

# Nutlet micromorphology of Indian *Leucas* (Lamioideae: Lamiaceae) with emphasis on its systematic implication

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## Abstract

Nutlet micromorphological characteristics in 40 taxa of Asian *Leucas* and two related species of African *Leucas* found in India were examined by light and scanning electron microscopy. Six types of basic ornamentation patterns can be distinguished: scalariform, verrucate, reticulate, ruminant, colliculate, and rugose. Of these, scalariform type was the most common and was found in all the sections of Asian *Leucas*, but the variation in alignment, size and shape of its composing cells provide further evidence in distinguishing the taxa. Verrucate, the second most frequent type was found in six species while other types were represented by only one to three species. However, the presence of colliculate sculpturing is more useful for separating African species from Asian *Leucas*. In Asian *Leucas*, nutlet size may be an important character rather than ornamentation for sectional division. Again, the type of glandular hairs on the nutlet apex can be used as a distinctive taxonomic character at specific level to separate *L. martinicensis* (Jacq.) R.Br. and *L. urticifolia* (Vahl) Sm. The results revealed high similarity of these taxa to the sect. *Leucas*. This study supports earlier observations on pericarp structure and subsequent phylogenetic analysis but is not in corroboration with the recent classification of Indian *Leucas*.

**Keywords:** Asian *Leucas*, India, micromorphology, nutlet

## Introduction

*Leucas* R.Br. comprises about 100 species distributed mainly on dry or disturbed ground in tropical to southern Africa, Arabia, tropical and subtropical parts of Asia and Australia (Harley *et al.*, 2004). Highest number of taxa is reported in the northeast tropical Africa (Ryding, 1998), and in India (Singh, 2001). Species prefers low ( $\pm 800$  m) to high (800–2600 m) altitudes and grows in ecologically divergent localities such as sea coasts, paddy fields, near wet lands, road sides, open habitats, farm land, plantations, grasslands, rocky hills, scrub jungles and shola grasslands.

Surface micromorphology of nutlets have become one of the strongest evidence reported to be useful at various taxonomic levels in Lamiaceae and related families, such as Verbenaceae and Scrophulariaceae (Wojciechowska, 1966; Sivarajan, 1981; Husain *et al.*, 1990; Budantsev & Lobova, 1997; Jamzad *et al.*, 2000; Attar *et al.*, 2007; Krawczyk & Glowacka, 2015). In addition to surface sculpturing, pericarp structure, types of exocarp cells and indumentum features were also considered to be taxonomically

important (Barthlott, 1981; Demissew & Harley, 1992; Ryding, 1993, 1995; Oran, 1996; Guerin, 2005; Moon & Hong, 2006; Kahraman *et al.*, 2011). In some genera of Lamiaceae, nutlet characters were potentially useful in infrageneric classification (Demissew & Harley, 1992) as well as at specific and subspecific levels (Marin *et al.*, 1994; Navarro & El Oualidi, 2000; Sampath Kumar & Murthy, 2012; Satil *et al.*, 2012; Krawczyk & Glowacka, 2015). In *Stachys* L., one of the largest genera of Lamiaceae, it has been shown that the sculpturing pattern of nutlet surface is more useful to separate species within sections, rather than characterizing large natural groups as sections (Salmaki *et al.*, 2008). Studies on *Salvia* L. showed that gross morphology of nutlets and their sculpturing pattern are variable and taxonomically useful for species delimitation (Oran, 1996; Kahraman *et al.*, 2011). The importance of nutlet morphological characteristics shown here has proven to be of great use in species discrimination and studying taxonomic relationships.

**Table 1.** Details of the voucher specimens examined for the present study

| Taxa  | Section            | Collection details   |
|---|--------------------|--|
| <i>L. angularis</i> Benth.                            | <i>Leucas</i>      | Kerala, Mukkali, way to Silent Valley, <i>Shinoj &amp; Vimal</i> CU135573      |
| <i>L. aspera</i> (Willd.) Link                        | <i>Plagiostoma</i> | Kerala, Alappuzha, Punnapra, <i>Shinoj</i> CU135568                            |
| <i>L. beddomei</i> (Hook.f.) Sunojk. & P.Mathew*      | <i>Astrodon</i>    | Wayanad, Chembra Peak, <i>Sunojkumar</i> CU139543                              |
| <i>L. biflora</i> (Vahl) R.Br. ex Sm.                 | <i>Leucas</i>      | Kerala, Munnar, <i>Shinoj</i> CU135590   |
| <i>L. cephalotes</i> (Roth) Spreng.                   | <i>Plagiostoma</i> | Maharashtra, Sant Gadge Baba Amravati University, <i>Prasad</i> CU290102       |
| <i>L. chinensis</i> (Retz.) R.Br. ex Sm.              | <i>Leucas</i>      | Kerala, Nelliampathy, Seetharkundu, <i>Smitha</i> CU135538                     |
| <i>L. ciliata</i> Benth.                              | <i>Astrodon</i>    | Kerala, Nelliampathy, Manpara, <i>Smitha</i> CU135542                          |
| <i>L. decemdentata</i> (Willd.) R.Br. ex Sm.          | <i>Leucas</i>      | Andhra Pradesh, <i>Vimal</i> CU139692  |
| <i>L. deodikarii</i> Billmore & Hemadri*              | <i>Leucas</i>      | Maharashtra, Junnar, <i>Vimal</i> CU139519                                     |
| <i>L. diffusa</i> Benth.*                             | <i>Plagiostoma</i> | Tamil Nadu, Tirunelveli, <i>Prasad</i> CU139564                                |
| <i>L. eriostoma</i> Hook.f.*                          | <i>Astrodon</i>    | Wayanad, Kurichiyarmala, <i>Shinoj</i> CU135577                                |
| <i>L. eriostoma</i> var. <i>lanata</i> Hook.f.*       | <i>Astrodon</i>    | Karnataka, Chikmagalur, Baba Budan Hills, <i>Sunojkumar</i> CU88101            |
| <i>L. helianthemifolia</i> Desf.*                     | <i>Astrodon</i>    | Kerala, Munnar, Devikulam, <i>Shinoj &amp; Vimal</i> CU135939                  |
| <i>L. helicterifolia</i> Haines*                      | <i>Leucas</i>      | Bihar, Valmiki Tiger reserve, <i>Vimal</i> CU139 652                           |
| <i>L. hirta</i> (B.Heyne ex Roth) Spreng.*            | <i>Astrodon</i>    | Palamala, Dhoni Hills, <i>Shinoj &amp; Vimal</i> , CU135557                    |
| <i>L. lamiifolia</i> Desf.*                           | <i>Astrodon</i>    | Tamil Nadu, Kodaikanal, <i>Alfred Joe</i> CU134225                             |
| <i>L. lanata</i> Benth.                               | <i>Leucas</i>      | Uttarakhand, Dehradun, Mussoorie lake, <i>Shinoj &amp; Vimal</i> CU139661      |
| <i>L. lanata</i> var. <i>candida</i> Haines*          | <i>Leucas</i>      | Tamil Nadu, Ooty, Burliar road, <i>Shinoj &amp; Vimal</i> CU135909             |
| <i>L. lanceaefolia</i> Desf.*                         | <i>Astrodon</i>    | Tamil Nadu, Ooty, Doddabetta, <i>Vimal</i> CU135924                            |
| <i>L. lavandulifolia</i> Sm.                          | <i>Plagiostoma</i> | Tamil Nadu, Ooty, Kodanad View point, <i>Shinoj &amp; Vimal</i> CU135922       |
| <i>L. longifolia</i> Benth.                           | <i>Plagiostoma</i> | Maharashtra, Junnar, <i>Prasad &amp; Sunojkumar</i> CU88173                    |
| <i>L. manipurensis</i> N.P.Singh*                     | <i>Astrodon</i>    | Meghalaya, Shillong, Mawphlang, <i>Shinoj &amp; Vimal</i> CU139635             |
| <i>L. marruboides</i> Desf.                           | <i>Leucas</i>      | Kerala, Wayanad, Muthanga forest, Ponkuzhi, <i>Shinoj &amp; Vimal</i> CU135519 |
| <i>L. marruboides</i> var. <i>pulneyensis</i> Hook.f. | <i>Leucas</i>      | Kerala, Nayikatty, on the way to Muthanga, <i>Smitha</i> CU290192              |

| Taxa                                     | Section            | Collection details  |
|--|--------------------|---|
| <i>L. martinicensis</i> (Jacq.) R.Br.    | <i>Hemistoma</i>   | Kerala, Marayoor, <i>Shinoj &amp; Vimal</i> CU135927                              |
| <i>L. mathewiana</i> Sunojk.*            | <i>Plagiostoma</i> | Andhra Pradesh, Ananthapur, Kalasamudram, <i>Sunojkumar</i> CU49664               |
| <i>L. montana</i> (Roth) Spreng.*        | <i>Leucas</i>      | Andhra Pradesh, Tirupati, Talakona waterfall, <i>Vimal</i> CU139682               |
| <i>L. mukerjiana</i> Subba Rao & Kumari* | <i>Leucas</i>      | Andhra Pradesh, Araku Valley, <i>Vimal</i> CU139694                               |
| <i>L. nepetifolia</i> Benth.*            | <i>Leucas</i>      | Tamil Nadu, Kanyakumari, Maruthwamalla, <i>Thoiba</i> CU138475                    |
| <i>L. nutans</i> (Roth) Spreng.          | <i>Plagiostoma</i> | Andhra Pradesh, Ananthapur Dist., SK University campus, <i>Sunojkumar</i> CU49659 |
| <i>L. prostrata</i> (Hook.f.) Gamble*    | <i>Astrodon</i>    | Tamil Nadu, Ooty, Kodanad View point, <i>Shinoj &amp; Vimal</i> CU135921          |
| <i>L. pubescens</i> Benth.*              | <i>Leucas</i>      | Kerala, Munnar, <i>Vimal</i> CU135751   |
| <i>L. rosmarinifolia</i> Benth.*         | <i>Astrodon</i>    | Tamil Nadu, Ooty to Coonoor road, Ketti town, <i>Shinoj</i> CU135788              |
| <i>L. sebalidiana</i> Sunojk.*           | <i>Astrodon</i>    | Kerala, Wayanad, <i>Shinoj &amp; Vimal</i> CU135504                               |
| <i>L. sivadasaniana</i> Sunojk.*         | <i>Astrodon</i>    | Karnataka, Kudajadri Hills, <i>Shinoj &amp; Vimal</i> CU135959                    |
| <i>L. stelligera</i> Wall. ex Benth.     | <i>Astrodon</i>    | Maharashtra, <i>Vimal</i> CU138401  |
| <i>L. stricta</i> Benth.                 | <i>Plagiostoma</i> | Maharashtra, <i>Prasad &amp; Vimal</i> CU290105                                   |
| <i>L. suffruticosa</i> Benth.*           | <i>Astrodon</i>    | Kerala, Silent Valley, <i>Prasad &amp; Vimal</i> CU135978                         |
| <i>L. urticifolia</i> (Vahl) Sm.         | <i>Hemistoma</i>   | Tamil Nadu, Coimbatore, TNAU, <i>Soumya</i> CU135321                              |
| <i>L. vestita</i> Benth.*                | <i>Astrodon</i>    | Karnataka, Kudachadri, <i>Sunojkumar</i> CU88122                                  |
| <i>L. wightiana</i> Wall. ex Benth.*     | <i>Plagiostoma</i> | Tamil Nadu, Tirunelveli, Kallidaikurichi, <i>Shinoj</i> CU138466                  |
| <i>L. zeylanica</i> (L.) R.Br. ex Sm.    | <i>Plagiostoma</i> | Kerala, Malappuram, <i>Sajna</i> CU138483   |

\*Species endemic to India

Based on Wallich's collections from Asian countries, Bentham (1832–1836, 1848) recognized six sections in *Leucas*. Recently, Scheen and Albert (2007) suggested segregation of this genus into two different groups, *Leucas s.s.* and 'African *Leucas*' based on their molecular phylogenetic studies (Scheen & Albert, 2009). *Leucas s.s.* is monophyletic whereas 'African *Leucas*' is an informal name given to the paraphyletic group comprising African *Leucas* species and related genera such as *Isoleucas*, *Leonotis*, *Otostegia* and *Acrotome*. Morphologically abrupt demarcation between Asian and African species was earlier suggested by Sebald (1980) and Ryding (1998). Both of these groups are apparently related, and resembling each other mainly in the absence of branched trichomes, and presence of a beard along the margin of upper lip of corolla (Hedge, 1992).

According to Bentham's classification (1832–1836, 1848), Asian *Leucas* consists of three natural sections: sect. *Astrodon* Benth., sect. *Plagiostoma* Benth., and sect. *Leucas* (*Ortholeucas*) Benth. In the morphology based revision of Indian *Leucas*, Singh (2001) added three new sections (sect. *Stricta*, sect. *Hyssopifolia*, and sect. *Diffusa*) to Bentham's classification and treated *Leucas* found in India under six infrageneric groups. Besides c. 41 Asian species, two African species, *L. martinicensis* and *L. urticifolia*, originally included in African *Leucas* sect. *Hemistoma* Benth. are also found as introduced weeds in India. In the revision on Indian *Leucas*, Singh (2001) treated these two African species in separate sections, keeping *L. urticifolia* in the sect. *Hemistoma* and *L. martinicensis* under sect. *Plagiostoma* intermixed with other species of *Leucas s.s.* Here we made an attempt to utilize nutlet micro morphological data in the systematic treatment of genus *Leucas s.s.* based on specimens gathered from India.

Majority of Asian *Leucas* are found in India and a few works are available on the taxonomy of this genus (Metcalf & Chalk, 1950; Abu-Asab & Cantino, 1987; Cantino, 1990; Ryding, 1993, 1995, 1998; Singh, 2001; Scheen & Albert, 2007, 2009; Sunojkumar & Mathew, 2008). However, there is no report yet addressing the significance of nutlet micromorphology in the systematics of Asian *Leucas*.

The present study attempts to investigate nutlet surface features of Asian *Leucas* found in India in a taxonomic context for the first time. In this study the authors used 60 accessions of 40 taxa of Asian *Leucas* and two species of African *Leucas* collected from India.

## Materials and Methods

The details on specimens analyzed, collection data and the vouchers used are provided in Table 1. Nutlets were collected from natural populations and the herbarium specimens deposited in the Calicut University Herbarium (CALI). Initially, nutlets were observed using Leica M80 stereomicroscope to check that they are of normal size and maturity. The dorsal surface of nutlet was considered for describing surface sculpturing, to ensure certainty about the stability of characters among different accessions of one species. The measurements were made on 20 nutlets per species randomly obtained from five to eight populations of each species. The ranges of nutlet measurements were presented in box plot graphics generated in R.

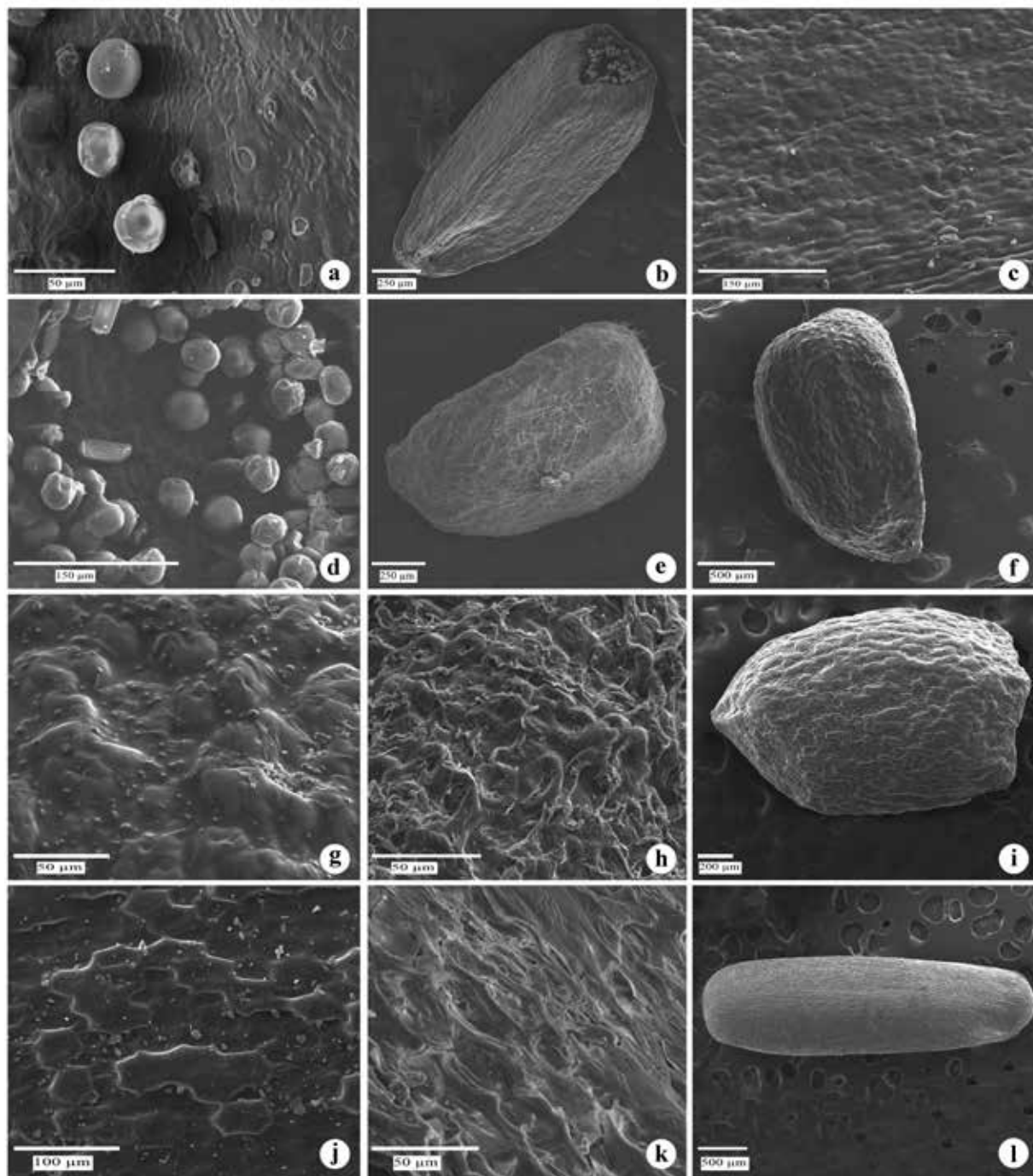
For scanning electron microscopy (SEM), dried mature nutlets were mounted directly on stubs using double-side adhesive tape, and sputter-coated with gold. Some micrographs were taken with a JEOL Model JSM-6390LV scanning electron microscope at 15 kV and others were taken with a Hitachi SU 6600 Horiba-Emax EDS scanning electron microscope at 10 kV.

Even though the terminology of Barthlott (1981) has been used in describing surface sculpturing in several species of seed plants, the authors found those of Stearn (1992) and Salmaki *et al.* (2008) to be more applicable to the patterns in *Leucas*.

## Results

The nutlet characters of all investigated taxa are listed in Table 2. Selected SEM micrographs of nutlets studied are illustrated in Figures 1–2. Among several characters of nutlets studied, the type of sculpturing, shape of anticlinal and periclinal walls, shape and size of nutlets and presence or absence of glandular and non glandular hairs were considered to be taxonomically most important. Nutlets show considerable variability among species in different sections, and therefore, afford valuable characters in delimitation of sections and species.

Nutlets have slightly elongate trigonous structure with two comparatively flat or sometimes even concave proximal sides and a broader, more rounded distal side. Nutlets in most of the studied species of *Leucas* are (Table 2) narrow oblong (Fig. 1i; Fig. 2b), oblong (Fig. 2k) to broad oblong (Fig. 2d), obovate (Fig. 2e) to broad obovate (Fig. 1i) and triquetrous in cross section. The apex is rounded (Fig. 2e) or truncate (Fig. 1i) and colour varies from blackish to light brownish.



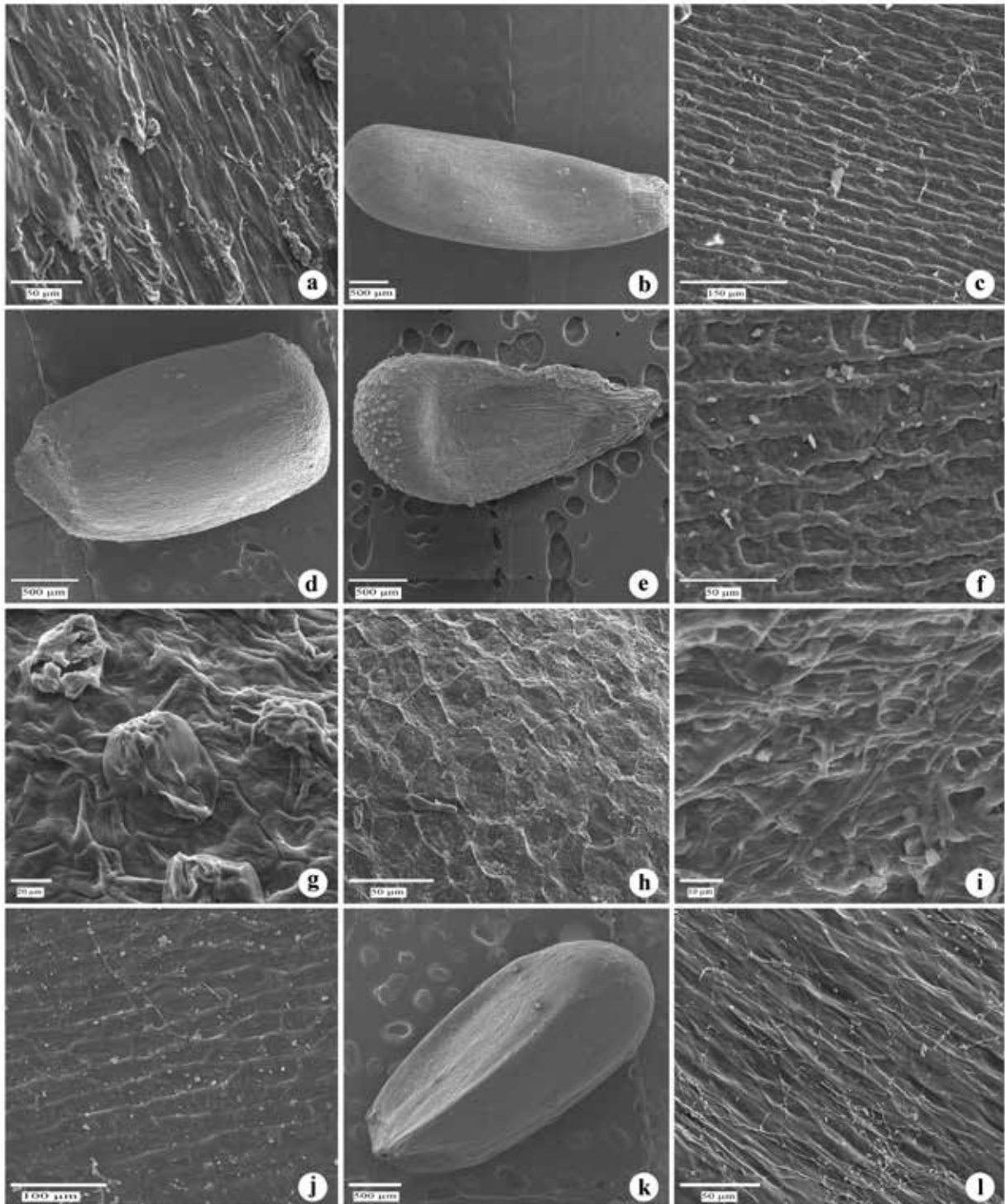
**Fig. 1.** SEM micrographs of nutlets in some taxa of *Leucas*: **a.** *L. martinicensis*; **b, c & d.** *L. urticifolia*; **e.** *L. angularis*; **f & g.** *L. biflora*; **h.** *L. chinensis*; **i & j.** *L. lanata*; **k.** *L. aspera*; **l.** *L. cephalotes*.

Nutlet length ranges from 1.37 mm (*L. angularis*) to 4.27 mm (*L. mathewiana*) and 0.79 mm (*L. nutans*) to 1.85 mm (*L. suffruticosa*) in width (Table 2).

The Asian *Leucas* usually do not possess hairs on nutlets, which is a common character seen in African *Leucas* including the two African species found in India: *L. martinicensis* and *L. urticifolia*. However, glandular hairs seen on *L. beddomei* and

non-glandular hairs on *L. angularis* (Fig. 1e) as exceptions in Asian taxa. The hairs are sessile in *L. beddomei* (Fig. 2g) and *L. martinicensis* (Fig. 1a) where as it is stalked in *L. urticifolia* (Fig. 1d).

Nutlet surface sculpturing pattern can broadly be classified in six types (Table 2): The most common is the scalariform type as in *L. beddomei* (Fig. 2f), *L. aspera* (Fig. 1k) and *L. lavandulifolia* (Fig. 2a). The



**Fig. 2.** SEM micrographs of nutlets in some taxa of *Leucas*: **a.** *L. lavandulifolia*; **b & c.** *L. mathewiana*; **d.** *L. stricta*; **e, f & g.** *L. beddomei*; **h.** *L. hirta*; **i.** *L. lanceaefolia*; **j.** *L. seabaldiana*; **k & l.** *L. stelligera*.

other types are colliculate as in *L. urticifolia* (Fig. 1c); reticulate as in *L. hirta* (Fig. 2h); verrucate as in *L. biflora* (Fig. 1g) and *L. lanata* (Fig. 1j); rugose as in *L. chinensis* (Fig. 1h); and ruminant as in *L. lanceaefolia* (Fig. 2i). The scalariform pattern was found in 27 species out of 42 species studied, followed by verrucate (6 species), reticulate and

ruminant pattern (each with 3 species), colliculate (2 species), and rugose (1 species).

In scalariform type, the species vary according to the alignment, size and shape of its composing cells, may be short celled as in *L. aspera* (Fig. 1k) and *L. beddomei* (Fig. 2f) or long celled as in *L. lavandulifolia* (Fig. 2a) and *L. mathewiana* (Fig. 2c).

Nutlets in studied species of African *Leucas* show relationship with the members of sect. *Leucas*. They are obovate in shape,  $\pm 1.97 \times 1.17$  mm (*L. martinicensis*) and  $\pm 1.76 \times 0.91$  mm (*L. urticifolia*) in size and show colliculate (Fig. 1c) type of sculpturing.

## Discussion

The results of present work show the utility of nutlet micromorphological characters for distinguishing species in *Leucas*. These kinds of characters have shown to be diagnostic and of systematic value as in previous studies of nutlet morphology in Lamiaceae (Husain *et al.*, 1990; Moon & Hong, 2006; Sampath Kumar & Murthy, 2012; Krawczyk & Glowacka, 2015). However, Navarro and El Oualidi (2000) found that the presence or absence of different type of glandular trichomes on the nutlet surface is the most important character in *Teucrium* L. whereas in tribe *Saturejeae*, nutlet shape and the nature of attachment scar are the significant characters in delimitation of taxa (Husain *et al.*, 1990).

Among different nutlet characters studied, the type of sculpturing has been considered to be taxonomically most valuable ones (Oran, 1996). The scalariform type is the most common type among the examined taxa, but the variations in alignment, size and shape of its composing cells provide further support for using nutlet morphology to distinguish species. For instance, in sect. *Plagiostoma*, *L. aspera* is morphologically similar to its close allies such as *L. lavandulifolia*, *L. stricta*, *L. wightiana* and *L. zeylanica*, all showing scalariform type of sculpturing, are characterized by concave periclinal and non-wavy raised anticlinal walls. But can easily be distinguishable by the pericarp cells of *L. aspera* being short (Fig. 1k). The pericarp cells of *L. lavandulifolia* (Fig. 2a), *L. stricta*, *L. wightiana* and *L. zeylanica* is long.

However, the type of sculpturing is more useful in the separation of Asian *Leucas* from African species. In terms of macromorphology, the former is characterized by having 8–10-toothed calyx with ciliate hairs at the mouth and different forms of habit such as annual and perennial herbs as well as sub shrubs and shrubs, while African *Leucas* are annuals with 5–10-toothed calyx without cilia at the mouth. Presence of colliculate sculpturing and glandular trichomes at nutlet apex has high systematic value for the segregation of this genus into *Leucas* s.s. and African *Leucas*. Moreover, Ryding (1993) pointed out that a combination of

three morphological characters, absence of glands on ovaries and nutlets, anterior corolla lip longer than posterior corolla lip, and dorsal side of calyx exceeding or equaling the ventral sides distinguish Asian species from the African species of *Leucas*. Even though they do not form a combination of three states in all taxa of African *Leucas*, but are similar in one of these characters (Ryding, 1998). So, these kinds of nutlet morphological characters are consistent with the macromorphological classification (Bentham, 1832–1836, 1848) and provide additional evidence to verify the segregation in *Leucas*.

As given in other Lamiacean genera (Salmaki *et al.*, 2008; Özkan *et al.*, 2009; Tarimcilar *et al.*, 2013; Sampath Kumar & Murthy, 2012; Kaya *et al.*, 2015), nutlet size also have valuable taxonomic significance in different sections of Asian *Leucas*. Based on nutlet length, sections of Asian *Leucas* are clearly separated (Fig. 3): sect. *Leucas* is characterized by very small nutlets (< 2 mm), sect. *Astrodon* with very large nutlets (> 2.5 mm) whereas sect. *Plagiostoma* possess intermediate length (2–2.5 mm). The occurrence of these three kinds of nutlets is also in accordance with leaf morphology in the three sections of Asian *Leucas*: sect. *Leucas* have ovate leaves, sect. *Astrodon* have oblong or linear-oblong leaves and sect. *Plagiostoma* is characterized by linear-lanceolate leaves.

Oran (1996) suggested that the shape of nutlets has low systematic importance in evaluating relationship among species. However, from a taxonomical perspective, the shape of nutlets can be used in separation of taxa in certain instances. For example, in sect. *Ortholeucas*, *L. pubescens* and *L. lanata* (Fig. 1i) with broad obovate nutlets are easily distinguishable from other species of this section. Similarly, *L. aspera*, *L. cephalotes*, *L. nutans*, *L. diffusa* and *L. mathewiana* in the sect. *Plagiostoma* are characterized by narrow oblong nutlets.

In *Leucas*, it has been suggested that the Asian species can be divided in to three natural groups based on pericarp structure (Ryding, 1993). The results obtained from the present study correlate with these features and are discussed below in a comparative context.

### *Leucas* sect. *Astrodon* Benth.

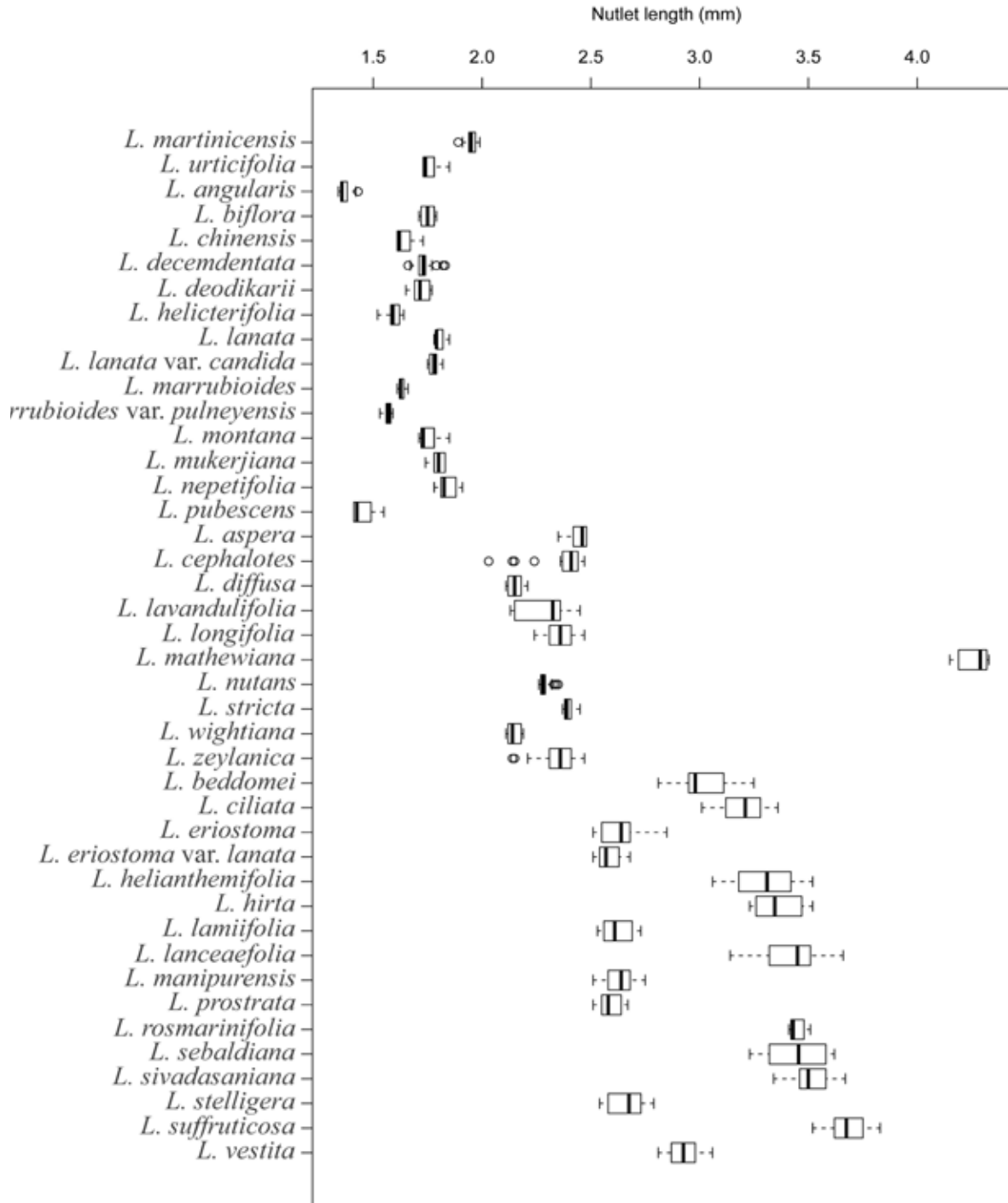
*Leucas* sect. *Astrodon* Benth. is one of the largest section of Asian *Leucas* and represents the most taxonomically diverse group of plants. Singh (2001) divided this section into three separate sections: *Astrodon*, *Diffusa* and *Hyssopifolia*, based

**Table 2.** Comparative chart of nutlet characters of the *Leucas* taxa examined in the present study

| Species investigated                           | Nutlet          |                       |         |                     | Anticlinal wall   |                         |
|--|-----------------|-----------------------|---------|---------------------|-------------------|-------------------------|
|  | Shape           | Size (mm) L × W       | Gland   | Surface sculpturing |                   | Outer periclinal wall   |
| <b>sect. <i>Hemistoma</i></b>                  |                 |                       |         |                     |                   |                         |
| <i>L. martinicensis</i>                        | Broadly obovate | 1.94±0.01 × 1.17±0.01 | Present | Colliculate         | Convex            | Represented by channels |
| <i>L. urticifolia</i>                          | Obovate         | 1.76±0.01 × 0.91±0.03 | Present | Colliculate         | Convex            | Represented by channels |
| <b>sect. <i>Leucas</i></b>                     |                 |                       |         |                     |                   |                         |
| <i>L. angularis</i>                            | Obovate         | 1.37±0.01 × 0.85±0.02 | Absent  | Reticulate          | Concave           | Raised                  |
| <i>L. biflora</i>                              | Obovate         | 1.75±0.01 × 1.05±0.01 | Absent  | Verrucate           | Convex projection | Represented by channels |
| <i>L. chinensis</i>                            | Obovate         | 1.64±0.01 × 0.92±0.01 | Absent  | Rugose              | –                 | –                       |
| <i>L. decemdentata</i>                         | Obovate         | 1.73±0.01 × 1.13±0.02 | Absent  | Scalariform         | Shallow concave   | Raised and wavy         |
| <i>L. deodikarii</i>                           | Obovate         | 1.72±0.01 × 1.16±0.01 | Absent  | Verrucate           | Convex projection | Represented by channels |
| <i>L. helicterifolia</i>                       | Obovate         | 1.59±0.01 × 0.98±0.02 | Absent  | Verrucate           | Convex projection | Represented by channels |
| <i>L. lanata</i>                               | Broadly obovate | 1.80±0.01 × 1.19±0.01 | Absent  | Verrucate           | Convex projection | –                       |
| <i>L. lanata</i> var. <i>candida</i>           | Obovate         | 1.78±0.01 × 1.13±0.01 | Absent  | Verrucate           | Convex projection | –                       |
| <i>L. marrubioides</i>                         | Obovate         | 1.63±0.01 × 0.89±0.02 | Absent  | Scalariform         | Shallow concave   | Raised and wavy         |
| <i>L. marrubioides</i> var. <i>pulneyensis</i> | Obovate         | 1.57±0.01 × 0.89±0.02 | Absent  | Scalariform         | Shallow concave   | Raised and wavy         |
| <i>L. montana</i>                              | Obovate         | 1.75±0.01 × 1.07±0.01 | Absent  | Ruminata            | Concave           | Raised                  |
| <i>L. mukerjiana</i>                           | Obovate         | 1.80±0.01 × 1.19±0.01 | Absent  | Ruminata            | Concave           | Raised                  |
| <i>L. nepetifolia</i>                          | Obovate         | 1.84±0.01 × 0.95±0.01 | Absent  | Reticulate          | Concave           | Raised                  |
| <i>L. pubescens</i>                            | Broadly obovate | 1.45±0.01 × 0.95±0.02 | Absent  | Verrucate           | Convex projection | Represented by channels |
| <b>sect. <i>Plagiosfoma</i></b>                |                 |                       |         |                     |                   |                         |
| <i>L. aspera</i>                               | Narrowly oblong | 2.43±0.01 × 0.93±0.01 | Absent  | Scalariform         | Shallow concave   | Raised                  |
| <i>L. cephalotes</i>                           | Narrow oblong   | 2.37±0.02 × 0.99±0.01 | Absent  | Scalariform         | Deep concave      | Raised                  |
| <i>L. diffusa</i>                              | Oblong          | 2.15±0.01 × 0.88±0.01 | Absent  | Scalariform         | Shallow concave   | Raised                  |
| <i>L. lavandulifolia</i>                       | Oblong          | 2.29±0.02 × 1.26±0.01 | Absent  | Scalariform         | Shallow concave   | Raised                  |



| Species investigated                   | Nutlet          |                       |         |                     |                 | Outer periclinal wall | Anticlinal wall |
|--|-----------------|-----------------------|---------|---------------------|-----------------|-----------------------|-----------------|
|  | Shape           | Size (mm) L × W       | Gland   | Surface sculpturing | Concave         |                       |                 |
| <i>L. longifolia</i>                   | Oblong          | 2.36±0.01 × 1.27±0.01 | Absent  | Scalariform         | Concave         | Raised                |                 |
| <i>L. mathewiana</i>                   | Narrowly oblong | 4.27±0.01 × 1.28±0.01 | Absent  | Scalariform         | Shallow concave | Raised                |                 |
| <i>L. nutans</i>                       | Narrowly oblong | 2.29±0.01 × 0.79±0.01 | Absent  | Scalariform         | Shallow concave | Raised                |                 |
| <i>L. stricta</i>                      | Broadly oblong  | 2.39±0.01 × 1.39±0.01 | Absent  | Scalariform         | Shallow concave | Raised                |                 |
| <i>L. wightiana</i>                    | Broadly oblong  | 2.15±0.01 × 1.13±0.01 | Absent  | Scalariform         | Concave         | Raised                |                 |
| <i>L. zeylanica</i>                    | Oblong          | 2.35±0.02 × 1.33±0.01 | Absent  | Scalariform         | Concave         | Raised                |                 |
| <b>sect. <i>Astrodon</i></b>           |                 |                       |         |                     |                 |                       |                 |
| <i>L. beddomei</i>                     | Obovate         | 3.01±0.02 × 1.24±0.01 | Present | Scalariform         | Shallow concave | Raised                |                 |
| <i>L. ciliata</i>                      | Obovate         | 3.19±0.01 × 1.46±0.01 | Absent  | Scalariform         | Shallow concave | Raised and wavy       |                 |
| <i>L. eriostoma</i>                    | Broadly oblong  | 2.63±0.02 × 1.15±0.01 | Absent  | Scalariform         | Shallow concave | Raised                |                 |
| <i>L. eriostoma</i> var. <i>lanata</i> | Narrowly oblong | 2.59±0.01 × 0.91±0.01 | Absent  | Scalariform         | Shallow concave | Raised and wavy       |                 |
| <i>L. helianthemifolia</i>             | Obovate         | 3.30±0.03 × 1.63±0.01 | Absent  | Scalariform         | Concave         | Raised                |                 |
| <i>L. hirta</i>                        | Oblong          | 3.36±0.02 × 1.44±0.01 | Absent  | Reticulate          | Concave         | Raised                |                 |
| <i>L. lamiifolia</i>                   | Obovate         | 2.62±0.01 × 1.35±0.01 | Absent  | Scalariform         | Shallow concave | Raised                |                 |
| <i>L. lancaefolia</i>                  | Oblong          | 3.18±0.08 × 1.74±0.01 | Absent  | Ruminant            | Concave         | Raised                |                 |
| <i>L. manipurensis</i>                 | Broadly obovate | 2.64±0.01 × 1.63±0.01 | Absent  | Scalariform         | Shallow concave | Raised                |                 |
| <i>L. prostrata</i>                    | Broadly obovate | 2.59±0.01 × 1.34±0.01 | Absent  | Scalariform         | Concave         | Raised                |                 |
| <i>L. rosmarinifolia</i>               | Oblong          | 3.45±0.01 × 1.77±0.01 | Absent  | Scalariform         | Shallow concave | Raised and wavy       |                 |
| <i>L. sebalidiana</i>                  | Oblong          | 3.44±0.02 × 1.35±0.01 | Absent  | Scalariform         | Shallow concave | Raised and wavy       |                 |
| <i>L. sivadasaniana</i>                | Oblong          | 3.51±0.02 × 1.73±0.01 | Absent  | Scalariform         | Concave         | Raised                |                 |
| <i>L. stelligera</i>                   | Oblong          | 2.66±0.01 × 1.33±0.01 | Absent  | Scalariform         | Concave         | Raised and wavy       |                 |
| <i>L. suffruticosa</i>                 | Oblong          | 3.68±0.02 × 1.85±0.01 | Absent  | Scalariform         | Concave         | Raised                |                 |
| <i>L. vestita</i>                      | Oblong          | 2.92±0.01 × 1.35±0.01 | Absent  | Scalariform         | Concave         | Raised                |                 |



**Fig. 3.** Box plot graphic for nutlet length in the studied species of *Leucas*.

on certain morphological characters he observed in certain herbarium specimens. Our observation of both live and herborized specimens confirms that *Leucas* sect. *Astrodon* (Bentham, 1832–1836, 1848) is characterized by having straight tubular to campanulate calyces, which are densely ciliate with dense shining hairs at mouth. Besides these features, all species attributed to this section

possess long non-glandular trichomes and lack capitate trichomes. Moreover, presence of many veins in the nutlets (veins over five and displaced from nutlet edges) and very high bone cells (Ryding, 1993) can be used as distinctive characters of this section. Among the species of this section, *L. beddomei* possess sessile glands on nutlet apex (Sunojkumar & Mathew, 2008). Morphologically,

this species is closely similar to *L. hirta* (subshrubs with elliptic leaf blades and many flowered verticils). But the latter can be distinguished by the presence of its unique reticulate sculpturing. The Western Ghats endemic species *L. lanceaefolia* is unique in this section due to the presence of ruminant type of sculpturing with concave periclinal and raised anticlinal walls.

#### ***Leucas* sect. *Plagiostoma* Benth.**

Most of the species in this section are medicinal herbs, widely distributed in Asian countries like Bangladesh, China, India, Malaysia, Myanmar, Philippines and Sri Lanka and exhibit their presence even up to Australian continent. This section is characterised by linear-lanceolate leaves, terminal compact verticils, oblique calyx and fan-shaped lower lip of corolla. Singh (2001) treated members of this section in three separate sections: *Diffusa*, *Plagiostoma* and *Stricta*. From nutlet micromorphological point of view, it is not possible to find any significant differences between the sections as mentioned above indicating the possible close relationship between them. According to Ryding (1993) presence of a more or less distinct foot-like structure at the base of nutlets and elongate or unequally thickened exocarp cells are one of the most important characteristic feature used for separating this section which is observed in our studies also. However, the Eastern Ghats endemic species *L. mathewiana* of this section possess the largest nutlets in the genus.

#### ***Leucas* sect. *Leucas* Benth.**

This section is characterized by small obovate nutlets and shows close similarity with the African *Leucas* in India but can be distinguished based on the glandular hairs at nutlet apex. Species of this section possess both pitted and ordinary type of exocarp cells in pericarp (Ryding, 1993) and is characterized by the presence of different types of sculpturing patterns. Amongst the species of this section, *L. angularis* and *L. pubescens* are very closely related in morphological features such as ovate leaves, long bracteoles, lesser number of flowers, coriaceous, long and cylindrical calyx. But the two can be distinguished based on sculpturing type, which is verrucate with convex periclinal walls in *L. pubescens* whereas reticulate with concave periclinal walls in *L. angularis*. Nutlet characters of *L. pubescens* provided a conclusive evidence to consider this species under the section *Leucas*, contrary to what has been suggested by Singh (2001) who placed *L. pubescens* in sect. *Hyssopifolia*.

Non-glandular hairs are noticed throughout on the nutlets of *L. angularis* (Fig. 1e) and on both end of nutlets in *L. mukerjiana* (Singh, 2001) as taxa specific characters.

#### **'African *Leucas*' sect. *Hemistoma* Benth.**

This section includes two species in India, which are widely distributed in Asia and Africa. Both species possess glandular hairs at nutlet apex, which, however, are sessile in *L. martinicensis* and stalked in *L. urticifolia*. Moreover, the pericarp thickness is lesser (20–25  $\mu\text{m}$ ) in *L. martinicensis*, but thicker (about 55  $\mu\text{m}$ ) in *L. urticifolia* (Ryding, 1993). Therefore, the type of indumentum and pericarp thickness supports close relationships of these two species. Our study on *L. martinicensis* and *L. urticifolia* found in Asia supported Sebold's (1980) and Ryding's (1993) inclusion of these two species in sect. *Hemistoma*, which is contrary to what has been suggested by Singh (2001) to include *L. martinicensis* in sect. *Plagiostoma*.

## **Conclusion**

Nutlet micromorphological observations provide strong support for the delimitation of African species from Asian *Leucas*. Among the characters studied, sculpturing pattern proved as useful character to separate African species from Asian *Leucas*. Within the Asian group, size of nutlets appears useful in separating species under three large natural groups as sections. However, the micromorphological data can be combined with a broad range of morphological characters in order to differentiate each taxa within the genus. Compared to other genera in Lamiaceae (Guerin, 2005) nutlet sculpturing in *Leucas* provides only low phylogenetic significance due to less variation among closely related species. Based on micromorphology, the Asian *Leucas* sect. *Leucas* shows possible relationships with African *Leucas*, providing a link between the two groups.

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