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Genetic Diversity and Variability among Populations and Ecological Characteristics of the *Uechtritzia Armena* Freyn (Asteraceae) Endemic to Turkey

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Research Article

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ABSTRACT

Uechtritzia armena Freyn (Asteraceae) which is an endemic species for Turkey grows in the provinces of Gumushane, Erzurum and Artvin. In this study, its distribution, ecological features and threats are discussed. Its local future is treated. The distribution of the species is related to *P. sylvestris* var. *hamata*. It grows in the northern aspect in *P. sylvestris* var. *hamata* forest. The results of the soil analysis showed that the soil type of the species is loamy with clay. The pH of the soil varies between 6-7.52. The percentage of organic matter in the soil is between 2.9 to 4.24. In addition, genetic diversity among populations was determined by RAPD. 16 primer generated a total of 127 bands, with a mean of 7.94 bands per primer. RAPD analyses showed various band sizes, which ranged from 250 to 3000 bp. Mean polymorphic locus ratios were determined as 92.13% considering four populations. The percentage of polymorphic bands for this species was 96.21%. The Nei's gene diversity (H) ranged from 0.166 to 0.183, Shannon's Information Index (I) differed from 0.258 to 0.299, while Nei's gene diversity (H) varied from 0.166 to 0.183. Genetic distances between populations ranged from 0.981 to 0.201. Cluster analysis showed correlation between genetic and geographic distance.

INTRODUCTION

Uechtritzia Freyn (Asteraceae) is represented by three species in the world: *U. armena* Freyn, *U. kokonica* (Rgl. and Schmalh.) Pobed, *U. lacei* (Watt) C.Jeffrey. *U. kokonica* is located in Central Asia and Afghanistan [1] while *U. lacei* is observed in N.W India and S. Jammu and Kashmir [2]. *Uechtritzia* is allied to the genus *Gerbera L*. which is grows in S. Africa. It appears to differ from *Gerbera* principally by its paleaceus receptacle [3].

Turkey has a very rich flora with about 11.000 taxa. About 3.700 of 11.000 taxa are endemic for Turkey [4]. There are many reasons why Turkey is so rich with flora. The soil types, variability in topography climatic conditions, geological diversity and geological history of the country and its location between the two continents can be can be considered as the main reasons of this plant diversity in Turkey [5]. A large number of endemic species has limited distribution like *U. armena*. It was first collected on Mount Spikor (Gümüshane-recorded as Erzincan in Turkish Flora) in 1892 by Sintenis. It was known from type specimen and evaluated as Endangered (EN) based on IUCN criteria [6]. This species were recorded from provinces of Erzurum, Artvin and Van for the past 15 years [7-9].

The species with narrow distribution like *U. armena* is more sensitive to extinction due to low genetic variation. Before planning conservation activities on such species, critical information related to distribution, biological relationships, threats, reproductive mechanisms, ecological properties, genetic diversity and habitat characteristics is required [10]. Although genus *Uechtritzia* was taxonomically revised [11], no information on abovementioned conservation planning has been detected.

Molecular markers are widely used to characterize genetic structures of plant populations. These contain polymerase chain

reaction (PCR) based markers like RAPD, ISSR, SSR, AFLP. RAPD and ISSR analyses have been used to describe population structures and genetic polymorphism especially in rare and endangered species such as. *Caldesia grandis* [12], *Buxus sinica* [13], *Magnolia officinalis* [14]. The aim of the present study is to determine the level of genetic diversity and genetic variation and differentiation within and among populations based on RAPD markers and to collect ecological data, type of reproduction and habitat characteristics. The results will show the degree of threat of *U. armena* and help to develop effective conservation strategies for this endangered species.

MATERIALS AND METHODS

Macro-Morphological Data: 15 plant samples were randomly collected from each population. Then, the samples were prepared as herbarium materials by using standard herbarium techniques. The fruit samples were also collected from each population. Skap and capitula length, and size of leaves were measured in centimeter (cm). In order to monitor the adaptation to external side of their natural zone, the living material was planted in the pot.

Soil analyses: The soil analyses were conducted in the Soil Laboratory of Erzincan Horticultural Research Institute. The saturation % and constitution, salt %, pH, lime %, organic matter %, phosphorus (P), and potassium (K) kg/ha in the soil samples were determined according to the methods described by Tüzüner [15] (total salt quantity), Hindistan and İnceoğlu [16] (determination of soil reaction (pH)), Çağlar [17] (lime ($CaCO_3$) determination), Ülgen and Ateşalp [18] (phosphorus (P_2O_5) determination), Doll and Lucas [19] (potassium (K_2O) determination), Ülgen and Ateşalp [18] (determination of organic matter), and Tuzuner [15] (classification of the soils).

Plant Materials and DNA extraction: The plant samples were collected from distribution areas in Gumushane (Sipikor: symbolized as "S" in table and map), Artvin (Mutlugun: symbolized as "M", and Demirkent: as "M") and Erzurum (Ormanagzı: symbolized as "O"). Leaf samples of 15 randomly selected individuals per population were collected. Each leaves were stored in zip-lock plastic bags with silica gel. The leaf tissue was then stored at ambient temperature until DNA extraction. Total DNA was extracted from 100 mg leaf tissue using the Qiagen DNA extraction Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The quantity of DNA was determined at 1% agarose gels and concentration was determined by UV spectrometer.

RAPD analysis: First, thirty-five randomly obtained RAPD primers were tested in DNA bulks. Sixteen primers were proved to be clear and reproducible. PCR amplification reactions were carried out in thirty µl final volume of reaction mixture containing 10x Buffer 3.0 µl, dNTPs (10mM) 1.2 µl, magnesium chloride (25mM) 1.2 µl, primer (5µM) 2.0 µl, Taq polymerase (5 units) 0.4 µl, water 19.2 µl sample DNA 3.0 µl (100ng/ µl). The thermal cycler (Eppendorf Company) was programmed to 2 min at 95°C; 2 cycles of 30 sec at 95°C, 1 minute at 37°C, 2 minutes at 72°C; 2 cycles of 30 sec at 95°C, 1 minute at 35°C, 2 minutes at 72°C; 41 cycles of 30 sec at 94°C, 1 minute at 35°C, 2 minutes at 72°C; followed by a final 5 minute extension at 72°C then brought down to 4°C .

Electrophoresis: The PCR products (27 µl) were mixed with 6x gel loading buffer (3 µl) and loaded onto an agarose (1.5% w/v) gel electrophoresis in 0.5XTBE (Tris-Borate- EDTA) buffer at 70 V for 150 min. The gel was stained in ethidium bromide solution (2 µl Etbr/100ml 1xTBE buffer) for 40 min and visualized under UV in Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge, UK).

Data analysis: All clearly detectable RAPD product bands were scored as either present (1) or absent (0), and a matrix of RAPDs data were assembled. Only reproducible and well-defined bands were scored. The matrix was then used for the following analyses: the percentage of polymorphic band (PPB), observed number of alleles (n_a), effective number of alleles (n_e), Shannon's information index (I) [20] and Nei's gene diversity (H) [21]. These measures were obtained at both species level and population level using the software package POPGENE [22]. To examine the genetic relationship among populations, a dendrogram was also constructed based on Ne''s genetic distance (D) using an unweiged paired group method of cluster analysis employing arithmetic averages (UPGMA) of NTSYS-pc version 2.02c [23].

RESULTS

Uechtritzia armena Freyn: Perennial. Rhizome brown, covered with brown scaly leaves. Scape 1, 40-72 cm long, white-tomentose, pubescent near capitulum. All leaves basal, 2-7; lamina, 7-18 x 6-14 cm, green and subglabrouse above, densely white-tomentose beneath, entire, acute to obtuse at apex, cordate at base; Petiole 6-20 cm long, weakly winged, floccose white tomentose. Capitula 1, 2.5-3 (to 4 cm when dried) cm broad; phyllaries oblong-lanceolate, acuminate at apex, purpish, white-tomentose. Flowers carmine. Marginals female, bilabiate; lower lobes with 3 apical teeth; upper lobes bifid, clavate; staminal tube short, 1-1,4 mm; style clearly exserted, bifid, papillate. Inner flowers hermaphrodite, lips ± in size; staminal tube clearly exserted; filaments attached in middle of tube, longer than anthers; style bifid, ± exserted. Achens 5-7 mm long, villose, immature; pappus, 11-15 mm long **(Figures 1 and 2).**

Ecological Properties: *U. armena* grows on top boundary of the *Pinus sylvestris* L. var. *hamata* forest on Mount Sipikor (Gümüşhane). The other populations are found at the aperture of *P. sylvestris* L. var. *hamata* forest in Artvin and Erzurum. The number of individuals in each populations except those on Sipikor are over 2000. It is approximately 200 on Sipikor. Leaf, skap and leaf size are the least among Sipikor (S) population. Sipikor population is also found at the highest altitude **(Table 1).**

Figure 1. *Uechtriztia armena* in nature.

Figure 2. Rhizomes of *Uechtriztia armena.*

Table 1. The findings of some properties of the populations.

Population	Altitude (m)	Population Size (as individual)	Skap Average (cm)	Leaf Average	Petiol Average (cm)
				(cm)	
M	1465	>100	72	10x8	7,3
D	1615	>100	69	15x11	12
O	1710	>100	69	14x11	15
S	2220	180-200	57	8,5x5,5	5,3
Achens are immature and members of the species reproduce by rhizomes in all populations (Figure 2)					

Considering the results of soil analysis, saturation value, electrical conductivity (Ec), organic matter (%), lime ($CaCO_3$) (%), salt concentration (%) phosphorus (kg/ha), potassium concentration (kg/ha) and texture are indicated in **(Table 2).**

Table 2. Soil analysis for each population.

Parameters	O	M	D	S
pH	6	6,4	7,52	6,95
Ec (milimhos)	0,38	0,2	0,2	0,24
Texture	69	58	78	63
Organic matter (%)	4,24	2,9	3,19	3,19
Lime (%)	0,97	1,36	1,71	1,36
Salt (%)	0,017	0,007	0,01	0,01
Phosphorus (kg/ha)	24,27	21,99	19,92	13,28
Potassium (kg/ha)	70,2	42,2	42,1	31,6

Genetic diversity and differentiation: In this study, 16 RAPD primers were selected and used to determine genetic diversity and population structure between and among four populations of *U.armena*. RAPD primer combinations produced 127 visible bands out of 250 to 3000 bp across 60 individuals **(Table 3).** The highest number of 12 bands was produced by OPW 6 and the least of 4 marker levels was produced by OPY 19. Banding patterns of D population genotypes using the primer OPA-01 are illustrated in **(Figure 3).**

Table 3. List of the selected primers and the degree of polymorphism obtained among the four populations.

Primer code	Sequence5'→3'	Size (bp) Min-Max	Total band	Polymorphic bands	Polymorphism ratio (%)
OPB-8	GTCCACACGG	500- 2600	10	9	90
OPY-1	GTGGCATCTC	400- 2300	8	8	100
OPW- 20	TGTGGCAGCA	600- 1900	6	5	83.3
OPK06	CACCTTTCCC	750- 2200	8	7	87.5
OPK19	CACAGGCGGA	300- 2700	10	9	90
OPBB13	CTTCGGTGTG	600- 1800	5	5	100
OPA-01	CAGGCCCTTC	400- 1900	6	5	83.3
OPA– 04	AATCGGGCTG	250- 2200	9	8	88.8
OPH- 16	TCTCAGCTGG	500-1900	7	7	100
OPH- 18	GAATCGGCCA	600-2800	8	7	87.5
OPW– 6	AGGCCCGATG	400- 1600	12	11	91.6
OPW– 7	CTGGACGTCA	300- 2700	10	10	100
OPW– 8	GACTGCCTCT	600- 1900	7	6	85.7
OPW– 13	CACAGCGACA	500-3000	8	7	87.5
OPW– 17	GTCCTGGGTT	750- 2200	9	9	100
OPY- 19	TGAGGGTCCC	500- 3000	4	4	100
Polymorphism		250-3000	127	117	92.13

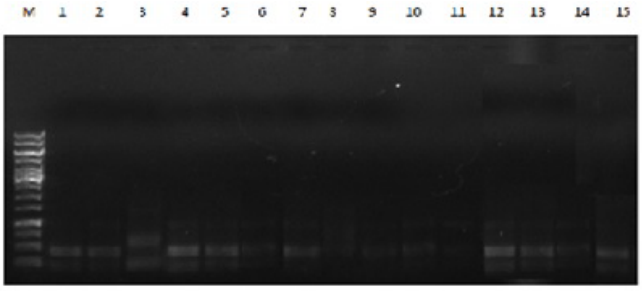

Figure 3. Ampliication products generated from 15 individuals of D populations of *Uechtritzia armena* using primer OPA-01. M = molecular marker.

The average numbers of bands and polymorphic bands per primer were 7.93 and 7.31 respectively. The percentage of polymorphic bands (PPB) per population varied from 53.85% to 69.23% with an average of 63.74%. The percentage of polymorphic bands for this species was 96.21%. The percentage of polymorphic loci, the average genetic diversity within populations of the four *U. armena* populations showed the following order S, O, M, D.

The Nei's gene diversity (H) ranged from 0.166 to 0.183, with an average of 0.171 and 0.192 at the population and species level respectively. Shannon's index (I) varied from 0.258 to 0.299 with an average of 0.275 and 0.333 at the population and species level respectively. *U. armena*, the highest and lowest levels of diversity occurred in populations D (PPB: 69.23%; H: 0.183; I 0.299) and S (PPB: 53.85%; H 0.166; I 0.258), respectively as shown in **(Table 4).** The coefficient of genetic differentiation among populations (Gst) was 0.321 with an observed effective gene flow between populations of (Nm) was 0.72. The overall genetic diversity of *U. armena*, based on RAPD analysis, is relatively high (PPB: 93.21%; I: 0.345; He: 0.265) at the species level, while it is relatively low (PPB: 63.74%; I: 0.275; He: 0.171) at the population level.

Table 4. Genetic variability within *Uechtriztia armena* populations detected by RAPD markers.

Population	No.of PB	PPB(%)	n_a	n_e	H	I
O	58	63.74	1.637	1.246	0.169	0.281
M	62	68.13	1.681	1.233	0.18	0.272
S	49	53.85	1.539	1.26	0.166	0.258
D	63	69.23	1.692	1.261	0.183	0.299
Population average	58	63.74	1.637	1.25	0.171	0.275
Species	91	96.21	2	1.463	0.192	0.333

PB: Polymorphic bands; PPB: Percentage of polymorphic bands; n_a = Observed number of alleles; n_e = Effective number of alleles; H = Nei's (1973) gene diversity; I = Shannon's Information index.

Cluster analysis: In order to represent the relationship among populations, cluster analysis (UPGMA) was used to generate a dendrogram based on Nei's genetic distance among the four populations studied. The dendrogram realized from the RAPD markers grouped four populations into two major clusters. Cluster 1 formed S population. Cluster 2 was divided into 2 sub-clusters: D and M populations formed sub-clusters. O populations formed other sub-clusters. The distance matrix showed that the highest genetic distance (0.981) was between S and O populations and D and M populations were found to be the most distinct with the lowest genetic distance (0.201) **(Figure 4).**

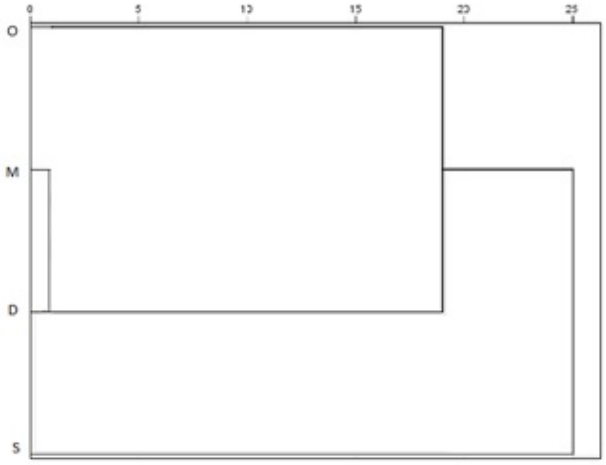

Figure 4. UPGMA clustering for 4 *U. armena* populations based on RAPD markers.

DISCUSSION

The species was first collected on Mount Sipikor in 1892 by Sintenis. It was indicated that the achens were immature in Sipikor population [3]. In this study, the same condition was detected for all populations. The individuals in the each population are dispersed in groups. Considering the distribution of populations in Turkey **(Figure 5)**, the critical question is if the species reproduces as vegetative and how its distribution can best be explained. Except the population in Van, the distribution of the species is related to *P. sylvestris* var. *hamata*. Distribution of *P. sylvestris* was limited in the lastglacial period [24,25]. Anatolia was a refuge for *P. sylvestris* and some conifers during the last glacial maximum (LMG) **(Figure 6).** After the last glacial period, the distribution of *P. sylvestris* has been changed. It moved back to north and higher altitude following colder climate [26]. During this movement, *Pinus* forests were fragmented. This fragmentation was accelerated by human destruction. According to Atalay [27], the most important factors of *Pinus* forests destruction in Anatolia are related to human-beings. It is estimated that *U. armena* was distributed in wider areas and its distribution was limited with habitat fragmentation after the last glacial period and during civilization in Anatolia.

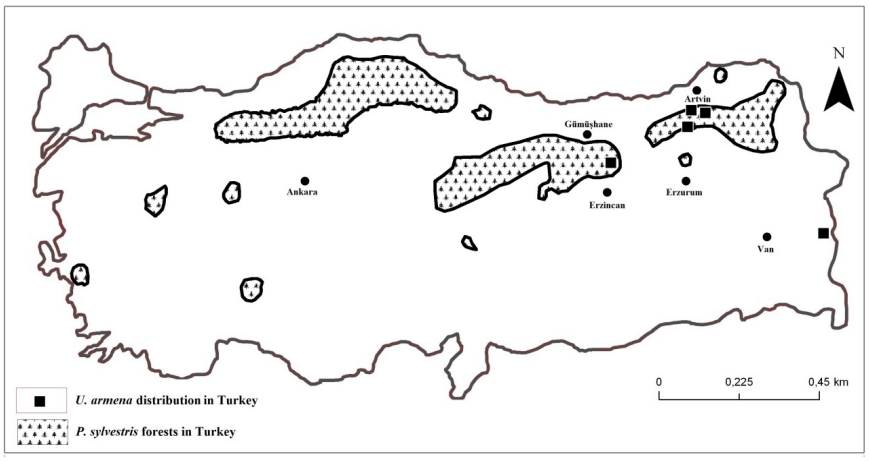

Figure 5. Distribution of *Pinus sylvestris* and *Uechtritzia armena* in Turkey.

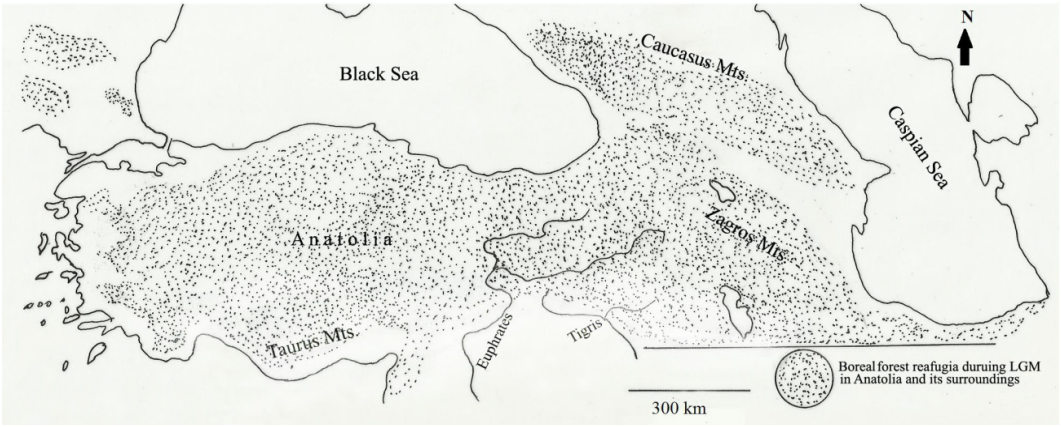

Figure 6. Distribution of Boreal forests including *Pinus sylvestris* during the Last Glacial Maximum in Anatolia and its surroundings.

The genus *Uechtritzia* is allied to the S. African *Gerbera* [3]. In addition, the species of the *Uechtritzia* extends up toKashmir (*U. kokonica* grows in Central Asia and Afghanistan; *U. lacei* is in N.W India and S. Jammu and Kashmir). The species of the genus are represented by isolated populations like *U. armena*. Probably, they were growing in larger geographical range in the past. The distribution scenario from past to present related to *U. armena* tells that the species is a paleoendemic.

Effective population size should consist of at least 4000-5000 members for sufficient genetic diversity in small populations. Loss of genetic variability and related problems of inbreeding and genetic drift are common in small populations [10]. Around 200 individuals were counted in Sipikor population. Moreover, the population is found on roadsides. It is estimated that the species may become locally extinct in the near future. The number of the species is quite high among Artvin and Erzurum populations. The members are very common in all of the gaps among the *P. sylvestris* in forests in Artvin and Erzurum. The total number cannot be determined due to a high number of individuals. The populations in Erzurum and Artvin are not under any threat yet. In other words, the future of the species is evaluated as good at the global level but *U. armena* may loss its natural habitats because of unexpected forestry activities in the future.

The results of the soil analysis showed that the soil type of the species is loamy with clay. It is slightly alkaline in Demirkent population while slightly asidic among Ormanağzı and Mutlugün populations. It is neutral in Sipikor population. While organic matter is in medium amount in Mutlugün population, it is higher in the rest of the populations. It is saltless for all populations. Whilst phosphorous is low in Sipikor population, it is medium in the others. Finally, potassium content is medium in Ormanağzı population butit is low in the others.

In terms of morphological characters, scap and petiole in Sipikor population are in average shorter than the others **(Table 1).** Two factors may be effective in this regard. First the soil is neutral and phosphorous content is low in Sipikor population. Second the population is distributed in more open areas and the light is sufficient in Sipikor population. The rest of the populations are surrounded by *P. sylvestris* which partially blocks sunlight.

Without any research of genetic variation in other *Uechtritzia* species, in comparison with other Asteraceae species, such as *Saussurea chabyoungsanica* (P: 90.5%; H: 0.352; I: 0.445 at the species level), (P: 45.6%; H: 0.173; I: 0.207) at the population level [28] and *O. longilobus* (PPB: 95.16%, H: 0.349, I: 0.517) at the species level, (P: 68.55%; H :0.271; I: 0.395) at the population level, O. *taihangensis* (PPB: 94.58%; H: 0.332; I: 0.504) at the species level, (P:71.50%; H: 0.208; I: 0.310) at the population level [29], *U. armena* was found to process high genetic diversity at the species level but lower genetic diversity at the population level.

Many studies demonstrate that endemic and endangered species tend to have a low level of genetic diversity [30]. The population and species average genetic diversity obtained in the present study was similar to that referred to other endangered and endemic species as *Sinojackia dolichocarpa* (PPB: 72.99%; H: 0.225; I: 0.3453), (PPB:36.32%; H:0.127; I; 0.189) [31], *Taihangia rupestris* (PPB: 80.43%; H: 0.248; I: 0.378), (PPB: 47.01%; H: 0.186; I: 0.272) [32], L. Sinense [33] at the population level and species level, respectively. *Saruma henryi Oliv* is an endangered perennial herb endemic to middle China, and the genetic diversity is quite low at the population level (in average, PPB = 22.8%, H= 0.083, I= 0.123) [34].

The level of genetic diversity, considering all *U. armena* populations, was similar to that obtained in studies conducted with the same molecular markers on *L.sinense* (PPB= 43.86, H= 0.169, I= 0.249) [33], *C. amonea* (PPB= 37.2, H= 0.120) [35].

In rare plants, demographic history, breeding system, geographic distribution range, somatic mutations, habitat fragmentation can significantly affect genetic diversity and it's partitioning within and among populations. Selfing species tend to have lower genetic diversity and heterozygosis within populations, as well as higher genetic differentiation among populations than mixed or outcrossing ones [36-38]. The loss in genetic diversity that is, extinction risk is resulted from reduction in population size of a species [39].

In conclusion, the present study showed that a great genetic diversity occurred among the *U. armena* populations due to their geographic and morphological differentiation. Morphology and RAPD markers are powerful tools for evaluating the genetic diversity and relationships in different *U. armena* populations.

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