On intraspecific variation of *Vavilovia formosa* (Stev.) Fed. (= *Pisum formosum* (Stev.) Alef.: Fabeae)

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The phylogeny and systematics of tribe Fabeae Rchb. are still somewhat unclear. One of the most enigmatic genera within this group is *Vavilovia* Fed. representing small highland plants with very specific features. It inhabits disrupted areas in Caucasus and Middle East and is commonly referred to as an endangered plant species. Numerous research reports dealing with its morphology, anatomy and taxonomical position (evidenced from results of both classical and molecular analyses) exist (1, 6 and works cited in reviews of these papers). Expressed interest in this genus is evidenced by the appearance of several publications during the last few years (7, 8, 9 etc.). Exhaustive surveys on the history of investigations on this plant species have recently been published (6, 7).

Since this genus was described by C. Steven in 1812 (see 7 for details), numerous data on its intrageneric differentiation were reported. Table 1 summarizes the history of microsystematics of *Vavilovia* with different synonyms listed. The present work together with (11) represent efforts to reveal any intrageneric variation within *Vavilovia* (regardless of whether we treat it as a *Pisum* species or as separate genus).

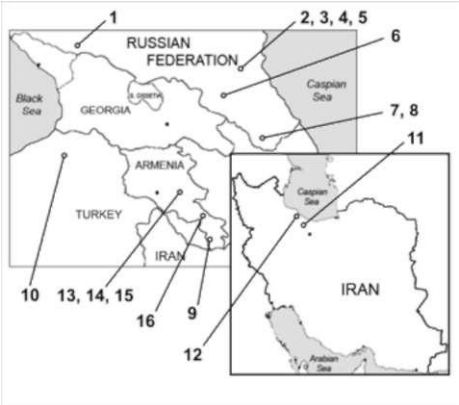
Table 1 Correspondence between different synonyms of intraspecific taxa within Vavilovia.

Source		
(4)	*Pisum formosum* var. *typicum* Gov.	*P. formosum* var. *microphyllum* Ser.
(2)	*Vaviloviaformosa* (Stev.) Fed.	*V. aucheri* Fed.
(3)	*V.formosa* (Stev.) Fed.	
(5)	*Alophotropis formosa* (Stev.) A. Grossh. | *A. aucheri* (Jaub. & Spach) A. Grossh.	

Materials and Methods

The morphometric analysis was performed using photo images of herbarium specimens (MW!, MHA!, ERE!, WIR!). In total 22 specimens were analyzed and 412 leaflets measured. The chosen samples represented collections of different years (1860-2007) from territories of the Kuban' region, Daghestan, Armenia, Turkey and Iran; for details see Figure 1 and Table 2.

Figure 1 Map depicting collection sites of herbarium samples. Numbers correspond to those in Table 2.



All samples were photographed or scanned. The leaves were measured with reference to two parameters, longitudinal and bilateral asymmetry of the leaflet, viz. distance between leaflet base and position of maximum width (l_p), distance between leaflet tip and position of maximum width (l_d), maximum distances from midvein to leaf margin from basiscopic (h_b) and acroscopic (h_a) sides (Fig. 3B). All measurements were performed using Meazure™ 2.0 program (C Thing Software). The obtained data were analyzed with the Statistica 8 (Statsoft Inc., Tulsa, OK, USA) software package.

Table 2. List of herbarium specimens used for analysis.

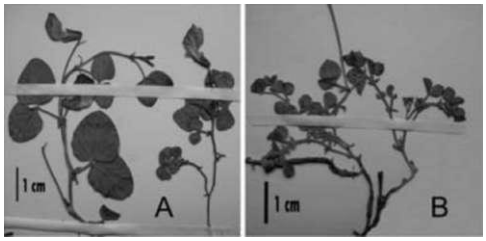
Specimen	Herbarium	No. on map (Fig. 2)
Kuban' region		
Ex herbario Horti botanici Jurjevensis. Headstreams of Kuban, Uchkulang. On rockslides. Very rare. VII-1900. Legit N. Desoulavy, det. W. Lipsky.	[MW!]	1
Daghestan		
Resp. Autonoma Daghestania. Districtus Akhty, p. Kurusch, ad fontes flumini Tschechitschaj. I/VII-1990. A.P. Khokhriakov.	[MW!, MHA!]	2
Resp. Autonoma Daghestania. Flumen Tscharyntschaj. 2/VII-1990. A.P. Khokhriakov.	[MW!, MHA!]	3
Daghestan, Akhty distr., Kurush township, Mular mountain. h - 2800 above sea level. 2/VIII-1991. A.P. Khokhriakov.	[MHA!]	4
Daghestan, Nesen-Dagh mountain. 5/VII 1990. A.P. Khokhriakov.	[MHA!]	5
Tsumada distr., near meteorological station "Sulak highland". Southern slope, on rockslides, 3300 m. 14/08/1997. R.A. Murtazaliev.	[MW!]	6
Eastern Caucasus, Daghestan, headstreams of riv. Usukh-Chai (feeder of riv. Samur), Bazar-dyuzi mountain, to south from village Kurush, on slate rockslides, h. 3000 m. 24 July 1970. V. Prima.	[MW!]	7
Nakhichevan region		
Respublica autonoma Nachiczevan, in monte Kapudzhikh jugi Zangezurici, inter schisti fragmenta, ca. 3200-3300 m. s. m. Leg. A. Takhtadzhian et S. Czerepanov. 1930 VIII 14.	[MW!]	9
Turkey		
E. Bourgeau. - Plantae Lyciae. 1860. In petrosis regionis alpinae montis Ak-Dagh. S. Julio.	[MW!]	10
Iran		
Hortus Botanicus "Ariamehr". Collector: H. Foroughi. Place: Nesa Karaj valley. Date: 13 May 1971. Alt.: 2110.	[MHA!]	11
Hortus Botanicus "Ariamehr". Collector: M. Fotovat. Place: Kelardasht. Kuhetakhtesoleyman. Slope: E. Soil: clay. Locality: rocky. 12 July 1973. Alt.: 3620.	[MHA!]	12
Armenia		
Armenian SSR, Kotayk district, Gegham ridge, lake Akna-litch, rubble rockslides on southern slope, 3300 m above sea level. 28.08.1947. Leg. A. Akhverdov, det. I. Arevshatyan.	[ERE!]	13
Armenian SSR, Kotayk district, Agmagan mountain, lake Akna-litch, NE shore, black rockslide, southern slope. 04.08.1960. Leg. et det. E. Gabrielyan.	[ERE!]	14
Gegham ridge, near Akna-litch lake, NW slope of mountain Eratumber I, 3200 m above sea level. High ascent strongly rank slope. 08.1968. Leg. A. Akhverdov, N. Mirzoeva. Det. A. Akhverdov.	[ERE!]	15
Armenia, Syunik province, Sisian distr., vicinity of Ughtasar mointain, alpine meadow, south slopes, 3320 m, N 39°41'15" E46°03'14". 02 August 2007. Leg. I. Gabrielyan, A. Bruch. Det. I. Gabrielyan.	[ERE!]	16

Results and Discussion

Morphological variations

When subdividing a single genus (regardless of *Vavilovia* Fed. or *Alophotropis* (Jaub. & Spach) Grossh.) into some intrageneric units, most authors agree that leaf features should be used as a key basis for division (4, 5). Two main distinguishing traits were cited, size and shape of the leaflet. According to A.A. Grossheim (5), plants from Southern Transcaucasia, Nakhichevan and Karabakh *(Alophotropis aucheri,* see Table 1) are characterized with smaller leaflets with keel-like basement (obovate shape). In contrast, plants from western and eastern Caucasus possess larger ovate leaflets with rounded basement *(A. formosa)* (Fig. 2).

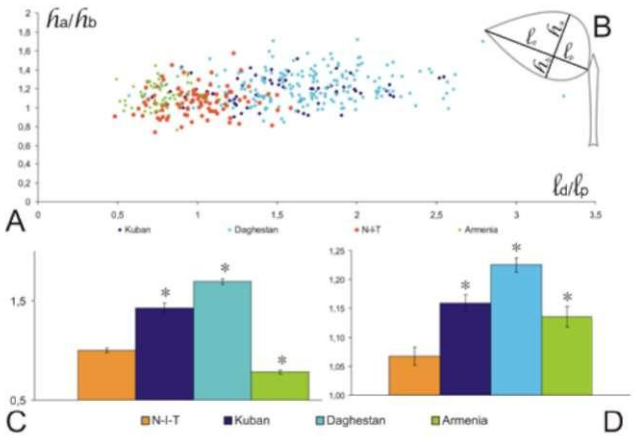
Figure 2. Herbarium specimens of V. formosa demonstrating a wide range of variation of leaf size andproportion. A: Daghestan, river Charyn-Chai, on rank rockslide, 2.07.1990. A.P. Khokriakov [M in monte Kapudzhikh jugi Zangezurici, inter schisti fragmenta, ca. 32000-3300m. s. m. Leg. A. VIII14** *IMHAIl.* ***Scale bar- 1cm.



Analysis of herbarium material available ensures that *Vavilovia,* similar to most legumes, exhibits expressed leaf anisophylly (Fig. 2). The first leaves of axillary sprouts bear leaves differing from leaves borne on upper nodes. For example, the first leaves on shoots of Daghestan plants possess tiny obovate leaflets while upper leaves are characterized with large ovate leaflets.

All Armenian plants possess clearly obovate leaftets ($l_d/l_p < 1$) with expressed acro-basiscopic asymmetry (Fig. 3A). The t-test reveals significant differences between Armenian samples and all other samples ($p < 0.01$). Daghestan samples are characterized with large ovate (sometimes almost triangular, $l_d/l_p > 1$) leaflets. The specimens from the Nakhichevan' region are remarkable with their tiny rhomboid (sometimes scarcely roundish, Fig. 1) leaflets. These plants together with those from Turkey and Iran form a transient group ("N-I-T group" on Fig. 3) between Daghestan-Kuban' and Armenian groups (Fig. 3A). The Iranian plants are characterized with comparatively large oblong "lathyroid" leaflets (although certain anisophylly is observed). Notably, many leaflets express "normal" acro-basiscopic leaflet asymmetry ($h_a/h_b < 1$), i.e. the basiscopic part is wider than acroscopic which is typical for most legumes.

Figure 3. Scatterplotdistribution of quantitative traits of s (Nakhichevan - Iran - Daghestan) group.



No distinct separation was observed for leaflet characters thus ensuring that leaf differences cannot be referred to as interspecific. Leaf traits seem quite variable in ontogeny and in different areas of the habitat. Possibly material from the same region collected on different elevations might enable research on the interrelation between leaf morphology and elevation where collections were made.

Earlier work (11) on DNA (RAPD) polymorphism reveal two supported subclades within a single *Vavilovia* clade, one of them being composed of Armenian and Nakhichevan samples and another of Daghestan and Kuban'. Samples from other regions of the habitat (e.g., Turkey) were not investigated in the presented molecular studies; therefore, no comparisons can be made.

It is problematic now to fully reconstruct the initial area of habitat of *Vavilovia.* To date, it inhabits few regions and seems to have no interchange of genetic information between them. This case represents the initial steps of allopatric speciation, and the first results of prolonged separation can be easily seen as morphological and DNA differences between populations along different sides of the Caucasus Mountains. One may propose that disjunction of *Vavilovia* populations occurred in stages: the initial area of habitat was first separated by a mountain ridge and then both halves split into other minor loci. The ancestral area of habitat may be connected with the Middle East, as all southern plants (Iran, Turkey and Nakhichevan) are significantly similar ($p < 0.01$) and differ from those from other regions. Kuban' specimens also exhibit proximity to the Daghestan samples according to molecular data (11); however, phenotypic differences between them are significant. The alternative scheme could include invasion of *Vavilovia* from ancient habitats (probably corresponding to southern part of contemporary area) throughout the Caucasus from the South to the North, expansion into new territory and then partial extinction in both primary and secondary areas. Regardless of the scenario chosen, one needs to assume that northern and southern populations of the Caucasus existed as separated entities for a long period, although morphological data (as compared to molecular ones) provide no estimation on longevity of such a separation. The most morphologically diverged group is located in Armenia.

The paucity of any arduous *Vavilovia* material leads to the fact that almost no data on intraspecific or intrageneric variability of this plant are available. Some reports on variability of flower color (pale pinkish or purple), pubescence *(P. formosum* var. *pubescens* C.C. Townsend (12)) and leaf morphology (see above) were cited. Some variation in anthocyanin pigmentation of vegetative parts was reported by R. Makasheva

et al. (6) and are clearly seen on fine photo images presented in (7). Flower and leaf pigmentation features are unfortunately lost in herbarium samples. However, in accordance with N.I. Vavilov's law of homological series (13), much wider variability can be expected in *Vavilovia* populations since much is known on interspecific (including mutational) variations in its close relative, *Pisum s.* str. As no specific status was attributed to different morphological novelties in pea (authors of (10) interpreted them as varieties), the existing differences in leaflet morphology can hardly be interpreted as interspecific or distinguishing subspecies of *Vavilovia.* Additional samples and collections of this plant are of great interest, especially material from other parts of hypothetical habitats. For example, absence of accessible collections from the territory of Georgia is remarkable and may be evidence for inadequate exploration in *Vavilovia* habitats.

On the other hand, the most widespread tool in taxonomical interpretations is the subjective will of specialists interested in a certain taxon. To date, no special investigations on heritability of various traits in *Vavilovia* have been conducted. For example, no crosses between different forms were performed and no clear data on morphological variation between plants from different areas of the habitat (with special reference to ontogenetic and environmental plasticity which overlap leaf differences) exist. It seems more correct to avoid conclusions on the status of *Vavilovia* forms. They can hardly be interpreted as different species, but probably correspond to different varieties or even subspecies. The level of molecular polymorphism distinguishing them is at least comparable to that between different subspecies of *Pisum s.* str.

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