Nutlet morphology and its taxonomic utility in *Salvia* (Lamiaceae: Mentheae) from Turkey

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Introduction

*Salvia* L. belongs to the family Lamiaceae, representing a diverse cosmopolitan assemblage of nearly 1000 species (WALKER et al. 2004, WALKER and SYTSMA 2007). The genus comprises at least 500 species in Central and South America, 250 species in Central Asia and the Mediterranean and 90 species in Eastern Asia (WALKER et al. 2004). Turkey is a major centre of diversity for *Salvia* in Asia (VURAL and ADIGÜZEL 1996). Since the most recent reviews of the genus in Turkey, four new species have been described and the total has now reached 90. Forty seven of these *Salvia* species in Turkey are endemic (HEDGE 1982, DAVIS et al. 1988, DUMAN 2000, DONMEZ 2001, HAMZAOĞLU et al. 2005). Five of the 12 taxa investigated here are endemic to Turkey.

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Salvia and its fruits have economic and cultural significance. In this paper we present results on investigation of the mericarps and answers to the question if there are variations among taxa and whether taxa can be distinguished by fruits. Furthermore, there is strong evidence about polyphyletic origin of Salvia from several different lineages that possess two fertile stamens and the staminal lever mechanism (WALKER et al. 2004, WALKER and SYTSMA 2007). Mericarp characters have been shown to be phylogenetically informative in other Lamiaceae genera (e.g. *Teucrium* L. (MARIN et al. 1994); *Hemigenia* R.Br. and *Microcorys* R.Br. (GUERIN 2005) and preliminary investigations sampling across groups in *Salvia* are needed to assess evolutionary patterns and phylogenetic utility. The mericarp anatomy and surface ultrastructure of some *Salvia* species from the Jordan region were reported by ORAN (1996, 1997). The variation observed was found to be useful at the species level but higher level utility was not assessed.

Some researchers correlated their major clades of *Salvia* with stamen types identified from examination of fresh material or obtained from the literature (WALKER and SYTSMA 2007). Three stamen types for Turkish *Salvia* species were identified (HEDGE 1982). Types A (with a reduced theca borne on the lower connective arm) and B (lower connective arm with variously shaped connective tissue blocking access to nectar) of both accounts are equivalent. Type C (lower connective arm reduced, connective and filament not articulated) of HEDGE (1982) has no equivalent in WALKER and SYTSMA (2007) although it is at least superficially similar to type C (*Rosmarinus* L.) and H (part of *Salvia* sect. *Audibertia*) in which the lower connective arm is entirely aborted. Given that the classification of *Salvia* has been shown to be inadequate and that stamen types appear to correlate to major clades, it will be important to determine whether the fruit types examined here correlate with stamen type, particularly since the phylogenetic placement of most of the species is unknown.

There are a number of accounts of the fruits of Lamiaceae including a small but growing number of micromorphological studies (see ISLEY 1947, HEDGE 1967, WOJCIECHOWSKA 1958, 1961, 1966, 1972, HUSAIN et al. 1990, REIDALI 1990, RYDING 1992a, b, MARIN et al. 1994, ORAN 1996, TURNER and DELPRETE 1996, ZHOU et al. 1997, DULETIĆ-LAŠEVIĆ and MARIN 1999, GUERIN 2005, MOON and HONG 2006, KAYA and DIRMENCI 2008). The observed morphological characters have consistently been found to be useful at various taxonomic levels. This is valuable in view of the need for identification of mericarps in economic and non-scientific contexts and for additional characters to assess the morphological radiation of a polyphyletic *Salvia* and potentially to aid in delimiting segregate genera in a revised classification of *Salvia* s.l.

Although mericarp characters, particularly exomorphic features, are used in many taxonomic treatments, they are far from being fully exploited. Seldom does one find keys or synopses for mericarp identification, and understanding of microcharacters requires detailed laboratory work. It has been pointed out that this is partly due to the rather scanty literature relating to mericarps and lack of a suitable descriptive terminology which can be applied universally (REIDALI 1990). Thus, while considerable literature is available on the seeds of some plant families, such as the Umbelliferae and Cyperaceae, little, other than incidental notes in manuals or species descriptions, has been published for many other families. Most of the groups dealt with are of economic importance, particularly the weedy and cultivated species. Wild plants have been relatively ignored. More recent account of species may be found in BARTHOLTT (1984).
The tribes *Ajuga*, *Scutellariae* and *Stachyeae* as represented in the USA have been distinguished, with the use of mericarp characters (ISLEY 1947). This author also noted that it was possible to recognize most species by mericarp characters. It may be possible to produce a key to species or species-groups based solely on the texture, size and areole (abscission scar) shape of the mericarps (REJDALI 1990).

This author reported that the mericarps of *Sideritis* L. were ovate to longitudinally elongate, oblong, with the surface varying tremendously between ridged, tuberculate or reticulate (REJDALI 1990).

He examined nutlets of *Hemigenia* and *Microcorys* using SEM and reported significant infrageneric variation in mericarp shape and the nature of the attachment (abscission) scar, surface sculpturing, exocarp cells and indumentum (GUERIN 2005). Typical nutlets were ovoidal, strongly reticulate or rugose, with the exocarp cells isodiametric and convex to papillate. Also common were cylindrical nutlets, often with longitudinal ridging and papillate exocarp cells. Surface pitting and concave exocarp cells were rare. The homologies of these characters were assessed and provide important data within phylogenetic analyses within the Westringieae and important generic and infrageneric diagnostic characters.

Detailed studies on the mericarps of most of the *Salvia* taxa investigated here have not been reported in the literature. There have been reports of the anatomical and surface ultrastructure studies on the mericarps of *Salvia* from the Jordan region, including three of the species examined here: *S. bracteata* Banks et Sol., *S. ceratophylla* L. and *S. verticillata* L., although few data were reported for the latter (ORAN 1996, 1997). To the extent possible, these results are compared with ours. Detailed comparisons with the surface ultrastructure study are problematic due to the lack of tabulation of characters and because some observed surface types were not categorised and some species were not assigned to listed types (e.g. *S. bracteata*). In ORAN (1996, 1997) there are some apparent inconsistencies between categories of surfaces and more detailed descriptions and images given in the plates. Observed shapes were reported as spherical, ovoid or ovoid-spherical. By contrast, HARLEY et al. (2004) described the mericarps of *Salvia* as trigonous, ovoid or sub-orbicular. The pattern of mericarp morphology across the infrageneric grouping of the genus is presently unknown.

The fruit of Lamiaceae is typically a schizocarp consisting of indehiscent locules which separate to form fruitlets (HARLEY et al. 2004). The entire fruiting body may remain entire or split into only two fruitlets (e.g. many taxa within tribe Chloantheae (sub-family Prostantheroideae), but typically, four separating, indehiscent fruitlets result that should properly be referred to as mericarps. Although the term ’nutlet’ has commonly been applied in reports of fruitlet morphology in Lamiaceae (MARIN et al. 1994, ORAN 1996, TURNER and DELPRETE 1996, GUERIN 2005, MOON and HONG 2006, KAYA and DIRMENCİ 2008), this is a somewhat informal term. Mericarps were the unit of study and this is strictly the correct term. We use the term ’mericarp’ throughout.

The point of attachment of the mericarps to the schizocarpic receptacle is here referred to as an ’abscission scar’ rather than an ’attachment scar’ (e.g. as in GUERIN 2005). The term ’areole’ is perhaps best applied to the attachment point of seeds rather than indehiscent fruits.
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Materials and methods

Material used for this study was collected from wild populations in Turkey (Tab. 1). The plant samples were dried as herbarium samples and stored in Celal Bayar University Herbarium (CBUH).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Voucher locality</th>
<th>Collection Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. blepharoclaena</em> Hedge et Hub.Mor. (E)</td>
<td>Kirşehir: Çiçekdağ, Ahmet Veli Mausoleum, in the forest, 1500 m, 28.V.2004.</td>
<td>Ozkan 4024</td>
</tr>
<tr>
<td><em>S. cryptantha</em> Montbret et Aucner ex Bentham (E)</td>
<td>Kirşehir: Çiçekdağı Road 50 km, Slopes of Mountain, 1150 m, 6.VI.2005.</td>
<td>Ozkan 5020</td>
</tr>
<tr>
<td><em>S. aethiopis</em> L.</td>
<td>Amasya: Merzifon, Tavsan Mountain, 950 m, 28.VI.1998.</td>
<td>Ozkan 1032</td>
</tr>
<tr>
<td><em>S. ceratophylla</em> L.</td>
<td>Kastamonu: Tosya, Roadside, 700 m, 25.V. 1998.</td>
<td>Ozkan 1014</td>
</tr>
<tr>
<td><em>S. candidissima</em> Vahl. subsp. candidissima</td>
<td>Kayseri: Develi, Roadside, 1150 m, 15.VII. 2004.</td>
<td>Ozkan 4054</td>
</tr>
<tr>
<td><em>S. cyanescens</em> Boiss et Bal. (E)</td>
<td>Kastamonu: Tosya, Gavurdagi, 1050 m, 17. VII. 1998.</td>
<td>Ozkan 1054</td>
</tr>
<tr>
<td><em>S. virgata</em> Jacq.</td>
<td>Amasya: Merzifon, Tavsan Mountain, West Slopes, 500 m, 25.VII.2001.</td>
<td>Ozkan 2018</td>
</tr>
<tr>
<td><em>S. halophila</em> Hedge (E)</td>
<td>Ankara: Eskisehir Road, 70 km, Riversi-des, 1000 m. 27.VII.2004.</td>
<td>Ozkan 4074</td>
</tr>
<tr>
<td><em>S. verticillata</em> L. subsp. <em>verticillata</em></td>
<td>Amasya: Merzifon, Between Büyük Radar and Derealan Village, 1000 m, 7.VII.1998.</td>
<td>Ozkan 1038</td>
</tr>
<tr>
<td><em>S. verticillata</em> subsp. amasiaca (Freyn et Bornm.) Bornm.</td>
<td>Amasya: Merzifon, Derealan Village, Roadside, 1100 m, 7.VII.1998.</td>
<td>Ozkan 1044</td>
</tr>
</tbody>
</table>

E: Endemic for Turkey.

In order to assess infraspecific variation, 9–10 mericarps from each taxon were measured. For SEM studies, mericarps were directly mounted on stubs and coated with gold. Micrographs were taken with a JEOL 5600 SEM. All the specimens were examined, but only the clearest photographs representing each mericarp sculpturing type were selected and illustrated. Terminology for descriptions of morphological characteristics of the mericarps follows STEARN (1978) and BOJNAŇSKÝ and FARGASOVÁ (2007).

Results

The characteristics of mericarp morphology (i.e., size, shape, surface sculpturing and colour) for the investigated taxa are given in table 2. Infraspecific variation was restricted
Tab. 2. Comparison of mericarp shape, size, sculpturing and colour in *Salvia* taxa

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Stamen type</th>
<th>Shape</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>L/W ratio</th>
<th>Sculpturing</th>
<th>Midridge</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>SD</td>
<td>Range</td>
<td>M</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td><em>S. bracteata</em></td>
<td>A</td>
<td>S</td>
<td>3.25</td>
<td>±0.17</td>
<td>3.0–3.5</td>
<td>2.55</td>
<td>±0.13</td>
<td>2.2–2.7</td>
</tr>
<tr>
<td><em>S. cadmica</em></td>
<td>A</td>
<td>S</td>
<td>3.06</td>
<td>±0.07</td>
<td>3.0–3.2</td>
<td>2.38</td>
<td>±0.12</td>
<td>2.2–2.6</td>
</tr>
<tr>
<td><em>S. blepharoclaena</em></td>
<td>A</td>
<td>S</td>
<td>2.50</td>
<td>±0.12</td>
<td>2.4–2.7</td>
<td>1.95</td>
<td>±0.11</td>
<td>1.8–2.1</td>
</tr>
<tr>
<td><em>S. cryptantha</em></td>
<td>A</td>
<td>S</td>
<td>3.12</td>
<td>±0.07</td>
<td>3.0–3.2</td>
<td>2.75</td>
<td>±0.14</td>
<td>2.5–2.9</td>
</tr>
<tr>
<td><em>S. aethiopis</em></td>
<td>B</td>
<td>T-PS</td>
<td>2.60</td>
<td>±0.12</td>
<td>2.1–2.9</td>
<td>1.67</td>
<td>±0.11</td>
<td>1.5–1.8</td>
</tr>
<tr>
<td><em>S. ceratophylla</em></td>
<td>B</td>
<td>S</td>
<td>2.64</td>
<td>±0.12</td>
<td>2.5–2.9</td>
<td>2.22</td>
<td>±0.14</td>
<td>1.9–2.3</td>
</tr>
<tr>
<td><em>S. candidissima</em></td>
<td>B</td>
<td>T-PS</td>
<td>2.40</td>
<td>±0.11</td>
<td>2.2–2.6</td>
<td>1.61</td>
<td>±0.14</td>
<td>1.4–1.8</td>
</tr>
<tr>
<td>subsp. candidissima</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cyanescens</em></td>
<td>B</td>
<td>T-PS</td>
<td>2.61</td>
<td>±0.09</td>
<td>2.5–2.8</td>
<td>1.85</td>
<td>±0.14</td>
<td>1.6–2.0</td>
</tr>
<tr>
<td><em>S. virgata</em></td>
<td>B</td>
<td>T-PS</td>
<td>2.31</td>
<td>±0.15</td>
<td>2.0–2.4</td>
<td>1.57</td>
<td>±0.12</td>
<td>1.4–1.7</td>
</tr>
<tr>
<td><em>S. halophila</em></td>
<td>B</td>
<td>S</td>
<td>1.77</td>
<td>±0.11</td>
<td>1.6–2.0</td>
<td>1.37</td>
<td>±0.14</td>
<td>1.2–1.6</td>
</tr>
<tr>
<td>subsp. verticillata</td>
<td>C</td>
<td>PS</td>
<td>1.88</td>
<td>±0.10</td>
<td>1.8–2.0</td>
<td>1.14</td>
<td>±0.11</td>
<td>1.0–1.3</td>
</tr>
<tr>
<td><em>S. verticillata</em></td>
<td>C</td>
<td>PS</td>
<td>2.25</td>
<td>±0.20</td>
<td>2.0–2.5</td>
<td>1.20</td>
<td>±0.13</td>
<td>1.0–1.4</td>
</tr>
<tr>
<td>subsp. amasiaca</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

M: Mean; SD: Standard Deviation

1A and B equivalent to HEDGE (1982) and WALKER and SYTSMA (2007); C equivalent to HEDGE (1982).
to minor differences in mericarp size. The studied *Salvia* mericarps ranged in length from 1.6 mm to 3.5 mm with the minimum value in *S. halophila* Hedge and the maximum value in *S. bracteata*, and in width from 1.0 mm to 2.9 mm with the minimum value in *S. verticillata* subsp. *verticillata* and the maximum value in *S. cryptantha* Montbret et Aucher ex Benth.

Mericarps of *Salvia bracteata*, *S. cadmica* Boiss., *S. blepharoclaena* Hedge et Hub.Mor., *S. cryptantha*, *S. ceratophylla* and *S. halophila* were spherical (Fig 1A-D, F, J), *S. aethiopis* L., *S. candidissima* Vahl. subsp. *candidissima*, *S. cyanescens* Boiss. et Bal. and *S. virgata* Jacq., were trigonous and prolate spheroidal (Fig 1E, G, H, I) and *S. verticillata* subsp. *verticillata* and *S. verticillata* subsp. *amasiaca* (Freyn et Bornm.) Bornm. were prolate spheroidal in shape (Fig 1K, L).

**Fig. 1.** Scanning electron micrographs of mericarp in *Salvia*. A – *Salvia bracteata*; B – *S. cadmica*; C – *S. blepharoclaena*; D – *S. cryptantha*; E – *S. aethiopis*; F – *S. ceratophylla*; G – *S. candidissima* subsp. *candidissima*; H – *S. cyanescens*; I – *S. virgata*; J – *S. halophila*; K – *S. verticillata* subsp. *verticillata*; L – *S. verticillata* subsp. *amasiaca*. 
Mericarps of investigated taxa exhibited three types of surface sculpturing, and are present in the following species:

**Type I.** Foveate: surface with small pits: *Salvia bracteata, S. cadmica, S. blepharoclaena, S. cryptantha, S. aethiopis* and *S. candidissima* subsp. *candidissima* (Figs. 2A-E, G).


**Type III.** Verrucate: surface with irregular projections or knobs: *S. cyanescens* (Fig 2H).

The mericarps of *Salvia bracteata, S. cadmica, S. cyanescens, S. halophila, S. verticillata* subsp. *verticillata* and *S. verticillata* subsp. *amasiaca* were dark brown while those
of *S. cryptantha*, *S. aethiopis* and *S. candidissima* subsp. *candidissima* were pale brown and those of *S. blepharoclaena*, *S. ceratophylla* and *S. virgata* were black.

**Discussion**

The shape, size, sculpturing and colour of mericarps examined varied, and character combinations identified or defined taxa or groups of taxa. The length to width ratio of mericarps varied with the perhaps obvious pattern of being larger in prolate mericarps than in spherical mericarps. Significantly, character combinations correlated with stamen type (i.e. type A, B or C) for each taxon. Taxa with stamen type A had spherical-foveate mericarps, taxa with stamen type B had spherical-reticulate mericarps or were trigonous and prolate spheroid with variable sculpturing diagnostic at lower levels; taxa with stamen type C had prolate-spheroidal-reticulate mericarps (although only the subspecies of a single species were examined for type C). This correlation is supported by molecular phylogenetic evidence, at least for *Salvia aethiopis* and *Salvia candidissima*, which have more or less identical mericarps, stamen type B and were shown to be closely related in all consensus trees of WALKER and SYTSMA (2007).

The mericarps of both subspecies of *Salvia verticillata* were prolate spheroidal, reticulate with small pits and were dark brown in colour. Mericarp size was given as 2.2 mm × 1.3 mm for both subspecies (HEDGE 1982). We measured the mericarp sizes as 1.8–2.0 × 1.0–1.3 mm for *S. verticillata* subsp. *verticillata* and 2.0–2.5 × 1.0–1.4 mm for *S. verticillata* subsp. *amasiaca*. Although the mericarps of both subspecies were reticulate with small pits, the size, particularly the length, differed and this character could be used to discriminate the subspecies.

The mericarps of *Salvia virgata* had a prominent mid-ridge extending distally from the abscission scar. The same characteristic were observed on the mericarps of *Sideritis* (REJDALI 1990). This apomorphic characteristic of *S. virgata* distinguishes it from all other investigated taxa and may remain an important character for identification when more taxa are sampled.

Although *Salvia* is the largest genus of Lamiaceae, its mericarp morphology and anatomy have been poorly reported. There has been a report of the only other known external micromorphological study on the mericarps of *Salvia* (ORAN 1996). Detailed comparisons with the conclusions of this previous study are hampered by the excessive number of surface types given, including two separate but very similar types listed for *S. viridis* L., the lack of types being assigned to all species in the study, the lack of tabulation of characters, and the lack of a clear conclusion as to the pattern of characters within and between groups and sections. The observed shapes, reported as spherical, ovoid or ovoid-spherical, are equivalent to our spherical and prolate-spheroidal respectively, with the third shape intermediate.

Exocarp cellular features such as cell shape and concavity/convexity are not reported here separately from surface sculpturing as the individual cells are somewhat obscured in the taxa examined. The mericarp surfaces were relatively smooth with sculpturing at lower levels created by the outer walls of the exocarp cells. The nature of the individual exocarp cells has shown to be an informative character in other groups and certainly varies significantly across the Lamiaceae with smooth or pitted cells common and convex to conical or papillose cells present in sub-family Prostantheroideae (GUERIN 2005).
The only available data on some of the investigated taxa are a few notes given in Flora of Turkey (HEDGE 1982). Our results showed some similarities and differences with these notes (Tab. 3). Some of the differences in the description of shape are apparently due to use of different terminology rather than any difference in observations or interpretation. For example, the »rounded-trigonous ovoid« of HEDGE (1982) is clearly equivalent to »trigonous and prolate spheroidal«. The differences in mericarp size are mostly insignificant and probably attributable to sampling error and differences in the accuracy of the measurement methods. However, a number of measurements are very different, for example for Salvia blepharoclaena, which had dimensions reported by HEDGE (1982) over 1.5× larger than in this study. The shape was given as rounded-trigonous ovate whereas the mericarps examined here were clearly spherical (see Figure 1C). The only available notes concerning the mericarps of S. cryptantha is a record of them being pale brown in HEDGE (1982), which agrees with our observations. We observed the same colour for S. cryptantha.

Some researchers found that the density of glands on mericarp was the most useful character in Teucrium (sub-family Ajugoideae) and HUSAIN et al. (1990) found that mericarp shape and the nature of the abscission scar were invariable in representative members of tribe Saturejaceae (MARIN et al. 1994). We found that in Salvia the mericarps were glabrous but that the shape was a significant character. The abscission scar of the taxa examined was very small, simple and positioned in the typical basal position, offering no utility at lower levels. Conversely, the abscission scar in some members of tribe Westringieae (sub-family Prostantheroideae) and Ajuga L. is baso-lateral and occupies a significant fraction of the length of the mericarp (often over half). The nature of the abscission scar is variable and phylogenetically informative at an infrageneric level in the Westringieae (GUERIN 2005). Clearly, even characters that are invariable within particular groups may be of systematic value at higher levels and hence worth reporting in the literature.

Some terms that appear to be analogous at best have been applied to characters across several sub-families and tribes of the Lamiaceae. For example, the reticulate surfaces of mericarps examined here are net-like at a very fine scale possibly relating to the walls of individual exocarp cells and the surface overall is not distinctly sculptured, whereas GUERIN (2005) applied the term to mericarps with relatively large, prominent ridges when the ridges joined into a net-like arrangement around depressed lacunae. Conversely, in some cases different terms have been applied to the same character. For example, HARLEY et al. (2004) described the mericarps of Salvia as trigonous, ovoid or sub-orbicular, terms which clearly describe shapes comparable to those reported here for the genus. The previous comparisons with the shape terminology of HEDGE (1982) and ORAN (1996) are further examples.

The above suggests a need for an acceptable and unifying descriptive terminology for mericarps to be applied across Lamiaceae. Author number four is currently collating terminology which has been used in the family or which should properly be used, but wider sampling of mericarp morphology across sub-families will be required to propose a comprehensive terminology.

In summary, we observed variation in the external morphology of mericarps in Salvia taxa representing the morphological diversity (particularly with regard to stamen types) of the genus in Turkey, including endemic taxa, and found that characters of shape, size, surface sculpturing and colour varied and were useful in distinguishing groups, species and
subspecies with regard to apomorphies but also with character combinations defining groups. Character combinations correlated with stamen types, suggesting mericarps may be useful phylogenetically and in classification. We conclude that a wider sampling of *Salvia* species from Turkey, indeed across the entire genus and even the tribe Mentheae, would be advantageous and informative. The mericarp micromorphology of taxa nested within *Salvia* s.l. such as *Rosmarinus* also needs to be examined and compared. This would provide data towards an understanding of the evolutionary and morphological radiation of the group but also towards building practical identification tools.

References


MERICARP MORPHOLOGY OF SALVIA


