Nordic Journal of Botany 30: 352–364, 2012
doi: 10.1111/j.1756-1051.2011.01306.x,

Subject Editors: Thomas Denk and Guido Grimm. Accepted 11 November 2011

Nutlet micromorphology of Turkish *Stachys* sect. *Eriostomum* (Lamiaceae) and its systematic implications

Fatih Satıl, Ayla Kaya, Ekrem Akçiçek and Tuncay Dirmenci

F. Satıl (fsatil@gmail.com), Dept of Biology, Faculty of Science and Art, Balıkesir Univ., TR-10145 Balıkesir, Turkey. – A. Kaya, Pharmaceutical Botany, Faculty of Pharmacy, Anadolu Univ., TR-26470 Eskişehir, Turkey. – E. Akçiçek, Dept of Biology Education, Faculty of Necatibey Education, Balıkesir Univ., TR-10100 Balıkesir, Turkey. – T. Dirmenci, Dept of Biology Education, Faculty of Necatibey Education, Balıkesir Univ., TR-10100 Balıkesir, Turkey.

The nutlet morphology of 32 taxa of *Stachys* sect. *Eriostomum* (Lamiaceae) has been studied by scanning electron microscopy (SEM), and a detailed description of the nutlet morphological features of all examined taxa is provided. We found some groups within *Stachys* sect. *Eriostomum* that present nutlet micromorphological characters that appear to be useful in the species-level taxonomy. The basic shape of nutlets in most taxa is obovoid or ± rounded and the size ranged between 1.5 and 3.0 mm in length and between 1.0 and 2.5 mm in width. Five basic types of sculpturing can be distinguished: reticulate–tuberculate, reticulate–smooth, reticulate–slightly furrowed, colliculate–tuberculate, colliculate–smooth. The reticulate type is the most common among the studied species. The colliculate type is characteristic for *S. minor* and *S. cretica* subsp. *vacillans*. Subsection *Spectabiles* with reticulate-smooth/slightly furrowed sculpturing is easily distinguished from the other subsections. In addition, nutlet micromorphology is useful for separating the subspecies of *S. cretica*. The systematic and biological implications of the nutlet characteristics are briefly discussed.

The genus *Stachys* L., one of the largest genera of Lamiaceae, includes about 300 species. It is a subcosmopolitan genus centred in the warm temperate regions of the Mediterranean and southwest Asia, with secondary centres in North and South America and southern Africa (Bhattacharjee 1982). The first revision of *Stachys* in Turkey was made by Bhattacharjee (1982) for the 'Flora of Turkey'. He treated 87 species (112 taxa) belonging to 15 sections and 2 subgenera. Of the 112 taxa, 52 (46%) are endemic to Turkey (Bhattacharjee 1982, Davis et al. 1988, Duman 2000, Akçiçek 2010). Most of the endemic taxa are east Mediterranean elements.

The section *Eriostomum* (Hoffmanns. & Link) Dumort. has 23 species (34 taxa) in Turkey. This section, which is homogenous with respect to general morphology, has a wide range throughout Europe, Asia and parts of northern Africa. It is divided into three sub-sections, one of which is subsect. *Spectabiles* R. Bhattacharjee, mainly distributed in the oriental and Irano–Turanian regions. Meanwhile, subsect. *Creticae* R. Bhattacharjee and subsect. *Germanicae* R. Bhattacharjee are widely distributed throughout Europe and Asia (Bhattacharjee 1974, 1980, Falciani 1997).

Nutlet surface anatomy provide some of the most useful taxonomic characters in some genera of Lamiaceae. The importance of scanning electron microscopy (SEM) for the study of nutlet surfaces and the taxonomic value of nutlet characters has been described in many genera of Lamiaceae (Husain et al. 1990, Demissew and Harley 1992, Marin et al. 1996, Budantsev and Lobova 1997, Jamzad et al. 2000). Nutlet morphology in Lamiaceae has proved useful to varying degrees at different levels in the taxonomic hierarchy (Budantsev and Lobova 1997).

Ryding (1992, 1993) studied nutlet characters in genera of Lamiaceae using SEM. Surfaces were typically smooth and sub-surface characters were used to distinguish taxa. Marin et al. (1994) characterised nutlets of *Teucrium* L. by the presence and density of oil glands. They concluded that nutlet characters were potentially useful within Lamiaceae at the level of section, genus and species. Oran (1996) found that gross nutlet morphology and surface sculpturing in species of *Salvia* L. was variable and taxonomically useful, and developed descriptive categories for shape, surface sculpturing pattern and cellular deposits. Husain et al. (1990) studied the micromorphology in the tribe Saturejeae and found that sculpturing patterns (most commonly reticulate) were the most useful characters. Demissew and Harley (1992) studied the seed epidermis and found that surface types correlated with the three infrageneric groups of *Stachys* in tropical Africa. The surfaces types recognized were coarsely reticulate, finely reticulate, reticulate and spinulose.

In Turkey, *Stachys yildirimlii* M. Dinç and *S. cydni* Kotschy ex Gemici & Leblebici (subgen. *Stachys* sect. *Ambleia*) were examined using SEM by Dinç and Doğan (2006). The nutlets of *S. yildirimlii* are brown, obovate-triangular, and on average 2.2–2.3 mm long and 1.1–1.2 mm wide. The surface ornamentation is reticulate-granulate. The nutlets of *S. cydni* are black, oblong-triangular and on average 2.1–2.2 mm long and 1.0–1.1 mm wide. The surface ornamentation is rugulate-granulate. Further, nutlet characters have recently been described in many other genera of Lamiaceae in Turkey (Kaya and Dirmenci 2008, Kaya et al. 2009, Özkan et al. 2009, Kahraman et al. 2010).

The taxonomy of *Stachys* is very difficult mainly due to great variation in macromorphological characters, particularly under different ecological conditions. Demissew and Harley (1992) found that variation in nutlet surface sculpturing and exocarp cellular morphology matched infrageneric groups of *Stachys*. They suggested that tropical African species of *Stachys* can be divided into three natural groups based on trichome features and nutlet microsculpturing patterns, which are to some extent in accordance with the subgeneric classification suggested by Bhattacharjee (1980). These groups are further supported by biogeographical and ecological data.

However, the species of *Stachys* sect. *Eriostomum* has so far not been investigated in detail and potentially informative microcharacters usefull for thier classification may have been overlooked. In spite of considerable morphological homogeneity among the species of the section, nutlet micromorphology may provide support for separating the species of this section. According to Salmaki et al. (2008), among the species attributed to this section, *S. byzantina* Boiss. and *S. spectabilis* Choisy ex DC. show similar microsculpturing pattern, but differ in nutlet shape. Other species of the section can be distinguished based on the type of microsculpturing.

The aim of this study is to present the surface micromorphology of the Turkish species of *Stachys* sect. *Eriostomum* species and to discuss their taxonomic values.

Material and methods

The plant material was collected in different regions of Turkey (Table 1). Voucher specimens were deposited in the Herbarium of the Necatibey Education Faculty of Balıkesir Univ., Turkey.

Nutlets were examined from 32 taxa of *Stachys* (Table 1). Two to four nutlets from different populations of each species were selected and examined when a number of additional specimens had been compared under stereomicroscope for similarity. Measurements and optical observation of nutlet colour were carried out under a stereomicroscope Wild M5. For scanning electron microscopy (SEM), dry, mature nutlets were mounted directly on stubs, using single-sided adhesive tape, coated with gold, and photographs were taken with EVO-50. Nutlet surface sculpturing terminology follow Stearn (1992) and Bojňanský and Fargašová (2007). For recording gross morphology and size parameters, at least 10 dry mature nutlets of each of the 32 taxa were analyzed.

Results

The main features of the investigated nutlets are summarized in Table 2. Selected SEM micrographs of nutlets are presented in Fig. 1–3.

The shape of nutlets showed three types of variation among the investigated taxa. Most nutlets were obovoid, but they were more or less rounded in *S. alpina* subsp. *macrophylla* and *S. balansae* (Fig. 1e–f), obovoid to ± rounded in *S. huber-morathii*, *S. pinetorum*, *S. obliqua*, *S. sericantha* (Fig. 1i–k, m), *S. vuralii* and *S. huetii* (Fig. 2n, 3d).

The apex of trigonous nutlets showed large variation, but most were obtuse-rounded. The apex was truncate-rounded in *S. tmolea*, *S. cretica* subsp. *kutahyensis* (Fig. 2a, l), rounded in *S. germanica* subsp. *heldreichii*, *S. balansae*, *S. minor* (Fig. 1a, f, l), *S. cretica* subsp. *cretica*, *S. cretica* subsp. *vacillans*, *S. vuralii* (Fig. 2g–h, n), *S. huetii* (Fig. 3d), truncate in *S. bithynica*, *S. tymphaea*, *S. cretica* subsp. *smyrnaea*, *S. cretica* subsp. *anatolica* (Fig. 1c, 2i, k), and obtuse-rounded in the others (Fig. 1–3).

Areoles were small and circular or triangular in shape. Most of the nutlets were distinctly winged towards the base. However, in some species (*S. bithynica*, *S. balansae*, *S. huber-morathii*, *S. pinetorum*, *S. minor*, *S. sericantha*, *S. cretica* subsp. *cassia*, *S. cretica* subsp. *garana*, *S. cretica* subsp. *anatolica*, *S. cretica* subsp. *kutahyensis*, *S. vuralii*, *S. thirkei* and *S. longispicata*) they were only slightly winged towards the base (Fig. 1–3).

Nutlets of all taxa were blackish–brown in colour. Nutlet size ranged between 1.5 and 3.0 mm in length and 1.0 and 2.5 mm in width. Among the examined species *S. huber-morathi* has the largest nutlets (2.5–3.0 × 2.0–2.5 mm), while species like *S. longispicata* (1.5–1.9 × 1.0 mm) possessed very small nutlets (Table 2).

Nutlet surfaces were found to be high diagnostic among taxa. Regarding the sculpturing pattern of nutlet surfaces, five basic types could be distinguished: reticulate-tuberculate in *S. germanica* subsp. *heldreichii*, *S. bithynica*, *S. tymphaea*, *S. balansae*, *S. pinetorum*, *S. sericantha*, *S. cretica* subsp. *cassia*, *S. cretica* subsp. *garana*, *S. cretica* subsp. *lesbiaca*, *S. cretica* subsp. *bulgarica*, *S. cretica* subsp. *cretica*, *S. cretica* subsp. *smyrnaea*, *S. cretica* subsp. *mersinaea*, *S. cretica* subsp. *anatolica*, *S. cretica* subsp. *kutahyensis*, *S. byzantina*, *S. vuralii*, *S. thirkei* (Fig. 1–2), reticulate-smooth in *S. thracica*, *S. alpina* subsp. *macrophylla*, *S. carduchorum*, *S. rizeensis*, *S. huber-morathii*, *S. obliqua* (Fig. 1d–e, g–i, k), *S. tmolea* (Fig. 2a), reticulate-slightly furrowed in *S. cretica* subsp. *trapezuntica* (Fig. 2e), *S. spectabilis*, *S. longispicata*, *S. viticina*, *S. huetii* (Fig. 3), colliculate-tuberculate in *S. cretica* subsp. *vacillans* (Fig. 2h), and colliculate-smooth in *S. minor* (Fig. 1l). The reticulate type was the most common among the studied species, but there were some specific differences in the sculpturing pattern between taxa (Table 2).

The five main nutlet surface types in sect. *Eriostomum* species distinguished based on surface ornamentation are (Bojňanský and Fargašová 2007): 1) reticulate: the reticulate pattern consists of large rounded-polygonal cells with more prominent walls; 2) tuberculate: tuberculate

Table 1. Nutlet specimens examined.

Taxa	Subsection	Collection data	Herbarium no.
S. germanica L. subsp. *heldreichii* (Boiss.) Hayek	*Germanicae*	Muğla: Ortaca, 5 m, 16 Aug 2009	EA 5374
S. bithynica Boiss.	*Germanicae*	Bursa: Uludağ, 2050 m, 6 Sep 2007	EA 4780
		Kayseri: Sarız, 2400 m, 7 Aug 2007	A. Duran 7667
S. tymphaea Hausskn.	*Germanicae*	Kırklareli: Dereköy, 450 m, 21 Jun 2009	EA 5292
		Tekirdağ: Saray, 150 m, 1 Aug 2007	Yıldız 16527
		Kırklareli: Kofcaz, 500 m, 2 Aug 2007	Yıldız 16611
S. thracica Dav.	*Germanicae*	Kırklareli: Armutveren, 380 m, 21 Jun 2009	EA 5291
		Kırklareli: Dereköy, 550 m, 2 Aug 2007	Yıldız 16522
		Tekirdağ: Saray, 165 m, 21 Jun 2009	EA 5294
S. alpina L. subsp. *macrophylla* (Albov) R. Bhattacharjee	*Germanicae*	Balıkesir: Alaçam Mountains, 800 m, 25 Jul 2007	EA 4771
		Bursa: Uludağ, ca 1000 m, 12 Jul 2008	EA 5217
S. balansae Boiss. & Kotschy	*Germanicae*	Rize: İkizdere, 2450 m, 1 Sep 2008	EA 5223
		Erzurum: Kop Mountain, 2450 m, 4 Sep 2008	EA 5248
		Ağrı: Tahir village, 2450 m, 12 Aug 2007	TD 3547
S. carduchorum (R. Bhattacharjee) Rech. f.	*Germanicae*	Van: Çatak, Kavuşşahap Mountain, 2750 m, 24 Jul 2009	EA 5335
		Hakkari: Uludere, 2400 m, 22 Jul 1974	Koyuncu 4455
S. rizeensis R. Bhattacharjee	*Germanicae*	Rize: Çamlıhemşin, 2500 m, 4 Sep 2008	EA 5235
		Artvin: Yusufeli, 2100 m, 18 Sep 2007	Yıldız 16703
S. huber-morathii R. Bhattacharjee	*Germanicae*	Çorum: Kırkdilim gorge, 1150 m, 10 Jul 2009	EE 1006
		Amasya: Gümüşhacıköy, 1300 m, 4 Jul 2008	Yıldırım 3488
S. pinetorum Boiss. & Bal.	*Germanicae*	Osmaniye: Amanos Mountains, 850 m, 9 Jul 2007	EA 4757
		Osmaniye: Hasanbeyli, 1400 m, 2 Sep 2006	Yıldız 16423
		Kahramanmaraş: Andırın: 1130 m, 10 Jul 2007	EA 4760
S. obliqua Waldst. & Kit.	*Germanicae*	Balıkesir: Madra Mountains, 300 m, 29 Jun 2007	EA 4659
		Balıkesir: Gökçeyazı, 250 m, 23 Jun 2009	EA 5316
		Burdur: Tefenni, 1200 m, 8 Jun 2007	EA 4616
S. minor (Boiss.) Akçiçek & Dirmenci	*Germanicae*	Hatay: Yayladağı, 500 m, 20 Jul 2009	EA 5319
		Mersin: Kuzucubelen, 550 m, 5 Jun 2009	EA 5273
S. sericantha P. H. Davis	*Germanicae*	Antalya: Kemer, Ovacık Village, 1200 m, 8 Jun 2007	EA 4624
		Antalya: Kumluca, 540 m, 7 Jun 2008	EA 5123
		Antalya: Beldibi, 10 m, 12 Jun 2010	EA 5496
S. tmolea Boiss.	*Creticae*	Balıkesir: Kaz Mountains, 1750 m, 27 Jul 2007	EA 4779
		İzmir, Bozdağ, 1600 m, 10 Jun 2007	EA 4642
		Kütahya: Murat Dağı, 1800 m, 27 Jun 2009	E. Erdoğan 1018
S. cretica L. subsp. *cassia* (Boiss.) Rech. f.	*Creticae*	Hatay: İskenderun, 50 m, 7 Jul 2007	EA 4741
		Osmaniye: Amanos Mountains, 850 m, 9 Jul 2007	EA 4758
		Hatay: Arsuz, 440 m, 11 Jun 2010	EA 5493
S. cretica L. subsp. *garana* (Boiss.) Rech. f.	*Creticae*	Kahraman Maraş: Başkonuş Mountain, 1250 m, 10 Jul 2007	EA 4763
		Malatya: Nemrut Mountain, 1400 m, 21 Jul 2009	EA 5334
		Hatay: Yayladağı, 750 m, 21 Jul 2008	EA 5195
S. cretica L. subsp. *lesbiaca* Rech. f.	*Creticae*	Çanakkale: Ayvacık, 360 m, 20 Jun 2009	EA 5288
		Balıkesir: Madra Mountain, 600 m, 26 Jul 2006	EA 4296
		Edirne: Uzunköprü, 110 m, 22 Jun 2009	EA 5302
S. cretica L. subsp. *trapezuntica* Rech. f.	*Creticae*	Trabzon: Maçka, 400 m, 7 Jul 2010	EA 5489
		Trabzon, Akçaabat, 80 m, 8 Jul 2010	EA 5490

S. cretica L. subsp. *bulgarica* Rech. f.	*Creticae*	Tekirdağ: Malkara, 250 m, 20 Jun 2009	EA 5287
		Tekirdağ: Şarköy, 250 m, 20 Jun 2009	EA 5287
S. cretica L. subsp. *cretica*	*Creticae*	Kırklareli: Pınarhisar, 260 m, 16 Jun 2010	EA 5498
		Çanakkale: Koru mountain, 50 m, 16 Jun 2010	EA 5498
S. cretica L. subsp. *vacillans* Rech. f.	*Creticae*	Antalya: Kemer, Ovacık village, 1240 m, 11 Sep 2008	EA 5244
		Burdur: Altınyayla, 1560 m, 12 Sep 2008	EA 5246
		Denizli: Pamukkale, 350 m, 1 Aug 2009	EA 5517
S. cretica L. subsp. *smyrnaea* Rech. f.	*Creticae*	Muğla: Marmaris, 130 m, 10 Jun 2007	EA 4640
		Balıkesir: Madra Mountain, 350 m, 5 Jul 2006	EA 4188
		Çanakkale: Ayvacık, 360 m, 20 Jun 2009	EA 5288
S. cretica L. subsp. *mersinaea* (Boiss.) Rech. f.	*Creticae*	Mersin: Kuzucubelen, 550 m, 16 Aug 2009	EA 5376
		Niğde: Ulukışla, 1050 m, 1 Jul 2001	A. Duran 5721
S. cretica L. subsp. *anatolica* Rech. f.	*Creticae*	Kütahya: Yoncalı, 1000 m, 6 Aug 2009	EA 5373
		Burdur: 900 m, 26 Jul 2007	EA 4774
		Malatya: Arapgir, 1300 m, 22 Jul 2009	EA 5331
S. cretica L. subsp. *kutahyensis* Akçiçek	*Creticae*	Kütahya: Tavşanlı, 850 m, 6 Jul 2007	EA 4726
		Balıkesir: Dursunbey, 235 m, 4 Jul 2008	EA 5099
S. byzantina C. Koch	*Creticae*	Çankırı: Ilgaz Mountain, 1850 m, 10 Jul 2009	EA 5338
		Bilecik: Muratdere, 900 m, 26 Jun 2007	EA 4658
		Balıkesir: Dursunbey, 600 m, 7 Jun 2007	EA 4615
S. vuralii Yıldız, Dirmenci, Akçiçek	*Creticae*	Kastamonu: Cide, 50 m, 5 Aug 2007	BY 16556
S. thirkei C. Koch	*Creticae*	Bursa: Uludağ, 1300 m, 28 Jul 2008	EA 5214
		Kütahya: Domaniç, 1500 m, 12 Jul 2008	EA 5210
		Çankırı: Kuzören village, 1020 m, 9 Jul 2009	E. Erdoğan 1016
S. spectabilis Choisy ex DC.	*Spectabiles*	Ardahan: 2060 m, 3 Sep 2008	EA 5236
		Tunceli: Ovacık, 1340 m, 23 Jul 2009	EA 5329
		Hakkari: Şemdinli, 1700 m, 5 Sep 2007	TD 3583
S. longispicata Boiss. & Kotschy	*Spectabiles*	Elazığ: Karakoçan, 1000 m, 20 Aug 2008	BY 16968
		Kahramanmaraş: Göksun, 1340 m, 21 Jul 2009	EA 5322
S. viticina Boiss.	*Spectabiles*	Hatay: Yayladağı, 400 m, 8 Jul 2007	EA 4748
		Hatay: Samandağı, 400 m, 8 Jul 2007	EA 4748
S. huetii Boiss.	*Spectabiles*	Erzurum: Palandöken Mountain, 2360 m, 12 Aug 2007	TD 3533
		Erzurum: Tortum, 2780 m, 10 Aug 2007	MFO 9692

Table 2. A comparision of examined characters for *Stachys* (sect. *Eriostomum*) nutlets. ± denotes slightly winged and + denotes distinctly winged. All species are blackish-brown.

Subsection	Taxa	Size (mm)	Shape	Surface sculpture		Apex	Wing
Germanicae	*S. germanica* subsp. *heldreichii*	1.7–2.0 × 1.0–1.2	obovoid	reticulate	tuberculate	tuberculate	+
Germanicae	*S. bithynica*	2.0–2.5 × 1.5–2.0	obovoid	reticulate	tuberculate	slightly tuberculate	±
Germanicae	*S. tymphaea*	2.0 × 1.2 (–1.5)	obovoid	reticulate	tuberculate	slightly tuberculate	+
Germanicae	*S. thracica*	2.5 × 2.0	obovoid	reticulate	smoth	slightly tuberculate	+
Germanicae	*S. alpina* subsp. *macrophylla*	2.0–2.5 × 1.7–2.2	± rounded	reticulate	smooth	slightly tuberculate	+
Germanicae	*S. balansae*	1.8–2.2 × 1.5–2.0	± rounded	reticulate	slightly tuberculate	tuberculate	±
Germanicae	*S. carduchorum*	2.5–3.0 × 1.8–2.0	obovoid	reticulate	tuberculate	tuberculate	+
Germanicae	*S. rizeensis*	2.0–2.5 × 1.8–2.0	obovoid	reticulate	smooth	slightly tuberculate	+
Germanicae	*S. huber-morathii*	2.5–3.0 × 2.0–2.5	obovoid or ± rounded	reticulate	smooth	slightly tuberculate	±
Germanicae	*S. pinetorum*	2.0–2.5 × 1.5–2.0	obovoid or ± rounded	reticulate	slightly tuberculate	smooth	±
Germanicae	*S. obliqua*	2.5 × 1.5–2.0	obovoid or ± rounded	reticulate	smooth	slightly tuberculate	+
Germanicae	*S. minor*	2.0–3.0 × 1.8–2.2	obovoid	colliculate	smooth	smooth	±
Germanicae	*S. sericantha*	2.0–2.5 × 1.5–1.8	obovoid or ± rounded	reticulate	slightly tuberculate	tuberculate	±
Creticae	*S. tmolea*	2.2–3.0 × 1.8–2.0	obovoid	reticulate	smooth	smooth	+
Creticae	*S. cretica* subsp. *cassia*	2.2–2.5 × 1.5	obovoid	reticulate	tuberculate	tuberculate	±
Creticae	*S. cretica* subsp. *garana*	2–3 × 1.5–2.0	obovoid	reticulate	tuberculate	tuberculate	±
Creticae	*S. cretica* subsp. *lesbiaca*	2.0–2.5 × 1.5–1.8	obovoid	reticulate	slightly tuberculate	tuberculate	+
Creticae	*S. cretica* subsp. *trapezuntica*	2.0–2.5 × 1.5–1.7	obovoid	reticulate	slightly furrowed	slightly tuberculate	+
Creticae	*S. cretica* subsp. *bulgarica*	2.5 × 1.5–1.8	obovoid	reticulate	tuberculate	tuberculate	+
Creticae	*S. cretica* subsp. *cretica*	2.0–2.5 × 1.8–2.0	obovoid	reticulate	slightly tuberculate	tuberculate	+
Creticae	*S. cretica* subsp. *vacillans*	2.5–3.0 × 2.0	obovoid	colliculate	tuberculate	tuberculate	+
Creticae	*S. cretica* subsp. *smyrnaea*	2.0–3.0 × 1.5–2.0	obovoid	reticulate	tuberculate	tuberculate	+
Creticae	*S. cretica* subsp. *mersinaea*	2.0–2.5 × 1.2–1.5	obovoid	reticulate	smooth	smooth	+
Creticae	*S. cretica* subsp. *anatolica*	2.0–3.0 × 1.5–2.0	obovoid	reticulate	tuberculate	tuberculate	±
Creticae	*S. cretica* subsp. *kutahyensis*	2.5–3.0 × 1.8–2.0	obovoid	reticulate	slightly tuberculate	tuberculate	±
Creticae	*S. byzantina*	2.5–3.0 × 1.8–2.0	obovoid	reticulate	slightly tuberculate	tuberculate	+
Creticae	*S. vuralii*	2.0–2.2 × 1.5–1.8	obovoid or ± rounded	reticulate	smooth	smooth	±
Creticae	*S. thirkei*	2.0–2.5 × 1.5–1.8	obovoid	reticulate	tuberculate	tuberculate	±
Spectabiles	*S. spectabilis*	2.0–2.2 × 1.0–1.5	obovoid	reticulate	smooth/slightly furrowed	slightly tuberculate	+
Spectabiles	*S. longispicata*	1.5–1.9 × 1.0	obovoid	reticulate	smooth/slightly furrowed	slightly tuberculate	±
Spectabiles	*S. viticina*	1.8–2 × 1.0	obovoid	reticulate	smooth/slightly furrowed	slightly tuberculate	+
Spectabiles	*S. huetii*	2.5–2.8 × 1.8–2.0	obovoid or ± rounded	reticulate	smooth/slightly furrowed	smooth	+

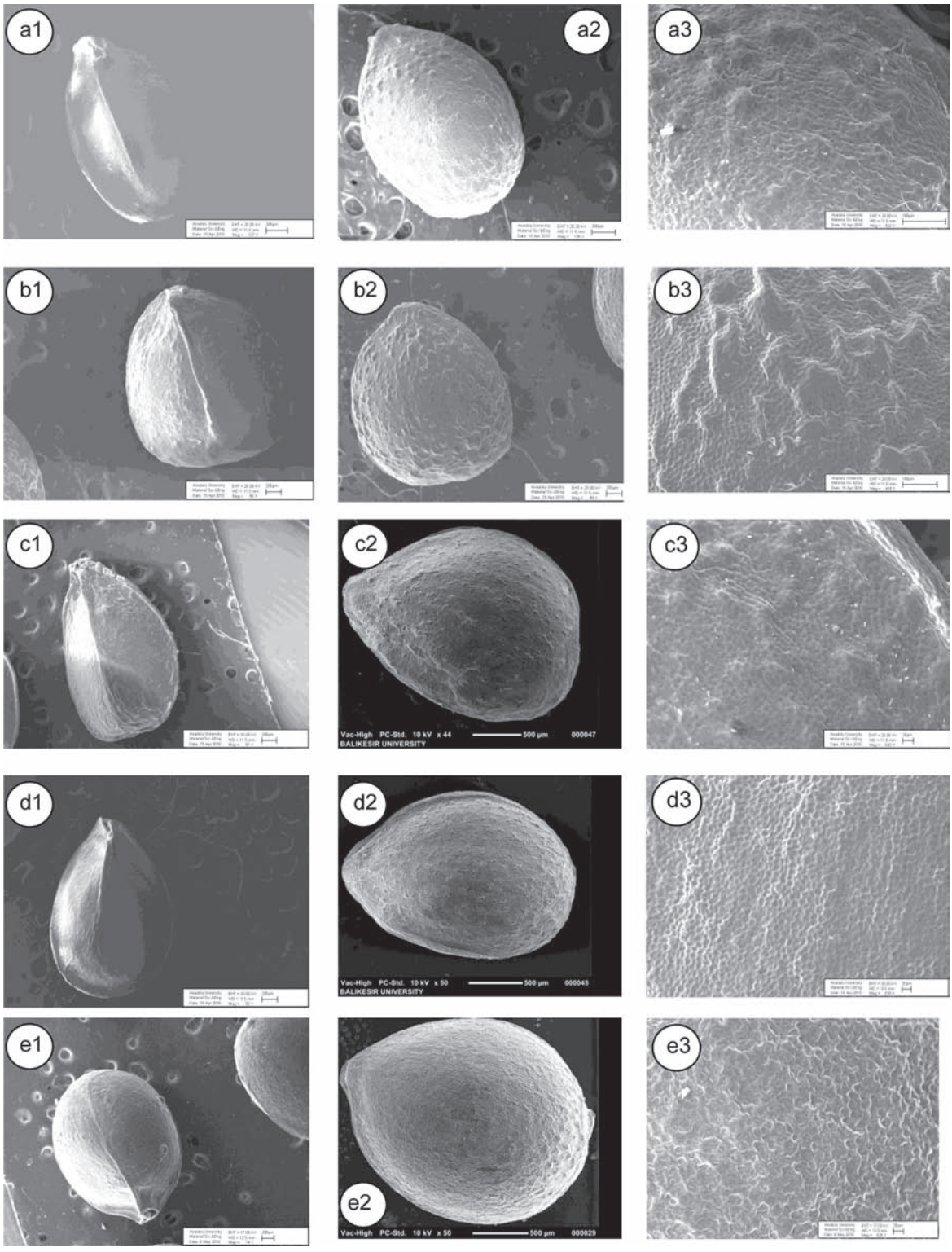

Figure 1. Scanning electron micrographs of nutlets of species of *Stachys* subsect. *Germanicae.* 1 = ventral, 2 = dorsal, 3 = surface sculpture. (a) *S. germanica* subsp. *heldreichii*, (b) *S. bithynica*, (c) *S. tymphaea*, (d) *S. thracica*, (e) *S. alpina* subsp. *macrophylla*, (f) *S. balansae*, (g) *S. carducorum*, (h) *S. rizeensis*, (i) *S. huber-morathii*, (j) *S. pinetorum*, (k) *S. obliqua*, (l) *S. minor*, (m) *S. sericantha.* Scale bars: a1, a2, b1, b2, c1, d1, e1, g1, g2, h1, i1, j1, k1, l1, m1 = 200 µm, a3, b3, g3, i3 = 100 µm, c2, d2, e2, f2, h2, i2, j2, k2, l2, m2 = 500 µm, c3, d3, e3, h3, j3, k3, m3 = 20 µm, f1 = 1 mm, f3, l3 = 50 µm.

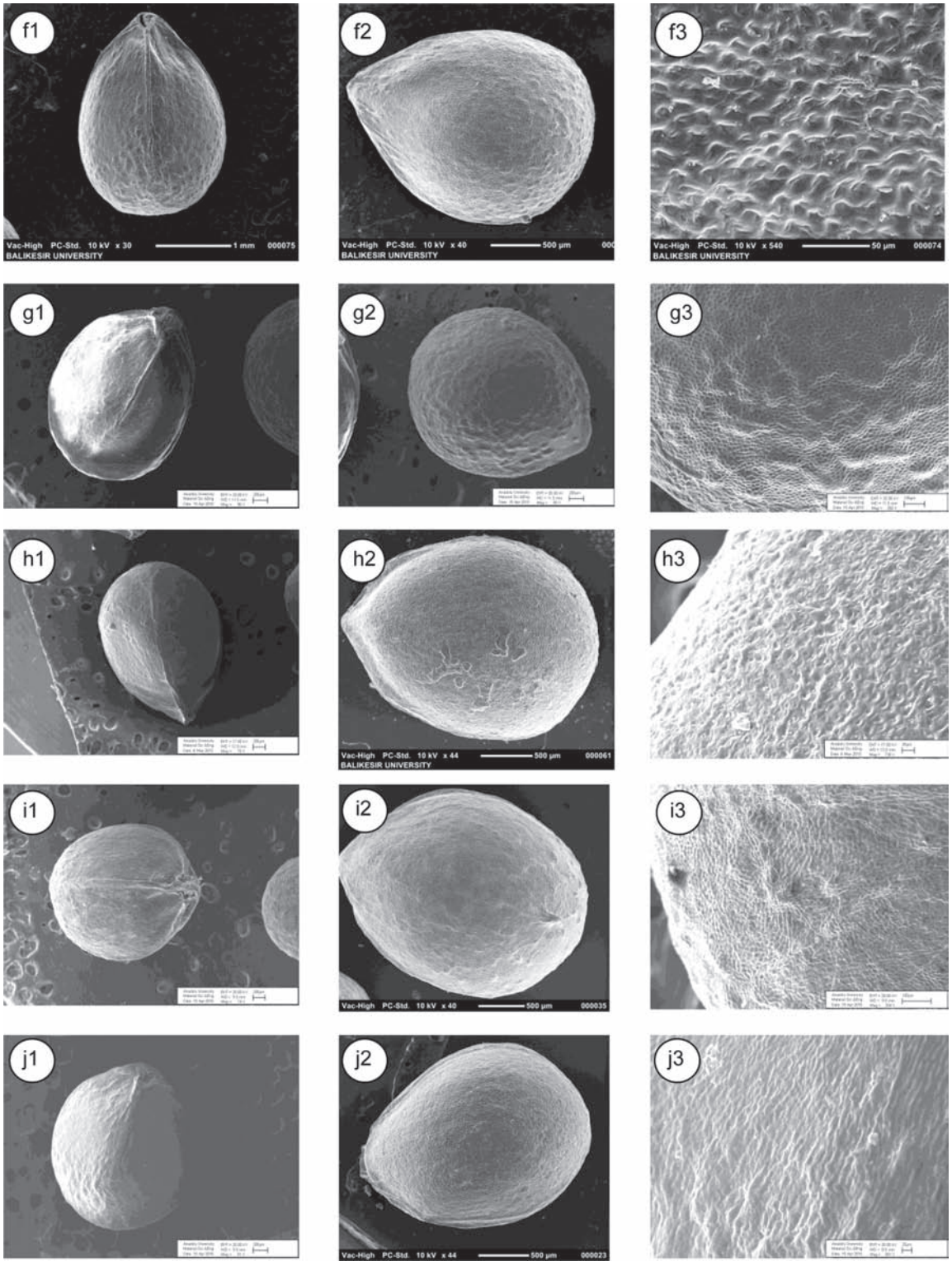

Figure 1. (Continued)

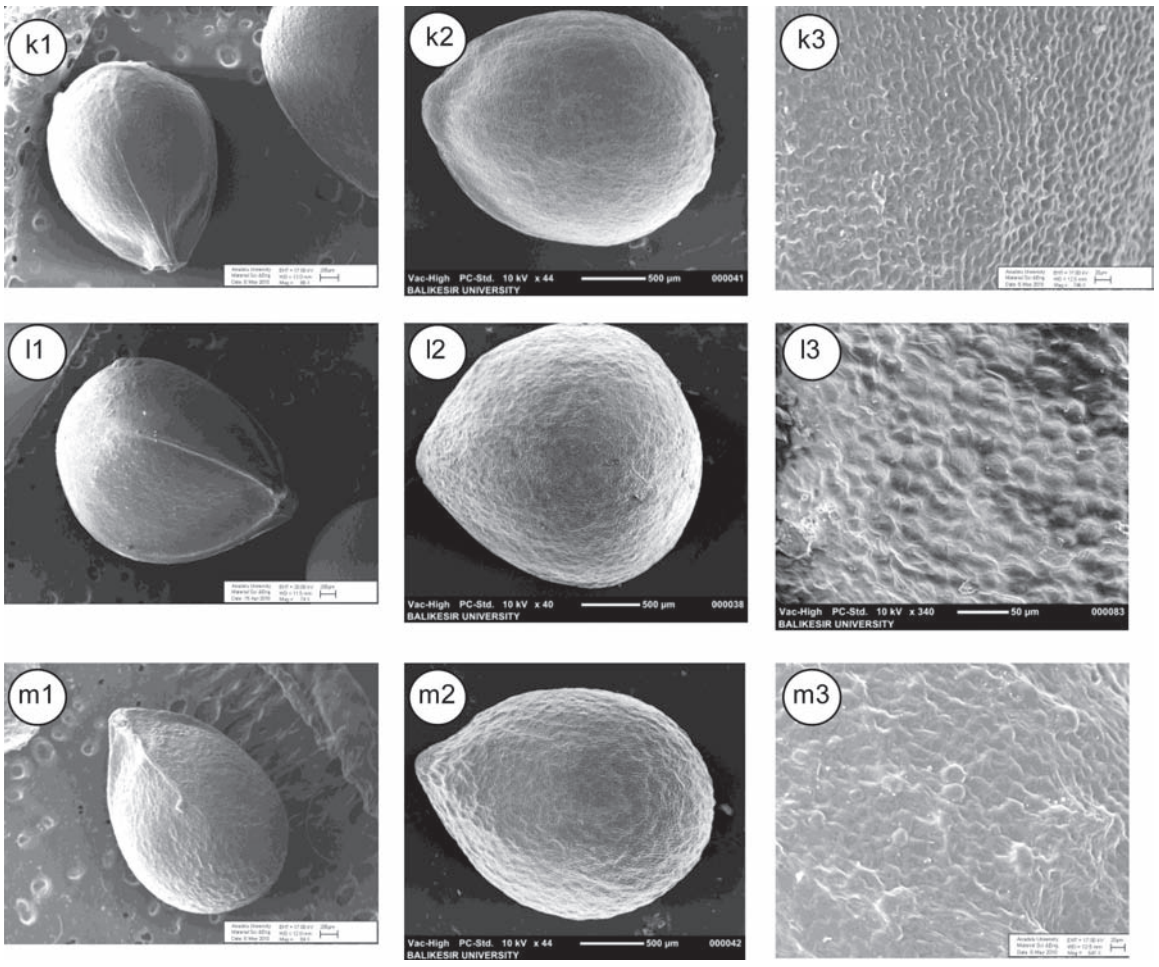

Figure 1. (Continued)

pattern is characterised by bearing small, warty, swelling, rounded or variously shaped projections, with small smooth rounded projections or knobs, covered with tubercles; 3) smooth: having an even surface, without irregularities or projections; 4) colliculate: with rounded broad elevations closely spaced and covering the seed-coat; 5) furrowed: having grooves, cracks, splits or narrow depressions, opposite of 'ridged'.

Discussion

Despite their stability as characters, the micromorphological characters of nutlet surfaces have either been totally ignored or only seldom mentioned in previous systematic studies on *Stachys*. In addition, the micromorphological characters of nutlets are largely unknown in the Turkish species, apart from nutlet shape, colour and size. In this work, the nutlet features of *S. rizeensis*, *S. minor*, *S. huetii*, *S. thirkei* and eleven subspecies of *S. cretica* are reported in detail for the first time. Among these, *Stachys vuralii* and *S. cretica* subsp. *kutahyensis* are recently described taxa (Akçiçek 2010, Dirmenci et al. 2011). In addition, *S. tymphaea* and *S. thracica* are new records for Turkey (Akçiçek et al. 2012).

The basic nutlet shape in most studied taxa was obovoid, however, ± rounded nutlets were found in a few species. Our results as far as nutlet shape is concerned are in accordance with Oran (1996) suggesting a relatively low importance of these characters when assessing relationships in *Stachys*. However, from a taxonomical point of view, nutlet shape can be used for separation of taxa in certain sections. For example, *S. alpina* subsp. *macrophylla* and *S. balansae* (Fig. 1e–f) with ± rounded nutlets are easily distinguished from other species of the section. In the same way, *S. balansae* can be distinguished from *S. bithynica* by nutlet shape alone. Nutlets of *S. bithynica* has an obovoid shape, while those of *S. balansae* are ± rounded (Fig. 1b, f).

Stachys balansae and *S. carduchorum* are morphologically similar (Rechinger 1982), but differ in nutlet size, shape and apex. The nutlets of *S. balansae* are smaller than those of *S. carduchorum* and are ± rounded, while they are obovoid in *S. carduchorum* (Table 2).

Many of the variable nutlet characters examined here are also of systematic value in other groups of Lamiaceae, particularly surface sculpturing (Husain et al. 1990, Oran 1996). The sculpturing of nutlets has been considered to provide the most valuable character (Oran 1996). In this study, regarding the sculpturing pattern of nutlet

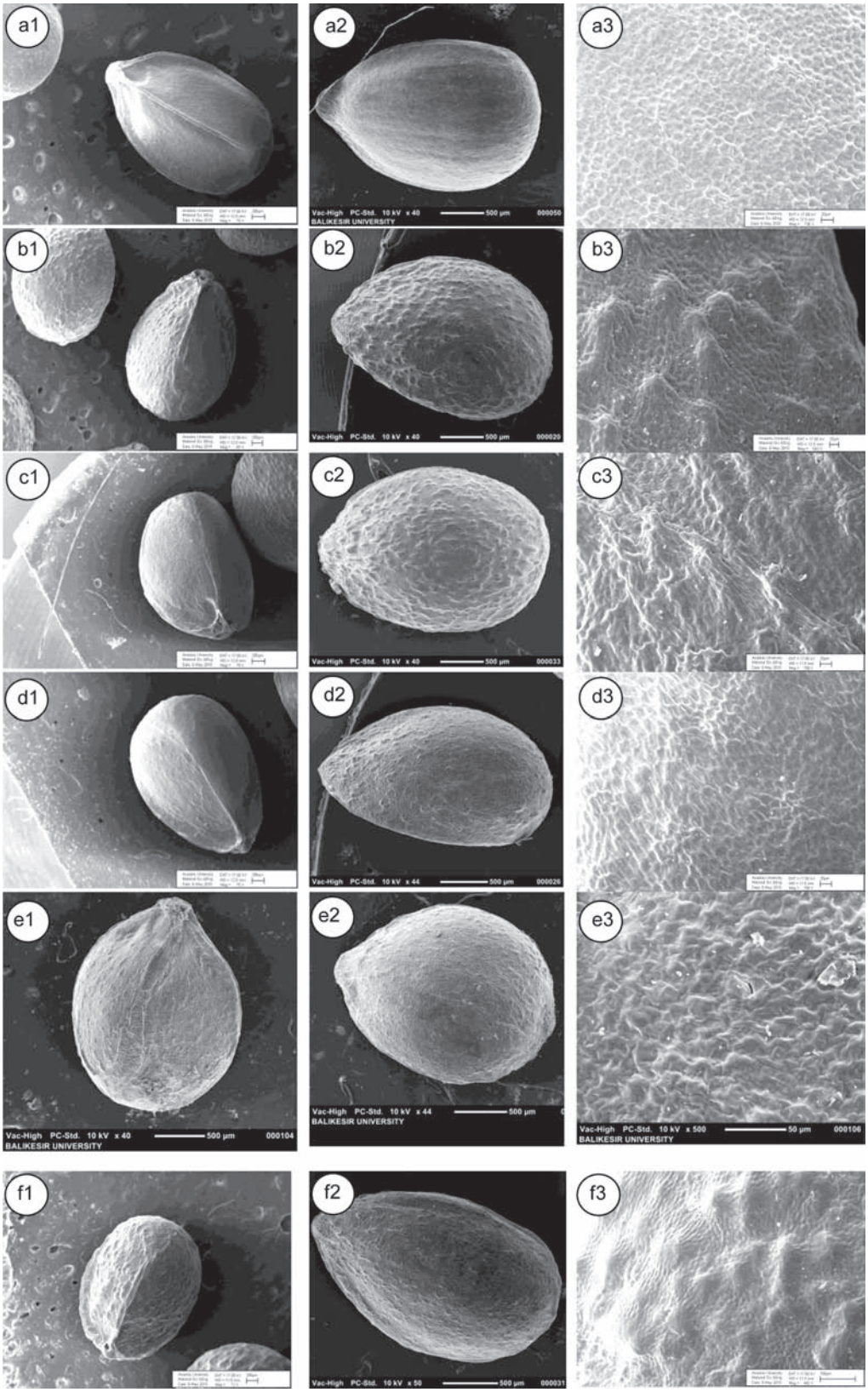

Figure 2. Scanning electron micrographs of nutlets of *Stachys* subsect. *Creticae.* 1 = ventral, 2 = dorsal, 3 = surface sculpture. (a) *S. tmolea*, (b) *S. cretica* subsp. *cassia*, (c) *S. cretica* subsp. *garana*, (d) *S. cretica* subsp. *lesbiaca*, (e) *S. cretica* subsp. *trapezuntica*, (f) *S. cretica* subsp. *bulgarica*, (g) *S. cretica* subsp. *cretica*, (h) *S. cretica* subsp. *vacillans*, (i) *S. cretica* subsp. *smyrnaea*, (j) *S. cretica* subsp. *mersinaea*, (k) *S. cretica* subsp. *anatolica*, (l) *S. cretica* subsp. *kutahyensis*, (m) *S. byzantina*, (n) *S. vuralii*, (o) *S. thirkei.* Scale bars: a1, b1, c1, d1, f1, h1, i1, j1, k1, l1, m1 = 200 µm, f3, j3, k3, m3 = 100 µm, a2, b2, c2, d2, e1, e2, f2, g1, g2, h2, i2, j2, k2, l2, m2, n1, n2, o1, o2 = 500 µml, a3, b3, c3, d3, h3, i3, l3 = 20 µm, e3, g3, n3, o3 = 50 µm.

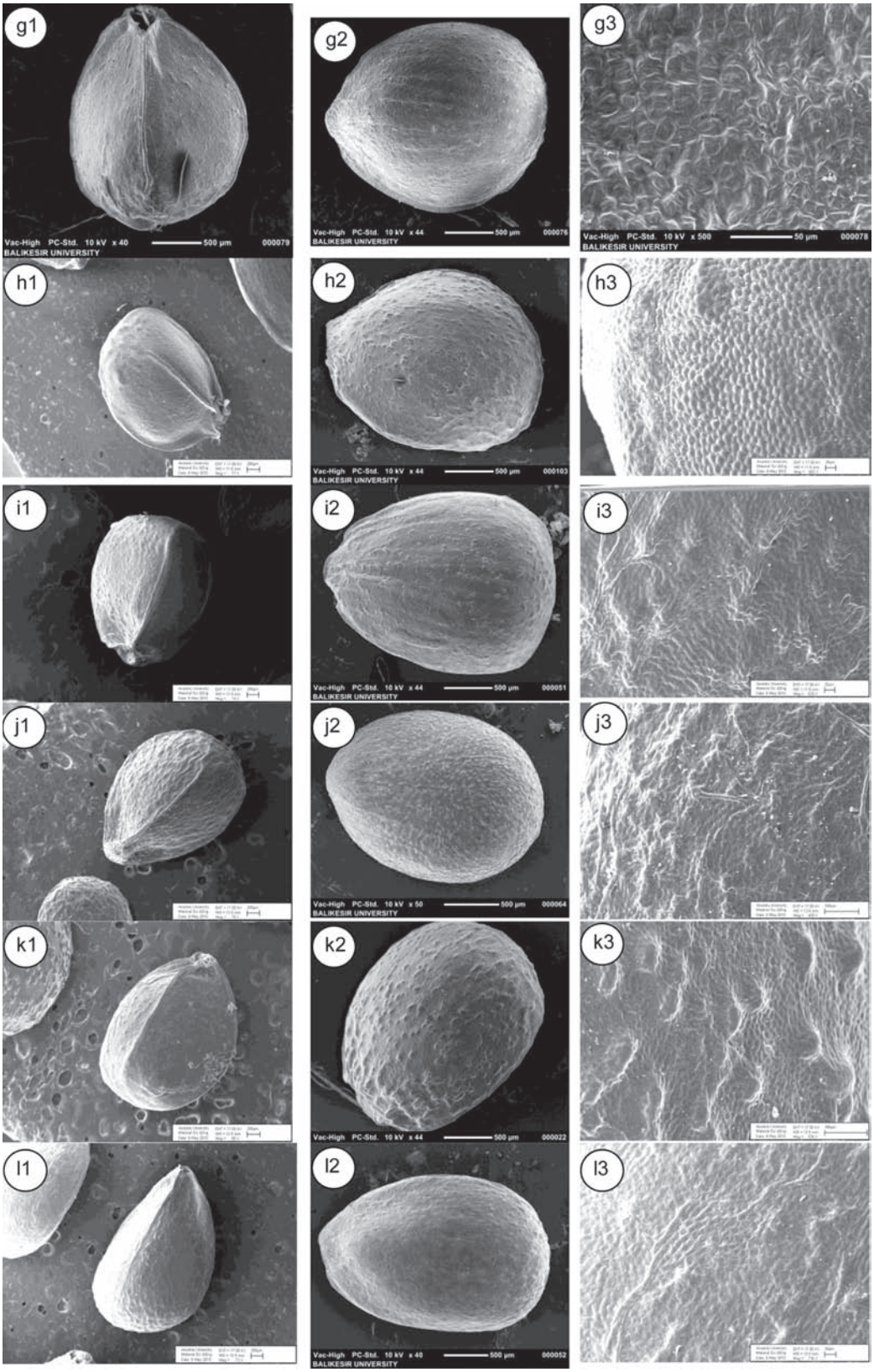

Figure 2. (Continued)

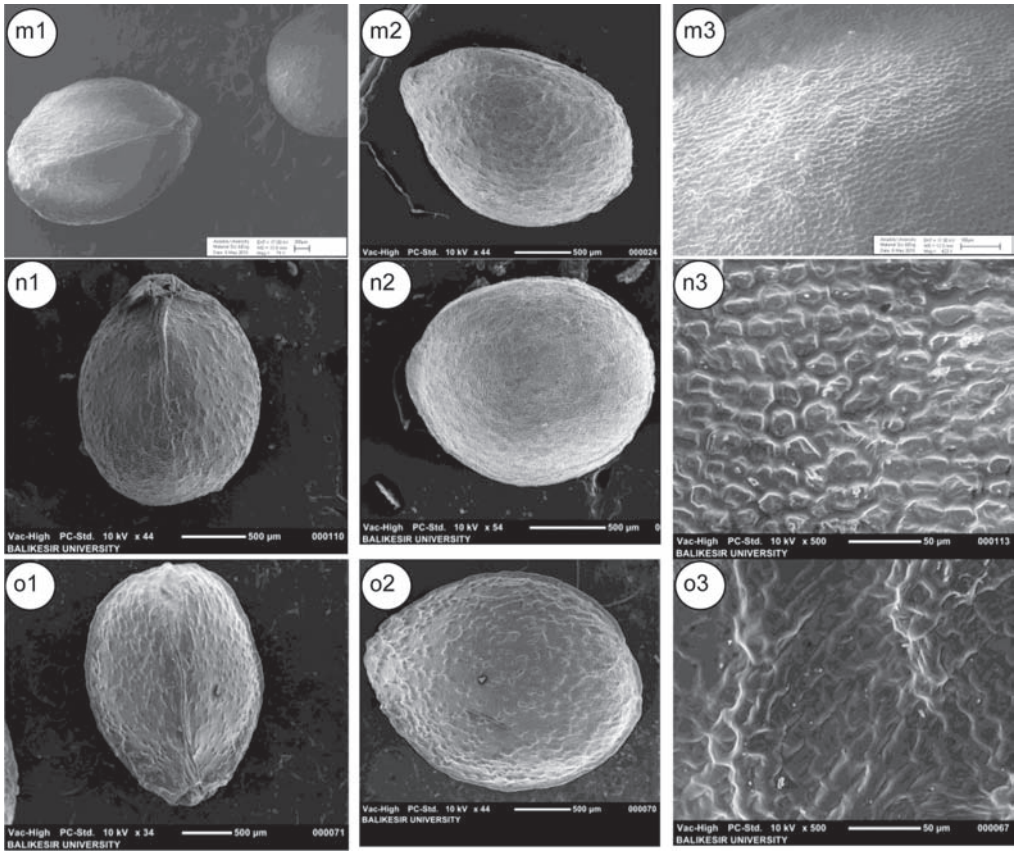

Figure 2. (Continued)

surfaces five basic types could be distinguished: reticulate-tuberculate, reticulate-smooth, reticulate-slightly furrowed, colliculate-tuberculate and colliculate-smooth. The reticulate type was the most common type among studied taxa. A colliculate sculpture was characteristic for *S. minor* and *S. cretica* subsp. *vacillans*. Further, subsect. *Spectabiles* could easily be distinguished from other subsections in *Eriostomum* based on theirs unique reticulate-smooth/slightly furrowed sculpturing (Fig. 3).

Sculpturing type may to some extent indicate taxonomic relationships. For example, in subsect. *Germanicae*, *S. minor* is morphologically closely related to *S. sericantha*. However, the nutlet surface of *S. minor* is colliculate-smooth, while in *S. sericantha* it is reticulate-slightly tuberculate (Fig. 1l–m). Furthermore, nutlet apex sculpture in *S. minor* is smooth, while in *S. sericantha* it is tuberculate (Fig. 1l2–m2). According to 'Flora of Bulgaria' (Jordanov 1989), *S. thracica* and *S. germanica* are morphologically very similar. Our surface sculpturing results show clear differences between *S. thracica* and *S. germanica* subsp. *heldreichii*. *Stachys thracica* has a smooth surface, while *S. germanica* subsp. *heldreichii* has a tuberculate surface (Fig. 1a, d).

The nutlet sculpture provides support for separating some of the subspecies of *S. cretica*. It is collicullate in subsp. *vacillans*, smooth in subsp. *mersinaea* and slightly furrowed in subsp. *trapezuntica*. *Stachys cretica* subsp. *vacillans*, *S. cretica* subsp. *anatolica* and *S. cretica* subsp. *kutahyensis* show similar morphological characters, however, nutlet surface sculpturing of these taxa can be used to separate them: nutlets of subsp. *vacillans* are colliculate-tuberculate, those of subsp. *kutahyensis* reticulate-slightly tuberculate and those of subsp. *anatolica* reticulate-tuberculate (Fig. 2h, k–l).

According to Salmaki et al. (2008), among species attributed to this section, *S. byzantina* and *S. spectabilis* show similar microsculpturing patterns, but differ in nutlet shape. However, their results for *S. byzantina* and *S. spectabilis* were not similar to ours. We found nutlets to be obovoid and reticulate in both species, and the nutlet surface was slightly tuberculate in *S. byzantina*, and smooth/slightly furrowed in *S. spectabilis* (Fig. 2m, 3a).

The nutlets of *S. byzantina*, *S. germanica* and *S. obliqua* have been examined by Bojňanský and Fargašová (2007) and our results are in accordace with their study.

As a result, despite considerable morphological homogeneity among species of the section, nutlet micromorphology do provide support for separating some subsections or species of this section, as well as for separating some subspecies of *S. cretica*. For example, subsect. *Spectabilis* is characterized by a smooth/slightly furrowed sculpture, and can be distinguished from the other studied subsections.

However, nutlet microsculpturing is not useful for separating large natural groups, like subsections of this section. It seems as if, in contrast to the situation in other genera of Lamiaceae, nutlet characters are of low phylogenetic value

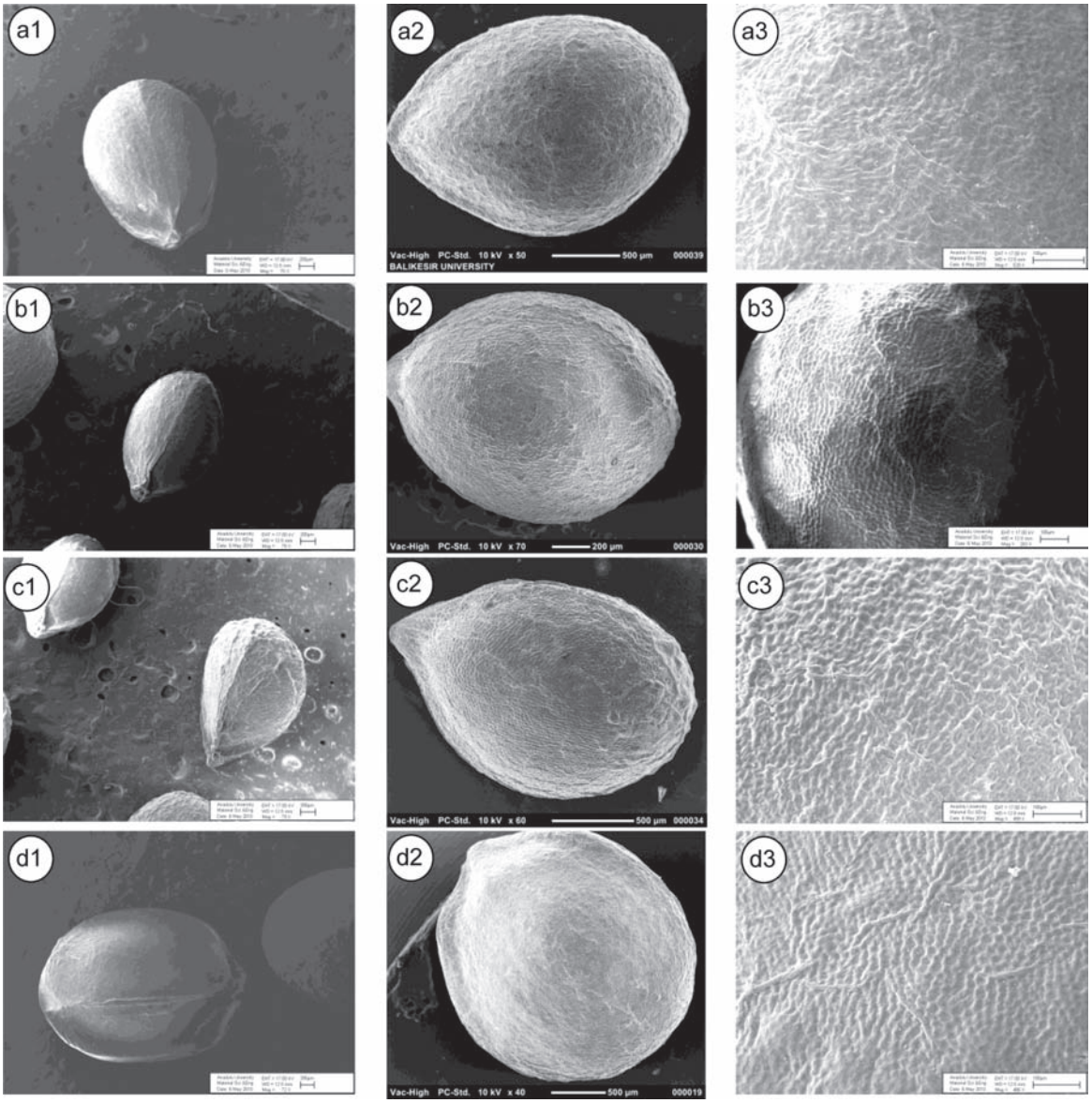

Figure 3. Scanning electron micrographs of nutlets of *Stachys* subsect. *Spectabilis*. 1 = ventral, 2 = dorsal, 3 = surface sculpture. (a) *S. spectabilis*, (b) *S. longispicata*, (c) *S. viticina*, (d) *S. huetii*. Scale bars: a1, b1, b2, c1, d1 = 200 µm, a2, c2, d2 = 500 µm, a3, b3, c3, d3 = 100 µm.

in *Stachys*, due to high variation even among closely related species (Salmaki et al. 2008).

Acknowledgements – The authors would like to thank TUBITAK (project no. 106T489) and SYNTHESYS program (project no. GB-TAF 4797) (financed by the European Community Research Infrastructure Action under the FPA 'Structuring the European Research Area programme') for financial support. We are also grateful to Basic Sciences Research and Applied Center of Balikesir Univ. (BUTAM).

References

Akçiçek, E. 2010. A new subspecies of *Stachys cretica* (section *Eriostomum*, Lamiaceae) from Turkey. – Turk. J. Bot. 34: 131–136.

Akçiçek, A. et al. 2012. Taxonomical notes on *Stachys* sect. *Eriostomum* (Lamiaceae) in Turkey. – Turk. J. Bot. 36: 217–234.

Bhattacharjee, R. 1974. Taxonomic studies in *Stachys* I: new species and infra-specific taxa from Turkey. – Notes R. Bot. Gard. Edinb. 33: 275–292.

Bhattacharjee, R. 1980. Taxonomic studies in *Stachys*: II. A new infrageneric classification of *Stachys* L. – Notes R. Bot. Gard. Edinb. 38: 65–96.

Bhattacharjee, R. 1982. *Stachys*. – In: Davis, P. H. (ed.), Flora of Turkey and the east Aegean Islands. Vol. 7. Edinb. Univ. Press, pp. 199–262.

Bojňanský, V. and Fargašová, A. 2007. Atlas of seeds and fruits of central and east-European Flora. – Springer.

Budantsev, A. L. and Lobova, T. A. 1997. Fruit morphology, anatomy and taxonomy of tribe *Nepeteae* (Labiatae). – Edinb. J. Bot. 54: 183–216.

Davis, P. H. et al. 1988. Flora of Turkey and the east Aegean Islands. Vol. 10, suppl. 1. – Edinb. Univ. Press, pp. 204–206.

Demissew, S. and Harley, M. M. 1992. Trichome, seed surface and polen characters in *Stachys* (Lamioideae: Labiatae) in tropical Africa. – In: Harley, R. M. and Reynolds, T. (eds), Advances in Labiatae science. R. Bot. Gard. Kew, pp. 149–166.

Dinç, M. and Doğan, H. 2006. *Stachys yildirimlii* (Lamiaceae), a new species from south Anatolia, Turkey. – Ann. Bot. Fenn. 43: 143–147.

Dirmenci, T. et al. 2011. *Stachys vuralii* (Lamiaceae), a new species from north Anatolia, Turkey. – Ann. Bot. Fenn. 48: 401–408.

Duman, H. 2000. *Stachys* L. – In: Güner, A. et al. (eds), Flora of Turkey and the east Aegean Islands. Vol. 11, suppl. 2. Edinb. Univ. Press, pp. 204–206.

Falciani, L. 1997. Systematic revision of *Stachys* sect. *Eriostomum* (Hoffmans. & Link) Dumort. in Italy. – Lagascalia 19: 187–238.

Husain, S. Z. et al. 1990. A micromorphological study of some representative genera in the tribe Saturejeae (Lamiaceae). – Bot. J. Linn. Soc. 103: 59–80.

Jamzad, Z. et al. 2000. Pollen exine and nutlet surface morphology of the annual species of *Nepeta* L. (Lamiaceae) in Iran. – In: Harley, M. M. et al. (eds), Pollen and spores: morphology and biology. R. Bot. Gard. Kew, pp. 385–397.

Jordanov, D. 1989. Flora reipublicae popularis bulgaricae. Vol. 9. – Aedibus Acad. Sci. Bulg. Serdicae, pp. 388–416.

Kahraman, A. et al. 2010. Morphology, anatomy, palynology and nutlet micromorphology of *Salvia macrochlamys* (Labiatae) in Turkey. – Biologia 65: 219–227.

Kaya, A. and Dirmenci, T. 2008. Nutlet surface micromorphology of the genus *Nepeta* L. (Lamiaceae) in Turkey. – Turk. J. Bot. 32: 103–112.

Kaya, A. et al. 2009. Nutlet surface micromorphology of Turkish *Satureja* L. (Lamiaceae). – Biologia 64: 902–907.

Marin, P. D. et al. 1994. Nutlet sculpturing of selected *Teucrium* species (Lamiaceae): a character of taxonomic significance. – Plant Syst. Evol. 192: 199–214.

Marin, P. D. et al. 1996. Nutlet ornamentation in selected *Salvia* L. species (Lamiaceae). – Flora Medit. 6: 203–211.

Oran, S. A. 1996. Ultrastructure of nutlet surface of the genus *Salvia* L. in Jordan and the neighbouring countries. – Dirasat. Nat. Eng. Sci. 23: 393–408.

Özkan, M. et al. 2009. Nutlet morphology and its taxonomic utility in *Salvia* (Lamiaceae: Mentheae) from Turkey. – Acta Bot. Croat. 68: 105–115.

Rechinger, K. H. 1982. Flora Iranica. – Akademische Druck- und Verlagsanstalt, pp. 354–396.

Ryding, O. 1992. The distribution and evolution of myxocarpy in the Lamiaceae. – In: Harley, R. M. and Reynolds, T. (eds), Advances in Labiatae science. R. Bot. Gard. Kew, pp. 85–96.

Ryding, O. 1993. Pericarp structure of *Leucas* and related genera (Lamiaceae subfam. Lamioideae). – Nord. J. Bot. 13: 637–646.

Salmaki, Y. et al. 2008. Nutlet morphology of *Stachys* (Lamiaceae) in Iran and its systematic implication. – Feddes Rep. 119: 631–645.

Stearn, W. T. 1992. Botanical Latin, 4th ed. – Timber Press.