



# Interspecific variability in morphological characters of species of the genus *Dasiphora* in Northeast Asia

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## ABSTRACT

The results of the study of the interspecific morphological variability of five species of the genus *Dasiphora* Raf. are presented. We found that some qualitative and quantitative characters of leaf and calyx of *Dasiphora* are species-specific and suitable for species identification. The level of variability of quantitative morphological parameters was estimated using the coefficient of variation. Similarities and differences between species of the genus *Dasiphora* established by morphological characters confirm the taxonomic diversity in this genus.

**Keywords:** Rosaceae, *Dasiphora*, morphological parameters, leaf, flower calyx, natural populations, North-East Asia

## РЕЗЮМЕ

**Андышева Е.В., Кислов Д.Е. Межвидовая изменчивость морфологических признаков видов рода *Dasiphora* Северо-Восточной Азии.** Приведены результаты исследования межвидовой морфологической изменчивости пяти видов и одной разновидности рода *Dasiphora* Raf. Установлено, что некоторые качественные и количественные признаки листа и чашечки цветка видов рода *Dasiphora* видоспецифичны и могут быть использованы для идентификации. С помощью коэффициента вариации оценен уровень изменчивости количественных параметров. Сходства и различия видов рода *Dasiphora*, установленные по морфологическим признакам, подтверждают таксономический состав этого рода.

**Ключевые слова:** Rosaceae, *Dasiphora*, морфологические параметры, лист, чашечка цветка, природные популяции, Северо-Восточная Азия

Genus *Dasiphora* Raf. (= *Pentaphylloides* Hill = *Potentilla* L. s. l.) belongs to the family Rosaceae Juss. (Wolf 1908, Baikov 2012). In the Asian part of Russia, taxonomic diversity of the genus comprises five species and one variety: *D. fruticosa* (L.) Rydb, *D. parvifolia* (Fisch. ex Lehm.) Juz., *D. manshurica* (Maxim.) Juz., *D. davurica* (Nestler) Kom. with two varieties: var. *flava* Gorovoi, Pshennikova et Volkova and *D. gorovoi* Pshennikova (Pshennikova 2006, 2016, Baikov 2012). Despite the small number of species in the genus, questions of taxonomy are still far from being resolved; the system of the genus is constantly being refined in connection with the revisions of its scope. When studying the interspecific and intraspecific variability of organisms, the choice of traits, the number of which can be very large, is of great importance. However, both fundamental taxonomical works (Takhtajan 2009, Simpson 2019) and most other works dealing with the analyzes of the structure of woody plant species (Bondareva 1987, Semkina 1999, Godin 2009, Banaev 2010) focus on morphological characters used in the systematics of a particular taxon.

A detailed study of systematically important characters of the genus *Dasiphora* made it possible to conclude that the characters of leaf structure most often serve as diagnostic markers (Komarov & Klobukova-Alisova 1932, Vorobyov et al. 1966, Voroshilov 1966, Kurbatskii 1988, Kharkevich 1996, Koropochinsky & Vstovskaya 2002, Li et al. 2003). This

choice is largely justified by the fact that the leaf is the most important photosynthetic organ ensuring the plant functioning. Thus, its traits should be subjected to considerable selection pressure and bear adaptive load in populations, and most clearly reflect plant responses to environmental factors (Krasilov 1979, Yamada & Suzuki 1996). However, despite the known parallelism and convergence of leaf traits, they are often more stable than traits of other organs. The belief that modification plasticity combines with rapid evolution does not justify at all with respect to leaves; they are evolutionarily very conservative (Krasilov 1979). On this basis, leaf morphology deserves considerable attention when studying the adaptive variability of species.

The aim of the present study is to compare leaf and calyx morphological parameters of *Dasiphora* species from natural populations of Northeast Asia

## MATERIAL AND METHODS

Plant specimens (leaves and calyxes) of five species and one variety of the genus *Dasiphora* from 25 coenotic populations (CPs) of Northeast Asia were used as the material for the study. Samples of *D. fruticosa* were collected from 15 geographically distant habitats, one from the Magadan Region (CP 2), two from the Irkutsk Region (CPs 10 and 11), four from the Republic of Buryatia (CPs 12–15), four from the Transbaikalian Region (CPs 17–20), three from the

Amur Region (CPs 25, 26, 40), and one sample from the Primorye Territory (CP 49). Specimens of *D. davurica* were collected in four (CPs 50–53), *D. mandshurica* in three natural populations (CPs 56–58), and *D. gorovoi* (CP 55) and *D. davurica* var. *flava* (CP 54) are represented by single populations from Primorye Territory and one population of *D. parvifolia* (CP 16) from the Republic of Buryatia. In each natural population, at least 20 individuals in the middle-aged generative stage were selected. Five shoots were selected from each individual evenly throughout the crown during the period of full leaf formation. One undamaged lower leaf and one flower calyx were taken from each shoot. Quantitative and qualitative morphological parameters of leaf and flower calyx were preliminarily selected for the study (Table 1). Measurements were carried out with an electronic caliper "Mitutoyo" with an accuracy of 0.1 mm. Parameters of length and width of the third pair of leaflets were measured only on specimens of *D. parvifolia*, in which an additional third pair of leaflets is formed.

Leaf and calyx pubescence were determined using a Nikon SMZ 745T binocular microscope. According to Zaitsev's method of scoring (1973), we used a five-point scale: practically naked (no hairs on the studied leaf area), 1; singly pubescent (hairs standing alone), 2; sparsely pubescent (hairs scattered over the surface), 3; densely pubescent (hairs on 50 % of the surface), 4; woolly pubescent (hairs covering 100 %), 5. For each qualitative parameter, based on the point estimate of Zaitsev (1973), a scale of pubescence with five steps (with percentage of hair coverage) was generated: O – absent, ORe – very rare (10–20 %), Re – rare (30–40 %), G – dense (50–70 %), OG – very dense (80–100 %).

For each qualitative trait of pubescence, we calculated the ratio of occurrence of pubescent types among all the populations studied for each species separately.

The coefficient of variation ( $Cv$ ) was used as a measure of variability; its estimation was carried out according to the scale of variability levels proposed by Mamaev (1975). A level of variability was considered very low if  $Cv \leq 7\%$ , low if  $Cv = 8\text{--}12\%$ , average  $Cv = 13\text{--}20\%$ , high if  $Cv = 21\text{--}40\%$  and very high if  $Cv > 40\%$ . All obtained quantitative and qualitative biometric data were processed by methods of variation statistics with the help of applied statistical programs "Excel 7.0" and "Statistica 7.0". To study interspecific variability, mean values between individuals in the population were calculated for all studied species, and in the samples of three species, *D. frutivosa* (15 CPs), *D. davurica* (4 CPs), *D. mandshurica* (3 CPs), mean values between available populations were additionally calculated.

For the construction of a decision tree for identification of *Dasiphora* spe-

cies classification purposes, data preparation operations included: 1) data cleaning, 2) quantitative features scaling / shifting, 3) qualitative features encoding. During automatic data cleaning all rows, that included not-a-number or non-existing values were removed from the dataset. The only one item with a non-existing value was removed during this stage. Scaling and shifting procedure was applied to all quantitative columns in the dataset. It consisted in removing the mean and scaling to unity standard deviation. To encode qualitative features, we used one-hot encoding transform from the Scikit-Learn package. This, in turn, led to extending the number of the data table columns related to qualitative features. All these stages were automatically performed by means of Scikit-Learn package (Pedregosa et al. 2011). We did not use any of dimensionality reduction approaches, because they lead to smoothing the feature meaning, that are important when dealing with building taxonomical hierarchy.

We used an optimized version of the CART algorithm implemented in the Scikit-Learn package to construct a binary decision tree. When all the morphological features were used, we led to a quite simple tree that exploited the set of qualitative features only.

It is common, when constructing a decision tree, to prune it to reduce the number of its sections and size. Moreover, the decision trees pruning approach is used to avoid classifier overfitting problem (Hastie et al. 2008). Due to internal structure of the dataset we obtained a perfect decision tree, that doesn't need any pruning. It is worth noting, that we could not estimate classification accuracy in this case. More precisely, if we tried to do this, classification

**Table 1.** Morphological parameters of leaves and flower calyx species of the genus *Dasiphora* of Northeast Asia

Type of data	Parameter	Parameter code
Quantitative	1. 1st pair length (1st leaflet)	L1p1
	2. 1st pair length (2nd leaflet)	L1p2
	3. 2nd pair length (3rd leaflet)	L2p3
	4. 2nd pair length (4th leaflet)	L2p4
	5. 1st pair width (1st leaflet)	W1p1
	6. 1st pair width (2nd leaflet)	W1p2
	7. 2nd pair width (3rd leaflet)	W2p3
	8. 2nd pair width (4th leaflet)	W2p4
	9. 3rd pair length (6th leaflet)	L3p6
	10. 3rd pair length (7th leaflet)	L3p7
	11. 3rd pair width (6th leaflet)	W3p6
	12. 3rd pair width (7th leaflet)	W3p7
	13. Displacement of the central vein 2nd pair (3rd leaflet)	S2p3
	14. Displacement of the central vein 2nd pair (4th leaflet)	S2p4
	15. Length of the terminal lobe	Lkd
	16. Width of the terminal lobe	Wkd
	17. Distance from apex to the widest part of the terminal lobe	Dvsh
	18. Distance from base to the widest part of the terminal lobe	Dosh
	19. Petiole length	Lp
	20. Distance between pairs of leaflets	Dpl
Qualitative	21. Pubescence of the upper part of the leaf	Dv1
	22. Pubescence of the lower part of the leaf	Dn1
	23. Pubescence of the petiole	Dp
	24. Pubescence of the calyx	Dc
	25. Pubescence of the pedicel	Dpc
	26. Pubescence of the upper side the outer sepal	Dvns
	27. Pubescence of the lower side the outer sepal	Dnns
	28. Pubescence of the upper side the inner sepal	Dvvs
	29. Pubescence of the lower side the inner sepal	Dnvs

accuracy would be 1.0, that means no misclassification cases can occur. A subset of qualitative features selected by the CART algorithm distinguishes the source dataset in a consistent way, with no contradictions. If we assume the dataset is representative, we don't need to make any further investigations: we have got a good decision tree, that can be used to precisely determine df-species by their morphological features.

In addition, we used Ward's method implemented in scikit-learn to perform hierarchical cluster analysis of data.

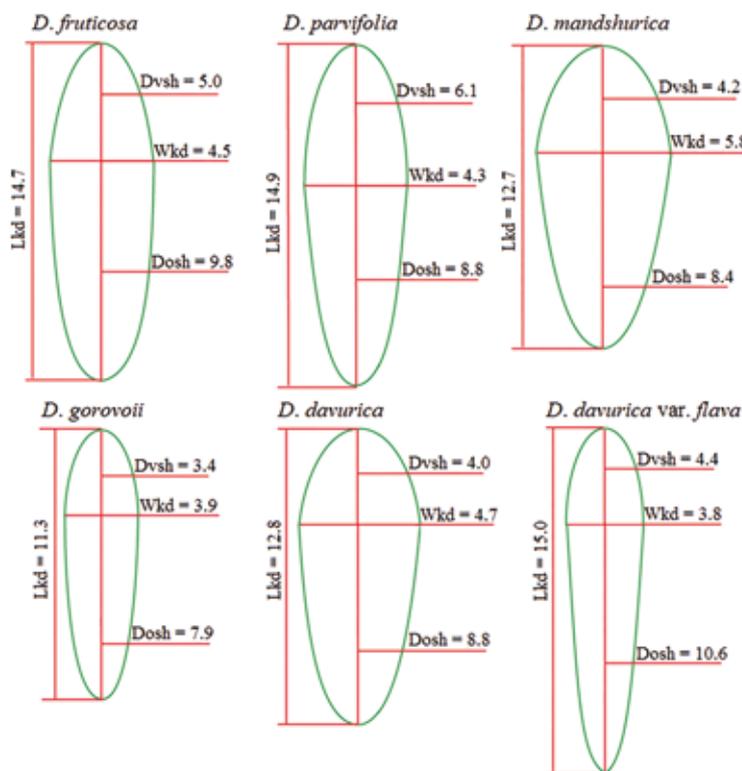
## RESULTS AND DISCUSSION

One of the tasks of morphological studies was to determine the variation of qualitative and quantitative morphological parameters of species of the genus *Dasiphora*, as well as to determine the most informative parameters for specimen identification at the interspecific level. In this connection, mean values and coefficient of variation for samples of all six species were calculated (Table 2).

Analysis of interspecific differentiation of quantitative characters showed that three taxa: *D. fruticosa*, *D. parvifolia* and *D. davurica* var. *flava* were the highest, *D. gorovoi* the lowest, and *D. mandshurica* and *D. davurica* the intermediate values of the coefficients of variation of the "length of 1–4 leaflets" and "length of the terminal lobe". At the same time, very low ( $Cv = 6–7\%$ ) and low coefficients of variation ( $Cv = 8–11\%$ ) were shown for these linear morphological parameters among all species (Table 2). According to the second parameter "width of 1–4 leaflets", some differences among leaflets were observed. Thus, the highest values of "width of 1–4 leaflets" are found in *D. mandshurica*, which

has the widest leaflets, the lowest values of width of 1 and 2 leaflets are found in *D. parvifolia*, and width of 3 and 4 in *D. davurica* var. *flava*, which has narrower leaflets (Fig. 1). According to the parameter "width of terminal lobe", the highest values were also noted in *D. mandshurica*, the lowest in two taxa: *D. gorovoi* and *D. davurica* var. *flava*. At the same time, the parameters "width of 1–2 leaflets" in two taxa: *D. gorovoi* and *D. davurica* var. *flava*, and "width of 3–4 leaflets" in *D. davurica* var. *flava* were recorded at the average level of variation ( $Cv = 13–17\%$ ). In other taxa the "width of 1–4 leaflets" is characterized by very low ( $Cv = 3–5\%$ ) and low levels of variation ( $Cv = 8–12\%$ ), as well as the parameter "width of terminal lobe" for all studied taxa ( $Cv = 4–12\%$ ) (Table 2). Thus, the length and width of individual five (1–4 leaflets, terminal lobe) leaflets of the whole leaf blade show that two taxa, *D. fruticosa* and *D. davurica* var. *flava*, have elongated leaflets of oblong-ovate shape. In *D. davurica*, the leaflets are somewhat shorter than in the previous two species, elongate, extending in the upper part. *D. parvifolia* has elongated narrower leaflets of linear or linear-lanceolate shape; *D. mandshurica* has shortened, broad leaflets of obovate shape; *D. gorovoi* has shortened narrowed leaflets of ovate-lanceolate shape (Fig. 1).

The greatest displacement of the central vein of the 3rd and 4th leaflets was noted in *D. davurica* and *D. mandshurica*, and the smallest in *D. parvifolia*. Judging by the very low coefficient of variation ( $Cv = 4–7\%$ ) in *D. davurica*, low ( $Cv = 11\%$ ) and medium ( $Cv = 16\%$ ) in *D. mandshurica*, the central vein deviation is a fairly stable feature in these taxa. In other species, this parameter is marked by a high level of variability ( $Cv = 21–30\%$ ) (Table 2). Parameters "distance from apex to the widest part of the terminal lobe" and "distance from base to the widest part of the terminal lobe" are quite stable and characterized by very low and low coefficients of variation ( $Cv = 6–12\%$ ). The exception is "distance from the apex to the widest part of the terminal lobe" in one species, *D. fruticosa*, where an average level of variability ( $Cv = 18\%$ ) was noted (Table 2). At the same time, the maximum value of "distance from apex to the widest part" was noted in *D. parvifolia* and *D. fruticosa*, the "distance from the base to the widest part" – in *D. davurica* var. *flava*, and the minimal values of these two parameters are noted in *D. gorovoi* (Fig. 1). Considering the parameter "petiole length", it should be noted that in *D. fruticosa* the maximum values of the petiole length and in *D. parvifolia* the smallest values are established. Values of the parameter "distance between pairs of leaflets" nearly for all species are at the same level, and the minimum is noted for *D. davurica* (Table 2). It should be noted that the variation coefficient of the parameter "petiole length" in *D. fruticosa* ( $Cv = 22\%$ ) and *D. gorovoi* ( $Cv = 21\%$ ) is at a high level, in *D. davurica* var. *flava* ( $Cv = 16\%$ ) at an intermediate level, in *D. parvifolia* and *D. mandshurica* ( $Cv = 11–12\%$ ) at low, and in *D. davurica* ( $Cv = 5\%$ ) at very low levels of variability. The parameter "distance between pairs



**Figure 1** The shapes of terminal lobe of leaf of species of the genus *Dasiphora* in Northeast Asia. See Table 1 for abbreviations of parameter codes

**Table 2.** Variability in morphological characters of species of the genus *Dasiphora* in Northeast Asia. The data are presented as the mean value ± standard deviation / the coefficient of variation (Cv, %), <sup>1</sup> – a space means that this morphological parameter is absent in taxa

Parameter	Taxon					
	<i>D. fruticosa</i>	<i>D. parvifolia</i>	<i>D. mandsburica</i>	<i>D. gorovoi</i>	<i>D. davurica</i>	<i>D. davurica var. flava</i>
L1p1	14.4±1.3 9	14.3±1.0 7	11.6±0.9 7	9.5±0.9 10	11.9±0.7 6	14.9±1.0 7
L1p2	14.5±1.3 9	14.8±1.0 7	11.6±0.9 8	9.5±0.9 10	12.0±0.7 6	14.8±1.0 6
W1p1	4.3±0.5 11	3.7±0.4 10	5.7±0.4 8	3.9±0.5 13	4.9±0.2 5	4.0±0.7 17
W1p2	4.3±0.5 11	3.6±0.4 10	5.7±0.5 9	3.9±0.5 13	5.0±0.2 3	4.0±0.7 17
L2p3	14.1±1.4 10	14.6±1.1 7	12.2±1.1 9	9.8±0.9 9	12.0±0.8 7	14.3±1.1 8
L2p4	14.2±1.4 10	14.7±1.2 8	12.5±1.2 9	9.9±0.9 9	12.2±0.9 8	14.2±1.4 10
W2p3	4.0±0.5 12	4.0±0.5 11	5.1±0.3 5	3.6±0.4 12	4.2±0.2 5	3.3±0.5 16
W2p4	3.9±0.5 12	4.0±0.5 13	5.1±0.3 5	3.6±0.4 11	4.2±0.2 5	3.3±0.5 15
L3p5	– <sup>1</sup>	11.9±1.5 13	–	–	–	–
L3p6	–	12.0±1.6 14	–	–	–	–
W3p5	–	3.5±0.6 17	–	–	–	–
W3p6	–	3.4±0.6 16	–	–	–	–
S2p3	0.4±0.1 22	0.3±0.1 26	0.5±0.1 16	0.4±0.1 22	0.5±0.0 4	0.4±0.1 30
S2p4	0.3±0.1 21	0.2±0.1 26	0.4±0.0 11	0.3±0.1 26	0.4±0.0 7	0.3±0.1 27
Lkd	14.7±1.3 9	14.9±0.9 6	12.7±1.1 9	11.3±1.2 11	12.8±0.8 7	15.0±0.9 6
Wkd	4.5±0.4 10	4.3±0.4 8	5.8±0.5 8	3.9±0.5 12	4.7±0.2 4	3.8±0.5 12
Dvsh	5.0±0.9 18	6.1±0.5 8	4.2±0.4 10	3.4±0.4 10	4.0±0.4 11	4.4±0.4 9
Dosh	9.8±1.0 11	8.8±0.7 8	8.4±0.7 8	7.9±1.0 12	8.8±0.5 6	10.6±0.6 6
Lp	9.5±2.1 22	5.8±0.7 12	9.0±1.0 11	8.7±1.8 21	6.7±0.3 5	7.4±1.4 19
Dpl	2.2±0.4 16	2.4±0.3 12	2.4±0.3 12	2.3±0.3 14	1.9±0.1 3	2.2±0.3 16

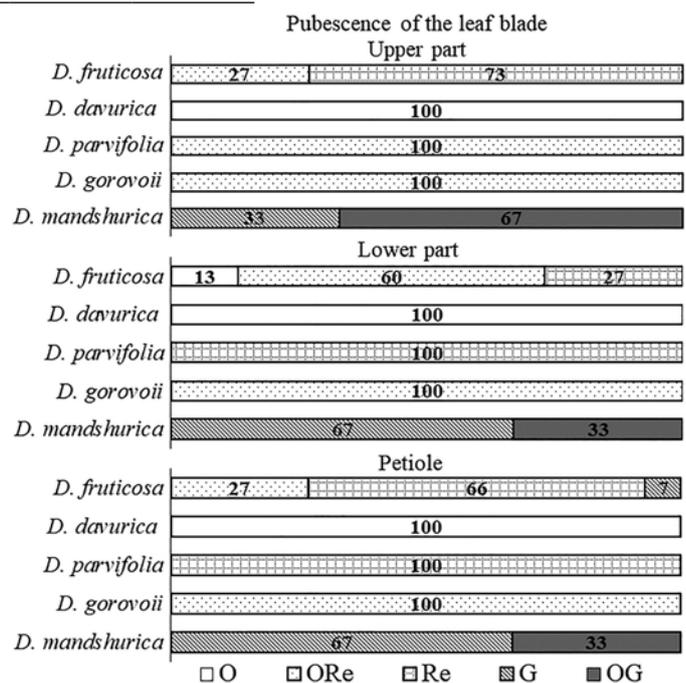
of leaflets" is most stable in the three species with very low in *D. davurica* (Cv = 3 %) and low in *D. parvifolia* and *D. mandsburica* (Cv = 12 %) coefficients of variation. The other three taxa, *D. fruticosa*, *D. davurica var. flava* (Cv = 16 %), and *D. gorovoi* (Cv = 14 %), the parameter "distance between pairs of leaflets" is at the average level of variability (Table 2).

According to the results of calculation of the share of occurrence of pubescence types, we found that for a number of leaf and calyx parameters some types of pubescence are species-specific and suitable to identify the species. According to the results of the analysis of interspecific variability of pubescence, we can see that the most informative features appear to be pubescence of the upper and lower parts of the leaf blade and pubescence of the petiole (Fig. 2).

Two taxa, *D. gorovoi* and *D. davurica var. flava* have stable monotypic pubescence in all characters, which is not variable at intraspecific level, which distinguishes them from all taxa (Figs 2, 3). In *D. davurica var. flava*, the leaf and petiole are not pubescent, and the pedicel,

calyx, and sepal have very rare (or single) pubescence. The leaf, petiole and sepals of *D. gorovoi* also have very rare pubescence, and the pedicel and calyx are rare. In *D. davurica*, the leaf, petiole, and inner sepal pubescence parameters are also monotypic and stable at the intraspecific level, with the outer sepal and pedicle having a combination of very rare and rare pubescence and only the calyx having a mixed type of very rare, rare, and dense pubescence. For *D. mandsburica*, the same combination of dense and very dense pubescence is noted for all leaf and calyx parameters. In two species, *D. fruticosa* and *D. parvifolia*, the mixed type of pubescence was noted, where the intraspecific variation of pubescence of all parameters of the leaf and flower cup is clearly traced (Figs 2, 3).

It should also be noted that for the taxa *D. davurica* and *D. davurica var. flava*, and for the species *D. mandsburica*, the combination of very dense and dense pubescence types of the lower and upper sides of the leaf blade are diagnostic and are considered the most stable and informative. Thus, a variation of the pubescence trait is observed in four species. At the same time, the pubescence character of *Dasiphora* species in many "keys" is diagnostic. It should also be noted



**Figure 2** Ratio (%) of occurrence of pubescence types of leaf of species of the genus *Dasiphora* among all the populations

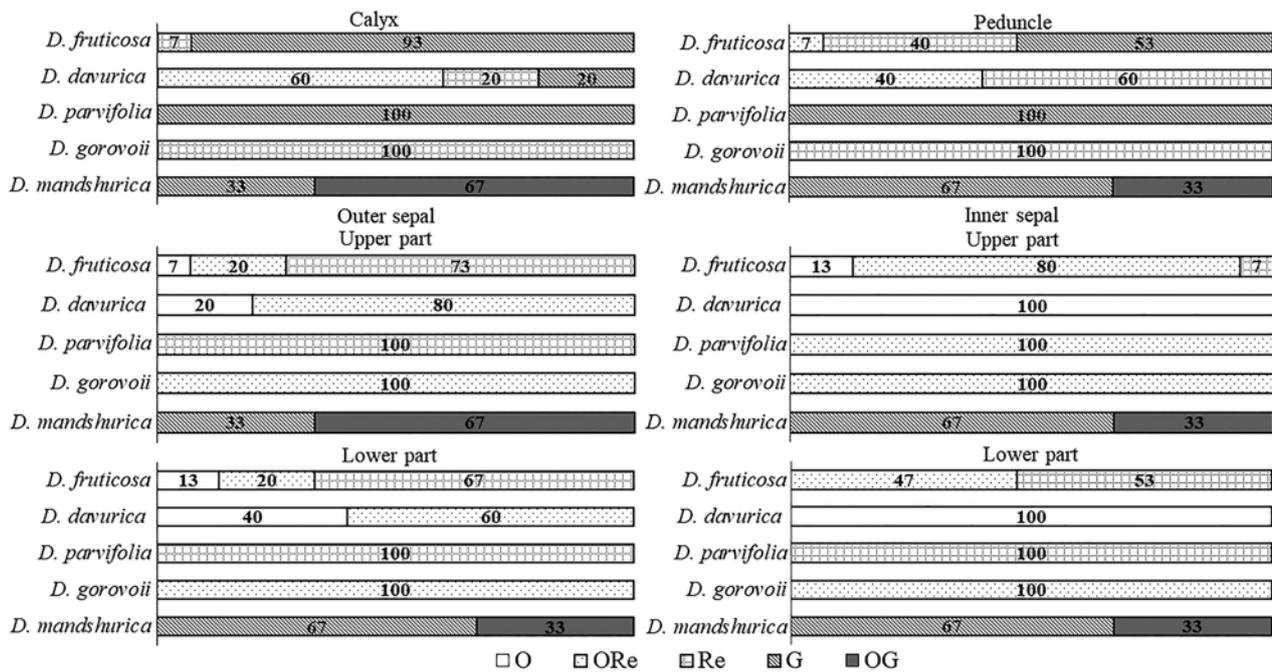


Figure 3 Ratio (%) of occurrence of pubescence types of calyx of species of the genus *Dasiphora* among all the populations

that the degree of variability of pubescence in different species of the genus *Dasiphora* varies, possibly depending on the influence of environmental factors.

The cluster analysis carried out on the whole set of quantitative characters by Ward's method showed that all species were divided into two main clusters (Fig. 4). The first cluster included all white-flowered taxa: *D. davurica*, *D. mandshurica*, and *D. gorovoi*. It should be noted that the species *D. davurica* and *D. mandshurica*, joined by *D. gorovoi*, which has been described as a hybrid taxon between the two latter (Pshennikova 2006), which is confirmed by the results of biochemical studies (Khranova et al. 2014, Andysheva et al. 2018). Thus, the closest related taxa for *D. gorovoi* are *D. davurica* and *D. mandshurica*. The second cluster included yellow-flowered taxa, of which *D. fruticosa* and the variety *D. davurica* var. *flava* are the closest, and *D. parvifolia* is adjacent to them. According to its morphological characteristics, *D. davurica* var. *flava* has similar qualitative characters (absence of pubescence, presence of glossy leaf blade) to the white-flowered taxon *D. davurica*, and *D. fruticosa* shares qualitative characters (yellow flower coloration). According to the results of cluster analysis of quantitative parameters, *D. davurica* var. *flava* can be considered a closely related taxon to *D. fruticosa*. Such a division of species indicates close relatedness in each of the groups.

Based on the results of the analysis of all 29 qualitative and quantitative morphological parameters, using an optimized version of the CART algorithm, the contribution of each trait at the interspecific level was automatically weighted and determined. As a result, the most optimal and relatively small subset of characters was selected, which, with a reliable sample of images, allowed to construct a classification tree (Fig. 5).

After a detailed analysis of the classification tree, a simplified key for determining species of the genus *Dasiphora* according to a certain set of qualitative characters was obtained.

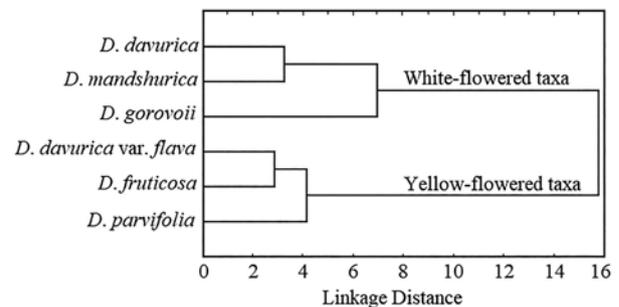


Figure 4 The dendrogram of species of the genus *Dasiphora* by quantitative parameters (Ward's method, Euclidian distances)

**The key for definition of species of genus *Dasiphora* on qualitative characters**

- Coloration of corolla, flowers white ..... 2
- Flowers yellow ..... 4
- 2. The upper side of the leaf is bare, leathery, glossy, pubescence is completely absent ..... ***D. davurica***
- The upper side of the leaf is pubescent ..... 3
- 3. Pubescence of the lower side of the inner sepals, single (very rare) ..... ***D. gorovoi***
- Pubescence of the underside of the inner sepal dense or very dense ..... ***D. mandshurica***
- 4. Petiole naked, no pubescence at all ..... ***D. davurica* var. *flava***
- The petiole is pubescent ..... 5
- 5. Presence of the third pair of leaflets (6, 7 leaflets) ..... ***D. parvifolia***
- Traits are different ..... ***D. fruticosa***

Thus, five statistically significant characters, which are the most suitable for species differentiations and which, partially, confirm characters used in keys of different keys (Vorobyov et al. 1966, Voroshilov 1966, Kurbatskii 1988, Koropachinsky & Vstovskaia 2002, Li et al. 2003) were selected.

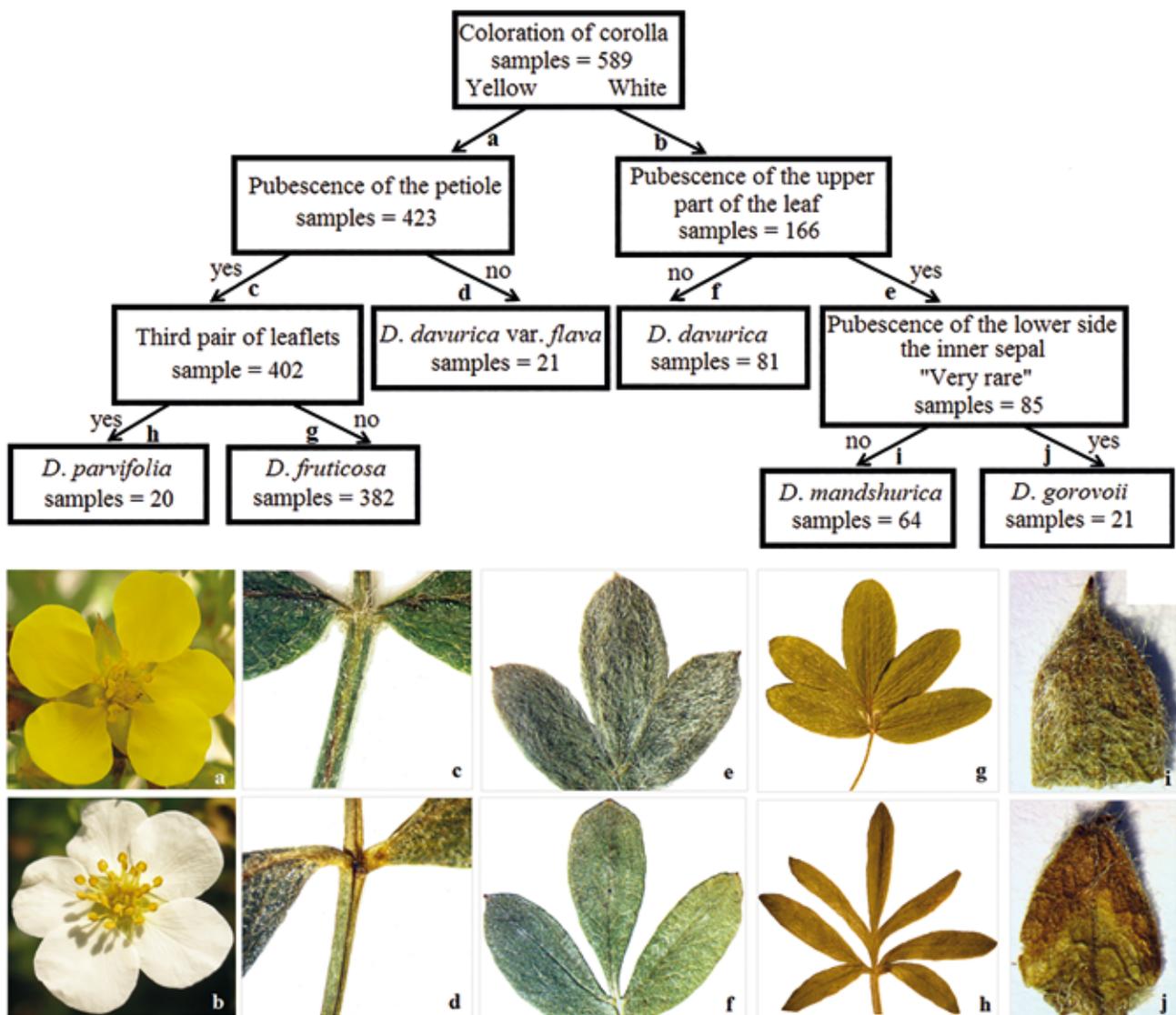


Figure 5 Classification tree of species of the genus *Dasiphora* by qualitative parameters

## CONCLUSION

An analysis of the interspecific morphological variability of species of the genus *Dasiphora*, based on the coefficient of variation, showed that most of the parameters: "length and width of leaflets and sepals", "distance from the apex to the widest part of the terminal lobe" and vice versa, "distance between pairs of leaflets" for all six species, for some species "petiole length" and "central vein mixing" are at very low, low and medium levels of variability. Thus, these parameters can be considered fairly stable parameters at the intraspecific level and can be used for species identification. The analysis of variance showed that qualitative parameters of pubescence for four taxa: *D. davurica*, *D. davurica* var. *flava*, *D. mandshurica* and *D. gorovoi* are sufficiently stable and can be used for identification. Species *D. parvifolia*, *D. fruticosa*, have a mixed type of pubescence, with a rather high level of variability, which may partly depend on a wide range of climatic conditions of growth throughout their distribution range. Using cluster analysis, all species are divided into two main clusters, white-flowered and yellow-flowered species, which indirectly confirms their most closely related relationships.

Using an optimized version of the CART algorithm, the contribution of each morphological trait at the interspecific level was automatically weighted and determined, which were interpreted in the form of decision trees, based on the analysis of which, a simplified key was constructed to identify species of the genus *Dasiphora*.

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