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MORPHOMETRIC ANALYSIS FOR THE EVALUATION OF DIVERSITY WITHIN AND AMONG VERONICA ANAGALLIS-AQUATICA L. POPULATIONS IN IRAN

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Water speedwells (*Veronica anagallis-aquatica* L.) mostly occur in damp freshwater places. They show a high level of phenotypic plasticity in response to variations in environmental factors, a characteristic that allows them to occur over a wide range of conditions. Populations of *V. anagallis-aquatica* are scattered in many parts of Iran and often have a wide variation in the same region while having a similar appearance in different regions. We attempt to provide an accurate estimate of diversity within and among populations. Also, using the analysis of habitat characteristics and morphological traits, we aim to determine the distribution pattern of specific morphotypes. Here, we present a morphometric study based on 39 morphological and four ecological characters for 576 individuals from the north, northwest, and center of Iran. The analyses of habitat factors and morphological traits showed that morphotypes were distributed in a specific habitat with a cline of diversity from the northeastern to the northwest, west, and center of Iran. Morphometric results showed a high level of intraspecific variability and diversity among populations. Seed weight, number of seeds per capsule, the length and width of the capsule, number of lateral racemes, number of pollen per flower, length of sepals, and peduncle position were the most important characters to predict the overall variation and diversity of the individuals and populations. Electrical conductivity and pH were important characters influencing the distribution of morphotypes.

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Keywords: Morphometry; intraspecific variation; Water speedwell; ecology; habitat

آنالیز مورفومتری برای برآورد تنوع درون و بین جمعیت‌های سیزاب آبی (Veronica anagallis-aquatica) در ایران

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سیزاب آبی (Veronica anagallis-aquatica L.) اغلب در مکان‌های مرطوب کنار آبهای شیرین می‌روید. این گیاه سطح بالایی از انعطاف پذیری فنوتیپیک را در مقابل تنوع فاکتورهای محیطی از خود نشان می‌دهد؛ وضعیتی که اجازه می‌دهد تا در گستره وسیعی از موقعیت‌ها رویش داشته باشد. جمعیت های سیزاب آبی در بسیاری از نواحی ایران پراکنده شده و اغلب سیمای ظاهری مشابهی در مناطق مختلف یا مورفولوژی متنوعی در یک منطقه نشان می‌دهند. اینجا در نظر است برآورد دقیقی از تنوع احتمالی درون و بین جمعیت‌های این گونه ارائه شود. همچنین، با استفاده از آنالیز ویژگی‌های زیستگاهی و صفات مورفولوژیک، الگوی پراکنش تیپ‌های مورفولوژیک مختلف را مشخص کنیم. در اینجا مطالعه مورفومتری ۳۹ صفت مورفولوژیک و چهار فاکتور اکولوژیک متعلق به ۵۷۶ فرد از شمال، شمال‌غرب و مرکز ایران انجام شد. نتایج تحلیل فاکتورهای رویشگاه‌ها و آنالیز

مورفومتریک نشان داد که مورفوتیپ‌ها در مناطق مشخصی پراکنده هستند و شیب در تنوع ویژگی‌های آن ها از شمال شرق تا شمال غرب و مرکز ایران دیده می‌شود. نتایج مورفومتری سطح بالایی از تنوع درون گونه‌ای بین جمعیت‌ها نشان داد. صفات وزن و تعداد دانه‌ها، طول و عرض کپسول، تعداد خوشه‌های جانبی، تعداد دانه‌های گرده، طول کاسبرگ‌ها و وضعیت دمگل بیشترین اهمیت را در نمایش تنوع و تفاوت افراد و جمعیت‌ها داشت. شاخص هدایت الکتریکی (EC) و pH شاخص مهمی هستند که پراکندگی مورفوتیپ‌ها را را تحت تاثیر قرار می‌دهند.

INTRODUCTION

Veronica L. is the largest genus of Plantaginaceae sensu APG (1998; 2016) with about 400-500 species. Based on molecular phylogenetic studies and analyses of morphological, karyological, and phytochemical character evolution (e. g., Albach & Chase 2001; Albach & al. 2004a; Taskova & al. 2004) Albach & al. (2004b) and Garnock-Jones & al. (2007) classified the genus *Veronica* into 12 subgenera, comprising from two to 150 species. Here, we focus on *V. anagallis-aquatica* L. of *Veronica* subg. *Beccabunga* (Hill) M. M. Martinéz-Ortega, Albach & M. A. Fischer, a subgenus which has been the focus of prior comprehensive analyses of genetic and morphological diversity (Ellmouni & al. 2017*)*. *Veronica anagallis-aquatica* is a semi-aquatic plant occurring in a variety of moist to aquatic habitats. It is cosmopolitan, notably rare only in subtropical and arctic deserts and tropical forests. This distribution also applies on a smaller scale to the distribution of the species in Iran, where it occurs up to 4000 m a.b.s. in any kind of at least seasonal wet habitat but is excluded from any kind of dry habitat. The species complex has been known to occur in different parts of Iran (Fischer 1981; Saeidi-Mehrvarz 2011). Its large range and high morphological variation typical for species occurring in habitats with a wide range of water availability, have led to the recognition of many infraspecific taxa but also species of unknown validity. Eleven different species are recognized in the *V. anagallis-aquatica* complex, four to seven of them are distributed in Iran but without a clear distribution pattern in any of them (Fischer 1981; Ellmouni & al. 2018). Apart from *V. anagallis-aquatica*, *V. oxycarpa*, *V. michauxii*, and *V. lysimachioides* discussed below, these are *V. anagalloides*, *V. heureka* and *V. scardica*, which are morphologically more clearly separated by the strong glandular indumentum and different leaf shape. Aquatic plant species are severely threatened by pollution through agricultural intensification (Steffen & Leuschner 2014). Therefore, understanding the reason for the variation is urgently needed, for understanding plant evolution and conservation of aquatic plants. Variation in *Veronica anagallis-aquatica* may have further implications since the species has been used as food and medicine. Leaves have been used raw or cooked as a uretic, spicy appetizer vegetable. *Veronica anagallis-aquatica* has been used in the treatment of scurvy due to its high vitamin C content. It has further been used in the elimination of blood contamination, wound healing, gastric ulcer treatment, and inflammation of the last finger knuckle (Duke& Ayensu 2006). *Veronica anagallis-aquatica* is also part of traditional Chinese medicine, as an herbal remedy for the treatment of influenza, pulmonary hemorrhage, throat and laryngeal, and hernia infections (Judd & al. 1994). This study presents results from the analysis of morphological characters in some *V. anagallis-aquatica* populations in Iran to investigate intraspecific variation and diversity among populations. It is also attempted to define qualitative and quantitative characters discriminating the taxa studied. With this data, we aim to examine the two following hypotheses for the underlying morphological variation in the *V. anagallis-aquatica* species complex: Are different taxa the result of phenotypic plasticity in response to varying availability in water? Or, are they caused by genetic differentiation in isolated aquatic habitats?

MATERIAL AND METHODS

Sampling

Veronica anagallis-aquatica is widely distributed in Iran. To reduce the effect of different ecological factors, we here mainly focus on the material collected from a relatively narrow region from NW to NE Iran. This study was based on collecting fresh material of 16 Iranian populations of *V. anagallis-aquatica* from their natural habitats during July-September 2015 (fig. 1; table 1). Thirty-six individuals from each population (576 individuals overall) were collected from springs and rivers sides and then examined visually with a stereomicroscope. For the morphometric studies, the complete organs of each sample were picked, dried, stored, and an herbarium specimen prepared. Voucher specimens are deposited at Islamic Azad University herbarium (IAUH).

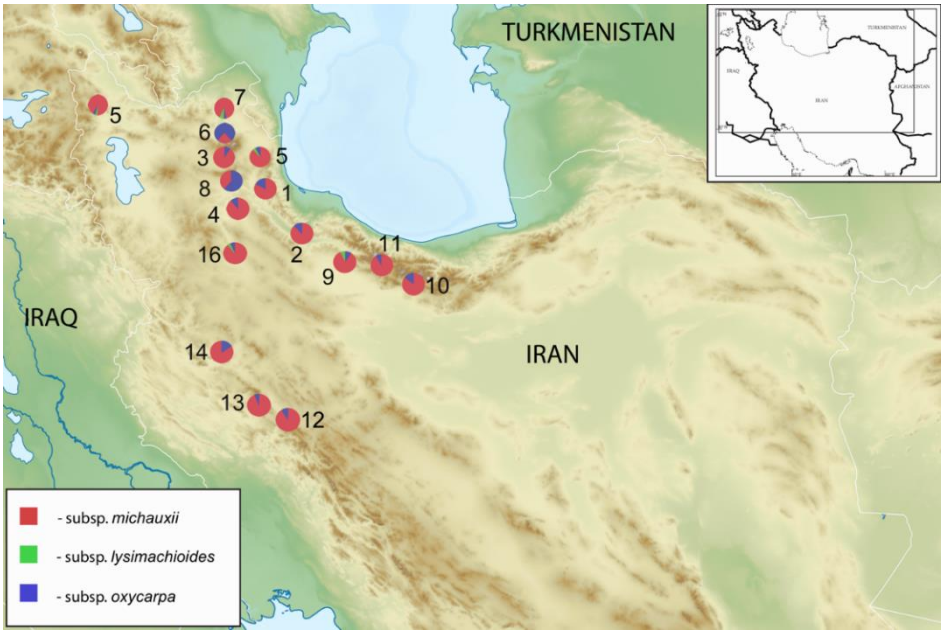

Fig. 1: Localities of 16 *Veronica anagallis-aquatica* populations collected for this study. Small Pie charts on the map show the proportion of three morphotypes of *V. anagallis-aquatica.* Numbers refer to *V. anagallis-aquatica* populations as in table 1.

Table 1. Collecting data and voucher specimens of *Veronica anagallis-aquatica* studied in this work. For each population, a voucher specimen is deposited at IAUH herbarium.

No.	Code	Province	City	Locality	Altitude (m)	Latitude	Longitude	Collector and Herbarium Code (IAUH)
1	VHM-1	Guilan	Masuleh	10 Km from Kalvaz to Khalkhal	3300	37° 36.93'N	48° 29.87' E	Azad 15152
2	VHA-2	Guilan	Aghdagh	10 Km from Aghdagh to Masuleh	1760	37° 9.52' N	49° 2.83' E	Azad 15153
3	VHS-3	Ardabil	Sarein	Alvares ski resort	2950	38° 13.53' N	47° 55.25' E	Azad 15154
4	VHK-4	Ardabil	Khalkhal	5 Km from Aznav spring to Khalkhal	1850	37° 34.9' N	48° 34.35' E	Azad 15155
5	VHG-5	Ardabil	Ghotour	Ghotour	2690	38° 19.67' N	48° 50.68' E	Azad 15156
6	VHV-6	Ardabil	Meshkinshahr	Velayat forest park	1520	38° 22.15' N	47° 40.8' E	Azad 15157
7	VHAG-7	Ardabil	Angout	Ghareh Aghaj	1940	38° 51.62' N	47° 45.6' E	Azad 15158
8	VHAA-8	Ardabil	Abgarm	Abgarm	1540	37° 41.73' N	48° 24.42' E	Azad 15159
9	VHR-9	Qazvin	Razjerd village	Razjerd village	1980	36° 21.38' N	50° 11.28' E	Azad 15160
10	VHGA-10	Alborz	Gachsar	Gachsar	2300	35° 47.22' N	51° 40.62' E	Azad 15161
11	VHD-11	Alborz	Dizin	Dizin ski resort	3040	36° 2.43' N	51° 25.63' E	Azad 15162
12	VHMZ-12	Hamadan	Malayer	20 Km from Malayer to Konjehdar village	1990	34° 8.15' N	49° 0.18' E	Azad 15163
13	VHHG-13	Hamadan	Hamadan	Ganjnameh	2040	34° 46.28' N	48° 27.58' E	Azad 15164
14	VHB-14	Kurdistan	Bijar	Changizghaleh	1900	35° 52.35' N	47° 32.1' E	Azad 15165
15	VHBJ-15	Azerbaijan (W)	Bazargan	Bazargan	1510	39° 24.52' N	44° 21.78' E	Azad 15166
16	VHMA-16	Azerbaijan(E)	Mianeh	44 Km from Achachi village to Mianeh	1690	37° 23.75' N	47° 47.65' E	Azad 15167

Habitat studies

For each habitat, the soil was sampled from a depth of 15 cm using a garden shovel. All samples were dried and sieved using a sieve (1 mm pores). In each habitat, six soil samples were randomly taken at a distance of at least 50 m. Then, a mixture of 100 g soil and 100 ml distilled water were shaken for 60 min. The resulting mixture was put steady for 24 hours. The soil solution was clarified using a funnel and filter paper. The CPD-65N portable multi-meter device was used to measure the pH, electrical conductivity (EC $_{\mu S/Cm}$), dissolved oxygen (DO), and salinity of the clear, obtained solution. Those data were used later alongside the morphometric data in a Principal Coordinates Analysis (PCoA) using PAST ver. 3.25 (Hammer & al. 2001) to draw distribution patterns of different *V. anagallis-aquatica* populations based on locality data.

Morphometric analysis

For the morphometric analysis, we scored 27 morphological quantitative and qualitative characters, the same as used by Ellmouni & al. (2017) plus an additional 12 characters (table 2). Measurements were made after Scalone, & Albach (2012) and Ellmouni & al. (2017). According to the Flora Iranica (Fischer 1981) and Flora of Iran (Saeidi-Mehrvarz 2011), there are three taxa recognized within *V. anagallis-aquatica*, here for simplicity called subspecies, *V. anagallis-aquatica* subsp. *lysimachioides* (Boiss.) M.A. Fischer, *V. anagallis-aquatica* subsp. *oxycarpa* (Boiss. in Kotschy) A. Jelen *and V. anagallis-aquatica* subsp. *michauxii* (Lam) A. Jelen. These are characterized by the following set of discriminative characters: 1) completely glabrous plants with sessile lower leaves and less than 3.5 mm long capsules identified as *V. anagallis-aquatica* subsp. *lysimachioides* (Boiss.) M.A. Fischer, 2), completely glabrous plants with petiolate lower leaves, longer than 3.5 mm capsules, identified as *V. anagallis-aquatica* subsp. *oxycarpa* (Boiss. in Kotschy) A. Jelen, and 3) plants with leaves at least partially gladular-puberolose identified as *V. anagallis-aquatica* subsp. *michauxii* (Lam) A. Jelen. (table 3).

To estimate intraspecific diversity, and help to solve taxonomic problems, we scored all morphological characters especially the states of 15 discriminative ones (table 3) for all individuals.

For more accurate estimation of inter-population and intraspecific variations, all analyses of the morphological data were computed using SPSS ver. 23 (IBM). The correlation of morphological characters and habitat traits was measured. Chi-square test and Pearson's coefficient of contingency were used (Antonius 2003). The nonparametric test was performed to test the correlation between qualitative characters. The software package PAST ver. 3.25 (Hammer & al. 2001) was used to analyze all qualitative characters and to draw a dendrogram of similarities between the individuals of *V. anagallis-aquatica*. "Nei's" distance was used for clustering (Weising & al. 2005, Freeland & al. 2011). "Ward's" algorithm and the "Euclidean" similarity index were used to generate a similarity-based dendrogram. Means, coefficients of variation, and ANOVA test were calculated for quantitative morphological characters (Muijs 2004). The principal component analysis (PCA) (Pearson 1901) was performed using the "KMO and Bartlett" tests as a coefficient. Hierarchical cluster analysis was performed by using the results of the PCA. Standardized data was used to compute a distance matrix based on Euclidean distances using Ward's method with an arithmetic averages (UPGMA) clustering algorithm (Ellmouni & al. 2017; Pearson 1901). The Kruskal-Wallis, One-way ANOVA, and U Mann-Whitney tests were used to rate the clusters and evaluate the main group differences. The "Z score's" method and agglomerative schedule were used for standardizing variable scales (Tyron & Baily 1970).

RESULTS AND DISCUSSION

Habitat characteristics

All samples were collected from mountainous regions and altitudes above 1000m with cold, semi-arid climates (Provinces Ardabil, Ghazvin, west Azerbaijan and east, Kurdistan), mild mountainous (Hamadan), or mild wet weather (Gilan), (Pourvahidi & al. 2013). Altitude, pH, EC, DO and the salinity of habitats were measured (table 4). Based on the altitude, it occurs predominantly in communities in the sub-alpine and alpine areas in Iran. Plants were rarely observed at altitudes below 1000 m. In contrast, populations of Masuleh (1), and Dizin (11) were found at altitudes above 3000 m. Populations Sarein (3), Gachasar (10), and Hamadan (13), were collected at 2000-3000 m. The rest of the populations were found at altitudes between 1000 m and 2000 m. In all localities, *V. anagallis-aquatica* appeared half-hidden in slow-moving streams, and leaves floating in the water were smaller and thicker. The soil was weakly acidic, neutral to weakly alkaline. Based on the pH, populations Ghotour (5), Abgarm (8), Dizin (11), and Hamadan (13) are located in a low alkaline range (pH=7.5 to 7.9). They are distinct from populations Aghdagh (2), Sarein (3), and Khalkhal (4), which grow on low acidic (pH = 6 to 7) soil. The other populations grow in the neutral range (pH= 7 to 7.4). Figure 2 shows the results of PCoA analysis of habitat characteristics of all 16 different populations.

Table 2. A list of 39 quantitative (1-17) and qualitative (18-39) morphological characters of *V. anagallis-aquatica* populations examined in this work. For the qualitative characters, states and coding are given.

No.	Characters	Abbreviation	State
1	Stem height (cm)	STL	-
2	Leaf blade length (cm)	LFBL	-
3	Leaf blade width (cm)	LFBW	-
4	Number of lateral racemes	NLR	-
5	Length of inflorescence (cm)	IFL	-
6	Length of peduncle (mm)	PDL	-
7	Length of bract (mm)	BRL	-
8	Number of pollen grains per flower	NPL	-
9	Number of flowers in racemes	NFR	-
10	Seed weight (mg)	SEW	-
11	Number of seeds per capsule	NSE	-
12	Length of seed (mm)	LSE	-
13	Width of seed (mm)	WSE	-
14	Length of style (mm)	STYL	-
15	Length of sepals (mm)	CXL	-
16	Length of capsule (mm)	CPL	-
17	Width of capsule (mm)	CPW	-
18	Shape of capsule	CPS	Orbicular (0), Elliptic (1)
19	Apex of capsule	CPA	Rounded (0), Acute (1)
20	Surface of capsule	CPSR	Glabrous (0), Hairy (1)
21	Life form	LIL	Annual (0), Perennial (1)
22	Root	ROH	Fibrous (0), taproot (1)
23	Stem	STH	Erect (0), Procumbent (1)
24	Stem indumentum	STT	Glabrous (0), Hairy (1)
25	Stem branching	STB	Absent (0), Present from middle (1) Present from Basal (2), Present from middle and Basal (3)
26	Leaf insertion	LFI	Cauline (0), Cauline and semi amplexicaul (1)
27	Leaf petiole	LFP	Sessile (0), Petiolate (1)
28	Petiole position on stem	PPAL	Lower leaves (0), Upper leaves (1)
29	Leaf blade shape	LFBS	Lanceolate (0), Elliptic-lanceolate (1) Linear-lanceolate (2), Oblanceolate to lanceolate (3), Ovate (4)
30	Leaf blade margin	LFBM	Entire (0) Serrate (1), Serrate to subentire (2) Sub-entire to crenate (3), Denticulate (4)
31	Leaf indumentum	LFSR	Glabrous (0), Hairy (1)
32	Inflorescence position	INP	Terminal (0), Axillary (1)
33	Inflorescence arrangement	INAR	Alternate (0), Opposite (1)
34	Inflorescence axis	INA	Glabrous (0), Hairy (1)
35	Density of inflorescence	IND	Dense (0), Lax (1)
36	Corolla color	CLC	Whitish (0), Pale red (1), Pale blue (2), Lavender (3), Pink (4), Lavender to pink (5), Purple lilac (6), Amethystine (7)
37	Peduncle indumentum	PPO	Glabrous (0), Hairy (1), Glabrous to slightly hairy (2)
38	Shape of bract	BRS	Linear lanceolate to lanceolate (0), Linear (1), Oblong to elliptic (2)
39	Shape of seed	SEP	Elliptic oblate (0), Flat convex (1)

Table 3. List of discriminative characters and states used in the morphological analysis of different subspecies of *V. anagallis-aquatica*.

Discriminative characters	**subsp. *lysimachioides***	**subsp. *michauxii***	**subsp. *oxycarpa***
Stem height	60-150	10-130	10-120
Number of lateral racemes	12-15	6-30	10-40
Length of peduncle	2.5-3.5	4-8	4-8
Length of style	1.5-3	1.8-3	2-4
Number of flowers in inflorescence	50-150	20-50	20-50
Shape of capsule	Orbicular	Elliptic	Elliptic
Apex of capsule	Rounded	Acute	Acute
Surface of capsule	Glabrous	Hairy	Intermediate
Stem indumentum	Glabrous	Hairy	Hairy
Inflorescence axis indumentum	Glabrous	Hairy	Hairy
Leaf indumentum	Glabrous	Hairy	Glabrous
Life form	Annual	Perennial	Perennial
Leaf petiole	Sessile	Sessile or petiolate	Sessile or petiolate
Leaf blade shape	Linear-Lanceolate	Lanceolate	Lanceolate
Corolla color	Whitish or pale red	Pale blue or lavender to pink	Pale blue or purple lilac or pink or bright blue

Table 4. Results of soil analysis were obtained from the samples of the natural habitats of *V. anagallis-aquatica* collected for this study. Abbreviations: EC: electrical conductivity, DO: dissolved oxygen, Sal.: salinity.

No.	**Locality**	**Mean values**			
		pH	**EC µS/cm**	**DO(mg/l)**	**Sal. (ppt)**
1	Masuleh	7.3	1762	6.6	0.92
2	Aghdagh	6.9	957	6.7	0.95
3	Sarein	6.9	1762	6.6	0.93
4	Khalkhal	6.2	1155	7	0.91
5	Ghotour	7.5	202	6.8	0.99
6	Meshkinshahr	7.2	1091	6.9	0.96
7	Angout	7.1	208	7	0.92
8	Abgarm	7.7	1158	7.1	0.94
9	Razjerd village	7.2	201	6.9	0.98
10	Gachsar	7.3	738	7.1	0.97
11	Dizin	7.6	650	7.2	0.93
12	Malayer	7.3	906	7.2	0.91
13	Hamadan	7.5	963	7.1	0.98
14	Bijar	7.4	804	6.9	0.95
15	Bazargan	7.4	285	6.4	0.92
16	Mianeh	7.2	1186	6.7	0.90

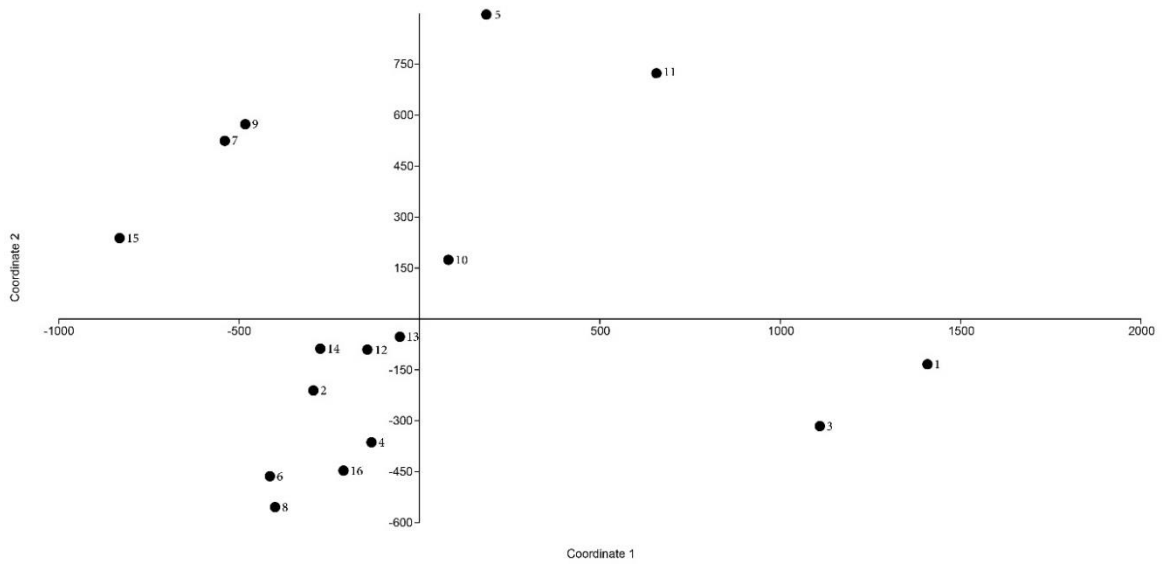

Fig. 2: Principal coordinates analysis (PCoA) of habitat parameters for Iranian *V. anagallis-aquatica* populations. Masuleh (1), Aghdagh (2), Sarein (3), Khalkhal (4), Ghotour (5), Meshkinshahr (6), Angout (7), Abgarm (8), Razjerd village (9), Gachsar (10), Dizin (11), Malayer (12), Hamadan (13), Bijar (14), Bazargan (15), Mianeh (16).

Morphometric analyses

The results of morphological data analysis divided the 576 samples into three morphotypes (following Fischer 1981): 1) the *lysimachioides*-type including 14 individuals, 2) the *oxycarpa*-type including 134 individuals, and 3) the *michauxii*-type including 428 individuals (figs. 3 & 4). The *michauxii*- and *oxycarpa*-types were predominant in all localities, whereas the *lysimachioides*-type individuals were observed only in Ghotour (5), Angout (7), Razjerd (9), and Bazargan (15) (fig. 1).

The results of the nonparametric tests for qualitative characters at a significance level of 99% (sig <0.01) are shown in table 5. table 6 shows the results of the Chi-square test and Pearson correlation at significance levels of 99% ($p<0.01$) and 95% ($p<0.01$). A significant correlation to altitude was found for LFBL (leaf blade length), NPL (number of pollens per flower). So, a significant correlation to STL (stem height) was found for LFBL, NLR (number of lateral racemes), and CXL (length of sepals). A significant correlation between NLR with PDL (length of peduncle), LSE (length of seed), and NFR (number of flowers in racemes) was also observed. In addition, we found significant correlations between LFBL with LFBW (leaf blade width), BRL (length of bract), CXL, and CPL (length of capsule) with NPL, NSE (number of seed), SEW (seed weight), PDL. Univariate ANOVAs showed that: Mean values of STL, LFBL, NLR, PPL, STYL, BRL (sig< 0.01; confidence 99%) and LFBW, IFL, PDL, NPL, SEW, NSE, WSE, CXL, CPL, CPW, LSE (sig< 0.05; 95%) had a significant difference for all populations (table 7).

The PCA analysis was performed based on both the quantitative morphological and the habitat characters. The Scree test criterion determines the number of suitable significant factors for analyzing and interpreting the PCA. UPGMA clustering analysis of morphological data allowed the separation of *V. anagallis-aquatica* populations into two main groups (table 8; fig. 5).

Veronica anagallis-aquatica is a semi-aquatic plant, mainly reproducing by selfing (Schlenker 1935) but occasionally pollinated by small insects (Ellis & Ellis-Adam 1994), seed dispersal in *Veronica* is by water, wind, or epi- or endozoochory (Bonn & Poschlod 1998; Grosskopf & Albach unpubl.). *Veronica anagallis-aquatica* produces a large number of fruits and seeds and reaches the age of sexual reproduction within one year (Duke & al. 2006). It occurs in permanently wet sites such as river margins, swamps, or springs to ephemeral habitats, drying out during parts of the year. Such habitats are numerous at all our sampling sites. The connectivity of these wet areas especially rivers and creeks might allow the seeds of *V. anagallis-aquatica* to spread over long distances by water (Santamaría 2002; Mozaffarian 2007). Apart from natural means, seeds can also be transported by humans in mud attached to shoes, clothing, vehicles, agricultural equipment, and construction equipment in

areas of high human frequency (Di Tomaso & Healy 2007). Based on these arguments, we assume high gene flow between populations of the species. However, it is not clear whether the distribution is produced by natural or anthropogenic vectors and whether it follows continuous range expansions or rather long-distance dispersal events.

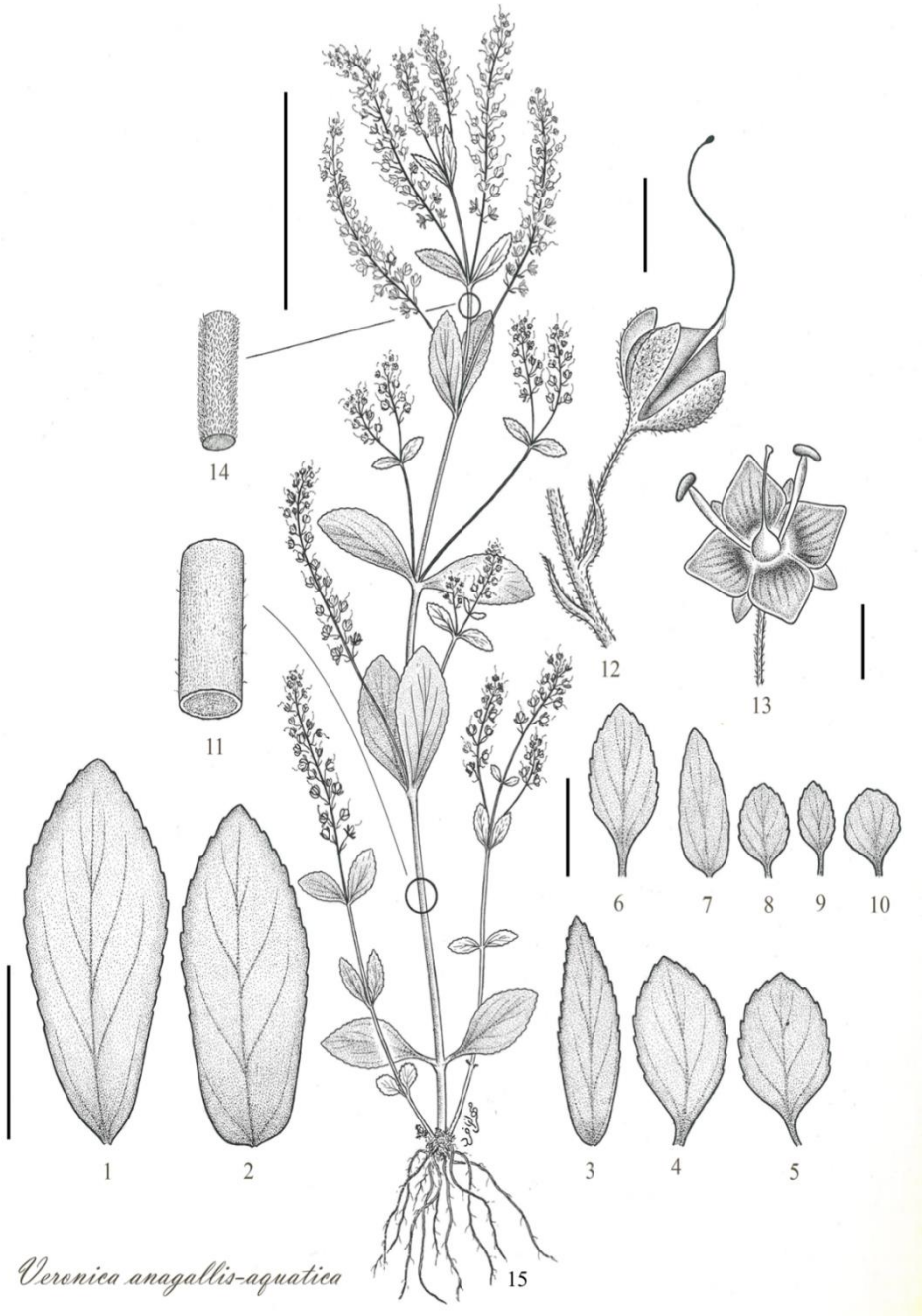

Fig. 3: Morphology of *Veronica anagallis-aquatica*. 1-10) Variation of leaf morphology, 11) stem cross section, 12) capsule with persistent style, 13) flower, 14) peduncle, 15) overall view. Scale bars: 1–10 = 20 mm, 12-13 = 2 mm, 15 = 50 mm.

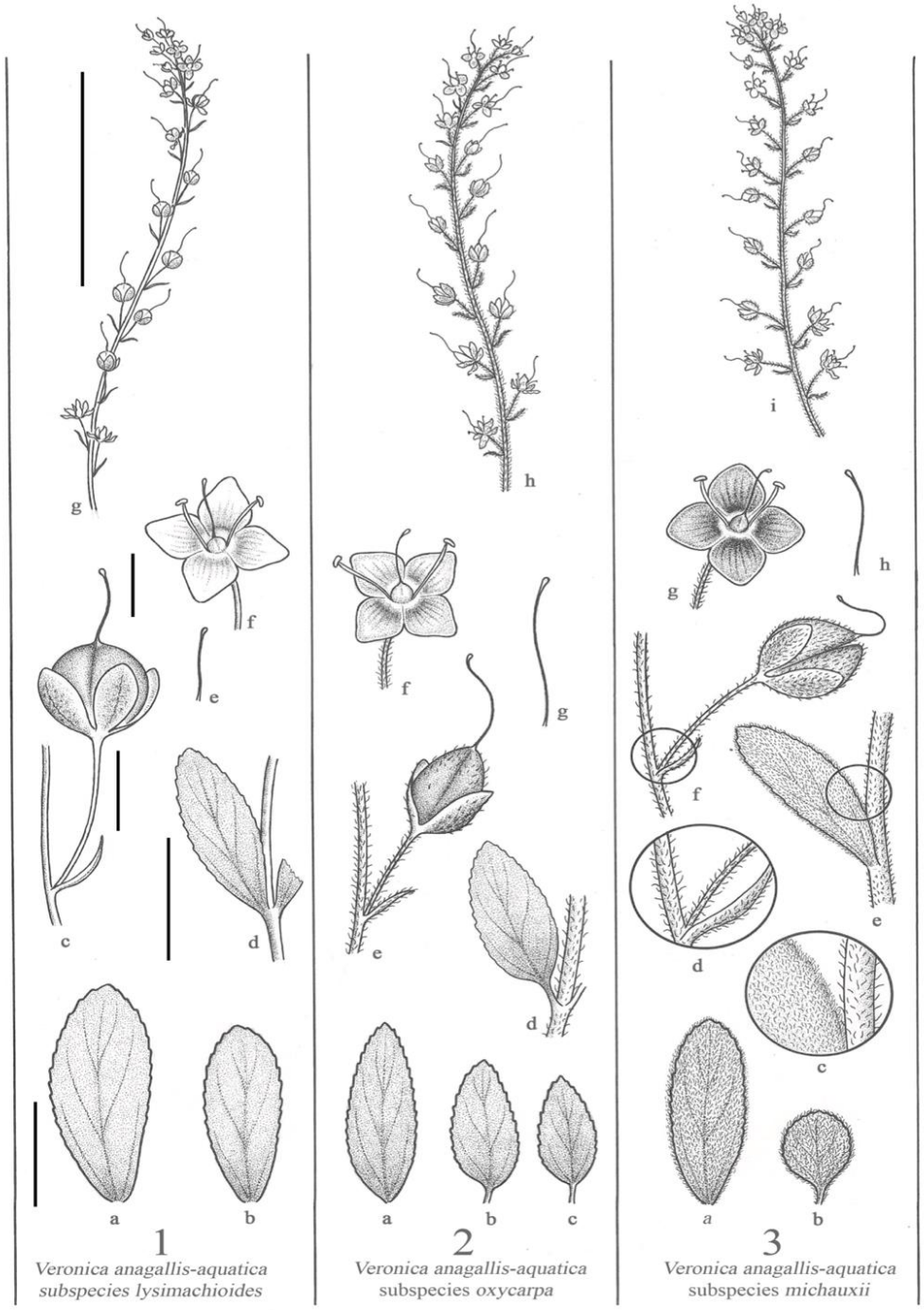

Fig. 4: Morphological characters of the three subspecies of *V. anagallis-aquatica* using discriminative characters (based on Fischer, 1981; Saeidi-Mehrvarz 2011). 1, *Veronica anagallis-aquatica* subsp. *lysimachioides*: a-b; lower leaves; c, fruiting pedicle; d, upper leaves; e, style; f, flower; g, fruiting peduncle. 2, *Veronica anagallis-aquatica* subsp. *oxycarpa*: a-c, lower leaves; d, upper leaves; e, fruiting pedicle; f, flower; g, style; h, fruiting peduncle. 3. *Veronica anagallis-aquatica* subsp. *michauxii*: a-b, lower leaves; c-d, indumentum; e, upper leaves; f, fruiting pedicle; g, flower; h, style; I, fruiting peduncle. Scale bars: lower and upper leaves = 20 mm; fruiting peduncle & flower = 2 mm; peduncle = 50 mm.

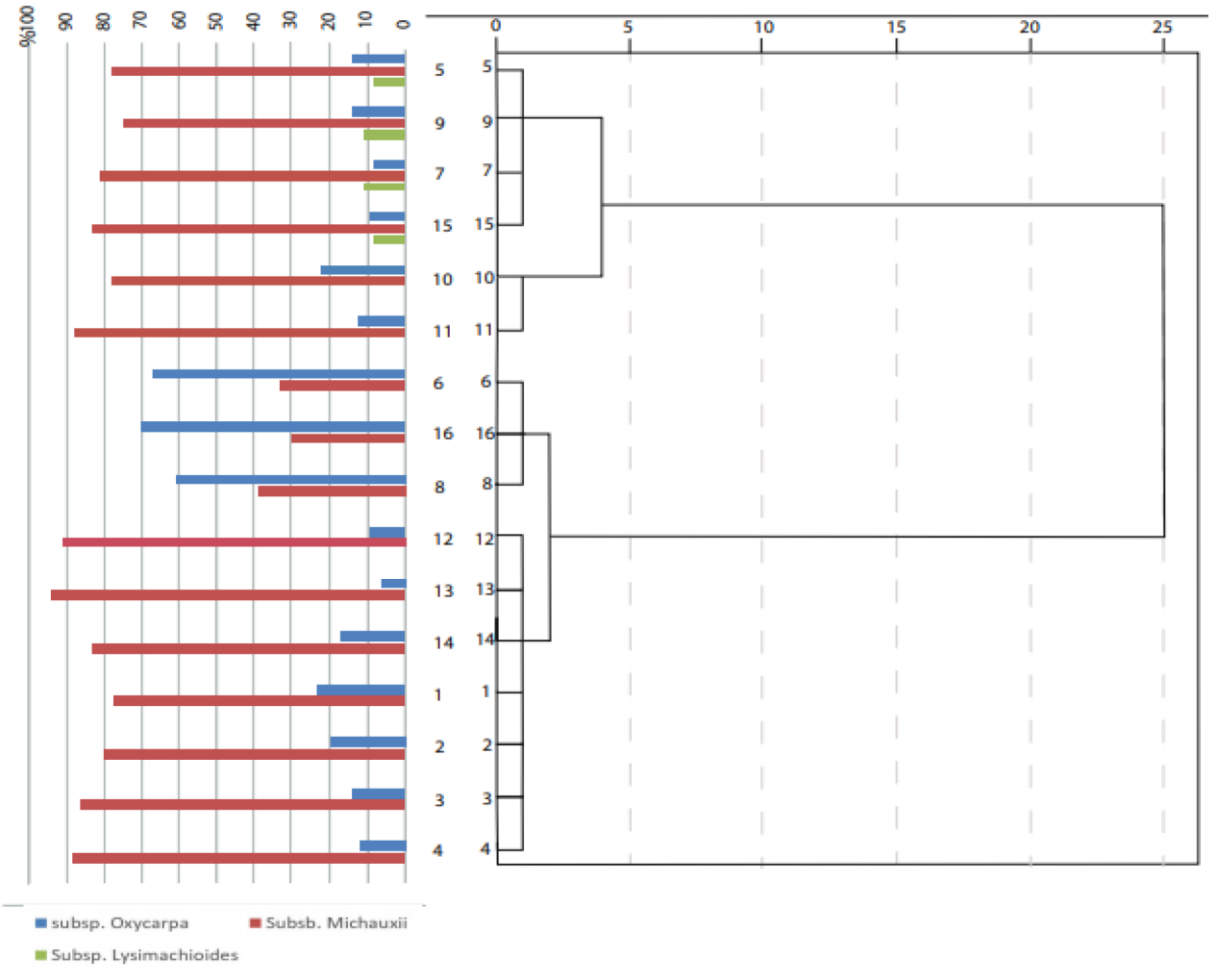

Fig. 5: Comparison of the relative contribution of morphotypes of *V. anagallis-aquatica* subspecies in the populations (left) with UPGMA clustering analysis (right). Populations: Masuleh (1), Aghdagh (2), Sarein (3), Khalkhal (4), Ghotour (5), Meshkinshahr (6), Angout (7), Abgarm (8), Razjerd village (9), Gachsar (10), Dizin (11), Malayer (12), Hamadan (13), Bijar (14), Bazargan (15), Mianeh (16).

Table 5. Results showing the relationships (Pearson correlation) between six qualitative variables (**: Correlation is significant at the 0.01 level (2-tailed)).

	Stem texture	**Stem branching**	**Leaf petiole**	**Petiole position at leaf**	**Leaf blade shape**	**Peduncle position**
Stem texture	1	0.179**	0.258**	-0.258**	-.0167**	0
Stem branching	0.179**	1	0.370**	-0.370**	-0.319**	-0.388**
Leaf petiole	0.258**	0.370**	1	-1.000**	0.258**	0.230**
Petiole position at leaf	-0.258**	-0.370**	-1.000**	1	-0.258**	-0.230**
Leaf blade shape	-0.167**	-0.319**	0.258**	-0.258**	1	0.069
Peduncle position	0	-0.388**	0.230**	-0.230**	0.069	1

Table 6. Data showing Pearson correlations between different ecological factors (1-4) and morphological characters (5-21) in the studied *V. anagallis-aquatica* populations (**: Significant at $p<0.01$, *: Significant at $p<0.05$). The numbers that appeared in the first row are corresponding to character numbers in the first column.

	Factor/Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	Altitude	1	.107**	.288**	-.051	.039	.430**	.072	-.152**	.141**	-.016	-.011	-.475**	.261**	0.13	.037	.024	.217**	-.038	-.041	.144**	-.085*
2	Soil pH	.107**	1	-.261**	.173**	-.182**	.024	.115**	.006	.180**	-.406**	.022	.360**	.173**	-.264**	.383**	.188**	.194**	-.057	.078	.464**	.229**
3	EC	.288**	-.261**	1	-.170**	.163**	.172**	.120**	-.170**	.221**	.132**	-.158**	-.521**	-.015	-.333**	.107*	.200**	.167**	.060	.089*	-.171**	-.041
4	DO	-.051	.173**	-.170**	1	-.029	-.091*	-.359**	-.396**	-.095*	-.549**	-.170**	.243**	.383**	0.72	.122**	-.511**	-.258**	.037	-.144**	.478**	.234**
5	Stem height	.039	-.182**	.163**	-.029	1	.517**	.213**	.443**	.300**	.173**	.151**	-.159**	.211**	.145**	-.002	.143**	-.330**	-.016	-.438**	.026	-.071
6	Leaf blade length	.430**	.024	.172**	-.091*	.517**	1	.615**	.188**	.383**	-.079	.554**	-.288**	.242**	-.138**	-.048	.216**	.227**	-.082*	-.511**	-.148**	-.053
7	Leaf blade width	.072	.115**	.120**	-.359**	.213**	.615**	1	.317**	.278**	.095*	.545**	.014	.197**	-.268**	.096*	.451**	.109**	-.028	-.302**	-.165**	-.034
8	Number of lateral racemes	-.152**	.006	-.170**	-.396**	.443**	.188**	.317**	1	-.018	.481**	.345**	.194**	-.358**	..337**	.236**	.551**	-.167**	-.006	.197**	.180**	-.013
9	Length of inflorescence	.141**	.180**	.221**	-.095*	.300**	.383**	.278**	-.018	1	-.128**	.118**	-.040	.300**	-.170**	-.077	.194**	-.021	.017	-.219**	-.044	.020
10	Length of peduncle	-.016	-.406**	.132**	-.549**	.173**	-.079	.095*	.481**	-.128**	1	.159**	-.347**	-.388**	..134**	.112**	.181**	-.141**	.055	.372**	-.159**	-.130**
11	Length of bract	-.011	.022	-.158**	-.170**	.151**	.554**	.545**	.345**	.118**	.159**	1	-.108**	.058	.-.063	-.219**	-.011	.231**	-.068	-.123**	-.324**	-.104*
12	Number of pollens per flower	-.475**	.360**	-.521**	.243**	-.159**	-.288**	.014	.194**	-.040	-.347**	-.108**	1	.187**	..136**	.027	.211**	-.297**	.064	-.134**	.406**	.213**
13	Seed weight	.261**	.173**	-.015	.383**	.211**	.242**	.197**	-.358**	.300**	-.388**	.058	.187**	1	.-.111**	-.222**	-.164**	-.333**	-.015	-.557**	.179**	.023
14	Number of flowers in racemes	0.13	.-.264**	.-.333**	.072	..145**	.-.138**	.-.268**	..337**	.-.170**	..134**	.-.063	..136**	.-.111**	1	.-.034	..123**	.-.445**	..035	..088*	..141**	.-.066**
15	Number of seeds per capsule	.037	.383**	.107*	.122**	-.002	-.048	.096*	.236**	-.077	.112**	-.219**	.027	-.222**	.-.034	1	.351**	-.269**	.040	.308**	.606**	.190**
16	Length of seed	.024	.188**	.200**	-.511**	.143**	.216**	.451**	.551**	.194**	.181**	-.011	.211**	-.164**	..123**	.351**	1	.003	.010	.091*	.189**	.061
17	width of seed	.217**	.194**	.167**	-.258**	-.330**	.227**	.109**	-.167**	-.021	-.141**	.231**	-.297**	-.333**	.-.445**	-.269**	.003	1	-.100*	.059	-.467**	-.056
18	Length of style	-.038	-.057	.060	.037	-.016	-.082*	-.028	-.006	.017	.055	-.068	.064	-.015	..035	.040	.010	-.100*	1	.072	.041	.048
19	Length of sepals	-.041	.078	.089*	-.144**	-.438**	-.511**	-.302**	.197**	-.219**	.372**	-.123**	-.134**	-.557**	..088*	.308**	.091*	.059	.072	1	.226**	.092*
20	Length of capsule	.144**	.464**	-.171**	.478**	.026	-.148**	-.165**	.180**	-.044	-.159**	-.324**	.406**	.179**	..141**	.606**	.189**	-.467**	.041	.226**	1	.291**
21	Width of capsule	-.085*	.229**	-.041	.234**	-.071	-.053	-.034	-.013	.020	-.130**	-.104*	.213**	.023	.-.066**	.190**	.061	-.056	.048	.092*	.291**	1

Table 7. Means and range of variation for quantitative morphological characters of *V. anagalis-aquatica* (**: Significant at $p<0.01$, *: Significant at $p<0.05$).

No.	Character	mean values±standard error of means															
		Masuleh	**Aghdagh**	**Sarein**	**Khalkhal**	**Ghotour**	**Meshkinshahr**	**Angout**	**Abgarm**	**Razjerd**	**Gachsar**	**Dizin**	**Malayer**	**Hamadan**	**Bijar**	**Bazargan**	**Mianeh**
1	Stem height (cm)	25.03±0.31	50.44±1.02	32.27±2.01	35.07±0.28	24.6±3.44	11.09±0.69	9.97±0.74	29.7±1.40	22.58±2.13	30.29±0.69	30.22±0.78	40.16±0.90	30.27±0.72	25.10±0.57	34.46±3.05	16.95±2.18
2	Leaf-blade length (cm)	5.32±0.22	5.06±0.17	5.10±0.15	5.06±0.17	5.10±0.15	4.05±0.14	2.04±0.11	3.51±0.11	3.50±0.15	3.06±0.15	6.06±0.19	4.35±0.24	5.33±0.22	5.33±0.22	5.05±0.15	1.63±0.19
3	Leaf-blade width (cm)	2.02±0.22	1.28±0.14	1.25±0.18	1.55±0.12	1.25±0.11	1.23±0.14	0.56±0.12	1.02±0. 11	1.01±0.15	1.49±0.11	1.04±0.15	2.01±0.14	2.17±0.84	2.46±0.15	1.005±0.12	1.40±0.56
4	Number of lateral racemes	7.36±2.14	27.52±2.07	22.91±1.74	27.80±1.93	28.19±1.95	7.36±2.08	5.00±0.47	22.83±1.71	7.27±2.9	28.08±2.00	27.36±2.25	12.72±2.10	16.47±1.66	12.47±1.94	57.05±2.32	28.38±1.91
5	Length of inflorescence (cm)	9.83±0.42	9.07±0.17	12.04±0.16	6.02±0.12	5.03±0.17	2.99±0.25	11.01±0.14	9.08±0.42	5.03±0.17	7.09±0.20	9.09±0.16	12.05±0.29	11.01±0.14	8.40±1.59	5.14±0.16	8.22±3.93
6	Length of the peduncle (mm)	3.71±0.88	3.71±0.88	2.75±0.49	4.16±0.77	2.09±0.29	1.74±0.88	2.19±0.83	2.26±0.68	2.65±0.49	2.73±o.43	2.26±0.68	1.00±0.001	1.10±0.16	1.04±0.15	4.41±0.76	3.93±0.88
7	Length of bract (mm)	4.01±0.17	4.08±0.22	2.44±0.14	5.06±0.23	5.06±0.23	4.05±0.14	2.04±0.17	3.03±0.22	4.05±0.14	2.04±0.17	5.06±0.23	3.02±0.16	5.03±0.18	4.02±0.25	5.04±0.15	4.06±0.21
8	Number of pollen grains per flower	0.40±0.18	0.69±0.012	0.78±0.01	0.68±0.01	0.87±0.03	0.86±0.009	0.96±0.14	0.86±0.017	0.90±0.05	1.11±0.021	0.80±0.006	0.90±0.012	1.11±0.045	1.12±0.045	1.07±0.23	0.97±0.004
9	Seed weight (mg)	0.04±0.0001	0.26±0.0009	0.028±0.0002	0.031±0.0002	0.026±0.0001	0.014±0.0002	0.022±0.0002	0.019±0.0002	0.057±0.0002	0.044±0.0001	0.040±0.0014	0.060±0.0002	0.055±0.0002	0.20±0.0003	0.027±0.0004	0.036±0.015
10	Number of seeds per capsule	43.30±1.16	34.90±1.08	34.90±0.826	32.33±1.09	24.70±1.136	35.16±2.035	44.02±1.46	51.55±1.84	26.75±1.59	44.66±1.06	50.41±2.143	33.19±2.20	36.58±1.72	36.11±1.76	46.63±1.62	36.02±1.66
11	Length of seed (mm)	0.30±0.16	0.20±0.013	0.50±0.016	0.20±0.015	0.21±0.017	0.30±0.015	0.20±0.016	0.30±0.014	0.20±0.015	0.30±0.015	0.30±0.015	0.30±0.016	0.31±0.017	0.31±0.018	0.60±0.015	0.30±0.107
12	Width of seed (mm)	0.51±0.015	0.20±0.014	0.30±0.015	0.10±0.015	0.70±0.016	0.81±0.020	0.10±0.015	0.20±0.015	0.11±0.001	0.12±0.016	0.20±0.017	0.21±0.018	0.21±0.019	0.21±0.017	0.20±0.014	0.27±0.020
13	Length of style (mm)	3.42±0.19	3.42±0.19	3.50±0.089	3.50±0.89	3.42±0.19	3.42±0.19	3.49±0.84	3.49±0.84	3.42±0.19	3.49±0.84	3.42±0.19	3.42±0.19	3.49±0.86	3.48±0.82	3.43±0.15	3.49±0.92
14	Length of sepals (mm)	3.01±0.115	2.01±0.145	3.005±0.128	3.02±0.136	3.01±0.115	3.01±0.115	3.01±0.122	4.08±0.159	3.05±0.138	3.03±0.161	3.02±0.115	2.03±0.145	2.008±0.151	2.03±0.194	3.03±0.122	4.008±0.08
15	Length of the capsule (mm)	3.01±0.11	2.01±0.11	3.005±0.12	3.02±0.13	3.01±0.11	3.01±0.12	4.008±0.15	3.05±0.13	3.03±0.16	3.02±0.11	2.03±0.14	2.008±0.15	2.03±0.19	3.03±0.12	4.008±0.08	2.89±0.61
16	Width of the capsule (mm)	2.44±0.122	2.49±0.12	3.03±0.167	2.70±0.073	2.80±0.53	3.40±0.421	3.05±0.102	4.15±0.090	3.03±0.095	3.68±0.072	3.69±0.044	3.13±0.104	3.41±0.131	3.58±0.082	3.01±0.148	3.02±0.128

Table 8. Distribution of quantitative variables in eight main factors in post-rotation PCA analysis. Measures ± 0.5 are shown only.

	Component							
	1	2	3	4	5	6	7	8
Altitude(m)			0.752					
Soil pH	0.585					0.565		
EC (µs/cm)			.758					
Do(mg/lit)								
stem height (cm)		0.503			-0.740			
Leaf blade length(cm)		0.725						
Leaf blade width(cm)		0.640						
Number of lateral racemes		0.648						
Length of inflorescence (cm)		0.627						
Length of peduncle (mm)								-0.781
Length of bract (mm)							0.914	
Number of Pollens per flower			-0.824					
Weight of seed				0.902				
Number of seeds per capsule	0.808							
Length of seed (mm)				0.606	0.911			
Width of seed						0.593		
Length of style (mm)						0-.503		
Length of sepals (mm)							0.564	
Length of capsule (mm)	.905							
width of capsule (mm)								

Members of the genus *Veronica* sect. *Beccabunga* shows a high level of phenotypic plasticity in response to variations of environmental factors, a potential that allows them to occur over a wide range of conditions (Ellmouni & al. 2017). In the present study, Principal Coordinates Analysis of habitat parameters differentiated all sampling sites into separate groups (fig. 2). Field observations and analysis of habitat factors showed that altitude and topographical factors play the most important role in population separation of Water Speedwell. Thus, populations are separated by dry, warm low mountain valleys as well as cold high mountain ranges, which also represent watersheds. Consequently, our analyses support the notion that mountains with diverse micro-climates and topographic complexity promote high biodiversity among and within species (Irl & al, 2015; Steinbauer & al, 2016). Furthermore, our results support the hypothesis of local adaptation to habitat factors in *V. anagallis-aquatica* (fig. 1).

Apart from habitat factors, our analysis of morphological characters also categorized individuals in separate *Veronica anagallis-aquatica* populations rather than according to subspecies (fig. 5). However, subspecies in *Veronica* are considered as groups of populations of homogeneous morphology, separated from other groups of populations by consistent morphological characters and by geography (Bardy & al. 2010), which is clearly not the case here. Therefore, we consider them in the following as morphotypes rather than subspecies. Differences in CXL (length of sepals), CPW (width of capsule), PPO (peduncle position), SEW (Seed weight), NSE (number of seed), CPL (length of capsule), NLR (number of lateral racemes), and NPL (number of pollen per flower) were most important to predict the overall variation and diversity of *V. anagallis-aquatica* populations (table 7). The survey of morphological data showed that each individual sample was different but can be grouped into one of the three morphotypes: the *lysimachioides*-type: 14 individuals, the *oxycarpa*-type: 134 individuals, and the *michauxii*-type: 428 individuals (table 3, fig. 1, 4), (Fischer 1981 & Saeidi-Mehrvarz 2011). However, there is no clear geographical pattern explaining the distribution of these morphotypes. Thus, there is the possibility that these morphotypes are three sympatric species, whose distinguishing characters are blurred by plastic characters, specific for the respective population, or these characters are responses of one common taxon to different environmental factors, variable within populations.

We exclude the possibility of in-situ origins of new distinct taxa in Iran based on the high interconnectivity of the habitats allowing gene flow between populations

and the absence of obvious geographic, environmental, or reproductive barriers. However, we cannot distinguish between high gene flow in three separate taxa or local differentiation due to phenotypic plasticity based on the diversity of water availability throughout the year. Such a pattern has been shown in Japanese populations of *Veronica anagallis-aquatica* (Matsubara & al. 2016). Our analysis, however, did not allow testing the influence of ploidy on the distribution patterns. On the global scale, the lysimachioides-type is associated with the diploid level whereas the other types are associated with the tetraploid level (Albach & al. 2008). Our analysis of habitat factors and morphological traits does allow us to infer some characteristics in distribution patterns (fig. 1, 4; table 4). For example, the lysimachioides-type is found in populations of the upper left corner of the PcoA (fig. 2) and restricted to habitats that are characterized by low electrical conductivity (EC <300 µs/cm; table 4). The oxycarpa-type is found in the lower right corner of the PcoA (fig. 2) and predominates in habitats characterized by high EC (>1000 µs/cm) and high pH (>7.0). In contrast, the michauxii-type is widespread, suggesting that this morphotype consists of multiple ecotypes or it forms a more general purpose-ecotype. Common garden experiments would be helpful to test these results further.

Comparing the UPGMA clustering analysis based on morphological data with the distribution chart of *V. anagallis-aquatica* morphotypes, there is a significant relationship between the distribution of *V. anagallis-aquatica* subspecies with the overall morphological data (not shown). This suggests that the morphological characters used to distinguish the three subspecies are indeed important morphological characters to delimit the intraspecific morphological variation. Future research should focus on the inheritance of characters distinguishing morphotypes, such as the dense glandular indumentum of the michauxii-type or the orbicular capsule of the lysimachioides-type. The rarity of intermediate morphotypes also raises the question about reproductive barriers between morphotypes with especially ploidy being a potentially overlooked aspect in our analysis.

Further, our groupings based on population-level analyses correspond partly with our previous analysis of genetic variation using ISSR (not published). However, that study did not support the genetic differentiation of the three subspecies since all populations formed independent genetic clusters. Therefore, as mentioned above, current evidence does not allow recognition of different species or subspecies, rather, these taxa are morphotypes that are partly ecologically separated, but further research is necessary to evaluate their taxonomic value.

We suggest that the studied *V. anagallis-aquatica* populations are characterized by adaptation to local conditions and phenotypic plasticity, which does not form along previously determined taxonomic limits but is not independent of these. The existence of some level of differentiation among the studied populations must be due to different environmental effects including geographical, hydrographical connection, soil, climate, and biotic factors from different districts (Shafie & al. 2011) or natural selection (Sultan 2003).

This study highlighted how morphometric, environmental, and bio-geographical information can help to understand intraspecific diversity and population variations. Phenotypic diversity observed can be used to derive valuable information about the genomic structure. On the other side, genetic information is useful to understand the control of phenotypic characters. Nevertheless, we still lack a clear scenario about the importance of phenotypic characters to distinguish genetic groups in *V. anagallis-aquatica*. This will likely require a range-wide phylogeographic analysis with common garden experiments. Here, we highlight especially the importance of electrical conductivity for such common garden experiments.

CONCLUSION

Here, we detected morphological and biogeographical, as well as partly ecological patterns of differentiation among populations of *V. anagallis-aquatica* in Iran. The main factors driving diversity and differences within and among *V. anagallis-aquatica* populations are local adaptation and ecological characteristics of the habitat. There is a clear geographical pattern for the distribution of *V. anagallis-aquatica* populations and morphotypes. Morphometric analyses helped to understand the taxonomic problems of *V. anagallis-aquatica* populations in Iran but did not reach a conclusive result. Common garden experiments and ploidy analyses, ideally accompanied by the transcriptomic or genomic study are needed to further understand the importance of the three morphotypes.

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