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ANATOMICAL CHARACTERIZATION OF VEGETATIVE ORGANS OF ENDANGERED HALOPHYTE *ODONTARRHAENA BORZAEANA* (NYÁR.) D. A. GERMAN

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Abstract

Odontarrhena borzaeana (Nyár.) D. A. German (= *Alyssum borzaeanum* Nyár.) is an endemic and endangered halophyte of the family Brassicaceae species grows on the sandy shores of the Black Sea. The aim of this research was to assess some anatomical features of vegetative organs of this species.

Roots had a secondary structure with less developed suber and secondary cortex and bundle-like arrangement of the primary xylem towards the centre of the root (alternating with pith tissue). Stem had a primary structure, with differentiated cortex (the innermost 2-3 cell layers having a flattened shape). Flattened blocks of phloem were followed by xylem. Well-developed sclerenchyma layers were found both within and between vascular bundles, forming a continuous ring around the stem. Pith was parenchymatous and abundant.

Leaf blades were amphistomatic and equifacial, with few layers of spongy parenchyma in the middle and well-developed palisade parenchyma towards both sides. Typical collateral bundles were each surrounded by a sheath of parenchymatous cells. Both stem and leaves were covered with massive stellate trichomes (larger on leaves), similarly to the ones reported in several related species.

Key words: Anatomy, Halophyte, Leaves, *Odontarrhena borzaeana*, Root, Stem, Trichomes.

Psammophilous plants are known to possess anatomical and physiological characteristics that allow them to colonize extreme habitats such as littoral sands, areas with extreme temperature and water variations, salinity, and sunlight exposure. Due to their specific habitat, these species are often found in limited populations. Drought, high osmotic pressure and high levels of mineral salts are the main limiting factors to plant growth in these areas. Adaptations to such conditions include both anatomical features and specific physiological mechanisms.

One of these littoral halophytic plants is an endemic species of Brassicaceae found on the sandy shores of the Black Sea. *Odontarrhaena borzaeana* (Nyár.) D. A. German was originally described under the genus *Alyssum* and later described under various names viz. (*Alyssum borzaeanum* Nyár., syn. *Alyssum tortuosum* subsp. *borzaeanum* (Nyár.) Stoj. & Stef., syn. *Alyssum tenderense* Kotov, syn. *Alyssum obtusifolium* Steven ex DC.) (Marhold, 2011; German, 2014), but was finally transferred to the genus *Odontarrhaena* in 2014, although recent genetic research shows that the entire genus could be joined with *Clypeola* (Salmerón-Sánchez *et al.*, 2018).

A perennial semi-shrub, it has a woody taproot, multiple hairy stems, 10-30 cm tall, plus some basal sterile shoots that form leaf rosettes. Leaves are small, rounded to broad-lanceolate, pubescent. Numerous yellow flowers are grouped in corymbs, blooming in April-May and often for a second time in September. Fruits are heart-shaped pubescent silicules. Each locule of the fruit hosts one unwinged seed and pollinated by insects, while there is a low degree of autogamy. Wind dispersal propagates seeds

The plant grows in lowland areas, such as grasslands on stony soils rich in limestone, sometimes rocky areas, but mostly on maritime sand dunes. The main populations are found along the coasts of the Black Sea (Romania, Bulgaria, Ukraine, Russia, Turkey), but it was also found in Transcaucasia and Northern Greece. In Romania, it is only found in the Constanța county, on the southern sector of the Romanian coast. Included in Romania's Vascular Plants Red List from Romania, it is considered as endangered and covered by the Bern Convention, Annex I (Păunescu, 2008; Anchev & Goranova, 2011; Red Book of Ukraine, 2023). Recent data, however, seem to indicate that its conservation status is improving, considering habitat and population status (Jianu, 2014).

Many members of the *Odontarrhaena* genus are known to be nickel hyperaccumulators, with potential application in bioremediation and biomining (Bettarini *et al.*, 2020). An interesting future research direction would be to investigate the potential of *Odontarrhaena borzaeana* for biomining.

Due to its rarity and endangered status, apart from ecological studies, the literature concerning the biology of this species is scarce. Little is known on its anatomy and physiology. The scope of the current paper was to further expand the knowledge on *O. borzaeana* by determining its anatomical features, that are possible adaptations to its particular environment.

Material and Method

Fresh *O. borzaeana* plant (root, stem and leaf) samples were collected from the small local population that grows on littoral sand dunes in Agigea Natural Reserve (Constanța County, Southeastern Romania). Care was taken to collect only small fragments needed for anatomical study, considering the threatened status of this species.

Organs were fixed and stored in FAA (mixture of formalin, concentrated acetic acid and ethyl alcohol, 5: 5: 90). Hand sections of selected samples were subjected to double staining using iodine green and alum carmine stains (Bercu & Jianu, 2003).

Bright field microscopy was performed with a microscope, equipped with an Omegon Telemikro electronic camera.

Results

Root cross section of *O. borzaeana* (Fig. 1), showed a regular secondary root structure, with a thin suber and a thin (2-3 cell layers) secondary cortex (Fig. 2A), followed by a thick primary cortex, showing peripheral lacunae. Such lacunae are known to be adaptations to poor air circulation in soil. Towards the centre, blocks of phloem were followed by secondary and primary xylem. Primary xylem had a bundle-like arrangement, organized in five radial blocks alternating with pith cells. Protoxylem vessels occupied the root central area (Fig. 2B).

The cross section of the stem was circular. A single-layered epidermis showed a thin cuticle, branched trichomes and stomata placed in line with the other epidermal cells (Fig. 3; Fig. 4A). The cortex was continuous, with no lacunae and small intercellular spaces. Inner cortical cells (2-3 layers) had a flattened shape, while the rest were oval.

Vascular tissue was organized in typical collateral bundles. Flattened blocks of phloem were located peripherally and covered with 2-3 layers of cellulosic, round sclerenchymatic cells. Towards the centre, the cambium, metaxylem and protoxylem followed (Fig. 4B, C). Above and between metaxylem blocks, areas of ligneous sclerenchyma were placed, joining the vascular bundles in a continuous ring. The centre was occupied by a compact parenchymatic pith, composed of large polygonal cells.

Leaf blades had slightly proeminent midribs and were covered in epidermis with thin cuticle, massive trichomes and stomata were in line with other epidermal cells, similar to that covering the stem (Fig. 5; Fig. 6A). The blade was equifacial, with thick layers of palisade parenchyma towards both upper and lower sides and few layers of poorly differentiated spongy parenchyma (Fig. 6A, B).

Vascular bundles were typical collateral bundles, with xylem towards the upper face, protected by layers of sclerenchyma. Both midrib and secondary bundles were surrounded by one layer of parenchymatous cells.

Leaves were amphistomatic, with numerous stomata on both sides.

Discussion

Perennial species related to our test species are known to possess a secondary root structure, such as *Odontarrhaena obtusifolia* (*Alyssum obtusifolium*) and *O. floribunda* (*A. floribundum*) – with typical concentric xylem rings (Orcan & Binzet, 2003; Orcan & Binzet, 2004) – while the annual species have a primary root structure, such as *Alyssum alyssoides* and *A. turkestanicum* (*A. desertorum*; Akyol *et al.*, 2017). A peculiar case is that of *A. strigosum*, an annual plant that has metaxylem rings, with protoxylem occupying part of the pith (Şirin *et al.*, 2022), a situation similar to our findings.

Concerning stem structure, the flattened cortical cell layers are also found in *O. obtusifolia*, *A strigosum* and *A. turkestanicum* and some authors consider them to be an endodermis (Orcan & Binzet, 2003; Akyol *et al.*, 2017). The continuous ring of vascular bundles, joined together by areas of ligneous sclerenchyma was observed by other authors in *O. floribunda*, *A. alyssoides* and *A. strigosum* (Orcan & Binzet, 2004; Akyol *et al*., 2017, Şirin *et al.*, 2022).

Known relatives of *O. borzaeana* either have a similar, equifacial structure of the leaf, like *O. floribunda*, *O obtusifolia*, *A. dasycarpum*, *A. strigosum* and *A. szovitsianum* (Orcan & Binzet, 2003; Orcan & Binzet, 2004; Sultanovna, 2018; Şirin *et al.*, 2022), or an unifacial one, with palisade and spongy tissue completely undifferentiated, such as *A. alyssoides* and *A. turkestanicum* (Akyol *et al.*, 2017; although the latter also possesses few rows of spongy tissue according to Sultanovna, 2018). Likewise, the layer of parenchymatous cells surrounding leaf vascular bundles is a feature that is also present in *O. obtusifolia* and *A. szovitsianum* (Orcan & Binzet, 2003; Sultanovna, 2018).

Amphistomatic leaves are similar to other related species, such as *O. obtusifolia* or *A. szovitsianum* (Orcan & Binzet, 2003; Sultanovna, 2018). A comparative analysis of the presence or absence of various key anatomical features in *O. borzaeana* and some related species is found in Table 1.

A feature shared with related species is the presence of large, branched, stellate trichomes (Fig. 7) on both stem and leaves. Similar in shape and structure, those found on leaves were more massive. Numerous and massive stellate hairs with 5-16 rays were also reported in *O. floribunda, O. obtusifolia*, *A. dasycarpum*, *A. szovitsianum*, *A. turkestanicum* (Orcan & Binzet, 2003; Orcan & Binzet, 2004; Sultanovna, 2018). Trichomes of Brassicaceae are often known to mineralize with Si, Ca (especially calcium phosphate), Mg. In metal-accumulating plants, including some species of *Odontarrhena* (*O. chalcidica*, *O. corymbosoidea*), they can sometimes accumulate high concentrations of Ni or Mn (Hopewell *et al.*, 2021).

Trichomes, along with a well-developed cuticle are considered common xerophytic adaptations. Among halophytic adaptations, lacunae found in *O. borzaeana* roots are one of the examples. This is because saline soils tend to have poor ventilation and water circulation. They can become marshy when wet and compact when dry (Grigore & Toma, 2006).

Table 1. Comparative table of some anatomical features found in *O. borzaeana* and other related species studied (according to Orcan & Binzet, 2003, Orcan & Binzet, 2004, Akyol *et al*., 2017, Sultanovna, 2018, Şirin *et al*., 2022).

Species	Secondary root structure	Protoxylem bundles alternating with pith	Root cortical lacunes	Stem endodermis	Xylem ring in stem	Equifacial leaf	Amphistomatic leaf	Stellate trichomes
O. borzaeana	+	+	+	+	+	+	+	+
O. floribunda	+	-	-	+	+	+	+	
O. obtusifolia	+	+	-	+	+	+	+	+
A. alyssoides	-	-	-	-	-	-	+	+
A. dasycarpum	?	?	?	?	?	+	+	+
A. strigosum	-	+	-	+	+	+	+	+
A. szovitsianum	?	?	?	?	?	+	+	+
A. turkestanicum	-	-	-	+	-	-/+	+	+

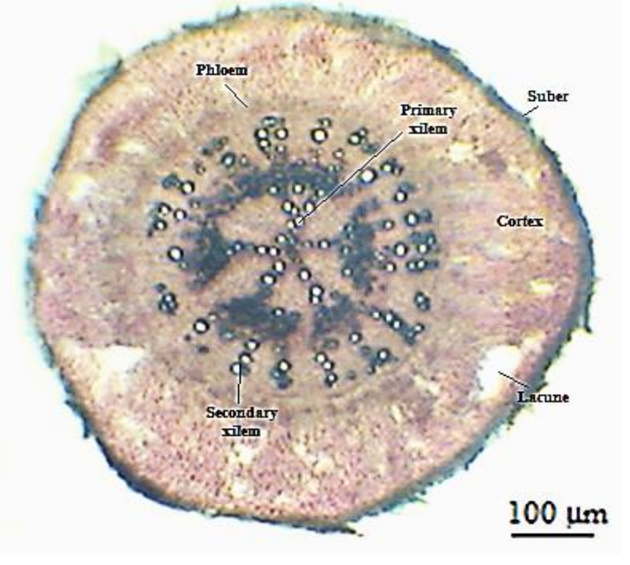

Fig. 1. Cross section of *Odontarrhaena borzaeana* root with secondary structure.

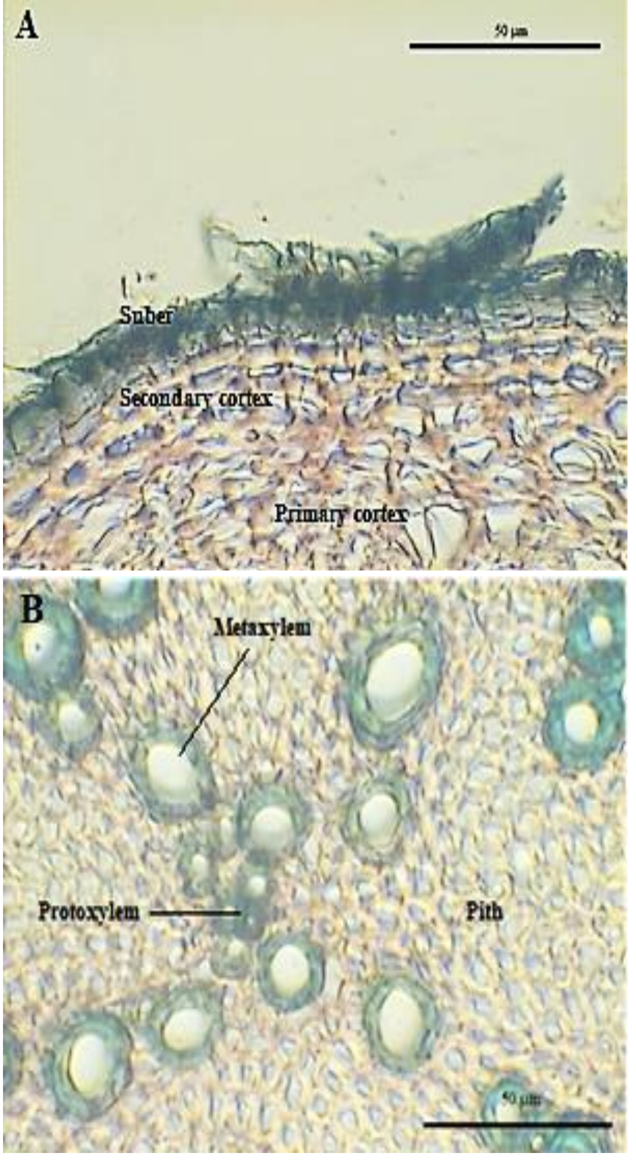

Fig. 2. Details of *O. borzaeana* root anatomy: A – Suber and cortex; B – Xylem and pith.

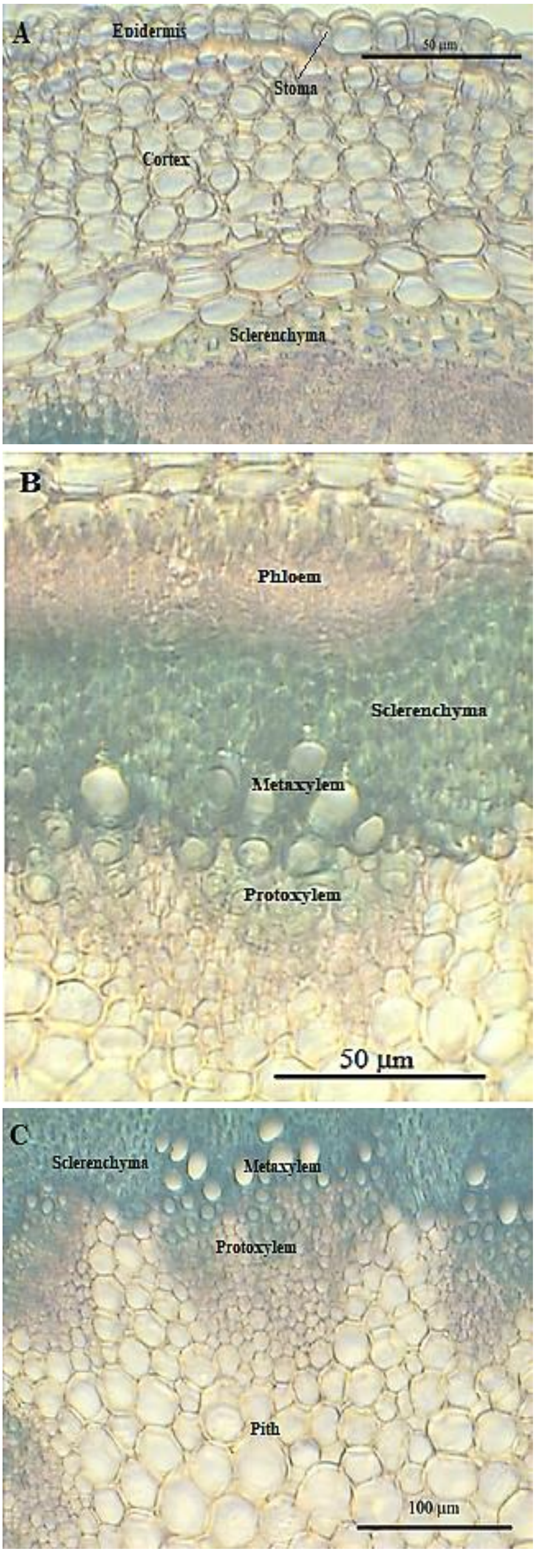

Fig. 4. Details of *O. borzaeana* stem anatomy: A – Epidermis and cortex; B – Vascular bundle; C – Xylem and pith.

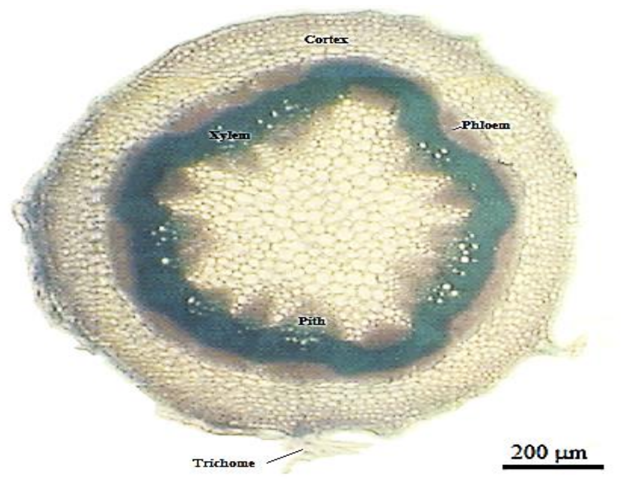

Fig. 3. Cross section of the *O. borzaeana* stem.

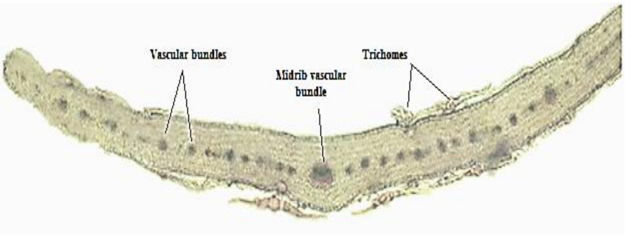

Fig. 5. Cross section of *O. borzaeana* leaf blade.

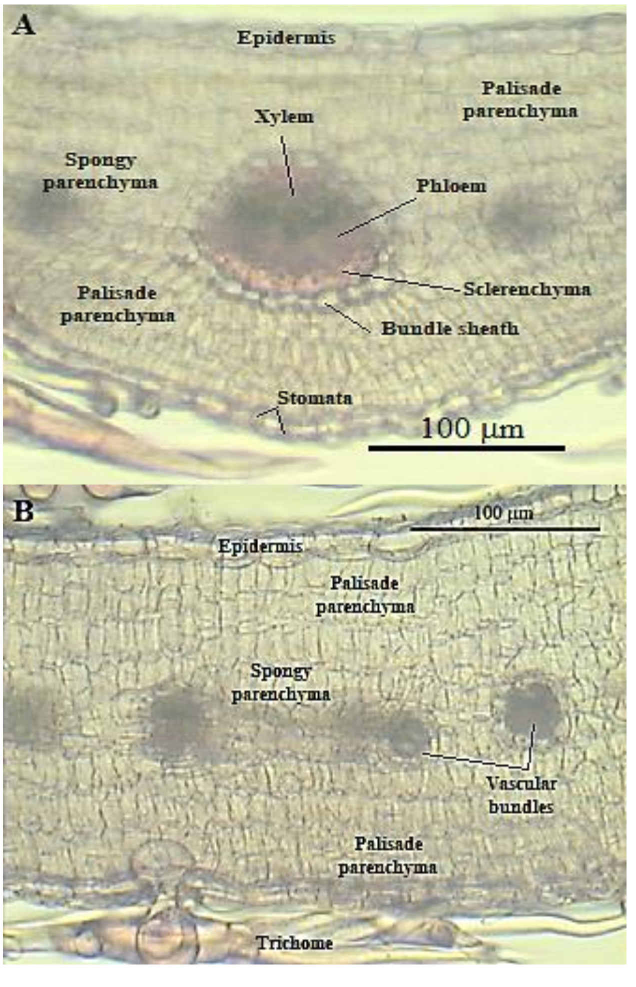

Fig. 6. Details of *O. borzaeana* leaf anatomy: A – Midrib; B – Secondary vascular bundles.

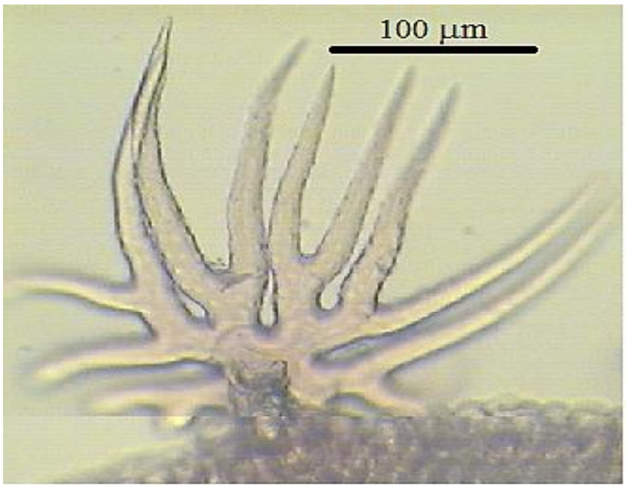

Fig. 7. Stellate trichome on *O. borzaeana* stem.

Conclusions

Cross sections on *Odontarrhena borzaeana* organs showed a root having a secondary structure with less developed suber and secondary cortex and bundle-like arrangement of the primary xylem towards the centre of the root (alternating with pith tissue), similarly to *Alyssum strigosum* root structure.

Stem had a primary structure, differentiated into cortex (the innermost 2-3 cell layers having a flattened shape), flattened blocks of phloem followed by xylem. Well-developed sclerenchymal layers were found both within and between vascular bundles, forming a continuous ring around the stem. Pith was parenchymatic and abundant.

Leaf blades were amphystomatic and equifacial, with few layers of spongy parenchyma in the middle and well-developed palisade parenchyma towards both sides. Typical collateral bundles were each surrounded by a sheath of parenchymatous cells, like in some other related species (*Odontarrhena obtusifolia*, *Alyssum szovitsianum*).

Both stem and leaves were covered with massive stellate trichomes (larger on leaves), similarly to the ones reported in several related species.

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