

Testing Hybridization Hypotheses with Morphometry: the Case of Eastern American Arctic Species of *Potentilla* sect. *Niveae* (Rosaceae)

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Abstract—Asexual reproduction, polyploidy and hybridization are well-known sources of taxonomic complexity in angiosperms. All these processes are believed to occur in *Potentilla* sect. *Niveae* (Rosaceae). Although it has been assumed that hybridization is common in section *Niveae*, this hypothesis has not been tested and recent studies suggest that phenotypic plasticity may sometimes better explain morphological intermediates in nature. To clarify the role of hybridization in the evolution of section *Niveae*, we tested two hybridization hypotheses for its eastern American Arctic species. The first is a potential hybrid between *Potentilla nivea* and *Potentilla arenosa*, and the second between *Potentilla arenosa* and *Potentilla vahliana* sensu lato. Twenty-four quantitative and 12 qualitative morphological characters were scored on specimens sampled from a representative range of the parental species and putative hybrids in the American Arctic east of the 100th meridian. Multivariate analyses showed that these two classes of characters give a congruent signal and that species form separate groups. Morphological evidence appears to give support to the hybridization hypothesis both between *Potentilla arenosa* and *Potentilla nivea* and between *Potentilla arenosa* and *Potentilla vahliana* sensu lato, although other explanations may also be conceivable. We discuss potential implications for the taxonomy of *Potentilla* and the study of hybridization in apomictic groups.

Keywords—Arctic-alpine, discriminant analysis, facultative apomixis, taxonomy, trichome morphology.

It is now well recognized that hybridization is and has been an important evolutionary process in plant evolution (Arnold et al. 1999; Rieseberg and Willis 2007; Soltis and Soltis 2009). Indeed, hybridization and introgression have had a strong impact on both evolutionary dynamics (Arnold et al. 1999; Rieseberg 2003; Arnold 2004) and taxonomy. At the other end of the reproductive spectrum is apomixis, a type of asexual reproduction that has evolved recurrently in the angiosperms (Whitton et al. 2008). Contrarily to hybridization, apomixis favours the rapid fixation of mutations and promotes morphological and genetic differentiation of populations. Apomixis can thus lead to the recognition of a great number of “microspecies”, the circumscription of which often vary among taxonomic treatments (Campbell and Dickinson 1990). In addition, apomixis has often been thought to be associated with hybridization in angiosperms (Asker and Jerling 1992). This reputation has certainly been significant in giving apomicts their notoriety for taxonomic instability and complexity (Richards et al. 1996). Many apomictic and hybridizing genera can be found in the Rosaceae, where, for instance, hundreds of taxa have been described in *Crataegus* L. (Camp 1942; Campbell and Dickinson 1990) and *Rubus* L. (Weber 1996), the majority of which are now relegated to synonymy.

Potentilla L. (Rosaceae) is representative of the types of problems brought about by hybridization and apomixis. This genus is mainly distributed in temperate, arctic and montane regions of the northern hemisphere and it is known to include several lineages reproducing by agamospermy and pseudogamy (Müntzing 1928; Asker 1970; Eriksen 1996; Nyléhn et al. 2003). Like most other agamosperms in the Rosaceae (Vamosi and Dickinson 2006), facultative apomixis in *Potentilla* appears to be associated with polyploidization and hybridization (Asker 1977; Töpel et al. 2011). This has led to complex taxonomic treatments in *Potentilla* sect. *Niveae*

(Rydb.) A. Nelson, where species boundaries sometimes differ substantially between authors (e.g. Soják 1989 and B. J. Ertter unpublished data).

Species of *Potentilla* sect. *Niveae* are easily recognized by their rosette habit and ternate leaves with an abaxial tomentum (Rydberg 1896). Identification at the species level is complex and relies mainly on petiole vestiture (Soják 1989; B. J. Ertter unpublished data) in part because of an apparent lack of other consistent characters. Artificial crosses have shown that at least some species of sect. *Niveae* are facultative pseudogamous apomicts that can be hybridized in the laboratory (Nyléhn et al. 2003). This may be indicative of inaccurate species limits or of a recent origin without acquisition of post-mating isolation mechanisms. However, artificial crosses are not informative of potential pre-mating barriers to reproduction that might prevent the formation of spontaneous hybrids in nature. In the field, specimens with more than three leaflets, but otherwise having the characteristics of sect. *Niveae* are classified as intersectional hybrids generally involving pinnate species of sect. *Pensylvanicae* Pöevertl., which are thought to hybridize readily with sect. *Niveae* species (Soják 1985). Although common garden experiments by Eriksen and Nyléhn (1999) indicate that the number of leaflets can be influenced by environmental conditions, AFLP and morphometric studies by Eriksen and Töpel (2006) show that (sub-)palmate leaves can be a good indicator of intersectional hybrid origin.

The situation is less clear in the case of hybridization within sect. *Niveae*. Currently, specimens of the section harboring an intermediate or mixed petiole vestiture type are considered intrasectional hybrids (Soják 1989). Some of them, such as *Potentilla arenosa* × *nivea* (*P.* × *prostrata* Rottb.), are apparently quite common (ÉLB pers. obs.). Yet, petiole vestiture does not appear consistently intermediate in experimental hybrids (Nyléhn et al. 2003), suggesting that

TABLE 1. Hybrids of *Potentilla* sect. *Niveae* and potential parental taxa of the American Arctic and Greenland, east of the hundredth meridian, following the Pan-arctic flora (Aiken et al. 2012). Indumentum terminology following Payne (1978), except for crispate being short, adpressed, irregularly curved trichomes (Eriksen and Yurtsev 1999). Floccose indumentum always is of flat, twisted trichomes, while crispate indumentum is of trichomes round in cross-section. Note: *Potentilla nivea* L. is a nomen conservendum following Eriksen et al. (1999).

Species	Section	Petiole vestiture	Straight trichome ultrastructure	Leaf
<i>Potentilla nivea</i> L. nom. cons.	<i>Niveae</i>	Floccose	–	Ternate
<i>Potentilla arenosa</i> (Turczaninow)	<i>Niveae</i>	Hirsute in two layers	Verrucose	Ternate
Juzepczuk subsp. <i>arenosa</i>				
<i>Potentilla arenosa</i> subsp. <i>chamissonis</i> (Hultén) Elven & D. F. Murray	<i>Niveae</i>	Villose-hirsutulous	(Sub-)Verrucose	Ternate
<i>Potentilla subvahliana</i> Jurtzev	<i>Niveae</i>	Villose to hirsute	Smooth	Ternate
<i>Potentilla vahliana</i> Lehmann (= <i>P. nivea</i> × <i>P. subvahliana</i> ?)	<i>Niveae</i>	Crispate/floccose, villose to hirsute	Smooth	Ternate
<i>Potentilla subgorodkovii</i> Jurtzev (= <i>P. crebridens</i> × <i>P. subvahliana</i> ?)	<i>Niveae</i>	Crispate/floccose, villose to hirsute	Smooth	Ternate
<i>Potentilla</i> × <i>prostrata</i> Rottbøll (= <i>P. arenosa</i> × <i>P. nivea</i>)	<i>Niveae</i>	Floccose, villose to hirsute	(Sub-)Verrucose	Ternate
<i>Potentilla arenosa</i> × <i>P. vahliana</i>	<i>Niveae</i>	Crispate, villose to hirsute	Subverrucose	Ternate
<i>Potentilla pulchella</i> R. Brown	<i>Pensylvanicae</i>	Crispate, villose	Smooth	Pinnate
<i>Potentilla uschakovii</i> Jurtzev (= <i>P. pulchella</i> × <i>P. vahliana</i>)	× <i>Rubricaulae</i>	Crispate, villose	Verrucose	Palmate
<i>Potentilla pedersenii</i> (Rydb.) Rydb. (= <i>P. pulchella</i> × <i>P. arenosa</i>)	× <i>Rubricaulae</i>	Crispate, villose	Verrucose	Palmate

this character alone is not sufficient for accurate hybrid identification. Although a number of morphological and genetic studies of variation have been made on sect. *Niveae* (Dansereau and Steiner 1956; Eriksen 1997; Hansen et al. 2000; Eriksen and Töpel 2006), there is still no unequivocal evidence of naturally occurring hybrids within this section. Nevertheless, while three “primary” species and one subspecies of *Potentilla* sect. *Niveae* are currently recognized in the American Arctic east of the 100th meridian, almost as many intrasectional hybrids or nothospecies are thought to exist in the region (Table 1). In order to clarify the status of some of these hybrids, we carried out a morphometric study of all eastern American taxa of *Potentilla* sect. *Niveae* to evaluate two hypotheses of intrasectional hybridization and understand their possible consequences on species boundaries.

In the region studied, the species of sect. *Niveae* can be divided into two groups on the basis of habit and trichome ultrastructure (Eriksen and Yurtsev 1999). The first group, called the “vahliana aggregate”, consists of *Potentilla vahliana* Lehm. and segregate species *P. subvahliana* Jurtzev and *P. subgorodkovii* Jurtzev. This group is easily recognized by a massive caudex with short internodes and marcescent leaves, leading to a “columnar” growth form (Dansereau and Steiner 1956), and is also characterized by the complete absence of verrucae on trichomes (Fig. 1A). The second group, here called the “nivea aggregate”, includes *P. arenosa* (Turcz.) Juz. and *P. nivea* L. (Elven and Murray 2012). These species are characterized by their verrucose terete trichomes (on the petiole in *P. arenosa* and on the adaxial face of leaves in both species, Figs. 1C, F) and by a more open growth form with longer petioles than in the “vahliana aggregate”.

The first hybrid studied is believed to occur between *Potentilla nivea* and *P. arenosa* (Table 1). The two parents differ to some degree in leaf abaxial tomentum density, teeth incision depth, number of flowers per inflorescence, size of petals and shape of the epicalyx bractlet (Aiken et al. 2012; B. J. Ertter unpublished data). All of these char-

acteristics are greatly variable, however, and no combination of them can accurately identify all specimens (ÉLB pers. obs.). Therefore, the main distinguishing trait in current use is petiole vestiture. *Potentilla nivea* exhibits a floccose pubescence of flat, twisted hairs on the petiole (Fig. 1D), while *P. arenosa* harbors only straight, verrucose hairs. There are two subspecies within *P. arenosa*. *Potentilla arenosa* subsp. *arenosa* is characterized by the presence of two dense layers of hirsute, conspicuously verrucose trichomes (Fig. 1C). In contrast, *P. arenosa* subsp. *chamissonis* (Hultén) Elven and D. F. Murray generally has a single layer of long, sparse, villosulo-hirsutulous vestiture of slightly verrucose to wavy-walled trichomes (Fig. 1F). Plants sharing the nivea-type and chamissonis-type trichomes on at least some petioles (Fig. 1E) are generally considered hybrids and can be referred to as *P. ×prostrata* (Soják 2004; Elven and Murray 2012). However, an almost continuous gradient in petiole vestiture appears to exist in nature (ÉLB pers. obs.). This kind of variation could also be considered akin to intraspecific variation with a genetic polymorphism maintained at a few loci. In consequence, an in depth study of quantitative and qualitative variation within this hybrid complex is needed to clarify the existence of distinct species and hybrids.

The second hybridization hypothesis tested involves *Potentilla vahliana* and *P. arenosa*, for which few hybrid specimens have been identified (JC pers. obs.; Table 1). Potential hybrids exhibit verrucose to at least slightly wavy trichome walls (Fig. 1B), but a more tussocky habit than is usual for *P. arenosa* (JC pers. obs.). These specimens were first found in northernmost Québec, but other similar hybrids were later identified from material collected on Baffin Island. Although hybridization is strongly suspected, one cannot exclude that these specimens may represent morphological extremes of either of the putative parents.

To validate the hybrid origin of the two putative hybrids, in-depth quantitative and qualitative morphological studies of the two hybrid complexes were undertaken. Potential

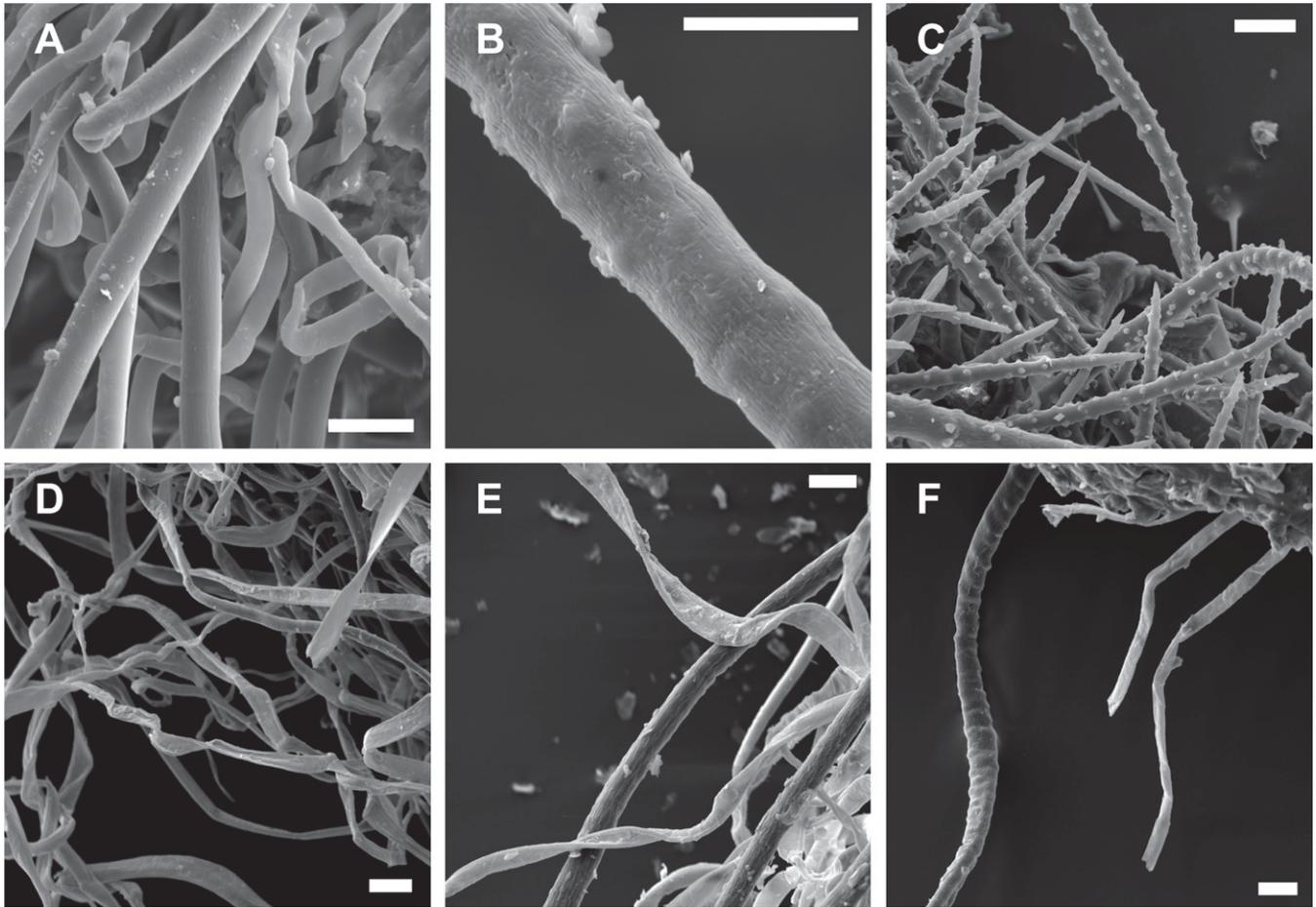


FIG. 1. Scanning electron microscope (SEM) micrographs of petiole vestiture and trichome ultrastructure of the eastern American Arctic species of *Potentilla* sect. *Niveae*. A. Long terete and smooth trichomes and short crispate indumentum of *P. vahliana* s.s. B. Long verruculose trichome of *P. vahliana* × *P. arenosa*. C. Two layers of straight verruculose trichomes of *P. arenosa* subsp. *arenosa*. D. Floccose indumentum of flat and twisted trichomes of *P. nivea*. E. Floccose indumentum with straight trichomes of *P. nivea* × *P. arenosa*. F. Long, sparse verruculose trichomes of *P. arenosa* subsp. *chamissonis*. Scale bar is 20 μ m.

implications for the taxonomy of *Potentilla* sect. *Niveae* and for the role of hybridization and apomixis in the evolution of the section is discussed.

MATERIALS AND METHODS

Plant Material—Herbarium specimens from CAN, DAO, MT, QFA and the private herbarium of Marcel Blondeau (“BLOM”) were selected to cover the known range of *Potentilla nivea* ($n = 18$), *P. arenosa* ($n = 36$) and *P. vahliana* s.l. ($n = 39$) in the eastern American Arctic and Greenland, east of the 100th meridian (Fig. 2). *Potentilla pulchella* R. Brown (sect. *Pennsylvanicae*) was also included ($n = 11$) to confirm that there was no evidence of intersectional hybrids in our dataset and to see whether the characters selected were sufficiently informative to separate a clearly distinct species. Localities where material is available in the Arctic are relatively few compared to southern Canada because there have been fewer botanical expeditions to the North. Nevertheless, CAN contains one of the most complete collections of Canadian Arctic plants, while DAO is the largest herbarium in Canada and MT has considerable material from Baffin Island due to the extensive collections by Dansereau. This made it possible to cover our entire study region. We aimed to include only complete, healthy-looking, ample specimens in flowering or early fruiting stage. Any evidently immature, deformed or unhealthy plants were not included. However, the limited number of collections available for some taxa (mainly *P. arenosa* subsp. *chamissonis*) made it impossible not to include incomplete specimens. We tried to avoid sympatric,

conspecific collections as much as possible. All the specimens of the *P. vahliana* s. l. group correspond to *P. vahliana* s. s., with the exception of one specimen from Axel Heiberg Island that appears closer to *P. subgorodkovii*, but is treated as *P. vahliana* s. l. in the statistical analyses. Most vouchers contained multiple distinct caudices, or “tufts”. Although it is impossible to reject the hypothesis that the “tufts” may have been connected by an underground caudex in nature, we considered every “tuft” on a sheet to be a distinct individual. Most individuals came from distinct collections, but the limited number of good specimens available made it difficult not to include, in some cases, individuals from the same herbarium sheet. Therefore, there is a certain amount of pseudo-replication in the sample. A total of 129 individuals were included in the analyses, coming from 83 herbarium sheets (Appendix 1). The two subspecies of *P. arenosa* were treated as distinct taxa in our analyses since they were found to be distinct in many quantitative characters (see Results).

Morphological Methods—Qualitative characters (Appendix 2) that appeared to vary among species were observed with the help of a dissecting or compound microscope and coded into binary variables or multistate ordered variables when binary coding seemed too coarse. Quantitative characters (Appendix 2) were selected to cover the greatest range of morphological structures, while remaining easy to measure reliably and showing some variability between species. Characters were measured with an electronic calliper with a precision of 0.1 mm or under a dissecting microscope at 12–50 \times using a calibrated eye-piece graticule, depending on the size of the structure. Values were estimated from the arithmetic means of at least two different leaves and flowers on each individual, whenever possible. The correct coding of indumentum characters of some individuals was verified using

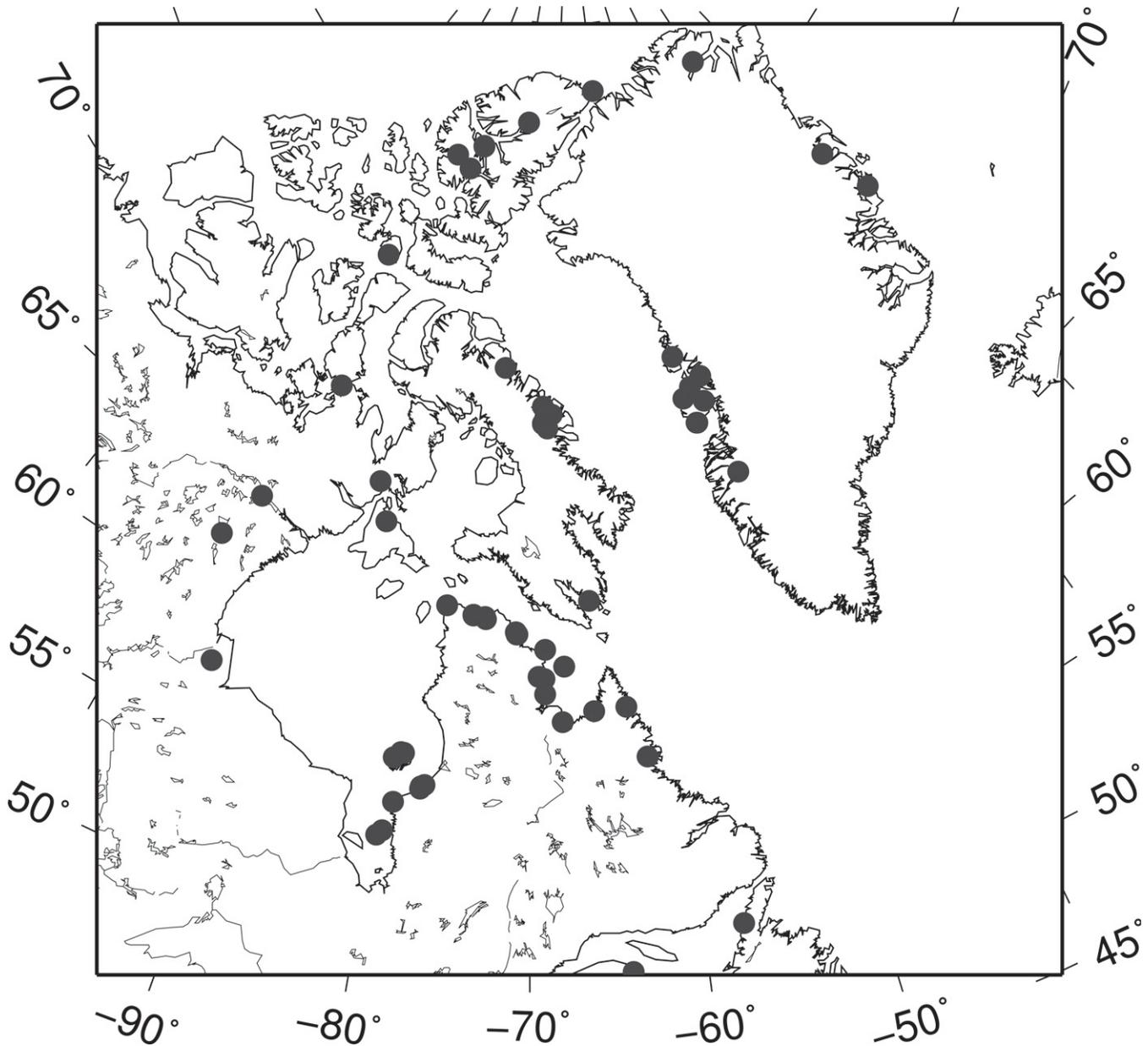


FIG. 2. Geographic sampling of the study.

environmental or high-vacuum scanning electron microscopy. Micrographs of petioles mounted with gold-palladium were taken with a Quanta 200 3D (FEI Company) (Fig. 1).

Transformation of the Quantitative Data Matrix—To eliminate the effect of scale on morphological measurements, most characters were analysed as ratios (Jungers et al. 1995). Leaf characters (PETIOLEN, LFLTWID, LFLTDIS, PETIOLULEN, TERMTEETHN, LATEETHN, TTOOTHLEN, TEETHLEN, SINUSLEN, and TEETHWID) were divided by the length of the terminal leaflet of their leaf, while flower characters, except style length (PETALEN, PETALWID, SEPALEN, SEPALWID, EPICLEN, and EPICWID), were divided by the radius of the hypanthium including the epicalyx bractlets (FLOWSIZE) of their flower (Appendix 2). The use of different variables for scaling the leaf and flower characters was necessary because size variation between these two organs was not highly correlated (data not shown). The length of the largest terminal leaflet (LFLLEN) and radius of hypanthium (FLOWSIZE) were included without scaling to account for size variation between species. This combination of scaled and raw characters allowed us to get the most information from the data by reducing the correlation between characters and size (with ratios), while still taking into account potentially informative variation in size (with raw charac-

ters). All quantitative characters were log transformed with the natural logarithm since Shapiro-Wilk tests of normality (Royston 1982) based on Mahalanobis generalized distances (Legendre and Legendre 1998) showed that this transformation was needed to achieve multivariate normality. Zero values were replaced by values equal to approximately half the measurement precision before computing the logarithms to avoid undefined values.

Missing Data—Given that a few flowering quantitative characters could not be measured because of the absence of some structures on some individuals, the initial matrix had about 2.4% missing values, with 24 individuals having at least one character missing. Excluding missing values is known to be problematic because it can lead to negative eigenvalues (Legendre and Legendre 1998; Jolliffe 2002). Excluding individuals with missing data is another option, but given that our sample size is limited and there are generally few missing characters per individual, we rejected this alternative. Instead, missing values were estimated by principal component analysis (PCA) imputation, a type of expectation-maximization (EM) algorithm, using the missMDA package (Husson and Josse 2010) in R (R Development Core Team 2012). Estimation of missing data with an EM algorithm has been shown to perform well with datasets that contain a low amount of

anatomically-biased missing data such as in the present study (Brown et al. 2012). Imputation by PCA works by first performing a PCA rotation on the complete matrix with missing values replaced by their column mean. The last k principal components are left out, and the scores of each missing value are calculated with the remaining $n-k$ components, where n is the number of characters. Another PCA is then done on the new matrix and the previous steps are iterated until convergence. The method assumes that most of the information in a PCA is contained in the first $n-k$ principal components, with the last k components being random noise due, in part, to the imputed missing values. The number of principal components to keep ($n-k$) is evaluated by running the iterations with different numbers of principal components, estimating the scores of known values, and assessing the difference between the real and estimated values (estimation error). The curve of estimation error as a function of the number kept components ($n-k$) follows approximately a negative exponential. The optimal number of principal components to retain is at the elbow of this curve. Missing values in our quantitative matrix were estimated with four principal components, and multiple imputations (Husson and Josse 2010) showed that the imputation error does not create instability of the PCA scores. This is to be expected given the small number of missing values in our dataset.

Morphometric Variation Within sect. *Niveae* and Intersectional Hybridization—To examine general morphometric variability between and within species, a PCA was done on the quantitative characters of all specimens. Eigenvalues of the first principal components were compared to the expected variance from a broken-stick distribution (Legendre and Legendre 1998). Individuals of *Potentilla pulchella* were included in this analysis.

Congruence Between Character Types—A procrustean co-inertia analysis (CoIA) was performed to test for congruence between the signal given by qualitative and quantitative characters. Using two configurations of the same objects coming from two prior ordinations, CoIA optimally rotates one configuration onto the other (Dray et al. 2003). The rotation aims to minimize the distance between each object from one ordination and its equivalent in the other. Quantitative variables were submitted to a PCA and rotated onto a principal coordinates analysis (PCoA) of the qualitative matrix based on Podani's (1999, eq. 2) modified Gower coefficient. The rows in both the PCA and PCoA were weighted by the number of other individuals included in the analysis coming from the same herbarium voucher. Thus, if two individuals came from the same sheet, then each one was given half the weight of an individual that was alone on its sheet. This was done to alleviate the obvious problem of pseudo-replication inherent in using individuals coming from a single herbarium accession. The significance of the relationship between the two ordinations in the CoIA was tested by using a non-parametric randomization test based on the RV coefficient, a multivariate analog of the Pearson's correlation coefficient (Heo and Gabriel 1998), as implemented in the R package ade4 (Dray and Dufour 2007). This coefficient tests for correlation between two tables and is more powerful than the commonly used Mantel test (Dray et al. 2003).

Test of Hybrid Hypotheses—If the selected morphological characters have a strong genetic component, hybrids are expected to be intermediate in a large number of these characters. Testing the hypothesis of hybridization therefore amounts to testing for intermediacy of individuals, assuming that the characters were correctly selected. Hence, linear discriminant analyses (LDA) of the parents of each hybrid complex were done to test the two hybridization hypotheses. This method searches for character combinations that best discriminate the different groups given a priori. Therefore, it should be more efficient than PCA in uncovering characters that best differentiate parents; characters that should be intermediate in hybrids. If the hypotheses of hybridization are supported, the projection of hybrids on the discriminant axes should place them midway between their parents. The log-transformed matrix of quantitative characters was used in LDA, excluding RACHISLEN, which proved to be invariable between species of sect. *Niveae*.

The efficiency of the LDA models in classifying parental specimens was assessed by leave-one-out cross-validation analysis. One by one, each individual was left out of the analysis and classified with a model estimated from the remaining individuals. Comparison of the a priori classification to the results of the cross-validation analysis gives an indication of the efficiency of the LDA models. Furthermore, to understand the contribution of each quantitative and qualitative variable to the discriminant axes, correlation analyses were done between each character and the axes of the LDA models. Spearman's rank correlation

was used for ordinal characters, while Pearson's product-moment correlation was used for continuous and binary variables. Boxplots of all quantitative characters can be found in the online supplementary appendices to this study (Fig. S1).

Distinctiveness of Lineages Within Sect. *Niveae*—To test whether currently defined species within sect. *Niveae* are morphologically distinct, a multivariate analysis of variance (MANOVA) was done on all taxa except *Potentilla pulchella* and putative hybrids. To avoid overfitting, only the first two principal components of a PCA of the species were used as variables in MANOVA since they were the only principal components that had greater eigenvalues than the broken-stick expectation. Wilk's lambda (Huberty and Olejnik 2006) was used to assess significance of MANOVA results. The first two principal components were not multinormal and the within-group variances were not homogeneous when verified with the R function betadisper of the vegan package (Oksanen et al. 2011), which provides a refined multivariate version of Levene's test for the homogeneity of variances (Anderson 2006). Therefore, we report the p value of a permutation-based MANOVA (permuting the species) in addition to the parametric MANOVA results.

RESULTS

Quantitative Variation Within Sect. *Niveae* and Intersectional Hybridization—Inspection of the PCA on quantitative characters including all species of the study (Fig. 3) showed that *Potentilla pulchella* is clearly differentiated from species of sect. *Niveae* on morphometric grounds. Two individuals of *P. arenosa* subsp. *arenosa* (Fig. 3, #39 and #77) had palmate leaflets and were therefore potential intersectional hybrids. Yet, these individuals were located within the sect. *Niveae* group in the PCA and were therefore treated as pure *P. arenosa* subsp. *arenosa* specimens in further analyses. Two individuals were found far from their respective species group in the PCA (Fig. 3, #71 and #88), but they did not show any sign of morphological intermediacy and were therefore not considered as potential hybrids. A PCA biplot is available in the online supplementary figures (Fig. S2).

Congruence of Quantitative and Qualitative Characters—Quantitative and qualitative morphological characters displayed a congruent signal and were significantly correlated ($RV = 0.51$, $p = 10^{-4}$, $nperm = 9999$). Inspection of the first two axes of co-inertia analysis, representing 95.2% of the common variance in the two datasets, showed that groups circumscribed with quantitative characters are found in the same relative positions in a PCoA of qualitative data (see Fig. S3 in online supplementary figures).

Test of Hybridization in the *Nivea-Chamissonis* Complex—Linear Discriminant Analysis (LDA) of the species from the *nivea-chamissonis* complex (*P. nivea*, *P. arenosa* subsp. *arenosa*, *P. arenosa* subsp. *chamissonis*) classified correctly 72.2% of all parental individuals (39 out of 54) in leave-one-out cross-validation. Since many individuals of what appeared to be *P. nivea* had some straight trichomes, the individuals entered as "pure *P. nivea*" were the 18 ones that had fewer straight trichomes on their petioles. "Hybrids" therefore also included ambiguous individuals that could be closer to *P. nivea*. Sixteen characters were significantly correlated with at least one of the discriminant axes. The variables most strongly correlated ($|r| > 0.6$) with the axes were, from high to low; presence of floccose indumentum on petiole (PETFLOCC), presence of flexuose terete trichomes (PETRICHORI), verrucosity of straight trichomes (VERRUC), number of layers of terete trichomes on petiole (PETSTRAIGHT), length from midvein to mid-leaf tooth

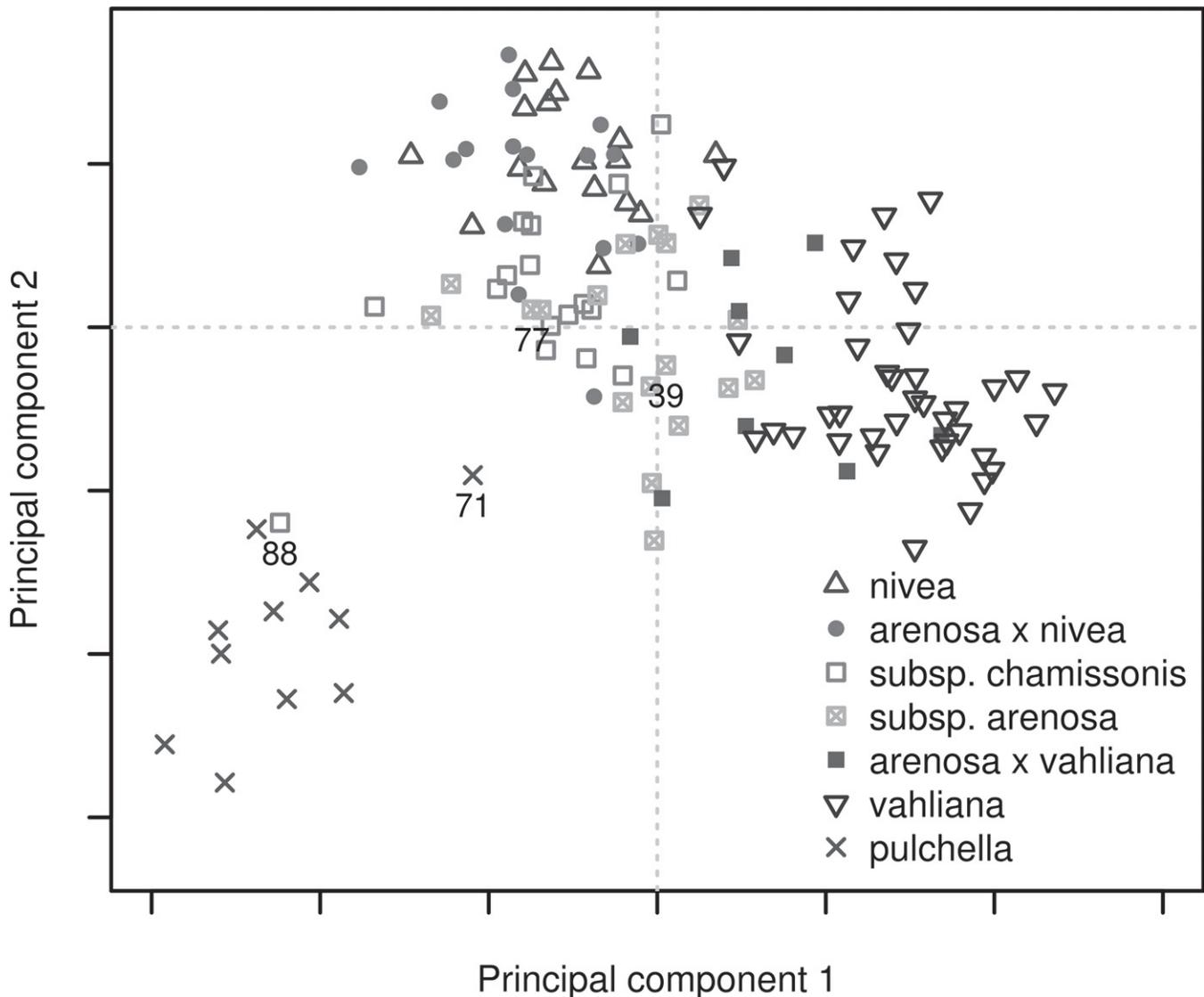


FIG. 3. First two principal components of a PCA of all species included in the study. The first axis represents 24.8% and the second 14.7% of the total variance of the quantitative dataset, with the first four axes having higher eigenvalues than expected under a broken-stick distribution.

sinus (SINUSLEN) and number of flowers per inflorescence (FLOWN). Flowering characters were the most helpful in discriminating the three parental taxa. Putative hybrids, when projected onto the discriminant axes, aggregated mostly between *P. nivea* and *P. arensa* subsp. *arensa*, although most remained closer to *P. nivea* (Fig. 4). Accordingly, about 75% of the 16 putative hybrids were classified as *P. nivea* by the model. A LDA biplot is provided in the online supplementary appendices (Fig. S4).

Test of Hybridization in the Arenosa-Vahliana Complex—Species from the arenosa-vahliana complex (*Potentilla arensa* subsp. *arensa*, *P. arensa* subsp. *chamissonis* and *P. vahliana* s.l.) were correctly classified 85.3% of the time by the LDA model in leave-one-out cross-validation (64 out of 75). Eighteen characters were significantly correlated with at least one of the discriminant axes. Of these, the most important ($|r| > 0.7$) from high to low, were verrucosity of straight trichomes (VERRUC), length of petioles (PETIOLEN), orientation of adaxial straight trichomes on leaflets (ADXTRICHORI), number of flowers per inflorescence (FLOWN), width of

terminal leaflet (LFLTWID), length of largest terminal leaflet (LFLTLEN) and number of epicalyx bractlet “lobes” (EPICN). A large number of quantitative characters were correlated with the first axis separating *P. vahliana* s.l. from both subspecies of *P. arensa*. Projection of the putative hybrids showed that they occupy an intermediate position on the discriminant axes, but remain closer to *P. vahliana* s.l. (Fig. 5). Accordingly, 66.7% of the putative hybrids were classified as *P. vahliana* s.l. by the model. A LDA biplot is available in the online supplementary appendices (Fig. S5).

Distinctiveness of Parental Lineages—Species and subspecies of *Potentilla* sect. *Niveae* were significantly different in quantitative morphology when tested by parametric MANOVA (Wilk’s lambda = 0.2256, $df_1 = 6$, $df_2 = 176$, $p < 10^{-15}$, $n = 93$). A non-parametric permutation-based MANOVA was also significant ($p = 10^{-4}$, $nperm = 9999$), indicating that the results are robust to non-normality in our dataset. The combined LDA of all sect. *Niveae* species (not shown) separated *P. vahliana* from the “nivea aggregate”

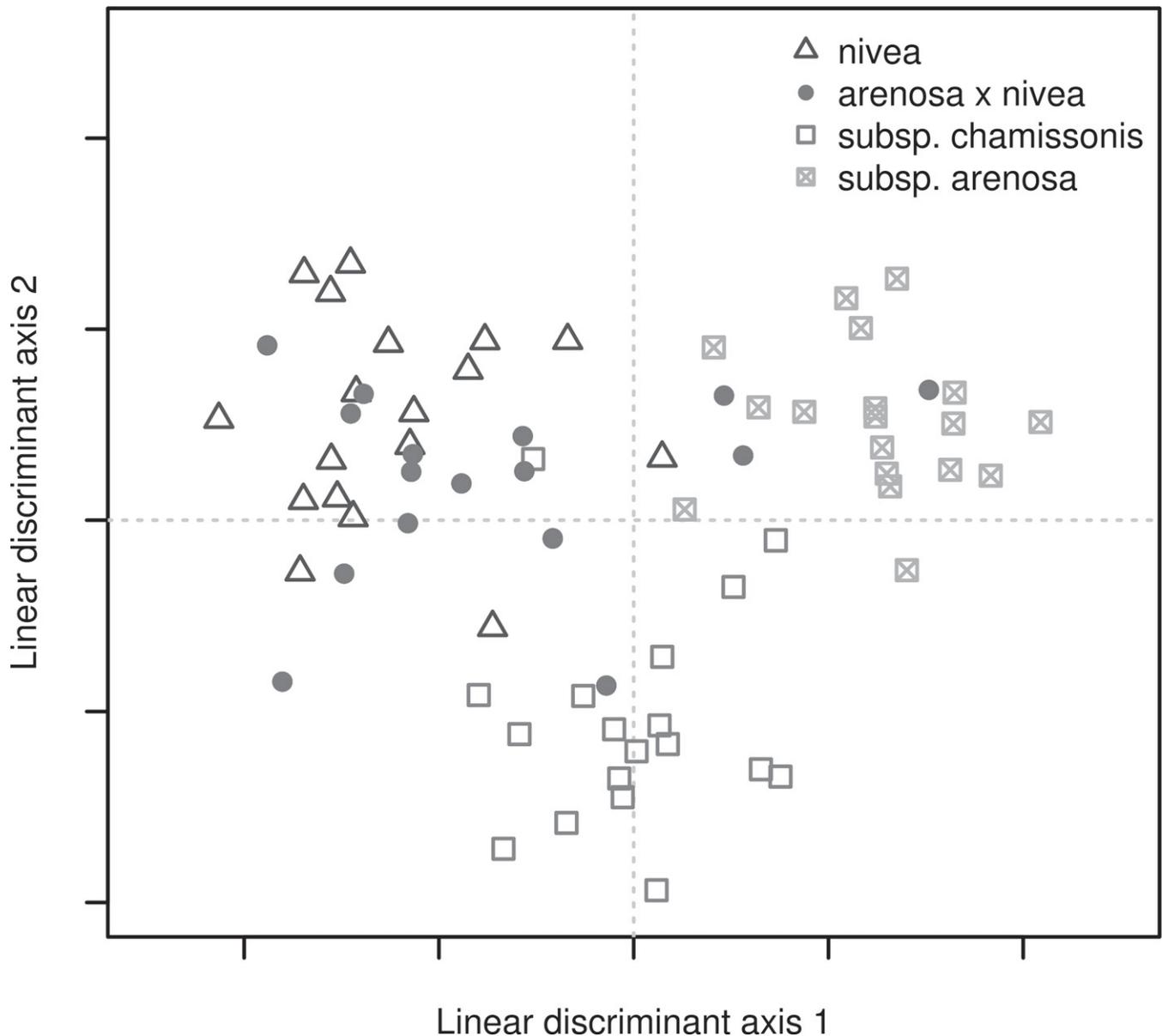


FIG. 4. Linear discriminant analysis of *P. nivea*, *P. arenosa* subsp. *arenosa* and *P. arenosa* subsp. *chamissonis*. Specimens classified as *P. arenosa* × *nivea* are projected onto the discriminant axes.

on the first axis, and *P. nivea* and the two subspecies of *P. arenosa* on the two subsequent axes.

DISCUSSION

Species Delimitation and Morphometry—Multivariate morphometry was highly successful in differentiating species of *Potentilla* sect. *Niveae* of the eastern American Arctic. When taken one by one, quantitative characters could not discriminate between closely related species. Combinations of characters proved much more effective, confirming the importance of looking at the joint distribution of a large number of characters when studying intricate species complexes. Other studies have likewise demonstrated the utility of multivariate analysis of morphology in apomictic species complexes (Dickinson and Phipps 1985; Smith and Phipps 1988; Chmielewski et al. 1990; Kołodziejek 2010;

Šuvada et al. 2012). These results are another indication that the approach of testing characters individually before inclusion in multivariate analyses may not always be appropriate. Indeed, it is known that characters can fail to differ significantly in univariate tests (e.g. *t* tests or ANOVAs) while still being able to discriminate the species well in multivariate space (Willig and Owen 1987).

Morphometric studies can also be helpful to find or to confirm the taxonomic potential of morphological characters and to identify potentially informative characters for phylogenetic analysis (Guerrero et al. 2003). In the case of *Potentilla* sect. *Niveae*, our analyses showed that incision of epicalyx bractlets (EPICN) is one of the most conspicuous features of *P. vahliana* and clearly separates it from all the species of the “*nivea* aggregate”. This character appears to have been neglected in the recent taxonomic literature, even though it was described by Lehmann (1856). Our

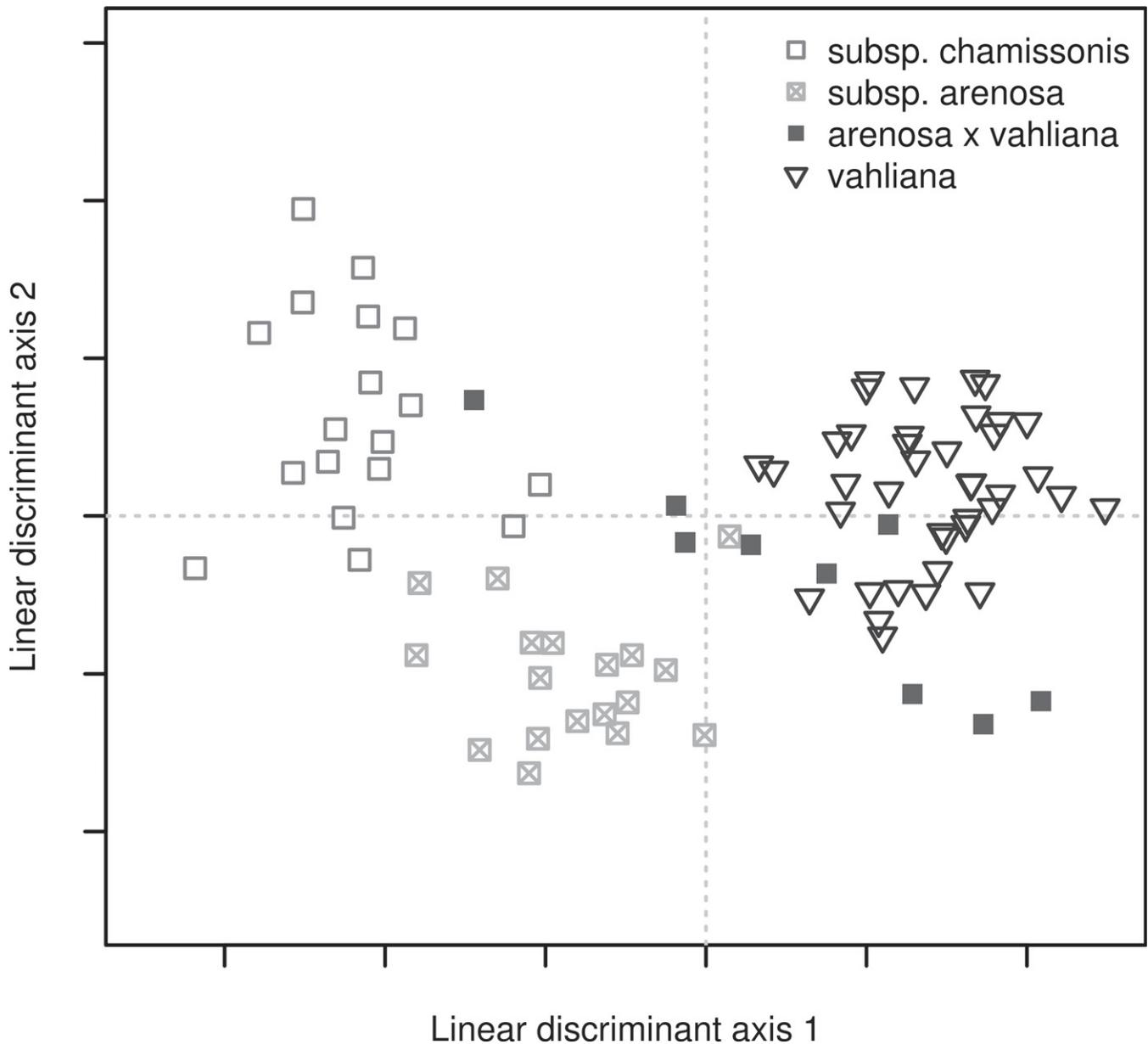


FIG. 5. Linear discriminant analysis of *P. vahliana* s.l., *P. arenosa* subsp. *arenosa*, *P. arenosa* subsp. *chamissonis*. Specimens classified as *P. arenosa* × *vahliana* are projected onto the discriminant axes.

study also confirms the taxonomic value of a number of quantitative characters in differentiating closely related species. For instance, *P. arenosa* subsp. *chamissonis* is distinguishable from *P. arenosa* subsp. *arenosa* by its higher petiole length to terminal leaflet length ratio (PETIOLEN / LFLTLEN), its lower epicalyx bractlet length/width ratio (EPICLEN / EPICWID) and its higher number of flowers (FLOWN) (Fig. S1). Other characters of interest are: largest terminal leaflet length (LFLTLEN), petiole length (PETIOLEN), number of leaf serrations (TERMTEETHN and LATEETHN), tooth length/width ratio (TEETHLEN / TEETHWID) and incision depth [TEETHLEN / (TEETHLEN+SINUSLEN)]. Combinations of these characters could be of help in classifying and identifying species within sect. *Niveae*.

It is interesting to note that species identified mainly with qualitative characters were also well supported by

quantitative morphology. The congruence between these two classes of characters suggests that the species and subspecies of *Potentilla* sect. *Niveae* are relatively well defined entities.

Distribution of Hybrids in the Eastern American Arctic—The distribution of putative hybrids between *P. arenosa* and *P. nivea* is intriguing since they are present almost throughout the range of *P. nivea* even where *P. arenosa* is not found. This fact might indicate that introgression has taken place, or that some putative hybrids are actually atypical *P. nivea* specimens. Alternatively, the present distribution of species and hybrids could reflect past population migrations, which would suggest that hybridization has been occurring for some time. Putative hybrids between *P. arenosa* and *P. vahliana* have been found on Baffin island, where both parents meet, and the adjacent northern tip of

the Ungava peninsula. *Potentilla arenosa* and *P. vahliana* are sympatric in many other arctic islands and mainland localities where additional hybrids should be sought (Soják 1985; Aiken et al. 1999; Aiken et al. 2012).

Hybridization Within *Potentilla* Sect. *Niveae*—Previous studies demonstrated that hybridization between *Potentilla* sect. *Niveae* and sect. *Pensylvanicae* is taking place in nature (Eriksen and Töpel 2006). However, hybridization within sect. *Niveae* has rarely been studied in the field, and evidence for its occurrence was tenuous (Dansereau and Steiner 1956; Hansen et al. 2000). Our results indicate that hybridization probably occurs among species of *Potentilla* sect. *Niveae*, at least in the eastern American Arctic.

The putative hybrids between *P. arenosa* and *P. nivea* (*P. ×prostrata*) behave as expected for hybrids; they are mostly positioned in an intermediate position between their parents in the multivariate analyses, even though they tend to be closer to *P. nivea* (Fig. 4). At least three different explanations may be proposed to account for an apparently closer affinity to *P. nivea*. First, non-intermediacy of hybrids could be expected, since it is known that hybrids are more likely to present a mixture of parental, extreme and intermediate characters than true intermediacy between parents, especially for second and higher generation hybrids (Rieseberg et al. 1993). Second, repeated back-crossing of the hybrids with *P. nivea* could also explain such a morphological signal. The high incidence of straight trichomes in what appears to be *P. nivea* would thus be due to introgression. This hypothesis could also explain the presence of hybrid specimens found in regions far from the current limits of the distribution of *P. arenosa*. Indeed, ancient introgression, presumably during the last Pleistocene glaciations when environmental changes reshaped the spatial distributions of arctic species (Abbott and Brochmann 2003), could leave traces of *P. arenosa* characteristics within current populations of *P. nivea* where *P. arenosa* is no longer found. Third, the irregular clustering of individuals classified as "*P. arenosa × P. nivea*" in the LDA (Fig. 4) could also be partially explained by our own very conservative bias in determining which individuals would be classified as *P. nivea* in the LDA. Only individuals devoid of any verrucose trichomes on the petioles were determined as pure *P. nivea*. Therefore, species labelled as "*P. arenosa × P. nivea*" for the LDA may have a wide range of genomic compositions, ranging from atypical *P. nivea* to hybrids and maybe possibly pure *P. arenosa*. In any case, it seems clear that the use of a single qualitative character like petiole vestiture for the identification of hybrids is less than ideal if we hope to attain a good understanding of intricate species groups like the nivea-chamissonis complex. Nevertheless, our results are mostly consistent with hybridization and introgression between *P. arenosa* and *P. nivea*, although more data would be needed before reaching definite conclusions.

Most putative hybrids between *P. arenosa* and *P. vahliana* were clustered near *P. vahliana* s.l. (Fig. 5). Despite this, the putative *P. arenosa × P. vahliana* hybrids exhibit extreme morphological characteristics compared to their putative parents, notably in terms of terminal leaflet width-length ratio (LFLTWID), leaflet distance (LFLTDIS) and tooth incision depth (TEETHLEN/SINUSLEN) (Fig. S1). In addition, they appear quite distinct from *P. vahliana* because they present no or few incised epicalyx bractlets (EPICN),

they have shorter and narrower petals (PETALEN and PETALWID), and longer and narrower epicalyx bractlets (EPICLEN and EPICWID) than typical *P. vahliana*. These characteristics make it unlikely that the putative hybrids belong to *P. vahliana*. It is possible, for instance, that hybridization is taking place, but that non-intermediacy of hybrids (Rieseberg 1995) or introgression causes such a morphological pattern. It is also possible that the wrong parental combination was studied. These different scenarios need to be studied more thoroughly and genetic information would be helpful in testing these alternative hypotheses.

Hybridization, Apomixis and Species-Level Taxonomy—It is now generally acknowledged that species boundaries might not always agree with limits to gene flow (Wu 2001; Feder et al. 2012). Indeed, there are many reported cases where introgression does not jeopardize the continued existence of hybridizing species (e.g. Machado et al. 2002; Joly and Bruneau 2007; Mallet et al. 2007; Yatabe et al. 2007). Likewise, complete lack of gene flow within species, such as expected in apomicts, is not inconsistent with morphologically and genetically cohesive and distinct groups that can be considered species (Templeton 1989; Hillis 2007; Birky et al. 2010). However, the current limited evidence available (Coyne and Orr 2004) seems to indicate that groups with intermediate amounts of sexual reproduction, such as facultative apomictic complexes, may be less prone to form distinct genotypic clusters (species sensu Mallet 1995). The problem of species delimitation could therefore be exacerbated in a group that combines both hybridization and asexual reproduction as found in *Potentilla* sect. *Niveae*. On the other hand, it is interesting to note that apomixis has the potential to reduce the impact of hybridization in such groups by reducing the background level of sexual reproduction. This also has the potential to multiply the number of successful genotypic clusters and maintain their discreteness. The relative amounts of sexual reproduction within clusters, hybridization between clusters and apomixis may thus be critical factors that determine the distinctness of "species" or "microspecies" in different apomictic groups. This might explain why taxonomists working on different apomictic complexes have tended not to agree on the treatment of their respective groups (Richards et al. 1996). Consequently, it is likely that the stabilization of the taxonomy of apomictic organisms will have to rely on pragmatic and statistical observation of genetic data (or genetically inherited traits) as well as on a species definition completely independent of any assumptions regarding the processes at work in a particular group (Mallet 1995).

Species of *Potentilla* sect. *Niveae* are facultative apomicts (Nylén et al. 2003) and hybridization is thought to be important in the group (Erter et al. in preparation; Eriksen and Töpel 2006; Töpel et al. 2011), despite the lack of clear evidence for it until now. Our results have shown that hybridization is likely to occur between *P. arenosa* and *P. nivea*, and between *P. arenosa* and *P. vahliana*, where putative hybrids were found to be mainly morphologically intermediate. Despite this evidence for hybridization, species in the section still formed distinct groups at the morphological level. Even if this study was performed on a regional scale, these findings reinforce the idea that cohesive species can be maintained despite the presence of gene flow, and even in groups associated with facultative apomixis.

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- Malte 119105* (CAN); *Malte 120249* (CAN); *Malte 126918* (CAN); *Malte 126921* (CAN); *Malte 126926* (CAN); *Rosenkrantz 10811* (DAO).
- Potentilla* cf. *arenosa* × *P. vahliana*. CANADA. Nunavut: *Dansereau 5007030365* (MT); *Dansereau 5007030453* (MT); *Dansereau 500710-0152* (MT); Québec: *Cayouette J81-751* (DAO); *Mantion 1328* (QFA).
- Potentilla nivea*. CANADA. Nunavut: *Manning 24-june-1973* (DAO); *Manning 17-july-1973* (DAO); *Potter 8351* (MT); West Greenland: *Porsild 11-july-1929* (MT); Manitoba: *Gillett 1822* (MT); Québec: *Blondeau SK89110* (QFA); *Blondeau 86177* (BLOM); *Blondeau 634a* (BLOM); *Blondeau KG91072* (BLOM); *Blondeau GR-88116* (BLOM); *Grandtner 8096* (DAO); *Lemieux 21493* (QFA); Newfoundland: *Hay 74-274* (MT).
- Potentilla arenosa* × *P. nivea*. CANADA. Nunavut: *Brisson 20121* (BLOM); *Manning 21-june-1971* (DAO); *Parker 16-august-1971* (DAO); *Parmelee 3867* (DAO); West Greenland: *Erlanson 3129* (DAO); East Greenland: *Bay 89-119* (DAO); Manitoba: *Gillett 2556* (MT); Québec: *Aubin 17-july-1968* (BLOM); *Brisson 20153* (QFA); *Deshaye 108* (QFA); *Dignard 03-95a* (DAO); *Goulet 70-30* (QFA); *Turcotte 91-59* (QFA); Labrador: *Jackson 11-july-1970* (DAO).
- Potentilla pulchella*. CANADA. Nunavut: *Brassard 1539* (MT); *Dansereau 5006283261* (MT); *Gadbois 36* (MT); *Manning 15-july-1971* (DAO); *Wilson 22* (MT); West Greenland: *Porsild 4-july-1929* (MT); North Greenland: *Holmen 6595* (MT); Manitoba: *Beckett ?-?-1957* (MT); Québec: *Blondeau 84051* (BLOM); *Blondeau AU-93267* (QFA); Newfoundland: *Bouchard 87140* (MT).
- Potentilla* cf. *subgorodkovii*. CANADA. Nunavut: *Parmelee 1037* (DAO).
- Potentilla vahliana*. CANADA. Nunavut: *Anderson QSF-8413* (QFA); *Anderson QSF-8414* (QFA); *Anderson QSF-8415* (QFA); *Bruggeman 22* (DAO); *Chillcott 38* (DAO); *Coombs 96* (DAO); *Dansereau 5001715-0593* (MT); *Dansereau 5006292190* (MT); *Dansereau 500716-0868A* (MT); *Dansereau 500722-0388* (MT); *Scotter 45238* (DAO); West Greenland: *Erlanson 3004* (DAO); *Holmen 28-june-1962* (DAO); Québec: *Blondeau SW89002* (BLOM); *Huckel 5* (DAO); *Polunin 1535* (CAN).

APPENDIX 2. Description of quantitative and qualitative characters.

Quantitative characters. **FLOWN:** Number of flowers in one inflorescence. **EPICN:** Average number of epicalyx bract lobes per flower, going from 5 (all epicalyx bractlets entire) to 10 (all incised). **LFLTMAX:** Maximum number of leaflets per leaf found on the plant. **HAIRLEN:** Length of the terminal tuft of hair of the terminal leaflet (mm). **RACHISLEN:** Ratio of the length of the rachis (from the base of the proximalmost leaflet to the base of the terminal leaflet) to the length of the petiole including rachis. **LFLTLEN:** Length from base to tip of the longest terminal leaflet, excluding petiolule (mm). **FLOWSIZE:** Flower size, measured as the length from the insertion of the pedicel to the tip of the epicalyx bract (mm). **STYLEN:** Length of the style, including basal cone and verrucae (mm).

Leaf quantitative characters, divided by the end leaflet length in the statistical analyses. **PETIOLEN:** Petiole length, from the upper end of the insertion of the stipules to the base of the proximalmost leaflet (mm). **LFLTWID:** Widest width of the terminal leaflet, perpendicular to the midvein (mm). **LFLTDIS:** Distance between leaflets, length from the margin of the terminal leaflet to the end of the lateral leaflets, measured on the LFLTWID line (mm). **PETIOLULEN:** Length of the terminal leaflet petiolule, from the base of the distalmost subterminal leaflet to the base of the lamina of the terminal leaflet, sometimes ambiguous (mm). **TERMTEETHN:** Number of teeth of the terminal leaflet. **LATEETHN:** Number of teeth of the subterminal leaflet. **TTOOTHLEN:** Length from the tip of the terminalmost subterminal tooth to the tip of the terminal tooth of the leaflet (mm), bigger values indicate a terminal tooth that exceeds the adjacent lateral teeth. **TEETHLEN:** Length of the teeth in the middle of the terminal leaflet, measured from the base of the distal adjacent sinus to the end of the tooth, parallel to the associated secondary vein (mm). **SINUSLEN:** Width from midvein to sinus, measured from midvein to the deepest point of the sinus used for TEETHLEN, parallel to the associated secondary vein (mm). **TEETHWID:** Width at the base of the teeth used for TEETHLEN, perpendicular to the associated secondary vein (mm).

Flower quantitative characters, divided by the length of the hypanthium including epicalyx in the statistical analyses. **PETALEN:** Length from the insertion of the petal to its furthestmost point, generally one of the two lobes of a reniform petal, parallel to the axis of symmetry of the petal (mm). **PETALWID:** Widest width of the petal, perpendicular to PETALEN (mm). **SEPALEN:** Length from insertion to tip of the sepal, parallel to the axis of symmetry of the sepal (mm). **SEPALWID:** Widest width of the sepal, perpendicular to SEPALEN (mm). **EPICLEN:** Length from insertion to tip of the epicalyx bractlet, measured parallel to the

APPENDIX 1. Specimens included in the analyses, classified by species and then by province or region, from west to east and then north to south. Only first collector is given (in italics), followed by specimen number (italics) or collection date, and then herbarium (in parentheses).

Potentilla arenosa subsp. *arenosa*. CANADA. Nunavut: *Bruggeman 590* (DAO); *Bruggeman 660* (DAO); *Bruggeman 767* (DAO); *Dansereau 5006-1854* (MT); *Dansereau 5006273156* (MT); *Dansereau 5007100363* (MT); *Reading 25-june-1985* (DAO); West Greenland: *Porsild 8-july-1929* (MT); North Greenland: *Holmen 6601a* (MT); East Greenland: *Seidenfaden 20-july-1930* (MT).

Potentilla arenosa subsp. *chamissonis*. CANADA. Nunavut: *Rev. Father Choque 7-july-1963* (MT); West Greenland: *Böche 804* (DAO); Québec: *Blondeau SW89085* (QFA); *Blondeau WB90128* (QFA); *Blondeau WB94-008* (QFA); *Blondeau WB94-008* (BLOM); *Cayouette C7658* (DAO);

axis of symmetry of the epicalyx bractlet, only non-incised bractlets were measured when possible (mm). **EPICWID**: Widest width of the epicalyx bractlet, perpendicular to **EPICLEN** (mm).

Qualitative characters. **HABIT**: 1: densely tufted growth with leaves close to the caudex, 0: loosely tufted growth. **MARCLEAF**: 1: leaves marcescent for many years and ensheathing the caudex, 0: leaves not marcescent for many years. **TOMENTUM**: 1: leaf with dense abaxial tomentum completely obscuring leaf surface and giving a white color, 0: abaxial surface visible through the sparse tomentum giving a greyish color. **ADXTRICHORI**: 1: straight trichomes on adaxial face of leaf erect, 0: appressed, ?: not applicable. **ADXFLOCC**: 1: twisted and flat-

tened trichomes (floccose) present on adaxial face of leaf, 0: none. **ADXSTRAIGHT**: 2: two layers of straight terete trichomes on adaxial face of leaf, 1: one layer, 0: none. **ADXCRISP**: 1: appressed and contorted short trichomes (crispate) present on adaxial face of leaf, 0: none. **PETFLOCC**: 1: twisted and flattened trichomes (floccose) present on petiole, 0: none. **PETSTRAIGHT**: 2: two layers of straight terete trichomes on petiole, 1: one layer, 0: none. **PETRICHORI**: 1: long terete trichomes flexuose and appressed, 0: straight and erect to ascending, ?: not applicable. **PETCRISP**: 1: appressed and contorted short trichomes (crispate) present on petiole, 0: none. **VERRUC**: 2: straight trichomes clearly verrucose, 1: verruculose to wavy-walled, 0: smooth.