



## POLLEN MICROMORPHOLOGICAL TRAITS OF THE TYRRHENIAN *STACHYS SALISII* JORD. & FOURR. (LAMIACEAE)

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**ABSTRACT** – Pollen micromorphology of plants from four populations of the Tyrrhenian insular endemic *Stachys salisii* is examined using light and scanning electron microscopy. The preliminary results show some differences between the populations analyzed as regards pollen surface micromorphology and dimensions. The basic shape of the pollen grains is subprolate, usually tricolpate, but also oblate-spheroidal and tetracolpate. The pollen surface is microreticulate, perforate or foveolate. Lumina are separated by smooth or sinuate muri, which make them polygonal and elongate, while the number of punctae for each lumen also show some variations.

**KEYWORDS:** WOUNDWORT, *LAMIOIDEAE*, MEDITERRANEAN VASCULAR FLORA, POLLEN MORPHOLOGY, SEM

### INTRODUCTION

*Stachys* L. (*Lamiaceae* Martinov, *Lamioideae* Harley) is one of the largest genera of mints, amounting to between approximately 275 (Bhattacharjee, 1980) and 300 (Willis & Airy-Shaw, 1973; Mabberley, 1997) species worldwide. It consists of annual and perennial herbs and subshrubs which show an extensive variation in morphological and cytological features (Lindqvist & Albert, 2002). The genus, which is absent only in Australia and New Zealand, has a subcosmopolitan distribution range, with centers of diversity in the warm temperate region of the Mediterranean and South Western Asia, Southern Africa, and North and South America (Salmaki et al., 2008).

*Stachys salisii* Jord. & Fourr. is an annual plant, 5-15-(25-30) cm tall with prostrate-procumbent stem. The leaves are opposite, orbicular, truncate-cordate at the base, obtuse at the apex and crenate at the margin. The inflorescence is made up of verticills of shortly pedunculate and opposite flowers at the axil of bracteal leaves and without bracteoles. The calyx

is tubular-campanulate with ovate-lanceolate and aristate teeth. The corolla extends well above the calyx, is pale rose to purple, with a slightly curved tube, a hairy gorge, upper lip bent down on stamens, and lower lip with central lobe wider than lateral ones. The fruit is a brown to black mericarp, generally ovate-deprexed (Borzatti De Loewenstern & Mannocci, 2008). From a preliminary examination, *S. salisii* results macromorphologically not much differentiated among the various populations despite a certain variability in the habitus has been reported regarding individuals from Elba and Capraia in cultivation (Borzatti De Loewenstern & Mannocci, 2008).

*S. salisii* has been reported to be cleistogamous (Borzatti De Loewenstern & Mannocci, 2008); the flowering occurs from February to May, the fruiting from April to June.

*S. salisii* grows on siliceous substrata (vulcanites, granites, metamorphytes, granodiorites and quartz-latites), and usually colonizes terophitic meadows in garrigues or glareicolous habitats. From the bioclimatic point of view, it is circumscribed to a Mediterranean pluviseasonal oceanic

bioclimate, while thermotype ranges between lower thermomediterranean and upper mesomediterranean; the ombrotype is found between lower dry and upper subhumid. This *taxon* has been found in proximity to the coast for the Sardinian populations of the island of Sant'Antioco (South-Western Sardinia), of the islands of Santo Stefano and Caprera (Arcipelago di La Maddalena, North-Eastern Sardinia), as well as of the island of Capraia (Arcipelago Toscano) and of the island of Corsica in Monte di a Trinità (Southern Corsica), and right in the inland of the island of Elba (Arcipelago Toscano) situated at 550 m. a.s.l. in Monte di Cote, as well as on Monte Liccioli in Northern Corsica at 735 m. a.s.l.

The taxonomic history of *S. salisii* has been particularly troublesome. Ulysses Salis-Marschlins in 1834 first described the Corsican plants as *S. marrubiiifolia* Viv. var. *pusilla* which, thereafter, was described by Bertoloni as *S. corsica* Pers. var. *micrantha* and then raised to the rank of species by Jordan & Forreau in 1868. In the 20<sup>th</sup> century Fiori (1929) included *S. marrubiiifolia* Viv. var. *pusilla* Salis-Marsch., *S. corsica* Pers. var. *micrantha* Bert. and *S. salisii* Jord. & Fourr. in the new var. *pusilla*. Briquet (1955) did not recognize any taxonomic significance in the case of the Corsican annual plants, attributing them to *S. corsica*. Camarda (1978) considered worthy to be mentioned var. *micrantha* Bert [incl. var. *pusilla* Fiori and f. *pusilla* (Salis-Marsch.) Litard.] for the Sardinian territory as probably being distinct from the typical *S. corsica*. Pignatti (1982) only mentioned that Sardinian and Corsican plants living at low elevation differ from typical *S. corsica* in their smaller size as well as in smaller and more deeply-colored corolla. Concerning the Tuscan plants, it was Bavazzano (1970) that reported *S. corsica* var. *pusilla* for the island of Capraia on the basis of some herbarium samples collected by Sommier who referred to them as a supposed Caprarian variety of *S. arvensis* (L.) L. (*S. arvensis* var. *capraria*). Subsequently, Soldano (1993) published the findings of *S. corsica* s.s. for Capraia, misidentifying the annual plant and confusing it with the perennial one, as confirmed later by Borzatti De Loewenstern & Mannocci (2008).

Gamisans (2005) described *S. aimerici* as a new *taxon* limited to Monte di a Trinità, shortly after synonymized with *S. salisii* by Borzatti De Loewenstern & Mannocci (2008) who also proposed that the Sardinian, Corsican and Tuscan plants should be included in the same *taxon* (*S. salisii*).

A number of studies have been carried out so far on the micromorphology of pollen grains in the genus *Stachys* (Basset & Munro, 1986; Demissew & Harley, 1992; Potoğlu Erkara & Koyuncu, 2007; Salmaki et al., 2008) but none of these focused on the analysis of the possible variability in pollen grains traits between populations of the same *taxon*. *S. salisii* is distributed in few, generally small and highly-fragmented populations, separated from each other

by at least 30 km. These circumstances, together with certain ecological dissimilarities between the various populations, could have led to a limited, but still accountable morphological differentiation. In this context of high taxonomical uncertainty and given the existence of possibly favorable assumptions for morphological divergence, pollen grain traits, such as dimensions and surface sculpturing, could prove some differentiation between the different island populations. The preliminary results of palynological analysis on plants from four populations are presented below.

## MATERIALS AND METHODS

Pollen grains from four island populations of *S. salisii* were examined: island of Caprera (CA), Monte di a Trinità (MT) and the islands of Capraia (CAP) and Elba (EL).

Pollen grains were taken from herbarium specimens deposited in the Herbaria of Cagliari (CAG) and Livorno (LIVOR).

Pollen was prepared for scanning electron microscopy (SEM) and light microscopy (LM), using the standard method of acetolysis proposed by Erdtman (1960).

The pollen grains were dried with ethanol (96%) after acetolysis and mounted in glycerine jelly for LM observation. The polar axis, equatorial axis, and the wall thickness taken from the interapertural area in polar view were measured under LM using an Olympus microscope model BX-41. About 20 pollen grains for each population were used for these measurements.

For SEM analysis, the dried pollen were mounted on stubs and then coated in a sputter coater with approximately 25 nm of gold-palladium. All the samples were examined and photographed with a SEM-Hitachi S-3400N.

The terminology of the pollen grain micromorphology mainly follows what was reported by Erdtman (1952) and Hesse et al. (2009).

## RESULTS

Figure 1 shows pollen grains SEM and LM micrographs, while the main features of the pollen grains investigated are summarized in Table 1. Pollen grains of *S. salisii* are single and isopolar. The mean of the polar axis varies from 27.8  $\mu\text{m}$  (EL) to 32.0  $\mu\text{m}$  (CA), while the equatorial axis length ranges from 21.6  $\mu\text{m}$  (EL) to 26.3  $\mu\text{m}$  (CAP). The shape of the

pollen grains is basically subprolate (Fig. 1- a, b, f, g, k, l, p, q), with a ratio of the length of the polar axis to the equatorial diameter (P/E) that is equal to 1.17–1.29. An oblate-spheroidal shape is found in some grains of the samples from MT and EL, although it is not found in great quantities in EL while it is more common in MT. Regarding the sculpturing of exine, two basic types can be distinguished, reticulate/perforate, which reticulate can in turn be subdivided into two subtypes: reticulate with polygonal muri (CAP and EL) and reticulate with elongated muri (MT), and reticulate/foveolate (CA) (Fig. 1- c, h, m, r). The wall thickness ranges from 0.8 to 1.0  $\mu\text{m}$ , and becomes reduced at the colpus margin, while the colpi converge close to the polar ends. The shape of the lumina is polygonal in CAP and EL

and elongate in CA and MT, whereas their length varies between 0.1–0.8. The number of punctae in the lumina shows major variation only in the samples from CAP (Fig. 1- c, h, m, r). The primary lumen size in the polar area (apocolpium) is smaller than in the interapertural area (mesocolpium).

The mean width of muri varies from 0.2  $\mu\text{m}$  to 0.4  $\mu\text{m}$  and their surfaces result smooth in CA and EL samples and sinuate in those from CA and MT. The pollen grain types are generally tricolpate, except in MT and EL, which show both a tricolpate and tetracolpate pollen pattern. Tetracolpate pollen grains are more frequent in MT samples than in EL. Moreover, pollen grains are generally radially symmetrical, while *S. salisii* from MT shows an asymmetrical shape in the tetracolpate pollen (Fig. 1- a, b, f, g, k, l, p, q).

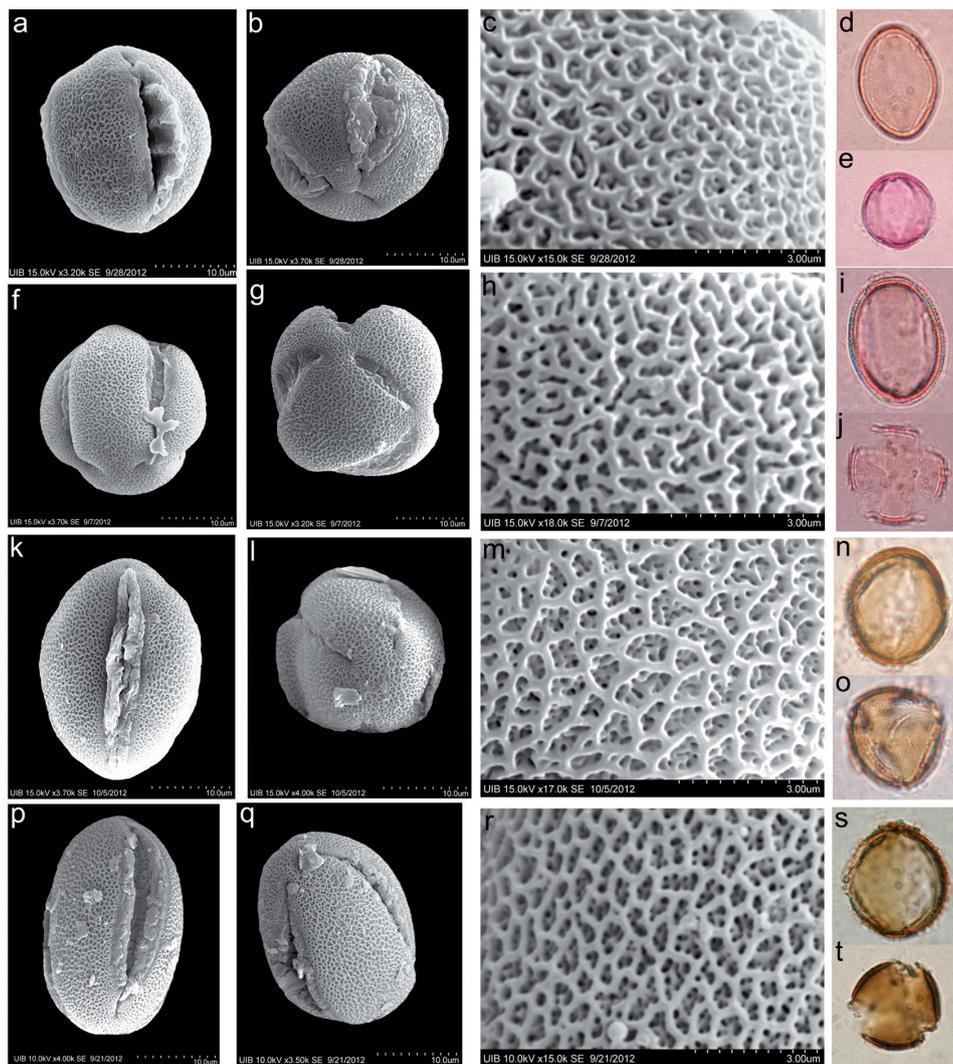


Fig. 1. SEM and LM pollen photography of the four populations of *Stachys salisii* investigated. Arcipelago di La Maddalena, Caprera Island (CA): a – e; Corsica, Monte dia Trinità (MT): f – j; Arcipelago Toscano, Capraia Island (CAP): k – o; Arcipelago Toscano, Elba Island (EL): p – t.

Table 1. Summary of pollen morphological data for the studied populations of *Stachys salisii*.

Population code	Polar axis (µm)	Equatorial axis (µm)	P/E	Shape	Sculpturing
CA	(27.2-) 32.0 ± 2.64 (-38)	(21-) 24.7 ± 1.80 (-27.5)	1.29	subprolate	reticulate/foveolate
MT	(26-) 28.33 ± 1.62 (-32)	(19.5-) 24.2 ± 3.25 (-30)	1.17	subprolate/oblate-spheroidal	reticulate/perforate
CAP	(26-) 30.9 ± 2.14 (-36)	(23.5-) 26.3 ± 1.78 (-32)	1.17	subprolate	reticulate/perforate
EL	(26-) 27.8 ± 1.98 (-31)	(16-) 21.6 ± 2.37 (-30)	1.28	subprolate/oblate-spheroidal	reticulate/perforate

Population code	Wall thickness (µm)	Shape of lumina	Length of lumina (µm)	Punctae in each lumen	Muri width (µm)	Muri surface	N. colpi
CA	0.8	elongate	0.25-0.6	1-6	0.2-0.4	sinuate	3
MT	1.00	elongate	0.4-0.8	1-5	0.25-0.35	sinuate	3/4
CAP	1.00	polygonal	0.1-0.6	3-12	0.2-0.4	smooth	3
EL	1.00	polygonal	0.1-0.5	1-5	0.2-0.3	smooth	3/4

## DISCUSSION

The main features of the pollen micromorphology analyzed are consistent with those reported earlier for some *taxa* belonging to *Lamioideae* (Abu-Asab & Cantino, 1992; 1994) and for what was reported later by Salmaki et al. (2008) concerning the genus *Stachys* in Iran. In accordance with the results of these previous studies, *S. salisii* generally displays symmetrical, isopolar monads, overall with a polar axis that is generally longer than the equatorial one and with an exine sculpturing that forms a net.

The basic shape of the pollen grains studied is subprolate, but oblate-spheroidal shape can also be found in the specimens from EL and MT. As far as the aperture system is concerned, the tricolpate type is the most frequent kind along with the subprolate shape. In this regard, the number of colpi seems to be correlated in some way with shape since the tetracolpate type is instead oblate-spheroidal in shape. This result is only partially in consonance with previous observations, since the tetracolpate aperture, together with a more oblate-spheroidal shape, should occur only in a few restricted *taxa* belonging to both *Stachys* and *Sideritis* genera, which in turn should be genetically related within the *Pontostachys* section (Abu-Asab & Cantino, 1992; 1994; Lindqvist & Albert, 2002). Moreover, the dimensions of pollen grain are not similar among the populations studied; CA and CAP show a greater size of the polar axis than MT and EL, along with a perfect tricolpate pattern and symmetrical grains. In MT and EL tetracolpate oblate-spheroidal pollen grains are present too, and an asymmetrical shape is observed in tetracolpate pollen grains from MT.

Concerning the sculpturing of the *S. salisii* pollen grain surface, this shows a reticulate type that concords with the type reported by Salmaki et al. (2008) for some Iranian *taxa* as well as for the patterns on the interapertural area and the polar area which are similar to each other. However, *S. salisii* shows a certain qualitative variability among the populations when studied for other micromorphological features that have to do with pollen grain sculpturing. The samples from CA are foveolate instead of perforate, the shape of the lumina is polygonal (CAP and EL) or elongate (CA and MT) and the muri surface is smooth (CAP and EL) or sinuate (CA and MT). There are other quantitative differences, especially in the length of lumina, the number of punctae per lumen, and the width of the muri.

## CONCLUSIONS

Our study on *S. salisii* shows that the various pollen micromorphological characters can be useful in characterizing the variability between the different populations.

On this basis, to define the variability inside this *taxon* in greater depth a broader morpho-biometrical study would need to include other features such as the mericarp and the indumentum as well as other macromorphological characters. In parallel, since the tendency to polyploidy has been already reported for this *taxon* (Borzatti De Loewenstern & Mannocci, 2008), a deeper karyotype analysis could reveal if there exist some differences in the chromosome number

among populations and if these could correlate with the observed variability in pollen grains micromorphology. Moreover, it could be useful to compare *S. salisii* with the closest *taxa*, such as *S. corsica* Pers., *S. arvensis* (L.) L. and *S. marrubiiifolia* Viv. in order to understand the possible relationship within these entities.

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