections of Schulz (1927) when they sequenced the internal transcribed spacers (ITS) of nuclear ribosomal DNA in combination with the chloroplastid *trn*L-*trn*F intergenic spacer and *trn*L intron from a large sample

TABLE 1. Species of *Draba* and outgroups sampled in the phylogenetic analysis with GenBank numbers for the sequence of ITS 1, ITS2, and the 5.8S subunit. Collection localities from Canadian Provinces are: AL = Alberta, NT = Northern Territory, NWT = Northwest Territory. Herbaria housing voucher specimens follow Holmgren et al. (1990).

Draba albertina Greene: UT, USA, *Windham & Rickart 95-185* (UT), AY047661. ***D. asprella*** Greene var. ***stelligera*** O.E. Schulz: AZ, USA, *Windham et al. 98-002* (UT), AY047662. ***D. breweri*** var. ***cana*** (Rydb.) Rollins: AL, Canada, *Mulligan & Crompton 3261* (DAO), AY047665. ***D. bruniifolia*** Stev.: Asia, *Chandjian s.n.* (UT), AY047664. ***D. crassifolia*** Graham: UT, USA, *Windham & Stone 95-203* (UT), AY047666. ***D. glabella*** Pursh: NT, Canada, *Cody 15818* (DAO), AY047668. ***D. juniperina*** Dorn: WY, USA, *Windham 96-152* (UT), AY047671. ***D. kassii*** Welsh: UT, USA, *Windham 98-211* (UT), AY047672. ***D. lonchocarpa*** var. ***exigua*** O.E. Schulz: UT, USA, *Windham 95-215* (UT), AY047673. ***D. lonchocarpa*** Rydb. var. ***lonchocarpa***: UT, USA, *Windham & Stone 95-199* (UT), AY047674. ***D. nemorosa*** L. var. ***nemorosa***: AL, Canada, *Mosquin & Mosquin 4685* (DAO), AY047676. ***D. nivalis*** Lilj.: NWT, Canada, *Parmelee & Seaborn 4177* (DAO), AY047677. ***D. oligosperma*** Hook.: AL, Canada, *Mulligan & Crompton 3221* (DAO), AY047678. ***D. sobolifera*** Rydb.: UT, USA, *Windham & Stone 95-201* (UT), AY047681. ***D. spectabilis*** Greene var. ***spectabilis***: UT, USA, *Windham 95-170* (UT), AY047682. ***D. subalpina*** Goodman & C.L. Hitchc.: UT, USA, *Windham 98-129* (UT), AY047683. ***D. ventosa*** A. Gray: AL, Canada, *Mulligan & Mulligan 3489* (DAO), AY047685. ***D. verna*** L. [=*Erophila verna* (L.) A.P. DC.]: UT, USA, (UT), AY047686. ***Drabopsis nuda*** (Belander) Stapf (=*Drabopsis verna* Koch): Iran, AF137577.

Outgroups: ***Arabis alpina*** L.: Europe, AJ232909. ***Aubrieta deltoidea*** (L.) DC.: Europe, AJ232923.

of primarily South American *Draba*. In the ITS phylogeny of Koch and Al-Shehbaz (2002) the majority of South American taxa fell into a single clade. The branches separating taxa within this almost exclusively South American clade were very short, suggesting a radiation in the New World (Koch and Al-Shehbaz 2002).

Our current understanding of relationships in the genus *Draba* is greatly improved since Schulz's (1927) account. However, the relationship of endemic North American species to other drabas is still unclear. Chromosomal data are lacking for almost half the taxa in North America, and two-thirds of *Draba* species worldwide. We generated a molecular phylogeny of western North American *Draba* species using both of the internal transcribed spacers and the 5.8S rRNA gene (collectively ITS), drawing on chromosome vouchers produced by Mulligan's (1970a, 1971a, 1971b, 1972, 1974b, 1975) studies in Canada and by Windham (2000, unpubl. data) in the western United States. The goals of this study were: 1) to assess the monophyly of the species groups proposed by Mulligan (1976) for western North American *Draba* and 2) to examine trends in the evolution of flower color, chromosome base number and growth habit in the sampled taxa.

MATERIALS AND METHODS

A total of 17 out of 104 North American *Draba* species, representing both widespread and endemic taxa, was sampled for this study (Table 1). This small cross-section of North American drabas includes five species from section *Leucodraba*, two species of yellow-flowered euploids (MG1), and eight species representing the yellow-flowered aneuploid group (MG2)(Table 2). Recent work in the western United States (Windham 2000, unpubl. data) has revealed the existence of at least five white-flowered aneuploids that defy classification under Mulligan's (1976) scheme. One of these species, *D. subalpina*, was included in our sample to determine its phylogenetic affinities.

The sample of *Draba* species was further augmented by three primarily Eurasian drabas (Table 1, 2). *Draba bruniifolia*, a taxon endemic to the eastern Mediterranean/Caucasus region was amplified from herbarium material. The widespread species *D. verna*, sometimes placed in the segregate genus *Erophila* DC. [*E. verna* (L.) A. P. DC.], was collected from Utah. ITS sequence data for *Drabopsis verna*, which falls with basal, annual drabas (e.g., *Draba verna* and *D. nemorosa*; Koch and Al-Shehbaz 2002) were obtained from GenBank (Table 1). Recent molecular work (Koch and Al-Shehbaz 2002) places *Draba* in a core group of Arabideae with the genera *Arabis* L. and *Aubrieta* Adans., which were used as outgroups in this study. Sequence data for species in these genera were obtained from GenBank (Table 1).

Seventeen drabas included in this analysis have been the subject of chromosome studies (Table 2). Fourteen of the DNA samples were extracted from the same individuals for which chromosome counts were made (Table 2). Chromosome voucher material sufficient for DNA extraction was not available for *D. lonchcarpa* var. *lonchocarpa* or *D. bruniifolia*, and DNA samples for these taxa were obtained from uncounted populations. Although we cannot be certain as to the chromosome numbers of these samples, there are enough counts available for *D. lonchocarpa* (Table 2) to warrant the assumption that chromosome base number is invariant in this species.

DNA was extracted from 10–20 mg of leaf tissue (mostly from herbarium specimens) using either a CTAB protocol (Hillis et al. 1996) or a rapid preparation (Edwards et al. 1991). Polymerase chain reaction (PCR) protocols included the amplification of the ITS region of all samples with the primers "ITSleu1" (5′-AACCTTATCATTTAG-3′; designed by Lowell Urbatsch) and "ITS4" (5′-CGTATAGTTATTCGCCTCCT-3′; White et al. 1990). Each 20 μL amplification reaction contained 13.7 μL H_20, 2.0 μL 10x buffer (Perkin Elmer), 1.4 μL $MgCl_2$ (5.0 μM), 0.4 μL dNTP's (1.0 μM), 0.12 μL AmpliTaq (Perkin Elmer), 1.0 μL of each primer (5.0 μM) and 0.75 μL of diluted DNA template (10:1 or 20:1), added in that order. PCR amplification began with 2 min 30 sec denaturation at 93°C followed by 35 cycles of denaturation for 20 sec at 95°C, annealing for 30 sec at 47°C, and extension for 1 min at 73°C. The program terminated with a final extension period of 4 min at 73°C. PCR products were purified using a QIAquick PCR purification kit (QIAGEN Inc.). Purified PCR-amplified templates were sequenced directly using dye-terminator chemistry (Perkin Elmer) and analyzed on an ABI 377 automated sequencer (Applied Biosystems).

Double-stranded sequences were assembled automatically using Sequencher 3.0 (Gene Codes Corp.) for the Power Macintosh and aligned by eye. The resulting alignment was edited visually in Sequencher 3.0 to remove ambiguities. Insertions/deletions (indels), all of which appeared in ITS 1, were treated as missing data. This approach retains phylogenetic information from taxa not

TABLE 2. Flower color, ploidy level, and chromosome counts for species of *Draba* and outgroups sampled in the phylogenetic analysis of ITS 1, ITS2, and the 5.8S subunit. Chromosome count citations followed by * are taken from the same population used in the phylogentic analysis of ITS. Chromosome citations (in parentheses after counts): 1 = Heilborn 1927; 2 = Winge 1933; 3 = Heilborn 1941; 4 = Rollins 1953; 5 = Löve & Löve 1956; 6 = Jørgenson et al. 1958; 7 = Löve & Löve 1961; 8 = Packer 1964; 9 = Merxmüller & Buttler 1965; 10 = Zhukova 1965; 11 = Böcher 1966; 12 = Knaben 1966; 13 = Mulligan 1966; 14 = Rollins 1966; 15 = Knaben & Engellskjøn 1967; 16 = Johnson & Packer 1968; 17 = Mulligan & Porsild 1969; 18 = Mulligan 1970a; 19 = Engelskjøn & Knaben 1971; 20 = Mulligan 1971a ; 21 = Mulligan 1971b; 22 = Zhukova & Petrovsky 1971; 23 = Zhukova & Tikhonova 1971; 24 = Mulligan 1972; 25 = Zhukova et al. 1973; 26 = Leute 1974; 27 = Mulligan 1974b; 28 = Mulligan 1975; 29 = Berkutenko & Gurzenkov 1976; 30 = Zhukova & Petrovsky 1977; 31 = Murín & Májorsky 1978; 32 = Dawe & Murray 1980; 33 = Maassoumi 1980; 34 = Zhukova & Petrovsky 1980; 35 = Löve & Löve 1982; 36 = vanLoons & Oudemans 1982; 37 = Petrovsky & Zhukova 1983; 38 = Berkutenko et al. 1984; 39 = Zhukova & Petrovsky 1984; 40 = Strid & Andersson 1985; 41 = Chinnappa & Chmielewski 1987; 42 = Strid 1987; 43 = Zhukova & Petrovsky 1987; 44 = Brochmann et al. 1992; 45 = Brochmann et al.1993; 46 = Windham 2000; 47 = Windham unpubl.

White-flowered euploids (*Leucodraba*): *D. breweri* var. *cana*: $2n = 32$ (20*, 35). *D. glabella*: $n = 32$, 40 (11); $2n = 64$ (18*, 35); $2n$ = ca. 75 (14); $2n = 80$ (18*). *D. lonchocarpa* var. *lonchocarpa*: $2n = 16$ (4, 13, 23, 27, 37, 39); $2n = 32$ (30). *D. lonchocarpa* var. *exigua*: $2n = 16$ (47*). *D. nivalis*: $n = 8$ (1, 12, 15); $2n = 16$ (5, 6, 7, 10, 16, 17, 22, 23, 27*, 28, 30, 34, 35, 38, 39, 41, 45, 46)

Yellow-flowered euploids (MG1): *D. nemorosa* var. *nemorosa*: $n = 8$ (26); $2n = 16$ (8, 9, 13, 25, 27, 28*, 31, 35, 38, 43). *D. oligosperma*: $2n = 32$ (41); $2n$ = ca. 60 (14); $2n = 64$ (24*)

Yellow-flowered aneuploids (MG2): *D. albertina*: $n = 12$ (28); $2n = 24$ (46*). *D. asprella* var. *stelligera*: $2n = 30$ (46*). *D. crassifolia*: $n = 20$ (3, 13, 19, 28, 45); $2n = 20$ (44); $2n = 40$ (13, 28, 37, 45). *D. juniperina*: $2n = 22$ (46*). *D. kassii*: $2n = 22$ (46*). *D. sobolifera*: $2n = 26$ (46*). *D. spectabilis* var. *spectabilis*: $n = 10$ (13); $2n = 20$ (46*). *D. ventosa*: $2n = 36$ (46*)

White-flowered aneuploids: *D. subalpina*: $2n = 26$ (46*)

Primarily Eurasian: *D. bruniifolia*: $2n = 32$ (42). *Draba verna*: $n = 7$, 12, 15, 16, 17, 18, 20, 26, 27, 29, 32 (2). *Drabopsis nuda*: $n = 8$ (33)

Outgroups: *Arabis alpina*: $2n = 16$ (40). *Aubrieta deltoidea*: $2n = 16$ (36)

missing data at the indel. The aligned sequences were analyzed using parsimony and maximum likelihood algorithms in PAUP* 4.0b10 (Swofford 1998).

Most parsimonious (MP) trees were generated using the heuristic search algorithm in PAUP*. This search employed tree bisection-reconnection (TBR) branch swapping, a default transition/transversion ratio of 2:1, with 1000 random additions of the sampled taxa. One thousand bootstrap replicates were generated in PAUP* using a full heuristic search to test the stability of particular nodes in the parsimony analysis. The data set is available on TreeBASE (study accession number = S901; matrix accession number = M1481).

We used Modeltest 3.06 (Posada and Crandall 1998) to evaluate the likelihood of 56 different models of sequence evolution and identify the least complex model of sequence evolution favored by the data. The likelihood ratio test option in Modeltest 3.06 was employed to compare likelihood scores in a nested design. We used the most likely model of evolution from Modeltest 3.06 as settings in a maximum likelihood (ML) reconstruction of phylogeny in PAUP*. The maximum likelihood heuristic search included 10 random additions of the data swapped to completion with TBR branch swapping.

RESULTS

Of the 624 aligned characters in the data matrix, 480 were constant, 80 were variable but not parsimony informative, and 64 were potentially parsimony informative. The ITS 1 region exhibited the most variation (93 variable sites of 271 total characters, 34%), followed by ITS 2, which contained 46 variable sites in 197 characters (23%). Only three percent (5 characters) of the 156 sites in the 5.8S gene showed any variation. Two taxa (*D. sobolifera* and *D. subalpina*) yielded identical sequences over the entire length of the alignment. We observed no ambiguous copies of ITS from any of the taxa we sampled. With the exception of two recognized subspecies of *D. lonchocarpa*, we never amplified variable copies of ITS, even when conspecifics were amplified from multiple populations.

The alignment required the introduction of several indels, all but one of which occurred in ITS 1. The longest ITS 1 region (268 bps excluding indels) occurred in *Drabopsis nuda*; the shortest (247 bps) occurred in both *Aubrieta deltoidea* and *Arabis alpina*. These two taxa also exhibited the longest indels in ITS 1 (12bps). The ITS 2 region (excluding indels) was 197 bps long in all sampled taxa except *Aubrieta deltoidea*, *D. verna* and *D. subalpina*, which had sequenced ITS 2 regions of 196 bps.

The parsimony analysis yielded 40 MP trees of 191 steps. The consistency index (CI) excluding uninformative sites was 0.62; the retention index (RI) was 0.75. This set of trees differed within, but not between, well-defined clades in the phylogeny. No additional islands of MP trees were found, consistent with the observation that data sets with an RI greater than 0.70 rarely contain multiple islands of MP trees (Maddison 1991).

The strict consensus of 40 MP trees (Fig. 1), with *Arabis alpina* and *Aubrieta deltoidea* identifed as outgroups, supported a monophyletic *Draba*, including the segregate genera *Drabopsis* and *Erophila* (= *D. verna*) (bootstrap support = 100%). Within this expanded *Draba*, a basal polytomy was formed by the three primarily Eurasian taxa *D. nemorosa*, *D. bruniifolia*, and *Drabopsis nuda* (Fig. 1). *Draba verna* was placed sister to the remaining sampled *Draba* taxa in all most parsimonious trees, although this node had only 51% bootstrap support (Fig. 1). Parsimony bootstrapping indicated three clades that were moderately to strongly supported among the remaining *Draba* sampled from

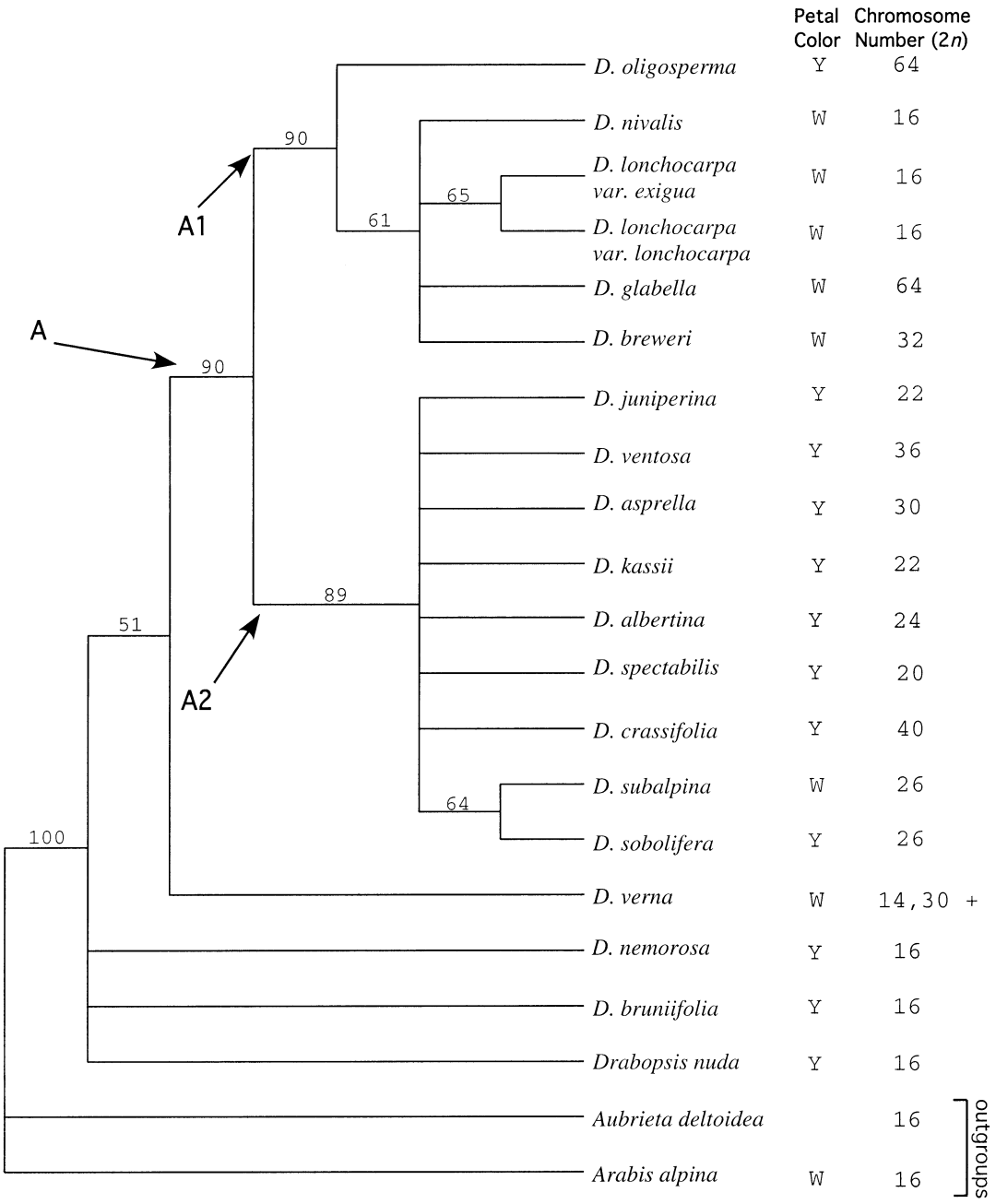

FIG. 1. Strict consensus of 40 most parsimonious trees. Bootstrap values from 1000 replicates appear above branches with >50% bootstrap support. Petal color is indicated by Y (yellow), W (white). Diploid chromosome numbers are given for counted taxa (references provided in Table 2).

North America. Clade A (bootstrap = 90%) included all *Draba* taxa endemic to North America plus *D. nivalis* and *D. crassifolia*, species native to both North America and Eurasia.

Two well-supported clades comprised the primarily North American grouping identifed above. Clade A1 (bootstrap = 90%) consisted of *D. oligosperma* (a member of MG1) positioned sister to a weakly supported (bootstrap = 61%) clade containing all species of section *Leucodraba*. Clade A2 (bootstrap = 89%) included all aneuploid taxa, encompassing the entirety of MG2, plus the only white-flowered aneuploid (*D. subalpina*). Within each of these clades, insufficient sequence variation resulted in extensive polytomies.

The simplest maximum likelihood model identified for this study assumes equal base frequencies, six substitution categories, and gamma distributed rate heterogeneity partitioned into four rate categories (i.e., general time reversible model, GTR+G; Heulsenbeck and Crandall 1997; Posada and Crandall 1998). No other, more complex model performed significantly better in a test of likelihood ratios. The resulting ML tree (Fig. 2) was perfectly congruent with the strict consensus MP tree (Fig. 1). *Draba verna* was placed sister to *Draba* Clade A, despite the fact that it is on one of the longest branches in the analysis (Fig. 2). In the ML tree, *Draba nemorosa*, *D. bruniifolia* and *Drabopsis nuda* (all with long branches) form a clade sister to the North American drabas. All of the well-supported clades (A, A1, A2) recovered in the MP analysis were also present in the ML tree, as well as a monophyletic section *Leucodraba*.

Discussion

Our study of ITS sequences in *Draba* has implications for the informal classification proposed by Mulligan (1976). The two ITS-based western North American subclades (A1 and A2) are consistent cytologically. Clade A1 contains only euploid ($x = 8$) taxa, whereas the members of Clade A2 are strictly aneuploid. These findings validate Mulligan's (1976) novel use of chromosome base number as an indicator of species relationships among North American taxa. One of Mulligan's species groups (section *Leucodraba*) appears to be monophyletic based on current sampling. Another group (the yellow-flowered aneuploids; MG2) is easily rendered monophyletic by the inclusion of white-flowered aneuploids (e.g., *D. subalpina*) unknown to Mulligan. The remaining species group (the yellow-flowered euploids; MG1) is not monophyletic. One of the assigned taxa, the strictly North American *D. oligosperma*, is sister to section *Leucodraba* in all trees, separated from the only other MG1 taxon sampled (*D. nemorosa*) by two well-supported nodes. The latter species regularly groups with Eurasian taxa, either as part of a polytomy, or as a member of a basal, primarily Eurasian clade. The observed lack of phylogenetic cohesion in MG1 is not surprising because both yellow flowers and euploid chromosome numbers have been considered ancestral in the genus (Mulligan 1976; Koch and Al-Shehbaz 2002).

Anueploidy appears to have originated twice in our phylogenetic tree, once in *D. verna* and again in the western North American Clade A2. *Draba verna*, which is sister to Clade A, is chromosomally diverse. Thirteen different numbers have been reported for the species, ranging from $2n = 14$ and 30 (the most common cytotypes) through $2n = 32$ (Winge 1933). This is quite different from the situation observed in Clade A2, where each morphologically defined taxon displays a single chromosome number (Mulligan 1976; Windham 2000). The value of aneuploidy for predicting relationships in this group is substantial, with *D. juniperina* and *D. oligosperma* providing a case in point. Rollins (1953) initially described white-flowered populations of *D. oligosperma* in Wyoming as the segregate species *D. pectinipila*. Dorn (1978) split *D. pectinipila* on the basis of flower color, habitat and geography, creating *D. juniperina*, a yellow-flowerd taxon restricted to the northeastern edge of Utah. Rollins (1993) decided there was little to be gained by splitting *D. oligosperma*, an apomictic euploid ($2n = 32, 64$), into three independent taxa and decided to synonymize *D. juniperina* and *D. pectinipila* with *D. oligosperma* based partly on the shared occurrence of doubly pectinate trichomes. *Draba juniperina* is a sexually reproducing aneuploid with a chromosome number of $2n = 22$ and should be maintained as a distinct species (Windham 2000). ITS data also provide support for this position, placing *D. oligosperma* in Clade A1 (all euploid) and *D. juniperina* in Clade A2 (all aneuploid). Futhermore, *D. juniperina* is sister to *D. kassii* in the ML reconstruction, the only other *Draba* species (worldwide) reported to have $2n = 22$.

There are very few records of aneuploid chromosome numbers among non-North American *Draba*. These include: *Draba crassifolia* Graham ($2n = 40$) distributed in Asia and North America, *D. olgae* Regel & Schmalh. ($2n = 12$; Zakharyera and Astanova 1968) and *D. hispida* Willd. ($2n = 14$; Sokolovskaya and Strelkova 1948) from central and southwestern Asia. However, counts for the latter two species represent a reduction in base number without polyploidy (unknown in Clade A2).

Our ITS Clade A2, containing *Draba crassifolia* and other aneuploid taxa from the western United States and Canada, appears to correspond to the monophyletic clade comprised of primarily South American endemic *Draba* in the phylogeny constructed by Koch and Al-Shehbaz (2002). Included in this clade are *D. jorullensis* Humb., Bonpl. & Kunth ($n = 12$; Beaman et al. 1962) from Mexico, *D. pickeringii* A. Gray ($2n = 24$; Fararger and Huynh 1965) from Peru, as well as *D. crassifolia* ($n = 20$) and *D. streptocarpa* A. Gray ($n = 20$; Price 1979) from the western United States. Still other South American species from the clade identified by Koch and Al-Shehbaz (2002) are euploid, such as *D. chionophila* S. F. Blake ($2n = 48$, Galland and Pfitsch 1986) and *D. mogollonica* Greene ($n = 16$, Ward 1983), and therefore indicate that if the majority of North and South American *Draba* fall into a single clade, that this clade is characterized by both euploid and aneuploid base chromosome numbers.

Ongoing chromosome studies (Windham unpubl. data) suggest that Clade A2 may encompass the majority of North American species. Because it includes a

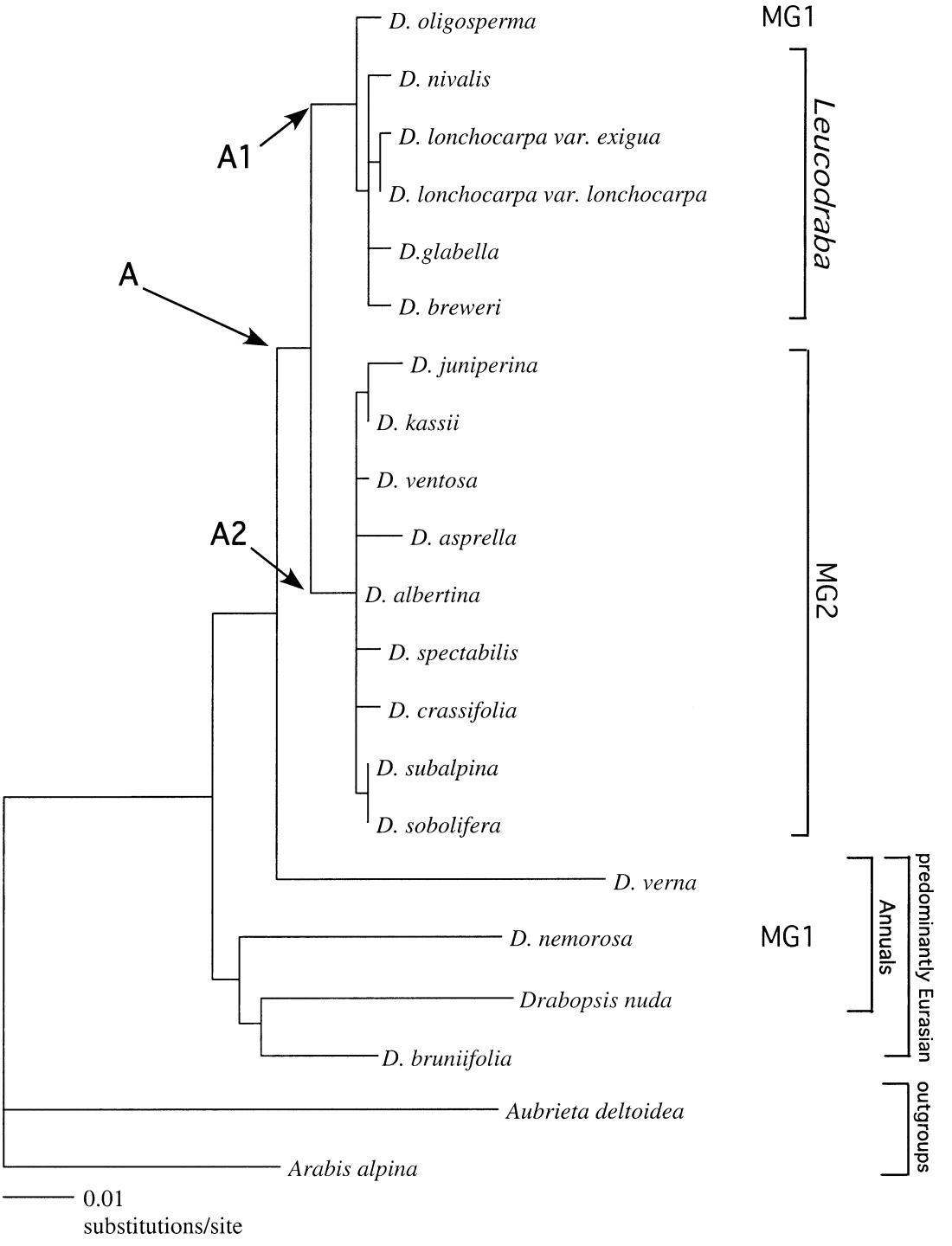

FIG. 2. Maximum likelihood tree of sampled taxa showing rates of evolution under the GTR+G model of sequence evolution. Previously proposed taxonomic groups are indicated to the right of relevant taxa: MG1 (Mulligan's group 1), MG2 (Mulligan's group 2), sect. *Leucodraba* L.

complete series of base numbers spanning the gap between diploids ($x = 8$) and tetraploids ($x = 16$) (Windham 2000), this clade provides a unique opportunity to study aneuploid speciation. Unfortunately, resolution in our ITS trees was insufficient to provide much information on the mechanisms of chromosomal evolution. What is clear, however, is that polyploid hybridization based on eight chromosomes, as described by Widmer and Baltisberger (1999) for *Draba* in the Alps, or recurrent processes typical of Nordic *Draba* (Brochman et al. 1992a,b) are unlikely to be involved in the formation of western North American aneuploid taxa.

The evolution of flower color in *Draba* is difficult to interpret. Conflicting reports regarding this character are not unusual, with *D. nemorosa* providing a good example. Mulligan (1975, 1976) assigned this species to his yellow-flowered euploid group (MG1), but Rollins (1993) described the flowers as ''pale yellow to whitish.'' The evolutionary lability of flower color is illustrated by *D. subalpina* and *D. sobolifera*, both endemic to southern Utah. The two taxa have identical ITS sequences and share an otherwise unique chromosome number of $2n = 26$. Despite their clear relationship, *D. sobolifera* is yellow flowered and *D. subalpina* is clearly and consistently white flowered. Both outgroup taxa, *Arabis alpina* and *Aubrieta deltoidea*, and their close relatives, have white flowers (Koch and Al-Shehbaz 2002), suggesting that this may be the pleisiomorphic character state for the broader clade that includes *Draba*. The white flowers of *D. verna*, not part of this basal white-flowered group, are likely the product of a reversal. Yellow flowers predominate within the western North American Clade A, and it is likely that the white flowers of section *Leucodraba* and *D. subalpina* also represent reversals, although it is possible that the sister groups of these taxa independently evolved yellow flowers. Either scenario indicates that flower color in *Draba* is subject to significant homoplasy, a deduction supported by the phylogeny of Koch and Al-Shehbaz (2002).

Growth habit also appears to be homoplastic in *Draba*. *Draba nemorosa* and *D. nuda*, both annual species, occur basally within *Draba*. The only other annual species sampled, *D. verna*, does not form a clade with these two taxa. Some of the aneuploids in Clade A2 (e.g., *D. albertina* and *D. crassifolia*) have been considered facultative annuals (Rollins 1993). However, Mulligan (1975, 1976), who grew and observed these species, considered them perennial or biennial. Koch and Al-Shehbaz (2002) found that the annual sections *Tomostima* (Raf.) O.E. Schulz and *Abdra* (Greene) O.E. Schulz form a clade sister to all other sampled *Draba* and might constitute a distinct genus.

The resolution provided by ITS in *Draba* is limited and other regions of the genome must be sampled to gain a better understanding of the relationships within some of the clades outlined in this study. Better phylogenetic resolution might be obtained by sequencing a more rapidly evolving DNA segment (such as the external transcribed spacer region; ETS) and analyzing it in conjunction with ITS (e.g., Clevinger and Panero 2000). Our ITS studies suggest that many of the North American species of *Draba* are recently derived, so the genus offers abundant opportunities to study the early stages of evolutionary divergence.

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LITERATURE CITED

AL-SHEHBAZ, I. A. 1987. The genera of Alysseae (Cruciferae; Brassicaceae) in the southeastern United States. Journal of the Arnold Arboretum 68: 185–240.

BEAMAN, J. H., D. C. D. DEJONG, and W. P. STOUTAMIRE. 1962. Chromosome studies in the alpine and subalpine floras of Mexico and Guatemala. American Journal of Botany 49: 41–50.

BERKUTENKO, A. A. and N. N. GURZENKOV. 1976. Chromosome numbers and distribution of Cruciferae in the south of the Magadan Region. I. Botanichesky Zhurnal 61: 1595–1603.

———, S. I. TZYTLENOK, and S. V. PULKINA. 1984. Chromosome numbers and dispersal of the Brassicaceae family in the Magadan district. Botanichesky Zhurnal 69: 75–80.

BÖCHER, T. W. 1966. Experminental and cytological studies on plant species. IX. Some Arctic and montane crucifers. Biologiske Skrifter 14: 1–74.

BROCHMANN, C., D. E. SOLTIS, and P. S. SOLTIS. 1992a. Electrophoretic relationships and phylogeny of Nordic polyploids in *Draba* (Brassicaceae). Plant Systematics and Evolution 182: 35–70.

———, P. S. SOLTIS, and D. E. SOLTIS. 1992b. Recurrent formation and polyphyly of nordic polyploids in *Draba* (Brassicaceae). American Journal of Botany 79: 673–688.

———, L. BORGEN, and B. STEDJE. 1993. Crossing relationships and chromosome numbers of Nordic populations of *Draba* (Brassicaceae), with emphasis on the *D. alpina* complex. Nordic Journal of Botany 13: 121–147.

CHINNAPPA, C. C. and J. G. CHMIELEWSKI. 1987. Documented plant chromosome numbers 1987:1. Miscellaneous counts from western North America. Sida 12: 409–417.

CLEVINGER, J. A. and J. L. PANERO. 2000. Phylogenetic analysis of *Silphium* and subtribe Engelmanniinae (Asteraceae: Heliantheae) based on ITS and ETS sequence data. American Journal of Botany 87: 565–572.

DAWE, J. C. and D. F. MURRAY. 1980. IOPB chromosome number reports LXIX, ed. Á. Löve. Taxon 29: 703–730.

DORN, R. D. 1978. A new species of *Draba* (Cruciferae) from Wyoming and Utah. Madroño 25: 101–103.

EDWARDS, K., C. JOHNSTONE, and C. THOMPSON. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research 19: 1349.

ENGELSKJØN, T. and G. KNABEN. 1971. Chromosome numbers of

Scandinavian arctic–alpine plant species III. Acta Borealia 28: 1–30.

FAVARGER, C. and K. L. HUYNH. 1965. IOPB chromosome number reports IV, eds. Á. Löve and O. T. Solbrig. Taxon 14: 86–92.

FERNALD, M. L. 1934. *Draba* in temperate northeastern North America. Rhodora 36: 241–261, 285–305, 314–344, 353–371, 392–404.

GALLAND, N. and W. A. PFITSCH. 1986. Chromosome number reports XC, ed. Á. Löve. Taxon 35: 195–198.

HEILBORN, O. 1927. Chromosome numbers in *Draba*. Hereditas 9: 59–68.

———. 1941. Some chromosome numbers in *Draba*. Svensk Botanisk Tidskrift 35: 141–142.

HILLIS, D. M., B. K. MABLE, A. LARSON, S. K. DAVIS, and E. A. ZIMMER. 1996. Nucleic Acids IV: sequencing and cloning. Pp. 343–344 in *Molecular systematics,* second edition, eds. D. M. Hillis, C. Moritz, and B. K. Mable. Sunderland: Sinauer, Associates.

HITCHCOCK, C. L. 1941. A revision of the *Drabas* of western North America. University of Washington Publications in Biology 11: 1–132.

HUELSENBECK, J. P. and K. A. CRANDALL. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annual Review of Ecology and Systematics 28: 437–466.

JOHNSON, A. W. and J. G. PACKER. 1968. Chromosome numbers in the flora of Ogotoruk Creek, N.W. Alaska. Botaniska Notiser 121: 403–456.

JØRGENSEN, C. A., T. SØRENSEN, and M. WESTERGAARD. 1958. The flowering plants of Greenland. A taxonomical and cytological survey. Biologiske Skrifter 9: 1–172.

KNABEN, G. 1966. Cytotaxonomical studies in some *Draba* species. Botaniska Notiser 119: 427–444.

——— and T. ENGELSKJØN. 1967. Chromosome numbers of Scandinavian arctic-alpine plant species II. Acta Borealia 21: 1–57.

KOCH, M. and I. A. AL-SHEHBAZ. 2002. Molecular data indicate complex intra- and intercontinental differentiation of American *Draba* (Brassicaceae). Annals of the Missouri Botanical Garden 89: 88–109.

LEUTE, G. H. 1974. IOPB chromosome number reports XLVI, ed. Á. Löve. Taxon 23: 801–812.

LÖVE, Á and D. LÖVE. 1956. Cytotaxonomical conspectus of the Icelandic flora. Acta Horti Gotoburg 20: 65–290.

——— and ———. 1961. Chromosome numbers of central and northwest European plant species. Opera Botanica 5: 1–158.

——— and ———. 1982. IOPB chromosome number reports LXXIV, ed. Á. Löve. Taxon 31: 119–128.

MAASSOUMI, A. A. R. 1980. Cruciferes de la flore d'Iran. Etude Caryosystematique. These, Strasbourg. 1–83.

MADDISON, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. Systematic Zoology 40: 315–328.

MERXMÜLLER, H. and K. P. BUTTLER. 1965. Die Chromosomenzahlen der mitteleuropäishen und alpinen *Draben*. Berichte der Deutschen Botanischen Gesellschaft 77: 411–415.

MULLIGAN, G. A. 1966. Chromosome numbers of the family Cruciferae III. Canadian Journal of Botany 44: 309–319.

———. 1970a. Cytotaxonomic studies of *Draba glabella* and its close allies in Canada and Alaska. Canadian Journal of Botany 48: 1431–1437.

———. 1970b. A new species of *Draba* in the Kananaskis Range of southwestern Alberta. Canadian Journal of Botany 48: 1897–1898.

———. 1971a. Cytotaxonomic studies of the closely allied *Draba cana*, *D. cinerea*, and *D. groenlandica* in Canada and Alaska. Canadian Journal of Botany 49: 89–93.

———. 1971b. Cytotaxonomic studies of *Draba* species of Canada and Alaska: *D. ventosa*, *D. ruaxes*, and *D. paysonii*. Canadian Journal of Botany 49: 1455–1460.

———. 1972. Cytotaxonomic studies of *Draba* species in Canada and Alaska: *D. oligosperma* and *D. incerta*. Canadian Journal of Botany 50: 1763–1766.

———. 1974a. Confusion in the names of three *Draba* species of the arctic: *D. adamsii*, *D. oblongata*, and *D. corymbosa*. Canadian Journal of Botany 52: 791–793.

———. 1974b. Cytotaxonomic studies of *Draba nivalis* and its close allies in Canada and Alaska. Canadian Journal of Botany 52: 1793–1801.

———. 1975. *Draba crassifolia*, *D. albertina*, *D. nemorosa*, and *D. stenoloba* in Canada and Alaska. Canadian Journal of Botany 53: 745–751.

———. 1976. The genus *Draba* in Canada and Alaska: key and summary. Canadian Journal of Botany 54: 1386–1393.

——— and A. E. PORSILD. 1969. Chromosome numbers of some plants from the unglaciated central Yukon plateau, Canada. Canadian Journal of Botany 47: 655–662.

MURÍN, A. and J. MÁJOVSKY. 1978. IOPB chromosome number reports LXI, ed. Á. Löve. Taxon 27: 375–392.

PACKER, J. G. 1964. Chromosome numbers and taxonomic notes on western Canadian and Arctic plants. Canadian Journal of Botany 42: 473–494.

PETROVSKY, V. V. and P. G. ZHUKOVA. 1983. Polyploids and diploids in the vascular flora of the Wrangel Island. Botanichesky Zhurnal 68: 749–760.

POSADA, D. and K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

PRICE, R. A. 1979. The *Draba crassa* complex (Brassicaceae): systematics and geography. M.S. Thesis. University of Wisconsin, Madison. 1–88.

ROLLINS, R. C. 1953. *Draba* on Clay Butte, Wyoming. Rhodora 55: 229–235.

———. 1966. Chromosome numbers of Cruciferae. Contributions to the Gray Herbarium 197: 43–65.

———. 1993. *The Cruciferae of continental North America.* Stanford: Stanford University Press.

SCHULZ, O. E. 1927. Cruciferae—*Draba* et *Erophila*. Pp. 1–396 in Das Pflanzenreich Series IV, Vol. 105, ed. A. Engler. Leipzig, Germany: Verlag von Willhelm Engelmann.

SOKOLOVSKAYA, A. P. and O. S. STRELKOVA. 1948. Distribution of polyploids III: studies of the flora of the alpine zone of the Greater Caucasian range. Uchenya Zapiski Pedagogicheskogo Instituta im Gerzena, Leningrad 45: 370–381.

STRID, A. 1987. Chromosome numbers of Turkish mountain plants. An annotated list of 34 taxa. Notes of the Royal Botanical Garden Edinburgh 44: 351–356.

———, and I. A. ANDERSSON. 1985. Chromosome numbers of Greek mountain plants. An annotated list of 115 species. Botanische Jahrbuch 107: 203–228.

SWOFFORD, D. L. 1998. PAUP*: Phylogenetic analysis using parsimony (* and other methods), beta test Version 4.0. Sunderland: Sinauer Associates.

VAN LOON, J. C. and J. J. M. H. OUDEMANS. 1982. IOPB chromosome number reports LXXV, ed. Á. Löve. Taxon 31: 342–368.

WARD, D. E. 1983. Chromosome counts from New Mexico and southern Colorado. Phytologia 54: 302–308.

WHITE, T. J., T. BRUNS, S. LEE, and J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in PCR protocols: a guide to methods and applications, eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White. London, England: Academic Press.

WIDMER, A. and M. BALTISBERGER. 1999. Molecular evidence for allopolyploid speciation and single origin of the narrow endemic *Draba ladina* (Brassicaceae). American Journal of Botany 86: 1282–1289.

WINDHAM, M. D. 2000. Chromosome counts and taxonomic notes

on *Draba* (Brassicaceae) of the Intermountain West. 1: Utah and vicinity. Madroño 47: 21–28.

WINGE, Ö. 1933. A case of amphidiploidy within the collective species *Erophila verna*. Hereditas 18: 181–191.

ZAKHARYEVA, O. I. and S. B. ASTANOVA. 1968. Chromosome numbers of some wild species of flowering plants of Middle Asia. Reports of the Academy of Science Tadzhik Soviet Socialistic Republic 11: 72–75.

ZHOKOVA, P. G. 1965. A caryological characterization of some plant species inhabiting Wrangel Island. Botanichesky Zhurnal 50: 1320–1322.

——— and V. V. PETROVOSKY. 1971. Chromosome numbers of certain flowering plants of the Wrangel Island. Botanichesky Zhurnal 56: 294–305.

——— and ———. 1977. Chromosome numbers of some western Chukotka plant species III. Botanichesky Zhurnal 62: 1215–1223.

——— and ———. 1980. Chromosome numbers and taxonomy of some species of the Anyui Mountains. Botanichesky Zhurnal 65: 651–659.

——— and ———. 1984. A cytotaxonomical study of some species of the family Brassicaceae in northern Asia. Botanichesky Zhurnal 69: 236–240.

——— and ———. 1987. Chromosome numbers and taxonomy of some plant species from the northern Asia regions. Botanichesky Zhurnal 72: 1617–1624.

——— and A. D. TIKHONOVA. 1971. Chromosome numbers of certain plant species indigenous to the Chukotskiy Province. Botanichesky Zhurnal 56: 868–875.

———, V. V. PETROVSKY, and T. V. PLIEVA. 1973. The chromosome numbers and taxonomy of some plant species from Siberia and far east. Botanichesky Zhurnal 58: 1331–1342.