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NEPETA MARGALLAICA (MENTHEAE: NEPETOIDEAE: LAMIACEAE): A NEW SPECIES FROM THE MARGALLA HILLS, PAKISTAN

A. Sultan [1]*, A. Khan [1], S. A. Shah [2], S. Naz [3] & Z. Jamzad [4]

A new species of *Nepeta* L. (Lamiaceae) from the Margalla Hills, in Pakistan, is described and illustrated. It belongs to *Nepeta* sect. *Cataria* sensu Bentham and is characterised by tuberous roots up to 3 cm in diameter, the presence of long multicellular flattened eglandular hairs, a spike-like inflorescence, an oblique calyx throat, unequal calyx teeth, and a 9 mm long white corolla. It is closely related to *Nepeta cataria* L., from which it differs in the root, indumentum, calyx and inflorescence characters. A distribution map of the new species is presented, as is an updated key to *Nepeta* sect. *Cataria*.

Keywords. Margalla Hills National Park, Nepetinae, section *Cataria*, taxonomy, tuberous roots
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Introduction

Nepeta L. (Lamiaceae, Nepetoideae, Mentheae, Nepetinae) comprises approximately 300 species distributed in Southwest and Central Asia, Europe, North Africa and North America, with Southwest Asia and the Western Himalayas being the main centres of diversity (Acar *et al.*, 2011). In the flora of Pakistan, *Nepeta* is the largest genus in the family Lamiaceae, comprising 55 species, 15 of which are endemic (Hedge, 1990; Jehan, 1996; Reshi *et al.*, 2012; Sultan *et al.*, 2021). In Pakistan, it is mainly recorded from Gilgit-Baltistan, Kashmir, Khyber Pakhtunkhwa, Balochistan, and northern and western Punjab.

Nepeta is a taxonomically complicated genus with morphological characters such as indumentum, calyx characters, leaf shape and size being highly variable even within a single species. Highlighting these taxonomic issues, Hedge (1990) noted that the genus contains both well-defined species and those that are exceedingly polymorphic. The lack of dependable morphological characters has contributed to the challenges in the delimitation of species in the genus. However, Hedge (1990) believed that nutlet size, shape, surface texture and areole length could provide more reliable characters. He also suggested that hybridisation and introgression probably play a role in the complexities of classification, and recommended comprehensive field studies and collation with the taxonomic situation

[1] National Herbarium of Pakistan (Stewart Collection), PARC-National Agricultural Research Centre, Islamabad, Pakistan.
[2] Department of Biological Sciences, National University of Medical Sciences, The Mall, Abid Majeed Road, Rawalpindi 46000, Pakistan.
[3] Plant Genetic Resources Institute, PARC-National Agricultural Research Centre, Islamabad, Pakistan.
[4] Research Institute of Forests and Rangelands, PO Box 13185-116, Tehran, Iran.
* Author for correspondence. E-mail: amirsultan_2000@yahoo.com.

in adjacent areas, particularly Kashmir, a rich area for *Nepeta* diversity in Pakistan (Hedge, 1990).

Previous infrageneric classifications of the genus based on morphological characters have been in conflict (Bentham, 1848; Briquet, 1896; Budantsev, 1993), largely due to differences in interpretation of the morphological variation of the characters used. However, molecular phylogenetic studies have greatly improved our knowledge of Lamiaceae and *Nepeta* (Jamzad *et al.*, 2003; Li *et al.*, 2016; Serpooshan *et al.*, 2018; Zhao *et al.*, 2021).

The results of molecular data analyses have supported the monophyly of *Nepeta* and the recognition of five major monophyletic groups within the genus, most of which included species from more than one section in previous classifications. These clades were: (i) sect. *Spartonepeta*, (ii) sects *Macronepeta* + *Spicatae*, (iii) sects *Nepeta* + *Micranthae* p.p. + *Oxynepeta* + *Schizocalyx* + *Macrostegiae*, (iv) sects *Capituliferae* + *Denudatae* + *Micranthae* p.p. + *Micronepeta* p.p., and (v) sect. *Psilonepeta* (Jamzad *et al.*, 2003). These clades were supported by some floral characters, including corolla shape, bract texture, colour, and pollen exine ornamentation (Jamzad *et al.*, 2003). The recent phylogenetic study in the subtribe Nepetinae (Serpooshan *et al.*, 2018) showed that the genera *Hymenocrater* Fisch. & C.A.Mey., *Lophantus* Benth. and *Marmoritis* Benth. are all included in *Nepeta*, and under this assumption, the genus *Nepeta* is monophyletic. The various molecular phylogenetic studies support Bentham's classification in some groups, and highlight that monophyletic groups are not based on their annual or perennial habit, but on floral, inflorescence and other morphological characters.

The Margalla Hills, a mountain range situated on the northern edge of Islamabad, starts near Tret and ends near Taxila and is an extension of the Murree Hills (Western Himalayas). The range nestles between an elevation of 685 m at its western end and 1604 m at its eastern end; it is covered by deciduous and evergreen trees and supports a diverse shrub growth on the southern slopes and pines and oak groves with undergrowth of *Myrsine africana* L. on the northern slopes. Recent botanical expeditions to the Margalla Hills have provided new collections, among which were specimens with diagnostic characteristics that did not match those of known *Nepeta* species.

Here, we describe a new species of *Nepeta* collected from the Margalla Hills, and provide illustrations, scanning electron microscopy (SEM) images, images of root anatomical sections, and a distribution map for the new species.

Materials and methods

A field expedition to the Margalla Hills was undertaken, during which a population of a *Nepeta* species with tuberous roots was observed. Collected herbarium specimens of this species were studied in further detail at RAW (herbarium codes follow Thiers, continuously updated), using identification keys in the *Flora of Pakistan* (Hedge, 1990), *Flora Iranica* (Rechinger, 1982), *Flora of China* (Li & Hedge, 1994) and other relevant literature (Reshi *et al.*, 2012), and were identified as belonging to a species new to science.

In addition to herbarium specimens, a tuberous root, detached from a healthy and mature plant, was examined to study its anatomical traits. The root was placed in formalin aceto-alcohol solution for 24 h. Thin serial transverse sections were then made using a Shandon microtome (Finesse 325; ThermoFisher Scientific, Waltham, MA, USA), using the paraffin wax method. Each section was passed through a series of 60%, 70% and 80% ethanol solutions and then stained following a method modified from that described by Mehdi *et al.* (2019), using 0.1% (w/v) toluidine blue, 0.5% (w/v) safranine O, and 0.2% (w/v) fast green solution. After staining, micrographs were taken of the root section at different magnifications, using a camera installed on the Primostar compound microscope (Zeiss 415500-0059-000; Oberkochen, Germany). Sections were labelled following the terminology of Moore (1987), and a full description of the root was produced following the terminology of Metcalfe & Chalk (1950).

Nutlets were removed from the herbarium specimens and studied directly under both a stereomicroscope (Olympus 605371; Tokyo, Japan) and a JEOL-5910 scanning electron microscope (Jeol, Tokyo, Japan) installed at the Centralized Resource Laboratory, Department of Physics, University of Peshawar. Material for examination by SEM was coated in gold and affixed to stubs, using double-sided adhesive tape. Based on the scanning electron micrographs, details of nutlet micromorphology were described, following Barthlott (1984) and Budantsev & Lobova (1997).

The conservation status of the species was assessed based on the *IUCN Red List Criteria and Categories* (IUCN, 2012).

Taxonomic treatment

Species description

Nepeta margallaica A.Sultan, Jamzad & A.Khan, **sp. nov.**

Resembles *Nepeta cataria* L. but differs from that species in having tuberous roots (vs non-tuberous), spike-like inflorescence (vs paniculate with distant lower verticillasters), calyx minutely pubescent not becoming urceolate in fruit (vs densely pubescent, curved, becoming urceolate in fruit), corolla uniformly white, lacking purple spots on the lower lip (vs corolla bearing purple spots on the middle lobe of the lower lip), and nutlets black and shiny (vs pale brown). It also differs from *Nepeta raphanorhiza* Benth., the other *Nepeta* species with tuberous roots, in having spike-like inflorescence with distant basal verticillasters (vs terminal ovoid spike), calyx herbaceous with an oblique throat (vs membranous with a suberect throat), and corolla white (vs violet-blue). – Type: Pakistan, Khyber Pakhtunkhwa, Haripur district, Margalla hills, near Barkot, Kohmal Paein, 980 m, 33°51'22.6"N, 73°09'43.4"E, 12 viii 2021, *Amir Sultan, Saeeda Naz, Muhammad Shafi & Zahfran Amjad* 212241 (holotype RAW [barcode 102046], isotype TARI). **Figures 1, 2, 3.**

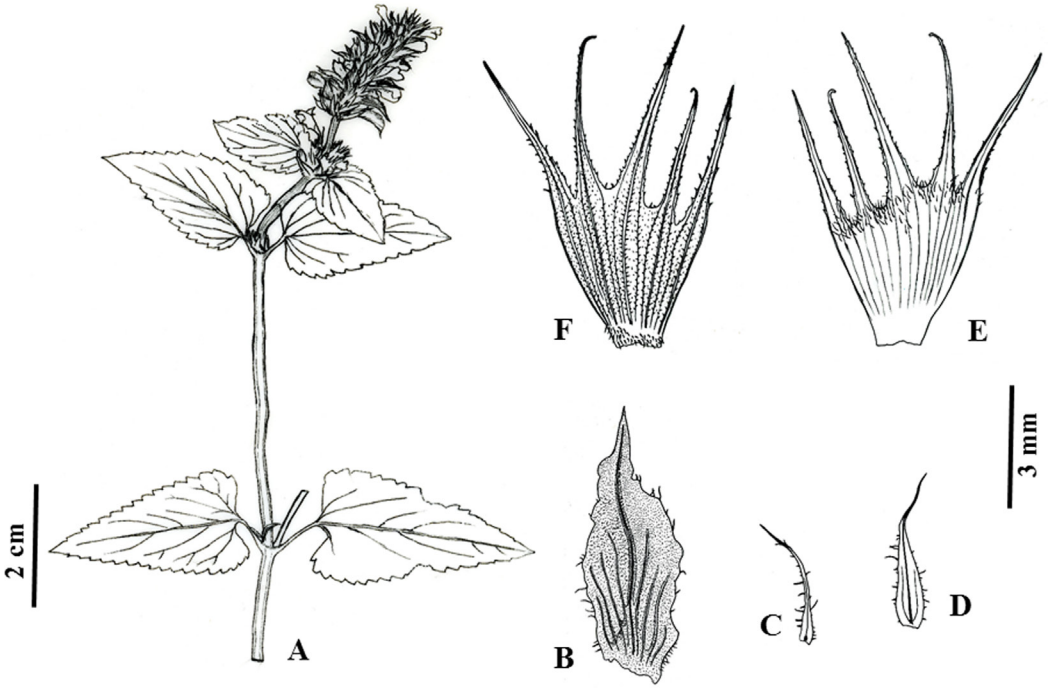

Figure 1. *Nepeta margallaica* A.Sultan, Jamzad & A.Khan, sp. nov. A, Habit; B, floral leaf; C, bracteole; D, bract; E, calyx (adaxial surface); F, calyx (abaxial surface). Drawn from the holotype, *Amir Sultan, Saeeda Naz, Muhammad Shafi & Zahfran Amjad* 212241, by Muhammad Saleem.

Perennial herb, roots tuberous, dark brown, up to 17 cm long, c.3 cm in diameter. *Stem* up to 65–85 cm, branched in upper part; pubescent, with retrorse, villous hairs, intermixed with some flattened multicellular hairs in upper parts. *Leaves* 3–5 × 1.5–2.5 cm, oblong-ovate to ovate-triangular, acute, crenate-serrate, truncate to shallowly cordate, adaxial surface glabrous to glabrescent, sparsely pubescent along veins, abaxial surface sparsely pubescent and densely pubescent along veins. *Petiole* 0.6–1.5 cm, pubescent, bearing few multicellular hairs. *Inflorescence* spike-like, basal verticillasters distant. *Lower floral leaves* leaf-like, broadly ovate to ovate-acuminate, serrate-crenate, 0.5–2.2 × 0.2–1.4 cm, ciliate with flattened, long multicellular hairs. *Bracts* lanceolate, acuminate, c.5 × 1 mm, bracteoles linear-lanceolate, long acuminate, 2.5–3 × 0.5 mm, ciliate. *Calyx* tubular-campanulate, 5.5–6 mm, 15-nerved, tube ± straight, pubescent, calyx throat oblique, hairy, teeth 2.5–3 mm, linear-triangular, the median tooth of the upper lip often recurved. *Corolla* white, 9 mm long, hairy, tube curved, shortly exerted from the calyx, abruptly dilated into the broad throat, upper lip 3–4 × 4 mm, lower lip 4–4.5 × 6 mm, middle lobe of the lower lip 3–4 × 3.5–5 mm, margin dentate. *Nutlets* black, shiny, ovate, 1.5 × 1 mm, trigonous, smooth, areole lobes short, horizontal.

Figure 2. *Nepeta margallaica* A.Sultan, Jamzad & A.Khan. A, Habitat; B, habit; C, tuberous root; D, inflorescence; E, flower in basal verticillaster; F, corolla (scale divisions, 1 mm); G, flower (scale divisions = 1 mm); H, nutlet (dorsal surface); I, nutlet (ventral surface). A, Photograph taken in the field; B, D–G, photographs of a pot-grown plant at the Botanical Conservatory of the National Agricultural Research Centre; C, H and I, photographs of the type collection (*Amir Sultan* et al. 212241). Photographs: A, Amjad Khan; B–G, M. Saleem and Faraz Ahmed; H and I, Saeeda Naz.

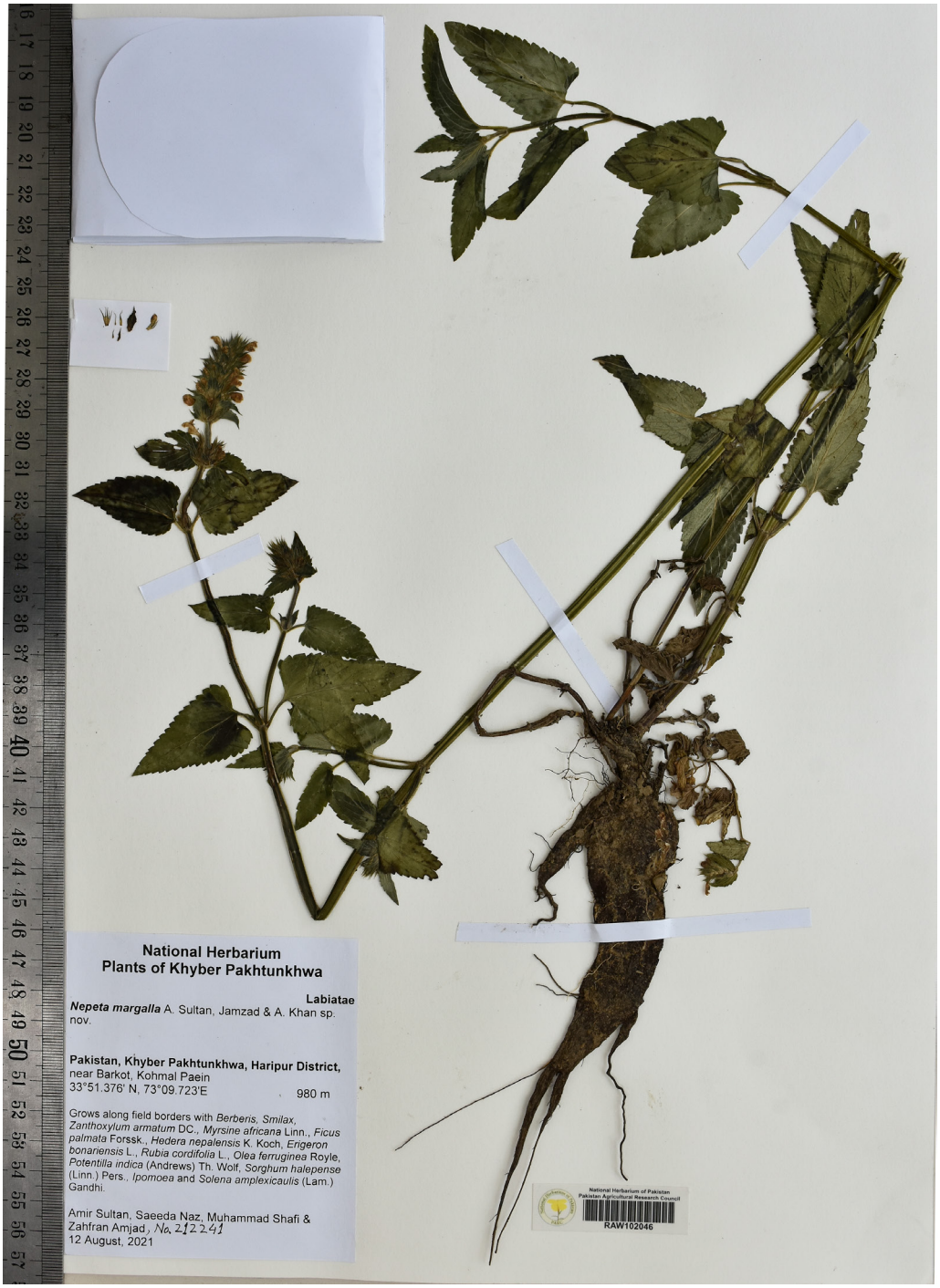

Figure 3. Holotype of *Nepeta margallaica* A.Sultan, Jamzad & A.Khan, sp. nov. (RAW [barcode 102046]).

Distribution. So far known only from two localities approximately 1 km apart, in the Barkot area of the Margalla Hills (Figure 4).

Habitat and ecology. Found growing along maize field borders in the type locality. Flowering July to October, fruiting August to October. The aerial plant parts die back during the winter, and the plant resprouts in spring from the tuberous roots.

Etymology. The species is named after the Margalla Hills, where it was originally found.

Proposed IUCN conservation category. Both documented collections of this species originate from locations adjacent to a road, situated within a 2 km radius of each other, and near human settlements. The AOO and EOO are each 4 km^2, it is recorded from two locations, there is evidence of a decline in at least the quality of habitat, and there are threats from overgrazing and uprooting by wild boars. As a precautionary measure, a provisional listing is provided as Critically Endangered (CR), based on B1ab(iii)+2ab(iii) criteria. Some plants are being maintained in the Botanical Conservatory at the National Agricultural Research Centre, Islamabad.

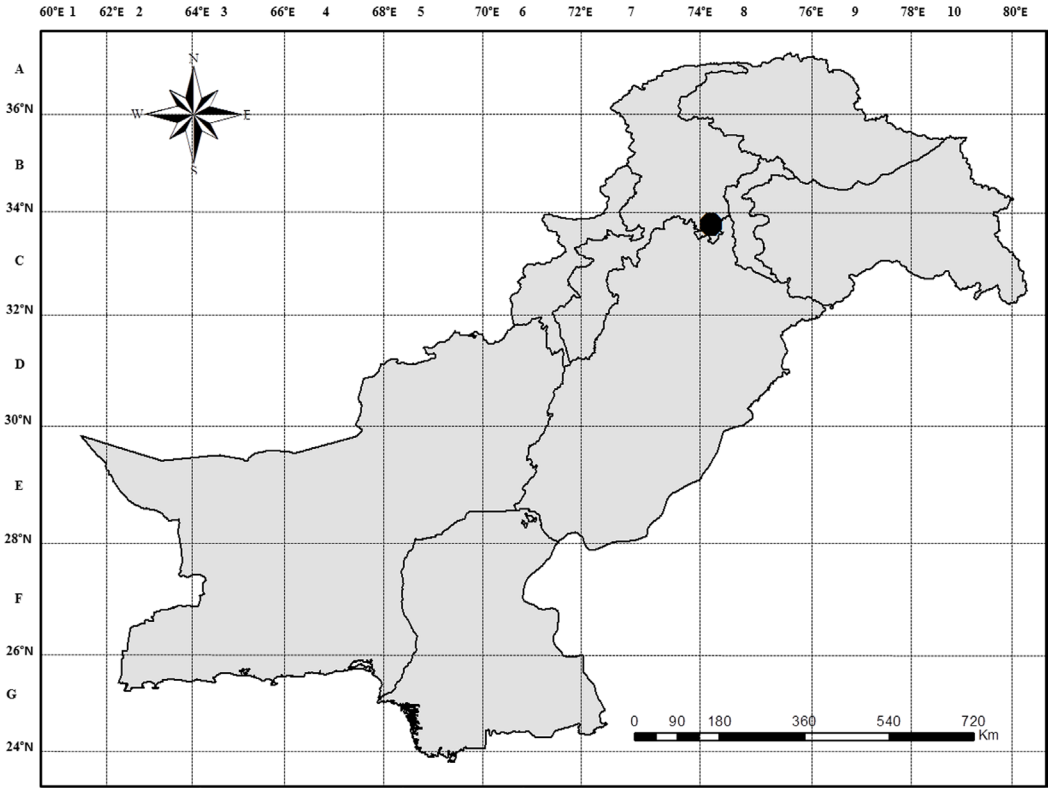

Figure 4. Map showing the distribution of *Nepeta margallaica* in Pakistan.

Notes. *Nepeta margallaica* belongs to *Nepeta* sect. *Cataria* Benth. and to the *N. cataria* species group (Jamzad, 2012), whose members are characterised by having a cymose or paniculate inflorescence, consisting of distant verticillaster or close verticillasters, spike-like in the upper part; and oblique calyx throat, unequal teeth, middle lobe of the lower corolla lip dentate-crenate, with the margin turned upwards. Rather *et al.* (2012) have recorded *Nepeta raphoanorhiza*, known locally in Kashmir as *kanz-gogal*, as having an edible root; its edibility was also reported by Stewart (1972). The edibility of the tuberous roots of *Nepeta margallaica* should be investigated.

Additional specimens examined. PAKISTAN. Khyber Pakhtunkhwa: Haripur district, Margalla hills, Barkot, 1005 m, 33°51'31.5"N, 73°10'08.6"E, 9 ix 2022, *Amir Sultan & Amjad Khan* 222521 (RAW [barcode 102320]).

Key to species of Nepeta *sect.* Cataria *sensu Bentham found in Pakistan (modified from Hedge, 1990)*

1a. Annual ____ 2
1b. Perennial ____ 3

2a. Calyx c.4.5 mm, nutlets clearly depressed-tuberculate, peduncles filiform ____ *N. graciliflora*
2b. Calyx 3.5–4 mm, nutlets inconspicuously depressed-tuberculate, peduncles not filiform *N. hindostana*

3a. Inflorescence of loose pedunculate cymes with clearly pedicellate flowers ____ 4
3b. Inflorescence of ± condensed pedunculate cymes, of distant verticillasters, ovoid heads or spikes ____ 7

4a. Nutlets with a prominent U- or V-shaped areole extending c.2/3 of nutlet length ____ 5
4b. Nutlets with an areole extending c.1/3 or less of nutlet length ____ 6

5a. Leaves c.7–10 × 7–10 mm, margin serrulate; peduncles up to 20 mm [northwestern Pakistan: Chitral] ____ *N. brachyantha*
5b. Leaves up to 25 × 25 mm, margin crenate-dentate; peduncles up to c.10 mm [West Pakistan] ____ *Nepeta* sp. aff. *hindostana*

6a. Calyx c.4.5 mm, nutlets clearly depressed-tuberculate, peduncles filiform ____ *N. graciliflora*
6b. Calyx 3.5–4 mm, nutlets inconspicuously depressed-tuberculate, peduncles not filiform *N. hindostana*

7a. Verticillasters ± clearly separated from each other or uppermost loosely congested ____ 8
7b. Verticillasters forming ± continuous spikes or ovoid heads, sometimes lowermost verticillasters distant ____ 9

8a. Inflorescence paniculate, calyx green ____ *N. cataria*

8b. Inflorescence unbranched or lower verticillasters shortly pedunculate, not paniculate, calyx often purplish _____ *N. rugosa*

9a. Rootstock with a distinct tuber, nutlets not tuberculate _____ **N. margallaica**
9b. Rootstock not tuberous, nutlets tuberculate _____ 10

10a. Calyx 9–10 mm, leaf apices obtuse _____ *N. schmidii*
10b. Calyx 6.5–8 mm, leaf apices ± acute _____ 11

11a. Perennial herb, 30–50 cm, leaves broadly ovate to triangular-ovate, 15–35 × 10–30 mm; inflorescence of numerous sessile or shortly pedunculate, many-flowered congested cymes forming an elongated or short spike interrupted below __ *N. griffithii*
11b. Dwarf suffruticose herb, c.10 cm high, leaves c.15 × 18 mm, cordate-reniform; verticillasters condensed into a ± loose head or spike _____ *N. amicorum*

Discussion

Nutlet micromorphology

In *Nepeta*, the micromorphological features of nutlets are important diagnostic characteristics. Previous studies have shown that there are two main types of exocarp ornamentation in *Nepeta*: smooth and sculptured (Jamzad *et al.*, 2000). *Nepeta margallaica*, *N. cataria* and *N. raphanorhiza* nutlets belong to the smooth type. The SEM images of the nutlet surface showed detailed ornamentation of the smooth surface. The exocarp consists of rounded-irregular cells with flat outer periclinal walls, with some striation on cell surfaces. The anticlinal walls are raised, following the cell shape (Table, Figure 5).

Table. A comparison of diagnostic characters of *Nepeta margallaica* A.Sultan, Jamzad & A.Khan, sp. nov. with *N. cataria* and *N. raphanorhiza*

Morphological character	*N. margallaica*	*N. cataria*	*N. raphanorhiza*
Roots	Tuberous, carotiform	Not tuberous	Tuberous, spheroidal
Inflorescence	Spike-like with distant basal verticillasters	Paniculate with distant lower verticillasters	Terminal ovoid spike
Calyx	5.5–6 mm long, minutely pubescent, herbaceous with oblique throat	5–6 mm long, densely pubescent, curved becoming urceolate in fruit	6–7 mm long, eglandular pilose to papillose-glandular, membranous with suberect throat
Corolla	9 mm long, uniformly white, lacking purple spots on the lower lip	6–8 mm long, bearing purple spots on the middle lobe of the lower lip	7–11 mm long, violet-blue
Nutlet	Black, shiny	Brown, dull matt	Light brown, shiny

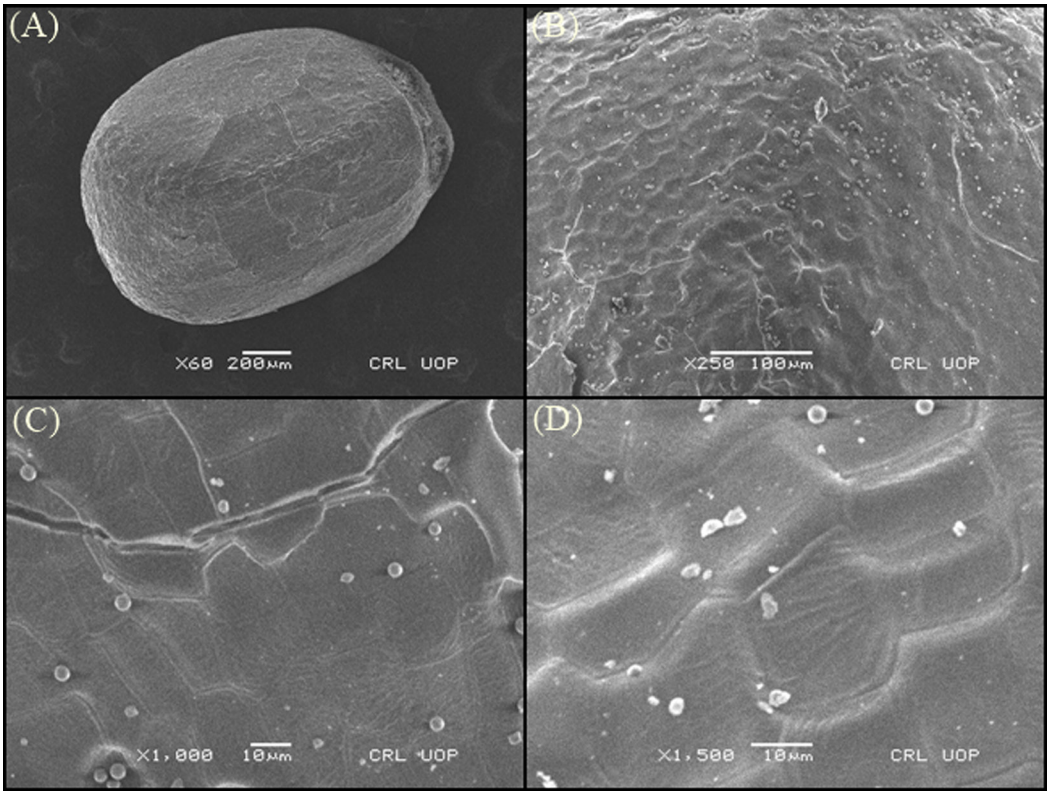

Figure 5. Scanning electron micrographs of *Nepeta margallaica*. A, Nutlet shape; B, nutlet surface; C and D, the exocarp, consisting of rounded-irregular cells with flat outer periclinal walls and raised anticlinal walls. All images of a nutlet from a pot-grown plant at the Botanical Conservatory of the National Agricultural Research Centre, taken by Abdullah Jan.

Root anatomy

The root of *Nepeta margallaica* is more or less circular in outline in cross-section (Figure 6). The outer wall consists of indistinguishable rupture tissues forming the exodermis, behind which lies a 6- to 9-layered periderm composed of thick-walled polygonal cells. The cortex is generally composed of 18–32 layers of slightly irregular thick-walled parenchyma cells. The parenchyma in the cortical region has distinct starch grains with oval to oblong shapes, and it appears reddish. Cambium is generally indistinguishable, but to some extent, phloem fibers form a ring as a procambium (see Figure 6). The vascular ring is composed of phloem fibers and radially arranged xylem vessels, along with the medullary rays towards the centre. The tuberous root in *Nepeta raphanorhiza* has a spheroidal shape, in contrast to that of *N. margallaica*, which has a longer, carotiform tuber. We do not have anatomical data on the root of *Nepeta raphanorhiza*; here, the comparison between it and *N. margallaica* is based on their root morphological traits.

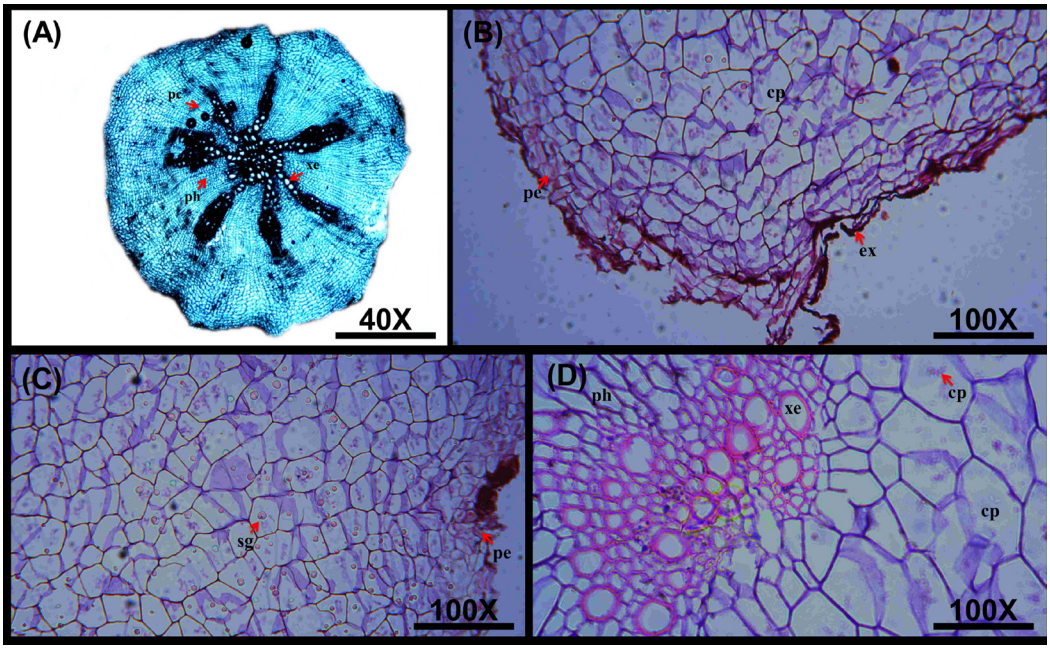

Figure 6. Cross-sections of the tuberous root of *Nepeta margallaica*. A–D, Sections stained with 0.1% toluidine blue. cp, cortical parenchyma; ex, exodermis; pc, procambium; pe, peridermis; ph, phloem; sg, starch grains in cortex; xe, xylem. All images of the type collection (*Amir Sultan, Saeeda Naz, Muhammad Shafi & Zahfran Amjad* 212241), taken by Amjad Khan.

Like the new species, *Nepeta connata* (sect. *Spicatae* Benth.) also has a woody or tuberous rootstock. However, it has entire, linear to linear-lanceolate leaves; an 11–16 mm long calyx; and a mauve to blue-violet, 20–25 mm long corolla. The other species with tuberous roots is *Nepeta tuberosa* L. (sect. *Pycnonepeta* Benth. subsect. *Subinterrupta* Benth.) from the Iberian Peninsula, Morocco and Algeria, which has slender upright spikes of purple flowers in dense interrupted verticillasters with pinkish-grey bracts. All parts of the plant are covered with fine hairs, giving it a silvery appearance.

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ORCID iDs

Amir Sultan http://orcid.org/0000-0003-2116-9502
Amjad Khan https://orcid.org/0000-0002-5337-7237

Sayed Afzal Shah https://orcid.org/0000-0001-5719-3139
Saeeda Naz https://orcid.org/0000-0002-4525-9222
Ziba Jamzad https://orcid.org/0000-0001-8129-3607

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