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RESEARCH PAPER

Achene morphology of the Far Eastern species of the genus *Dasiphora* Raf. (Rosaceae): Systematic implications

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ABSTRACT

This paper presents results of morphological comparison between five Far Eastern taxa in the genus *Dasiphora*. Such morphological characteristics as size and pubescence of achenes show significant difference of *D. davurica* var. *flava* from other Far Eastern taxa of this genus. These data and our previous studies demonstrate taxonomic peculiarities of the *D. davurica* var. *flava* and bring to the idea of its consideration at the level of species. The new nomenclatural combination *Dasiphora flava*: (Vorosch.) Gorovoj, Pshenn. et S. Volkova is proposed.

Keywords: *Dasiphora*, *Potentilla*, *Pentaphylloides*, the Russian Far East, morphology, achene, taxa

РЕЗЮМЕ

Пшенникова Л.М. Морфология орешков дальневосточных видов рода *Dasiphora* Rafin (Rosaceae): систематическое значение. Приведены результаты морфологических исследований орешков пяти таксонов дальневосточных представителей рода *Dasiphora*. Показано, что таксон *D. davurica* var. *flava* размерами орешков и их опушением значительно отличается от других дальневосточных представителей рода. Полученные данные указывают на необходимость придания разновидности *D. davurica* var. *flava* статуса вида. Приводится новая номенклатурная комбинация *Dasiphora flava* (Vorosch.) Gorovoj, Pshenn. et S. Volkova.

Ключевые слова: *Dasiphora*, *Potentilla*, *Pentaphylloides*, российский Дальний Восток, морфология, орешек, таксон

Plants of the genus *Dasiphora* belong to shrubs. Representatives of this genus are also considered as *Pentaphylloides* Duham (Yakubov 1996, Koropachinskiy & Vstovskaya 2002) or *Potentilla* L. (Voroshilov 1982, 1985, Kamelin 2001). In recent floristic summaries (Kozhevnikov & Probatova 2006, Baikov 2012) the shrubby potentillas were treated under the name *Dasiphora* Rafin. The genus *Dasiphora*, family Rosaceae includes 10 species distributed in Europe, Asia and North America (Shipchinsky 1954). Species of the genus are well known for their ornamental and medicinal properties (Triel & Sokolenko 2010). The genus *Dasiphora* is represented in the Russian Far East by 5 taxa: *D. davurica* (Nestl.) Kom. et Alis., *D. mandshurica* (Maxim.) Juz., *D. fruticosa* (L.) Rydb. (Probatova & Barkalov 2006), *D. gorovoii* Pshenn. (Pshennikova 2006) and *D. davurica* var. *flava* (Vorosch.) Gorovoj, Pshenn. et S. Volkova (Volkova et al. 2009). However, the systematic position of *D. davurica* var. *flava* remains unclear. Under the name *Potentilla davurica* Nestl. var. *flava* Vorosch. this plant was first identified and described from Olginsky district, Primorsky Krai by Voroshilov (1972) more than 40 years ago. Voroshilov (1972) noted that *Potentilla davurica* plants collected from this site have unusually dark-yellow flowers, small, bare and narrow leaflets with a slightly bluish color, so he described these plants as *P. davurica* var. *flava* Vorosch. Later, this author proposed a new combination for *P. glabrata* Willd – *P. fruticosa* L. subsp. *glabrata* (Willd.) Vorosch. and indicated *P. davurica* as its synonym (Voroshilov 1982, 1985).

To compare *D. davurica* var. *flava* from Olginsky district with other Far Eastern taxa of this genus, we studied its chromosome numbers and anatomical structure of leave epidermis and petioles (Volkova et al. 2009, Volkova & Pshennikova 2011, Pshennikova & Volkova 2013) and came to conclusion that this taxon can be the result of natural hybridization.

Morphological characteristics of seeds are important for classification of individual species into groups within genera (Belyaev 1984, Kovtonyuk 1994, Kozhanchikov 1967). Sculpture of achenes' surface and the presence of trichomes are important systematic features for the genus *Potentilla*. Since *Dasiphora* plants show high variability in this feature (Kurbatsky 1984, 2008), our objective was to identify taxonomic specialty and morphology of achenes of representatioves of the genus *Dasiphora* of the Russian Far East.

MATERIAL AND METHODS

We studied morphological features of achenes of 5 taxa from the genus *Dasiphora* using plants from living collections of the Botanical Garden-Institute FEB RAS (Vladivostok, Russia) that were grown under ecologically similar conditions. Plants of *D. davurica*, *D. davurica* var. *flava*, *D. mandshurica*, *D. gorovoii*, and *D. fruticosa* were transplanted from their natural habitat in the Russian Far East (Fig. 1). In living collection, *D. fruticosa* was represented by plants from two collection areas: Mt. Olkhovaya (1669 m), Sikhote-Alin Mountains, Primorsky Krai and upper reaches of

Herbie river, Badzhalsky Mountain Range, Khabarovsky Krai. Achenes of each taxon were collected from 3–5 plants in October, 2013. A total of 50 achenes from each taxon were examined. We measured the length and maximum width of 10–12 achenes and also measured the length of trichomes. The structure of the achene surface was studied using a SEM EVO 40 (Carl Zeiss SMT) and a SM Stemi 2000-C (Carl Zeiss, Axiovision 4.8) microscopes. The data were processed using Statistica 9.0. The results are presented as mean values with standard errors. We used one-way ANOVA algorithm, consisted of following steps: 1) testing for normality (Shapiro-Wilk test); 2) testing the equality of variances (Bartlett's test) 3) applying standard parametric (Fisher's ANOVA) or non-parametric (Kruskal-Wallis test) ANOVA procedure; 4) performing pairwise comparison of averages (Tukey test or Welch test with Bonferroni correction). All computations were performed with an aid of SciPy (Jones 2001-2016).

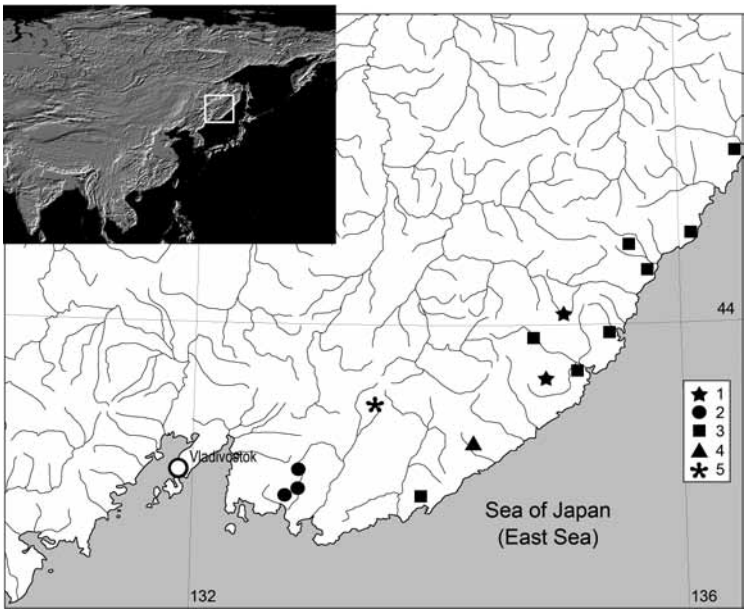

Figure 1 Location of sapmling sites of *Dasiphora* taxa in Primorsky Krai. 1 – *D. davurica* var. *flava*, 2 – *D. davurica*, 3 – *D. mandshurica*, 4 – *D. gorovoii,* 5 – *D. fruticosa*

RESULTS AND DISCUSSION

The achenes from five taxa were studied by SEM. This technique showed that the ultrastructures of the achenes of all studied species were formed by reticulate cells (Fig. 2).

D. fruticosa

According to our data, the achenes from both populations are similar. They vary from 1.24 to 1.71 mm in length (Table 1) and from 0.60 to 0.77 mm in width, have ovoid shape, mostly with slightly flattened sides, light brown to brown in colour, and densely villous. The villi consist of simple long trichomes, and the trichomes of plants from alpine populations are the longest compared with those of the other studied taxa (Table 1) and significantly longer (0.5 mm) than those of the eponymous species from Khabarovsky Krai. The middle part of the achene may be densely or sparsely villous. On achenes with pointed tops, there is a roundish scar. Two flat protuberances are noticeable on the ventral side. On the dorsal side they are rounder, narrowinging to form a scar. There are also short protuberances that appear as ledges of the pericarp. Our study coincides with published morphological features of achenes of *D. fruticosa* (Triel 1985, Triel & Sokolenko 2010).

D. mandshurica

The morphological features were investigated for the first time. Achenes varied from 1.58 to 2.0 mm in length and from 0.65 to 0.91 mm in width, with an ovoid shape, mostly with slightly flattened sides, with a pointed apex, villous, almost yellow or light brown in colour. The villi consist of simple long trichomes and are evenly distributed on the surface. Two flat ptotuberances are noticeable on the ventral side, and the dorsal side is rounder, narrowing to form a scar. There are also short protuberances on the sides that appear as ledges of the pericarp. Achenes do not differ in form from the two types described above. Achenes of *D. mandshurica* are longer and wider than achenes of *D. fruticosa*, but its values are close to those of *D. gorovoii* (Table 1). There is a statistically significant difference between lengths and widths of achenes from different species.

D. gorovoii

The morphological features of these achenes were studied for the first time. Achenes vary from 1.39 to 1.8 mm in length and from 0.60 to 0.91 mm in width, have an ovoid shape, mostly with slightly flattened sides, with a pointed apex, villous, and yellowish or light brown in color. The villi consist of simple long trichomes and are evenly distributed on the surface. Two flat protuberances are noticeable on the ventral side; the dorsal side is rounder, narrowing to form a scar. There are also short protuberances on the sides that appear as ledges of the pericarp. Achenes do not differ in form or colour from those of *D. mandshurica*, but are smaller in size (Table 1). There is a statistically significant difference between lengths and widths of achenes of *D. mandshurica* and *D. gorovoii*.

Table 1. Morphometric characteristics of *Dasiphora* achenes

Taxon (specimen)	Length, mm	Width, mm	Length of trichomes, mm
D. davurica	1.54 ± 0.28	0.71 ± 0.14	1.25 ± 0.32
D. davurica var. *flava*	2.19 ± 0.35	1.09 ± 0.04	1.33 ± 0.06
D. mandshurica	1.81 ± 0.05	0.80 ± 0.03	1.36 ± 0.04
D. gorovoii	1.52 ± 0.03	0.73± 0.002	1.41 ± 0.04
D. fruticosa (Mt. Olkhovaya)	1.50 ± 0.04	0.76 ± 0.01	1.56 ± 0.05
D. fruticosa (Khabarovsk)	1.52 ± 0.06	0.64 ± 0.02	1.08 ± 0.03

D. davurica

Achenes vary from 1.35 to 1.7 mm in length and from 0.62 to 0.77 mm in width, with an ovoid shape, primarily with slightly flattened sides, with a pointed apex, villous, yellowish

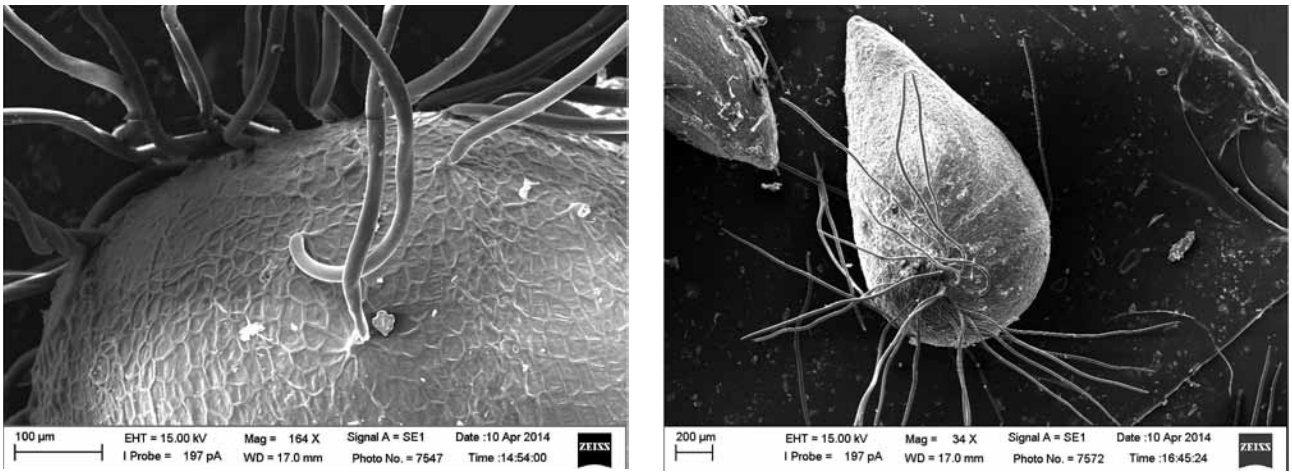

Figure 2 SEM micrographs of the surface of an achene of *Dasiphora davurica* var. *flava*. Sculpture of achene's surface is on the left and general view of achene on the right images

or light brown in colour. The villi consist of simple long hairs or trichomes and are concentrated primarily around the scar at the base of the achene and at the top. The middle part of the achene may be villous, rarely covered with villi or sometimes naked. Achenes with pointed tops have roundish scar. Two protuberances are noticeable on the flatter ventral side, narrowing to form a scar on the rounder dorsal side. There are also short protuberances on the sides that appear as ledges of the pericarp.

D. davurica* var. *flava

The morphological features of achenes were studied for the first time, revealing a fairly clear distinction between this taxon and the other studied species. The achenes vary from 2.04 to 2.35 mm in length and from 0.9 to 1.41 mm in width. They are larger, almost yellow or light brown in colour, ovoid or pear-shaped, with villi concentrated at the achene's base, the rest of the achene is naked (Figs. 2 and 3). It is believed that immature achenes exhibit denser villi

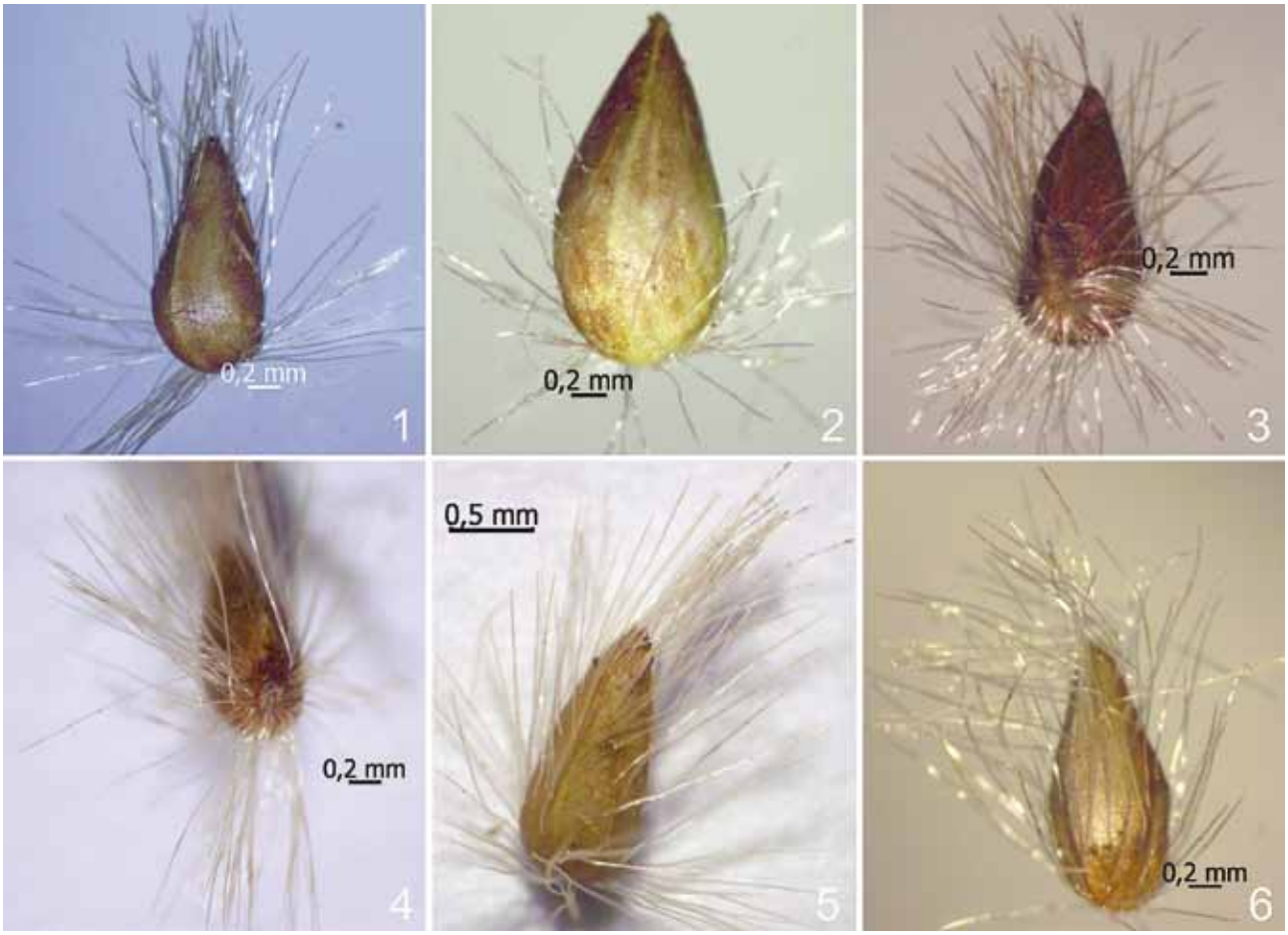

Figure 3 General view of the achenes of *Dasiphora*. 1 – *D. davurica*, 2 – *D. davurica* var. *flava*, 3 – *D. fruticosa* (Khabarovsk population), 4 – *D. fruticosa* (Mt. Olkhovaya population), 5 – *D. gorovoii,* 6 – *D. mandshurica*

coverage (Trill 1985, Trill & Sokolenko 2010); therefore, we compared both immature and mature achenes. Villi in this taxon is the same in both phases.

Taxonomically significant characters

The results of studying achenes' length applying the one-way ANOVA algorithm are following:

- sample distribution is normal: *D. fruticosa* (Shapiro-Wilk test, p = 0.699); *D. davurica* (p = 0.607); *D. davurica* var. *flava* (p = 0.037);
- Bartlett's test shows that sample variances could be treated as equal (p = 0.824).

Therefore classical one-way ANOVA was performed. One-way ANOVA (Fisher's ANOVA) showed significant differences between groups ($p < 0.001$). So, next step is to uncover these differences by using pairwise comparison (Tukey's test). Applying Tukey's test with two levels of significance ($\alpha = 0.01$ and $\alpha = 0.05$) leaded to following (at both levels): *D. davurica* and *D. fruticosa* do not differ significantly; pairs *D. davurica* and *D. davurica* var. *flava* and *D. davurica* var. *flava* and *D. fruticosa* are significantly different (Fig. 4).

The results of studying achenes' width by the same procedure are:

- data normality: *D. fruticosa* (p = 0.142); *D. davurica* (p = 0.107); *D. davurica* var. *flava* (p = 0.450);
- Bartlett's test to the data shows that samples have unequal variances ($p < 0.001$).

Thus we used non-parametric one-way ANOVA to handle this case correctly. Using Kruskal-Wallis test yields to significant differences between groups ($p < 0.001$). Due to unequal variances we used Welch's test with Bonferroni correction to make parwise comparison of averages in this case. From this computational step we got the following (at the levels of significance $\alpha = 0.01$ and $\alpha = 0.05$) (Fig. 4): *D. davurica* and *D. fruticosa* do not differ significantly; pairs *D. davurica* vs *D. davurica* var. *flava* and *D. davurica* var. *flava* vs *D. fruticosa* are significantly different.

According to our recent observations, important peculiarity of *D. davurica* var. *flava* is tomentose inner side of the stipules, which is not typical for other four taxa from this genus (Fig. 5, 6). This variety combines morphological characteristics from both *D. fruticosa* and *D. davurica*. *D. davurica* var. flava has the same number of chromosomes (2n = 14) as *D. fruticosa* (Volkova et al. 2009). Both *D. davurica* var. *davurica* and *D. davurica* var. *flava* have common features in the structure of epidermis (Volkova & Pshennikova 2011). Three taxa, *D. fruticosa*, *D. davurica* var. *davurica* and *D. davu-*

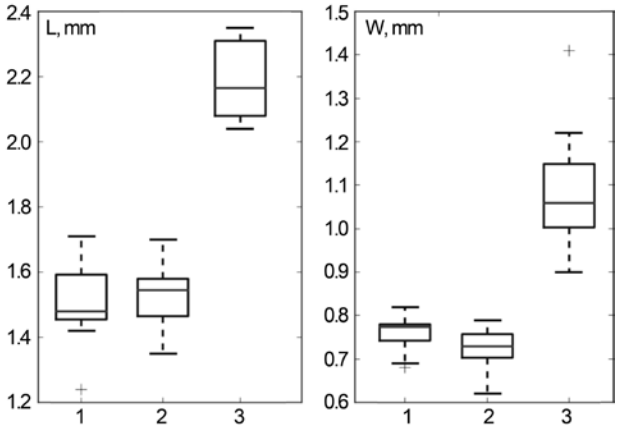

Figure 4 Box plots showing the differences of achene length (left) and width (right) for taxa 1 – *D. fruticosa*, 2 – *D. davurica*, 3 – *D. davurica* var. *flava*

Figure 5 The inner side of the stipules *D. davurica* var. *flava*

Figure 6 The inner side of the stipules *D. davurica*

rica var. *flava*, have common features in anatomical structure of the petioles: single-rowed collenchyma occasionally turning into distich in adaxial protuberances and in the central part of abaxial side (Pshennikova & Volkova 2013). Since *D. davurica* var. *flava* has common features with both *D. davurica* var. *davurica* and *D. fruticosa*, we can assume that this taxon is the result of natural hybridization.

Morphometric studies of achenes of the five Far Eastern taxa from the genus *Dasiphora* revealed that the general shape of a mature achene is quite steady. The achenes have unified structure and many common features indicating the evolutionary closeness of these taxa. The only significant differences that were identified separate *D. davurica* var. *flava* from other Far Eastern species of the genus. The main morphological distinctive properties of achenes are their size, trichome length, and location of villi on its surface. These data confirm the results of our previous study (Volkova et al. 2009, Volkova & Pshennikova 2011, Pshennikova & Volkova 2013) showing the taxon *D. davurica* var. *flava* merits species rank. Therefore the new combination is proposed: *Dasiphora flava* (Vorosch.) Gorovoj, Pshenn. et S. Volkova comb. et stat. nov. (*Potentilla davurica* Nestl. var. *flava* Vorosch. 1972, in Byull. Glavn. Bot. Sada (Moscow) 83: 36). **Holotype:** Russia, prov. Primorskensis, distr. Olga, prope pagum Novo-Nikolajevka, monticulus cretaceous, 27. lX 1969, V.N. Voroshilov (MHA) (Fig. 7).

Figure 7 Type specimen (Typus) of *Potentilla davurica* var. *flava* (MHA)

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