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MORPHOLOGY AND ECOLOGY OF *ANCHUSA LIMBATA* (BORAGINACEAE) – NATIVE TO ANTALYA (TURKEY)

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Abstract

In this study, morphological and ecological characteristics of *Anchusa limbata* Boiss. & Heldr., a rare species endemic to Antalya, have been investigated. The morphological features of the species were examined in detail; measurements were made with a digital caliper, ruler, light microscope, stereomicroscope and scanning electron microscope (SEM). Detailed description has been given by specifying the minimum and maximum ranges, and morphological notes have been provided. Ecological data were obtained from field and laboratory studies. In the field studies, the species growing with *A. limbata* were recorded and the locality characteristics, slope and aspect ranges were also noted with a clinometer and a lensatic compass, and numerical data about the population status of the species in the areas of its occurrence were recorded as ranges and threatening factors were identified by making counts in 15 sample areas. The relationship between the distribution of the species and soil properties was examined. *A. limbata* grows in chalky soils of the redpine forest and travertine slopes. Populations of this species are found between 24° and 355° exposures (commonly ENE), between 0° and 45° inclinations. Another feature is some indicator species that indicate the presence (presence/absence) of the species in the fields such as *Pinus brutia, Verbascum leptocladum, Thymelaea tartonraira, Alkanna pinardi* etc. are indicators of *A. limbata*'s presence. In contrast to the literature, both biennial and perennial individuals of the species have been recorded. The threatening factors for the species are urbanization, climatic changes, tourism activities, field and road opening, landscape arrangement. The current research has shown that *A. limbata* must be protected together with its natural habitat using various in situ conservation strategies.

Key words: *Anchusa limbata*, Conservation, Ecology, Endemic, Plant morphology, Turkey.

Introduction

Pine forests are considered to be one of the most important elements of Natural Mediterranean forest ecosystems. These forests have been severely affected by anthropogenic disturbances and consequences of global change. To achieve an appropriate and complete management of these ecosystems key species or endemic species, beyond dominant ones that support vulnerable ecosystem functions, should be taken into consideration (López-Jurado *et al.*, 2019). Few (2-3) species become extinct each year for the past two and half centuries in the World and extinction of seed plants is at a faster rate than the normal turnover of species (Humphreys *et al*., 2019). According to Pimm & Raven (2000), the leading cause of species extinction is habitat destruction. They opined that humanity had been rapidly destroying rich habitats. Unless there is immediate action to salvage the remaining unprotected hotspot areas, the extinction of species will be more than double in the years to come. On the other hand, global climate change has been threatening biodiversity. Extreme climatic shifts could increase extinction risk and alter the distribution of species and significantly decrease the population size of rare species on small, fragmented, restricted to fine-scale geologic formations or limited dispersal ability (Maschinski *et al*., 2006). Small populations are especially sensitive to extinction and the consequences of local extinction are greatest for rare endemic species, for which local losses can be considered as equivalent to global extinction (Maschinski *et al*., 2006; Honnay & Jacquemyn, 2007; Dirnböck *et al*., 2011).

Recently, many species under the threat of extinction have been ensured the sustainability and conservation of populations and biodiversity through the species action plans that is supported by the General Directorate of Nature Conservation and National Parks of Turkish Republic Ministry of Agriculture and Forestry and one of these plans is the Action Plan of *Anchusa limbata* (NCNP, 2017).

Genus *Anchusa* L. (1753, 133) with about 170 species is a member of the "Boragineae" tribe of the family Boraginaceae. The genus *Anchusa*, mainly distributed in the Mediterranean Basin and Middle East Countries includes 30-40 species (Hilger *et al.*, 2004; Akcin *et al.*, 2010; Yıldırım, 2016). The genus was monographed by Guşuleac (1927, 1929). The genus was treated in the Flora of Turkey by Chamberlain (1978) and later by Davis *et al*., (1988); Seçmen *et al*., (1998); Valdés (2011); Güner *et al*., (2012). In Turkey, the genus *Anchusa* is represented by 19 taxa, 5 of which are endemic. These endemic taxa are *A. konyaensis* Yıld. var. *konyaensis, A. konyaensis* var. *selcukensis* Yıld., *A. leptophylla* Roem. & Schult. subsp. *incana* (Ledeb.) D.F. Chamb., *A. leptophylla* subsp. *tomentosa* (Boiss.) D.F. Chamb. and *A. limbata* Boiss. & Heldr. (Chamberlain, 1978; Davis *et al*., 1988; Seçmen *et al*., 1998; Valdés, 2011; Güner *et al*., 2012). Heldreich collected a specimen for the first time from Antalya (Pamphylia region of Anatolia) and this unusual specimen belonged to genus *Anchusa* L. and was described by Boissier (1849) as *A. limbata* on Heldreich's specimen (Baytop & Tan, 2008). Until the study of Bigazzi *et al*., (2003), it had only been known from the type specimen of Heldreich for approximately 150 years. Ekim *et al*., (2000) and Bigazzi *et al*., (2003) classified this threatened species under the category Critically Endangered (CR). After completion of the Flora of Turkey, there were a few studies on the *Anchusa* species in Turkey. A basic taxonomic research on *A. limbata* was published by Bigazzi *et al*., (2003). Also, the systematic information about *A. limbata* and some of the factors that threaten its natural environment based on Bigazzi *et al*., was briefly provided by Yıldırım (2016). In the current study,

considerable new information about ecological and morphological features of this species in Turkey have been collected and discussed in detail.

Materials and Methods

Research data were obtained from literature, research area and laboratory studies conducted between 2017 and 2021. Field studies were carried out primarily in Antalya and then in the neighboring provinces (Burdur, Isparta). The studies were carried out in 304 different localities, especially in the Red Pine forest zones. *A. limbata* was found in 91 localities and the data were collected. The data of those localities where the plant was present/absent were processed with the QGIS 3.16 program and a map was created using them (QGIS.org, 2021).

Morphological measurements of the collected specimens of *A. limbata* were carried out (minimum 15 samples). The measurements were made with a digital caliper and a ruler, and they were shown as intervals. Pollen preparations were prepared according to the Wodehouse method (Wodehouse, 1935). Palynological characters of 20 pollen of *A. limbata* were measured under a Nikon binocular light microscope with a micrometer. Besides, SEM studies were conducted in Akdeniz University, Faculty of Medicine, Department of Histology and Embryology. The pollen prepared for SEM studies were collected through a special tape, covered with gold palladium using the Polaron SC7620 Sputter Coater tool, examined with the LEO 14320 Scanning Electron Microscope (SEM) and the pictures were taken with a Polaroid brand camera. The terminology used for the pollen morphological characters were of Erdtman (1943), Díez (1994), Bigazzi & Selvi (1998), Binzet & Akcin (2011), Halbritter (2016), Halbritter *et al*., (2018).

Besides, the study contains ecological relationships of *A. limbata*. Plant species present in the habitat of *A. limbata* were identified with the help of Flora of Turkey and East Aegean Islands (Davis, 1965-1985; Davis *et al*., 1988; Güner *et al*., 2000, 2012). Presences, endemism status, threatened categories, chorotypes of these taxa were given according to Davis (1965-1985), Bern Convention (1979), Davis *et al*., (1988), Ekim *et al*., (2000). For the identification of insects, the study conducted by Demirsoy (2003) was used. In the areas where the individuals of the species have spread and not observed six soil samples were analyzed and compared. The analysis of the soil samples was done in the Ministry of Food, Agriculture and Livestock, Fruit Research Institute Directorate, Agricultural Analysis Laboratory (Eğirdir/Isparta). In addition, the ecological relationships of the plant with other plants and animals during the field studies were recorded through observations. Plants existing in 15 natural sample areas with variable area were counted in 2018, 2019, 2020 and 2021. Accordingly, the population trend of the species was also noted.

Results and Discussion

Anchusa limbata Boiss. & Heldr. (1849, 99) belongs to the family Boraginaceae.

Type: Hab. rarissima in colle calcareo pinetis consito Pamphyliae inter Adalia et Jenidjè Khan (Heldr.) Fl. Mart. Heldreich 468 (holo.: G; iso.: B)

A. limbata was first collected from Antalya during a botanical trip on 12 March 1845 by Theodor von Heldreich (Bigazzi *et al*., 2003; Baytop & Tan, 2008). This species was described by Boissier (1849). He also gave a detailed description again in the "Flora Orientalis" (Boissier, 1879). According to Bigazzi *et al*., (2003), Boissier could not give the morphology of the fruits, because the plant was collected by Heldreich in the early flowering period and without mature fruits but was able to identify the unique structure of the flowers of the plant.

Chamberlain (1977; 1978), recognized a monotypic subgenus *Anchusa* subg. *Limbata* Chamb. & R. Mill. based on the distinctive features of highly reduced corolla lips and transitional attachments. There was no other collection besides Heldreich's original specimen of *A. limbata,* Heldreich's specimens remained lone specimens for description and diagnosis. Therefore systematic relationship and better understanding of taxonomic position remained unclear (Bigazzi *et al*., 2003). However, with the rediscovery of this species by Bigazzi *et al*., (2003), some of its morphological characteristics, chromosome features, some ecological characteristics and conservation status were discussed.

Distribution: This species is found mainly in Kepez district and partially in the borderline of Döşemealtı-Kepez districts in Antalya (Fig. 1).

A. limbata, as stated in Chamberlain (1978) and Güner *et al.*, (2012), is a plant of the Eastern Mediterranean Phytogeographical region.

In Turkey, the local names that have been used for *Anchusa* species are Gövrek, Sığırdili, Arı çiçeği, Arı otu, Güriz, Ballı Emzik, Tatlı Emzik and Balıcak.

Specimens examined: Turkey, C3 Antalya: Kepez district, between Fatih and Duacı neighborhood, Kepez Urban Forest, redpine forest, travertine ledges, 201 m, 10.04.2017, Çinbilgel 10388 & Muca (AKDU 6223); ibid., 206 m, 10.04.2017, Çinbilgel 10400 & Muca; ibid., 209 m, 01.05.2017, Çinbilgel 10418 & Muca; ibid., 213 m, 01.05.2017, Çinbilgel 10427 & Muca; ibid., 206 m, 01.05.2017, Çinbilgel 10428 & Muca; ibid., 243 m, 19.05.2017, Çinbilgel 10451 & Muca; Kepez district, between Odabaşı and Kirişçiler villages, redpine forest, 265 m, 02.05.2017,Çinbilgel 10431 & Muca; ibid., 278 m, 02.05.2017, Çinbilgel 10433 & Muca; Kepez district, Varsak town, forest border-roadside, 228 m, 11.04.2017, Çinbilgel 10407 & Muca; Kepez district, between Kirişçiler village and Varsak town, forest border-roadside, 224 m, 20.05.2017, Çinbilgel 10483 & Muca; Döşemealtı district, Urban Forest, redpine forest, 222 m, 29.05.2017, Çinbilgel 10485 & Muca; Kepez district, Varsak town, chalky soils, 121 m, 30.05.2017, Çinbilgel 10489 & Muca; Kepez district, Varsak town, Karşıyaka neighborhood, redpine forest, 137 m, 02.06.2017, Çinbilgel 10492 & Muca; Kepez district, Varsak town, Ünsal neighborhood, redpine forest, 180 m, 03.06.2017,Çinbilgel 10493 & Muca; Kepez-Döşemealtı districts, between Fatih and Duacı neighborhoods, Kepez Urban Forest, redpine forest, 277 m, 12.06.2017, Çinbilgel 10507 & Muca (GPS records of the localities where the species is distributed are located in the NOAH's Ark National Biodiversity Database).

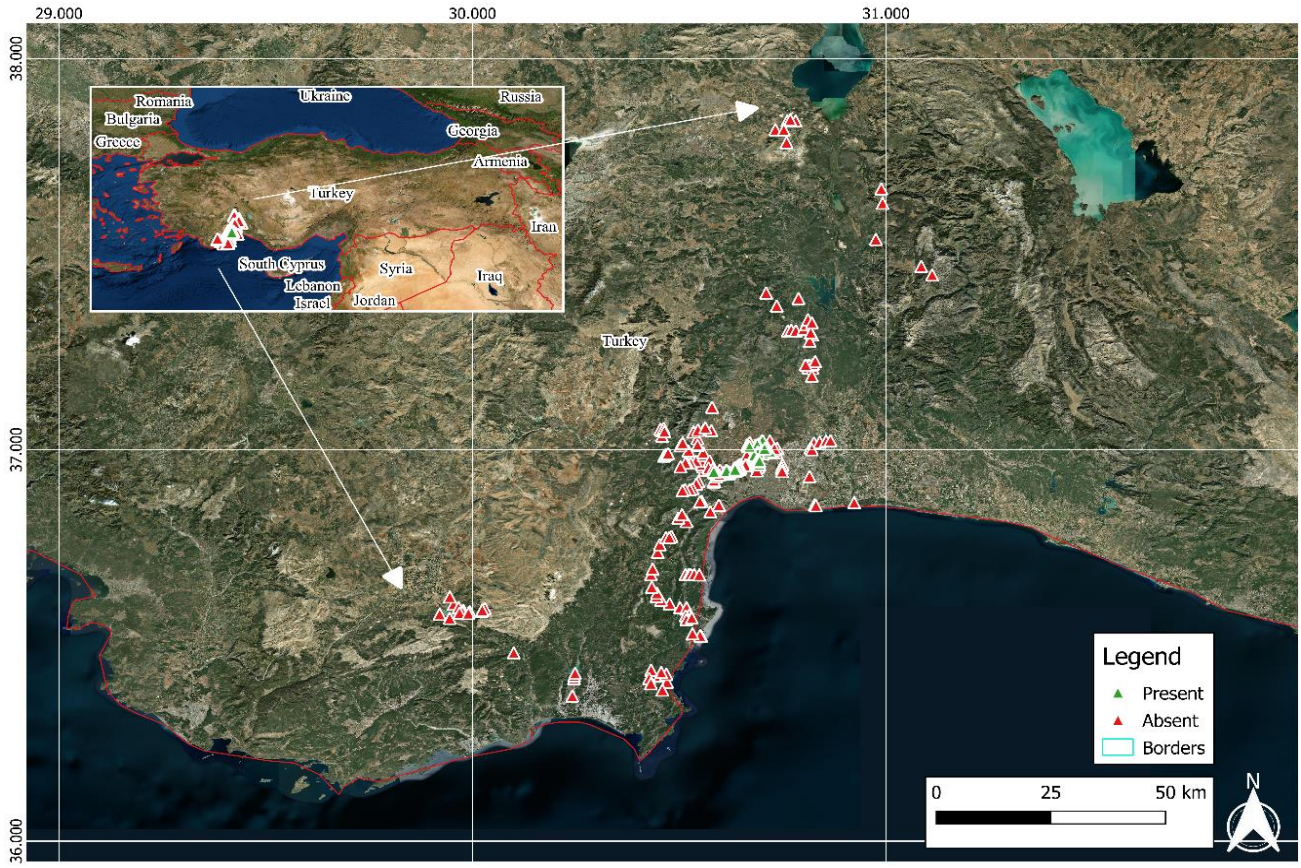


Fig. 1. Distribution of *A. limbata* (green marks: present, red marks: absent).

Additional specimen examined: *A. limbata*-Turchia C3 Antalya: collina di Kepez (periferia N di Antalya), pineta rada di *P. brutia*, in punti su suolo calcareo sbriciolato, 245 m, 5.6.2002, Bigazzi, M.; Duman, H., Selvi, F. 02.01. (E00163619, photos!).

Phenology: *A. limbata* is a biennial to perennial plant. Flowering time is from February to mid-June. Fruit formation begins in April when the flowering continues. Besides, mature fruits can be found in April, as well. However, immature fruits have also been observed. It is possible to observe its young seedlings from February to August.

Seedlings and young individuals die due to severe intraspecific competition for water or the influence of sun rays. This type of death generally occurs in May. Secondly, plants have been found to die altogether with dried flower at the end of generative period. This type of death is observed in June (Table 1). Climatic changes may cause changes in phenology of the species.

Morphology: The extended recent updated description of *Anchusa limbata* Boiss & Heldr.:

Monomorphic, patent-hispid biennial or perennial herbs; root to 50 cm; stems decumbent, ascending or erect, to 30 cm, branches to 9; basal leaves linear to narrowly lanceolate, 4–10 × 0.4–0.8 cm, widest in ¾ towards apical, entire to slightly undulate; stem leaves become small upward, 1.8–7.7 × 0.4–0.9 cm, amplexicaule, lower stem leaves similar to basal leaves, upper stem leaves wide at base and narrow towards the end. Cymose generally dense with 13-69 (-90) flowers (sterile flowers can also be present), pedisel 3–12 mm, drooping in fruit; bracts foliaceous, triangular, wide at base and amplexicaul, with slightly S shape in apical, 5.8–20 × 2.8–6.5 mm, ± densely whitish patent-hispid. Calyx ellipsoid in flower, swollen at middle, 7–11 × 3.5–5 mm (generally 9 mm long), swollen in fruit, broadly ovoid, 10–13 × 5.2–11 mm, patent-hispid, divided to 1/3, lobes triangular, 3 mm (to 4.5 mm in fruit). Corolla actinomorphic, tube 9–11 mm, pinkish at upper part and whitish-pale pinkish, limb greatly reduced with crenulate margin, reddish, with 1–2 mm short lobes. Corolla deciduous and upper part of corolla tube and lobes dark purple, lower part of corolla tube straw colored when dry. Stamens inserted above middle of tube; scales conspicuous, to 1–1.5 mm, exserted, densely long-papillate. Anthers 1.17–1.57 mm. Stigma capitate or slightly bilobed, style exserted, persistent. Young nutlets green, mature nutlets to dark brown from pale brown, oblique and helmet shaped, sometimes fewer by abortion, 2–2.2 × 2.2–3 mm, opening from scar by splitting. Fl. (2)3–6. Chalky soils on travertine under and openings *Pinus brutia* forest, 79–294 m (Fig. 2).

This species grows in calcareous soils on the travertine rocks under the Red Pine forest or in its openings, at 79–294 meters. Boissier (1849) described this plant species as biennial in the original protologue, but later stated it as a perennial in his 1879 publication. With the phenological observations in recent field studies, it was found that this species had both biennial and perennial individuals. The monomorphic hairs of *A. limbata* are a systematically important diagnostic character. Besides, characters such as petals color and short lobes are also important characters in distinguishing

A. limbata from other species. It was observed that *A. limbata*'s stigma was capitate or slightly lobed similar to the findings of Bigazzi *et al.*, (2003).

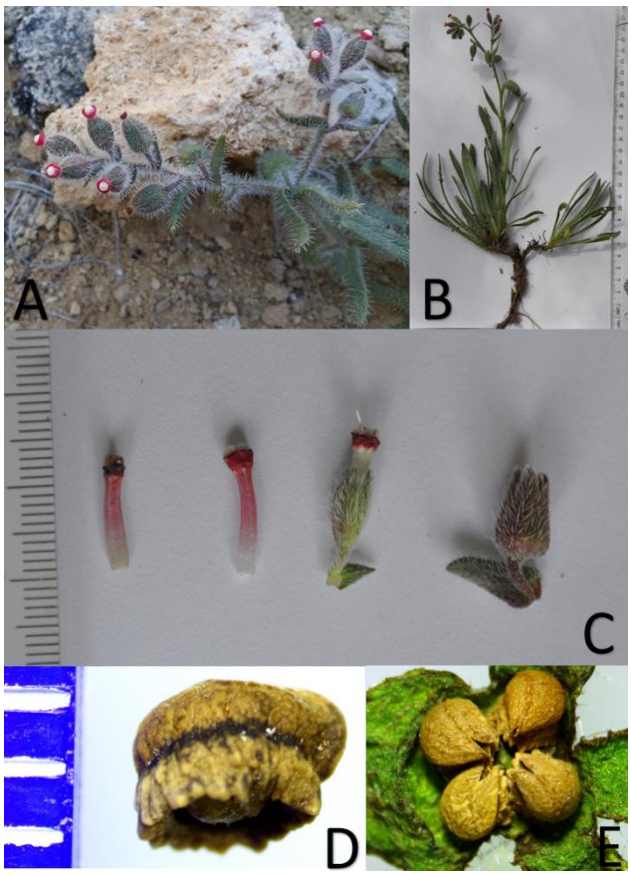


Fig. 2. *A. limbata*; A-B. Habit, C. Flower parts, D-E. Fruit.

Pollen morphology: Pollen unit monad, size: medium sized, polar axis (P) average 39 ± 2.36 µm, equatorial axis (E) average 29.05 ± 2.19 µm, polar axis/equatorial axis ratio shape index (P/E ratio) 1.34, prolate in shape, pollen class: colporate, pollen diameter average in polar 29 ± 1.50 µm, polar shape: spheroidal, tricolporate brevicolporus/ brevicolporate, with an average equatorial diameters of pores 3.75 ± 0.71 µm, average diameters of annulus 6.5 ± 0.69 µm, with exine ornamentation perforate; (micro-) reticulate-perforate in equatorial bant (Fig. 3).

According to Bigazzi *et al.*, (2003), equatorial reticulum of *A. limbata* was smooth while equatorial reticulum of other *Anchusa* taxa was distinctly sculptured-granulose. However, equatorial reticulum is (micro-) reticulate-perforate. The aperture number of *A. limbata* was 3. Bigazzi & Selvi (1998) also reported 3 apertures. Dièz (1994) recorded 4 apertures in Cetik's specimen. However, the species was wrongly identified and now currently treated as *A. hybrida*.

Habitat selection and ecological requirements: *A. limbata* grows in the red pine forest. Generally, it is found under red pine forests, in the openings of red pine forest, along forest paths, in refuges, in areas between road and the edges of red pine forests and ruderal areas in destroyed red pine forests.

It is found in the habitats where the rock and rock fragments appear on the surface on the main rock of travertine, which is highly permeable, spongy, loose and rich in lime, usually with white or sometimes gray colored soil. It can be seen in natural or artificially striped surfaces. Stripping or exiting the chalky soil could be due to the activities of some mammals (digging nest of foxes) and forest road construction by humans. Thus, it is observed that these activities have positive effects on the germination of seeds and the habitat of the species. Besides, after the trenches that are planned for the forest road, the plant grow on the heaps of soil along the roadside.

Specifically, the species does not grow in the Red Mediterranean (Terra-Rossa) Soil and Red-Brown Forest Soil which is the largest and most important soil group of the Mediterranean Region. Therefore, according to the study, it is believed that the biggest factor affecting the distribution of species is soil type. Most important factors controlling the species distribution are soil and travertine rock structures, special shape and structure of its fruits and the red pine forest vegetation. The species is not found in maquis and other impermeable marl lands.

A soil sample was taken from the area close to the distribution of the species and 5 soil samples taken from the regions representing the best examples of the populations were analyzed. The analyses were made randomly by choosing five chalky soil samples where populations were best represented and one soil sample from terra rossa soil in the red pine forest that was close to the distribution of the populations but that didn't have the aforementioned species (Tables 2-3).

According to soil analysis results, the soils in which the plant grows are quite chalky. The color of these soils is white-gray. The amount of lime in these soils is very high. However, the lime in the terra rossa soil sample is medium. The saturation of chalky soils is between loamy and clayey loamy soils. No significant difference has been found in saturation compared to terra rossa soil. Salinity of the chalky soils is between 0.004-0.006% (0.051). Accordingly, these soils are saline. Similarly, in the terra rossa soil sample, it has been observed that the salinity is 0.027%. When pH has been evaluated, it is observed that the pH ranges of chalky soil are between 7.6-8.3 and slightly alkaline. However, the terra rossa soil sample is neutral with 6.9 pH. The amount of organic matter in chalky soils is very low-middle range. On the contrary, the amount of organic matter is high in the terra rossa. The amounts of P, K, Ca, Mg, Fe, Cu, Mn, Zn in chalky soils range from very low to middle, while in the terra rossa soil they are between high and middle.

Table 1. Phenology of *A. limbata*.

	Months		1	2	3	4	5	6	7	8	9	10	11	12
Phenology	Vegetative period													
	Generative period	Flowering time												
		Fruit and Seed time												
	Deaths													

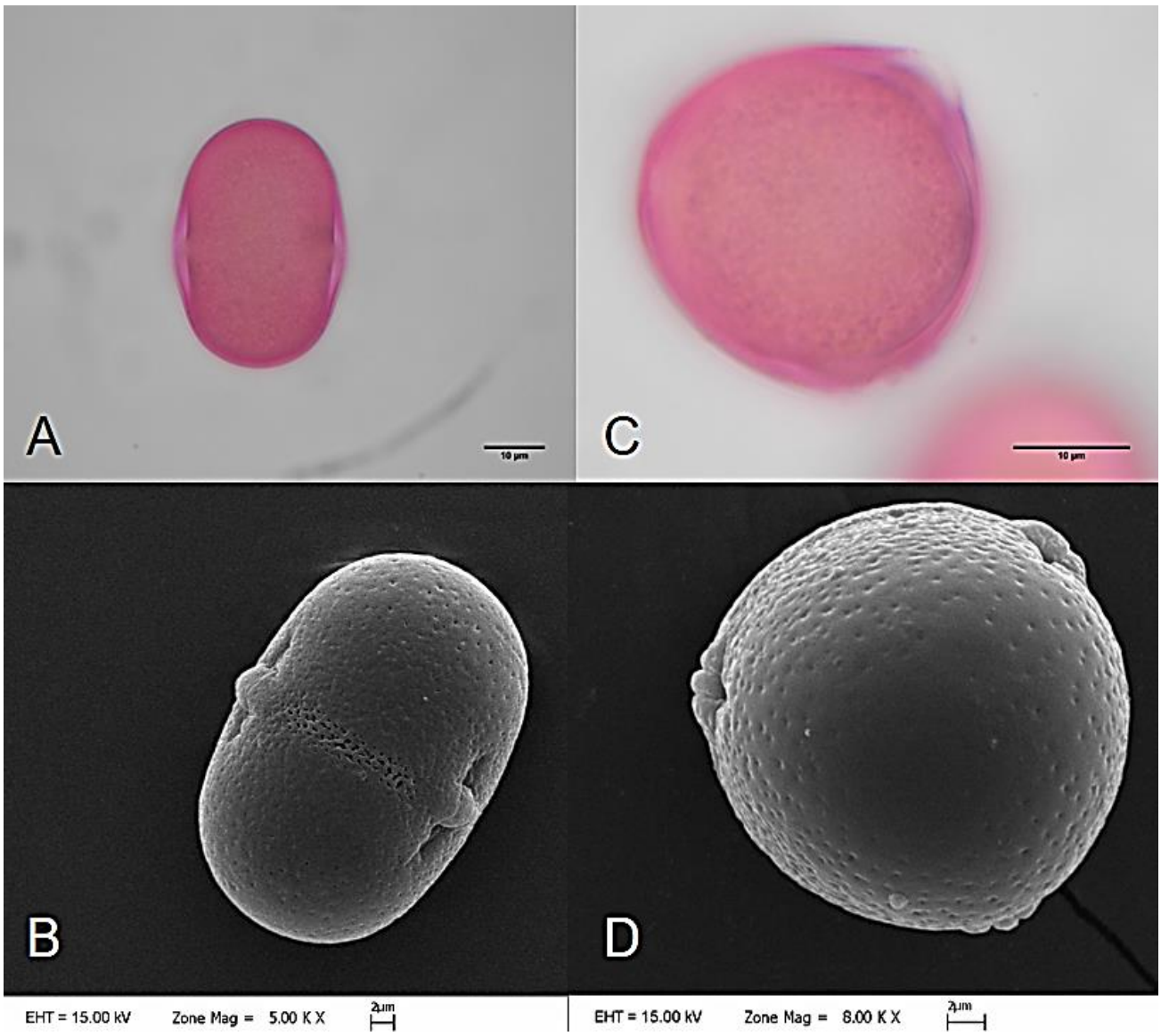


Fig. 3. A-D. Pollen grains of *A. limbata* (Light microscope images, scale bar: 10 µm, SEM microscope images, scale bar: 2 µm).

Table 2. Feature comparison of soils where species grows and not.

Sample number	Date (2017)	Soil color	Saturation (%)	Salinity (Saturation mud) (%)	pH (Saturation mud)	Lime (Calcimetric) (%)	Organic matter (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
1.	June, 02	White	39.05	0.004	8.1	>50	0.89	4	47	2743	46	1.75	0.17	0.34	0.17
2.	May, 30	White	42.9	0.051	7.6	>50	0.85	5	47	2767	46	1.21	0.24	1.91	0.41
3.	May, 29	White	56.65	0.004	8.3	>50	0.38	3	47	2452	46	0.46	0.13	0.14	0.14
4.	May, 02	White	53.9	0.006	7.8	>50	2.16	4	47	3272	46	3.86	0.14	0.68	0.38
*5.	June, 13	Red	57.2	0.027	6.9	5.45	4.48	64	770	5529	315	10.85	1.3	31.58	2.47
6.	May, 01	White	45.65	0.005	7.9	>50	0.58	1	47	2579	46	0.78	0.15	0.87	0.26

*Sample 5 is Terra Rossa soil

Table 3. Commentatived comparison of soil properties where species grows and not.

Sample number	Saturation	Salinity	pH	Lime	Organic matter	P	K	Ca	Mg	Fe	Cu	Mn	Zn
1	Loam	Unsalted	Light Alkaline	Extremely	Very few	Extremely	Very few	Medium	Very few	Few	Few	Few	Very few
2	Loam	Unsalted	Light Alkaline	Extremely	Very few	Few	Very few	Medium	Very few	Few	Medium	Medium	Few
3	Clay loam	Unsalted	Light Alkaline	Extremely	Very few	Few	Very few	Medium	Very few	Few	Few	Few	Very few
4	Clay loam	Unsalted	Light Alkaline	Extremely	Medium	Few	Very few	Medium	Very few	Medium	Few	Few	Few
5	Clay loam	Unsalted	Neutral	Medium	High	Much	Much	Much	Medium	Sufficient	Much	Sufficient	Sufficient
6	Loam	Unsalted	Light Alkaline	Extremely	Very few	Very few	Very few	Medium	Very few	Few	Few	Few	Few

According to these results, it is evident that both organic matter and inorganic substances are generally low in the chalky soils. Whereas organic matter and inorganic substances are more in terra rossa soil. So, chalky soils where the plant grows are very poor in terms of organic and inorganic nutrients.

The sociability scale of the *A. limbata* is in single or small groups. In terms of plant sociology, it grows in the unit of *Pinus brutia* community which develops in the Hot Mediterranean Vegetation Zone. According to Akman (1995), *Pinus brutia* communities in the Hot Mediterranean Vegetation Zone belong to the phytosociological units of *Quercetea ilicis* Br. – Bl. 1947 class and *Quercetalia ilicis* Br. – Bl. 1947 ordo.

Apart from these factors, it is necessary not to ignore the factors such as altitude, exposure and inclination. Based on the altitude measurements of 91 localities where *A. limbata* is located, it is distributed between 79 and 294 m. The average of the altitudes is 183.8 m. Standard deviation is ±50.7 m. Therefore, it can easily be seen between 133.1 m and 234.5 m. In the study, 40 measurements were evaluated with a lensatic compass and 44 measurements with a clinometer. In terms of exposure, populations are present between 24° and 355°. The average of measurements is 150.8°. The standard deviation is 83.9°. Thus, the species could be easily seen at the range between 66.9° and 234.7°. The species is most commonly found in the east-northeast. The most dense population is located in east-northeast. In terms of inclination, the populations of species is located between 0° and 45°. The average of inclination measurements is 17.45°. The standard deviation is 12.4°. Thus, species can easily be seen in the inclination degrees between 5.1° and 29.8°. It is most commonly found in the inclination degrees between 15°-25° preferring places with partial slopes than flat ground.

Plant species in the close surroundings of the points of *A. limbata* are listed in Table 4. Plant species indicating the presence of *A. limbata* are *Pinus brutia* Ten. var. *brutia*, *Verbascum leptocladum* Pančić, *Thymelaea tartonraira* (L.) All., *Alkanna pinardi* Boiss., *Anthemis hyalina* DC., *Erica manipuliflora* Salisb., *Hypericum montbretii* Spach, *Acantholimon acerosum* (Willd.) Boiss. subsp. *brachystachyum* (Boiss.) Doğan & Akaydın, *Iberis carica* Bornm., *Muscari neglectum* Guss. ex Ten., *Crepis micrantha* Czerep. etc. Also, *Alkanna macrophylla* Boiss. & Heldr. and *Onosma strigosissima* Boiss. were found in the close areas of *A. limbata.*

A. limbata was not listed in the CITES 2005 Flora list (Inskipp and Gillett, 2005) and in the Annex-1 list of the BERN Convention (1979). When *A. limbata* was evaluated according to IUCN criteria (Anon., 2012) in this study; because of the inadequacy of previous studies, no data could be obtained to indicate a decrease or increase in population size. So, it had not been evaluated in a category of CR (Critically Endangered). It was included in the CR-B1 category because the geographic range was less than 100 km^2. It belonged to B1a category because it was overly fragmented or known to exist only in one place. According to Bigazzi *et al.*, (2003), *A. limbata* was in the CR. Because, the number of its individuals was less than 500 individuals in an area of 2 hectares, although there were plenty of young and adult individuals with fruits indicating no decrease in the population, it was very close to the region where there were intense human population and rapid urbanization, and they were of the opinion that the future of the species was uncertain.

Chamberlain (1978) gave additional locality record such as “Antalya to Elmali, Gümüş Bucağı”, *Cedrus libani* forest, 1100-1300 m, Chetik 1990 (E!)” as a different locality from its type sample. As a result of the current study, the plant was encountered in Gümüşbucağı. This locality was in the old road of Elmalı district, between Elmalı and Avlan Lake. The region was close to the Elmalı Cedar Research Forest. Specimens from Gümüşbucağı Region and Elmalı Cedar Research Forest were collected and these specimens were identified as *A. hybrida*. *A. limbata* had not been found in these localities. Therefore, the samples collected by Çetik 1990 (Chamberlain, 1978), Deniz 1281 (Deniz & Sümbül, 2004) were not *A. limbata*. In Bigazzi *et al.*, (2003), when they looked at the record of Çetik, they realized that there was no *A. limbata*, but instead it was an incomplete sample of few and withered flowering *A. undulata*. This finding was supported in June 1997 by the field studies in Elmalı, the collection area indicated on the label, and confirmed only the presence of *A. hybrida* (*A. undulata* subsp. *hybrida*). A record of *A. limbata* was found in some other publications (Özçelik & Korkmaz, 2002; Fakir, 2006; Özçelik *et al.*, 2006; Arıtuluk *et al.*, 2014; Aslan & Alkan, 2015). It wasn’t found in the field studies carried out in these areas. Also, Yilmaz & Yilmaz (2009) recorded *A. limbata* from Erzurum (Eastern Anatolia) and reported only the flower color as blue. However, the corolla color of *A. limbata* is not blue.

Table 4. Other Plant Species in the Habitat of *Anchusa limbata*.

Family	Taxon	Presence	Value status	IUCN status	Chorotype
Pinaceae	*Pinus brutia* Ten. var. *brutia*	49	-	-	E.Medit.
Scrophulariaceae	*Verbascum leptocladum* Pančić	41	End.	EN	E.Medit.
Thymelaeaceae	*Thymelaea tartonraira* (L.) All. subsp. *argentea* (Sm.) Holmboe	35	-	-	-
Boraginaceae	*Alkanna pinardi* Boiss.	34	End. (BERN)	EN	E.Medit.
Asteraceae	*Anthemis hyalina* DC.	26	-	-	-
Cistaceae	*Cistus creticus* L.	24	-	-	-
Cistaceae	*Fumana thymifolia* (L.) Spach	22	-	-	-
Ericaceae	*Erica manipuliflora* Salisb.	18	-	-	E.Medit.
Poaceae	*Poa bulbosa* L.	16	-	-	-
Poaceae	*Aegilops biuncialis* Vis.	16	-	-	-
Poaceae	*Brachypodium distachyon* (L.) P.Beauv.	14	-	-	-
Hypericaceae	*Hypericum montbretii* Spach	14	-	-	-
Plumbaginaceae	*Acantholimon acerosum* (Willd.) Boiss. subsp. *brachystachyum* (Boiss.) Doğan & Akaydın	10	End.	LC	Ir.-Tur.
Asparagaceae	*Asparagus acutifolius* L.	10	-	-	Medit.
Fabaceae	*Trigonella corniculata* Sibth. & Sm.	10	-	-	-
Caryophyllaceae	*Silene macrodonta* Boiss.	9	-	-	-
Apiaceae	*Torilis arvensis* (Huds.) Link subsp. *heterophylla* (Guss.) Hayek	9	-	-	Medit.
Amaryllidaceae	*Allium rotundum* L.	8	-	-	-
Lamiaceae	*Satureja thymbra* L.	7	-	-	E.Medit.
Plantaginaceae	*Plantago cretica* L.	7	-	-	E.Medit.
Poaceae	*Avena sterilis* L.	7	-	-	-
Euphorbiaceae	*Euphorbia peplus* L.	6	-	-	-
Brassicaceae	*Iberis carica* Bornm.	6	End.	NT	E.Medit.
Asparagaceae	*Muscari neglectum* Guss. ex Ten.	6	-	-	-
Lamiaceae	*Thymbra spicata* L. subsp. *spicata*	6	-	-	Medit.
Amaryllidaceae	*Allium flavum* L. subsp. *tauricum* (Besser ex Rchb.) K.Richt.	6	-	-	Medit.
Fabaceae	*Ononis reclinata* L.	6	-	-	Medit.
Asteraceae	*Crepis micrantha* Czerep.	6	-	-	-
Poaceae	*Bromus rigidus* Roth	6	-	-	-
Caryophyllaceae	*Minuartia mesogitana* (Boiss.) Hand.-Mazz. subsp. *kotschyana* (Boiss.) McNeil	5	-	-	-
Poaceae	*Festuca sipylea* (Hack.) Markgr.-Dann.	5	-	-	E.Medit.
Rubiaceae	*Crucianella latifolia* L.	5	-	-	Medit.
Lamiaceae	*Ajuga chamaepitys* (L.) Schreb. subsp. *cuneatifolia* (Stapf) P.H.Davis	4	-	-	-
Caprifoliaceae	*Valerianella vesicaria* (L.) Moench	4	-	-	-
Asteraceae	*Phagnalon rupestre* subsp. *graecum* Batt.	4	-	-	E.Medit.
Anacardiaceae	*Pistacia palaestina* Boiss.	4	-	-	E.Medit.
Asteraceae	*Hedypnois rhagadioloides* (L.)F.W.Schmidt subsp. *cretica* (L.)Hayek	4	-	-	Medit.
Rubiaceae	*Galium peplidifolium* Boiss.	4	-	-	E.Medit.
Asparagaceae	*Drimia maritima* (L.) Stearn	4	-	-	-
Asteraceae	*Crepis reuteriana* Boiss.	4	-	-	E.Medit.
Asteraceae	*Crepis foetida* L.	1	-	-	-
Fagaceae	*Quercus coccifera* L.	3	-	-	Medit.
Campanulaceae	*Campanula propinqua* Fisch. & C.A.Mey.	3	-	-	-
Rubiaceae	*Rubia tenuifolia* subsp. *brachypoda* (Boiss.) Ehrend. & Schönb.-Tem.	3	-	-	E.Medit.
Xanthorrhoeaceae	*Asphodelus aestivus* Brot.	3	-	-	-
Capparaceae	*Capparis orientalis* Veill.	3	-	-	Medit.
Lamiaceae	*Teucrium polium* L. subsp. *polium*	3	-	-	-

Table 4. (Cont'd.).

Family	Taxon	Presence	Value status	IUCN status	Chorotype
Santalaceae	*Thesium billardierei* Boiss.	3	-	-	Ir.-Tur.
Geraniaceae	*Erodium cicutarium* (L.) L'Hér. subsp. *cicutarium*	3	-	-	-
Euphorbiaceae	*Euphorbia taurinensis* All.	2	-	-	-
Asteraceae	*Centaurea benedicta* (L.) L.	2	-	-	-
Lamiaceae	*Salvia viridis* L.	2	-	-	Medit.
Caryophyllaceae	*Minuartia picta* (Sm.) Bornm.	2	-	-	-
Poaceae	*Bromus squarrosus* L.	2	-	-	-
Fabaceae	*Medicago rigidula* (L.) All. var. rigidula	2	-	-	-
Poaceae	*Poa annua* L.	2	-	-	Multi-regional
Poaceae	*Piptatherum coerulescens* (Desf.) P.Beauv.	2	-	-	-
Brassicaceae	*Matthiola longipetala* (Vent.) DC. subsp. *bicornis* (Sm.) P.W.Ball	2	-	-	-
Caryophyllaceae	*Silene colorata* Poir.	2	-	-	Medit.
Euphorbiaceae	*Euphorbia exigua* L.	2	-	-	-
Asteraceae	*Echinops ritro* L.	1	-	-	-
Santalaceae	*Osyris alba* L.	1	-	-	Medit.
Malvaceae	*Malva neglecta* Wallr.	1	-	-	-
Asteraceae	*Erigeron canadensis* L.	1	Invader	-	-
Fabaceae	*Lathyrus setifolius* L.	1	-	-	Medit.
Primulaceae	*Anagallis arvensis* L. var. *caerulea* (L.) Gouan	1	-	-	-
Iridaceae	*Gladiolus anatolicus* (Boiss.) Stapf	1	-	-	E.Medit.
Scrophulariaceae	*Scrophularia canina* L. subsp. *bicolor* (Sm.) Greuter	1	-	-	E.Medit.
Asteraceae	*Filago eriocephala* Guss.	1	-	-	E.Medit.
Selaginellaceae	*Selaginella denticulata* (L.) Spring	1	-	-	-
Euphorbiaceae	*Euphorbia characias* L. subsp. *wulfenii* (Hoppe ex W. D. J. Koch) Radcl.-Sm.	1	-	-	E.Medit.
Lamiaceae	*Micromeria myrtifolia* Boiss. & Hohen.	1	-	-	-
Linaceae	*Linum corymbulosum* Rchb.	1	-	-	Medit.
Brassicaceae	*Alyssum mouradicum* Boiss. & Balansa	1	-	-	-
Brassicaceae	*Odontarrhena muralis* (Waldst. & Kit.) Endl.	1	-	-	-
Crassulaceae	*Sedum pallidum* M.Bieb.	1	-	-	Euro.-Sib.
Fabaceae	*Onobrychis oxyodonta* Boiss.	1	-	-	-
Boraginaceae	*Anchusa hybrida* Ten.	1	-	-	Medit.
Caprifoliaceae	*Knautia integrifolia* (Honck. ex L.) Bertol. var. *bidens* (Sm.) Borbás	2	-	-	E.Medit.
Asteraceae	*Sonchus asper* (L.) Hill subso. *glaucescens* (Jord.) Ball	1	-	-	-
Rhamnaceae	*Rhamnus lycioides* subsp. *graeca* (Boiss. & Reut.) Tutin	1	-	-	-
Boraginaceae	*Echium italicum* L.	1	-	-	Medit.
Brassicaceae	*Hirschfeldia incana* (L.) Lagr.-Foss.	1	-	-	-
Asteraceae	*Crupina crupinastrum* (Moris) Vis.	1	-	-	-
Caprifoliaceae	*Scabiosa reuteriana* Boiss.	1	End.	LC	E.Medit.
Poaceae	*Lagurus ovatus* L. subsp. *ovatus*	1	-	-	Medit.
Fabaceae	*Ononis pubescens* L.	1	-	-	Medit.
Caryophyllaceae	*Velezia tunicoides* P.H.Davis	1	End.	VU	E.Medit.
Boraginaceae	*Heliotropium hirsutissimum* Grauer	1	-	-	E.Medit.
Euphorbiaceae	*Chrozophora tinctoria* (L.) A.Juss.	1	-	-	-
Zygophyllaceae	*Tribulus terrestris* L.	1	-	-	-
Plantaginaceae	*Plantago afra* L.	1	-	-	-
Asteraceae	*Senecio vulgaris* L.	1	-	-	-

End.: Endemic, EN: Endangered, LC: Least concern, NT: Near threatened, VU: Vulnerable, E.Medit.: East mediterranean element, Medit.: Mediterranean element, Ir.-Tur.:Irano-Turanian element, Euro.-Sib.: Euro-Siberian element

In the research region, some individuals of *A. limbata* and *A. hybrida* were found very close to each other. One individual of *A. hybrida* in the area looked very similar to *A. limbata*. Corolla limb of this sample was highly reduced. Both the variation in *A. hybrida* and the seemingly close appearance to *A. limbata* raised the question of whether there was a gene exchange between *A. limbata* and *A. hybrida*. Therefore the genetic diversity of *A. limbata* populations should also be investigated.

Bigazzi *et al.*, (2003), based on herbarium researches, stated that *A. limbata* was known from a very narrow area in Antalya, a coastal city at the foot of the southwestern Anatolian plateau. This area is probably the Kepez hills, the same point of Heldreich's original collection. This population is located in the *Pinus brutia* Forest of the Hot Mediterranean belt, growing in calcareous soils at 230-250 m altitude. Here, *A. limbata* was colonized on finely crumbled substrates, avoiding both hard limestone outcrops and layered areas with thick pine needles.

When Bigazzi *et al*., (2003) visited the area in June 2002 found that the population was less than 500 plants of varying concentrations in the area over 2 hectares. They observed no demographic decline in the population when they saw the abundant fruiting adult plants and many young individuals. However, they observed plants as natural and rare in a stable demographic structure for a long time. But, according to them, the rapidly expanding urbanization in the immediate vicinity of plant population put the future of this species quite uncertain. Therefore, they included in the CR category of the IUCN Red List (Anon., 2001). They suggested that seeds should be collected for sowing in botanical gardens for conservation to store in the seed banks as a practical action.

The species population was clustered at 5 polygons. The sizes of these polygons were 1583.83 hectares, 228.28 hectares, 144.73 hectares, 33.32 hectares and 16.57 hectares respectively. As mentioned earlier, in 2002 there was a population of less than 500 individuals with varying concentrations within 2 hectares. *A. limbata* was identified in 91 localities and 73 of them were counted. A total of 5904 individuals were counted in 73 localities, of which 3782 were in juvenile stage or flowerless, 923 were flowering in the generative period, 852 were in unspecified forms (seedling or flowered), 16 were with dried flowers but alive, and approximately 40 were completely dry. In addition, 50 (30 flowering, 20 seedlings) were hurt by animals. There was an average of 80 living individuals per point. The minimum number of individuals varied between 1-20 and 175-499 at maximum in the points.

Threats and limiting factors: Some species, distributed in Turkey can be endangered because of growing in special habitats and having limited areal. Endemic species that have narrow areal or low density are more at risk than other species. A variety of reasons that can cause habitat destruction as agriculture, animal husbandry, urbanization, road construction and mines may cause the existence in danger. It can also be affected by the climate change and severe climatic conditions.

To identify threats to this species; the areas factors affecting the population indirectly in their habitats were investigated. Factors threatening the species distribution were various such as overgrazing pressure, *Cercopis* spp. (Homoptera, Cercopidae), red spider mite (*Panonychus ulmi*, Acarina, Arachnida), snails, ants, deforestation (road building, landscaping and other activities), organic waste and solid waste, extreme temperature and drought (climatic change), urbanization and settlement.

In the field observations were made on *A. limbata* in 2018, it was observed that significant climatic changes and shifts, as well as the pressure of drought on species were more effective than in 2017. The effect of direct sunlight and drought on the young seedlings was observed to be quite high. These effects cause the young seedlings to dry out and be eliminated from the habitats.

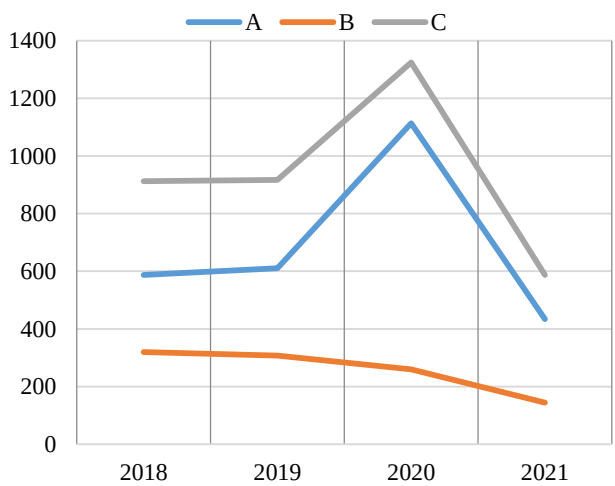
An essay covering 15 sample areas was established during the field studies in 2018 (Table 5). In these sample areas, counts were made between 2018 and 2021, observations and differences were compared in terms of the number of individuals (Table 6, Fig. 4). It was observed that the pressure of factors especially such as climate changes, urbanization, deforestation (especially road widening activities) on the species increased. Interspecific competition was also observed.

Table 5. The features of the sample areas in the assay.

Sample area no	Locality (Antalya)	Size of sample areas (m^2)	Altitude (m)	Exposure	Notes and pressure factors
1	Zoo	40	192	SW	inclination 20°, picnickers, pig
2	Zoo	40	194	S	inclination <5°, picnickers, patway
3	Zoo	52	204	SW	inclination 15°, picnickers, roadsides
4	Zoo	40	200	SW	inclination <5°, picnickers, roadsides
5	Park Orman	210	237	S	inclination 25°, waste, pasturage
6	Park Orman	110	257	W	inclination 35°, openings
7	Fatih Neighborhood	190	209	SE	inclination 10°, waste, rubble, partially pasturage
8	Fatih N.	180	228	NW	inclination 25°, waste, rubble, partially pasturage
9	Fatih N.	280	259	SW	inclination 10°, waste, rubble, partially pasturage
10	Fatih N.	345	227	SW	inclination 35°,waste, rubble, antropogenic effects
11	Fatih N.	224	226	…	inclination 35°, roadsides
12	Varsak Neighborhood	70	143	…	inclination 0°, pasturage pressure
13	Varsak N.	64	126	NE	inclination 35°, antropogenic and pasturage pressure
14	Varsak N.	100	127	…	inclination 0°, antropogenic and pasturage pressure, waste
15	Varsak N.	208	225	SE	inclination <5°, roadsides, antropogenic pressure, waste

Table 6. Individual number of the sample areas in the assay.

Sample area no.	2018 (April, 20)			2019 (May, 28)			2020 (June, 03)			2021 (May, 21)		
	A	B	C	A	B	C	A	B	C	A	B	C
1.	13	16	29	9	14	23	15	8	23	11	4	16
2.	8	9	17	56	1	57	54	1	55	12	1	13
3.	32	44	79	Damaged because of road regulation								
4.	23	11	34	21	0	21	90	0	90	2	1	3
5.	16	23	39	16	12	28	32	4	36	22	4	26
6.	65	18	83	103	2	105	51	7	58	20	2	22
7.	80	50	130	68	39	107	86	29	115	17	21	38
8.	37	28	65	40	21	61	96	26	122	50	19	70
9.	97	33	130	35	20	55	101	16	117	63	20	88
10.	66	15	84	100	42	142	170	25	195	79	26	105
11.	99	55	154	91	69	160	166	22	188	90	2	93
12.	39	29	68	12	47	59	67	26	93	4	0	4
13.	7	15	22	1	27	28	45	22	67	21	14	35
14.	11	10	24	12	8	20	65	17	82	8	27	36
15.	26	8	34	46	5	51	75	8	83	35	3	38
Total	619	364	992	**610**	**307**	**917**	**1113**	**260**	**1324**	**434**	**144**	**587**
Except area 3	**587**	**320**	**913**									



A: Number of flowerless individuals, B: Number of flowering individuals, C: Total (including dead individuals)

Fig. 4. Annual change graph of the number of individuals.

A total of 992 individuals of *A. limbata* were counted in 15 sample areas in the field studies conducted in 2018. According to the findings, the number of dead individuals were 3, flowerless individuals were 619 and the flowering individuals were 364. In 2019, one of the sample areas (3rd) was destroyed by road construction. Therefore, the 3rd sample area was excluded from the study. A total of 917 individuals of *A. limbata* were counted at remaining 14 sample areas. While no dead individuals were observed among them, the number of flowerless individuals was 610 and flowering individuals were 307. At this point, it was also observed that the road construction factor was not only responsible for the loss of habitat but also the loss of individuals. In this case, as a result of the two-year observation, the population trend appeared to be partially stable. So, it is predicted that habitat destruction (road construction etc.) factor is the most important factor. In the observations and censuses made in 2020, the number of dead individuals were 2, the flowerless individuals were 1113, and the flowering individuals were 260. In 2021, 9 dead individuals, 434 flowerless individuals, 144 flowering individuals and in total 587 individuals were observed (Fig. 4). This result showed that the reproductive potential was good but there was a decreasing tendency in the flowering potential. In the field studies in 2021, the effects of the arid climate were clearly observed. Due to the arid climate, the number of individuals had decreased considerably. In 2021, the 4th sample area was partially damaged by road construction. In the same period, it was also determined that the landscaped areas threatened the natural habitat of the species. So, the greatest threat to the species was determined to be primarily drought, followed by road improvements, landscaping and urbanization.

It is obvious that the pollination of the *Anchusa* species is carried out by insects. Many insects were seen on *A. limbata*, but it was not possible to identify the insect which facilitated the pollination.

The fruits of this species are dispersed through rolling with the help of its peculiar structure on the inclined places, rain water or wind. The species shows a distribution in the form of clusters rather than random distribution. Terra-rossa soil is one of the factors that determine the distance of the clusters.

The permeability of the chalky soil in which the species thrives enables the roots to move to the deep for easy access to water, therefore; the plant grows well in chalky soil which is much poorer than terra-rossa in terms of water. Besides, the hairy structure of the species is considered to be an example of its adaptation to drought.

Conclusion

As a result of field studies, a total of 304 localities of which 91 localities were recorded the presence of *A. limbata*. The total area occupied by 91 localities was 3708.48 ha. It was observed that *A. limbata* clustered in

5 polygons within this distribution area. Soil is the most important factor affecting the distribution of the species. The chalky soil where the plant grows is quite calcareous, poor in nutrients and organic matter. The presence of this species on the Antalya travertines, in the red pine forest or its edges, in white-gray chalky soil, at the altitude-exposure degrees and the inclination and the presence of indicator species are other characteristic factors affecting the distribution.

In contrast to the literature, both biennial and perennial individuals were recorded. *A. limbata* grows in lime soils of the redpine forest and travertine slopes. The populations of the species are found between 24° and 355° exposures (commonly ENE), between 0° and 45° inclinations. In the field, some plant species (*Pinus brutia, Verbascum leptocladum, Thymelaea tartonraira, Alkanna pinardi* etc.) are indicator of presence of *A. limbata*. The threatening factors of the species are climatic changes, anthropogenic activities including urbanization, tourism activities, field and road openings, landscape arrangements, etc. This research has shown that *A. limbata* must be protected together with its natural area.

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