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ORIGINAL ARTICLE

Palynological evolutionary trends within the tribe Mentheae with special emphasis on subtribe Menthinae (Nepetoideae: Lamiaceae)

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Abstract The pollen morphology of subtribe Menthinae sensu Harley et al. [In: The families and genera of vascular plants VII. Flowering plants·dicotyledons: Lamiales (except Acanthaceae including Avicenniaceae). Springer, Berlin, pp 167–275, 2004] and two genera of uncertain subtribal affinities (*Heterolamium* and *Melissa*) are documented in order to complete our palynological overview of the tribe Mentheae. Menthinae pollen is small to medium in size (13–43 µm), oblate to prolate in shape and mostly hexacolpate (sometimes pentacolpate). Perforate, microreticulate or bireticulate exine ornamentation types were observed. The exine ornamentation of Menthinae is systematically highly informative particularly at generic level. The exine stratification in all taxa studied is characterized by unbranched columellae. Orbicules are consistently absent in Menthinae. Our palynological data are interpreted in a phylogenetic context at tribal level in order to assess the systematic value of pollen characters and to evaluate the existing molecular phylogenies for this group. Pollen morphology suggests *Heterolamium* as a close relative of subtribe Nepetinae and supports the molecular affinity of *Melissa* to subtribe Salviinae.

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Introduction

The pollen morphology of Lamiaceae has proven to be systematically valuable since Erdtman (1945) used the number of nuclei and the aperture number to divide the family into two subfamilies (i.e. Lamioideae: bi-nucleate and tricolpate pollen, Nepetoideae: tri-nucleate and hexacolpate pollen). While the circumscription of subfamily Lamioideae has been dramatically changed after Erdtman's treatment due to progress in molecular systematics, including for instance many genera previously placed in Verbenaceae, the subfamily Nepetoideae is a consistently supported monophyletic group based on both molecular and morphological evidence (Cantino 1992; Harley et al. 2004; Wagstaff et al. 1995). Examples of morphological synapomorphies for the latter subfamily are the presence of six colpi, although some variation in aperture number occurs sporadically, exalbuminous seeds and an investing embryo type (Cantino 1992). According to the most recent classification (Harley et al. 2004), Nepetoideae comprise three tribes: Elsholtzeae, Mentheae and Ocimeae.

Palynological studies in Nepetoideae have been restricted geographically or have been based on a limited number of taxa using mainly light microscopic observations (Afzal-Rafii 1983; Henderson et al. 1968; Varghese and Verma 1968; Vij and Kashyap 1975; Waterman 1960). In contrast to the subfamily Lamioideae in which many palynological data are published (Abu-Asab and Cantino 1992, 1993, 1994), only few genera of Nepetoideae are described using detailed scanning and transmission electron microscopic observations (Harley 1992; Harley et al. 1992; Rudall 1980; Wagstaff 1992).

Tribe Mentheae includes about 65 genera and 2,000 species, and represents almost one fourth of the currently recognized genera in Lamiaceae. Mentheae is the largest and economically most important tribe including many culinary herbs such as mint (*Mentha*), oregano (*Origanum*), savory (*Satureja*), sage (*Salvia*) and thyme (*Thymus*). Mentheae, which was paraphyletic or polyphyletic as circumscribed by Briquet (1897) and Wunderlich (1967), was greatly expanded by Cantino et al. (1992) to include Glechoneae, Hormineae, Lepechinieae, Meriandreae, Monardeae, Nepeteae, Prunelleae, Rosmarineae and Salvieae and is strongly supported as a monophyletic group by molecular data (Wagstaff et al. 1995, Table 1). More recently, traditionally segregated tribes were recognized at subtribal level by Harley et al. (2004), i.e., Salviinae, Menthinae and Nepetinae. Although tribe Mentheae is a monophyletic group, the monophyly of its subtribes is still questionable based on the existing molecular phylogenies (Trusty et al. 2004; Walker and Sytsma 2007).

Since a thorough palynological study of the entire tribe Mentheae was lacking (Wagstaff 1992), we have presented a detailed pollen study of subtribe Salviinae and Nepetinae with a strong emphasis on the type genera *Salvia* and *Nepeta* (Moon et al. 2008a, b). In addition, we have assessed the possible occurrence of orbicules in Salviinae and Nepetinae. Orbicules are small sporopollenin particles that can be produced in species with a secretory tapetum (Huysmans et al. 1998, 2000). The presence or absence of orbicules and their morphology can provide additional palynological characters that might have a phylogenetic significance (e.g., Huysmans et al. 1998; Raj and El-Ghazaly 1987; Vinckier and Smets 2002). All taxa studied in Salviinae and Nepetinae have smooth tapetal membranes without any patterned sporopollenin deposition, meaning that orbicules were not observed in our two previous studies.

In order to present a detailed palynological overview of the entire tribe Mentheae sensu Harley et al. (2004), the present study focuses on the third subtribe Menthinae including two unplaced genera *Heterolamium* and *Melissa*. We, therefore, aim to document and illustrate the pollen morphology as well as the ultrastructure of the pollen wall, and we want to trace the possible occurrence of orbicules in subtribe Menthinae sensu Harley et al. (2004) using LM, SEM and TEM. These observations are discussed in the light of our previous pollen studies in the two other subtribes Salviinae and Nepetinae, with special emphasis on the systematic significance of pollen characters within the entire tribe Mentheae. Additionally, palynological data of two currently unplaced genera *Heterolamium* and *Melissa* are incorporated to comment on their enigmatic taxonomic position.

Materials and Methods

Material

The present study is based on herbarium material of 58 species (66 specimens) from 42 genera of the subtribe Menthinae, collected from the following herbaria: BR, GH, K, LV, MO and S (acronyms follow Holmgren et al. 1990; for a complete list of specimens, see Appendix) and also in part from plants recently collected by the first author. Two monotypic genera *Eriothymus* and *Kurzamra* could not be studied because of lack of material. Since the genus *Lycopus* has already been studied by Moon and Hong (2003), it was also excluded from the present study.

SEM observations

Standard acetolysis (Erdtman 1960; Reitsma 1969) destroys the colpus membranes, which may influences the natural pollen shape and size (Demissew and Harley 1992; Moon et al. 2008a, b). The subtribes Salviinae and Nepetinae showed a striking size difference according to treatment: critical point dried pollen grains were always smaller than acetolysed pollen grains (Harley 1992; Lens et al. 2005; Moon et al. 2008a, b; Schols et al. 2004). Taking this in consideration, all samples were critical point dried in this study for optimally preserving their natural size and shape.

For pollen and orbicule observations, dried flowers or buds were rehydrated for 1–2 h in the wetting agent Agepon® (Agfa Gevaert, Leverkusen, Germany; Agepon wetting agent:distilled water = 1:200). Anthers were separated from the flowers and the tips were removed with a razor blade to facilitate rehydration. After dissection, the anthers remained for one more hour in the wetting agent. Following dehydration in a graded acetone series, the material was critical point dried (CPD 030, Balzers). The dried anthers were mounted on stubs with double-sided adhesive tape. The locules were opened and the pollen grains were carefully removed with a cactus needle. The removed pollen grains were collected on the same stub for observation. The stubs were coated with gold (SPI-MODULE™ Sputter Coater, SPI Supplies, West Chester, PA, USA) prior to observation with a JEOL JSM-6360 scanning electron microscope at 10–20 kV. The size measurements of 15–20 fully developed pollen grains on SEM images were made using Carnoy 2.0 (Schols et al. 2002).

Ultrastructure

For transmission electron microscopy (TEM), the anthers from living material were directly fixed in 2.5% glutaraldehyde while dried anthers were rehydrated in 0.05 M

Table 1 Genera of tribe Mentheae sensu Harley et al. (2004) and their previous taxonomic positions

Tribe Mentheae sensu Harley et al. (2004)	Bentham (1876)	Briquet (1897)	Wunderlich (1967)
Subtribe Menthinae			
Acanthomintha (A.Gray) Benth. & Hook. f.	Tribe Satureineae, subtribe Melisseae	Tribe Glechoneae	Tribe Glechoneae
Blephilia Raf.	Tribe Monardeae	Tribe Monardeae	Tribe Monardeae
Bystropogon L'Hér.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Thyminae	Tribe Saturejeae, subtribe Thyminae
Cleonia L.	Tribe Stachydeae	Tribe Stachydeae, subtribe Brunellinae	Tribe Stachydeae, subtribe Prunellinae
Clinopodium L.	Tribe Satureineae, subtribe Melisseae (as *Calamintha*)	Genus *Satureia* sect. *Clinopodium*	Genus *Satureja*
Conradina A. Gray	Tribe Satureineae, subtribe Melisseae	Tribe Satureieae, subtribe Melissinae	Tribe Saturejeae, subtribe Melissinae
Cuminia Colla	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Menthinae	Tribe Saturejeae, subtribe Menthinae
Cunila D. Royen ex L.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Thyminae	Tribe Saturejeae, subtribe Thyminae
Cyclotrichium (Boiss.) Manden. & Scheng.	*Genus Calamintha* (see *Clinopodium*)	Genus *Satureja* sect. *Cyclotrichum*	N.I.
Dicerandra Benth.	Tribe Satureineae, subtribe Melisseae (as *Ceranthera*)	Tribe Satureieae, subtribe Melissinae (as *Ceranthera*)	Tribe Saturejeae, subtribe Melissinae (as *Ceranthera*)
Eriothymus (Benty.) Schmidt	Tribe Satureineae, subtribe Melisseae (as *Keithia*)	Genus *Hedeoma*	N.I.
Glechon Spreng.	Tribe Satureineae, subtribe Melisseae	Tribe Glechoneae	Tribe Glechoneae
Gontscharovia Boriss.	N.I.	N.I.	N.I.
Hedeoma Pers.	Tribe Satureineae, subtribe Melisseae	Tribe Satureieae, subtribe Melissinae	Tribe Saturejeae, subtribe Melissinae
Hesperozygis Epling	N.I.	N.I.	N.I.
Hoehnea Epling	N.I.	N.I.	N.I.
Horminum L.	Tribe Satureineae, subtribe Lepechineae	Tribe Hormineae	Tribe Hormineae
Hyssopus L.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Hyssopinae	Tribe Saturejeae, subtribe Hyssopinae
Kurzamra Kuntze	Tribe Satureineae, subtribe Melisseae (as *Soliera*)	Tribe Satureieae, subtribe Melissinae	N.I.
Lycopus L.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Menthinae	Tribe Saturejeae, subtribe Menthinae
Mentha L.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Menthinae	Tribe Saturejeae, subtribe Menthinae
Micromeria Benth.	Tribe Satureineae, subtribe Melisseae	Genus *Satureia*	Genus *Satureja*
Minthostachys (Benth.) Spach	Genus *Bystropogon*	Genus *Bystropogon*	N.I.
Monarda L.	Tribe Monardeae	Tribe Monardeae	Tribe Monardeae
Monardella Benth.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Thyminae	Tribe Saturejeae, subtribe Thyminae
Neoeplingia Ramamoorthy, Hiriart & Medrano	N.I.	N.I.	N.I.
Obtegomeria P.D. Cantino & Doroszenko	N.I.	N.I.	N.I.
Origanum L.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Thyminae	Tribe Saturejeae, subtribe Thyminae
Pentapleura Hand.-Mazz.	N.I.	N.I.	N.I.

Table 1 continued

Tribe Mentheae sensu Harley et al. (2004)	Bentham (1876)	Briquet (1897)	Wunderlich (1967)
Piloblephis Raf.	Genus *Satureja* (sect. *Pycnothymus*)	Genus *Satureja* (sect. *Pycnothymus*)	N.I.
Pogogyne Benth.	Tribe Satureineae, subtribe Melisseae	Tribe Satureieae, subtribe Melissinae	Tribe Saturejeae, subtribe Melissinae
Poliomintha A. Gray	Tribe Satureineae, subtribe Melisseae	Genus *Hedeoma*	N.I.
Prunella L.	Tribe Stachydeae (as *Brunella*)	Tribe Stachydeae, subtribe Brunellinae (as *Brunella*)	Tribe Stachydeae, subtribe Prunellinae
Pycnanthemum Michx.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Thyminae (as *Koellia*)	Tribe Saturejeae, subtribe Thyminae
Rhabdocaulon (Benth.) Epling	Tribe Satureineae, subtribe Melisseae (as *Keithia*)	Genus *Hedeoma*	N.I.
Rhododon Epling	N.I.	N.I.	N.I.
Saccocalyx Coss. & Durieu	Genus *Satureja*	Tribe Satureieae, subtribe Melissinae	Tribe Saturejeae, subtribe Melissinae
Satureja L.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Melissinae	Tribe Saturejeae, subtribe Melissinae
Stachydeoma Small	Genus *Hedeoma*	Genus *Hedeoma*	N.I.
Thymbra L.	Tribe Satureineae, subtribe Melisseae	Tribe Satureieae, subtribe Melissinae	Tribe Saturejeae, subtribe Melissinae
Thymus L.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Thyminae	Tribe Saturejeae, subtribe Thyminae
Zataria Boiss.	Tribe Satureineae, subtribe Menthoideae	Tribe Satureieae, subtribe Thyminae	Tribe Saturejeae, subtribe Thyminae
Ziziphora L.	Tribe Monardeae	Tribe Satureieae, subtribe Melissinae	Tribe Saturejeae, subtribe Melissinae
Subtribe Salviinae			
Chaunostoma Donn. Sm.	N.I.	Incertae Sedis	N.I.
Dorystaechas Boiss. & Heldr.	Tribe Monardeae	Tribe Meriandreae	Tribe Meriandreae
Lepechinia Willd.	Tribe Satureineae, subtribe Lepechineae	Tribe Lepechinieae	Tribe Lepechinieae
Meriandra Benth.	Tribe Monardeae	Tribe Meriandreae	Tribe Meriandreae
Perovskia Kar.	Tribe Monardeae	Tribe Meriandreae	Tribe Meriandreae
Rosmarinus L.	Tribe Monardeae	Tribe Rosmarineae	Tribe Rosmarineae
Salvia L.	Tribe Monardeae	Tribe Salvieae	Tribe Salvieae
Zhumeria Rech.f. & Wendelbo	N.I.	N.I.	N.I.
Subtribe Nepetinae			
Agastache J.Clayton ex Gronov.	N.I.	Tribe Nepeteae	Tribe Nepeteae
Cedronella Moench	Tribe Nepeteae	Tribe Nepeteae	Tribe Nepeteae
Dracocephalum L.	Tribe Nepeteae	Tribe Nepeteae	Tribe Nepeteae
Drepanocaryum Pojark.	N.I.	N.I.	N.I.
Glechoma L.	Genus *Nepeta*	Tribe Nepeteae	Tribe Nepeteae
Hymenocrater Fisch. & C.A.Mey.	Tribe Nepeteae	Tribe Nepeteae	Tribe Nepeteae
Lallemantia Fisch. & C.A.Mey.	Tribe Nepeteae	Tribe Nepeteae	Tribe Nepeteae
Lophanthus Adans.	Tribe Nepeteae	Tribe Nepeteae	Tribe Nepeteae
Marmoritis Benth.	Genus *Nepeta*	N.I.	N.I.
Meehania Britton	N.I.	Tribe Nepeteae	Tribe Nepeteae

Table 1 continued

Tribe Mentheae sensu Harley et al. (2004)	Bentham (1876)	Briquet (1897)	Wunderlich (1967)
Nepeta L.	Tribe Nepeteae	Tribe Nepeteae	Tribe Nepeteae
Schizonepeta (Benth.) Briq.	Genus *Nepeta*	Tribe Nepeteae	Tribe Nepeteae
Incertae Sedis			
Heterolamium C. Y. Wu	N.I.	N.I.	N.I.
Melissa L.	Tribe Satureineae, subtribe Melisseae	Tribe Satureieae, subtribe Melissinae	Tribe Saturejeae, subtribe Melissinae

N.I. not indicated

sodium cacodylate buffer (pH 7.3) prior to fixation. Fixed anthers were rinsed with 0.05 M sodium cacodylate buffer (pH 7.3), followed by post fixation in 2% OsO_4. Prior to embedding in LR-White Resin (Polysciences Inc., Warrington, PA, USA), the material was dehydrated in a graded ethanol series. Semi-thin sections (±1 µm) were cut with a microtome (Reichert Jung Ultracut E), stained with 0.1% thionin–0.1% methylene blue, and observed with a Leitz Dialux 20 microscope. The ultra-thin sections (70 nm) on copper grids were stained with uranyl acetate and lead citrate in an ultrastainer (LKB 2168), and observed with a Zeiss EM 900 transmission electron microscope at 50 kV.

Pollen terminology follows the Glossary of Pollen and Spore Terminology (Punt et al. 2007; http://www.bio.uu.nl/~palaeo/glossary/glos-int.htm).

Results

The pollen morphology of subtribe Menthinae is highly uniform. Therefore, we prefer to give a general pollen description for the subtribe with references to particular species when necessary. The major pollen features of all taxa investigated are summarized in Table 2. Representative pollen grains are illustrated in Figs. 1–4.

Size and shape

Pollen grains are shed as monads and their size varies from small to medium ($P = 13.0$–43.3 µm). *Mentha pulegium* possesses the smallest pollen grains while *Cleonia lusitanica* and *Monarda punctata* have the largest pollen grains (Table 2). At infrageneric level the taxa studied have a rather narrow size range (Table 2).

The shape of pollen grains in equatorial view ranges from oblate to prolate ($P/E = 0.67$–1.72; Fig. 1a–f). Suboblate to prolate-spheroidal shapes are common even within the same taxa (Table 2). The outline in polar view is circular, sometimes mixed with elliptic grains because of width of the mesocolpium area (Fig. 1g–l).

Apertures

All taxa studied have hexacolpate pollen grains. Pollen aperture heteromorphism only occurs in *Prunella vulgaris* and *Pycnanthemum incanum* with a few pentacolpate pollen grains (Fig. 1j).

Simple colpi are distributed symmetrically. The range of colpi lengths is 10.6–37.1 µm. Colpus length is strongly correlated with length of polar axis. Colpi ends are acute (Fig. 1) and the apocolpium index (AI) varies between 0.10 and 0.29 (Table 2).

Exine ornamentation

Three distinct exine ornamentation types are observed in Menthinae: bireticulate, microreticulate or perforate (Fig. 2). Based on the detailed configuration of the exine ornamentation three subtypes can be defined for the bireticulate pattern, and two subtypes for the microreticulate pattern.

Type I: bireticulate

A bireticulum consists of a non-congruent, two-layered reticulum. The main reticulum is referred to as the primary reticulum and the substratum as the secondary reticulum. Three subtypes can be defined based on the number of secondary lumina in each primary lumen. Type I-1: The primary muri are more than twice as thick as the secondary muri. Primary lumina are continuous and irregular to rounded and slightly shallow. The secondary reticulum is microreticulate and the number of secondary lumina does not exceed ten in each primary lumen. Observed in: *Gontscharovia*, *Horminum*, *Melissa*, *Monardella*, *Thymus*, *Ziziphora* (Fig. 2a, b). Type I-2: The primary muri are thicker than the secondary muri. The shape of the primary lumina is rounded and continuous. The secondary lumina

Table 2 Overview of major palynological characters of all species studied within Menthinae

Species	*P* (μm)	*E* (μm)	Shape							AI	CL (μm)	Type	OW/NW	Figures
			O	SO	OS	S	PS	SP	P					
Acanthomintha ilicifolia	24.5–25.4–26.0	25.4–29.4–32.2	−	++	+	−	++	−	−	/	20.2–21.6–23.2	II-2	NW	
Acanthomintha obovata	25.3–27.1–28.7	23.7–26.0–27.1	−	−	+	−	++	−	−	0.12–0.17	21.8–23.7–25.8	II-2	NW	
Blephilia ciliata	22.3–23.4–24.8	24.8–26.5–28.3	−	+	++	−	−	−	−	0.11–0.17	18.3–19.7–21.7	II-1	NW	1j
Blephilia hirsuta	22.2–24.5–27.5	18.4–25.0–31.0	−	+	++	−	+	+	−	/	18.7–21.1–24.7	II-1	NW	
Bystropogon canariensis	18.4–19.0–19.2	18.5–21.2–22.6	−	++	−	−	+	−	−	/	14.7–15.4–16.0	II-1	OW	
Bystropogon canariensis var. *smithianus*	17.8–19.0–19.7	19.5–20.1–21.0	−	−	++	−	−	−	−	0.17–0.22	15.4–16.0–17.0	II-1	OW	
Cleonia lusitanica	31.4–39.6–43.3	30.2–36.0–40.2	−	+	++	−	+	++	+	0.15–0.23	26.3–34.0–37.1	I-2	OW	2d
Clinopodium vulgaris	30.3–31.3–33.1	29.6–33.7–36.9	−	++	+	−	+	−	−	0.18–0.23	24.2–24.6–24.9	II-2	OW	
Conradina canescens	20.3–22.1–23.5	23.7–26.7–28.7	+	++	−	−	−	−	−	0.13–0.20	17.0–18.7–20.0	II-2	NW	1k
Conradina grandiflora	25.1–29.3–32.0	27.5–28.6–31.0	−	−	+	−	++	−	−	/	22.4–25.2–28.2	II-2	NW	
Cuminia eriantha var. *fernandezia*	28.3–31.5–34.3	25.2–27.1–28.2	−	−	−	−	++	+	−	0.19–0.24	24.2–25.9–28.2	III	NW	
Cunila origanoides	22.0–23.4–25.0	22.0–26.3–28.6	−	+	+	−	+	−	−	/	18.4–19.8–21.4	II-1	NW	
Cyclotrichium origanifolium	23.6–27.9–33.9	22.6–25.8–27.6	−	−	+	−	++	+	−	/	19.5–23.6–30.5	I-2	OW	1f
Dicerandra christmanii	29.4–30.6–31.6	29.7–33.8–36.1	−	++	++	−	+	−	−	0.14–0.18	24.6–26.3–28.0	II-2	NW	
Dicerandra odoratissima	22.5–24.4–27.0	26.9–28.0–29.2	−	++	+	−	+	−	−	/	18.3–20.4–23.1	II-2	NW	
Glechon marifolia	25.2–31.6–34.4	25.0–32.2–37.0	+	+	++	−	+	++	−	0.14–0.22	22.1–26.9–29.0	II-2	NW	1d
Gontscharovia popovii	24.9–29.6–32.5	27.4–32.1–36.0	−	+	++	−	−	−	−	0.12–0.18	21.1–24.9–28.0	I-1	OW	
Hedeoma ciliolata	24.8–29.8–31.1	32.2–36.0–38.7	−	++	+	−	−	−	−	0.15–0.24	20.5–25.1–28.0	II-1	NW	
Hesperozygis nitida	25.9–28.4–30.1	28.7–33.9–37.5	−	++	+	+	−	−	−	/	21.0–23.6–26.0	II-2	NW	1h
Heterolamium debile	30.4–36.8–43.2	27.0–31.9–35.9	−	−	+	−	+	++	+	0.17–0.23	23.2–28.7–32.0	I-3	OW	1e
Heterolamium debile	25.7–31.8–37.8	27.0–34.2–39.7	−	++	++	−	+	−	−	0.16–0.24	21.1–24.6–30.0	I-3	OW	
Hoehnea epilobioides	30.0–30.4–31.0	20.9–26.8–34.9	−	++	−	−	−	+	++	0.15–0.22	20.9–23.2–27.0	II-1	NW	
Hoehnea minima	26.3–29.4–33.5	30.4–31.9–34.2	−	++	+	−	+	−	−	/	22.0–24.2–26.4	II-1	NW	4a
Horminum pyrenaicum	20.8–28.3–34.3	22.3–30.8–35.6	−	+	++	−	−	−	−	0.20–0.27	17.3–23.5–28.7	I-1	OW	
Hyssopus officinalis	21.1–21.7–23.1	23.5–25.9–27.3	−	++	+	−	−	−	−	0.10–0.21	17.6–18.8–19.6	I-2	OW	1l, 2c, 3b, d
Melissa flava	29.0–31.5–35.7	31.0–36.1–39.1	−	++	+	−	−	−	−	0.11–0.24	25.5–27.3–29.2	I-1	OW	
Melissa officinalis	26.6–30.9–35.1	26.9–30.3–33.8	−	−	++	−	++	+	−	0.12–0.19	23.5–26.4–30.2	I-1	OW	2b
Mentha pulegium	13.0–15.0–16.8	11.8–12.5–13.8	−	−	++	−	+	++	−	/	10.6–11.8–12.8	II-2	OW	
Micromeria marginata	25.0–26.6–28.9	32.7–35.2–39.1	++	++	+	−	−	−	−	0.10–0.17	20.1–22.4–24.3	II-1	OW	1a
Minthostachys molis	24.3–26.7–30.0	30.8–24.9–30.1	−	+	+	−	++	++	−	/	18.9–21.2–22.7	II-1	NW	2f
Monarda fistulosa	30.6–32.3–34.6	31.5–37.4–41.3	−	++	+	−	+	−	−	0.16–0.24	24.9–27.6–29.8	III	NW	1b, 2k
Monarda punctata	35.9–39.1–42.9	31.7–41.4–47.8	−	+	+	−	+	−	−	0.12–0.20	29.9–33.3–36.8	II-1	NW	2l
Monardella macrantha	26.4–27.5–28.6	31.7–33.7–36.3	−	++	−	−	−	−	−	0.12–0.19	22.8–24.0–25.1	I-1	NW	4d
Monardella nana	21.8–22.9–24.0	25.1–27.3–29.6	−	++	+	−	−	−	−	0.12–0.18	18.4–20.0–21.4	I-1	NW	

Table 2 continued

Species	*P* (µm)	*E* (µm)	Shape							AI	CL (µm)	Type	OW/NW	Figures
			O	SO	OS	S	PS	SP	P					
Monardella odoratissima	21.4–22.6–24.0	25.4–26.1–27.4	−	++	+	−	−	−	−	0.14–0.21	17.3–19.1–21.8	I-1	NW	1e, h
Neoplingia leucophylloides	18.6–21.4–23.3	22.7–27.5–29.6	+	++	−	−	+	−	−	/	13.9–17.6–19.2	II-1	NW	
Obtegomeria caerulescens	22.7–24.3–26.1	26.8–27.6–28.0	−	++	++	−	−	−	−	0.16–0.21	20.0–21.4–23.4	III	NW	
Origanum rotundifolium	22.5–25.4–28.9	17.2–19.4–25.8	−	−	−	−	+	++	++	/	16.5–18.4–19.4	II-2	OW	
Pentapleura subulifera	18.1–21.4–23.8	17.9–23.0–26.8	++	+	+	−	+	+	−	0.14–0.25	15.3–18.3–21.1	II-2	OW	
Piloblephis rigida	18.8–20.2–21.1	22.6–23.8–24.8	−	++	+	−	−	−	−	/	14.6–17.0–18.6	II-2	NW	2j, 4e
Pogogyne douglasii	25.4–27.2–30.9	26.0–28.8–30.9	−	−	++	−	+	−	−	/	22.1–23.7–25.7	II-2	NW	
Pogogyne serpylloides	24.7–25.1–25.6	26.7–28.2–29.8	−	+	++	−	−	−	−	0.20–0.29	19.7–20.8–22.0	II-2	NW	
Poliomintha incana	26.5–28.6–30.0	30.1–31.1–33.2	−	+	++	−	−	−	−	0.15–0.25	21.8–24.3–26.2	II-1	NW	
Poliomintha longiflora	25.3–29.1–33.8	30.2–32.6–36.5	+	+	++	−	+	−	−	0.14–0.17	20.7–24.7–28.8	II-1	NW	2g
Prunella vulgaris	26.5–31.1–35.5	19.4–22.4–25.8	−	−	−	−	+	++	++	/	18.0–23.3–26.3	I-3	OW	
Pycnanthemum albescens	22.8–24.2–25.2	25.1–28.0–30.5	−	++	++	−	−	−	−	0.16–0.21	17.3–19.9–21.5	II-1	NW	1g[a]
Rhabdocaulon coccineum	29.3–33.8–39.0	33.2–41.0–44.4	+	++	+	−	−	−	−	0.15–0.21	25.4–28.6–34.5	II-2	NW	
Rhabdocaulon strictum	21.8–24.7–27.0	14.7–17.6–20.3	−	−	−	−	+	+	++	/	15.7–18.9–21.0	II-2	NW	
Rhododon ciliatus	19.9–20.7–21.7	21.8–23.7–25.2	−	++	++	−	−	−	−	0.18–0.23	16.1–17.6–18.3	III	NW	
Saccocalyx satureioides	20.6–22.5–23.5	22.7–25.5–27.1	−	+	++	−	+	−	−	0.18–0.26	16.6–18.6–20.1	II-1	OW	1i
Satureja thymbra	28.4–29.5–30.5	34.5–35.7–36.9	−	++	++	−	−	−	−	/	23.9–24.8–26.4	II-1	OW	3c[b]
Stachydeoma graveolens	22.7–25.5–28.9	24.0–28.6–32.5	−	++	++	−	+	−	−	0.12–0.19	18.4–22.0–26.0	II-2	NW	2i, 4c
Thymbra spicata	22.2–24.6–26.1	28.1–29.5–31.6	−	++	+	−	−	−	−	/	19.6–21.4–23.4	I-2	OW	
Thymbra spicata	27.0–27.9–30.5	31.9–35.0–39.0	−	++	−	−	−	−	−	0.15–0.21	22.4–23.6–25.4	I-2	OW	
Thymus pallasianus	20.7–23.2–26.7	25.2–29.0–36.8	+	++	+	−	−	−	−	0.11–0.20	17.7–19.7–22.4	I-1	OW	
Thymus serpylum	23.5–27.5–30.1	27.4–32.3–37.9	+	++	++	+	−	−	−	0.13–0.18	21.0–23.8–27.0	I-1	OW	2a, 3e
Zataria multiflora	17.2–19.0–20.9	18.6–19.5–21.6	−	+	++	−	++	−	−	/	12.8–15.1–16.4	II-1	OW	3a
Ziziphora clinopodioides	24.2–25.8–27.2	29.9–32.3–35.3	+	++	−	−	−	−	−	0.14–0.24	20.4–22.2–24.8	I-1	OW	1c, 4b, f

Numbers refer to minimum–mean–maximum except for AI (apocolpium index) values that correspond to a range

P polar axis, *E* equatorial diameter, *O* oblate, *SO* suboblate, *OS* oblate spheroidal, *S* spherical, *PS* prolate spheroidal, *SP* subprolate, *P* prolate, *CL* colpus length, *OW* Old World, *NW* New World. −, absent; +, present; ++, dominance; /, no data

[a, b] Micrographs of *Pycnanthemum incanum* and *Satureja spinosa* used for references which are not included taxa in the table as lacking of enough pollen grains to calculate mean value

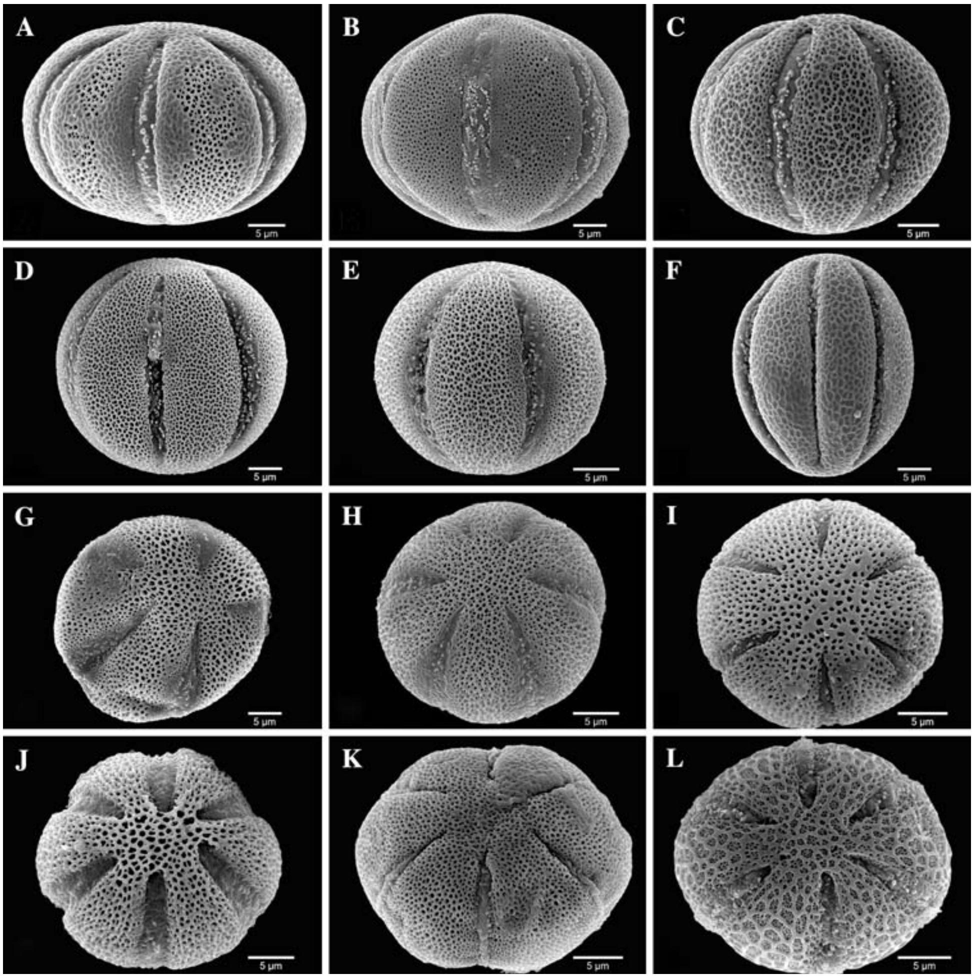

Fig. 1 SEM micrographs of pollen grains of Menthinae. **a–f** Variation of pollen shape in the equatorial view. **g–l** Polar view with variations of shape and aperture number. **a** Oblate pollen grain with elliptic outline of *Micromeria marginata*. Suboblate pollen grain, **b** *Monarda fistulosa*, **c** *Ziziphora clinopodioides*. **d** Oblatespheroidal pollen grain with circular outline, *Glechon marifolia*. *Monardella odoratissima*, **e** oblate spheroidal pollen, **h** circular outline. **f** Subprolate pollen grain of *Cyclotrichium origanifolium*. **g** Pentacolpate pollen grain of *Pycnanthemum incanum*. Hexacolpate pollen grain with circular shape, **h** *Monardella odoratissima*, **i** *Saccocalyx satureioides*, **j** *Blephilia ciliata*. Hexacolpate pollen grain with elliptic shape, **k** *Conradina canescens*, **l** *Hyssopus officinalis*

are regular and at the mesocolpia over ten secondary lumina per primary lumen were counted. The secondary lumen size and number decreases towards the poles and apertures. Observed in: *Cleonia*, *Cyclotrichium*, *Hyssopus*, *Thymbra* (Fig. 2c, d). Type I-3: The primary muri are irregular in pattern and sometimes discontinuous. Each primary lumen contains more than 20 rounded secondary lumina. Observed in: *Heterolamium*, *Prunella* (Fig. 2e).

Type II: microreticulate

Two subtypes are recognized based on the possible presence of tectal connections in the lumina. Type II-1: Microreticulate without secondary tectal connections. Observed in: *Blephilia*, *Bystropogon*, *Cunila*, *Hedeoma*, *Hesperozygis*, *Hoehnea*, *Micromeria*, *Minthostachys*, *Monarda punctata*, *Neoplingia*, *Poliomintha*, *Pycnanthemum*, *Saccocalyx*, *Satureja*, *Zataria* (Fig. 2f–h). Type II-2: The basic exine ornamentation is microreticulate, but secondary tectal connections are present in this subtype. Some perforations are elongated and subdivided into two or three smaller units by muri at a slightly lower plane. Observed in: *Acanthomintha*, *Clinopodium*, *Conradina*, *Dicerandra*, *Glechon*, *Mentha*, *Origanum*, *Pentapleura*, *Piloblephis*, *Pogogyne*, *Rhabdocaulon*, *Stachydeoma* (Fig. 2i, j).

Type III: perforate

The exine ornamentation is perforate. The distance between the edges of adjacent perforations is greater than the diameter of perforations. Observed in: *Cuminia*, *Rhododon*, *Monarda fistulosa* and *Obtegomeria* (Fig. 2k).

Pollen wall stratification and ultrastructure

All taxa studied show the same exine stratification (Fig. 3). The tectum is thicker than the foot layer. Columellae are simple, unbranched and densely spaced. The foot layer is continuous or distinctly discontinuous (Fig. 3c–e), relatively thin and supported by a very thin, often hardly discernible endexine (Fig. 3c–e). The intine is thicker below the colpi than at the mesocolpia (Fig. 3b, e). Pollenkitt is accumulated in the infratectum (Fig. 3e).

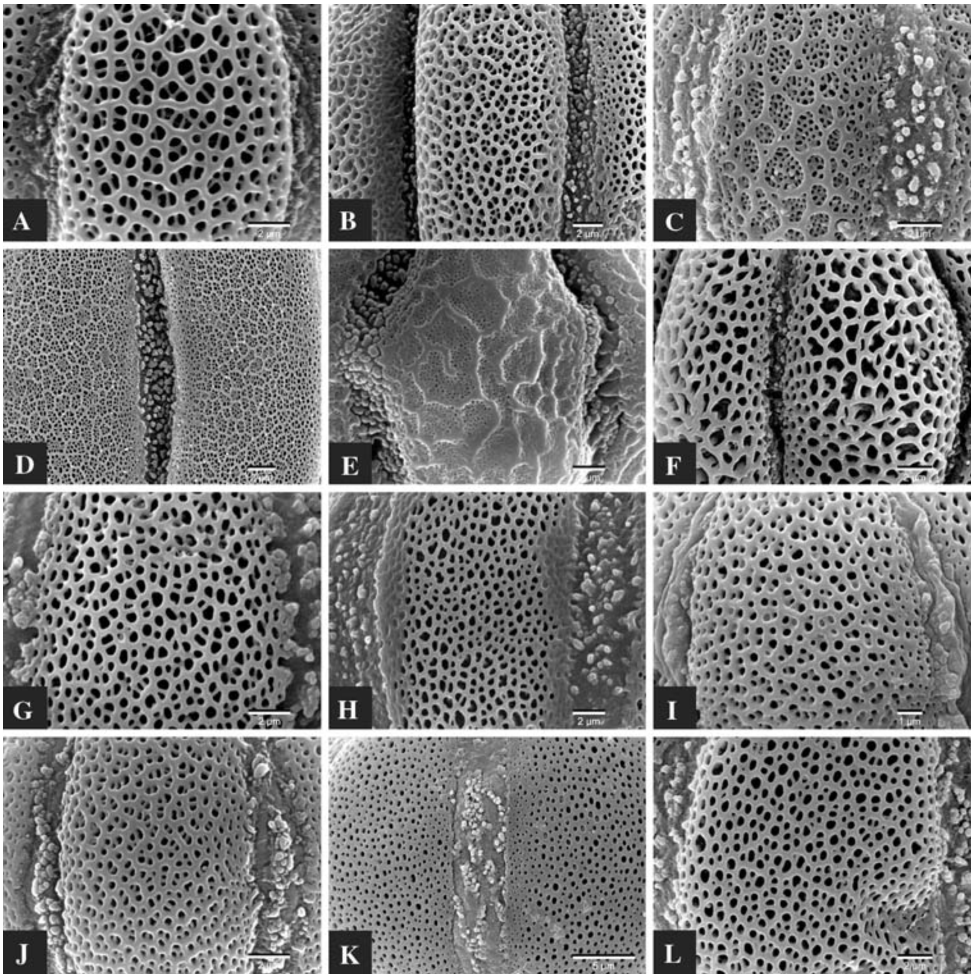

Fig. 2 SEM micrographs of variation of exine ornamentation in Menthinae. Variations of bireticulate exine ornamentation; **a** *Thymus serpyllum*, **b** *Melissa officinalis*, **c** *Hyssopus offiicinalis*, **d** *Cleonia lusitanica*, **e** *Heterolamium debile*. Reticulate exine ornamentation; **f** *Minthostachys mollis*, **g** *Poliomintha longiflora*, **h** *Hesperozygis nitida*. Reticulate exine ornamentation with possible secondary tectal connections visible; **i** *Stachydeoma graveolens*, **j** *Piloblephis rigida*. Variation of exine ornamentation in the genus *Monarda*; **k** perforate—*Monarda fistulosa*, **l** reticulate—*Monarda punctata*

Orbicules

Orbicules were absent in all species investigated (Fig. 4). The inner locule wall displays a characteristic annular or star shaped pattern because of the prominent underlying endothecium thickenings (Fig. 4d). The tapetal membrane is completely smooth without any sporopollenin deposition (Fig. 4d–f).

Discussion

Menthinae are a stenopalynous group. Their pollen is small to medium size ($P = 13.0$–43.3 μm), hexacolpate (mixed with few pentacolpate pollen grains in *Prunella vulgaris* and *Pycnanthemum incanum*) with an oblate to prolate shape, and a perforate, microreticulate or bireticulate exine ornamentation. The colpus membranes are beset with granules and the exine stratification typically shows simple columellae. Orbicules are consistently absent.

Pollen features of Menthinae compared to other members of tribe Mentheae

There is ample evidence in the literature that the size of pollen grains is greatly affected by different preparation treatments and generally critical point-dried pollen is smaller than acetolysed pollen grains in SEM (Harley 1992; Lens et al. 2005; Moon et al. 2008a, b; Reitsma 1969; Schols et al. 2004). The present results are based on critical point dried anthers in order to conserve optimally the natural size and shape. Although the size varies between taxa of Menthinae, it is notable that the range of size variation is more or less constant within the same genus (Table 2).

In Menthinae, we found some pentacolpate pollen grains in *Prunella vulgaris* and *Pycnanthemum incanum*. This aperture heteromorphism was known in 17 species belonging to eight genera in tribe Mentheae (hexacolpate is always dominant but mixed together with tetra-, penta- or octocolpate pollen; Moon et al. 2008a, b; Trudel and Morton 1992). Even if aperture heteromorphism might offer an evolutionary advantage due to an increased

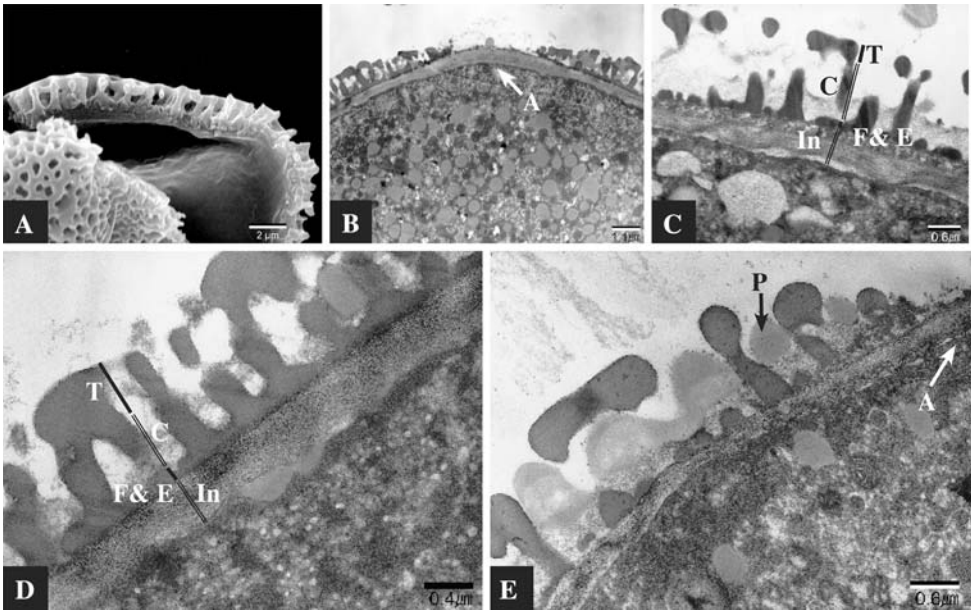

Fig. 3 SEM and TEM micrographs of pollen wall of Menthinae. **a** Cross section through exine of *Zataria multiflora*. **b–h** TEM observations of the ultrastruture of the pollen wall. **b** Exine thickness decreases towards the apertures (*A*) while the intine is thicker below the colpi than at the mesocolpia; *Hyssopus officinalis*. **c–e** Magnified part of the pollen wall showing tectum (*T*), columellae (*C*), foot layer (*F*), endexine (*E*) and intine (*In*). **c** Foot layer is discontinuous and endexine is hardly divided with foot layer; *Satureja spinosa*. **d** Foot layer is continuous; *Hyssopus officinalis*. **e** Exine is thicker below the mesocolpia than at the apertures (*A*) and deposition of pollenkitt (*black arrows*) in the infratectum; *Thymus serpyllum*

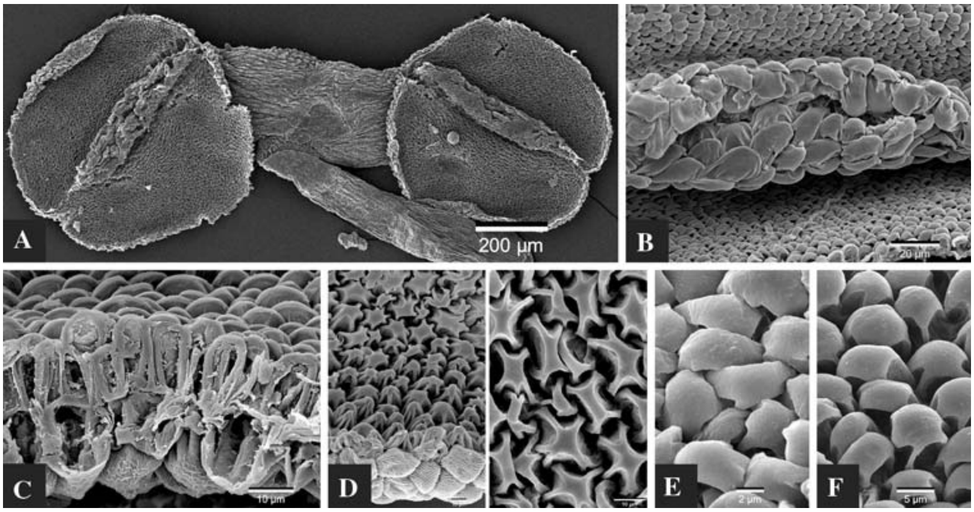

Fig. 4 SEM micrographs of Menthinae. **a** Anther with two thecae of *Hoehnea minima*. **b** The completely dehisced stamen showing the inner locule wall without orbicules; *Ziziphora capitata*. **c** Structure of the endothecium thickenings in cross section of *Stachydeoma graveolens*. **d** Inner locule wall with no traces of orbicules of *Monardella macrantha*, *left*; inner locule wall, *right*; detail of *star-shaped* endothecium cells. Detail of annular shaped endothecium cells without orbicules; **e** *Piloblephis rigida*, **f** *Ziziphora clinopodioides*

germination success (Dajoz et al. 1995; Furness and Rudall 2004; Mignot et al. 1994), it occurs rarely and only a very limited number of pollen grains are affected (less than 5%). In addition, aperture heteromorphism occurs often in cultivated individuals (A.J. Paton, personal communication), and it could therefore be induced by atypical ecological circumstances. Our results show that all taxa studied in Menthinae have predominantly hexacolpate pollen, which supports hexacolpate pollen as a synapomorphy for Nepetoideae. Simple columellae are plesiomorphic and found in most gynobasic-styled Labiatae (Abu-Asab and Cantino 1992). All taxa studied in Menthinae have simple columellae and a continuous or discontinuous foot layer with hardly observable endexine (Fig. 3c–e). Such an exine stratification also occurs in subtribes Salviinae and Nepetinae, and is common in subfamily Nepetoideae (Harley 1992; Harley et al. 1992; Moon and Hong 2003; Moon et al. 2008a, b; Nabli 1976).

Exine ornamentation variation in Menthinae

The observed variation in exine ornamentation could be defined as bireticulate, microreticulate and perforate. Microreticulate exines are most common in Menthinae while a bireticulum is common in Salviinae and Nepetinae and in other Lamiaceae as well (Abu-Asab and Cantino 1992, 1994; Jamzad et al. 2000; Moon et al. 2008a, b; Trudel and Morton 1992; Wagstaff 1992). The shared subtype of bireticulate exine suggests a close relationship between *Cleonia* and *Cyclotrichium*, *Hyssopus* and *Thymbra*, while *Gontscharovia* has a similar exine pattern as *Horminum*, *Monardella*, *Thymus* and *Ziziphora*. Historically, *Cleonia* was considered related with *Prunella* (Table 1; Briquet 1897; Wunderlich 1967). In addition the molecular phylogeny suggested *Horminum* as a sister genus of *Cleonia* and *Prunella* (Walker and Sytsma 2007). The exine type corroborates this assumption, since all three genera possess bireticulate pollen even though we can find slight intergeneric differences in ornamentation (type I; Fig. 2d, e). *Minthostachys* has once been included in *Bystropogon* (Table 1; Bentham 1876; Briquet 1897); their similar exine ornamentation indeed confirms a close relationship between these genera. A similar example could be found in the genera *Poliomintha*, *Rhabdocaulon* and *Stachydeoma*, which possess a similar exine as *Hedeoma* (Table 1). Perforate exine ornamentations were found in *Cuminia*, *Obtegomeria*, *Rhododon* and *Monarda fistulosa*. It should be noted that bireticulate pollen with 10–20 secondary lumina is restricted to Old World taxa (*Cleonia*, *Cyclotrichium*, *Hyssopus*, *Thymbra*) while perforate pollen was found only in New World taxa (*Cuminia*, *Monarda fistulosa*, *Obtegomeria*, *Rhododon*; Table 2). In fact, bireticulate pollen occurs generally in Old World taxa except for the New World genus *Monardella* (Table 2).

At generic level, exine sculpturing appears as a stable character, except for *Monarda* (Table 2; Fig. 2k, l). The variation in exine patterns found in *Monarda* seems to correlate with its subgeneric classification. *Monarda fistulosa* (subgenus *Monarda*) for instance possesses perforate grains and *Monarda punctata* (subgenus *Cheilyctis*) reticulate grains (Fig. 2k, l; Prather et al. 2002). However, our sampling for the genus *Monarda* is insufficient (2/20 species) to address the infrageneric relationships based on pollen data alone.

Systematic implications of exine ornamentation at tribal level

The most recent accepted classification of tribe Mentheae (Harley et al. 2004) included many genera from different, previously recognized tribes (Table 1). Although tribe Mentheae is a well-supported monophyletic group, the subtribal delimitation and the relationships between genera are still poorly resolved (Cantino 1992; Harley et al. 2004; Trusty et al. 2004; Wagstaff et al. 1995; Walker and Sytsma 2007). In the most recent phylogenetic hypothesis produced by Walker and Sytsma (2007), subtribe Salviinae appears as a monophyletic group. However, subtribe Menthinae splits into two major lineages and genus *Lycopus* of the Menthinae is sister to subtribe Nepetinae. It should be mentioned that this molecular study was focused on genus *Salvia* with related genera and includes only 35 out of 65 Mentheae genera (Walker and Sytsma 2007). Nevertheless, to date this phylogeny is the most comprehensive one of the Mentheae (Walker and Sytsma 2007; Fig. 5).

The different exine patterns recognized evolved independently several times throughout the Mentheae (Fig. 5). Subtribe Salviinae possesses mainly bireticulate pollen except for *Lepechinia* and *Chaunostoma*, which have perforate pollen. The perforate exines of *Lepechinia* and *Chaunostoma* of Salviinae support that these genera are sister to all other genera within Salviinae (Epling 1948; Moon et al. 2008a). According to molecular topologies, *Salvia* is not a monophyletic group (Walker et al. 2004; Walker and Sytsma 2007) and pollen characters provide further evidence for this finding (Moon et al. 2008a). Interestingly bireticulate exines with thin primary muri or with a secondary reticulum with large lumina are restricted to *Salvia* clade I (Moon et al. 2008a). In addition, these features could be interpreted as more derived based on a reduced tectal surface (Walker and Doyle 1975). Reticulate exine patterns occur mainly in Menthinae clade I. The sister group of Menthinae clade I consists of subtribe Nepetinae and Menthinae clade II (*Lycopus*, *Cleonia*, *Prunella* and *Horminum*). Menthinae clade II has problematic subtribal delimitations. Trusty et al. (2004) demonstrated conflicting results with traditional taxonomy showing that *Hyssopus* (Menthinae) is closely related with *Lallemantia* (Nepetinae), while *Horminum* (Menthinae), *Nepeta* (Nepetinae) and *Prunella* (Menthinae) grouped together based on macromolecular data. The shared bireticulate exine ornamentation supports that Menthinae clade II and Nepetinae clade are closely related (Fig. 5). The taxonomic position of *Lycopus* has been problematic because of contradictory placements in different molecular phylogenies (Bräuchler et al. 2005; Trusty et al. 2004; Wagstaff et al. 1995; Walker and Sytsma 2007). For instance, the results of cpDNA restriction site analyses by Wagstaff et al. (1995) showed that *Lycopus* is sister to the major Menthinae clade, while combined analysis of the chloroplast regions *trn*L-F, *psb*A-*trn*H and the nuclear rDNA ITS suggested *Lycopus* as a sister of the Nepetinae clade (Walker and Sytsma 2007). However, the palynological data of *Lycopus* provide evidence for a relationship

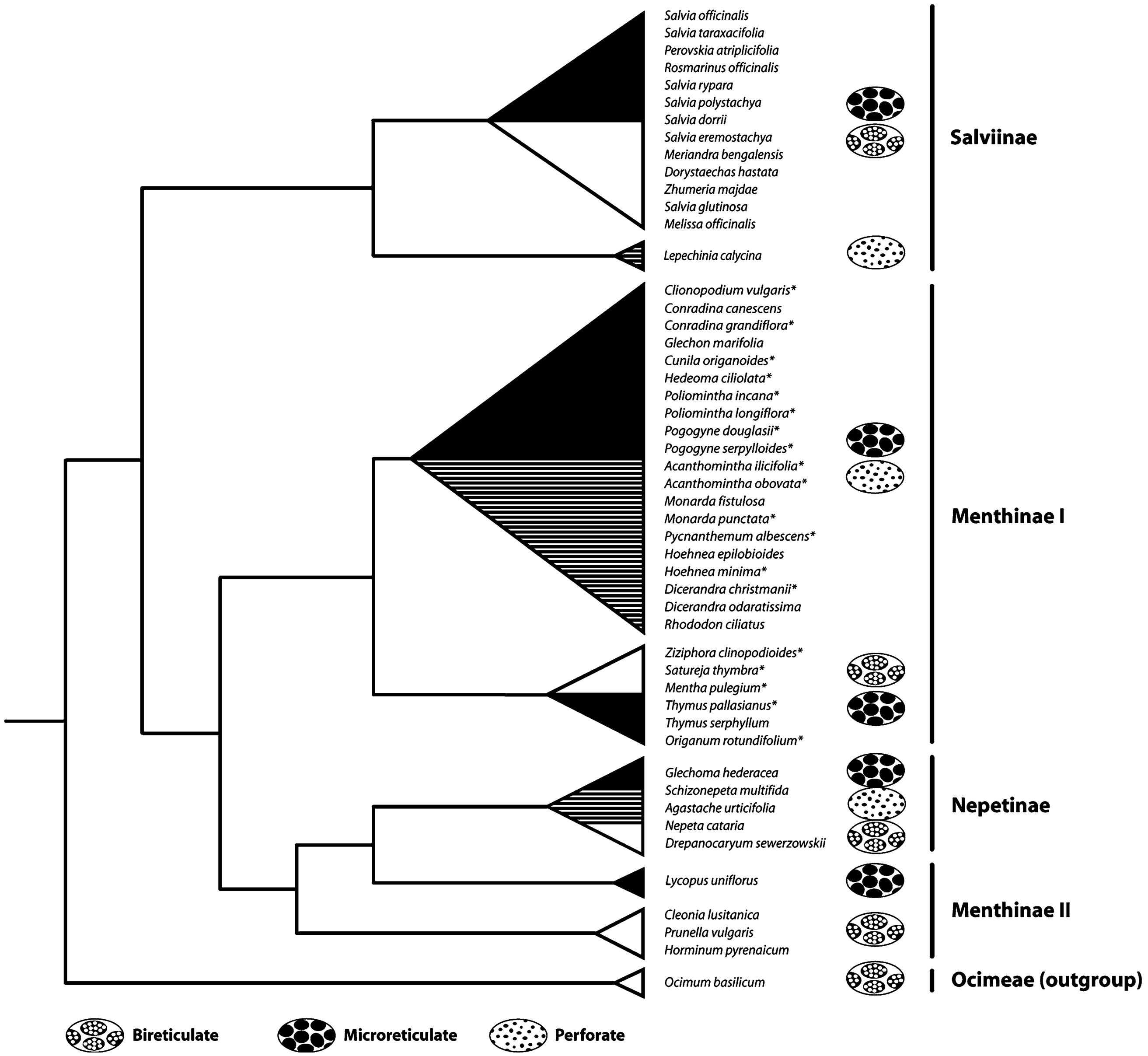

Fig. 5 Diagrams of different exine patterns in Mentheae and their distribution on the most recent molecular phylogenetic tree (simplified tree based on Walker and Sytsma 2007; their Figs. 3, 4). The three major types of exine ornamentation recognized were plotted on the topology; their occurrence in each clade is indicated as follows without reference to individual species (*white* bireticulate, *black* reticulate, *stripe* perforate). The taxa indicated by an *asterisk* were included in the present study but lacking in Walker and Sytsma (2007)—their phylogenetic position is estimated by assuming that genera are monophyletic

with Menthinae clade I (Moon and Hong 2003). Unfortunately, the present molecular framework of Lamiaceae has a very poor sampling of Mentheae taxa and particularly the monophyly of Nepetinae awaits confirmation. Even within Nepetinae the relationships among genera are conflicting (Bräuchler et al. 2005; Jamzad et al. 2003; Trusty et al. 2004; Wagstaff et al. 1995; Walker et al. 2004; Walker and Sytsma 2007). From a palynological point of view it is interesting to note that bireticulate exine patterns in Salviinae show a clear distinction between primary and secondary reticula, while bireticulate pollen of Nepetinae has a rather vague distinction between the two incongruent reticula (Moon et al. 2008a, b).

Genera of uncertain affinity

The present study included two genera of uncertain subtribal affinity, *Heterolamium* and *Melissa*. *Heterolamium* is endemic to China and historically this genus belonged to *Orthosiphon*. However, *Orthosiphon debile* Hemsl. has been accepted as an independent genus *Heterolamium* based on its distinct anther and corolla characters (Li and Hedge 1994). According to the most recent classification of Lamiaceae (Harley et al. 2004) *Heterolamium* belongs to tribe Mentheae without any indication of its subtribal position. *Heterolamium* has bireticulate pollen with discontinuous primary lumina consisting of over 20 secondary lumina (type I3, Fig. 2e). Although *Prunella* possesses the same exine type as *Heterolamium*, *Prunella* belongs to the problematic Menthinae clade within subtribe Nepetinae. Furthermore, its exine ornamentation resembles species of *Drepanocaryum*, *Lophanthus* and *Nepeta*, which are all members of Nepetinae (Moon et al. 2008b). In addition, *Heterolamium* is characterized by 15 calyx nerves and four stamens with the posterior pair longer than the anterior, both features being synapomorphies of Nepetinae (Harley et al. 2004). In conclusion, our results strongly support a position for *Heterolamium* in subtribe Nepetinae.

The genus *Melissa* consists of four species, distributed through Europe, North Africa, Macaronesia and Asia. *Melissa* was previously placed in subtribe Melissinae of tribe Satureieae together with several other genera, which currently belong to subtribe Menthinae (Table 1; Bentham 1876; Briquet 1897; Wunderlich 1967). Recent molecular phylogenies suggest *Melissa* as a member of subtribe Salviinae in Mentheae (Trusty et al. 2004; Walker and Sytsma 2007). *Melissa* has bireticulate pollen grains. The primary muri are thick with rounded primary lumina and the number of secondary lumina is 5–10 per primary lumen (Fig. 2b). This kind of exine ornamentation is very similar to the ornamentation pattern in the genera *Dorystaechas*, *Meriandra*, *Perovskia* and *Rosmarinus* of the subtribe Salviinae (Moon et al. 2008a), supporting the hypothesized relationship between these genera (Walker et al. 2004; Walker and Sytsma 2007). Thus, palynological data support the inclusion of *Melissa* into the Salviinae. However, within subtribe Menthinae, a similar exine ornamentation was found in taxa from genera *Gontscharovia*, *Horminum*, *Monardella*, *Thymus* and *Ziziphora* (Table 2).

Lack of orbicules

In general, the presence of orbicules is considered as a plesiomorphic trait. Indeed, in angiosperms orbicules are mainly restricted to species possessing a secretory tapetum, which is predominantly present in the early diversified lineages of flowering plants (i.e., Furness and Rudall 1998; Huysmans et al. 1998). In Chloanthaceae (currently tribe Chloantheae of Lamiaceae sensu Harley et al. 2004), variation in shape and surface patterns of orbicules was found to be taxonomically useful (Raj and El-Ghazaly 1987). All genera investigated of Mentheae are lacking orbicules (Moon et al. 2008a, b). The locule wall is smooth, with no traces whatsoever of orbicule-like sporopollenin deposition. The absence of orbicules in Mentheae and their presence in tribe Chloantheae actually confirms the plesiomorphic nature since tribe Chloantheae belongs to the basal Lamiaceae subfamily Prostantheroideae (Harley et al. 2004; Raj and El-Ghazaly 1987). However, a study on *Lavandula dentata* (tribe Ocimeae of subfamily Nepetoideae sensu Harley et al. 2004; Suarez-Cervera and Seoane-Camba 1986) showed that this species has a secretory tapetum without orbicule formation. The tapetum type of Mentheae is unknown and therefore further ontogenetical studies of both pollen and tapetal cells will be necessary to provide insight in the relationship between tapetum type and orbicule occurrence.

Conclusion

Mentheae is a stenopalynous group with hexacolpate pollen, perforate/microreticulate/bireticulate exine ornamentation and a pollen wall stratification with simple unbranched columellae. Variation in exine ornamentation may have systematic importance particularly at generic level. Our results suggest *Heterolamium* to be a member of subtribe Nepetinae and *Melissa* to belong to subtribe Salviinae. The exine variation observed in *Monarda* might have potential taxonomic value at subgeneric level. However, we found variability in some pollen characters between closely related species and a remarkable association between specific exine ornamentation types and the major geographic areas. A phylogenetic study of Mentheae (combining morphology and macromolecules) based on a relevant sampling will be necessary to illuminate the enigmatic intergeneric relationships. The present study provides pollen morphological characters that hopefully will be added in future phylogenetic analyses.

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Appendix

Table 3

Table 3 Voucher specimens of subtribe Menthinae that are examined in the present study

Species	Voucher specimens
Acanthomintha ilicifolia A.Gray	USA, 26–29.05.1915. Dutton & Walker 3819 BR
Acanthomintha obovata Jeps.	USA, 18.05.1919. Walker 5094 BR
Blephilia ciliata (L.) Benth.	USA, 13.06.1966. Radford 44758 BR
Blephilia hirsuta (Pursh) Benth.	USA, 12.07.1966. Radford 44922 BR
Bystropogon canariensis (L.) L'Hér	Canary Is., without date, Bullemont 1855 BR
Bystropogon canarensis var. *smithianus* H.Christ	Canary Is., 15.05.1901. Bornmüller 2765 BR
Cleonia lusitanica (L.) L.	Morocco, 13.05.1934. Wall 45 S
Clinopodium vulgare L.	Cultivated in KEW accession no.: 1994–2824
Conradina canescens A. Gray	USA, 30.11.1969. Godfrey 69283 BR
Conradina grandiflora Small	USA, 24.10.1956. Ahles & Bell 21395 BR
Cuminia eriantha var. *fernandezia* (Colla) *Harley*	Chile, 23.11.1991. Billiet & Jadin 5631 BR
Cunila origanoides (L.) Britton	USA, 07.09.1897. Anonymous 323b BR
Cyclotrichium origanifolium (Labill.) Manden.	Lebanon, 06.07.1897. Bornmüller 1260, BR
Dicerandra christmanii Huck & Judd	USA, 11.09.1987. Skean, Jr. 2130 MO
Dicerandra odoratissima R.M.Harper	USA, 17.09.1967. Radford & Leonard 11479 BR
Glechon marifolia Benth.	Uruguay, 12.02.1955. Pedersen 3627 BR
Gontscharovia popovii (B. Fedtsch. & Gontsch.) Boriss.	URSS, 31.08.1931. Anonymous 206 K
Hedeoma ciliolata (Epling & W.S.Stewart) R.S.Irving	Mexico, 01.10.1954. Rzedowski 5003 GH
Hesperozygis nitida (Benth.) Epling.	Brazil, 22.09.1976. Dombroswski 6442 K
Heterolamium debile (Hemsl.) C. Y. Wu	China, 03.1889. Henry s.n. K
	China, 1885–1888. Henry 5770 GH
Hoehnea epilobioides (Epling) Epling	Brazil, 23.10.1974. Kummrow 688 K
Hoehnea minima (Schmidt) Epl.	Brazil, 26.01.1916. Dusén 17542 GH
Horminum pyrenaicum L.	Italy, 26.06.1969. Cnops 21169 BR
Hyssopus officinalis L.	Spain, 22.09.1974. Bondía et al., 1242 GF BR
	Cultivated in KEW accession no.: 1975–1170 K[a]
Melissa flava Benth.	India, without date, Strachey and Winterbottom 1 BR
Melissa officinalis L.	France, 1986. Sotiaux s.n. BR
	Spain, 1869. Bourgeau s.n. BR[a]
	Cultivated in KEW accession no.: 1994–2690 K[a]
Mentha pulegium L.	Cultivated in KEW accession no.: 1994–1897 K[a]
Micromeria marginata (Sm.) Chater	Cultivated in KEW accession no.: 1995–1960 K[a]
Minthostachys mollis Griseb	Ecuador, 10.08.1939. Asplund s.n. BR
Monarda fistulosa L.	USA, 06.08.1973. Bouharmont 8498. BR
Monarda punctata L.	USA, 26.10.1957. Ahles & Haesloop 38096 BR
Monardella macrantha A.Gray	Cultivated in KEW accession no.: 1980–998 K[a]
Monardella nana A. Gray	Cultivated in KEW accession no.: 1999–270 K[a]
Monardella odoratissima Benth.	USA, 29.08.1969. Howell 46064 BR
Neoplingia leucophylloides Ramamoorthy	Mexico, 05.08.1982. Hiriart & Medrano 12792 K
Obtegomeria caerulescens (Benth.) Doroszenko	USA, 16.08.1986. Hermes, Cuadrov & Gentry 2706 MO
Origanum rotundifolium Boiss.	Cultivated in KEW accession no.: 1968–19106 K[a]
Pentapleura subulifera Hand.-Mazz.	Iraq, 4–9. 07. 1957. Rechinger 12085 K
Piloblephis rigida (Bartram ex Benth.) Raf.	USA, 13.02.1995. Holst et al. 4543 MO
Pogogyne douglasii Benth.	USA, California, 31.05.1892. Bioletti s.n. BR

Table 3 continued

Species	Voucher specimens
Pogogyne serpylloides (Torr.) Gray	USA, California, 28.04.1964. Rose 64044 BR
Poliomintha incana (Torr.) A.Gray	USA, 18.06.1985. Whiting 756/731 GH[a]
Poliomintha longiflora A.Gray	Mexico, 11.09.1955. Rzedwskiz 6583 GH
Prunella vulgaris L.	UK, 03.07.1886. Bailey 1132 LV[a]
	Belgium, 17.06.2005. Moon LV
	Cultivated in KEW accession no.: 1994–2824 K[a]
Pycnanthemum albescens Torr. & A.Gray.	USA, 27.08.1982. Kessler et al. 2648 BR
Pycnanthemum incanum (L.) Michx.	USA, 10.09.1966. Bradley et al. 3491 BR
Rhabdocaulon coccineum (Benth.) Epling	Brazil, 12.04.1977. Harley 20332 K
Rhabdocaulon strictum (Benth.) Epl.	Argentina, 18.04.1979. Persen 12455 GH
Rhododon ciliatus (Benth.) Epl.	USA, 06.06.1969. Correll 37399 GH
Saccocalyx satureioides Coss. & Durand	Algeria, 26.05.1965. Faurel et al. 5650 BR
Satureja thymbra L.	Cultivated in KEW accession no.: 2001–823 K[a]
Satureja spinosa L.	Cultivated in KEW accession no.: 1989–3009 K[a]
Stachydeoma graveolens (Chapm. ex A. Gray) Small	USA, 18.08.1962. Godfrey 62494 BR
	USA, 18.08.1962. Godfrey 62494 BR
Thymbra spicata L.	Italy, 18.06.1883. Ascherson 470 BR
	Cultivated in KEW accession no.: 2001–825 K[a]
Thymus pallasianus	Cultivated in KEW accession no.: 2001–4194 K[a]
Thymus serpyllum	Cultivated in KEW accession no.: 1973–21043 K[a]
Zataria multiflora Boiss.	Iran, 16.05.1892. Bornmüller 4274 GH
Ziziphora clinopodioides Lam.	Turkey, 01.09.1993. Vašák s.n. BR

All taxa were investigated by scanning electron microscopy

[a] The selected taxa also observed by transmission electron microscopy

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