

NUTLET MICROMORPHOLOGY IN SELECTED SPECIES OF *NEPETA* L. (LAMIACEAE) IN KASHMIR HIMALAYA

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The nutlet micromorphology of thirteen species of *Nepeta* was investigated by scanning electron microscopy. Two main patterns i.e. reticulate and indistinct reticulate are described. Both patterns are of smooth type. Reticulate type is found in both Kashmir and Ladakh regions of the study area and indistinct reticulate type is exclusively confined to cold desert region of Ladakh. The nutlets of *N. govaniana* are largest and that of *N. raphanorhiza* are smallest among the species studied. Out of 13 studied species, 11 belong to reticulate type and 2 belong to indistinct reticulate. Only smooth pattern is found in our species and no sculptured pattern is recognised.

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Key words. *Nepeta*, *Lamiaceae*, nutlet, macromorphology, micromorphology.

ریزریخت‌شناسی فندقه‌های گونه‌هایی منتخب از جنس *Nepeta* L. (*Lamiaceae*) در منطقه کشمیر هیمالیا
توحیدا حسن و غلام حسن دار، اعضای هیئت علمی دپارتمان گیاهشناسی دانشگاه کشمیر، هندوستان.

ریزریخت‌شناسی فندقه ۱۳ گونه از جنس *Nepeta* با استفاده از میکروسکوپ الکترونی اسکن مورد بررسی قرار گرفت و دو الگوی مشبک و مشبک نامشخص تشخیص داده شد که هر دو الگو از نوع صاف بودند. الگوی مشبک در هر دو منطقه کشمیر و لاداخ و الگوی مشبک نامشخص فقط در بیابانهای سرد منطقه لاداخ مشاهده گردیدند. فندقه‌های گونه *Nepeta govaniana* بزرگترین و فندقه‌های گونه *N. raphanorhiza* جزو کوچک‌ترین فندقه‌ها بودند. از بین ۱۳ گونه مطالعه شده ۱۱ گونه از الگوی مشبک و ۲ گونه از الگوی مشبک نامشخص بودند و از طرف دیگر فندقه‌ها از نوع صاف و بدون تزئینات تشخیص داده شدند.

Introduction

Nepeta L. approximately contains 300 species (Jamzad *et al.*, 2000), most of which are herbaceous perennials. It is one of the largest genera in family *Lamiaceae*, subfamily *Nepetoideae*, tribe *Mentheae* (Pojarkova, 1954; Başer *et al.*, 2000; Jamzad *et al.*, 2003; Jamzad *et al.*, 2005). Its species are distributed throughout Southwest and Central Asia, Europe, North Africa, North and Central America, Japan, Korea, China, and the Canary Islands (Pojarkova, 1954; Hedge, 1978, 1986; Jamzad *et al.*, 2000). The highest number of taxa is found in the Southwestern Asia, especially Iran [where species representative of most sections (12) occur (Rechinger, 1982)] and the Western Himalayas including the adjacent Hindu-Kush (Pojarkova, 1954).

Variations in many morphological characters such as indumentum, calyx characters, leaf shape and size have been observed among closely related species. As leaf morphology varies within a same species, so its diagnostic use in classification above the species level

is uncertain. Various species have also been recognized on the basis of nutlet characters (Hedge and Lamond, 1968; Budantsev and Lobova, 1997; Jamzad *et al.*, 2000; Jamzad, 2001; Jamzad *et al.*, 2003a,b) but some species belonging to different sections have same type of nutlet surface ornamentation, so the utility of nutlet characters at infrageneric level is doubtful. The micromorphological features of nutlets which are considered to be stable characters are investigated little (Kaya & Dirmenci, 2008), but in recent times the ultrastructural features have been given importance. Some external features such as morphology, shape, colour, areole form & location and size of the nutlets were also used as diagnostic characters in classification of different species of *Nepeta*.

Studies on nutlet morphology and pericarp structure in *Lamiaceae* are increasing (Husain *et al.*, 1990; Demissew & Harley, 1992; Cantino, 1992; Ryding, 1993, 1994; Marin *et al.*, 1994; Orafi 1996). In recent years, the importance of scanning electron microscopy

Table 1: Species of *Nepeta*, source of investigated materials and phylogeographic province.

S.No.	Taxa	Collection site	Collector	Voucher no.	Section	Phytogeographic province
1	<i>N. coerulescens</i> Maxim.	Zanskar	Tauheeda	2776	<i>Pycnonepeta</i>	Ladakh
2	<i>N. connata</i> Royle ex Benth.	Baltal, Sonamarg	Tauheeda & Dar	2781	<i>Pycnonepeta</i>	Kashmir
3	<i>N. discolor</i> Royle ex Benth.	Hemis, Ladakh	Tauheeda & Bilal	2710	<i>Pycnonepeta</i>	Ladakh
4	<i>N. eriostachya</i> Benth.	Zojila	Tauheeda & Dar	2785	<i>Pycnonepeta</i>	Ladakh
5	<i>N. floccosa</i> Benth.	Khalsi, Kargil	Tauheeda & Dar	2749	<i>Capituliferae</i>	Ladakh
6	<i>N. glutinosa</i> Benth.	Zanskar, Ladakh	Tauheeda & Dar	2788	<i>Glechomanthe</i>	Ladakh
7	<i>N. govaniana</i> (Wall. ex Benth.) Benth.	Gulmarg, Kashmir	Tauheeda & Dar	2729	<i>Macronepeta</i>	Kashmir
8	<i>N. kokanica</i> Regel	Nubra, Ladakh	Tauheeda	2794	<i>Pycnonepeta</i>	Ladakh
9	<i>N. longibracteata</i> Benth.	Khardungla, Leh	Tauheeda & Bilal	2739	<i>Glechomanthe</i>	Ladakh
10	<i>N. paulsenii</i> Briq.	Dras, Zojila	Tauheeda	2757	<i>Pycnonepeta</i>	Ladakh
11	<i>N. podostachys</i> Benth.	Leh, Ladakh	Tauheeda & Bilal	2791	<i>Pycnonepeta</i>	Ladakh
12	<i>N. raphanorhiza</i> Benth.	Pampore, Pulwama	Tauheeda & Dar	2705	<i>Pycnonepeta</i>	Kashmir
13	<i>N. leucolaena</i> Benth.	Hemis, Ladakh	Tauheeda & Bilal	2717	<i>Macronepeta</i>	Kashmir & Ladakh

(SEM) in the study of nutlet surfaces, and the taxonomic value of nutlet characters has been ascertained for various genera of *Lamiaceae* (Barthlott, 1984; Stace, 1989; Husain *et al.*, 1990; Ryding 1993; Budantsev 1993a, b; Marin *et al.*, 1996; Budantsev and Lobova, 1997; Jamzad *et al.*, 2000; Salmaki *et al.*, 2008). Nutlet morphology in the *Lamiaceae* has proved useful to varying degrees at different levels of the taxonomic hierarchy. Some nutlet characters were used by Bentham (1848) in the diagnosis of some tribes (Budantsev and Lobova, 1997). Furthermore, micro-morphological characters of nutlet can be used at generic, specific, and even at variety level (Kaya & Dirmenci, 2008; Husain *et al.*, 1990). However, the importance of ultrastructural features of pericarp surface has already been demonstrated for *Nepeta* (Hedge, 1962, 1992; Rechinger 1982; Ubera, 1982; Ubera and Valdes, 1983; Budantsev 1993a; Budantsev and Lobova, 1997; Jamzad *et al.*, 2000; Mosquero *et al.*, 2002; Padure, 2004; Padure and Toma, 2004).

The genus *Nepeta* in Kashmir consists of 22 species belonging to 6 different sections. The main objective of this study is to provide reliable characters useful in separation of species within certain groups known as taxonomically difficult in Kashmir. Some species are recognized only morphologically and some are recognized morphologically as well as micro-morphologically

Materials and Methods

This study is based on dry herbarium specimens deposited in the Herbarium of the Centre for Biodiversity & Taxonomy of Kashmir University

(Table 1). Measurements and optical observations on nutlet colors were carried out under a Stereomicroscope Olympus SZ-STB2. About eight populations were studied for each species. For SEM, dry mature nutlets were mounted directly on stubs, using single-sided adhesive tape and coated with gold. Photographs were taken with Hitachi S-3000H Scanning Electron Microscope. The terminology used in nutlet coat-surface ornamentation is after Budantsev and Lobova (1997). The nutlet micromorphology of 13 taxa of *Nepeta* species was worked out and the pictures of these nutlets are given below.

Result

The main features of the investigated nutlets (nutlet size, shape, colour, areole type and surface pattern) are summarized in Table 2. Selected SEM micrographs of nutlets studied are presented in (Figs. A-M). The nutlets are glabrous, rarely pilose apically in surface and black, bluish-brown or brown in color. The size of nutlets ranges from 1.13 mm (in *N. raphanorhiza*) to 2.32 mm (in *N. govaniana*) in length and 0.55 mm (in *N. paulsenii*) to 1.42 mm (in *N. govaniana*) in width. The nutlets of *N. govaniana* are largest and that of *N. raphanorhiza* are smallest among the species studied. The nutlets are ellipsoid (*Nepeta eriostachya*), oblong (*N. longibracteata*), ovoid (*N. raphanorhiza*), to obovoid (*N. connata*), trigonous, areole bilobed or straight (Table 2; Figs. 1-13). Based on nutlet surface ornamentation, two main types of nutlets i.e. I) Reticulate and II) Indistinct reticulate are recognized in the present samples.

1. Reticulate: The reticulate type is smooth type of

nutlets characterized by polygonal elongated or irregular cells. The anticlinal walls are straight, thickened or thinned. Species having this pattern are as follows:

A. *N. glutinosa*: It consists of polygonal elongated cells with depressed both anticlinal and external periclinal walls.

B. *N. raphanorhiza*: It consists of polygonal elongated cells covered with white granules. The anticlinal walls are depressed and external periclinal walls are flat.

C. *N. podostachys*: It is characterized by irregular or rounded cells with depressed anticlinal walls and slightly concave periclinal walls.

D. *N. paulsenii*: It is recognized by discontinuous elongated cells with anticlinal walls depressed. The external periclinal walls are flat or somewhat ridged.

E. *N. discolor*: It is characterized by elongated cells with depressed anticlinal walls. The external periclinal walls are flat.

F. *N. coerulescens*: It is characterized by polygonal cells with raised anticlinal walls but less prominent than *N. govaniana* and *N. kokanica*. The EPW are depressed.

G. *N. govaniana*: It is recognized by polygonal or rounded cells with raised anticlinal walls. The external periclinal walls are depressed or unevenly concave.

H. *N. kokanica*: It consists of polygonal cells, with more prominent AW and depressed EPW.

I. *N. connata*: It consists of irregular sometimes discontinuous cells with less prominent AW.

J. *N. eriostachya*: It is characterized by reticulate-cellular pattern, which is formed by rounded or polygonal cells covered with trichomes. The anticlinal walls are straight and prominent. The external periclinal walls are flat or slightly concave.

K. *N. floccosa*: It is also a smooth pattern consisting of primary and secondary reticulate cells. The surface of reticulate nutlets has conjugate cells with straight and raised anticlinal walls. The external periclinal walls are depressed.

2. Indistinct reticulate: This pattern is characterized by incomplete reticulation with undefined borders. Species with this pattern are as follows:

L. *N. leucolaena*: It consists of cells with an irregular shape and indistinct borders. The anticlinal walls are sinuous, external periclinal walls are less prominent and discontinuous.

M. *N. longibracteata*: It is characterized by incomplete borders like *N. leucolaena* but the reticulation is smooth than the latter.

Discussions

The nutlets of *Nepeta* are generally glabrous, rarely pilose apically in surface, trigonous and black,

blackish-brown or brown in color, areole straight or bilobed. The nutlets have generally prominent U- or V-shaped areole. But different species are distinguished on the basis of areole form and its location. In some species areole is present at lateral position and in some it is present at basal position. Likewise some species have bilobed areole and some have straight areole. Since the position and location of areole varies, it is useful for the delimitation of the species. The species studied belong to four different sections. In Sect. *Pycnonepeta*, areole is basal in *N. coerulescens*, *N. connata* & *N. kokanica* and sub-basal in *N. eriostachya* & *N. raphanorhiza* and lateral in *N. discolor*, *N. paulsenii* & *N. podostachys*. Areole is basal in Sect. *Macronepeta* & lateral in *Capituliferae*. In Sect. *Glechomanthe*, areole is sub-basal in *N. glutinosa* & basal in *N. longibracteata*.

In section *Pycnonepeta* reticulate surface ornamentation is found, but there are some differences in terms of nutlet shape, color, areole form and its location in different species of this section, e.g., nutlets of *N. discolor* are oblong, areole is bi-lobed and lateral in position and in *N. eriostachya*, nutlets are ellipsoid, areole is straight and sub-basal in position. In this section, the anticlinal walls are depressed except in *N. coerulescens*, *N. eriostachya* & *N. kokanica*, where anticlinal walls are raised and prominent. Section *Capituliferae* share same kind of surface ornamentation to section *Pycnonepeta*, however, there are some differences among the species belonging to two different sections e.g., *N. floccosa* belonging to Sect. *Capituliferae* have primary and secondary reticulations which are not found in the species of Sect. *Pycnonepeta*. In section *Glechomanthe*, two patterns, i.e., reticulate and indistinct reticulate were observed. Reticulate pattern is found in *N. glutinosa* and indistinct reticulate pattern in *N. longibracteata*. Even though the two species have different nutlet shape, color, areole form and location. Section *Macronepeta* also observed same two patterns mentioned above. Reticulate pattern were observed in *N. govaniana* and indistinct pattern in *N. leucolaena*. However, differences in other characters in nutlets of these species were also observed. In *N. govaniana*, the nutlet shape is broadly obovoid and areole form is straight, but the nutlets of *N. leucolaena* have linear-oblong shape and bi-lobed areole form. In addition, the anticlinal walls are raised in *N. govaniana*. Based on selected examined taxa in *Nepeta* two main groups can be distinguished in the genus: first group characterized by smooth nutlet surface and the second type recognizable by sculptured surface (Budantsev and Lobo, 1997; Padure, 2004). Among the studied species in the present study, only *Nepeta leucolaena* and *N. longibracteata* shows indistinct reticulate

Table 2: Comparison of the nutlet characters in *Nepeta* species vis-à-vis their sectional distribution and occurrence in two provinces of the Kashmir Himalaya.

S.No	Taxa	Size (mm)	Shape	Colour	Areole form	Areole location	Nutlet surface
1	<i>N. coerulescens</i>	1.80±0.07×0.96±0.04	ovoid-broadly obovoid	brown	bi-lobed	basal	reticulate
2	<i>N. connata</i>	1.58±0.08×1.20±0.10	obovoid-ellipsoid	brown	bi-lobed	basal	reticulate
3	<i>N. discolor</i>	1.47±0.12×0.91±0.05	oblong	dark-brown	bi-lobed	lateral	reticulate
4	<i>N. eriostachya</i>	1.43±0.15×0.82±0.05	ellipsoid	dark-brown	straight	sub-basal	reticulate
5	<i>N. floccosa</i>	2.17±0.10×1.06±0.13	narrow oblong	dark-brown	bi-lobed	lateral	reticulate
6	<i>N. glutinosa</i>	1.22±0.10×0.72±0.15	broadly ellipsoid	greenish-brown to brown	straight	sub-basal	reticulate
7	<i>N. govaniiana</i>	2.32±0.13×1.42±0.04	broadly obovoid	greenish-brown	straight	basal	reticulate
8	<i>N. kokanica</i>	1.20±0.10×0.96±0.04	oblong-ellipsoid	brown	straight	basal	reticulate
9	<i>N. longibracteata</i>	2.02±8.26×0.82±0.05	oblong	brown	bi-lobed	basal	indistinct reticulate
10	<i>N. paulsenii</i>	1.16±0.04×0.55±0.04	ellipsoid	brown	bi-lobed	lateral	reticulate
11	<i>N. podostachys</i>	1.19±0.10×0.84±0.07	ellipsoid	brown	bi-lobed	lateral	reticulate
12	<i>N. raphanorhiza</i>	1.13±0.07×0.72±0.15	ovoid	light-brown	bi-lobed	sub-basal	reticulate
13	<i>N. leucolaena</i>	1.81±0.07×0.89±0.07	linear oblong	black	bi-lobed	basal	indistinct reticulate

pattern of nutlet surface, but the most common type was reticulate pattern (reticulate-cellular in *N. eriostachya*; Table 2). Our samples of *N. govaniiana*, *N. kokanica* and *N. coerulescens* show a similar pattern to *N. formosa*, *N. sibirica* and *Lophanthus schrenkii* as mentioned in a previous study are the same as that of our samples like. Jamzad *et al.* (2000) observed the surface sculpturing of annual species of *Nepeta* in Iran, the reticulate patterns of which are the same as that of our species.

When a comparison is made between our samples and that of others, it is clear that only smooth pattern is found in our species. As *Nepeta podostachys*, *N. paulsenii* and *N. discolor* are very similar to each other morphologically and in habitat, they also exhibit similarities with respect to the nutlet morphology (i.e. reticulate type of ornamentation). It is clear that the species of different sections share same kind of surface ornamentations, even though there are some minor differences among these species. e.g., surface

ornamentations in *N. govaniiana* and *N. kokanica* belonging to different sections are almost alike. Two different types of nutlet surfaces i.e., reticulate & indistinct reticulate occur in two different phytogeographic provinces of the study area. Reticulate pattern is found in both Kashmir and Ladakh regions whereas; indistinct reticulate pattern is exclusively confined to Ladakh region. Reticulate surface ornamentation found in both the regions does not correlate with the ecology of the species. Both regions have different ecological conditions so as the case with their species, but their nutlet surface ornamentation is the same. However, indistinct reticulate surface ornamentation found in only Ladakh region showed correlation with the ecological conditions of the species. Because Ladakh is a cold desert having different climate that may favour the formation of nutlets having such pattern. However, such pattern is not found in Kashmir region having temperate climate.

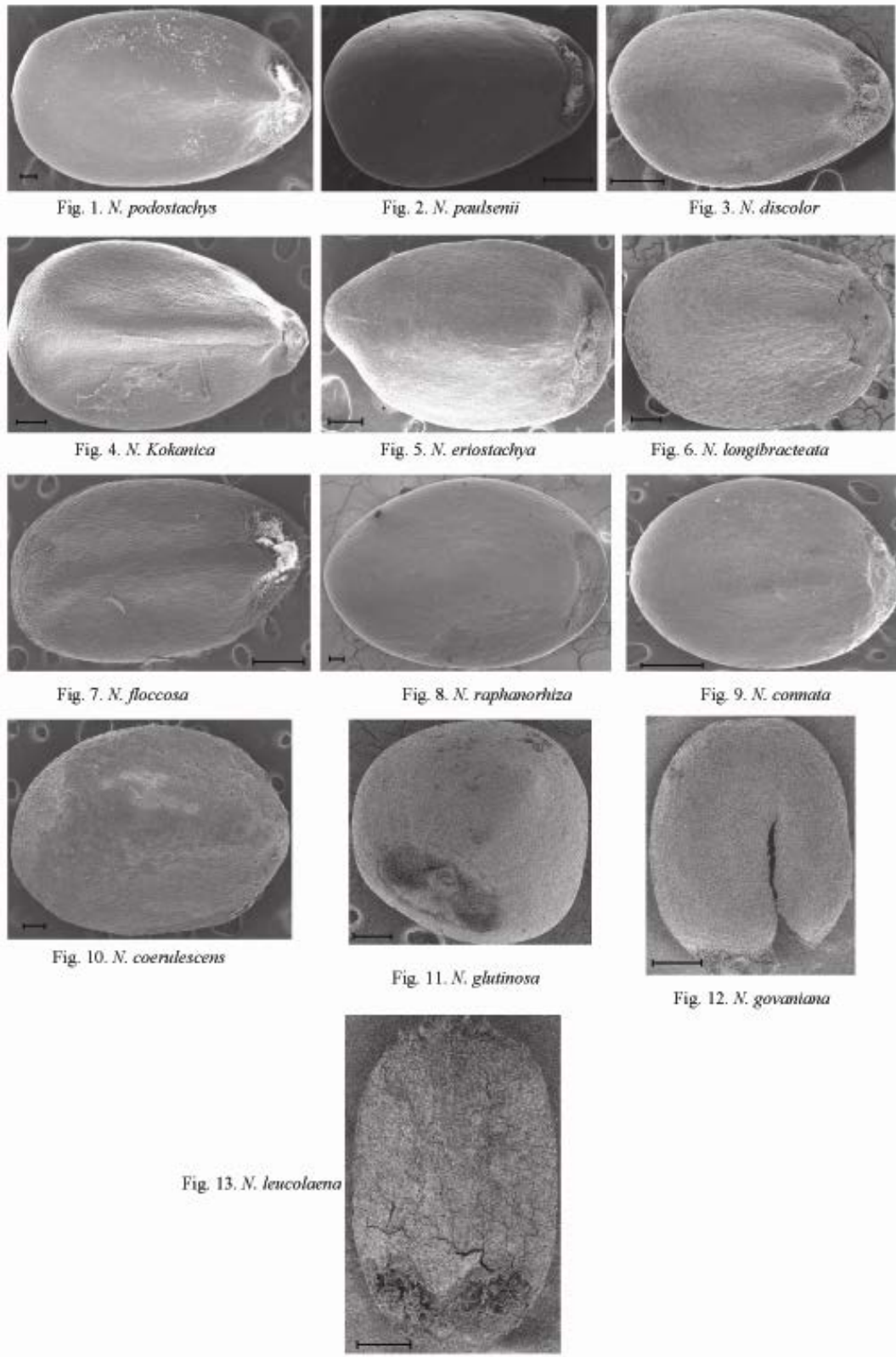
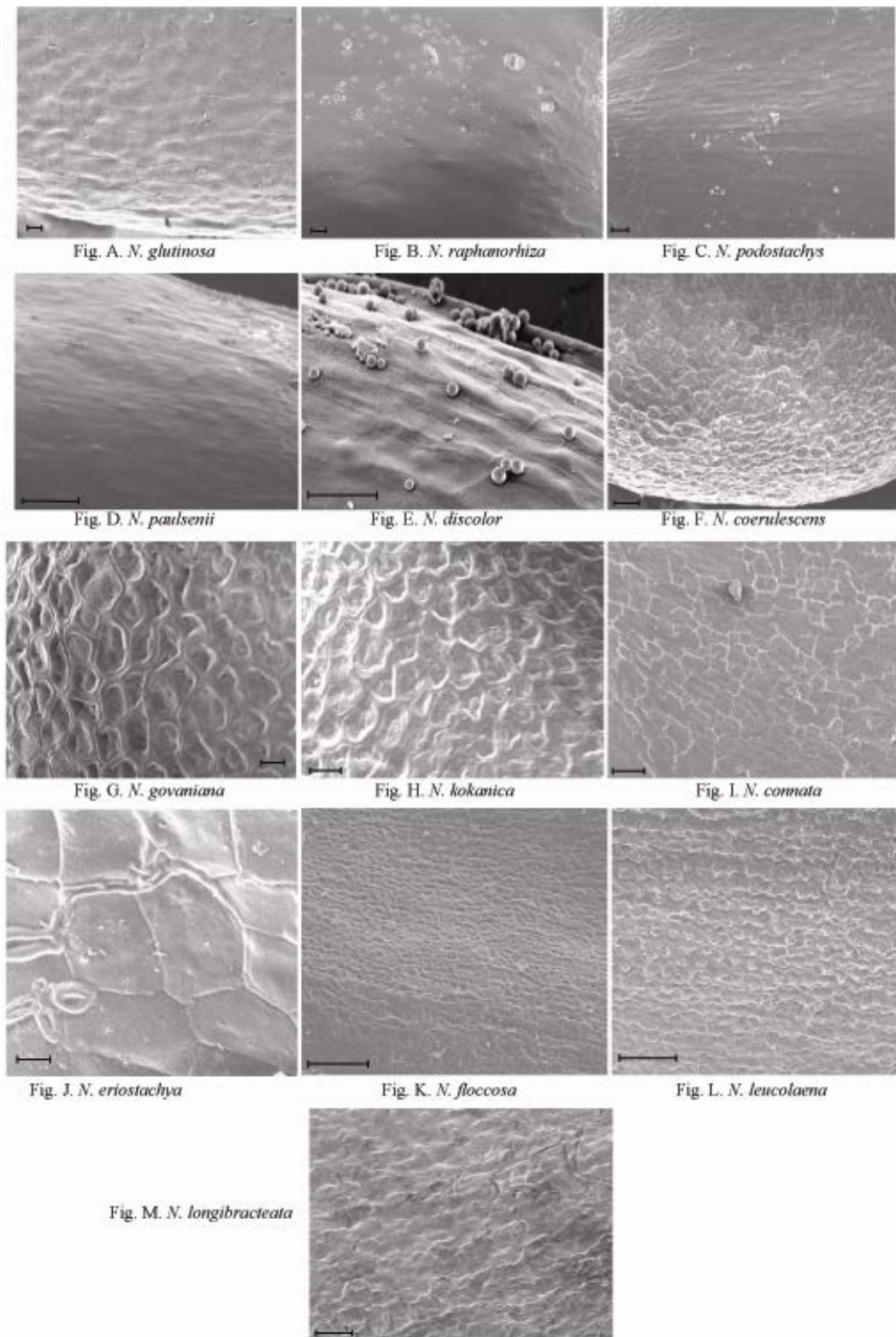


Fig. 1-13. Nutlets of *Nepeta* species (Scale figs. 1-8, 10, 11 = 500µm; 9, 12, 13 = 1mm).



Figs. A-M: SEM micrographs of nutlets in *Nepeta* species (Scale figs. B, D, H = 100µm; A, E, G = 50µm; C, F, I, K-M = 200µm; J = 30µm).

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