

A Scanning Electron Microscope Study: Investigation of Some *Convolvulus* L. Taxa (Convolvulaceae) as Morphological and Palynological

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Abstract: Detailed morphological data provide useful information about the classification of plants such as *Convolvulus* taxa and play an important role in classification systems. A scanning electron microscope (SEM) study of three *Convolvulus* taxa were carried out in this research. The morphological characteristics of three *Convolvulus* species are compared with them. The pollen characteristics of endemic *Convolvulus holosericeus* subsp. *macrocalycinus* Hausskn. & Bornm. ex Bornm (Endemic), *Convolvulus betonicifolius* Mill. subsp. *peduncularis* (Boiss.) Paris and *Convolvulus arvensis* L. were examined by means of SEM. *Convolvulus arvensis* pollen is smaller than the other two species and plays a role in species distinction. *Convolvulus holosericeus* Bieb. subsp. *macrocalycinus* is systematically distinguished from the other two species in that seed shape is oblong. In this study, pollen, leaf, stem, seed morphology of three *Convolvulus* taxa, one of them endemic to Turkey, were worked out in with SEM to contribute systematic position of studied taxa.

Key words: Convolvulaceae, *Convolvulus*, micromorphology, systematic, SEM.

Taramalı Elektron Mikroskopu Çalışması: Bazı *Convolvulus* L. Taksonlarının (Convolvulaceae) Morfolojik ve Palinolojik Olarak İncelenmesi

Özet: *Convolvulus* taksonları gibi bazı bitkilerin sınıflandırılmasında detaylı morfolojik veriler sınıflandırma sistemlerinde önemli bir rol oynar ve taksonomileri hakkında faydalı bilgiler sağlar. Bu çalışmada üç *Convolvulus* taksonunun morfolojik özellikleri taramalı elektron mikroskopu (SEM) ile araştırılmıştır. *Convolvulus holosericeus* subsp. *macrocalycinus* Hausskn. & Bornm. ex Bornm (Endemik), *Convolvulus betonicifolius* Mill. subsp. *peduncularis* (Boiss.) Paris ve *Convolvulus arvensis* L. türlerine ait polen özellikleri, gövde, yaprak ve tohum morfolojik özellikleri SEM aracılığıyla incelenmiştir. *Convolvulus arvensis* poleni diğer iki türden daha küçük olup tür ayırımında rol oynamaktadır. *Convolvulus holosericeus* Bieb. subsp. *macrocalycinus*, tohum şeklinin dikdörtgen olmasıyla diğer iki türden sistematik olarak ayırt edilir. Bu çalışmada, taramalı elektron mikroskopu (SEM) ile incelenen taksonların sistematik konumuna katkı sağlamak amacıyla, biri Türkiye'ye endemik olan üç *Convolvulus* taksonunun polen, yaprak, gövde, tohum morfolojisi çalışılmıştır.

Anahtar Kelimeler: Convolvulaceae, *Convolvulus*, Mikromorfoloji, Sistematik, SEM.

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INTRODUCTION

The *Convolvulus* L. genus is one of well known genera of Convolvulaceae family (Austin, 1998). Convolvulaceae family is consisting of herbs, vines and shrubs. Convolvulaceae family spreads from Asia to America, North and South temperate regions, subtropics and tropics (Saensouk, 2007). Convolvulaceae is one of the large family of plant kingdom, including more than 1900 species and around 50 genera in the world (Staples, 2018). Convolvulaceae taxa are mainly tropical plants and most of the genera are endemic to tropical regions of individual ingredients (Austin, 1998). Convolvulaceae taxa, which can live in both temperate regions and tropical, show rich morphological features within the family (Wood et al., 2015). Generally, Convolvulaceae taxa are more common in arid regions with less vegetation. Some of the Convolvulaceae members are used as folk medicine. This family, which is medically and economically important, is used to treat various ailments (Ashfaq et al., 2019). *Convolvulus pilosellifolius* and *C. oxyphyllus* are reported to be important for grazing in desert conditions in Arabia (Dickson, 1955). The roots and stems are known as rosewood or rhodium of *C. scammonia* has roots that produce a brown exudate known as scammony, which is used as a laxative (Grigoriev, 1953). *C. arvensis* was traditionally applied to treat epilepsy in north Pakistan (Ashfaq et al., 2019). *C. prostratus* is used as the best brain tonics and it used as a nerve tonic (Waheed et al., 2020, Sethiya et al., 2009), *C. arvensis* is used to treat boils and inflammation and treat constipation (Kılıç and Bağcı, 2013). Aerial parts of *C. betonicifolius* Mill. subsp. *peduncularis* used to treat hemerroid and the public uses it as animal feed (Kılıç, 2016).

Convolvulus includes close to 200 species following its most recent circumscription and that includes annual, perennial herbs, completely herbaceous, shrubs and subshrubs (Wood et al., 2015). The high rate of variation in the phenotypic traits of *Convolvulus* made identification difficult. Variations that are sometimes found in the same species in response to environmental conditions. Phenotypic flexibility as a result of environmental changes is a challenging aspect of systematics (Tod, 2009). Pollen grain morphology is very important for the identification of plants. According to Arora and Modi (2008), morphological and palynological evidence provides important information about closely related genera and species. Both scanning electron microscopy and light microscopy provide solid evidence to identify different taxonomic and morphological features of plant taxa (Qureshi et al., 2019).

There are more than 32 *Convolvulus* species, and 9 of them are endemic to Turkey in Flora of Turkey (Davis et al., 1988). Aykurt and Sümbül (2014) had been revision the genus *Convolvulus*, which is represented with 33 species (36 taxa) in the Flora of Turkey, was specified to be contain of 39 taxa (3-hybrid). Carine et al. (2004), in their evaluation of the phylogenetic associations of the *Convolvulus* species spreading in Makaronesia, from a molecular perspective, that Acanthocladia would not be a monophyletic group as Sa'ad suggested, but rather sprout observation and spiny bush species in the Canary Islands, Western and Eastern Mediterranean. Their characteristics are not homologous, allowing them to consider such species under two taxonomic headings (Carine et al., 2004). Aykurt and Sümbül (2014) stated that IUCN threat categories of the *Convolvulus* taxa. *C. arvensis* is LC, *C. holosericeus* subsp. *macrocalycinus* is NT, *C. betonicifolius* subsp. *peduncularis* is LC. And they reported another study that a new natural hybrid a *Convolvulus* x *turcicus* and it's between *C. compactus* and *C. holosericeus* subsp. *holosericeus* from central Anatolia. Many of these features of undisputed taxonomic status can be viewed with the SEM more conveniently than by conventional microscopy (Newton, 1972). It is possible to examine the systematics of plants both in traditional ways and with various molecular markers and to determine their taxonomies (Kursat et al., 2014, Sancar et al., 2021). In taxonomic studies, therefore, the SEM has become a routine tool for assessing and identifying plant material by comparative methods.

In this study, it is aimed to explain the pollen, leaf, stem and seed morphology of three *Convolvulus* taxa on the basis of traditional classifications by supporting them with SEM studies. The aim of the study is to evaluate the systematic importance of taxa. In addition, informative characters that can be useful in identifying and distinguishing species-subspecies in *Convolvulus*, which is based on Turkish taxa, will also be explained.

MATERIALS and METHODS

Plant Materials

Plant taxa collected from their habitats in Turkey were dried for morphological and palynological observations and stored as herbarium samples. The voucher specimens have been deposited at the Adıyaman University Pharmacy Faculty herbarium. The taxonomic definitions were prepared according to Flora of Turkey, and the collection places and collector numbers of the examined samples are given in Table 1 (Davis, 1972). And the photographs of the plants taken from the field are shown in Figure 1.

Table 1. The locality informations of collected *Convolvulus* taxa.

<i>Convolvulus arvensis</i> L.	B7: Elazığ, Keban, South of the village Aşağıçakmak, <i>Pinus</i> forest clearing, sandy steppe areas, 1200-1300 m, 25.05.2012 Kılıç 2095.
<i>Convolvulus betonicifolius</i> Mill. subsp. <i>peduncularis</i> (Boiss.) Paris	B7: Elazığ, Keban, West of Dürümlü village, steppe slopes, field edge, 1100-1200 m, 25.05.2012, Kılıç 2104.
<i>Convolvulus holosericeus</i> Bieb. subsp. <i>macrocalycinus</i> Hausskn. et Bornm. ex Bornm. (Endemic)	B7: Elazığ, Northern parts of Harput, towards Obuz Village, forest clearing, sandy, calcareous, steppe areas, 1200-1300 m, 18.06.2012, Kılıç 2132

Morphology

The morphological description of *Convolvulus* taxa is given in the following sections and compared with the Flora of Turkey (Davis, 1972). Detailed morphological characteristics of these taxa were presented (Table 2). Seed representing the general seeds structure were selected from the samples with seed. Seed belonging to *Convolvulus* species were placed on prepared stubs. Surface photographs of the seed were taken with a Leo S420 SEM (Figure 2).

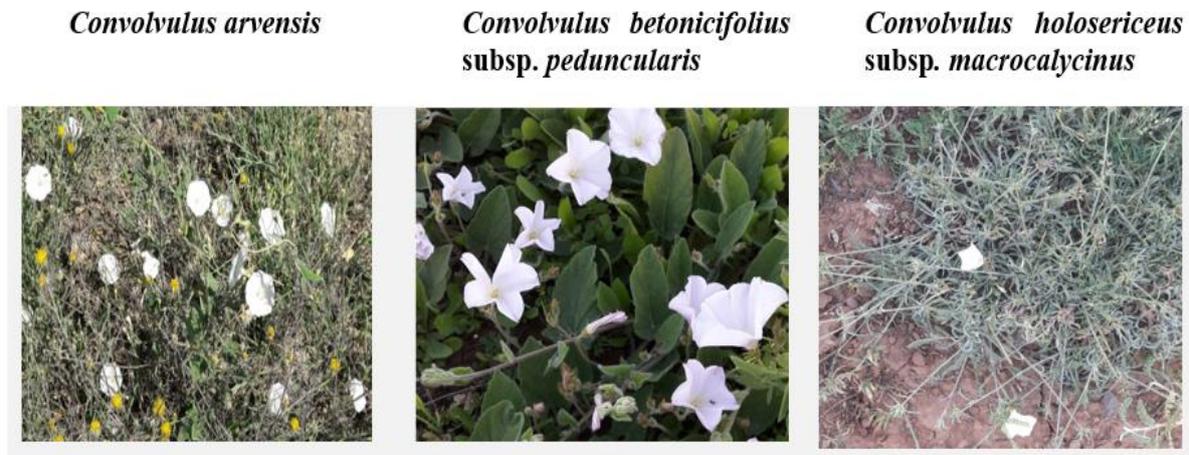


Figure 1. Habitats and general view of *Convolvulus* taxa

Pollen morphology with SEM

Pollen grains of 3 taxa belonging to the genus *Convolvulus* were studied with SEM. Polar axis (P), equatorial axis (E) were measured from at least 30 pollen grains in the experiment with SEM. These measures are given in Table 2 and in Figure 3. The pollen grains were examined with a Leo S420 SEM operated at 15kV. SEM micrographs are shown in Figure 2. For the pollen terminology and the pollen shape classification available resources were used (Faegri and Iversen, 1975; Punt, 1984, 2007; Erdtman, 1969) (Table 2).

Preparation of samples for SEM

SEM studies were conducted by direct mounting of stem, seed, leaf samples on stubs attached with sticky tape. The surface patterns are compared under the details set out below (Table 2). The samples were then studied and photographed by a Leo S420 SEM (Figure 2-3).

RESULTS

Morphological characters including leaf, stem, pollen sizes, seed, colour, shapes and surface characteristics are summarized in Table 1.

Morphological Results

Convolvulus arvensis

C. arvensis is perennial grasses with twigs or climbers. Climbing stems that do not branch above the ground can be 80 cm. The leaves are at the base with auricles or herbaceous extensions, usually 3-5 cm, glabrous or sparsely short. The flowers are usually single on the leaf seats. Corolla is white or pink. Flowering time is between May and September and fruiting between July and September. The seed's shape is ovoid and 3-4 × 2.5-4 mm size. There are rough projections on the seed surface. The habitat is sandy steppes, field sides and roadsides. The life form is hemicryptophyte and it also shows distribution between 200-1050m. It has a very wide spread in Iran-Turan.

Convolvulus betonicifolius* subsp. *peduncularis

Plant hirsute, climber or cuddly, short-haired herbaceous perennials. Stem and leaves are stalked or with an auxiliary extension at the base and lobes cordate, ovoid and narrowly ovoid. *C. betonicifolius* Mill. subsp. *peduncularis* is distinguished from the other subspecies of *C. betonicifolius* ssp. *betonicifolius* in that it has leaves cordate. The flowers are in the leaf seats, each stem has 1 or 3 flowers. Corolla is pink or white in color. Flowering time is between May and July. Seed shape is oblong and 3-4 × 2-3 size. As habitats, empty or cultivated fields are road sides. It spreads at a 300-1700m height. The life form is hemicryptophyte. General and regional distribution in Turkey, Syria, Lebanon.

***Convolvulus holosericeus* subsp. *macrocalycinus* (Endemic)**

They are perennials with woody base, ascending form, with horizontal gray hair. The lower leaves are narrowly inverted ovoid and lanceolate-inverted lanceolate. The flowers are sometimes single and terminal or in leaf axes or terminal panicles. The corolla is white to pale yellow. Outer sepals pouched at base. Flowering time is between April and July and its habitat is arid steppes, rocky, sandy, eroded and calcareous hills, limestone fields. It shows distribution in 250-1700m. height. The life form is hemicryptophyte. Seed shape is ovoid, 3-5 × 2-4 mm. It is the type with a less rough surface. The stem and leaves are less hairy. General and regional distribution in Turkey.

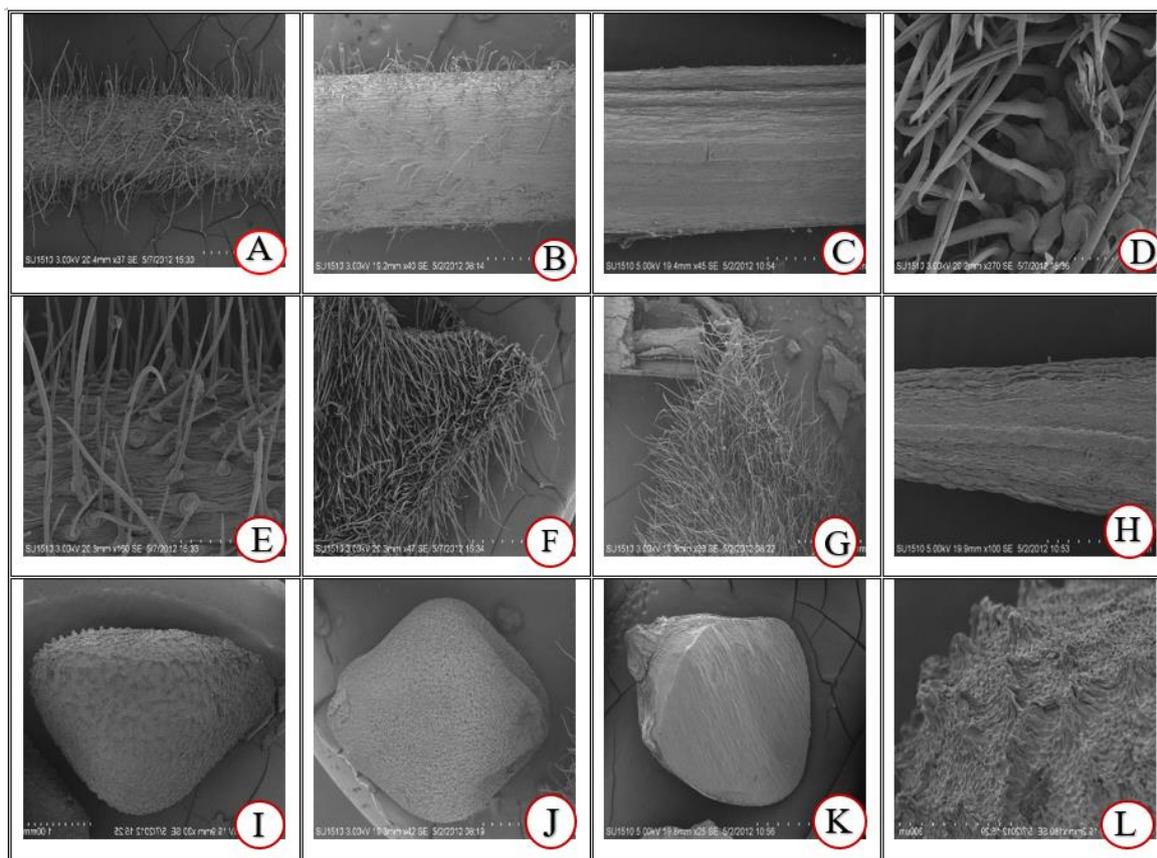


Figure 2. SEM images of taxa of *Convolvulus*: *C. arvensis* Stem (A-D), leaf (F), Seed (I-L), *C. betonicifolius* subsp. *peduncularis* Stem (B-E), leaf (G), Seed (J), *C. holosericeus* subsp. *macrocalycinus* Stem (C), leaf (H), Seed (K).

Palynological Results

Convolvulus species have a isopolar pollen shape. Pollen grains a radially symmetrical and they are oblate-spheroidal in *C. arvensis*, *C. holosericeus*. subsp. *macrocalycinus* and *C. betonicifolius* subsp. *peduncularis*. And this taxas a pollen type three- zonocolpate. The pollen grains were symmetrical, isopolar. Pollen shape is sub-oblate. Exine tectate, tectum densely perforate. Apertüre is a tricolpate of *C. holosericeus*. subsp. *macrocalycinus* that colpi borders and tapering ends distinct. *C. arvensis* and *C. betonicifolius* subsp. *peduncularis* have a colpate, tricolpate apertures colpus area gemmate and granulate. Pollen shape (P/E) of *C. arvensis* is oblate-spheroidal, *C. betonicifolius* subsp. *peduncularis* is sub-oblate and *C. holosericeus* subsp. *macrocalycinus* is prolate-spheroidal.

Table 2. Morphological and palynological characters of *Convolvulus* taxa

	<i>C. arvensis</i>	<i>C. betonicifolius</i> subsp. <i>peduncularis</i>	<i>C. holosericeus</i> . subsp. <i>macrocalycinus</i>
Morphological Characters			
Leaves size average	3x5 cm	2x6	2x6
Leaves shape	Ovate-Deltoid	Ovate	Linear
Corolla size	1- 2.5 cm	3- 4.5 cm	2 - 3 cm
Colors of corolla	White	White-pink	White
Stem length	8-80cm	15-90 cm	10–65 cm
Stem hairs	Pilose hairs	Tomentose hairs	Glabrous to pubescent

Seed shape	Ovoid	Oblong	Ovoid
Seed size average	3-4 × 2.5-4 mm	3-4 × 2-3 mm	3-5 × 2-4 mm
Seed colour	Brown	Brown	Black
Seed surface	Rough	Rough	Rough
Pollen Morphological Characters			
Polar axis (P, µm)	35-(44)-50	47-(52)-56	47-(53)-60
Equatorial axis (E, µm)	40-(45)-50	53-(60)-68	52-(49)-60
Pollen type	3-zonocolpate	3-zonocolpate	3-zonocolpate
Pollen ornamentation	Microechinate-Perforate	Microechinate-Perforate	Microechinate-Perforate
Aperture (Pollen)	Colpate, Tricolpate	Colpate, Tricolpate	Tricolpate
Pollen Shape (P/E)	Oblate-Spheroidal	Suboblate	Prolate-Spheroidal

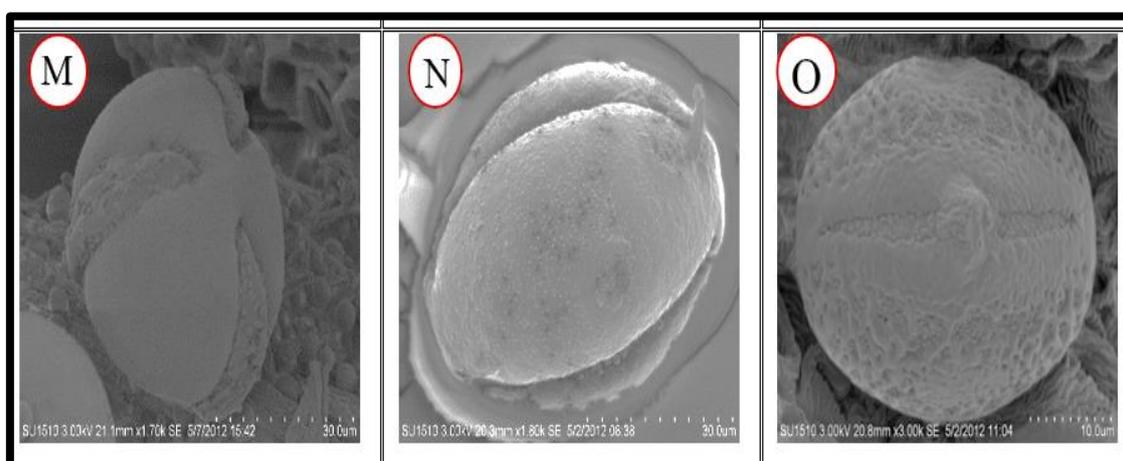


Figure 3. SEM images of pollen grains of: *C. arvensis* (M), *C. betonicifolius* subsp. *peduncularis* (N), *C. holosericeus* subsp. *macrocalycinus* (O)

DISCUSSION

The morphological characteristics of three *Convolvulus* taxa were compared with each other. The pollen characteristics of endemic *Convolvulus holosericeus* subsp. *macrocalycinus*, *Convolvulus betonicifolius* subsp. *peduncularis* and *C. arvensis* were examined by SEM. Wood et al. reported that very variable trailing or twining perennial herb up to 1 m high, vegetative parts hirsute, thinly to densely pubescent and generally pilose or tomentose for *C. betonicifolius*. According to our study, *Convolvulus betonicifolius* subsp. *peduncularis* has a height up to 90 cm. Wood et al. said that the gibbous sepals are very distinctive in this species. *C. holosericeus* var. *macrocalycinus* was originally lectotypified by Sa'ad (1967) but as she used the erroneous name *macrosepalus*, valid lectotypification dates from the Flora of Turkey (Wood et al., 2015).

Eraghi et al., reported that polar axis length 37-53 mm of *C. arvensis* (Eraghi et al., 2015). Our study is this length 35-50 mm. Tekin and Yilmaz (2016) stated that pollen shape is a sub-oblate of *Convolvulus betonicifolius* subsp. *betonicifolius*. This result is consistent with *Convolvulus holosericeus* subsp. *macrocalycinus* in our study. Aykurt and Sümbül (2011) is based on trikolpat all types *Convolvulus*. All three species in our study are tricolpate. They stated that polar axis is 59.45-66.62 and equatorial axis is

61.5-67.65 of *Convolvulus holosericeus* subsp. *macrocalycinus* and we determined that polar axis 47-(53)-60, equatorial axis 52-(49)-60. As a result, our studies are similar in terms of palynological characters.

The pollen morphologies of some *Convolvulus* L. taxa were examined using scanning electron microscopy, and the pollen of the taxa were radially symmetrical, isopolar and tricolpate. The largest pollen is *C. armenus*, *C. persicus*; the smallest pollen belongs to *C. leptocladus*. However, most species have subprolate and prolate-spheroidal pollen. The tectum is perforated (most common), micro-reticular or reticulate (Ranjbar et al., 2020). In this study, pollen morphological characters were compared with our results. Although our results were similar in general terms, it was observed that we had pollen of different sizes when compared to other species in Iran.

Khalik and Osman (2007) stated that *Convolvulus arvensis* seeds are pyramidal and $3.2-4.4 \times 2.7-3.4$ mm, brown. We have studied *C. arvensis* seeds are average size $3-4 \times 2.5-4$ mm. And it is shape ovoid, brown and seeds surface rough. There does not appear to be a large difference between the two studies. In another study reported that *Convolvulus holosericeus* seeds ovoid-elliptical and $4.5-5 \times 3-3.5$ mm, bright, white-sericeous (Aykurt and Sumbul 2011). We stated that $3-5 \times 2-4$ mm and its ovoid and black color of *Convolvulus holosericeus*. subsp. *Macrocalycinus*. The seed surface of this endemic species is rough.

CONCLUSION

In this study, pollen morphology of three *Convolvulus* taxa, one of them endemic to Turkey, were studied in detail with SEM. Besides, leaf and stem morphologic structures of these taxa with SEM were revealed for the first time in this study. *C. arvensis* pollen is smaller than the other two species and plays a role in species distinction. *Convolvulus holosericeus* Bieb. subsp. *macrocalycinus* is systematically distinguished from the other two species in that seed shape is oblong.

REFERENCES

- Arora A, Modi A. (2008). An acetolysis technique for pollen slide preparation. *Indian J. Aerobiol.*, 21(2), 90-1.
- Ashfaq, S., Ahmad, M., Zafar, M., Sultana, S., Bahadur, S., Abbas, N., (2019). Medicinal plant biodiversity used among the rural communities of arid regions of Northern Punjab, Pakistan. *Indian J. Tradit. Knowl.*, 18, 226-241.
- Austin, D. F. (1998). Parallel and convergent evolution in the family Convolvulaceae: 201– 234. -In: Biodiversity and taxonomy of tropical flowering plants. – Mentor Books, Culicut, India.
- Aykurt, C., Sümbül, H. (2011). New natural hybrids of *Convolvulus* (Convolvulaceae) from Turkey. *Nordic Journal of Botany*, 39(4), 408-416.
- Aykurt, C., Sümbül, H. (2014). A new natural hybrid of *Convolvulus* L. (Convolvulaceae) from the Central Anatolia. *Annales Botanici Fennici*, 48(5): 428–434.
- Carine, M.A., Ruseel, S.J., Santos-Guerra, A., Francisco-Ortega, J. (2004). Relationships of the Macaronesian and Mediterranean Floras: Molecular evidence for multiple colonisations into Macaronesia and subsequent back-colonisation of the continent in *Convolvulus* (Convolvulaceae). *American Journal of Botany*, 91, 1070-1085.
- Davis, P. H., Mill, R. R., Tan, K. (1988). *Convolvulus* L. in: Davis, P. H., Mill, R. R., Tan, K. (eds), Flora of Turkey and the East Aegean Islands 10: 182. Edinburgh Univ. Press, Edinburgh.
- Dickson, V. (1955). *The wild flowers of Kuwait and Bahrain*. George, Allen and Unwin, London, 1–144.
- Erdtman, G. (1952). *Pollen Morphology and Plant Taxonomy, Angiosperms*. Almquist and Wiksell, Stockholm, 150-170.

- Erdtman, G. (1969). *Handbook of palynology*. Munksgaard, Copenhagen, pp 486.
- Faegri, K., Iversen, J. (1975). *Textbook of Pollen Analysis*. New York, Hafner Publishing Co.
- Grigoriev, C. (1953). Convolvulaceae. In: Shishkin BK et al. (Eds) Flora USSR volume 19: Tubiflorae. USSR Scientific Academy, Moscow and Leningrad, 1–37.
- Kilic, O. (2016). An ethnobotanical survey from Bingol (Turkey). *Journal of Applied Res.*, 2(10), 685-691.
- Kilic, O., Bagci, E. (2013). An ethnobotanical survey of some medicinal plants in Keban (Elazığ). *JMPR.*, 7(23), 1675-1684.
- Kursat, M., Sancar, P.Y., Civelek, S. (2014). New record for the flora of Turkey, *Artemisia fragrans* Willd (Asteraceae). *Ot Sistematiik Botanik Dergisi*, 21(2), 49-58.
- Stant, M.Y. (1973). The role of the scanning electron microscope in plant anatomy. *Kew Bulletin*, 28(1), 105-115.
- Newton, L. E. (1972). Taxonomic use of the cuticular surface features in the genus *Aloe* (Liliaceae). *Bot. Journ. Linn. Soc.*, 65, 335-339.
- Punt, W. (1984). *Umbelliferae*. In: Punt W, Blackmore S, Clarke GCS (eds). The Northwest European Pollen Flora, IV. Elsevier, Amsterdam.
- Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S., Le Thomas, A. (2007). Glossary of pollen and spore terminology. *Rev. Palaeobot. Palynol.*, 143(1–2), 1-81.
- Qureshi, M.N, Talha, N., Ahmad, M., Zafar, M., Ashfaq, S. (2019). Morpho-palynological investigations of natural resources: A case study of Surghar mountain district Mianwali Punjab, Pakistan. *Microsc Res Tech.*, 82(7), 1047-56.
- Ranjbar M., Ezazi, A., Ghahremannejad, F. (2020). Contribution to the pollen morphology of *Convolvulus* (Convolvulaceae). *Phytotaxa*, 439(3).
- Sa'ad F. (1967). *The Convolvulus species of the Canary Isles, the Mediterranean region, and the Near and Middle East*. Mededelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit Utrecht. Bronder Off set, Rotterdam, 1–287.
- Saensouk, S. (2007). *Family Convolvulaceae in Muang district, Nong Khai province, Thailand*. Warasan Wichai Mokho.
- Sancar, P.Y., Wahi, C.F., Civelek, S., Kursat, M. (2021). An investigation on the chloroplast and nuclear genomes of taxa belong to the subgenus *Dracunculus* (Bess.) Rydb. of *Artemisia* L.(Asteraceae) in Turkey. *Brazilian Journal of Biology*, 82, e242403.
- Sethiya, N.K, Nahat, A., Mishra, S.H., Dixit, V.K. (2009). An update on Shankhpushpi, a cognition-boosting Ayurvedic medicine. *Zhong Xi Yi Jie He Xue Bao*, 7(11), 1001-22.
- Staples, G. (2018). *Flore du Cambodge, du Laos et du Viêt Nam: (révision de la Flore Générale de L'Indochine)*. Convolvulaceae. Publications Scientifiques du Muséum (in French).
- Tekin, M., Yilmaz, G. (2016). Palynological Studies of the Genus *Convolvulus* L. (Convolvulaceae) from Turkey. *Botanical Sciences*, 94(3), 543-549.
- Tod, F.S. (2009). *Plant taxonomy: the systematic evaluation of comparative data*. Columbia university press, Vienna, Austria.
- Waheed, K., Muhammad, S.K., Ashfaq, S., Zafar, M., Ullah, I., Ullah S. (2020). Antimicrobial Activity and Phytochemical Screening of *Euphorbia helioscopia*. *Planta Daninha.*, 38, 45-54.
- Wood, J.R.I., Williams B.R.M., Mitchell T.C., Carine, A.M., Harris, D.J. Scotland RW. (2015). A foundation monograph of *Convolvulus* L. (Convolvulaceae). *Phytokeys*, 51, 1-282.