

# Comparative Morphological, Anatomical, and Palynological Studies on the Genus *Stachys* L. sect. *Ambleia* Bentham (Lamiaceae) Species in Turkey

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**Abstract:** In this study, comparative morphological, anatomical and palynological studies of *Stachys* L. sect. *Ambleia* Bentham species (Lamiaceae) in Turkey are carried out on the plants collected from their type localities. These species are *Stachys cydni* Kotschy ex Gemici & Leblebici and *S. yildirimlii* M. Dinç, which are highly local endemics. Morphologically, micromorphological features of the trichomes are studied. Trichome morphology appears to have a taxonomic value with respect to the results. The anatomical studies on cross-sections of stems, leaves, and surface sections of leaves are presented. According to the anatomical comparison between the 2 species, the pith structure of the stems, the cuticle thickness of the leaves, and stomatal distribution on the leaves are the distinguishing features of the species. Scanning electron microscope studies on the pollen grains show that exine ornamentation is clearly different in the 2 species. Whereas the pollen grains of *S. yildirimlii* have a retipilate exine ornamentation, those of *S. cydni* have a reticulate ornamentation.

**Key Words:** *Stachys*, *Ambleia*, anatomy, micromorphology, palynology, taxonomy, Lamiaceae, Turkey

## Türkiye *Stachys* L. Cinsi *Ambleia* Bentham Seksiyonu Türleri Üzerine Karşılaştırmalı Morfolojik, Anatomik ve Palinolojik Çalışmalar

**Özet:** Bu çalışmada, tip lokalitelerinden toplanan örnekler üzerinde, Türkiye *Stachys* L. cinsi *Ambleia* Bentham seksiyonu türleri üzerine karşılaştırmalı morfolojik, anatomik ve palinolojik çalışmalar gerçekleştirilmiştir. Bu türler, oldukça lokal endemik olan *Stachys cydni* Kotschy ex Gemici & Leblebici ve *S. yildirimlii* M. Dinç'dir. Morfolojik olarak tüylerin mikromorfolojik özellikleri çalışılmıştır. Çalışma sonuçlarına göre, tüy morfolojisinin taksonomik değeri olduğu gözükmektedir. Gövde ve yapraklardan alınan enine kesitler ile yapraklardan alınan yüzeysel kesitler üzerine yapılan anatomik çalışmalar sunulmuştur. Bu iki tür üzerinde yapılan anatomik karşılaştırmaya göre, gövdedeki öz bölgesinin yapısı, yapraklardaki kutikula kalınlıkları ve stomaların dağılımı türleri ayırıcı özelliklerdir. Polen taneleri ile yapılan taramalı elektron mikroskobu çalışmaları, ekzin süslerinin bu türde açıkça farklı olduğunu göstermektedir. *S. yildirimlii* polenleri retipilat ekzin süslerine sahip iken, *S. cydni* polenleri retikulat ekzin süslerine sahiptir.

**Anahtar Sözcükler:** *Stachys*, *Ambleia*, anatomi, mikromorfoloji, palinoloji, taksonomi, Lamiaceae, Türkiye

## Introduction

*Stachys* L., one of the largest genera of the Lamiaceae, is a subcosmopolitan genus centred in the warm temperate regions of the Mediterranean and Southwest Asia, with secondary centres in North and South America and southern Africa, and contains about 300 species (Heywood, 1993; Hickey & King, 1997). With 81 species, Turkey is one of the richest countries in the world in *Stachys* diversity. Sect. *Ambleia* is represented in

Turkey by only 2 local endemic species, *S. cydni* Kotschy ex Gemici & Leblebici and *S. yildirimlii* M. Dinç (Bhattacharjee, 1982; Davis *et al.*, 1988; Gemici & Leblebici, 1998; Duman, 2000; Dinç & Doğan, 2006).

There are many anatomical studies on the family Lamiaceae in Turkey (Kaya *et al.*, 2000; Kandemir, 2003; Uysal, 2002, 2003; Erken, 2005). However some of them are related with the *Stachys* species, there has been no investigation of sect. *Ambleia* species as yet.

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Investigations of pollen morphology in Lamiaceae have been essential as an aid to classification within this family (Abu-Assab & Cantino, 1994). However, the pollen morphology of the genus *Stachys* is not well known, and those of some Turkish *Stachys* taxa have been given by Moore et al. (1991) and Abu-Assab & Cantino (1994).

The taxonomic value of the indumentum and its importance in systematic and phylogenetic relationships are well known in Lamiaceae (Abu-Assab & Cantino, 1987). Trichomes are among the most useful taxonomic characters in some genera of the family. Their absence or presence and their typology can be used as taxonomic markers in the infrageneric classification of the genus *Teucrium* L., while the infrasectional classification of sect. *Polium* (Mill.) Schreb. is based almost totally on the typology of the trichomes (Navarro & El Oualidi, 2000). Trichome morphology is an essential taxonomic character for delimitation of the sections *Amblesia* Bentham and *Zietenia* (Gled.) Bentham in the genus *Stachys* as well. The section *Amblesia* is characterized by dendroid indumentum and it is isolated from other sections of *Stachys* by this feature (Bhattacharjee, 1980, 1982).

Although general morphological features of the 2 species have been previously given (Gemici & Leblebici, 1998; Dinç & Doğan, 2006), taxonomic value of trichome morphology for interspecific classification in the section *Amblesia* has been emphasized neither in these studies nor in the other studies on this section (Bhattacharjee, 1980, 1982; Rechinger, 1982). Here, we report on the comparison of the trichome morphologies of the 2 species. The aim of this paper is also to present the anatomical and palynological features of the 2 species and to discuss their taxonomic values.

## Materials and Methods

Plant samples of the 2 species were collected from their type localities. The specimens are dried according to standard herbarium techniques and stored at KNYA and GAZI herbaria. The collecting localities of the species:

*S. yildirimlii*. C5 Mersin: Aslanköy, Cocakdere, Şahinlik Tepe, rocky places, 1950 m, 12.VI.2005, M.Dinç 2336 & H.H. Doğan (KNYA).

*S. cydni*. C5 Mersin: Aslanköy, Cocakdere, Şahinkayası mevkii, *Pinus nigra* forest, clearings, rocky slopes, 1500 m, 08.VI.2003, M.Dinç 1794 & H.H. Doğan (GAZI); C5

Mersin: Çamlıyayla, Payam, Manastır area, limestone rocks, 1700 m, 12.VII.2004, M.Dinç 2188 & H.H. Doğan (KNYA); C5 Mersin: Aslanköy; Cocakdere, *Pinus nigra* forest, clearings, rocky slopes, crevices of limestone rocks, 1700 m, 14.VII.2004, M. Dinç 2275 & H.H. Doğan (KNYA).

These specimens were used for morphological and palynological studies. For the morphological studies, at least 20 individuals of each species were investigated. All measurements were determined on 30 trichomes for each species.

Some plant samples belonging to the 2 species (M.Dinç 2336 & H.H.Doğan, M. Dinç 2275 & H.H. Doğan) were fixed in 70% alcohol. Anatomical investigations were performed on the cross-sections of the herbaceous stems, woody stems and leaves, and the surface sections of leaves. The cross sections were painted with basic fuchsine and covered with glycerin-gelatin (Vardar, 1987). Their photographs were taken with an Olympus BX-50 microscope. The stomatal index and stomatal index rate were calculated as described by Meidner & Mansfield (1968). Similar features of *S. cydni* and *S. yildirimlii* were not emphasized during the presentation of the anatomical results.

Palynological investigations were made by both light microscope and scanning electron microscope. For light microscope studies, the pollen slides were prepared according to the Wodehouse (1935) technique. Pollen grains were dissected from herbarium specimens and placed on a clean microscope slides. Glycerin-gelatin with basic fuchsine was placed on pollens and allowed to melt and mixed by a clean pin to get scattered pollen grains. All measurements were determined on at least 30 pollen grains. The pollen grains were also directly placed on prepared stubs and covered with gold for SEM studies. Photographs were taken with SEM. Pollen terminology followed Erdtman (1952) and Punt et al. (1994).

## Results

### I- Trichome Morphology

General appearance of *S. yildirimlii* and *S. cydni* are presented in Figures 1 and 2. As seen in the figures, they are morphologically distinguished. These taxa differed in several morphological traits, especially in leaf shape and in patterns of indument. The trichomes of the 2 species



Figures 1 and 2. General appearance of *Stachys* sect. *Ambleia* species in Turkey: 1. *S. yildirimlilii* 2. *S. cydni*.

are dendroid. *S. yildirimlilii* has a greyish-green appearance throughout because of its sparser trichomes. The trichomes are 0.4-0.6 mm long and 6-10-branched. Its branches are 1-3-celled (Table 1; Figure 3).

Except for the upper surfaces of the leaves, *S. cydni* has a greyish-white appearance owing to densely felted trichomes. The trichomes are 0.6-0.8 mm long and 14-20-branched. Its branches are always 1-celled in *S. cydni* (Table 1, Figure 4).

## II- Anatomical Properties

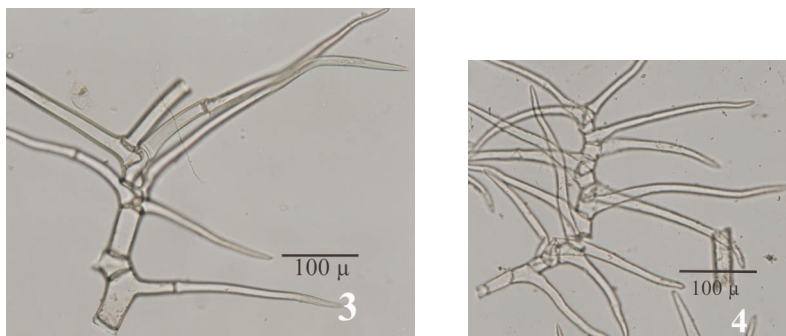
### Herbaceous Stem

The transverse section of the herbaceous stem is rectangle shaped. The epidermis consists of single layer rectangular cells, and is surrounded by a cuticle layer. There are multi-cellular dendroid hairs on the epidermis. Underneath the epidermis, there is collenchyma with single layered cells between the corners, but 6-7 layers of collenchyma can be seen below the epidermis at the corner of the stem. The shape of collenchyma cells is ovoid. Along the stem radius, the cortex (20-40  $\mu$ ) consists of 3-4 layers of oval and rectangular cells. The vascular bundles are of collateral type and surrounded by 1-3 layers of sclerenchyma fibres. The vascular bundles at the corners are larger than the others. Cambium is distinguishable in *S. yildirimlilii*, but not in *S. cydni*. Phloem and xylem members are clear. In the large vascular bundles, there is a parenchyma with 3-5 layered oval and

rectangular cells. In *S. cydni*, the pith is hollow in the centre and the outer part consists of deformed cells, whereas in *S. yildirimlilii*, the pith is completely filled with large orbicular parenchymatic cells (Table 1, Figures 5 and 6).

### Woody Stem

The transverse section of the woody stem is circular shaped. The surface is covered with periderm. During the early stages of the periderm, deformed epidermal cells and the trichomes are visible. Periderm is multilayered and its cells are flattened. The cortex is 10-15 layered and parenchymatic. Parenchymatic cells are rectangular and polygonal. There are sclerenchyma fibres between the cortex and vascular tissue. Sclerenchyma fibres form bouquets consisting of 2-8 cells in *S. cydni*, but in *S. yildirimlilii*, the sclerenchyma fibres are seen as an uninterrupted ring. Phloem elements are present under the sclerenchyma. Cambium is indistinguishable. Primary and secondary xylem can be differentiated. Tracheae in the secondary xylem are denser and larger than in the primary xylem. They are 10-30  $\mu$  in *S. yildirimlilii* and 20-50  $\mu$  in *S. cydni*. Pith rays are 1-2 layered. In *S. yildirimlilii*, the pith is completely filled with large orbicular parenchymatic cells as in its herbaceous stem. In *S. cydni*, it is hollow in the centre and the outer part consists of deformed parenchymatic cells as in its herbaceous stem (Table 1, Figures 7 and 8).



Figures 3 and 4. Trichome morphologies of the species: *Amblesia* species: 3. *S. yildirimlii* 4. *S. cydni*.

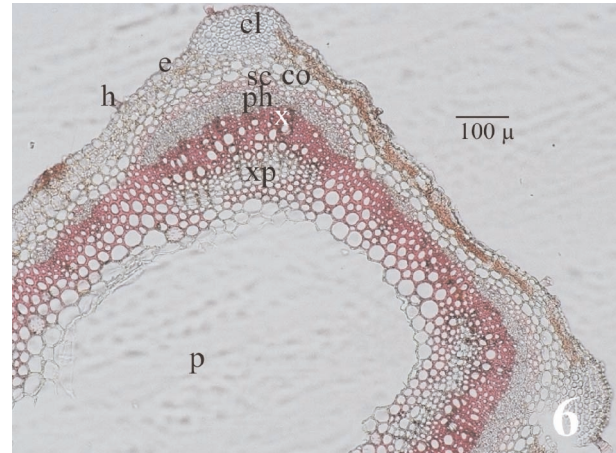
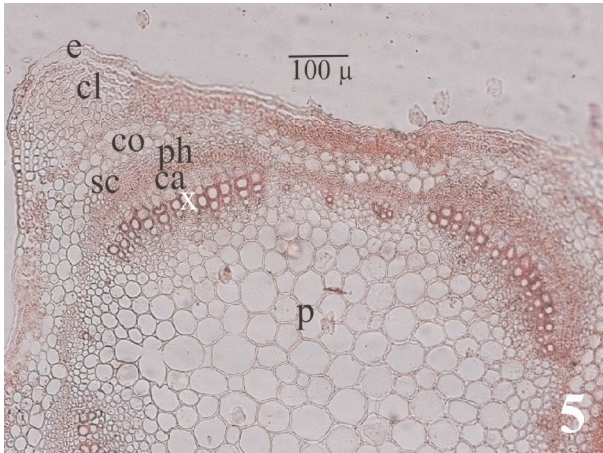
Table 1. Micromorphological and anatomical properties of *Stachys yildirimlii* and *S. cydni*.

Character	<i>Stachys yildirimlii</i>	<i>S. cydni</i>
Trichomes	0.4-0.6 mm, 6-10 branched, branches 1-3-celled	0.6-0.8 mm, 14-20 branched, branches 1-celled
Leaf	Bifacial, amphistomatic	Bifacial, hypostomatic
Stomata	Anomocytic	Anomocytic
Upper leaf surface	Cuticle 5-6 µ thickness, number of stomata 12 ± 2 and number of epidermis cells 180 ± 6 in mm <sup>2</sup> , stomata 23-25 x 18-20 µ, stomata index 6.25	Cuticle 8-10 µ thickness, lacking stoma, number of epidermis cells 360 ± 6 in mm <sup>2</sup>
Lower leaf surface	Cuticle 5-6 µ thickness, number of stomata 58 ± 4 and number of epidermis cells 143 ± 6 in mm <sup>2</sup> , stomata 19-21 x 17-19 µ, stomata index 29.2	Cuticle 6-8 µ thickness, number of stomata 78 ± 4 and number of epidermis cells 152 ± 5 in mm <sup>2</sup> , stomata 21-23 x 18-20 µ, stomata index 33.9
Herbaceous stem	Tetragonal with 6-7 layers of collenchyma and a large vascular bundle at the corners, cambium distinguishable, pith consists of large orbicular parenchymatic cells	Tetragonal with 6-7 layers of collenchyma and a large vascular bundle at the corners, cambium indistinguishable, pith hollow with deformed parenchymatic cells outside
Woody stem	Terete, phloem is surrounded by uninterrupted scleranchymatic ring, trachea of secondary xylem 10-30 µ in diameter, pith consists of large orbicular parenchymatic cells	Terete, phloem is surrounded by scleranchymatic bouquets, trachea of secondary xylem 20-50 µ in diameter, pith hollow with deformed parenchymatic cells outside

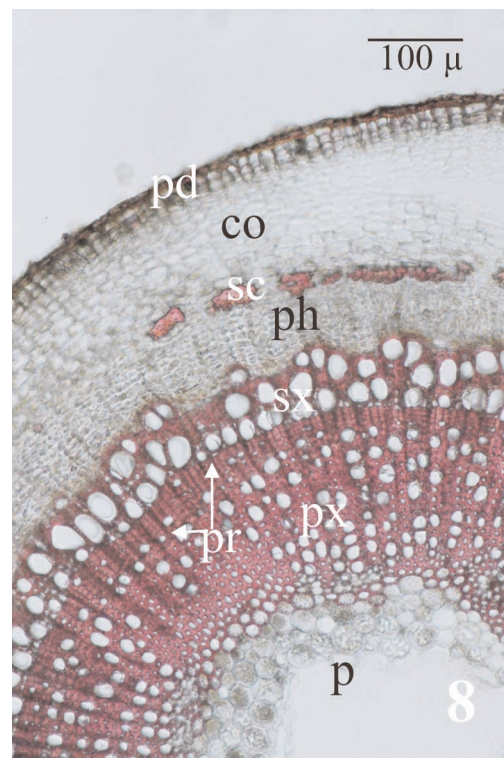
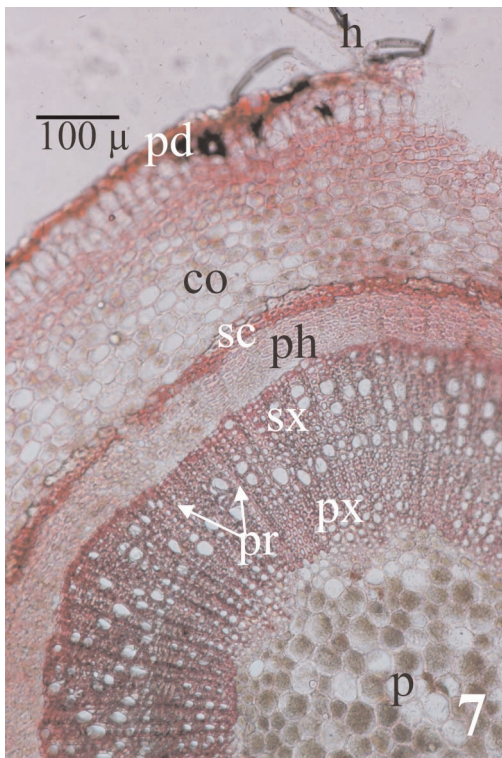
### Leaf

In transverse section, the upper and lower epidermises comprise uniseriate, oval, and rectangular cells. Both epidermises are covered with a cuticle. The upper cuticle layer is thicker than the lower one in *S. cydni*. But, the cuticle thickness is approximately the same on both epidermises in *S. yildirimlii*. There are dendroid trichomes on both epidermises. In *S. cydni*, the trichomes are fairly dense on the lower surface. In *S.*

*yildirimlii*, the density on the upper and lower surfaces is approximately the same. Mid-rib is triangle shaped and has a 4-6 layered collenchyma located below both epidermises. Vascular bundles are surrounded by a parenchymatic bundle sheath. Leaves are bifacial (dorsiventral). Palisade parenchyma cells are 2-layered under the upper epidermis. Spongy parenchyma cells are 3-4 layered under the lower epidermis (Table 1, Figures 9 and 10).



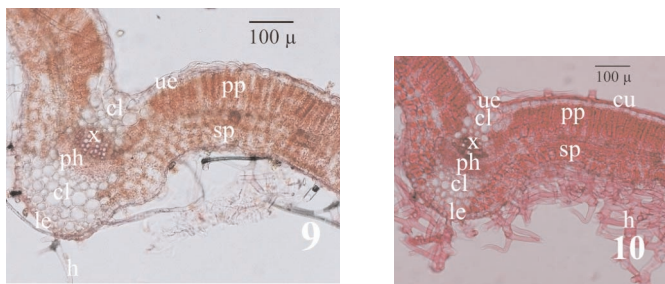
Figures 5 and 6. Transsections of herbaceous stems: 5. *S. yildirimlii* 6. *S. cydni*. e: epidermis, h: hair, cl: collenchyma, co: cortex, sc: sclerenchyma, ph: phloem, x: xylem. xp: xylem parenchyma, ca: cambium, p: pith.



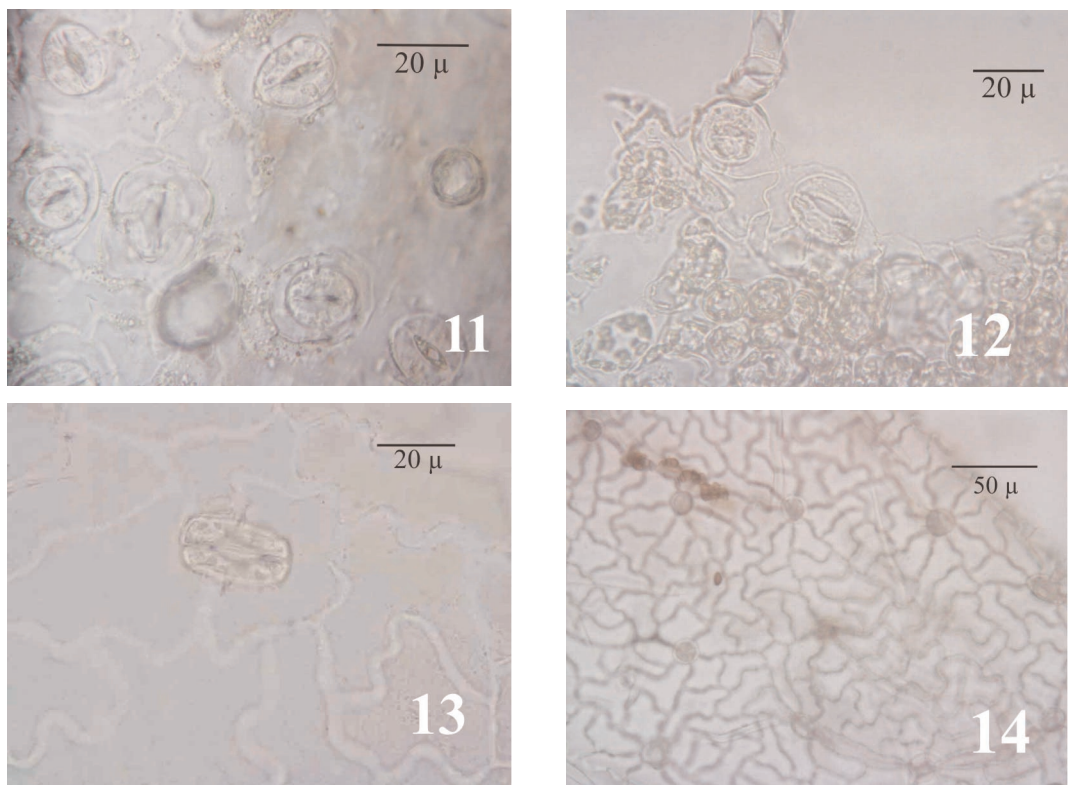
Figures 7 and 8. Transsections of woody stems: 7. *S. yildirimlii* 8. *S. cydni*. pd: peridermis, h: hair, co: cortex, sc: sclerenchyma, ph: phloem, pr: pith ray, sx: secondary xylem, px: primary xylem, p: pith.

The leaves of *S. yildirimlii* are amphistomatic with anomocytic stomata. But, upper surface has a few stomata. The number of stomata is  $12 \pm 2$  per  $\text{mm}^2$  on the upper epidermis and  $58 \pm 4$  on the lower epidermis of the leaf. The stomata index is 6.25 for the upper

epidermis and 29.2 for the lower epidermis. The leaves of *S. cydni* are hypostomatic with anomocytic stomata. The number of stomata is  $78 \pm 4$  per  $\text{mm}^2$  on the lower epidermis of the leaf. The stomata index is 33.9 for the lower epidermis (Table 1, Figures 11-14).



Figures 9 and 10. Transections of the leaves: 9. *S. yildirimlii* 10. *S. cydni*. cu: cuticle, ue: upper epidermis, le: lower epidermis, cl: collenchyma, ph: phloem, x: xylem, pp: palisade parenchyma, sp: spongy parenchyma, h: hair.



Figures 11-14. Surface sections of the leaves: 11. Lower leaf surface of *S. yildirimlii* 12. Lower leaf surface of *S. cydni*; 13. Upper leaf surface of *S. yildirimlii*; 14. Upper leaf surface of *S. cydni*.

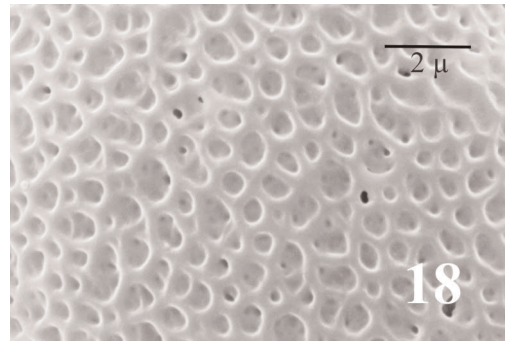
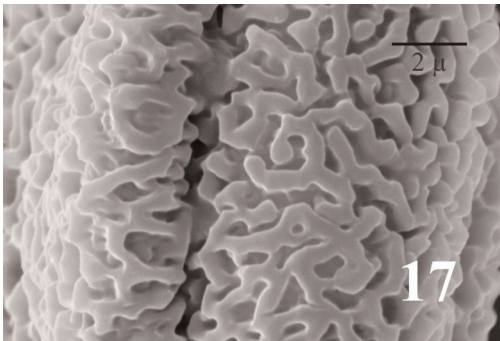
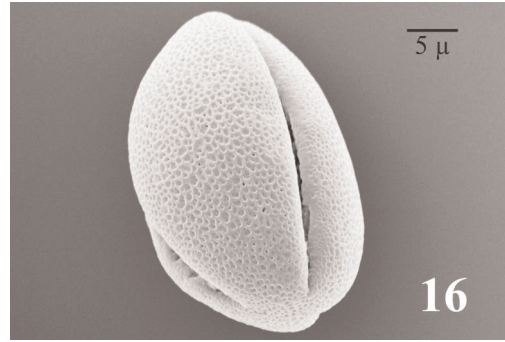
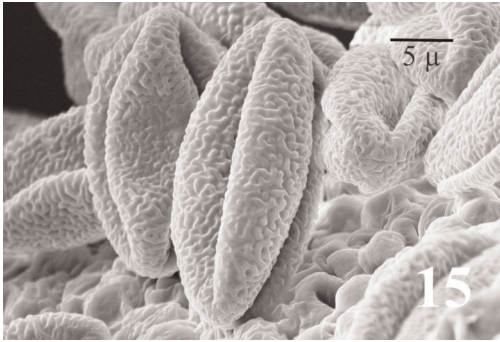
### III- Pollen Morphology

The pollens of *S. yildirimlii* are monad, medium sized, and 3-zonocolpate. Polar axis (P) is between 24 and 28 μm, equatorial axis (E) 14 and 17 μm, and P/E rate 1.56 and 1.73. In the equatorial view, the shape of pollen grain is prolate. The exine thickness is between 1.30 and 1.45 μm. Intine thickness is 0.4-0.6 μm. The ornamentation is retipilate. The width of the muri is 0.6-0.7 μm and that of lumina is 0.9-1.5 μm.

The pollens of *S. cydni* are monad, medium sized, and 3-zonocolpate. Polar axis (P) is between 28 and 32 μm, equatorial axis (E) 18 and 21 μm, and P/E rate 1.54 and 1.68. In equatorial view, the shape of pollen grain is prolate. The exine thickness is between 1.40 and 1.50 μm. Intine thickness is 0.5-0.7 μm. The ornamentation is reticulate. The width of the muri is 0.4-0.5 μm, and that of lumina is 0.6-0.8 μm (Table 2, Figures 15-18).

Table 2. Pollen morphology of *Stachys yildirimlii* and *S. cydni*.

Species	Polar Axis (µm)	Equatorial Axis (µm)	Pollen Shape	Exine Thickness (µm)	Intine Thickness (µm)	Exine Ornem.	Muri Width (µm)	Lumina Width (µm)	Aperture Type
<i>S. yildirimlii</i>	24-28	14-17	prolate	1.3-1.45	0.4-0.6	retipilate	0.6-0.7	0.9-1.5	3-colpate
<i>S. cydni</i>	28-32	18-21	prolate	1.4-1.50	0.5-0.7	reticulate	0.4-0.5	0.6-0.8	3-colpate



Figures 15-18. SEM micrographs of pollen grains: 15-17. *S. yildirimlii*; 16-18. *S. cydni*. 15-16. General appearances of the pollen grains; 17-18. The exine ornamentalions in detail.

## Discussion

Morphological features of sect. *Amblesia* species in Turkey have been given previously (Gemici & Leblebici, 1998; Dinç & Doğan, 2006). Our observations agree with the literature. One of the morphological characteristics of sect. *Amblesia* is ovate to oblong-lanceolate cauline leaves with entire margins (Bhattacharjee, 1980). *S. cydni* has always entire-margined leaves, whereas *S. yildirimlii* displays cauline leaves crenations, as seen in the Iranian endemic *S. obtusirena* Boiss. of sect. *Amblesia* (Rechinger, 1982). This character, peculiar to the 2 species in the section, might indicate that *S. yildirimlii* is most closely related to *S. obtusirena*, which is geographically isolated.

Morphological investigations on the trichomes show that micromorphological features of the trichomes are definitive for separating the 2 species. The branch number of the trichomes and the cell number of the branches are significantly different between the 2 species. Taxonomic value of the trichome morphology for separating the 2 closely allied species may also be useful for other closely related species of sect. *Amblesia*.

Metcalf and Chalk (1950) pointed out that the stems of the family Lamiaceae species are rectangular and the collenchymatic tissue covers broad area at the corners, and a developed scleranchymatic tissue surrounds the vascular tissue. The anatomical studies on some of the family Lamiaceae species showed that they

had the same anatomical characteristics (Kaya *et al.*, 2000; Kandemir, 2003; Uysal, 2002, 2003). These characteristics are observed in both species as well. The sclerenchyma generally forms a continuous ring-shaped tissue in the woody stems of *S. yildirimlii*, but it forms separate bouquets in those of *S. cydni*. The ring shaped sclerenchymatic tissue outside the vascular tissue was reported in *Cyclotrichium origanifolium* (Labill.) Manden. & Scheng., *Salvia hypargeia* Fich. & C.A.Mey., *Stachys thirkei* C.Koch and *Stachys cretica* L. subsp. *smyrnaea* Rech. fil., which were previously investigated (Kaya *et al.*, 2000; Uysal, 2002, 2003; Kandemir, 2003). On the other hand, *Thymbra sintenisii* Bornm. & Aznav. has a separate bouquet shaped sclerenchyma tissue like in *S. cydni* (Erken, 2005).

Anatomical studies show that the leaves of the both species are bifacial. However, the stomatal distribution on the leaves is certainly different between the 2 species. There are no stomata on the upper surfaces of the leaves (hypostomatic) studied in *S. cydni*. The stomata occur on the lower surfaces that are densely covered by the trichomes. Although the stomata are present on the surfaces of the leaves of *S. yildirimlii* (amphistomatic), they are denser on the lower surface than the upper one.

The pith structure of the stem is anatomically different between the 2 species in all preparations studied. It is hollow in the herbaceous and woody stems of *S. cydni*, but completely filled with large orbicular parenchymatic cells in the stems of *S. yildirimlii*.

All members of the sect. *Ambleia* in the world are xerophytic perennials (Bhattacharjee, 1980). The thick cuticle layer on the leaves, hypostomatic leaves on which stomata are concealed by trichomes, and dense and white trichomes reflecting sunlight are some signs of the xeromorphy (Metcalf & Chalk, 1983). Both species exhibit xeromorphy. However, *S. cydni* with more densely and felted white hairy, thicker leaf cuticle, the hypostomatic leaves on which the stomata completely concealed by trichomes appears to be more xerophytic

than *S. yildirimlii*. In *S. yildirimlii*, the cuticle thickness and the trichome density are not as much of *S. cydni*, and are approximately the same on the both epidermises. The leaves of *S. yildirimlii* are also amphistomatic.

Although pollen morphology supports the segregation of some genera of Lamiaceae such as *Phlomis* L., *Marrubium* L. and *Stachys* L. (Abu-Asab & Cantino, 1994), and infrageneric classification of some genera such as *Teucrium* L. (Dönmez *et al.*, 1999), it is often regarded as insufficient criteria for separating the species (Moore *et al.*, 1991). Moore *et al.* (1991) classified the pollen morphologies of *Stachys sylvatica* L., *S. palustris* L., *S. arvensis* (L.) L., *S. annua* (L.) L., *S. germanica* L., *S. alpina* L., *S. recta* L. and *S. officinalis* (L.) Trevisan under the group called *Stachys sylvatica* type. In this group, the pollen morphologies are trizonocolpate, pollen exines have reticulate ornamentation, and lumina are more or less uniform in size. Pollen morphologies of *S. cydni* exhibit the features of *Stachys sylvatica* type as well. But, the pollen exine ornamentation of *S. yildirimlii* is retipilate in all preparations examined by SEM. Consequently, pollen exine ornamentation appear to have significant taxonomic value in segregation of the 2 species.

The present study points out that some micromorphological, palynological, and anatomical characters have taxonomic value like the morphological characters separating the 2 taxa. Consequently, these characters may be used for separating sect. *Ambleia* species, especially close relatives.

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