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# Morphological and anatomical studies on nutlets of *Stachys* (Lamiaceae) from south-west Spain

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Abstract: Scaning electron microscope and light microscope examinations of *Stachys'* nutlets features from south-west Spain have been made. Morphological and anatomical studies have shown that these taxa can be differentiated on the basis of nutlet features. Nutlets of different taxa are described, illustrated and compared. A key to distinguish the different taxa is provided.

Keywords: *Stachys* nutlets - morphology - anatomy - seed-coat – SEM – pericarp - sclerenchymatal cell-layer.

## Introduction

The genus *Stachys* L., is mainly distributed in warm temperate areas of the Mediterranean and south-west Asia, with secondary centres in north and south America and southern Africa (Ball 1972). In the south-west of Spain this genus is represented by *S. officinalis* (L.) Trevisan, *S. germanica* L. subsp. *cordigera* Briq., *S. circinata* L'Hér., *S. ocymastrum* (L.) Briq. and *S. arvensis* (L.) L. (Ubera 1987).

In agreement with Battacharjee (1980), there is little knowledge about speciation, evolutionary tendencies, relationships and biogeography of this genus. The same author indicates the variability of this genus and the numerous infrageneric classifications that have resulted (Dumortier 1827; Reichenbach 1830; Bentham 1834; Boissier 1879 sec. Battacharjee 1980; Briquet 1895 sec. Battacharjee 1980). Ball (1972) subdivided the genus in four sections: *Betonica* (L.) Bentham, *Eriostomum* (Hoffmanns. & Link) Dumort., *Stachys* and *Olisia* Dumort. According to Battacharjee (1980), the genus *Stachys,* is

divided into two subgenera: *Stachys* and *Betonica*, with 21 sections and numerous subsections.

The genus comprises annual or perennial herbs with petiolate basal leaves and sessile caulinar leaves. Inflorescence with verticillasters condensed together or lax spike like. Calyx campanulate, subactinomorphic, with 5 veins. Corola 2-lipped: posterior lip flat or concave, entire or emarginate; the anterior lip with a big central convex lobe and two small lateral lobes. Four parallel stamens and anthers with thecae dehiscent by a common opening. Most species grow in rocky place, mainly on limestone and other basic rocks. Rocks habitats have evidently had an influence on the evolution of general habit and life form (BATTACHARJEE 1980).

*Stachys* has been the subject of several recent studies. Thus, MARIN (1992) studied the fatty acids of the subfamily Lamioideae (Stachyoideae) nutlets; DEMISSEW & HARLEY (1992) studied the nutlet morphology, trichomes and pollen of this genus in Africa tropical, underlining the systematic importance of these characters. Moreover FALCIANI (1995, 1997) carried out respectively studies on trichomes in Italian species included in *S. germanica* group and a revision of the section *Eriostomum* also in Italian taxa. As soon as Lamiaceae anatomy, WAGNER (1914) and WOJCIECHOWSKA (1966) give very interesting data about nutlets of the *Stachys* genus.

This paper reports results of an anatomical and morphological study of the nutlets of the *Stachys* from south-west Spain.

## Material and Methods

Material used for this study was collected from 30 wild populations belonging to 5 taxa. Collectors and localities are shown in the Appendix. Voucher specimens of the plants are deposited in the Herbarium of the Departamento de Biología Vegetal y Ecología de la Universidad de Sevilla (SEV).

The nutlets were fixed in FAA at least for 48h and later transferred to 70% ethanol for storage.

The length and width data are based on a sampling from 120-240 nutlets per taxon.

For scaning electron microscopic observation, nutlets were dehydrated in an ethanol series and then treated with xylene for several days. After drying, samples were mounted on stubs using double-sided adhesive and coated with gold-palladium in a sputter coater. Observations were made in a Philips LX-20 Autoscan SEM.

For anatomical investigation, mature nutlets of FAA-preserved material were rinsed in 70% ethanol prior to dehydration. Dehydration and embedding were carried out using the tertiary butyl alcohol method (JOHANSEN 1940), infiltrated with paraffin and sectioned at a thickness of 7 - 10 μm. The sections were then attached to glass slides, stained with safranin (1%) and fast-green (0.1%) solutions and permanently mounted.

The terminology basically follows STEARN (1992) and FONT QUER (1993).

## Results

The nutlets in all examined taxa are woody. They show a small ventral hilum prolongated on a longitudinal fold to spread 2/3 of the ventral middle line length (Figs 2, 9, 17 and 21), except in *S. circinata* where this is extending all along one (Fig. 14). In this fold, commonly, the cells are not differentiable, only *S. officinalis* presents thin elongated rectangular cells arranged in parallel files.

The nutlets are matt and with dorsiventral symmetry in most of taxa, only in *S. officinalis* they are asymmetric. Hairs are not frequent, only *S. officinalis* sometimes, shows eglandular hairs at the apex (Figs 6 & 7). Also, in *S. officinalis*, *S. germanica* subsp. *cordigera* and *S. circinata* (Figs 4 & 19) some papillae have been observed. In the insertion area appears a great quantity of sphaerical waxes.

The epicarp is formed by irregular and radially elongate cells, with thin walls and normaly thick tangential ones. The mesocarp shows two different regions. The outer region of mesocarp is made up of more or less rectangular cells with thin walls. The inner region of mesocarp is formed by a sclerenchymatal cell-layer with a width lumen that contains a calcium oxalate crystal in the upper area. The endocarp is constituted by a layer of cells (from isodiametric to slightly elongate tangential) with thin walls. In some cases the inner tangential ones appear very thickened (Figs 25 – 29).

Seed-coat is formed by 1 or 2 cell-layers with a thickened from 2 to 5 μm. The inner layer, when appears, is colourless and formed by more or less rectangular cells with thin walls. Endosperm is constituted by more or less polygonal cells with a variable size which contain egg-shaped to almost sphaerical starch grains and it is located around the embryo. The embryo is straight, sometimes, slightly curved.

### *Stachys officinalis*

Nutlets (2.2) 2.9 - 4.1 x 1.4 - 2.2 mm (length x width). Oblong, slightly trigonous and more or less compressed (Figs 1 & 2). They have irregularly cristate apex and obtuse base. Hilum subcircular to triangular in outline. Colour brown-greyish. Nutlet surface smooth formed by oblong cells very variable in size with obscure radial walls and smooth tangential ones (often collapsed) (Fig. 3). In the ventral side, there are light thickened like small pustules with an irregularly catened disposition and with scattered pits (Fig. 5). Towards the apex eglandular hairs (135 - 200 μm long) of 1 - 4 cells can appear, with or without papillae with variable distribution and abundance; sometimes only papillae have been observed (Figs 4, 6 and 7).

Pericarp 88 - 90 μm thick. Epicarp 35 - 36 μm thick, with cuticle 2 - 3 μm thick. Outer region of mesocarp 28 - 30 μm thick, composed of 3 - 4 layers of more or less rectangular cells with sometimes little crystals inside (druse). Inner region shows a sclerenchymatal cell-layer (around 23 μm thick) with a calcium oxalate crystal from 7 to 12 μm of diameter. Endocarp 4 - 5 μm thick, with a layer formed

by slightly tangentially elongate cells with thin walls except for tangential inner one (Fig. 25).

Seed-coat around 5 μm, formed by a layer of more or less rectangular cells with thickened radial walls and thin tangential ones (Fig. 25).

### *Stachys germanica* subsp. *cordigera*

Nutlets 2.4 - 3.6 x 1.5 - 2.9 mm. From subellipsoid to ellipsoid (Figs 8 & 9). They have rounded apex and acute base. Hilum more or less elliptical in outline. Colour brown-greyish. Nutlet surface smooth formed by polygonal cells with obscure radial walls and smooth tangential ones (Fig. 12). At the dorsal side appear some small papillae with an irregular distribution.

Pericarp 110 - 115 μm thick. Epicarp 40 - 43 μm thick, with cuticle 4 - 7 μm thick. Outer region of mesocarp around 30 μm thick, composed of 4 - 6 layers of polygonal cells, some rectangular, in which cytoplasm can appear small crystals (druse). Inner region shows a sclerenchymatal cell-layer (around 38 μm thick) with a calcium oxalate crystal around 6 μm of diameter. Endocarp 9 - 10 μm thick, formed by a layer of cells from isodiametric to rectangular with a double membrane (Fig. 26).

Seed-coat from 3 to 4 μm thick, formed by a layer of tangentially elongate cells with thickened radial walls and thin tangential ones (Fig. 26).

### *Stachys circinata*

Nutlets 2.0 - 3.1 x 1.6 - 2.5 mm. From broadly ovoid to suborbicular (Figs 13 & 14). They have from slightly acute to rounded apex and emarginate base. Hilum circular or triangular in outline. Colour from dark brown to black. Nutlet surface smooth formed by polygonal cells with obscure radial walls and smooth tangential ones (Fig. 15). In the dorsal side appear papillae with a variable size (2 - 10 μm) irregularly distributed. Also can be observed at whole the surface scattered pits, generally two by cell.

Pericarp 94 - 96 μm thick. Epicarp around 50 μm thick, with cuticle 1 - 3 μm thick. Outer region of mesocarp 16 - 19 μm thick, composed of 2 - 3 layers of more or less rectangular cells. Inner region shows a sclerenchymatal cell-layer of 20 - 21 μm thick with a calcium oxalate crystal from 6 to 9 μm of diameter. Endocarp 5 - 6 μm thick, with an only layer constituted by rectangular cells (Fig. 27).

Seed-coat from 2 to 4 μm thick formed by a layer of more or less rectangular cells with thin walls (Fig. 27).

### *Stachys ocymastrum*

Nutlets 1.1 - 1.6 x 0.7 - 1.2 mm. From obovoid to pyriforms (Figs 16 & 17). They have rounded apex and acute base. Hilum from circular to slightly elliptic in

outline. Colour dark brown with clearer spots. Nutlet surface with small tubercles, sometimes continuous, from 18 to 50 μm long, formed by polygonal cells with almost obscure radial walls and rugulate tangential ones. In the union between cells, sometimes appear groups of papillae (3 - 4) from 7 to 8 μm of diameter (Fig. 19). Also can be observed at whole the surface scattered pits, generally in a variable number.

Pericarp 87 - 91 μm thick. Epicarp around 20 μm, constituted by rectangular cells with very variable size and thickness. Generally, in the biggest size cells can be observed grooves parallel to surface, with cuticle 1 - 2 μm thick. Outer region of mesocarp 10 - 14 μm thick, composed of 2 - 3 layers of rectangular cells with variable size. Inner region shows a sclerenchymatal cell-layer (around 37 μm thick) with a calcium oxalate crystal from 6 to 11 μm of diameter. Endocarp 2 - 3 μm thick, with an only layer constituted by oblong cells with the inner wall very thick (Fig. 28).

Seed-coat from 3 to 5 μm thick, constituted by two layers: the outer one 1 - 3 μm thick, formed by from isodiametric to rectangular cells, with radial walls very thick, the inner one around 2 μm thick, formed by more or less rectangular cells with thin walls (Fig. 28).

### *Stachys arvensis*

Nutlets 1.1 - 1.4 x 0.6 - 1.1 mm. From obovoid to pyriforms (Figs 20 & 21). They have rounded apex and acute base. Hilum from circular to slightly elliptic in outline. Colour dark-brown. Nutlet surface with small tubercles, sometimes continuous from 25 to 225 μm long, formed by polygonal cells with obscure radial walls and warty (sometimes smooth) tangential ones (Figs 22 - 24).

Pericarp 48 - 50 μm thick. Epicarp 1 - 24 μm thick, constituted by radially elongate cells with very variable size show it in the external morphology, with cuticle 1 - 3 μm thick. Outer region of mesocarp 4 - 7 μm thick, composed by 1 - 2 layers of rectangular cells. Inner region shows a sclerenchymatal cell-layer 20 - 21 μm thick, with a calcium oxalate crystal from 5 to 6 μm of diameter. Endocarp 3 - 4 μm thick, with an only layer constituted by rectangular cells (Fig. 29).

Seed-coat from 3 to 4 μm thick, constituted by two layers: the outer one 2 - 3 μm thick, formed by tangentially elongate cells with radial walls very thick, the inner one around 1 μm thick, formed by more or less rectangular cells with thin walls (Fig. 29).

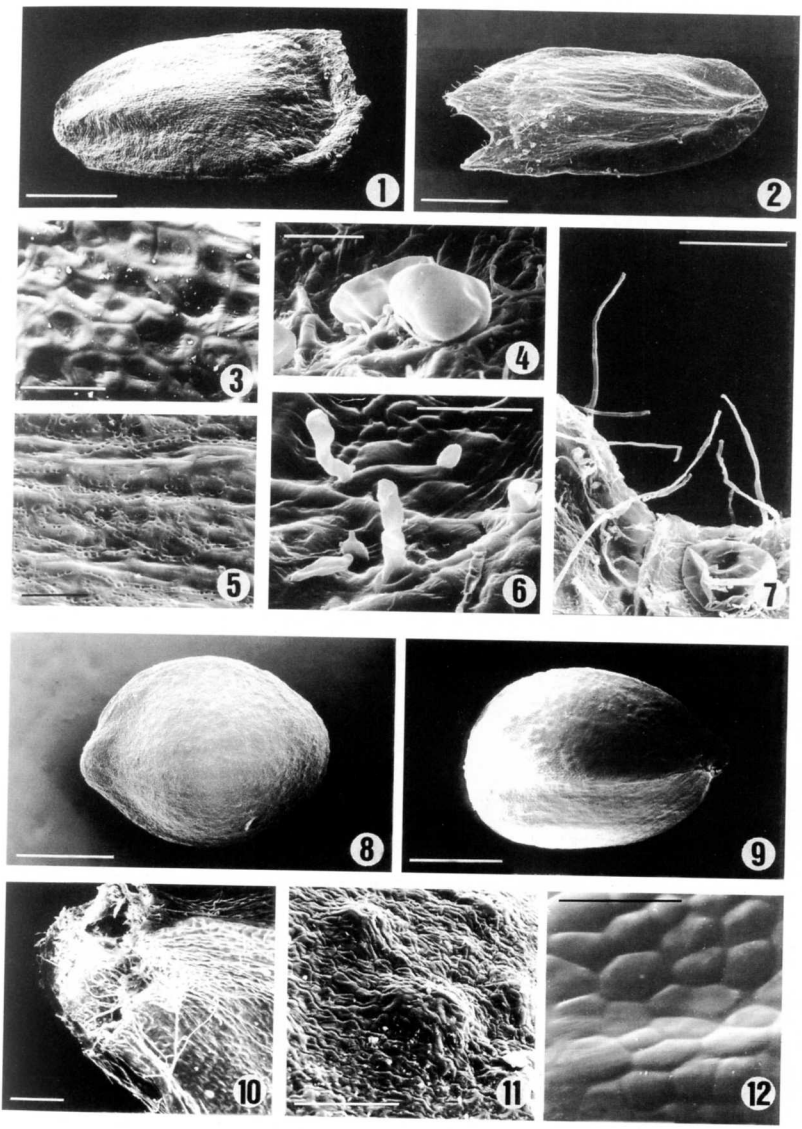
## Discussion

Nutlets convey sufficient information to allow identification of the taxa in the study area.

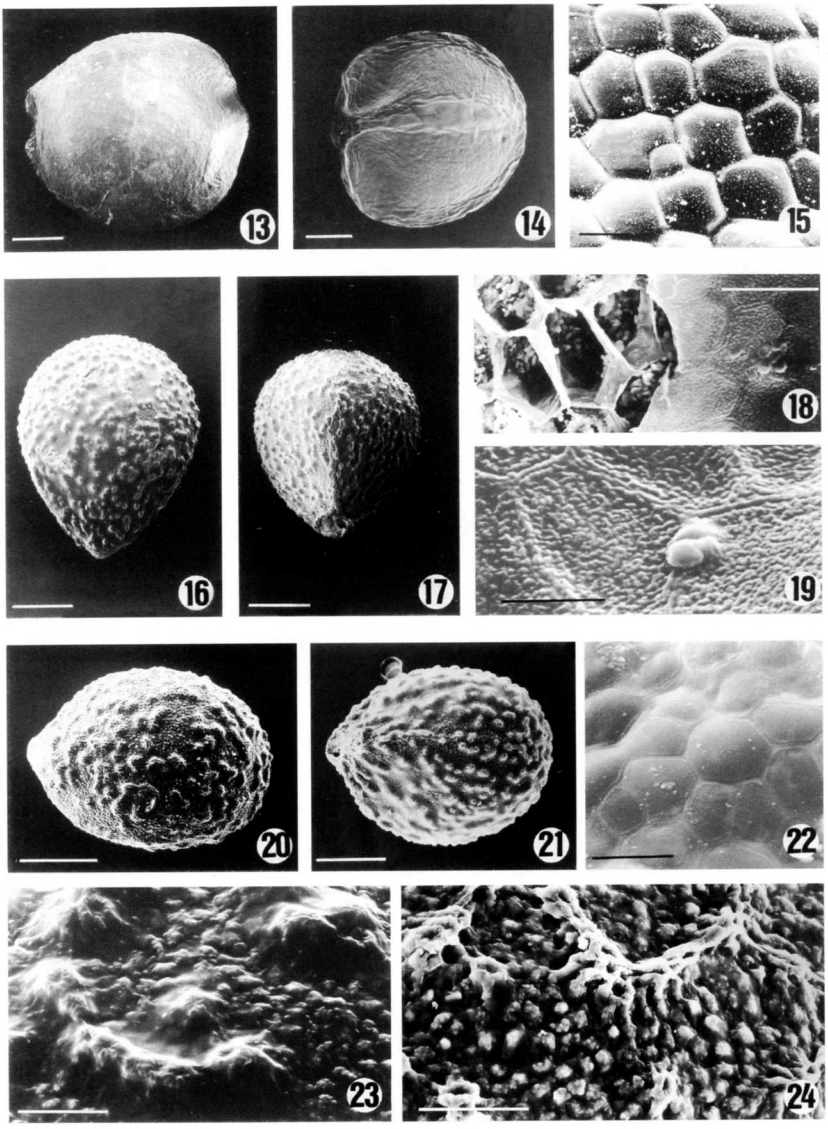
Attending to morphological characters, DEMISSEW & HARLEY (1992) used the reticulate pattern of the nutlet surface as essential character to differentiate the

*Stachys'* species present in tropical Africa, thus, they established three groups. However, in this study, the observations have been made on cell micro-characters which shape that reticulate pattern, using them to distinguish the different species studied. Thus, *S. officinalis* is different to the other species because the nutlets show a irregularly cristate apex and it's the only one which has eglandular hairs. This species is included in sect. *Betonica* by some authors as HEGI (1964), BALL (1972), ZANGHERI (1976) or FOUNIER (1977). However, others authors as CLAPHAM et al. (1962), based on several characters such as leaves well-marked basal rossette or upper lip of corolla nearly flat, consider this taxon belinging to genus *Betonica* L. In this work, in agreement with most authors is considered within sect. *Betonica*. In the rest of species which have a rounded apex, there are two groups: the first one is formed by *S. germanica* subsp. *cordigera* and *S. circinata* included in the sects. *Eriostomum* and *Stachys* respectly, both with smooth nutlets and the second one with *S. ocymastrum* and *S. arvensis* belonging to sect. *Olisia*, has small tubercles on the surface. In the first group, *S. circinata* and *S. germanica* subsp. *cordigera* are differentiated by the presence or absence of papillae, respectively. In the second group *S. ocymastrum* and *S. arvensis* are distinguished by the outer tangencial wall; rugulate in *S. ocymastrum* and warty (sometimes smooth) in *S. arvensis*.

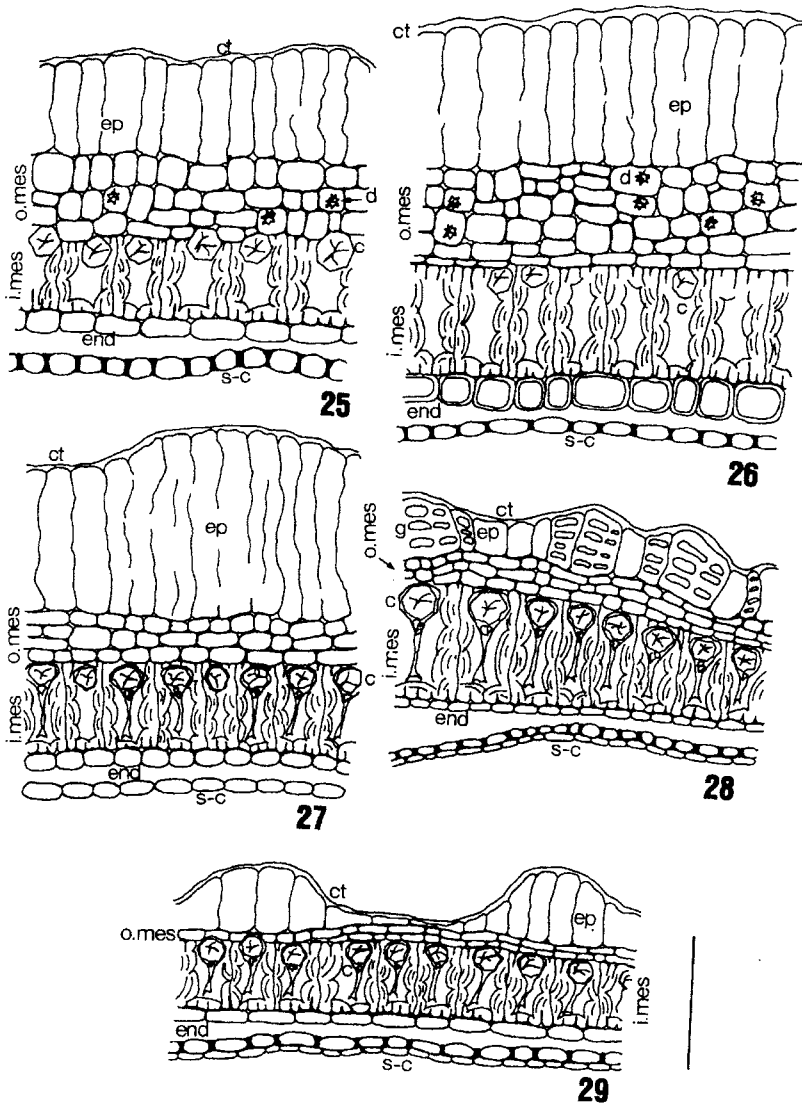
Anatomical characters, in particular the mesocarp, can be used to identify the examined species. It has been observed that the internal region, also known as sclerenchymatal cell-layer, is very constant on a intraspecific level and gives a good character to differentiate the species. So, two groups can be differentiated; one constituted by *S. officinalis*, *S. circinata* and *S. arvensis* with a sclerenchymatal cell-layer that doesn't exceed 23 µm thick, and the other integrated by *S. germanica* subsp. *cordigera* and *S. ocymastrum* with a sclerenchymatal cell-layer's thickness of 37 - 38 µm. In this layer there are crystals which morphology and volume can be related with the size and shape of the cell lumen in which the crystal has been formed. Thus, the crystals that appear in the outer region of mesocarp (*S. officinalis* and *S. germanica* subsp. *cordigera*) are smaller and more irregular than those which originated in the inner region and they can be included in the "Type II" proposed by RYDING (1992) in his study of the Nepetoideae subfamily (Tribe Ocimeae). In this type, the author includes principally smooth crystals. In all taxa studied, the endocarp shows only one cell-layer, although in *S. germanica* subsp. *cordigera* is very characteristic by the presence of cells with double membrane. With respect to the epicarp also has been useful in some species as *S. ocymastrum* by its characteristics grooves or *S. arvensis*, which shows crests and lobulations constituting a reflect of its external morphology. In all cases, the presence of a cuticle has been observed, although it's very important in *S. germanica* because it can to reach 7 µm thick and, sometimes, can difficult the visualization of the epidermal cells. In none of the species studied, it has been observed mucilaginous cells in the epicarp, a notable characteristic in some genera of Lamiaceae. This absence of mucilage seems to be a characteristic of Lamioideae (Stachyoideae) tribe, like WOJCIECHOWSKA (1966) and GRUBERT (1974) made evident before.




Figures 1 - 12. SEM of *Stachys* nutlets. Figs 1 - 7. *S. officinalis*. Figs 8 - 12. *S. germanica* subsp. *cordigera*. Figs 1 & 8. Whole nutlet, dorsal face. Figs 2 & 9. Whole nutlet, ventral face. Figs 3, 5, 11 & 12. Detail of nutlet surface. Figs 4, 6 & 7. Detail of hairs. Fig. 10. Detail of insertion area. In Figs 1, 2, 8, 9, scale bars = 1 mm. In Figs 7, 10, 11, scale bars = 100 μm. In Figs 3, 4, 5, 12, scale bars = 50 μm. In Fig. 6, scale bar = 20 μm.




Figures 13 - 24. SEM of *Stachys* nutlets. Figs 13 - 15. *S. circinata.* Figs 16 - 19. *S. ocymastrum.* Figs 20 - 24. *S. arvensis.* Figs 13, 16 & 20. Whole nutlet, dorsal face. Figs 14, 17 & 21. Whole nutlet, ventral face. Figs 15, 18, 19, 22, 23 & 24. Detail of nutlet surface. In Figs 13, 14, 16, 17, 20, 21, scale bars = 500 μm. In Figs 23, 24, scale bars = 100 μm. In Figs 15, 18, 22, scale bars = 25 μm. In Fig. 19, scale bar = 10 μm.




Figures 25 - 29. Drawings of *Stachys* pericarp and seed-coat anatomy. Fig. 1. *S. officinalis*. Fig. 2. *S. germanica* subsp. *cordigera*. Fig. 3. *S. circinata*. Fig. 4. *S. ocymastrum*. Fig. 5. *S. arvensis*. Scale bar = 100 μm, ct, cuticle; ep, epicarp; o.mes, outer mesocarp; c, oxalate calcium crystal; d, druse; i. mes., inner mesocarp; end, endocarp; s-c, seed-coat; g, grooves.

At last, the bearing of this plants together the presence of erect calyces seems to indicate an anemobalistic dispersion as in other groups like Caryophyllaceae and some species of *Scrophularia* or *Antirrhinum* (PIJL 1972). BOUMAN & MEEUSE (1992), in their study about dispersal in Lamiaceae, include *Stachys* in the ballistic dispersion group, where as dispersal agents can act, the rain, the wind or even animals. Nevertheless, these authors indicate that *Stachys*, among others, shows an evidently anemobalistic dispersal and that this system is more effective when the fruiting branches are thin and stick out of leaves like take place in the species of *Betonica* section.

## Key to species from south-west Spain based on nutlets features

**1a.** Surface with small tubercles. Nutlets < 2 mm long ..... 2
**1b.** Surface smooth. Nutlets ≥ 2 mm long ..... 3
**2a.** Sclerenchymatal cell-layer > 30 µm thick. Epicarp with grooves. Outer tangential wall rugulate ..... *S. ocymastrum*
**2b.** Sclerenchymatal cell-layer ≤ 25 µm. Epicarp without grooves. Outer tangential wall warty, sometimes smooth ..... *S. arvensis*
**3a.** Apex irregularly cristate. Surface with oblong cells ..... *S. officinalis*
**3b.** Apex obtuse. Surface with polygonal cells ..... 4
**4a.** Sclerenchymatal cell-layer ≤ 25 µm thick. Outer region of mesocarp without crystals. Papillae present ..... *S. circinata*
**4b.** Sclerenchymatal cell-layer > 30 µm thick. Outer region of mesocarp with crystals. Papillae absent ..... *S. germanica* subsp. *cordigera*

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## Appendix

*S. arvensis.* SPAIN: Cádiz, Los Barrios, 4.III.96, *Garrido, Hidalgo & Martín Mosquero,* SEV 153297 and SEV 153298. Córdoba, between Espejo and Montilla, 21.III.96, *Martín Mosquero & Ocaña,* SEV 153317, La Rambla: joined farming, 21.III.96, *Martín Mosquero & Ocaña,* SEV 153314. Sevilla, La Corchuela, 29.II.96, *Juan, Martín Mosquero & Ocaña,* SEV 153290, Espartinas: in olive plantation, 4.III.96, *Juan, Martín Mosquero & Ocaña,* SEV 153294.

*S. circinata.* SPAIN: Cádiz, between Coripe and Olvera: Peñón de Zaframagón, 10.VI.96, *Martín Mosquero & Ocaña,* SEV 153393, Algodonales, 10.VI.96, *Martín Mosquero & Ocaña,* SEV 153401, between Alcalá de los Gazules and Paterna, 17.VI.96, *Martín Mosquero & Ocaña,* SEV 153429, Grazalema, 26.VI.96, *Martín Mosquero & Ocaña,* SEV 153489. Córboba, Sª de Rute, 25.VI.96, *Martín Mosquero & Ocaña,* SEV 153465, between Priego de Córdoba and Rute: "Loma de las Chozas", 30.VI.97, *Carmona & Martín Mosquero,* SEV 153908.

*S. germánica* subsp. *cordigera.* SPAIN: Cádiz, Crossroads of Alcalá de los Gazules, 4.VI.96, *Juan & Martín Mosquero,* SEV 153375, Sª de Algeciras, 4.VI.96, *Fernández, Juan & Martín Mosquero,* SEV 153386, Grazalema: Ascent of the Pto de las Palomas, 26.VI.96, *Fernández, Juan & Pastor,* SEV 153481. Córdoba, Rute: "Fuente Alta", 25.VI.96, *Martín Mosquero & Ocaña,* SEV

153468. Huelva, Galaroza; on the banks of Múrtiga river, 11.VII.96, *Martín Mosquero* & *Ocaña,* SEV 153589. Sevilla, Cazalla de la Sierra, 3.VII.96, *Martín Mosquero* & *Pastor,* SEV 153561.

*S. ocymastrum.* SPAIN: Cádiz, between El Bosque and Prado del Rey, 22.V.96, *Martín Mosquero* & *Ocaña,* SEV 153882, Puerto de Santa María; direction to "Laguna Salada", 31.V.96, *Martín Mosquero* & *Ocaña,* SEV 153361, between Medina Sidonia and Algeciras, 4.VI.96, *Fernández, Juan* & *Martín Mosquero,* SEV 153377. Córdoba, between La Victoria and La Guijarrosa: joined to a stream, 15.V.96, *Martín Mosquero* & *Ocaña,* SEV 153343. Huelva, between Hinojos and Almonte, 19.V.96, *Martín Mosquero,* SEV 153344. Sevilla, Sevilla-Cádiz road, 29.IV.96, *Díaz* & *Martín Mosquero,* SEV 153337.

*S. officinalis.* SPAIN: Cádiz, Algeciras: Sª del Bujeo, 17.VII.96, *Garrido* & *Martín Mosquero,* SEV 153541. Algeciras: "Arroyo de la Miel", 1.VIII.96, *Martín Mosquero,* SEV 153657. Huelva, Arroyo de la Rocina. "Paso del Fresnillo", 8.VIII.97, *Martín Mosquero* & *Pérez Porras,* SEV 153933.

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