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Preservation and ecology of a living relict shrub in South Caucasus as a eco-genetic heritage from Tertiary: *Epigaea gaultherioides* (Boiss. & Bal.) Takht.

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Abstract

Aim: *Epigaea gaultherioides* is an euxine element with a tertiary origin as a relict species facing threat of extinction. In this study, an attempt was made to explore the ecological characteristics by taking into consideration the soil-plant interactions of this taxon.

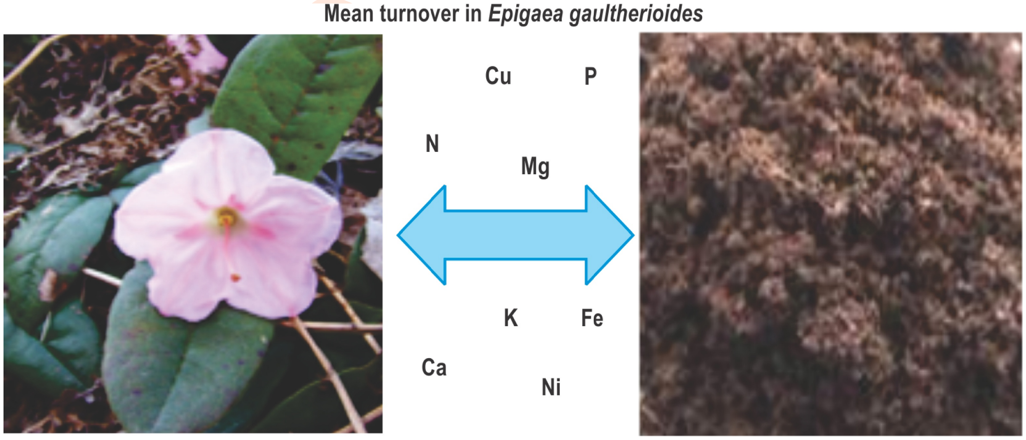
Methodology : Soil and plant samples were collected and analysed using Kjeldahl, Spectrophotometer, Flame photometer and ICP-OES.

Results : The findings showed that generally grows on sandy and / or clayey soils, of strong acidic nature, non-calcified, non-saline and high in organic matter. The plant samples collected from the study area were rich in iron, aluminium, zinc and nickel; but their percentage in the soils was low.

Interpretation : The findings of this study will assist to better understand the ecological requirements of this relict taxon for its possible future successful conservation programs.

Key words : Eco-genetical heritage, Endemic, *Epigaea gaultherioides*, Relict, Shrub

Mean turnover in *Epigaea gaultherioides*



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Introduction

Colchic region is one of the most important refuges for Tertiary relict biota in western Eurasia (Mai, 1995; Denk *et al*., 2001). The climate of the area is mild, with evenly distributed high precipitation and lack of winter frosts (Denk *et al*., 2001). Tertiary relict taxa present here are extant species which showed extensive distribution ranges in the region during different periods of the Tertiary, but are currently confined to small isolated areas (Denk *et al*., 2001). The relict taxa are mostly disjunctly distributed found in the Iberian Peninsula at the western margin of west Eurasia and in the Euxinian area (northern Turkey, western Georgia) in the East. These taxa also show small disconnected ranges throughout western Eurasia, but some are confined to only one of the potential relict areas of western Eurasia, whose relict nature is deduced from their fossil record only (Denk *et al*., 2001). The identification of relict populations, or of refugia can prove to be helpful in the conservation of such taxa (El-Bana *et al*., 2010). In addition to this the naturally gowing rare species or "old rare" species are regarded as endemics and relicts with a narrow range, restricted habitat or small population size (Huenneke, 1991; Aguilar *et al*., 2008; Šmídová *et al.*, 2011; Wei and Jiang, 2012). There is an urgent need to investigate the history of such populations using evidence from different fields (Pott, 1995; Šmídová *et al*., 2011).

Despite its ecological value and threatened status, no detailed ecological studies on the current status of *Epigaea gaultherioides* (Boiss. and Bal.) Takht. in the vulnerable ecosystems have been carried out. In view of the above, the present study provides insights into some ecological characteristics of this relict taxon.

Materials and Methods

Study species: *Epigaea* has three species each distributed on one of three land masses. *Epigaea repens* is found broadly in the eastern United States, *E. asiatica* is found in Japan and nearby islands, and *E. gaultherioides* is found in the Caucasus region of Eurasia (Gillespie and Kron, 2013). The Caucasus region has also been identified as a biodiversity 'hotspot' and possible Pleistocene refugium (Gillespie and Kron, 2013).

E. gaultherioides is an evergreen prostrate dwarf shrub, sharing the habitats, together, with *Fagus orientalis* and *Picea orientalis* forests as well as *Vaccinium arctostaphylos* and different taxa of *Rhododendron*. It rarely occurs on open north-facing slopes, generally found between 900-2300 m altitudes in the Northern parts of Turkey, the Caucasus and Georgia (Davis, 1978; Serdar *et al*., 2010). It is found in the Rize and Artvin provinces in Turkey (northeast Anatolia). The phytogeographical origin of this tertiary relict and rare taxon is presented as Euxine element (Davis, 1978).

Study sites: The plant and soil samples were collected from five different locations in the Northern areas of Turkey. The information on the collection sites is given below:

Location 1: Artvin-Şevvaltepe, Sazlıkdere 1700 m; Location 2: Rize-İkizdere, Çağırankaya 1850 m; Location 3: Artvin-Murgul, Tiryal Mountain 2000 m; Location 4: Rize-İkizdere, 2000 m and Location 5: Artvin-Murgul, Tiryal Mountain 2100 m.

Data analyses: Soil samples were collected from 0-30 cm depth air dried, and sieved using 2 mm mesh. The physical/chemical characteristics were determined following the methods outlined by Ozturk *et al*. (1997). The plant samples were also collected during same time, brought to the laboratory, left under direct air on the table, then dried at 80 ºC for 24 hrs and hand crushed. Total nitrogen content was determined by the Kjeldahl method; phosphorus content was estimated spectrophotometerically and total potassium and sodium content by flame photometer (Ozturk *et al*., 1997). The concentrations of potassium, calcium, magnesium, zinc, iron, copper, nickel, cadmium, lead, cobalt and aluminium were determined in the plant samples as well as the soils supporting the plant by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Ozturk *et al*., 2017).

Statistical analyses: All calculations were based on parameters of plant species and soil samples. Pearson's correlation statistical analyses was performed using IBM SPSS Statistics 20 software. Statistical significance were expressed as *p<0.05 and **p<0.01 level (Aldrich, 2018; Ozturk *et al*., 2019).

Results and Discussion

The results of analysis of plant samples are presented in Table 1. The nutrient concentrations measured in the plant samples of different localities ranged from 0.82-0.99% for nitrogen, 0.07-0.09% for phosphorus, 0.6-0.8% for potassium, 0.52-0.63% for calcium, 0.23-0.30% for magnesium, 145-620 mg kg^{-1} for aluminium, 0.0-1.5 mg kg^{-1} for cobalt, 1.5-4.5 mg kg^{-1} for copper, 70-230 mg kg^{-1} for iron, 3-15 mg kg^{-1} for lead, 3.5-6.0 mg kg^{-1} for nickel, and 18-37 mg kg^{-1} for zinc, respectively. Cadmium was not present in any plant sample (Table 1). In the soil, the concentrations of nitrogen, phosphorus, potassium, calcium, magnesium, copper, iron, and nickel varied between 0 and 0.003%, 0.11 and 0.18%, 0.003 and 0.006%, 0 and 1.08%, 0 and 0.011%, 0 and 1.49 mg kg^{-1}, 11.2 and 30.8 mg kg^{-1} and 2.06 mg kg^{-1}, respectively (Table 2).

In general, the plant samples collected from the study areas were rich in iron, aluminium, zinc and nickel; however, the percentages of these elements in the soils were low. The results of soil analyses are presented in Table 2. The mean values of soil samples revealed that *E. gaultherioides* prefers soils with sandy and / or clayey texture, strongly acidic in nature, non-calcified, non-saline but rich in organic matter.

Table 1 : Concentrations of some major and trace elements in the plant samples of different locations

Plant samples	Location 1	Location 2	Location 3	Location 4	Location 5
N (%)	0.82	0.82	0.99	0.93	0.96
P (%)	0.08	0.07	0.07	0.09	0.09
K (%)	0.6	0.6	0.7	0.7	0.8
Ca (%)	0.63	0.58	0.52	0.59	0.53
Mg (%)	0.29	0.30	0.26	0.23	0.29
Fe (mg kg^{-1})	150	70	135	230	120
Cu (mg kg^{-1})	2.5	1.5	4.5	3.5	4.5
Ni (mg kg^{-1})	5.5	5.5	3.5	6.0	5.5
Cd (mg kg^{-1})	0	0	0	0	0
Zn (mg kg^{-1})	18	18	29	24	37
Pb (mg kg^{-1})	12	6	15	3	8
Co (mg kg^{-1})	1.0	1.5	1.5	0.5	0.0
Al (mg kg^{-1})	205	235	275	145	620

Table 2 : Physical and chemical parameters in soil samples of different locations

Soil samples	Location 1	Location 2	Location 3	Location 4	Location 5
Sand (%)	78.55	90.81	74.10	78.66	73.77
Clay (%)	7.07	4.02	11.33	7.01	9.36
Silt (%)	14.38	5.18	14.58	14.26	16.87
pH	4.7	4.9	5.0	5.5	5.6
Organic matter (%)	1.60	21.76	3.79	4.42	2.38
$CaCO_3$ (%)	0	0	0	0	0
Na (mg kg^{-1})	20.03	15.18	22.72	15.09	28.61
N (%)	0.003	0.002	0	0	0
P (%)	0.11	0.12	0.12	0.17	0.18
K (%)	0.006	0.003	0.004	0.003	0.003
Ca (%)	1.08	0.02	0.09	0	0.07
Mg (%)	0.011	0.001	0.001	0.002	0
Fe (mg kg^{-1})	25.2	11.2	28.0	22.4	30.8
Cu (mg kg^{-1})	0.44	0.00	0.00	0.00	1.49
Ni (mg kg^{-1})	2.06	2.06	2.06	2.06	2.06

The correlation coefficients between the elemental values determined in the plant samples from different altitudes (Table 3) showed positive significant correlation between zinc and available potassium as well as copper (>0.97, >0.89). High positive significant correlations were found between the available copper and nitrogen (>0.95). However, highly negative significant correlations were found between the available of cobalt and phosphorus (->0.96).

The correlation coefficients between the elemental values determined in the soil samples from different altitudes (Table 4) showed that positive significant correlation existed between magnesium and available of potassium as well as calcium (>0.93, >0.98). High positive significant correlations were found between the available of organic matter and sand (>0.93); between available iron and clay (>0.88); iron and silt (>0.97); phosphorus and pH (>0.99); and finally between available calcium and potassium (>0.96). However, highly significant negative correlations were found between sand and available clay, silt and iron (->0.91, ->0.97, ->0.99), between organic matter and available of silt and iron (->0.97, ->0.92).

The correlation coefficients between the elemental values determined in the plant and soil samples from different altitudes (Table 5) showed that positive significant correlation existed between plant Cu and available of soil Fe (>0.88). However, highly significant negative correlations were found between plant N and available soil N (->0.93).

Habitat loss is the major factor for biodiversity loss which leads towards species extinction. The basis for conservation is the investigation of ecology and distribution of species. Many endemic and/or relict taxa show restricted distribution. This is one of the major topics of several ecological and phylogenetic researches (Ozturk *et al.*, 2004). Nearly 25 to 100 species are lost everyday as a result of habitat loss, alien species introductions, over exploitation of species and environmental pollution. Extinction of taxa is proceeding at such a high rate that our

Table 3 : Pearson's correlation coefficients of major and trace elements in plant samples in different locations

Correlation matrix (R)	P	K	Ca	Mg	Fe	Cu	Ni	Cd	Zn	Pb	Co	Al
N	0.251	0.841	-0.826	-0.543	0.315	**0.950***	-0.513	.0.115	0.846	0.200	-0.328	0.423
P		0.598	0.110	-0.347	0.624	0.383	0.641	.0.078	0.435	-0.525	**-0.959***	0.340
K			-0.726	-0.270	0.211	0.871	-0.092	.0.053	**0.973****	-0.075	-0.733	0.760
Ca				0.077	0.261	-0.720	0.623	.0.084	-0.817	-0.267	0.085	-0.619
Mg					-0.876	-0.439	0.053	.0.193	-0.123	0.244	0.160	0.421
Fe						0.350	0.249	.0.026	0.037	-0.279	-0.409	-0.381
Cu							-0.452	.0.204	**0.887***	0.314	-0.471	0.528
Ni								.0.261	-0.261	-0.824	-0.551	-0.046
Cd									.0.301	.0.273	.0.066	.0.088
Zn										0.119	-0.615	0.833
Pb											0.435	0.103
Co												-0.593

**Correlation is significant at 0.01 level (2-tailed); *Correlation is significant at 0.05 level (2-tailed)

Table 4 : Pearson's correlation coefficients of major and trace elements in soil samples in the different locations

Correlation matrix (R)	Clay	Silt	pH	OM	Na	N	P	K	Ca	Mg	Fe	Cu	Ni
Sand	**-0.913***	**-0.970****	-0.414	**0.933***	-0.728	0.547	-0.384	-0.193	-0.100	-0.011	**-0.985****	-0.467	.0.036
Clay		0.785	0.271	-0.735	0.724	-0.612	0.184	0.105	-0.075	-0.185	**0.881***	0.293	.0.049
Silt			0.463	**-0.974****	0.673	-0.459	0.470	0.233	0.200	0.130	**0.966****	0.536	.0.093
pH				-0.285	0.334	-0.813	**0.986****	-0.716	-0.634	-0.635	0.383	0.487	.0.070
OM					-0.558	0.301	-0.303	-0.422	-0.375	-0.326	**-0.924***	-0.406	.0.162
Na						-0.349	0.324	0.051	0.035	-0.161	0.816	0.835	.0.205
N							-0.709	0.678	0.769	0.781	-0.458	-0.167	.0.308
P								-0.651	-0.528	-0.528	0.371	0.554	.0.218
K									**0.959****	**0.932***	0.241	-0.066	.0.316
Ca										**0.976****	0.177	0.078	.0.082
Mg											0.061	-0.081	.0.064
Fe												0.593	.0.028
Cu													.0.227

**Correlation is significant at 0.01 level (2-tailed); *Correlation is significant at 0.05 level (2-tailed); OM: Organic matter

coming generations will face serious consequences if steps are not taken today.

A great emphasis has been laid on the conservation of relict taxa and populations (Habel *et al*., 2010a; Hampe and Petit, 2005; Vogler and Reisch, 2013). The reason has being their evolutionary significance. Such populations sharing our planet today have existed successfully under severe ecological stresses for long durations (Ronikier *et al*., 2012; Vogler and Reisch, 2013). In view of this, they have got adapted to their harsh environment, mostly located at the edge of their distribution range and are exposed to strong selection processes. Therefore, relict taxa may be the best examples to challenge future climatic changes (Habel *et al*., 2010b; Vogler and Reisch, 2013). These plant taxa are natural laboratories fitting the models for testing how natural plant populations will respond to climate change (Hampe and Petit, 2005; Lázaro-Nogal *et al*., 2016). Their antiquity renders them important for the conservation of genetic diversity (Hampe and Petit, 2005; Hampe and Jump, 2011; Lázaro-Nogal *et al*., 2016). The relicts; in particular when these are also endemics; are of great importance from the point of view of conservation biology as they can be highly informative for knowing the past diversity and have a high patrimonial value (Grandcolas *et al*., 2014). The relicts are more informative in terms of diversity of todays groups than their original ancestral characters. Their importance would increase more during the coming decades because of the major extinction crisis currently under way. Latter will make it necessary to conserve species not only for ecological services but also as representatives of a patrimony nested in the Tree of Life. Relicts include valuable taxa, clading extinctions and need conservation as representatives of large and mainly extinct groups (Grandcolas *et al.,* 2014).

Table 5 : Pearson's correlation coefficients of major and trace elements in plant and soil samples in different locations

Correlation Matrix (R)

	Plant N	Plant P	Plant K	Plant Ca	Plant Mg	Plant Fe	Plant Cu	Plant Ni
Soil N	**-0.933***	-0.354	-0.845	0.781	0.614	-0.350	-0.813	0.272
Soil P	0.533	0.849	0.830	-0.341	-0.375	0.411	0.533	0.435
Soil K	-0.400	-0.192	-0.504	0.551	0.226	0.069	-0.176	-0.157
Soil Ca	-0.546	-0.022	-0.501	0.686	0.330	0.058	-0.292	0.109
Soil Mg	-0.604	0.002	-0.594	0.805	0.192	0.190	-0.381	0.198
Soil Fe	0.688	0.463	0.708	-0.409	-0.218	0.347	0.880*	-0.323
Soil Cu	0.226	0.577	0.664	-0.284	0.415	-0.182	0.426	0.230
Soil Ni	.0.303	.0.155	.0.222	.0.099	.0.106	.0.037	.0.052	.0.091

**Correlation is significant at 0.01 level (2-tailed); *Correlation is significant at 0.05 level (2-tailed)

In order to secure the survival of threatened relicts, international coordination among the scientists needs to be significantly improved (Kozlowski *et al*., 2012). For this purpose, living *ex-situ* collections will be an ideal solution as a part of overall conservation strategies. It must also include participation by local administration, national parks, foresters together with other *ex-situ* and *in-situ* approaches for successful conservation (Kozlowski *et al*., 2012).

Kozlowski *et al.* (2012) have presented some recommendations for relict plants conservation via living *ex-situ* collections:

1. Prepare a global priority list of most important relict taxa with their global conservation status; 2. "Botanic Gardens" and "Arboreta" in the countries with emblematic relict taxa in particular woody ones should integrate their *ex-situ* conservation into their conservation strategies and action plans. They need to develope their public awareness and outreach programmes, particularly in the regions where such woody species are most at threat; 3. Conservation priority should be given to the most threatened taxa, rare and/or to narrow endemics in the case of relict genera; 4. Involvement of local communities and organisations is a must and critical for long term conservation of such taxa. This needs to be encouraged and supported from the earliest stages of conservation planning; 5. In the case of new *ex-situ* collections only well-documented plant material with detailed information on its origin should be used; 6. Detailed investigations of the provenance data for all relict plants in cultivation should be undertaken in the case of existing, often very old collections; 7. Genetic studies at genus level should be carried out for verifying and/or clarifying of provenance of ex situ collections of threatened relict woody plants in cultivation; 8. Conservation of maximum genetic diversity for a given taxon should be undertaken concerning the minimum number of cultivated woody plants per botanic garden; 9. Well-coordinated specialist groups should be established for highly threatened relict woody species and/or genera, so as to act globally and to develop their long term *ex-situ* conservation strategy.

Epigaea gaultherioides, *Liquidambar orientalis, Quercus pontica* and other relict plants are ecologically important for Turkish relict diversity. They need special attention from the point of biodiversity as well as for the global diversity as a relict heritage with us from tertiary period. We must protect their current distribution area as much as we can.

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