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NUTLET SCULPTURING OF SELECTED SPECIES FROM *AJUGOIDEAE, SCUTELLARIOIDEAE AND STACHYOIDEAE (LAMIACEAE)*

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Nutlet ornamentation of four species of *Ajuga (Ajugoideae),* two species of *Scutellaria (Scutellarioideae),* two species of *Marrubium, Prasium majus* and *Sideritis hyssopifolia (Stachyoideae)* were analysed, using scanning electron microscopy. The study provides additional stable micromorphological characters to include in classification at tribal and generic levels within the *Lamiaceae.*

Key words: *Lamiaceae, Ajuga, Prasium, Sideritis, Marrubium, Scutellaria,* nutlet sculpturing, SEM, taxonomy.

Ključne reči: *Lamiaceae, Ajuga, Prasium, Sideritis, Marrubium, Scutellaria,* skulpturiranost orašica, SEM, taksonomija.

INTRODUCTION

Lamiaceae is a large family with about 220 genera and more than 3500 species. The family has an almost cosmopolitan distribution and wide range of morphological diversity. The species are mainly herbaceous or shrubby growing usualy in warm open habitats. Areas of major diversity of *Lamiaceae* species are Mediterranean region, Asia and Northern America, including Mexico (Hedge, 1992).

Many species are of economic importance (aromatic, medicinal or culinary herbs). A number of *Labiatae* species were investigated by botanists, from different aspects.

Taxonomy of this interesting and important family is still unresolved. First of all, there is not clear difference between *Lamiaceae* and *Verbenaceae* within the order *Lamiales* (Cronquist, 1981; Olmstead et al. 1992). In spite of several major reviews dealing with infrafamilial classification of *Lamiaceae* (Bentham, 1876; Briquet, 1895-97; Erdtman, 1945; Wunderlich, 1967; Cantino, 1992), many problems still remains. Those classifications are based on different characters (morphological, palynological, embryological). An attempt of application of chloroplast DNA sequences in *Asteridae* as well as *Lamiales* has been done (Olmstead et al. 1992).

In earlier reviews of any group of *Lamiaceae,* nutlet microcharacters were ignored or seldom mentioned in spite of their stability (Davis & Heywood, 1963). Recent studies of selected taxa, have shown that nutlet microcharacters can be used as additional taxonomic parameters in delimitation of such taxa (Krestovskaja, 1988; Husain et al. 1990; Marin et al. 1994).

In this survey nutlet ornamentation of selected genera from the *Ajugoideae, Scutellarioideae* and *Stachyoideae* as a potential valuable taxonomical character is described.

MATERIAL AND METHODS

Plant material was collected from different parts of Yugoslavia. Some samples were collected from herbarium specimens from the Herbarium of Botanical Institute and Garden „Jevremovac" in Belgrade, where voucher specimens are deposited. Places of collection of specimens are given in Tab. 1.

Tab. 1. – Places of collection of analysed species

Taxa	Place of collection
Subfam. *Ajugoideae*	
Ajuga reptans L.	Beograd (Yu)
Ajuga genevensis L.	Kukavica (Yu)
A. laxmanii (L.) Bentham	Lisac planina (Yu)
A. chamaepitys (L.) Schreber	Bistrica (Yu)
Subfam. *Scutellarioideae*	
Scutellaria alpina L.	Košice (Chech Republic)
Scetellaria altissima L.	Fruška Gora (Yu)
Subfam. Stachyoideae	
Prasium majus L.	Barcelona (Spain)
Sideritis hyssopifolia L.	Bern (Swiss)
Marrubium incanum Desr.	Barcelona (Spain)
Marrubium vulgare L.	Barcelona (Spain)

For scanning electron microscopy (SEM) at least three samples of mature nutlets were used. The samples were previously observed by light microscope. The nutlets were mounted on stubs and coated with 30 nm of gold – palladium (85:15) in a JEOL JEE 4B vacuum evaporator and observed with a JEOL JSM T.35 scanning electron microscope.

RESULTS AND DISCUSSION

The nutlet sculpturing of 10 species from selected genera of *Ajugoideae, Scutellarioideae* and *Stachyoideae* subfamilies are presented (Figs 1-27). Infrafamilial classification was according to W u n d e r l i c h (1967).

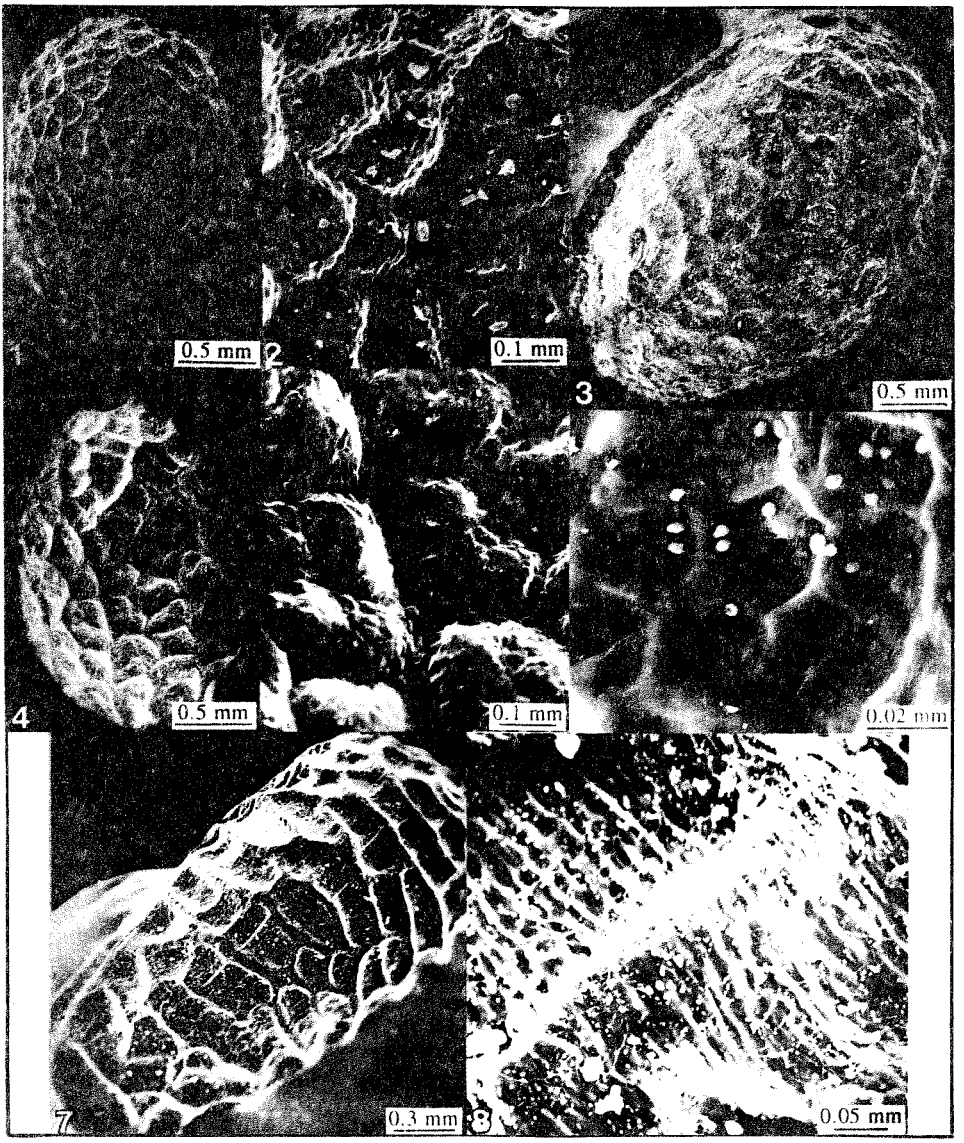
From the *Ajugoideae* subfamily, four *Ajuga* species are analysed (Figs 1-8). The *Ajuga* nutlets showed quite high similarities in size, shape and ornamentation between the species. Especially, *Ajuga genevensis* (Figs 1, 2), *A. laxmanii* (Fig. 3) and *A. reptans* (Figs 4-6) were very similar with characteristic more or less ovate or angular sunken fields. The epicarp cells were 5-6 angular with thick walls. The ornamentation of *A. chamaepitys* (Figs 7, 8) is also with sunken fields, but they are transversaly elongated. The epicarp cells are striate. Species from the first group showed similarities with some *Teucrium* species *(T. polium, T. bracteatum)* (M a r i n et al., 1994). *Teucrium* is close genus to *Ajuga,* also with some similarities in nutlet micromorphology. But, according to micromorphological, as well as other characters, *Teucrium* is very heterogenous. In number of species oil glands were found on nutlets, and some of them consists of trichomes. Those structures were not found in any *Ajuga* species analysed in this paper. Some authors suggest to establish a new subfamily *Teucrioideae,* which includes *Teucrium,* some genera from *Ajugoideae* (but not *Ajuga)* and some genera which were so far placed in *Verbenaceae* family (C a n t i n o et al., 1992). Micromorphological data can not resolve completely these problems. However, there are some similarities in general microcharacters between these to genera which shows possibility of their close relationships.

Recent investigation of fatty acid composition in *Ajugoideae* nutlets showed high similarities of *Teucrium* and *Ajuga* (M a r i n et al., 1991). The main constituents in both genera were octadecadienoic (18:2) and octadecatrienoic acid (18:3).

From the *Scutellarioideae* subfamily, two *Scutellaria* species were analysed (Figs 9-16). According to nutlet ornamentation, *Scutellaria* species are very distinguishable from all other analysed genera. *Scutellaria altissima* possess very characteristic bumps, which are multicelled (Figs 9-12). The cells forming those bumps are very small, with more or less expressed apices. In the center of bumps small trusses of trichomes were present. In *Scutellaria alpina* similar pattern was found. The bumps were not expressed, but a number of trichome trusses were dispersed all over the nutlets (Figs 13-16). Trichomes were long and unicellulate. In addition, very small papillae were distributed on epicarp cells of nutlets. (See higher magnification, Figs 15, 16).

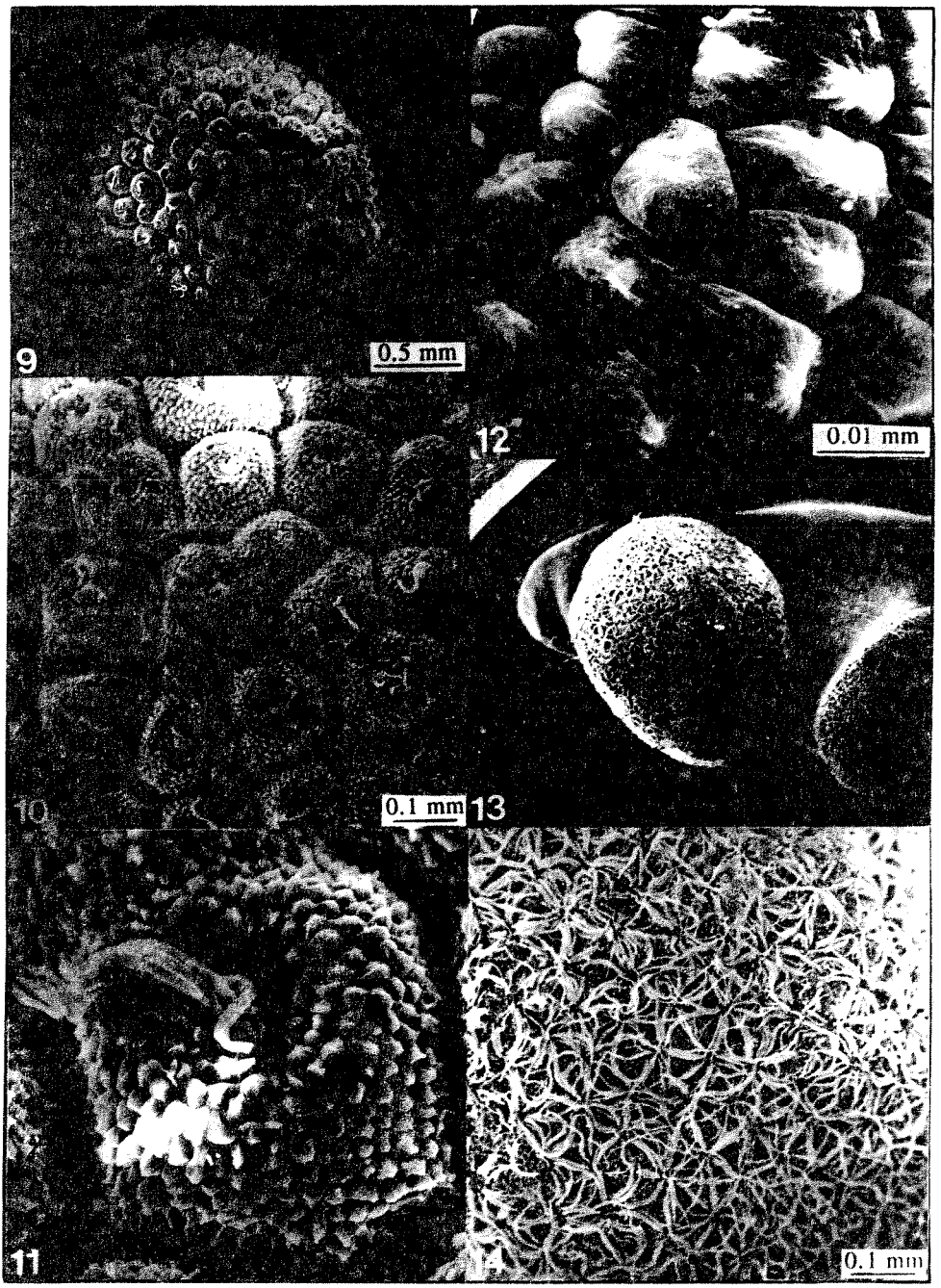
Nutlet lipid analysis in four *Scutellaria* species showed quite high uniformity in fatty acid composition (M a r i n et al., 1991). The octadecadienoic and octadecenoic were dominant fatty acids. Content of the octadecatrienoic acid was very low.

Recent investigation of global taxonomy of *Scutellaria* showed that nutlet morphology varies enormously within the sections (P a t o n, 1990). Some of species showed papillatae nutlets, in others the papillae were fused into a basal wing. In some species the hooks on the nutlet surface are present. P a t o n (1992) was hypothesized that the morphology of the fruiting calyx in *Scutellaria* is subjected to two main selection pressures, to the protection of immature fruit and the dispersal of the mature nutlets. According to this author, nutlet ornamentation probably plays a role in dispersal (adaptation to water, wind or animal dispersal). He suggested that nutlet sculpturing is more variable than calyx morphology because fewer constraints are placed on its evolution during development.

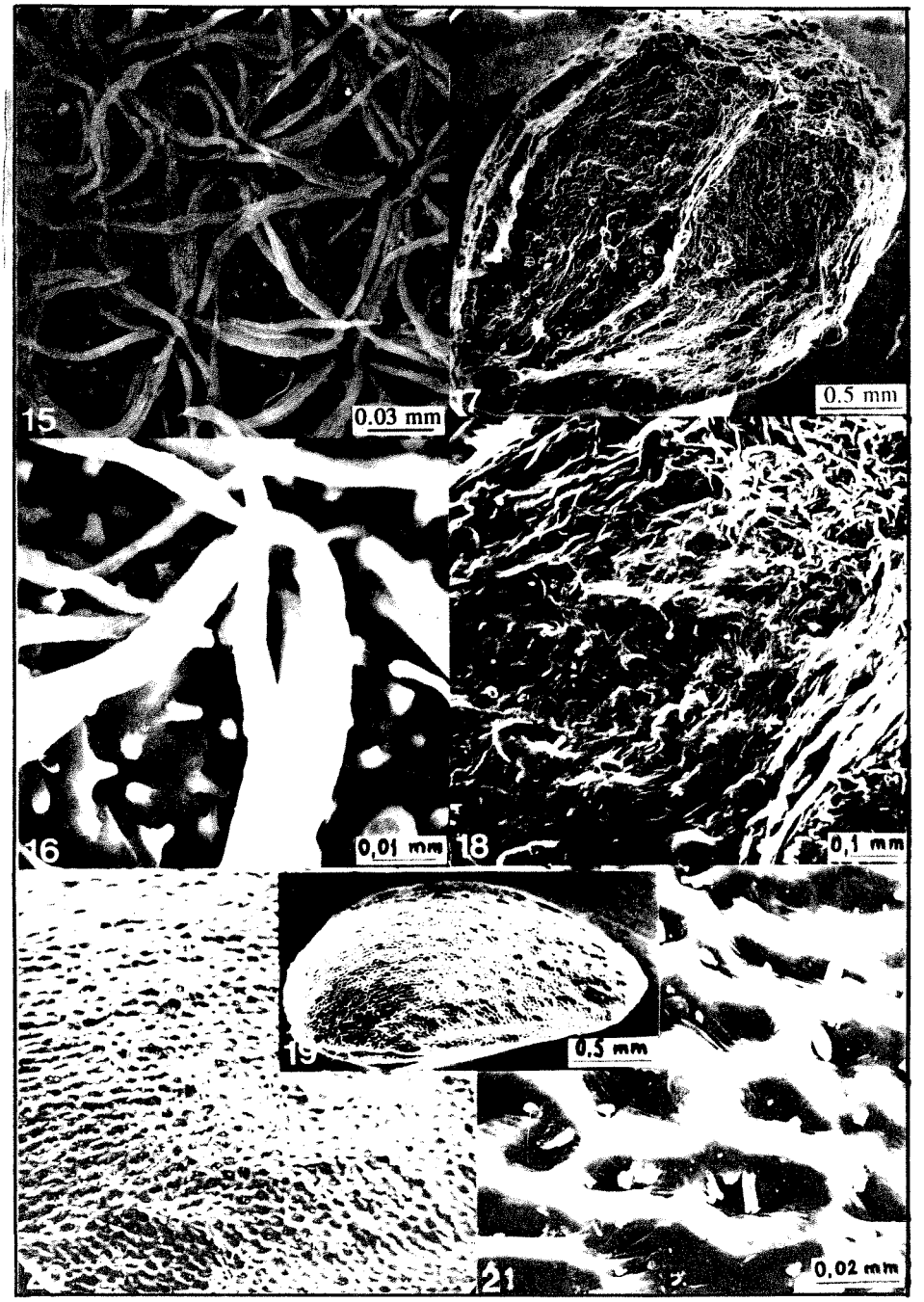


Figs. 1 - 2. – *Ajuga genevensis*; 3. – *A. laxmanii*;
4 - 6. – *A. reptans*; 7 - 8. – *A. chamaepitys*

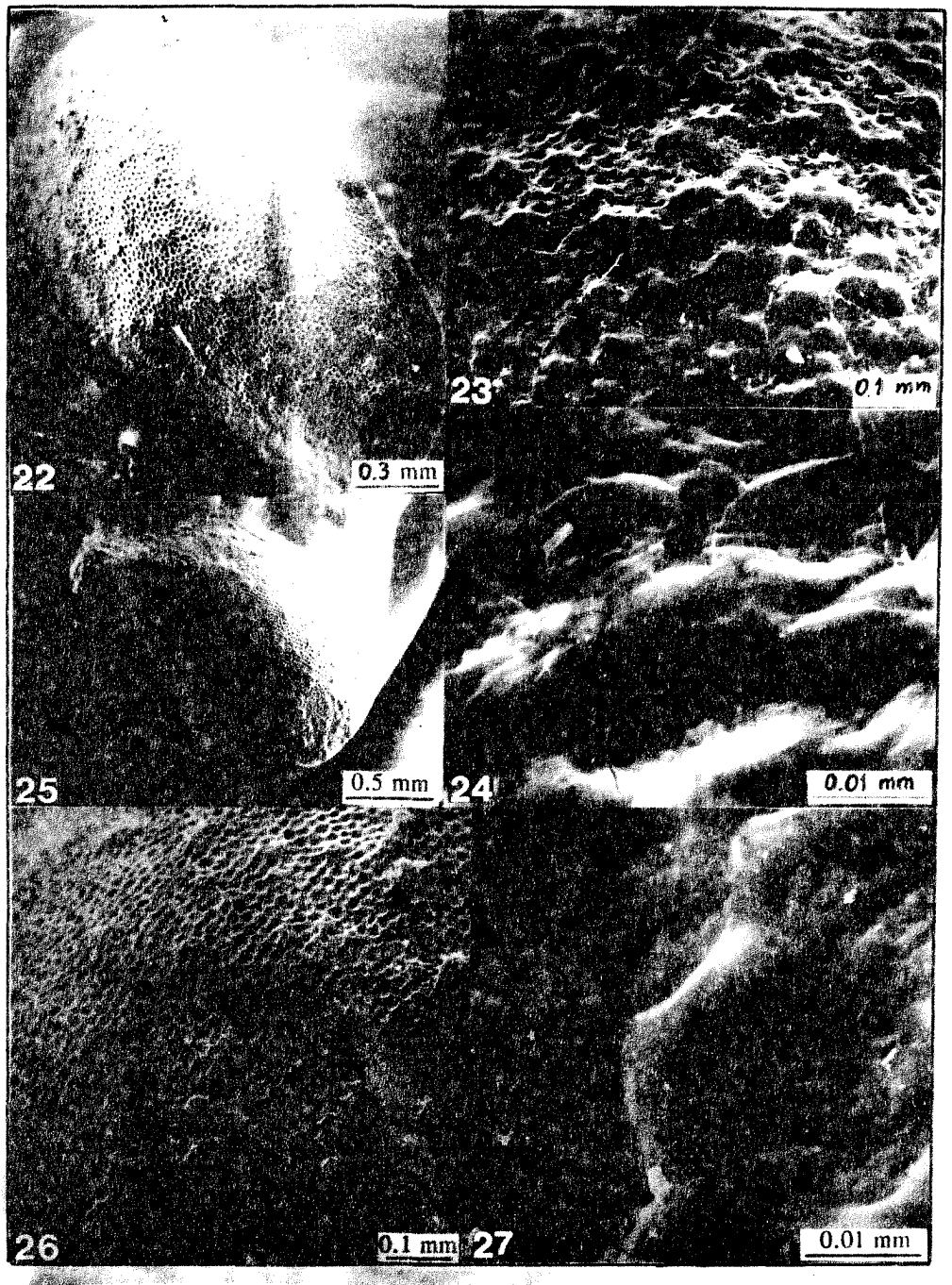
It should be mentioned that *Scutellaria* is subcosmopolitan genus consisting about 360 species, which are heterogenous because of great diversity in their ecological habitats. Probably, it is very old and „well defined" genus, derived from other taxa of *Lamiaceae* (separate subfamily!). The taxonomical significance of nutlet ornamentation, in spite of their great diversity, can not be ignored. The problem is how to explain



Figs 9 - 12. – *Scutellaria altissima;* 13 - 14. – *S. alpina*



Figs. 15 - 16. - *Scutellaria alpina*; 17 - 18. – *Prasium majus*; 19 - 21. – *Sideritis hyssopifolia*



Figs 22 - 24. – *Marrubium incanum*
Figs 25 - 27. – *M. vulgare*

such a diversity, their evolution and phylogenetical relationship. However, explanation of less variation in calyx morphology and their probable phylogenetic relationship is much easier and more evident.

From the *Stachyoideae* subfamily (sensu W u n d e r l i c h) four selected species were analysed. From the tribe *Prasieae, Prasium majus* was analysed. Nutlet ornamentation of this species has shown nonspecialized pattern (Figs 17, 18). It could be explained by the fact that *Prasium* species are endozoochorous. The exocarp become fleshy (endocarp becoming sclerotic) and attracting for the animals (B o u m a n et M e e u s e , 1992). The mature nutlets are dryed and as result of drying of fleshy exocarp become crowded.

From the tribe *Marrubieae Sideritis hyssopifolia* and two *Marrubium* species are presented. Nutlets of the *Sideritis hyssopifolia* are characterised by low protrusions sparsely distributed. The epicarp cells were angulare and thick-walled (Figs 19-21). Nutlet ornamentation of *Marrubium incanum* (Figs 22-24) and *M. vulgare* (Figs 25-27) showed very high similarities. Low protrusions formed by one or few cells were sparsely distributed on nutlets. Epicarp cells were angular or ovate, thick – celled. In spite of the facts that these species belongs to the different genera, nutlet ornamentation showed very similar structures. It could be suggested that those genera are very close related.

Marrubium and *Sideritis* showed similar pattern in fatty acid composition in nutlets (M a r i n et al., 1992). In both genera the dominant fatty acid was linoleate. *Sideritis* species consists of slightly higher content of this acid, and lower content of oleate.

Upon the whole the various forms of dispersal in *Lamiaceae* family do not reflect the taxonomic relationships (B o u m a n & M e e u s e , 1992). Those different types of dispersal are connected with different ecological factors. In a single genus it is possible to find different types of primary or secondary dispersal. In most cases the dispersal is result of parallelism and convergence.

However, nutlet ornamentation of species analysed in this survey showed that these parameters have to be included in further taxonomical revisions of any taxa of *Lamiaceae*. It seems that these microcharacters are of the best taxonomical value at generic and tribal level, and in some cases in infrageneric classification of *Lamiaceae*.

In addition, results of recent investigation of fatty acid composition suggest that these parameters can be used as taxonomic markers at different levels within the family.

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Rezime

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SKULPTURIRANOST ORAŠICA KOD ODABRANIH VRSTA IZ *AJUGOIDEAE, SCUTELLARIOIDEAE* I *STACHYOIDEAE (LAMIACEAE)*

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U ovom radu izvršena je mikromorfološka analiza ornamentacije orašica kod 10 odabranih vrsta iz različitih podfamilija *(Ajugoideae, Scutellarioideae* i *Stachyoideae)* familije *Lamiaceae,* pomoću skenirajućeg elektronskog mikroskopa (SEM). Dobijeni rezultati su pokazali da ovi parametri treba da budu uključeni i ravnopravno tretirani prilikom taksonomskih revizija različitih taksona kod usnatica. Izgleda da ovi mikrokarakteri imaju najveću vrednost kao markeri na nivou tribusa i rodova, a u nekim slučajevima i u infrageneričkoj klasifikaciji unutar familije *Lamiaceae.*