

# Phylogenetic significance of leaf micromorphology and anatomy in the tribe Mentheae (Nepetoideae: Lamiaceae)

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A comparative micromorphological study of leaves was carried out on 102 species of Mentheae; 61 species were selected for the anatomical study. Mentheae possessed both amphistomatic and hypostomatic leaves. The diversity of leaf epidermal characteristics was based on the variation in morphology of epidermal cells, stomata types and trichome types. Although each characteristic on its own has rather limited systematic value, the combination of some of these features may be systematically relevant, especially for the identification of species. For example, branched multicellular nonglandular trichomes were a diagnostic characteristic for all genera investigated of the subtribe Salviinae; however, this trichome type was also observed in *Hedeoma ciliolata* and *Neoepplingia leucophylloides* of the subtribe Menthinae. Capitulate glandular trichomes with pear-shaped heads were only observed in *Salvia dorrii*. Subsessile glandular trichomes with multicellular heads (more than ten cells) were an apomorphy for *Perovskia*. The anatomical leaf structure was consistent throughout the tribe. In some species, the vascular bundles in the midrib were modified into a mechanical tissue, which is an adaptation to xerophytic environments. The observed variations are discussed in an ecological context and their phylogenetic significance is evaluated. © 2009 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2009, 160, 211–231.

ADDITIONAL KEYWORDS: epidermis – phylogenetics – stomata – systematics – trichome – vascular bundle.

## INTRODUCTION

Lamiaceae (Lamiales, euasterids I; APG II) are rich in herbs and medicinal plant species, which are of great economic importance. Lamiales formerly had a restricted circumscription that included the families Lamiaceae, Verbenaceae, Boraginaceae and Lenoaceae (Cronquist, 1988), but recent phylogenetic work has shown that Lamiales is monophyletic with the inclusion of former orders Bignoniales, Hippuridales, Plantaginales and Scrophulariales (Stevens,

2001; Judd *et al.*, 2008). Consequently, Lamiales has become one of the larger angiosperm groups, containing about 12% of the eudicot diversity (23 families with 1059 genera and 23 275 species; Stevens, 2001). Lamiaceae has a cosmopolitan distribution and consists of 236 genera and about 7000 species (Stevens, 2001).

Many species of Lamiaceae produce essential oils which are secreted by glandular hairs on aerial vegetative organs and some reproductive organs. These hairs have been investigated from structural, ultrastructural and biochemical viewpoints for their commercial value in various members of the family (Amelunxen, 1964, 1965; Amelunxen, Wahlig &

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Arbeiter, 1969; Heinrich, 1973; Bosabalidis & Tsekos, 1982, 1984; Heinrich *et al.*, 1983; Werker, Putievsky & Raavid, 1985; Dudai *et al.*, 1988; Bosabalidis, 1990; Autunes & Sevinete-Pinto, 1991). Although leaf epidermal morphology has received less attention than fruit morphology and seed anatomy as a potential systematically informative characteristic, the surface of the leaves is commonly covered by various nonglandular and glandular trichomes which, with characteristics of the stomatal complex, may have systematic value (Stace, 1984; Webster, Del-Arco-Aguilar & Smith, 1996; Moon & Hong, 2003; Beilstein, Al-Shehbaz & Kellogg, 2006).

In Lamiaceae, the systematic value of trichome types was demonstrated by Abu-Asab & Cantino (1987) in the subtribe Melittidinae (Dumort.) Endlicher. In addition, Cantino (1990) performed a comprehensive study of Lamiaceae and Verbenaceae with emphasis on the morphology of the stomatal complex and the sessile glandular trichomes, including their systematic importance. However, this study focused mainly on the subfamily Lamioideae *sensu* Erdtman (1945) in order to elucidate the relationships with Verbenaceae, rather than subfamily Nepetoideae (Dumort.) Luerssen. Since Erdtman (1945) suggested two subfamilies in Lamiaceae (Lamioideae and Nepetoideae) on the basis of pollen characteristics, the monophyly of subfamily Lamioideae has been questioned because of similarity with Verbenaceae (Cantino & Sanders, 1986). Consequently, in the most recent classification of Lamiaceae (Harley *et al.*, 2004), Lamioideae *sensu* (Erdtman, 1945) is segregated into six subfamilies, Symphorematoideae Briq., Viticoideae Briq., Ajugoideae Kostel., Prostantheroideae Luerss., Scutellarioideae (Dumort.) Caruel and Lamioideae Harley, which include many genera of former Verbenaceae. Nepetoideae is always supported as a monophyletic group in both molecular and morphological analyses (Cantino & Sanders, 1986; Cantino, Harley & Wagstaff, 1992; Kaufmann & Wink, 1994; Wagstaff, Olmstead & Cantino, 1995; Harley *et al.*, 2004).

A comparative anatomical study of leaves has been reported only for subtribe Hyptidinae Endl. of Nepetoideae (Rudall, 1980). Rudall (1979) also showed that the variation in leaf structure is correlated with ecological constraints, but anatomical data are useful for infrageneric delimitation in Nepetoideae (Hyptidinae: Rudall, 1979, 1980; *Origanum* L.: Bosabalidis & Kokkini, 1997).

Mentheae Dumort. is the largest tribe of Nepetoideae, including 65 genera and approximately 2000 species, and these species are known for their high content of essential oils, which are widely used in pharmaceutical preparations, perfumery and cosmetics. According to the most recent classification, Mentheae can be divided into three subtribes:

Salviinae (Dumort.) Endl., Nepetinae (Dumort.) Coss. & Germ. and Menthinae (Dumort.) Endl. (Harley *et al.*, 2004). Although Mentheae is a well-supported monophyletic group within Nepetoideae, the relationships among the genera are poorly resolved. In particular, the relationship of *Heterolamium* C.Y.Wu and *Melissa* L. to other members of Mentheae remains obscure (Wagstaff *et al.*, 1995; Harley *et al.*, 2004). The lack of morphological support for the molecular phylogenetic hypothesis of Mentheae illustrates the necessity of thorough morphological research. Our previous research has focused on the systematic relationships of Mentheae *sensu* Harley *et al.* (2004) based on pollen and nutlet characteristics. These features proved to be systematically important at various taxonomic levels, and especially so at the generic level (Moon, 2008; Moon *et al.*, 2008a, 2008b). These results encouraged us to explore other morphological characteristics, such as leaf features, in order to further our understanding of the phylogenetic relationships within the study group.

The objective of this paper is therefore to present a detailed description of leaf micromorphology in Mentheae using scanning electron microscopy (SEM). In addition, we aim to describe the leaf anatomy based on the midrib structure of selected taxa. The variation in leaf characteristics is discussed with respect to their potential systematic value and in relation to our previous work in Mentheae.

## MATERIAL AND METHODS

This study was conducted mainly on material taken from herbarium specimens on loan from the following herbaria (abbreviations according to Holmgren, Holmgren & Barnett, 1990): BR, G, GH, K, LV, MO, S and SNU. Plants recently collected in formalin–acetic acid–alcohol (FAA) by H.-K. Moon were also used. A list of all species and specimens investigated is provided in the Appendix. This study included all genera of Mentheae *sensu* Harley *et al.* (2004), except for the monotypic *Eriothymus* J.A.Schmidt, which is known only from the type collection and is possibly extinct (Harley *et al.*, 2004). *Lycopus* L. was studied only anatomically in this study because the detailed leaf morphology was carried out by Moon & Hong (2003).

For leaf epidermal observations, leaves were first examined using a stereomicroscope (Leica MZ6) in order to select fully mature leaves. The dried material was rehydrated overnight in the wetting agent Agepon® (Agfa Gevaert, Leverkusen, Germany; Agepon wetting agent : distilled water, 1 : 200) prior to dehydration. Leaves were separated from the specimen/individual and the mid-part of the leaf was dissected with a razor blade to fit the size of the aluminium stub. All samples were dehydrated

through a graded ethanol series (in 70, 90, 95 and 100% ethanol) prior to critical point drying (CPD 030, Balzers). The dried leaves were mounted on stubs with double adhesive tape. The stubs were coated with gold (SPI-MODULE™ Sputter Coater, SPI Supplies, West Chester, PA, USA) and observed with a JEOL JSM-6360 scanning electron microscope at 5–15 kV.

For the anatomical observations, the material was dehydrated in a graded ethanol series, embedded in paraffin, sectioned (8–10 µm) with a rotary microtome (Zeiss HM360), stained in aniline blue and safranin solutions and permanently mounted using Entellan. Sections were examined and photographed using a Leitz Dialux 20 light microscope equipped with a PL-B622CF microscopy camera (PixeLink) and Microscopica v1.3 (Orbicule, Belgium).

Size measurements on SEM and light microscopy images were made using Carnoy 2.0 (Schols *et al.*, 2002). Terminology for the stomatal complex, trichomes and surface sculpturing follows Cantino (1990) and Moon & Hong (2003).

## RESULTS

Significant variation was observed in epidermal cells, the stomatal complex and trichomes (Figs 1–4). The midrib structure of the leaf was similar throughout the tribe (Fig. 5), but xeromorphic adaptations were found in a few species (Fig. 5H, I). Representative leaf characteristics are summarized in Table 1. In some cases, the stomatal complex and cell surface pattern could not be recognized because of extremely dense nonglandular trichomes on the epidermis.

### EPIDERMAL CELLS

Leaf epidermal cells were irregular or isodiametric (Table 1). The cell outlines were described for adaxial (AD) and abaxial (AB) leaf surfaces separately, although in many taxa the patterns were similar on the same leaf. Irregular cells were rather common and distributed evenly on both sides. Isodiametric cells were more common on AD surfaces (c. 62%). Undulate cell outlines were found in *Bystropogon canariensis* (L.) L'Hér (AD), *Dicerandra odoratissima* R.M. Harper (AB), *Hoehnea minima* (Schmidt) Epling (AB), *Meehaniania urticifolia* (Miq.) Makino (AD), *Salvia polystachya* Ort. (AB) and *Salvia sclarea* L. (AB and AD). Sometimes, the epidermal cells were invisible because of a dense layer of trichomes (Figs 1D, E, I, 2E). The isodiametric epidermal cells usually had straight to curved anticlinal walls, whereas irregular cells had undulate to sinuate anticlinal walls. The anticlinal walls of undulate cell outlines were hardly discernible (Figs 1, 2).

Striation occurred commonly on irregular cells and in some taxa on isodiametric cells (Table 1). Striation often occurred on only one side of the leaf (AB, 14 species; AD, eight species), except in 16 species where striation was observed on both sides of the leaf. Striae were restricted to the stomatal areas in *Hesperozygis nitida* (Benth.) Epling (AB), *Micromeria marginata* (Sm.) Chater (AD; Fig. 2J, K), *Poliomintha glabrescens* A. Gray ex Hemsl. (AD) and *Satureja thymbra* A. Gray ex Hemsl. (AD; Fig. 2O).

### STOMATA

Representatives of Mentheae had both amphistomatic and hypostomatic leaves, but amphistomatic (69 species) were more common than hypostomatic (25 species; Table 1) leaves. *Meriandra bengalensis* (Roxb.) Benth. may be amphistomatic with a few stomata on the AD surface, although stomata were invisible on the AB surface. *Bystropogon canariensis*, *Conradina canescens* A. Gray, *Conradina grandiflora* Small, *Hedeoma ciliolata* (Epling & Stewart) Irving, *Poliomintha longiflora* A. Gray, *Rosmarinus officinalis* L. and *Salvia dorrii* (Kellogg) Abrams are possibly hypostomatic as stomata were absent on the AD side. In Mentheae, five types of stomatal complex were observed (definitions adapted from Cantino, 1990; and references cited therein): actinocytic (stoma surrounded by a single ring of five or more radially elongated cells enclosing the guard cells); anisocytic (stoma surrounded by three subsidiary cells, one of which is markedly smaller than the other two); anomocytic (stoma surrounded by cells that are indistinguishable from other epidermal cells); diacytic (stoma enclosed by a pair of subsidiary cells with common walls perpendicular to the guard cells); diallelocytic (stoma enclosed by an alternating complex of three subsidiary cells of graded size oriented perpendicular to the guard cells). In some cases, more than one type of stoma may be present on the same surface, for example, anomocytic, anisocytic and diacytic in *Glechoma hederacea* (Fig. 1P). Moreover, diacytic and diallelocytic types often occurred simultaneously (Table 1; Fig. 2G, M, O). The most common types of stomata in the tribe were diacytic and anomocytic. In the amphistomatic taxa, stomata were more frequent on the AB surface. Stomatal size varied considerably in Mentheae (10–36 × 7–27 µm). The smallest stoma was recorded in *Zataria multiflora* Boiss. on the AD surface (10 µm in length), and *Thymus serpyllum* L. possessed the largest stoma on the AB surface (36 µm in length).

### TRICHOMES

The leaf epidermis of all species investigated was covered by various hairs. We defined two categories of

Table 1. Characterization of the leaf components in Mentheae

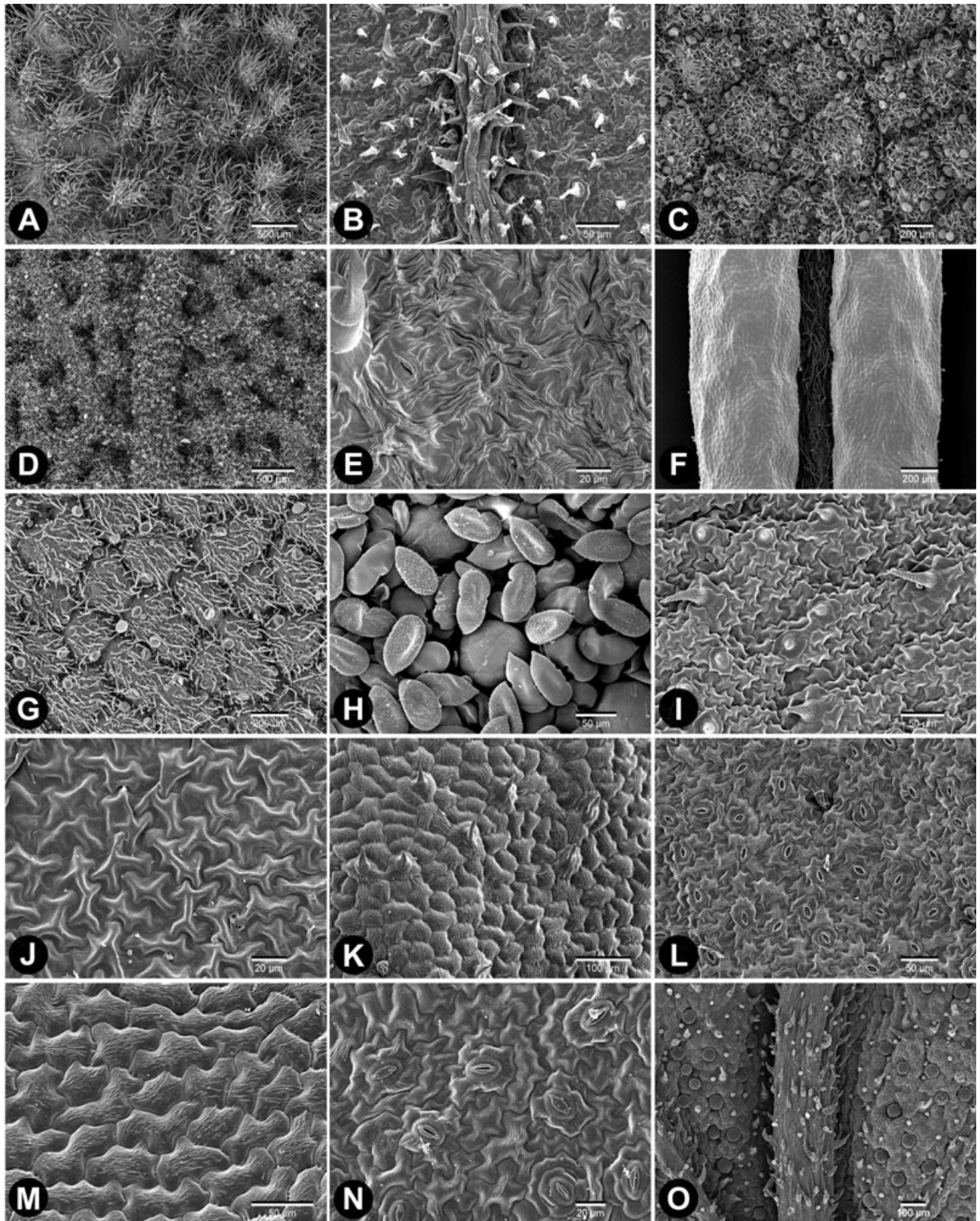
Taxon	SP	Stomatal type	Size of stomata (µm)	Surface cell	AW	SS	SL	US	UL	B	C	P	S
Subtribe Salviinae													
<i>Chaenostoma meciastandrum</i>	AD	/	13–16 × 12–17	Isometric	st/cur	+	-	+	-	+	+	-	+
	AB	/	22–24 × 18–23	Striated	/	-	-	+	-	+	+	-	+
<i>Dorysphaechas hastata</i>	AD	/	24–28 × 21–23	Striated isometric	st/cur	+	-	+	+	+	+	+	-
	AB	/	17–20 × 13–15	Isometric	cur/un	+	-	+	-	+	+	-	+
<i>Lepechinia calycina</i>	AD	Ani/dia/dialleloctyic	18–20 × 16–18	Irregular	sin	+	-	+	-	+	+	-	+
	AB	Anomocytic	20–25 × 15–16	Irregular	cur	+	-	+	-	+	+	-	+
<i>Lepechinia caulescens</i>	AD	Diacytic	18–23 × 16–18	Irregular	un	+	-	+	+	+	+	-	+
	AB	Anomocytic	15 × 9	#		-	-	+	-	+	+	-	+
<i>Meriandra bengalensis</i>	AD	#	16–19 × 14–16	Irregular	un	-	-	+	-	+	+	-	+
	AB	Dia/dialleloctyic	16–20 × 13–15	Irregular	un	+	-	+	-	+	+	-	+
<i>Perovskia abrotanoides</i> (thrum)	AD	Dia/dialleloctyic	19–22 × 15–19	Irregular	un	+	-	+	-	+	+	-	+
	AB	Dia/dialleloctyic	18–22 × 15–17	Striated irregular	sin	+	-	+	-	+	+	-	+
<i>Perovskia abrotanoides</i> (pin)	AD	/	20–24 × 15–18	Irregular	st/cur	+	-	+	-	+	+	-	+
	AB	Anomo/diacytic	16–21 × 15–18	Irregular	un	-	-	+	-	+	+	-	+
<i>Perovskia scrophularifolia</i>	AD	Diacytic		Isometric	cur	-	-	+	-	+	+	-	+
	AB	#		Irregular	st	-	-	+	-	+	+	-	+
<i>Rosmarinus officinalis</i>	AD	Diacytic	16–19 × 13–16	Irregular	un	-	-	+	+	+	+	-	+
	AB	/	13–18 × 13–15	Isometric	/	-	-	+	+	+	+	-	+
<i>Salvia aethiopsis</i>	AD	Dia/dialleloctyic	20–24 × 14–17	Isometric	cur/un	+	-	+	-	+	+	-	+
	AB	/	15–21 × 11–15	/	/	-	-	+	+	+	+	-	+
<i>Salvia canariensis</i>	AD	/	19 × 12	/	/	+	-	+	-	+	+	-	+
	AB	Anomo/actinocytic	19–22 × 16–20	Partially striated irregular	sin	+	-	+	-	+	+	-	+
<i>Salvia coccinea</i>	AD	/	18–22 × 20–22	Partially striated	/	-	-	+	-	+	+	-	+
	AB	/	20–25 × 18–20	/	/	-	-	+	-	+	+	-	+
<i>Salvia dorrii</i>	AD	/	16–24 × 13–16	Irregular	sin	-	-	+	-	+	+	-	+
	AB	Diacytic	12–15 × 12–15	Isometric	st/cur	-	++	+	-	+	+	-	+
<i>Salvia glutinosa</i>	AD	#		#		-	++	+	-	+	+	-	+
	AB	/		Irregular	sin	+	-	+	-	+	+	-	+
<i>Salvia officinalis</i>	AD	/	13–17 × 13–15	Irregular	/	-	-	+	-	+	+	-	+
	AB	/	16–18 × 14–16	Isometric	cur/un	+	-	+	-	+	+	-	+
<i>Salvia polystachya</i>	AD	Diacytic	20–25 × 14–18	Irregular	un/sin	+	-	+	+	+	+	-	+
	AB	/	22–27 × 19–20	/	/	+	-	+	-	+	+	-	+
<i>Salvia pratensis</i>	AD	Anomocytic	17–20 × 13–19	Isometric	st/cur	+	-	+	+	+	+	-	+
	AB	/	13–17 × 13–14	/	/	-	-	+	-	+	+	-	+
<i>Salvia rypara</i>	AD	Anomocytic	18–22 × 16–18	Irregular	un	+	-	+	-	+	+	-	+
	AB	/	19–24 × 16–18	Irregular	un	+	-	+	-	+	+	-	+
<i>Salvia sclarea</i>	AD	Diacytic	20–25 × 15–19	Isometric	st/cur	+	-	+	-	+	+	-	+
	AB	Diacytic	21–25 × 18–20	Irregular	st/cur/un	+	-	+	+	+	+	-	+
<i>Salvia taraxacifolia</i>	AD	Anomocytic	12–15 × 6–10	Partially striated irregular	sin	-	-	+	+	+	+	-	+
	AB	Anomocytic	12–16 × 10–14	Partially striated irregular	sin	-	-	+	+	+	+	-	+
<i>Salvia verbenaca</i>	AD	#		#		-	-	+	-	+	+	-	+
	AB	#		#		-	-	+	-	+	+	-	+
<i>Salvia verticillata</i>	AD	Diacytic	20–26 × 15–20	Irregular	st/cur	+	-	+	+	+	+	-	+
	AB	Anomo/dia/dialleloctyic	16–22 × 16–20	Irregular	cur	+	-	+	+	+	+	-	+
<i>Zhumeria majdae</i>	AD	Anomocytic	17–20 × 10–13	Irregular	sin	+	-	+	+	+	+	-	+
	AB	Anomo/diacytic	14–20 × 12–14	Irregular	sin	+	-	+	-	+	+	-	+
Subtribe Menthininae													
<i>Acanthomintha lanceolata</i>	AD	Diacytic		Irregular	st/cur	+	-	+	+	+	+	-	+
	AB	Anomo/dia/dialleloctyic		Irregular	cur	+	-	+	+	+	+	-	+
<i>Acanthomintha obovata</i>	AD	Anomocytic		Irregular	sin	+	-	+	+	+	+	-	+
	AB	Anomo/diacytic		Irregular	sin	+	-	+	-	+	+	-	+



Table 1. Continued

Taxon	SP	Stomatal type	Size of stomata ( $\mu\text{m}$ )	Surface cell	AW	SS	SL	US	UL	B	C	P	S
<i>Monardella nana</i>	AD	Dia/dialleloctytic	27–31 × 22–24	Isometric	st/cur	+	-	+	-	-	+	-	+
	AB	Dia/dialleloctytic	28–37 × 19–22	Partially striated irregular	cur/un	+	-	+	-	-	+	-	+
<i>Monardella odoratissima</i>	AD	Anomocytic	13–14 × 11–13	Irregular	cur	+	-	+	+	-	+	-	+
	AB	Anomocytic	15–18 × 13–17	/	/	+	-	+	+	++	+	-	+
<i>Neoptilingia leucophylloides</i>	AD	/	13 × 14	Irregular	st/cur	-	-	-	-	++	+	-	+
	AB	/		Irregular	st/un	-	-	-	-	++	+	-	+
<i>Obegomeria caernlescens</i>	AD	/		Striated isometric	cur	+	-	-	+	-	+	-	+
	AB	/		Striated isometric	cur	-	-	-	+	-	+	-	+
<i>Origanum rotundifolium</i>	AD	Aniso/dia/dialleloctytic	20–24 × 15–20	Isometric	st/cur	-	-	-	-	+	+	-	+
	AB	Dia/dialleloctytic	17–23 × 13–18	Isometric	st/un	-	-	-	-	-	+	-	+
<i>Origanum vulgare</i>	AD	Anomocytic	12–15 × 10–12	Isometric	cur/un	-	-	-	+	-	+	-	+
	AB	Anom/dia/dialleloctytic	18–21 × 12–16	Irregular	un/sin	-	-	+	+	-	+	-	+
<i>Pentapleura subtilifera</i>	AD	Diacytic	17–23 × 12–16	Partially striated irregular	un	-	-	-	+	-	+	-	+
	AB	Diacytic	12–18 × 12–16	Irregular	cur	-	-	-	+	-	+	-	+
<i>Pioblophus rigida</i>	AD	/		Isometric	st/cur	+	-	-	+	-	+	-	+
	AB	/		Striated isometric	st/cur	+	-	-	+	-	+	-	+
<i>Pogogyne douglasii</i>	AD	Diacytic	24–27 × 16–20	Partially striated isometric	cur	-	-	-	-	+	+	-	+
	AB	Diacytic	21–24 × 16–18	Partially striated irregular	un/sin	-	-	-	-	-	+	-	+
<i>Pogogyne serpylloides</i>	AD	Anomocytic	19–22 × 14–17	Partially striated irregular	sin	+	mo	-	-	-	+	-	+
	AB	Anomocytic	13–16 × 12–14	Partially striated irregular	sin	+	m-vf	-	-	-	+	-	+
<i>Poliomintha glabrescens</i>	AD	Striated diacytic	24–28 × 16–19	Irregular	un	-	-	+	+	+	+	-	+
	AB	Anomocytic	21–26 × 16–19	/	/	++	-	+	+	+	+	-	+
<i>Poliomintha incana</i>	AD	/	14–28 × 14–17	Striated	/	-	-	+	+	-	+	-	+
	AB	/		Striated	/	-	-	++	+	-	+	-	+
<i>Poliomintha longiflora</i>	AD	/		Irregular	sin	+	-	+	+	-	+	-	+
	AB	/		#	/	+	-	+	+	-	+	-	+
<i>Prunella vulgaris</i>	AD	Dia/dialleloctytic	27–30 × 19–22	Striated isometric	un	-	-	-	+	-	-	-	-
	AB	Dia/dialleloctytic	20–25 × 17–20	/	/	-	-	-	+	-	-	-	-
<i>Pycnanthemum albescens</i>	AD	/	18–21 × 14–18	#	/	+	-	++	+	-	+	-	++
	AB	/		#	/	+	-	++	+	-	+	-	++
<i>Pycnanthemum incanum</i>	AD	Anomocytic	19–24 × 18–20	Irregular	un	-	-	+	+	-	+	-	+
	AB	Anomocytic		/	/	-	-	+	+	-	+	-	+
<i>Rhabdocaulon coccineum</i>	AD	Dia/dialleloctytic	22–27 × 15–20	Isometric	st/cur	+	-	-	-	-	+	-	+
	AB	Dia/dialleloctytic		Irregular	un	-	-	-	-	-	+	-	+
<i>Rhabdocaulon strictum</i>	AD	/		Striated irregular	st/cur	+	mo	-	-	-	+	-	+
	AB	/		Isometric	st/cur	-	-	-	-	-	+	-	+
<i>Rhododon ciliatus</i>	AD	Dialleloctytic	23–29 × 18–21	Isometric	sin	+	-	-	-	-	+	-	+
	AB	Anomocytic	22–25 × 17–21	Partially striated irregular	sin	+	-	-	-	-	+	-	+
<i>Saccocalyx saturoioides</i>	AD	Anomocytic	18–21 × 13–18	Partially striated irregular	sin	+	-	-	-	-	+	-	+
	AB	Diacytic	17–24 × 13–15	/	/	+	-	-	+	-	+	-	+
<i>Satureja thymbra</i>	AD	Striated dialleloctytic	15–19 × 12–15	Irregular	/	+	-	-	+	-	+	-	+
	AB	Dia/dialleloctytic	25–28 × 18–20	Irregular	un	-	-	+	+	-	+	-	+
<i>Stachydeoma graveolens</i>	AD	/	21–25 × 17–19	Irregular	un	-	-	+	+	-	+	-	+
	AB	Diacytic	16–20 × 15–18	Irregular	un/sin	+	-	-	+	-	+	-	+
<i>Thymbra spicata</i>	AD	/	16–19 × 14–17	/	/	+	-	-	-	-	+	-	+
	AB	/	23–28 × 20–24	Partially striated isometric	cur/un	+	vo	-	-	-	+	-	+
<i>Thymus pallasiensis</i>	AD	Diacytic/	24–27 × 17–22	Isometric	cur/un	+	-	-	-	+	+	-	+
	AB	/	21–23 × 15–17	Striated irregular	cur/un	+	mf	-	-	-	+	-	+
<i>Thymus serpyllum</i>	AD	/	20–23 × 13–16	Striated irregular	cur	+	-	-	-	-	+	-	+
	AB	Diacytic	26–30 × 21–23	Irregular	un	-	-	-	-	-	+	-	+
<i>Zataria multiflora</i>	AD	/	30–36 × 18–20	Striated irregular	cur/un	+	-	-	-	-	+	-	+
	AB	Dia/dialleloctytic	10–13 × 8–12	Irregular	sin	+	-	+	-	-	+	-	+
	AB	Dia/dialleloctytic	15–18 × 10–14	/	/	+	-	+	-	-	+	-	+







**Figure 1.** Scanning electron micrographs of leaf surfaces of subtribes Salviinae and Nepetinae. A, *Dorystaechas hastata*. Adaxial surface showing cluster of branched nonglandular trichomes. B, *Melissa officinalis* L. Abaxial surface with simple nonglandular trichomes and anomocytic stomata. C, D, *Meriandra bengalensis* showing clear venation border with trichome distribution (C, adaxial surface; D, abaxial surface). E, *Perovskia abrotanoides*. Abaxial surface showing striate irregular epidermal cells. F, *Rosmarinus officinalis*. Abaxial surface of tightly revolute leaf. G, *Salvia officinalis*. Adaxial surface. H, *Salvia dorrii*. Adaxial surface with sessile glandular trichomes and pear-shaped head attached excentrically to the stalks. I, *Agastache urticifolia* Kuntze. Adaxial surface showing simple nonglandular trichomes with irregular epidermal cells and sinuate cell walls. J, *Cedronella canariensis* (L.) Webb & Berthel. Adaxial surface showing irregular epidermal cells with sinuate cell walls. K, L, *Dracocephalum ruyschiana* L. K, Adaxial surface showing isometric epidermal cells with well-developed striations and simple nonglandular trichomes. L, Abaxial surface showing irregular epidermal cells with striations and actinocytic/diacytic stomata. M, N, *Glechoma hederacea*. M, Adaxial surface with partially striated irregular epidermal cells with sinuate anticlinal walls. N, Abaxial surface with anomocytic/anisocytic/diacytic stomata. O, *Nepeta nuda* L. Abaxial surface showing distribution of trichomes on leaf blade and vein.

these, nonglandular and glandular, according to the absence or presence of a secretory head on the trichome. For the glandular trichomes, we distinguished between capitate and sessile trichomes following Cantino (1990).

#### Nonglandular trichomes

Three types of nonglandular trichome were observed: simple unicellular trichomes, uniseriate trichomes and branched (= dendriform) trichomes (Fig. 3). In the simple unicellular and uniseriate trichomes, we defined two subtypes according to their length. The long simple unicellular trichomes (> 100 µm) were only found in *Conradina grandiflora*, *Salvia officinalis* L. and *Salvia taraxacifolia* Coss. & Bal. on both sides with similar frequency. Long uniseriate trichomes (> 200 µm) occurred throughout Mentheae and were found together with short uniseriate trichomes (Table 1). The simple unicellular and uniseriate trichomes were usually distributed evenly on the leaf, but sometimes occurred more frequently on the margin or vein, or were only found on the margin or vein (Table 1; Fig. 3H). Branched trichomes were found in all genera of Salviinae and also in *Hedeoma ciliolata* and *Neoplegingia leucophylloides* Ramamoorthy of Menthinae (Table 1; Fig. 3I–L). Simple unicellular and uniseriate trichome types were common and widespread in Mentheae.

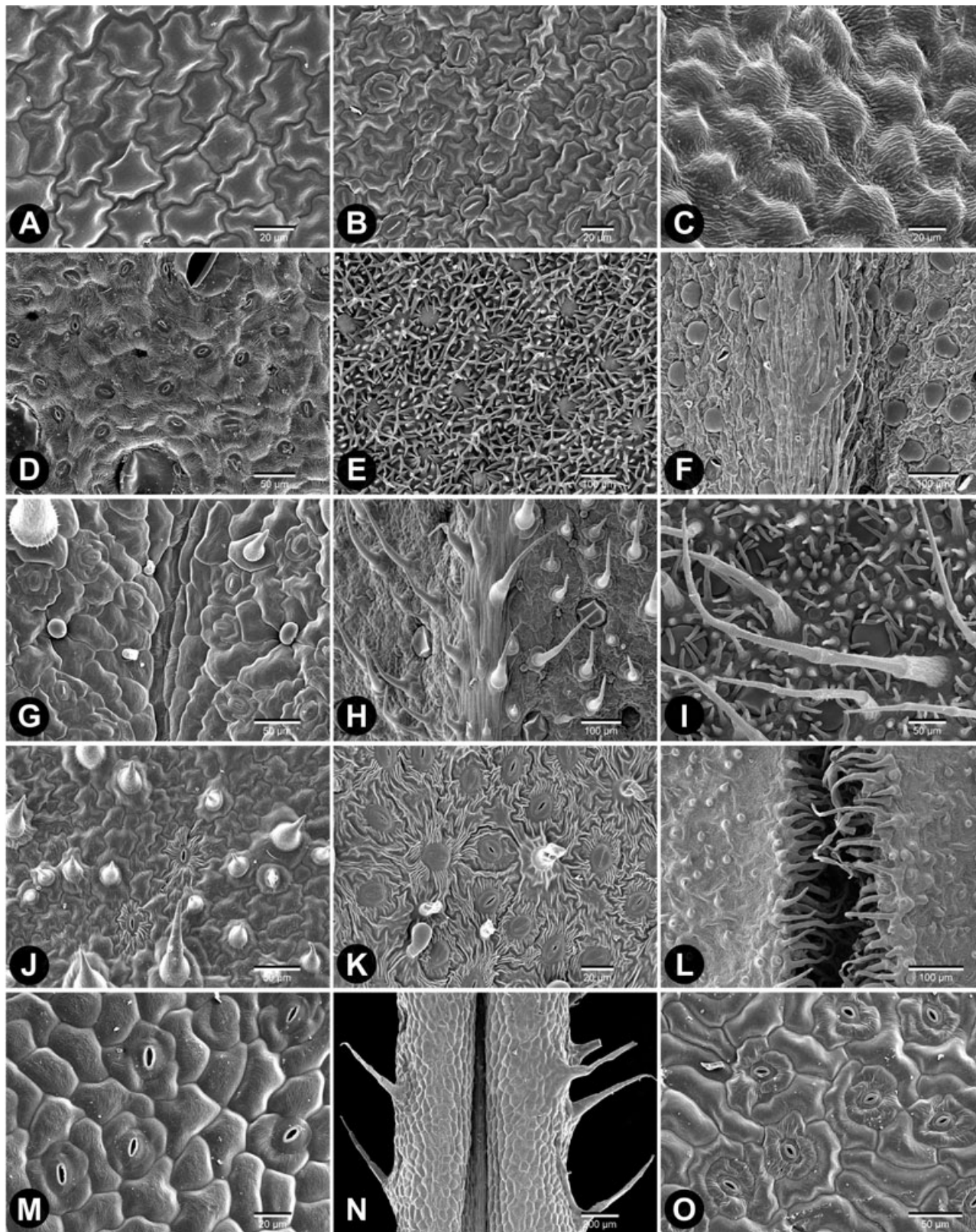
#### Glandular trichomes

Three different glandular trichomes were present: capitate trichomes, pilate trichomes and sessile glandular trichomes (= peltate glandular trichomes; Fig. 4). In capitate glandular trichomes, the head cell was attached to a single cell stalk, whereas, in pilate glandular trichomes, the stalk consisted of more than one cell (Fig. 4A–F). Capitate glandular trichomes occurred in most taxa of Mentheae, but were not found in *Conradina canescens*, *Conradina grandiflora*, *Dicerandra christmanii* Huck & Judd, *Drepanocaryum sewerzowii* (Regel) Pojark., *Gontscharovia*

*popovii* (B.Fedtsch. & Gontsch.) Boriss., *Hedeoma ciliolata*, *Lallemantia peltata* (L.) Fisch. & C.A.Mey., *Marmoritis rotundifolia* Benth., *Prunella vulgaris* L., *Schizonepeta multifida* (L.) Briq., *Zataria multiflora* and *Ziziphora clinopodioides* Lamarck. Pilate glandular trichomes were found in *Acanthomintha lanceolata* Curran, *Acanthomintha obovata* Jeps., *Dorystaechas hastata* Boiss. & Heldr. ex Benth., *Salvia canariensis* L., *Salvia rypara* Briq. and *Salvia verbenaca* L. Capitate and pilate glandular trichomes had a spherical head attached centrally to the stalks (Fig. 4A–F). *Salvia dorrii* was characterized by capitate glandular trichomes with a pear-shaped head attached excentrically to the stalk (Fig. 1J). Following Cantino (1990), sessile glands could be divided into several subtypes based on the number of cells and the cell wall configurations in the head of the gland. However, in Mentheae, the number of cells in the head of the gland often varied within the same species, and sometimes the number of head cells could not be counted. Therefore, we focused on the presence or absence of sessile glands and their density. Glands with four- or eight-celled heads were rather common in Mentheae (Fig. 4G, H). In addition, multicellular head (more than ten cells) glands occurred only in *Perovskia* Karel. (Fig. 4L). Sessile glandular trichomes were distributed throughout Mentheae, like capitate glandular trichomes, except in *Bystropogon canariensis*, *Clinopodium vulgare* L., *Conradina canescens*, *Hymenocrater bituminosus* Fisch. & C.A.Mey., *Lallemantia peltata*, *Lophanthus tschimganicus* Lipsky, *Prunella vulgaris*, *Rosmarinus officinalis* and *Salvia taraxacifolia*.

#### LEAF ANATOMY

Laminas were dorsiventral or isobilateral (Fig. 5). The epidermis was composed of a single cell layer, and the cells were rectangular or oval on both sides of the leaf. The mesophyll was differentiated into a two-seriate palisade and a one- to four-seriate spongy



**Figure 2.** Scanning electron micrographs of leaf surfaces of subtribe Menthinae. A, B, *Clinopodium vulgare*. A, Adaxial surface showing irregular epidermal cells. B, Abaxial surface showing distribution of anomocytic/diacytic stomata. C, *Cuinia eriantha* var. *fernandezia*. C, Adaxial surface showing striated isometric epidermal cells. D, *Dicerandra christmanii*. D, Adaxial surface showing striated isometric epidermal cells. E, *Hedeoma ciliolata*. E, Abaxial surface showing distribution of subsessile glandular trichomes and branched nonglandular trichomes. F, *Hoehnea minima*. F, Abaxial surface showing distribution of trichomes. G, H, *Mentha pulegium* L. G, Adaxial surface showing irregular epidermal cells with diacytic/diallelocytic stomata. H, Abaxial surface showing distribution of trichomes. I, *Monarda fistulosa*. I, Abaxial surface showing distribution of trichomes. J, K, *Micromeria marginata*. J, Adaxial surface showing irregular epidermal cells and striated stomata. K, Abaxial surface showing striated irregular epidermal cells. L, *Obtegomeria caerulea* (Benth.) Doroszenko & P.D.Cantino. L, Abaxial surface of tightly revolute leaf. M, *Origanum rotundifolium*. M, Adaxial surface showing isometric epidermal cells with diacytic stomata. N, *Piloblephis rigida* (W.Bartram ex Benth.) Raf. N, Abaxial surface of tightly revolute leaf. O, *Satureja thymbra*. O, Adaxial surface showing irregular epidermal cells and striated diallelocytic stomata.

parenchyma. Palisade tissue was present below the upper and lower epidermis (Fig. 5H, J, K), and cells were cylindrical in transverse section. The spongy parenchyma cells, circular or ovoid, were located between the palisade tissues (Fig. 5G, H, J). The midrib region was well developed and projected outwards (Fig. 5A–I). Vascular bundles were collateral. The xylem faced the AD side, and the phloem the AB side. Vascular bundles were covered with parenchymatous cells. Collenchymatous cells occurred below the upper and lower epidermis in the midrib region. In *Micromeria marginata*, *Monardella odoratissima* Benth., *Saccocalyx satuireioides* Coss. & Durand and *Thymus serpyllum*, vascular bundles in the midrib functioned as a mechanical tissue composed of compact thick-walled sclerenchymatous cells (Fig. 5H, I).

## DISCUSSION

### LEAF MICROMORPHOLOGICAL CHARACTERISTICS OF MENTHEAE

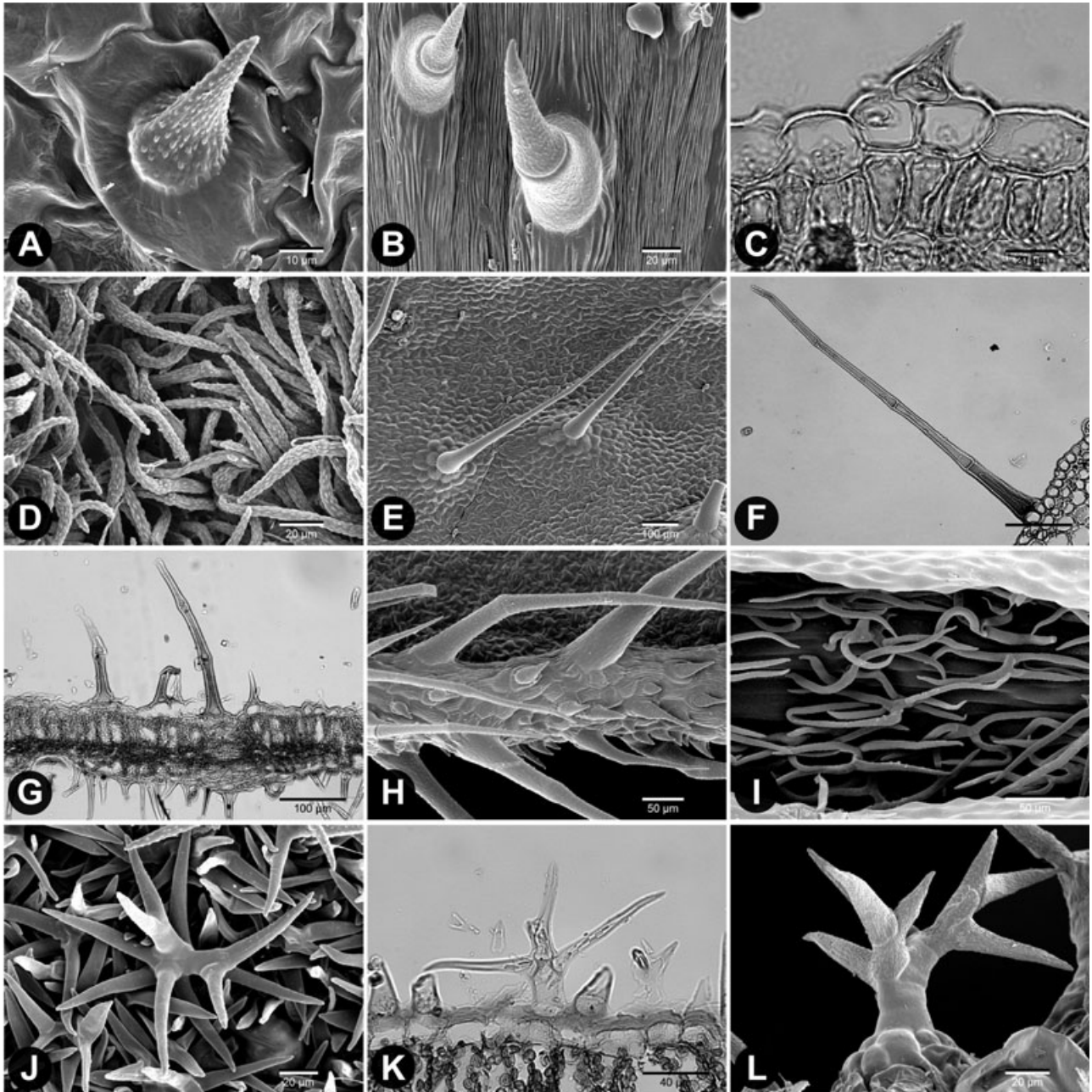
The leaf characteristics of Mentheae show a great diversity in epidermal cell outline, stomatal complex and structure and density of trichomes, and the pattern of epidermal anticlinal cell walls may be closely related to ecological conditions. In general, straight or curved walls are characteristic of species growing in drier conditions, whereas undulate walls are found in species inhabiting more humid areas (Stace, 1965). However, our results do not support this hypothesis. Undulate or sinuate anticlinal walls often appeared in species occurring in xeric habitats, including *Hedeoma ciliolata*, *Poliomintha glabrescens* and *Ziziphora clinopodioides*. In addition, more than one anticlinal wall type was sometimes found within the same leaf (Table 1).

Cantino (1990) found five different types of stomata in Mentheae. El-Gazzer & Watson (1968) investigated stomatal configurations in a wide range of Lamiaceae, but listed only the predominant type in each genus.

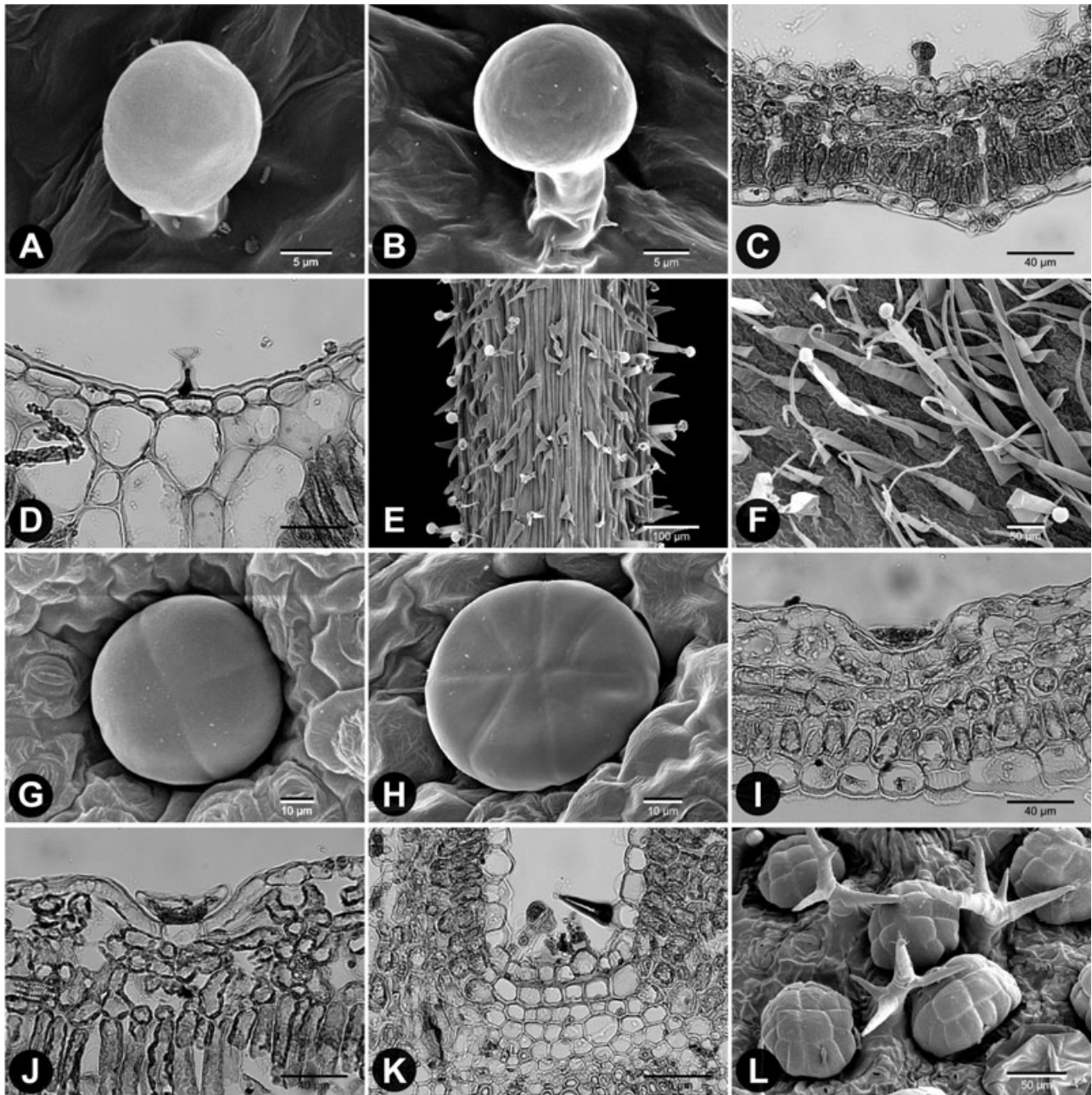
Furthermore, their observations regarding many genera of Lamiodeae conflicted with Cantino's findings (1990). Stomatal position and types from the present study are comparable with those in the study of Cantino (1990). Anomocytic, diacytic and diallelocytic stomatal types were most common in Mentheae, but anisocytic and actinocytic stomata were sometimes found together with anomocytic or diacytic stomata (Table 1). It is notable that all examined taxa had mainly anomocytic or diacytic stomata. Mentheae commonly had amphistomatic leaves, which are believed to occur more frequently in xeric habitats (Parkhurst, 1978). In the amphistomatic species, stomata were more numerous on the AB surface. We found only one incongruent case in *Monarda fistulosa* L., which was reported as hypostomatic (Cantino, 1990), but was amphistomatic with a few stomata on the AD surface in the present study.

Trichome diversity in Lamiaceae may be taxonomically significant at various taxonomic levels (Rudall, 1979, 1980; Abu-Asab & Cantino, 1987; Cantino, 1990; Demissew & Harley, 1992; Ayodele & Olowokudejo, 2006). However, the structure and density of trichomes are diverse, and sometimes too variable for phylogenetic use (Guerin, 2005). Trichomes are one of the most important traits contributing to passive resistance of plants to pathogens, pests and drought (Levin, 1973), and may play an important role in the adaptation to environments with high levels of irradiance (Stenglein *et al.*, 2005). The types of trichome, however, are usually constant in species groups (Stace, 1965; Okpon, 1969). The leaf epidermis of *Pistacia atlantica* Desf. (Anacardiaceae), for instance, has been shown to have the same trichome type across several populations under different climatic conditions, although the density of trichomes differed among the populations according to the altitude (Belhadj *et al.*, 2007).

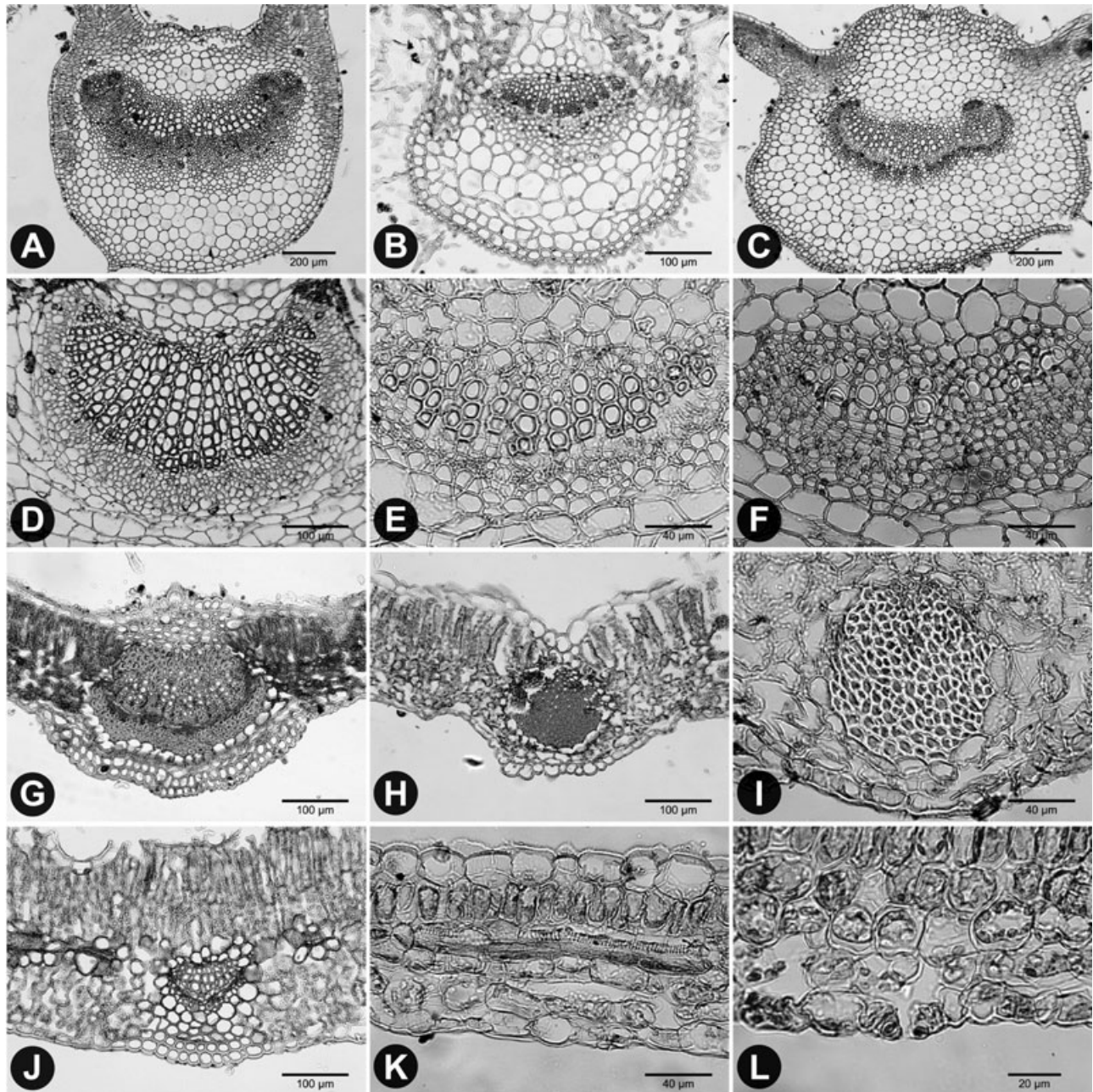
In Mentheae, the types of trichome recognized were consistent with previously published data (Bokhari & Hedge, 1971; Cantino, 1990; Kaya *et al.*, 2000; Jang &



**Figure 3.** Light and scanning electron micrographs of nonglandular trichomes of tribe Mentheae. A, *Blephilia ciliate* Raf. Simple short nonglandular trichomes (< 100 µm). B, *Micromeria marginata*. Short uniseriate nonglandular trichomes (< 200 µm). C, *Glechoma hederacea*. Structure of simple unicellular nonglandular trichome. D, *Conradina grandiflora*. Simple long nonglandular trichomes (> 100 µm). E, *Clinopodium vulgare*. Long uniseriate nonglandular trichomes (> 200 µm). F, *Minthostachys mollis* (Kunth) Griseb. Structure of long uniseriate nonglandular trichome. G, *Conradina canescens*. Cross-section of leaf with structure of simple short nonglandular trichomes and short uniseriate nonglandular trichomes. H, *Clinopodium vulgare*. Distribution of simple short and long uniseriate nonglandular trichomes on the leaf margin. I, *Rosmarinus officinalis*. Branched nonglandular trichomes at abaxial surface. J, K, *Hedeoma ciliolata*. J, Branched nonglandular trichomes. K, Structure of branched nonglandular trichome. L, *Perovskia abrotanoides*. Branched nonglandular trichome.



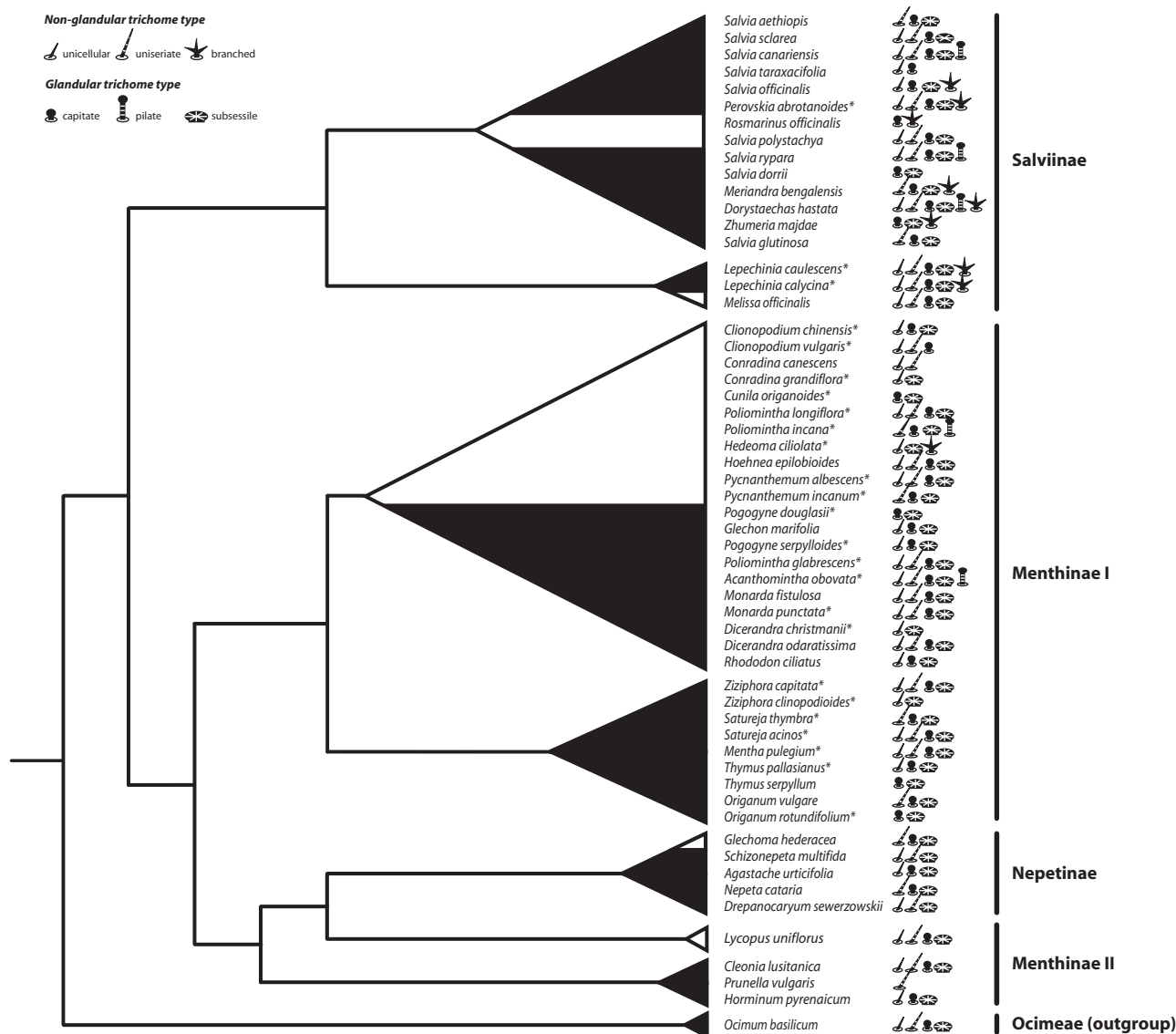
**Figure 4.** Light and scanning electron micrographs of glandular trichomes of tribe Mentheae. A, *Clinopodium acinos* Kuntze. Short stalked capitate glandular trichome. B, *Lepechinia calycina* Epling. Capitate glandular trichome. C, *Nepeta cataria*. Cross-section of leaf; capitate glandular trichome on the abaxial surface with stoma on the adaxial surface. D, *Rosmarinus officinalis*. Cross-section of leaf showing structure of capitate glandular trichome on the adaxial surface. E, F, *Acanthomintha obovata*. E, Pilate glandular trichomes on petiole. F, Pilate glandular trichomes on the adaxial surface. G, *Nepeta nuda*. Glands with four-celled head on the abaxial surface. H, *Blephilis ciliata*. Glands with eight-celled head on the abaxial surface. I, J, Cross-section of leaf showing structure of subsessile glandular trichome on the abaxial surface. I, *Glechoma hederacea*. J, *Origanum vulgare*. K, *Nepeta cataria*. Cross-section of leaf showing capitate glandular trichome and simple trichome on the midrib on the adaxial surface. L, *Perovskia abrotanoides*. Multicellular head (more than ten) glands with branched nonglandular trichome on the abaxial surface.



**Figure 5.** Light micrographs of cross-sections of leaves in Mentheae. A–J, Midrib structure with vascular bundle. A, *Perovskia scrophulariifolia*. B, *Rosmarinus officinalis*. C, *Salvia glutinosa*. D, *Agastache urticifolia*. E, *Mentha pulegium*. F, *Prunella vulgaris*. G, *Cunila organoides*, showing xylem fibres and phloem fibres. H, I, Vascular bundle modified into a mechanical tissue. H, *Micromeria marginata*. I, *Thymus serpyllum*. J, *Dicerandra christmanii* showing a vascular bundle with an extension area towards the abaxial surface. K, L, *Glechoma hederacea*. K, Leaf structure with longitudinal section of vein. L, Stomata on the abaxial surface.

Hong, 2007; Satil, Ünal & Hopa, 2007). To address the distribution patterns of each trichome type, we used the most comprehensive phylogeny of Mentheae (Walker & Sytsma, 2007; Fig. 6). Non-glandular trichomes, both simple-unicellular and uniseriate, occurred widely in Mentheae. The presence of both

kinds of trichome is a common condition. The presence of only uniseriate or unicellular trichomes may represent a diagnostic characteristic. The presence of branched trichomes in most Chloanthoideae Briq. was suggested as a possible synapomorphy for this former subfamily, although branched nonglandular



**Figure 6.** Diagrams of trichome types in Mentheae and their distribution on the most recent molecular phylogenetic tree (simplified tree based on Walker & Sytsma, 2007, Figs 3, 4). For each taxon the occurrence of stomata on the adaxial side of leaves is shown within the clades: Black, amphistomatic leaves, white, hypostomatic leaves. The taxa indicated by an asterisk were included in the present study but lacking in Walker & Sytsma (2007), and their phylogenetic position is estimated by assuming that the genera are monophyletic.

trichomes were also found in Ocimeae Dumort. and Lamioideae (Cantino, 1990). However, our results show that the presence of branched trichomes evolved at least twice, even within Mentheae (Fig. 6). Branched trichomes occurred mainly in Salviinae, but this type of trichome was also found in *Hedeoma ciliolata* and *Neoepplingia leucophylloides* of subtribe Menthinae (Table 1).

Glandular trichomes were almost universally present in Mentheae (Fig. 6); their absence is a characteristic of only *Conradina canescens* and *Prunella*

*vulgaris*. Capitate glandular trichomes were observed in all taxa of Salviinae and were also present in Mentheae. The absence of capitate glandular trichomes is a possible synapomorphy for certain species. Subsessile glandular trichomes have been widely reported in Lamiaceae (Solereider, 1908; Metcalfe & Chalk, 1950; Huang & Cheng, 1971; Bosabalidis & Tsekos, 1982; Werker *et al.*, 1985; Cantino, 1990). Our results also show a wide distribution of subsessile glandular trichomes in Mentheae (Fig. 6). Subsessile glandular trichomes with multicellular

heads were restricted to *Perovskia* (Fig. 4L), whereas four- to eight-celled head sessile glands were rather common throughout the tribe (Fig. 4G, H). Glandular trichomes normally co-occurred with nonglandular trichomes, but, in *Cuminia eriantha* var. *fernandezia* (Colla) Harley, *Cunila organoides* (L.) Britton, *Hoehnea minima*, *Lophanthus tschimganicus*, *Origanum rotundifolium* Boiss., *Pogogyne douglasii* Benth. and *Thymus serpyllum*, glandular trichomes were observed alone.

The subtribal delimitation of Mentheae has been questioned by molecular studies (Wagstaff *et al.*, 1995; Walker & Sytsma, 2007). Although all published molecular phylogenetic analyses are largely congruent with the subtribal delimitation of Mentheae *sensu* Harley *et al.* (2004), the monophyly of Menthinae and Nepetinae is still questioned. Because the phylogenetic significance of pollen and nutlet features, including sexine ornamentation, morphology of abscission scar and surface pattern of nutlets, was demonstrated (Moon, 2008; Moon *et al.*, 2008a, b), we might expect that additional morphological characteristics could help to solve the remaining taxonomic problems in Mentheae, especially at the subtribal level. Although the leaf morphological characteristics are too variable to be used phylogenetically, it is too early to draw conclusions on the systematic importance of leaf morphology in Mentheae. Indeed, a thorough molecular phylogeny of Mentheae is still lacking. Our results confirm that leaf morphological characteristics are useful for the identification of species in Mentheae.

#### LEAF MICROSTRUCTURE AND FLORAL DIMORPHISM

The present study included the gynodioecious *Glechoma hederacea* L. (separate hermaphroditic and female plants coexist in a single population) and the distylic *Perovskia abrotanoides* (two types of flower with different style lengths). Gynodioecy is rather common in Lamiaceae, whereas heterostyly has been reported in only a few species (Owens & Uebera-Jiménez, 1992; Moon *et al.*, 2008b). Floral size dimorphism between two flower types within gynodioecy (hermaphrodite + female) and heterostyly (pin + thrum) is well known in flowering plants (Delph, 1996). Significant size dimorphism between different floral morphs has been found in *Glechoma hederacea* (Widén, 1992) and *Perovskia abrotanoides* Kar. (H.-K. Moon, unpubl. data). However, we could not find micromorphological differences between different flower morphs. This is consistent with the former study of a gynodioecious species *Lycopus maackianus* Makino (Hong & Moon, 2003). Indeed, the leaf micromorphological features were almost identical, except for the density of certain trichome types (Table 1).

This result might suggest that the micromorphological characteristics of leaves are more closely associated with species circumscription than with gender dimorphism.

#### LEAF ANATOMICAL STRUCTURE

The midrib vasculature and anatomical structure of leaves were generally uniform throughout the tribe. In *Micromeria marginata*, *Monardella odoratissima*, *Saccocalyx satureioides* and *Thymus serpyllum*, the vascular bundle of the midrib was modified into a mechanical tissue. This is often found in plants which grow in seasonally dry or nutrient-poor environments (Rudall, 1980). *Micromeria marginata*, *Saccocalyx satureioides* and *Thymus serpyllum* are tiny shrubs and have small leaves with revolute margins. These are all typical xeromorphic adaptations (Rudall, 1979, 1980). The presence of glandular trichomes is another xeromorphic characteristic (Rudall, 1980), although glandular trichomes occur throughout the tribe (Table 1). Further leaf anatomical studies of Mentheae with expanded taxon sampling will be necessary to ascertain whether the variation in leaf microstructure reflects phylogenetic relationships among species, or whether it may be influenced significantly by ecological factors.

#### CONCLUSIONS

The systematic value of leaf epidermal micromorphological characteristics was restricted to the subtribal level in Mentheae. Branched nonglandular trichomes were found throughout subtribe Salviinae, but it was not an apomorphic trait. The great diversity of stomata and trichomes in Mentheae was useful at rather lower taxonomic levels (genus or species). Subsessile glandular trichomes with multicellular heads are diagnostic for the genus *Perovskia*. *Salvia dorrii* is easily recognized by trichomes with pear-shaped heads attached excentrically to the stalks. *Rosmarinus officinalis* is characterized by a few capitate glandular trichomes at the AD surface with a cluster of branched nonglandular trichomes at the AB surface. Therefore, the combination of leaf micromorphological characteristics could be helpful in the identification of species. Nonetheless, the value of these features might be better appreciated in Mentheae by means of a phylogenetic approach when used in conjunction with other morphological and molecular characteristics.

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## APPENDIX

## VOUCHER SPECIMENS OF TRIBE MENTHEAE EXAMINED IN THE PRESENT STUDY

Subtribe Salviinae		
<i>Chaunostoma mecistandrum</i> Donn. Sm.	A, S	Mexico, 06.xi.1939. Matuda 3915, GH
<i>Dorystaechas hastata</i> Boiss. & Heldr. ex Benth.	A, S	Turkey, 05.xi.1998. Verlooue 3102, BR
<i>Lepechinia calycina</i> (Benth.) Epling ex Munz	S	USA, 24.v.1986. Dechamps 4201, BR
<i>Lepechinia caulescens</i> (Ortega) Epling	A, S	Mexico, 04.ix.1890. Pringle 3264, BR
<i>Meriandra bengalensis</i> (Roxb.) Benth.	A, S	Yemen, 12.ix.1977. Lavranos & Newton 15796, MO
<i>Perovskia abrotanoides</i> Kar. – thrum type	S	Nepal, 04.vii.1976. Billiet and Leonard 6805, BR
<i>Perovskia abrotanoides</i> Kar. – pin type	A, S	Nepal, 11.vii.1976. Billiet and Leonard 6888 BR
<i>Perovskia scrophulariifolia</i> Bunge	A, S	Cultivated in Gent: 09.vi.2005. Moon, LV
<i>Rosmarinus officinalis</i> L.	S	Spain, 18.ix.1983. Cnops 83.56, BR
<i>Rosmarinus officinalis</i> L.	A	Belgium, 09.vi.2005. Moon, LV
<i>Salvia aethiopsis</i> L.	S	Hungary, viii.1876. Rechter s.n., BR
<i>Salvia canariensis</i> L.	A, S	Spain, 04.x.1995. Hanson GC95-62, BR
<i>Salvia coccinea</i> Buc'hoz ex Etl.	A, S	Spain, 25.xii.1973. Lewalle 7322, BR
<i>Salvia dorrii</i> (Kellogg) Abrams	S	USA, 23.v.1965. Cronquist 10171, BR
<i>Salvia glutinosa</i> L.	A, S	France, 02.ix.1985. Dechamps 2810, BR
<i>Salvia officinalis</i> L.	S	Spain, 1915. Elías 2450, LV
<i>Salvia officinalis</i> L.	A	Cultivated in BR: 22.vi.2005. Moon, LV
<i>Salvia polystachya</i> Ort.	A, S	Mexico, 03.x.1980. Rodriguez 5019, BR
<i>Salvia pratensis</i> L.	S	France, 1973. Witte 17388, LV
<i>Salvia rypara</i> Briq.	A, S	Bolivia, 07.xi.1993. Billiet & Jadin 6123, BR
<i>Salvia sclarea</i> L.	S	France, 24.vii.1953. Andre 11, BR
<i>Salvia taraxacifolia</i> Coss. & Bal.	A, S	Morocco, 09.vii.1984. Lewalle 11087, BR
<i>Salvia verbenaca</i> L.	S	Without locality, 1919 Sennen s.n., LV
<i>Salvia verticillata</i> L.	S	The Netherlands, 14.vii.1949. Bakhuizen & Van den Brink 6794, BR
<i>Zhumeria majdae</i> Rech.f. & Wendelbo	S	Iran, 08.iv.1975. Wendelbo & Foroughi 15731, K
Subtribe Menthinae		
<i>Acanthomintha lanceolata</i> Curran	A, S	USA, 05.v.1919 Mason 12284, BR
<i>Acanthomintha obovata</i> Jeps.	S	USA, 18.v.1919. Walker 5094, BR
<i>Blephilia ciliata</i> (L.) Benth.	S	USA, 13.vi.1966 Radford 44758, BR
<i>Bystropogon canariensis</i> (L.) L'Hér	A, S	France, without date, Bullemont 1855 (hermaphrodite), BR
<i>Cleonia lusitanica</i> (L.) L.	A, S	Morocco, 13.v.1934. Wall 45, S
<i>Clinopodium acinos</i> (L.) Kuntze	S	Cultivated in KEW 1989-3009: 04.vii.2006. Moon, LV
<i>Clinopodium chinense</i> (Benth.) Kuntze	S	South Korea, without date 1931 Mori s.n., SNU
<i>Clinopodium vulgare</i> L.	S	Cultivated in KEW 1994-2824: 04.vii.2006. Moon, LV
<i>Conradina canescens</i> A. Gray	A, S	USA, 30.xo.1969. Godfrey 69283, BR
<i>Conradina grandiflora</i> Small	S	USA, 24.x.1956. Ahles & Bell 21395, BR
<i>Cuminia eriantha</i> var. <i>fernandezia</i> (Colla) Harley	S	Chile, 23.xi.1991. Billiet & Jadin 5631, BR
<i>Cunila origanoides</i> (L.) Britton	S	USA, 07.ix.1897 Anonymous 323b, BR
<i>Cunila origanoides</i> (L.) Britton	A	USA, 09.vi.1972. Kral 48356, K
<i>Cyclotrichium oranifolium</i> (Labill.) Manden.	A, S	Lebanon, 06.vii.1897. Bornmüller 1260, BR
<i>Dicerandra christmanii</i> Huck & Judd	A, S	USA, 11.ix.1987. Skeeon, Jr. 2130, MO
<i>Dicerandra odoratissima</i> R.M.Harper	S	Turkey, 17.ix.1967. Radford & Leonard 11479, BR
<i>Glechon marifolia</i> Benth.	A, S	Uruguay, 12.ii.1955. Pedersen 3627, BR
<i>Gontscharovia popovii</i> (B.Fedtsch. & Gontsch.) Borris.	A, S	Russia, 31.viii.1931. Anonymous s.n., K
<i>Hedeoma ciliolata</i> (Epling & Stewart) Irving	A, S	Mexico, 01.x.1954. Rzedowski 5003, GH
<i>Hesperozygis nitida</i> (Benth.) Epling	A, S	Brazil, 22.ix.1976. Dombrowski 6442, K
<i>Hoehnea epilobioides</i> (Epling) Epling	A, S	Brazil, 23.x.1974. Kummrow 688, K
<i>Hoehnea minima</i> (Schmidt) Epling	S	Brazil, 26.i.1916. Dusén 17542, GH
<i>Horminum pyrenaicum</i> L.	A, S	Italy, 26.vi.1969. Cnops 21169, BR
<i>Hyssopus officinalis</i> L.	S	Ecuador, 22.ix.1974. Bondía <i>et al.</i> , 1242GF, BR
<i>Hyssopus officinalis</i> L.	A	Cultivated in Kew 1975-1170: 03.vii.2006. Moon, LV

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<i>Kurzamra pulchella</i> (Clos) Kuntze	S	Chile, i.1924. Werdermann 263, GH
<i>Lycopus europaeus</i> L.	A	Belgium, 07.viii.2005. Moon, LV
<i>Mentha pulegium</i> L.	A, S	Cultivated in Kew 1994-1897: 04.vii.2006. Moon, LV
<i>Micromeria marginata</i> (Sm.) Chater	A, S	Cultivated in Kew 1995-1960: 04.vii.2006. Moon, LV
<i>Minthostachys mollis</i> Griseb	S	Ecuador, 10.viii.1939. Asplund s.n., BR
<i>Minthostachys andina</i> (Britton ex Rusby) Epling	A, S	Bolivia, 22.viii.1993. Audiovio 585, BR
<i>Monarda fistulosa</i> L.	S	USA, 06.viii.1973. Bouharmont 8498, BR
<i>Monarda punctata</i> L.	S	USA, 26.x.1957. Ahles & Haesloop 38096, BR
<i>Monardella macrantha</i> A.Gray	S	Cultivated in Kew 1980-998: 03.vii.2006. Moon, LV
<i>Monardella nana</i> A.Gray	S	Cultivated in Kew 1999-270: 03.vii.2006. Moon, LV
<i>Monardella odoratissima</i> Benth.	S	USA, 29.viii.1969. Howell 46064, BR
<i>Neoplingia leucophylloides</i> Ramamoorthy	A, S	Mexico, 05.viii.1982. Medrano 12792, K
<i>Obtegomeria caerulescens</i> (Benth.) Doroszenko	A, S	USA, 16.viii.1986. Cuadrov & Gentry 2706, MO
<i>Origanum rotundifolium</i> Boiss.	S	Cultivated in Kew 1968-19106: 04.vii.2006. Moon, LV
<i>Origanum vulgare</i> L.	S	France, 1971. Witte 17047, LV
<i>Origanum vulgare</i> L.	A	Belgium, 22.vi.2005. Moon, LV
<i>Pentapleura subulifera</i> Hand.-Mazz.	A, S	Iraq, 4-9.vii.1957. Rechinger 12085, K
<i>Piloblephis rigida</i> (Bartram ex Benth.) Raf.	A, S	USA, 13.ii.1995. Holst <i>et al.</i> 4543, MO
<i>Pogogyne douglasii</i> Benth.	S	USA, 31.v.1892. Bioletti, BR
<i>Pogogyne serpylloides</i> (Torr.) A.Gray	A, S	USA, 28.iv.1964. Rose 64044, BR
<i>Poliomintha glabrescens</i> A.Gray ex Hemsl.	S	Mexico, 18.viii.1937. Wynd 696, GH
<i>Poliomintha incana</i> (Torr.) A.Gray	S	USA 18.vi.1985. Whiting 756/731, GH
<i>Poliomintha longiflora</i> A.Gray	S	Mexico, 11.ix.1955. Rzedwskiz 6583, GH
<i>Prunella vulgaris</i> L.	A, S	Belgium, 17.vi.2005. Moon, LV
<i>Pycnanthemum albescens</i> Torr. & A.Gray.	S	USA, 27.viii.1982 Kessler <i>et al.</i> 2648, BR
<i>Pycnanthemum incanum</i> (L.) Michx.	A, S	USA, 10.ix.1966. Bradley <i>et al.</i> 3491, BR
<i>Rhabdocalon coccineum</i> (Benth.) Epling	A, S	Brazil, 12.iv.1977. Harley 20332, K
<i>Rhabdocalon strictum</i> (Benth.) Epling	S	Argentina, 18.iv.1979. Persen 12455, GH
<i>Rhododon ciliatus</i> (Benth.) Epling	A, S	USA 06.vi.1969. Correll 37399, GH
<i>Saccocalyx satureioides</i> Coss. & Durand	A, S	Algeria, 26.v.1965. Faurel <i>et al.</i> 5650, BR
<i>Satureja thymbra</i> L.	A, S	Cultivated in Kew 2001-823: 04.vii.2006. Moon, LV
<i>Stachydeoma graveolens</i> (Chapm. ex A.Gray) Small	S	USA, 18.viii.1962. Godfrey 62494, BR
<i>Thymbra spicata</i> L.	S	France, 18.vi.1883. Ascherson 470, BR
<i>Thymbra spicata</i> L.	A	Cultivated in Kew 2001-825: 03.vii.2006. Moon, LV
<i>Thymus pallasianus</i> Heinr.-Braun	S	Cultivated in Kew 2001-4194: 04.vii.2006. Moon, LV
<i>Thymus serpyllum</i> L.	A, S	Cultivated in Kew 1973-21043: 04.vii.2006. Moon, LV
<i>Zataria multiflora</i> Boiss.	S	Iran, 16.v.1892. Bornmüller 4274, GH
<i>Ziziphora capitata</i> L.	S	Moldova, 03.vi.1971. Diaconescu s.n., BR
<i>Ziziphora clinopodioides</i> Lam.	A, S	Turkey, 01.ix.1993. Vašák s.n., BR
Subtribe Nepetinae		
<i>Agastache urticifolia</i> (Benth.) Kuntze	A, S	USA, 26.vi.1996. Bouharmont 26820, BR
<i>Cedronella canariensis</i> (L.) Webb & Berthel	A, S	Spain, Canary Is., 29.vi.1926. Linder 2670, GH
<i>Dracocephalum parviflorum</i> Nutt.	A, S	Canada, 22.vi.1978. Collet 111, BR
<i>Dracocephalum ruyschiana</i> L.	S	Russia, 28.vii.1981. Vašák s.n., BR
<i>Drepanocaryum sewerzowii</i> (Regel) Pojark.	A, S	Tajikistan, 23.v.1974. Vašák and Ziatník s.n., BR
<i>Glechoma hederacea</i> L. – hermaphrodite	A, S	Belgium, 19.v.2005. Moon, LV
<i>Glechoma hederacea</i> L. – female	S	Belgium, 19.v.2005. Moon, LV
<i>Hymenocrater bituminosus</i> Fisch. & C.A.Mey.	S	Iran, 28.vi.1942. Kyilbynacob s.n., BR
<i>Lallemantia peltata</i> (L.) Fisch. & C.A.Mey.	A, S	Without locality, vi.2002. Dagh s.n., BR
<i>Lallemantia royleana</i> (Benth.) Benth.	A, S	Iran, 04.v.1972. Léonard 5385, BR
<i>Lophanthus tschimganicus</i> Lipsky	S	Uzbekistan, 09.viii.1926. Baranov & Raikova s.n., BR
<i>Marmoritis rotundifolia</i> Benth.	S	China, 20.vii.1979. Hartmann 2464, G
<i>Meehanian urticifolia</i> (Miq.) Makino	A, S	Japan, viii.1936. Makino s.n., BR
<i>Nepeta cataria</i> L.	S	France, 18.vii.1975. Kapp s.n., LV
<i>Nepeta cataria</i> L.	A	Cultivated in Kew 1994-1793: 04.vii.2006. Moon, LV
<i>Nepeta grandiflora</i> L.	A, S	Cultivated in BR: 22.vi.2005. Moon, LV

APPENDIX *Continued*


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<i>Nepeta nuda</i> L.	S	Switzerland, Valais, 06.vii.1973. Lawalree 18115, LV
<i>Nepeta fissa</i> C.A.Mey.	S	Armenia, 04.vii.1894. Sintenis 6096, BR
<i>Schizonepeta multifida</i> (L.) Briq.	A, S	Russia, 20.vii.1974. Amebyehko and ChnpnIehko s.n., MO
<i>Schizonepeta tenuifolia</i> (Benth.) Briq.	A, S	Japan, without date, Makino s.n. 1932, BR
Unplaced genera		
<i>Heterolamium debile</i> (Hemsl.) C.Y.Wu	A, S	China, A. Henry s.n., March, 1889, K
<i>Melissa flava</i> Benth.	S	Nepal, without date, Kumaon <i>et al.</i> 1, BR
<i>Melissa officinalis</i> L.	S	France, 1986. Sotiaux s.n., BR
<i>Melissa officinalis</i> L.	A	Cultivated in Kew 1994-2690: 03.vii.2006. Moon, LV

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A, anatomical observation; S, scanning electron microscopy observation.