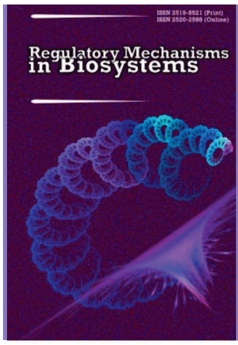

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Palynological, karyological and eco-physiological features of *Microcnemum coralloides* of Armenian flora

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The nature of pollen grains and karyotype morphology are important bases in the task of clarifying relationships and they are considered to be of significant value in observing the effects of environmental and climatic factors on plants. The investigations of the palynological, as well as karyological and physiological data regarding to *M. coralloides*, as one of the rarest species of the Armenian flora, were conducted for the first time using light and scanning electronic microscopy. In Armenia the only remaining population of this species is currently located in the Ararat valley salt marshes to the south-east of Ararat town. In the Red Book of Plants of the Republic of Armenia the species *M. coralloides* is presented in the category EN (Endangered species). Description of the pollen grain and karyotype of the Armenian population of *M. coralloides* subsp. *anatolicum* collected from the vicinity of Ararat town are provided in the article. The study of the water regime, transpiration intensity and photosynthesis, as well as plastid pigments were also conducted. Comparative analysis between Armenian and Iranian specimens of the subspecies *M. coralloides* subsp. *anatolicum* showed some discrepancies in pollen features, as well as karyological data, which indicate some specificity of the population growing on the territory of Armenia. The diversity and intersections in pollen traits can be attributed to either natural variability within a population (plasticity), or they may be influenced by genetic differences among populations and closely related species. The physiological data highlights specialized adaptations of *M. coralloides* subsp. *anatolicum* for managing water resources, reducing transpiration, and performing photosynthesis in the high salinity conditions of the Ararat Plain. This showcases the plant's remarkable resilience and adaptability to its specific ecological niche, emphasizing the interplay between environment and biology.

Keywords: pollen grains; chromosome number; karyotype; water regime; photosynthesis intensity; plastid pigments.

Introduction

Microcnemum Ung.-Sternb. (Salicornioideae, Chenopodiaceae) is a monotypic mostly Eurasian genus containing the species *M. coralloides* (Loscos & J. Pardo) Font Querwith. *M. coralloides*, as well as all the other members of the subfamily Salicornioideae is a hygro-halophyte plant with succulent, predominantly articulate stems, reduced leaves and inflorescences of spikelets comprised of minute, reduced flowers (Kühn et al., 1993).

The species *M. coralloides*is represented by two subspecies, namely subsp. *coralloides*, endemic to Spain and subsp. *anatolicum* Wagenitz, occurring in West Asia (Armenia, Central and Northwestern Iran, Central and Southwestern Anatolia, Syria) (Hedge, 1997). According to the literature data, the main diagnostic features that allow the subspecies of *M. coralloides* to be distinguished are the plant colour and seed testa ornamentation.

In particular, one should note the crimson or blue-greenish coloration for *M. coralloides* subsp. *coralloides* and yellowish green for subsp. *anatolicum* (Aellen, 1967). On the other hand, the colour of the plant found in Iran is purple and hence identification of its subspecies is difficult (Akhani, 1988). The coloration of the two subspecies of *Microcnemum* does not allow one to reliably distinguish differences between the two subspecies and is probably changeable (Kadereit & Yaprak, 2008).

Observations of the Armenian population of *M. coralloides* subsp. *anatolicum* confirmed this opinion, since during the growing season there was a significant change in the colour of the plants: from reddish-green or light green in seedlings, dark or light yellow-green during flowering and reddish during fruiting, which indicates a dependence upon the influence of environmental conditions in the process of seasonal development (Akopian, 2022). The seed testa is the only consistent character to distinguish two subspecies (Kadereit & Yaprak, 2008): in subsp. *coralloides* the testa is obscurely papillose (Aellen, 1967) or granulate (Kadereit & Yaprak, 2008; Piirainen et al., 2017) and in subsp. *anatolicum* (West Asia) the testa is densely papillose (Aellen, 1967) or papillose (Kadereit & Yaprak, 2008; Piirainen et al., 2017).

The molecular data (ITS and ML analysis of the trnT/F region) indicate that *M. coralloides* subsp. *coralloides* originated from within *M. coralloides* subsp. *anatolicum*, which implies an East Mediterranean origin and subsequent westward dispersal (Kadereit & Yaprak, 2008). Also noted, there is a slight difference between two subspecies of *M. coralloides* in habit (height, succulence and branching), which is evaluated as dependent on influence by seasonal fluctuation of rainfall and salinity (Kadereit & Yaprak, 2008). Both subspecies *M. coralloides* subsp. *anatolicum*, collected from Iran and *M. coralloides* subsp. *coralloides*, collected from Spain show close pollen morphological characters, these data in the two subspecies do not support morpho-molecular findings that suggest they are genetically distinct taxa" (Dehghani et al., 2020).

In the Red Book of Plants of the Republic of Armenia (Tamanyan et al., 2010) the species *M. coralloides* is presented in the category EN (Endangered species), in Armenia, the only remaining population of the annual *M. coralloides* subsp. *anatolicum* is currently located in the Ararat valley salt marshes to the south-east of Ararat town (Akopian, 2022). The reason for the study was the lack of palynological, as well as karyological and eco-physiological data regarding *M. coralloides*, as one of the rarest species of the Armenian flora.

Fig. 1. *Microcnemum coralloides* subsp. *coralloides* from the vicinity of Ararat town (Armenia)

Material and methods

Pollen morphology of *M. coralloides* subsp. *coralloides* was studied using light microscopy LM (AmScope 2000X LED, China, 2015) and scanning electron microscopy SEM (JEOL JCM-7000, Japan, 2019). The descriptions of pollen grains using LM are based on acetolyzed material (Avetisyan, 1950) and also on grains stained with basic fuchsine. The studies were carried out at x200, x400 and x1000 magnifications, and the measurements were carried out on 20 pollen grains.

The total number of pores for each grain was carried out by calculating the length of the apothem (Angelini et al., 2014). According to the authors, in pantoporate pollen grains "apothem … is half of the sum of interpore distance plus mean pore diameter" and "…in order to obtain total number of pores, total area of pollen grain surface was divided by the area of one hexagon" (p. 249).

For SEM, non-acetolyzed dry pollen grains were placed in a drop of alcohol and then mounted on aluminum stubs and sputter-coated with gold. The specimens were examined and photographed under SEM at the Center for Ecological Noosphere Studies NAS RA (Yerevan, Armenia), scanning electron microscope (JEOL JCM-7000, Japan, 2019) was run at 15 kV. Five morphological characters, namely diameter of pollen grains, number and diameter of pores, exine thickness, mesoporium width (using LM), and the number of spinules on a unit area (1 μm²) of the mesoporium surface (on SEM level), were measured.

The morphological terminology used mainly follows (Punt et al., 2007; Hesse et al., 2009).

Pollen material was gathered from the living plants: Armenia, Ararat district. In the vicinity of Ararat town, salt marshes. 39°49'40.36" N, 44°43'30.39" E. 25.06.2018. Leg. J. Akopian (personal collections).

Karyological investigations were conducted using mitotic chromosome preparations from metaphase stages, obtained from meristematic cells in root tips. The seeds were germinated on damp filter paper within Petri dishes in a laboratory setting (19–21 °C). The root tips underwent a pretreatment in a 0.4% colchicine solution for a duration of 2 hours, followed by fixation in a mixture of alcohol and glacial acetic acid at a 3:1 ratio, held at room temperature for at least 2 hours. Following a hydrolysis step in 1 N HCl at 60 °C for 10–15 minutes, the root tips were subjected to staining with Schiff reagent for 1.5 hours. Subsequently, the root tips were gently squashed onto a glass slide along with 45% acetic acid. The prepared slides were sequentially immersed in butyl alcohol for 5 minutes, followed by xylene for another 5 minutes, before being embedded in Canadian balsam. For chromosome counting, a minimum of 10 plates of chromosome preparations were meticulously examined. The determination of chromosome numbers and the characterization of species karyotypes were performed using an Photomicroscope (AmScope 2000xLED Lab Trinocular Compound Microscope SKU: T120B-5M, China, 2020) equipped with an oil immersion objective (×100). Photomicrographs were captured using the same microscope setup. We follow the scheme of chromosome morphology and terminology presented by (Agapova & Grif, 1982).

Specimens examined: Armenia, Ararat district, near Ararat town, salt marshes, 810 m a. s. l., 08.08.2016. Leg. J. Akopian, A. Ghukasyan, Z. Hovakimyan, Z. Paravyan. Det. J. Akopian. ERE 194199; Armenia, Ararat district, near Ararat town, salt marshes in the vicinity of Ararat town, at an altitude of 900–1000 m a. s. l., 39°49'28.9" N 44°43'53.2" E. 09.08.2020. Leg. J. Akopian, A. Ghukasyan, Z. Hovakimyan, Det. J. Akopian.

The investigation of the ecophysiological characteristics (water regime, water deficiency, transpiration, and photosynthesis intensity) of *M. coralloides* subsp. *anatolicum* in Armenia has been carried out for the first time. The studies of water regime, the intensity of transpiration and photosynthesis were conducted according to Salnikov & Maslov (2014). All the measurements were done in the period between 11:00 and 13:00. Each measurement was done with 3 repetitions and in 3 options (7–10 shoots were chosen for each sample). The data presented are the average results of the performed analysis which were subjected to statistical development. Water deficiency was determined based on the principle of water saturation of leaves. The fresh sample was promptly weighed and dried at a thermostat of 105 ºC for determining the total water content. The intensity of transpiration of the leaf was determined by rapid weighing with a torsion scale.

The intensity of photosynthesis was determined by the change in the dry weight of the leaf sample. The amount of dry material synthesized during photosynthesis was determined by the difference between the initial and the final measurements of the dry weight. The intensity of photosynthesis in mg/dm^2, per hour, was expressed by dividing the amount of synthesized dry material by the time period between the measurements.

The content of photosynthetic pigments (chlorophylls "a" and "b", carotenoids) was determined by a modified method based on the use of an organic solvent of dimethyl sulfoxide, which allows one to obtain stable extracts necessary for performing extra laboratory studies (Mezhunts & Navasardyan, 2010). The measurement was carried out on a spectrophotometer (UV-6300PC Double Beam Spectrophotometer, China, 2020), and the quantitative accounting of chlorophylls "a", "b" and carotenoids was carried out according to the Mackini-Arnon and Wettstein formulas (Mezhunts & Navasardyan, 2010). The cell sap density was determined with Refractometer (Digital HI96801, Romania, 2020).

Results

Pollen grains 17–25-pantoporate, spheroidal, 17.4–20.6 μm in diameter, the outline rounded or angular-rounded (Fig. 2, 3). Pores operculate (Fig. 2a, 2b, 3c), more or less rounded, submerged, 1.6–2.3 μm in diameter; mesoporium width 2.6–3.5 μm; on the level of SEM, operculum surface regularly densely spinulose (Fig. 3c). Exine thickness 1.1–1.3 μm, the columellae layer poorly expressed. Exine ornamentation densely granular (Fig. 2a); exine ornamentation perforate-spinulose, number of spinules per 1 μm^2 of pollen grain surface 4–7, spinules conical, with pointed or slightly rounded ends, several times smaller than spinules noted on pores opercula (Fig. 3b, 3c); the surface of the pollen grains wavy (SEM).

As a result of karyological investigations, the diploid cytotype (2n = 18) with basic number x = 9 was found for *M. coralloides* subsp. *anatolicum* from Armenia (Fig. 4).

The karyotype description on the material of the Armenian population of *M. coralloides* ssp. *anatolicum* collected from the vicinity of Ararat town is given for the first time. The karyotype of *M. coralloides* ssp. *anatolicum* is symmetric, consisting of 2 pairs of submetacentric chromosomes (I & III), with IC = 37.4%, 6 pairs of metacentric chromosomes, with IC = 40.4–42.0%, and 1 pairs of acrocentric chromosomes with IC = 12% (VII) (Fig. 5). Total chromosome length Σ = 34.64 μm.

Karyotype formula: 2n = 18 = 4SM + 12M + 2A.

Studying the water regime, intensity of photosynthesis and transpiration in fresh leaves of *M. coralloides* subsp. *anatolicum* revealed a high content of total (62.3 ± 1.7%), free (23.8 ± 0.5%) and bound (38.5 ±

1.2%) water, as well as low levels of water deficiency (1.6 ± 0.3%) and intensity of transpiration (123.7 ± 1.3 mg/g wet weight per hour) and high intensity of photosynthesis (16.5 ± 0.2 mg CO_2/dm^2 per hour).

In order to study the variability of the photosynthetic apparatus, our attention was focused also on the amount of chlorophylls "a" and "b" and carotenoids, which are important internal factors of plant adaptation (Table 1).

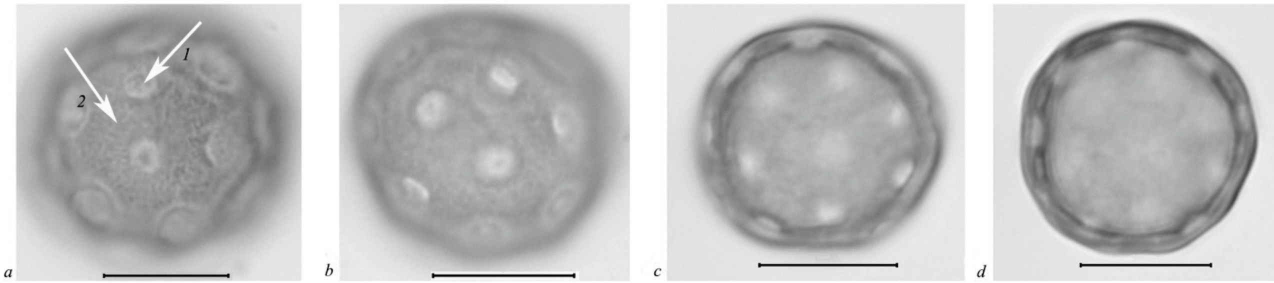

Fig. 2. Pollen grains of *Microcnemum coralloides* subsp. *anatolicum* LM micrographs: *a*, *b* – overall view of pollen grains, operculate pore (*1*), exine ornamentation (*2*), *c* – pore areas on the reverse side of pollen grain, *d* – structure of the exine; scale bar 10 µm

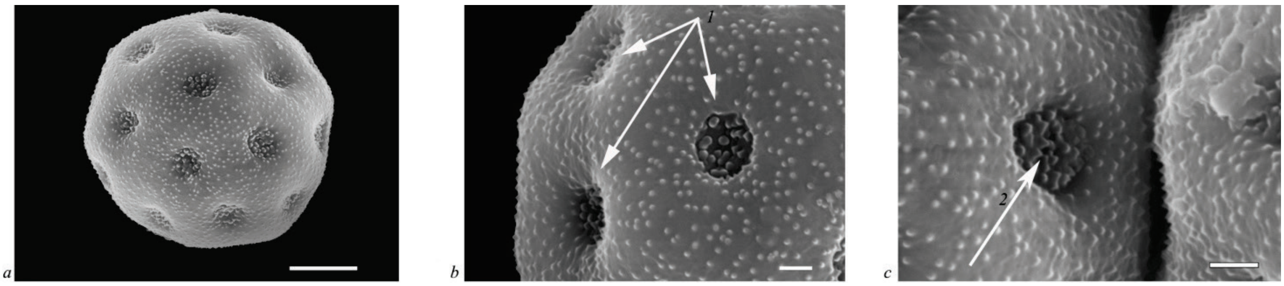

Fig. 3. Pollen grains of *Microcnemum coralloides* subsp. *anatolicum* SEM micrographs: *a* – overall view of pollen grain, *b* – pores (*1*), *c* – pore with operculum (*2*); scale bar *a* – 5 µm, *b*, *c* – 1 µm

Fig. 4. Mitotic metaphase plate of *M. coralloides* subsp. *anatolicum* (2n = 18)

Fig. 5. Karyotype of *M. coralloides* subsp. *anatolicum*

Microcnemum coralloides subsp. *anatolicum* a content chlorophyll "a"; chlorophyll "b"; chlorophyll ("a" + "b"); chlorophyll "a"/"b", carotenoids, fresh substance indicates their sufficient internal reserve for the implementation of photosynthesis processes (Table 1). The cell sap density was 11.0% in 1 g of the substance under investigation, which is also an important internal factor of plant adaptation.

Discussion

In comparison with palynological data provided for the *M. coralloides* subsp. *anatolicum* collected from Iran (Dehghani et al., 2020), the data on pollen morphology in the sample collected in the territory of Armenia differ considerably from these. In particular, Iranian investigators noted somewhat larger sizes of pollen grains (25.3–25.9 µm as against 17.4–20.6 µm in diameter, identified by us), as well as the size of individual pores (approximately 2.4–2.9 and 1.6–2.3 µm in diameter, respectively), a significantly larger number of pores (36 ± 2.5 and 17–25 respectively), differences (up to two times) between the mesoporium width (6.7–6.8 and 2.6–3.5 µm, respectively), as well as the thickness of the exine layer (about 2.1 and 1.1–1.3 µm, respectively). The variations in pollen traits and overlapping of pollen traits can be due to natural variation (plasticity) or genetic differences among populations.

Table 1
The content of plastid pigments in fresh leaves of *Microcnemum coralloides* subsp. *anatolicum*

Name of plastid pigments	Quantity of plastid pigments in fresh leaves, mg/g
Optical density of chlorophyll "a", λ* 663	0.65 ± 0.02**
Optical density of chlorophyll "b", λ 645	0.25 ± 0.01
Optical density of carotenoids, λ 440.5	1.22 ± 0.01
Chlorophyll "a" content, per wet leaf, mg/g	7.65 ± 0.40
Chlorophyll "b" content, per wet leaf, mg/g	2.69 ± 0.10
Chlorophyll "a" + "b"	10.34 ± 0.50
Chlorophyll "a"/"b"	2.80 ± 0.11
Carotenoids content, per wet leaf, mg/g	5.68 ± 0.32

Note: *λ – the length of the wave; ** – standard deviation SD.

The observed chromosome number (2n = 18) with basic number x = 9 is in agreement with previous data from Armenia (Ghukasyan & Akopian, 2017; Fayvush & Ghukasyan, 2023), Spain (Castroviejo & Coello, 1980) and from Iran (Ghaffari et al., 2006). Only the diploid cytotype has been published for this species. Karyotype of *M. coralloides* ssp. *anatolicum* from Armenian flora is symmetrical, consists of 2 pairs of submetacentric chromosomes, 6 pairs of metacentric chromosomes, while according to Ghaffari et al. (2006), the karyotype of the Iranian population of this species is represented by 2 pairs of metacentric chromosomes, 3 pairs of submetacentric chromosomes and 4 pairs of subtelocentric chromosomes. The existing discrepancies in karyotype can indicate specificity of the population of the subspecies *M. coralloides* subsp. *anatolicum* growing on the territory of Armenia.

From an eco-physiological point of view, the study of the water regime is crucial for halophytic plants, as it is the environment where all the main reactions of plant life take place (Genkel, 1982). Highly mineralized water is absorbed by plants with great difficulty precisely due to the high concentration of salts. At the same time, the degree of accumulation of salts by plants depends on both their specific features and the quantity of salts in the soil. In plants growing in saline soils, transpiration intensity is generally low and depends not only on soil water content and the absorption capacity of the root system, but also on plasma hydrophilicity and leaf salt content. Low transpiration rates in the subspecies *M. coralloides* subsp. *anatolicum* indicate a high water-holding capacity of the leaves and the whole plant. The high osmotic pressure results in a high water holding capacity and enabling efficient water usage. Changes in transpiration intensity and photosynthesis are influenced by both heredity and structural characteristics of plants (Rozentsvet et al., 2016).

The ratio of the content of different forms of water is best expressed in the indicator of natural water deficit, which in *M. coralloides* subsp. *anatolicum* (typical succulent) turned out to be the lowest of all the halophytes of salt marshes in the vicinity of Ararat town (Hovakimyan, 2018). On the other hand, in a comprehensive change in the water regime caused by external factors, shifts in the intensity of transpiration are important, since it, together with the intensity of water inflow, determines the water balance of plants. In *M. coralloides* subsp. *anatolicum* of the flora of Armenia, the intensity of photosynthesis is considerably higher than in other halophytes of salt marshes in the vicinity of Ararat town (Hovakimyan, 2018). The main role of chlorophyll "a" is to absorb light from the orange-red-purple-blue colours of the spectrum, and chlorophyll "b" – to increase the absorption spectrum of organisms and to convert more energy into chemical energy, which is responsible for the adaptation of plants to extreme growing conditions.

Conclusion

The variations in pollen traits can be influenced by both natural variation (plasticity) and genetic differences among populations and closely related taxa. Natural variation or plasticity refers to the range of traits that can be expressed by individuals within a population in response to environmental conditions. Thus, from a palynological point of view, the existing discrepancies in pollen features between the specimens from Armenia and Iran can indicate some specificity of the population of the subspecies *M. coralloides* subsp. *anatolicum* growing on the territory of Armenia.

From a karyological standpoint, even though both specimens of *M. coralloides* subsp. *anatolicum* from Armenia and those from Iran have the same diploid chromosome number (2n = 18), there can still be observed variations in their karyotypes. This suggests that there are distinct chromosomal differences between these populations. This kind of variation in karyotypes can be indicative of population specificity and adaptation to specific environmental conditions within the territory of Armenia. It suggests that these populations have potentially undergone genetic differentiation and may be evolving separately from one another. This could be due to a variety of factors, including environmental conditions, and genetic drift.

Studying these karyotypic differences provides valuable insights into the genetic diversity and evolutionary dynamics of this subspecies, and it helps us understand how it is adapted to their respective habitats.

The given data on the regulation of the water regime, the intensity of transpiration and photosynthesis are a characteristic feature of *M. coralloides* subsp. *anatolicum* of the flora of Armenia. This species has evolved a suite of physiological and morphological features that enable it to effectively manage water resources, reduce water loss through transpiration, and carry out photosynthesis in conditions where salinity levels are high, and to grow in the challenging environment of the Ararat Plain's saline soils. Additionally, the internal factors such as root system structure, leaf anatomy, and other physiological mechanisms further contribute to the plant's successful growth and development in this extreme environment. This adaptation showcases the remarkable resilience and adaptability of plants to their specific ecological niches. It also highlights the intricate interplay between environmental conditions and the biological traits of organisms.

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The authors consider that there is no conflict of interest.

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