Analele ştiinţifice ale Universităţii "Al. I. Cuza" Iaşi
Tomul LII, s. II a. Biologie vegetală, 2006

MORPHO-ANATOMY OF FLOWER AND INFLORESCENCE IN *NEPETA* L. (*LAMIACEAE, NEPETOIDEAE*)

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Abstract: The paper presents a morphological and anatomical study of flower and inflorescence of *Nepeta* L. species in Romania. Original data regarding the inflorescence type and sexual distribution of flower in *Nepeta* are discussed. The flowers of *Nepeta* are hermaphrodite in *N. cataria*, *N. nuda* ssp. *nuda* is a gynomonoecious plant and *N. parviflora* and *N. ucranica* ssp. *ucranica* are gynoecious. Morphology of glandular and non-glandular trichomes in calyx, corolla and bracts are studied for the first time. Three identification keys based on morpho-anatomical characteristics and sexual distribution of flower were elaborated.
Keywords: *Nepeta*, *Lamiaceae*, flower, inflorescence, bracts, trichomes, gynomonoecious, gynoecious.

Introduction

Nepeta is a large widely distributed genus with numerous species around the world. Perennial herbs, usually aromatic, occasionally gynomonoecious or gynodioecious. Verticillasters in spikes or opposite cymes in racemes or panicles; floral leaves bractlike; bracts narrow, shorter than or longer than flowers. Calyx 13-15-veined, tubular, slightly curved or straight, throat oblique or regular; teeth 5, equal or unequal, subulate or narrowly lanceolate to oblong-triangular, apex acuminate to spiny-acuminate. Corolla 2-lipped; tube basally narrow, ± abruptly dilated into an ample throat; upper lip ± flat or concave, 2-lobed or emarginate; lower lip large, 3-lobed, with middle lobe larger, concave or ± flat, margin undulate or dentate; lateral lobes small, ovate to semicircular. Stamens 4, nearly parallel, glabrous, ascending under upper lip of corolla, posterior 2 longer than anterior, included or exserted, fertile; stamens of pistillate flowers rudimentary, included; anther cells 2, ellipsoid, divaricate, apex not confluent. Style exserted, apex subequally 2-cleft [11].

Nepeta comprises four species in Romania, two extant and rare species like *N. ucranica* and *N. parviflora*, and two common species represented by *N. cataria* L. and *N. nuda* L. The gynodioecius phenomenon is well known in *Lamiaceae* and *Nepeta* genus, till 1889, and it was noticed like an uncommon among the genera and species of the Old World [7]. Lewis and Crowe (1956) started for the first time the studies regarding the genetics and evolution of gynodioicy [6]. *N. cataria* presents hermaphrodite flower and *N. nuda* is a monoecious plant with obvious difference in the size of corolla [5]; *N. parviflora* M. Bieb. and *N. ucranica* L. are gynoecious (have both hermaphrodite and female structures) (fig. 1).

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Regarding the flower morphology, Budantsev (1992) realized an important study about the morphological transformations of inflorescences in species of the genus *Nepeta* [1]; anatomically, the polygamy phenomenon was studied by Daskalova (2005) [2].

Material and methods

The mature plants were collected from different populations with *Nepeta* (tab I) from Romania. The vegetal material (calyx, corolla, bracts, bracteoles) was preserved in alcohol 70% or FAA and free hand sectioned. The material were cleared with chloral hydrate, stained in carmine red and green iodine and mounted in gelatinized glycerin. Numerical characteristics were undertaken at ML-4M IOR microscope. The prepared material was viewed and photographed with a Nikon camera; morphological draws and photos are presented in fig. 2-7.

Table I - *Nepeta* L. taxa and the source of investigated materials

Taxa	Provenience
N. cataria L.	Caraş-Severin: Băile Herculane; Constanţa: Basarabi (Fântânița-Murfatlar); Bucureşti („I. Todor" Bot. Garden)
N. nuda L.	Caraş-Severin: Băile Herculane; Jud. Constanţa: Basarabi, rezervaţia Fântânița-Murfatlar; Cluj: Cluj Napoca (Dl. Sf. Gheorghe); Vâlcea: Frăsinet; Bucureşti („I. Todor" Bot. Garden)
N. parviflora M. Bieb.	Constanţa: Fântânița-Murfatlar
N. ucranica L.	Cluj: (Dl. Sf. Gheorghe; Ploscoş-Valea Florilor; Frata-Vişinelu („Faţa Terţiului" and „Dealul Părului"); Suatu (Dl. Banffy); Mureş: Zau de Câmpie, Dealul Bota; Miheşu de Câmpie, Dealul Mare; Şăuliţa.

Results and discussions

Morphology of flower and inflorescence. There are a lot differences between the species regarding the morphology of flower and inflorescence in *Nepeta* (fig. 1).

Next section presents data about the reproductive structures distributions on plant and original characteristics of flower and inflorescence with taxonomic significance in *Nepeta* (tab. II). The original morphological features of *Nepeta* flowers and inflorescence and macro-images of flowers are presented in fig. 2-4.

There are many differences in *Nepeta* species regarding the morphological aspects in flower: the color of flowers; corolla length related to calyx size; the shape of middle lobe of lower corolla lip; calyx shape, teeth and tube length ratio in calyx; type anastomosis of veins in calyx; the presence or absence of bract in central flower of cyme; the shape of bracts and bracteoles; the inflorescence type; bracts and calyx length ratio, etc.

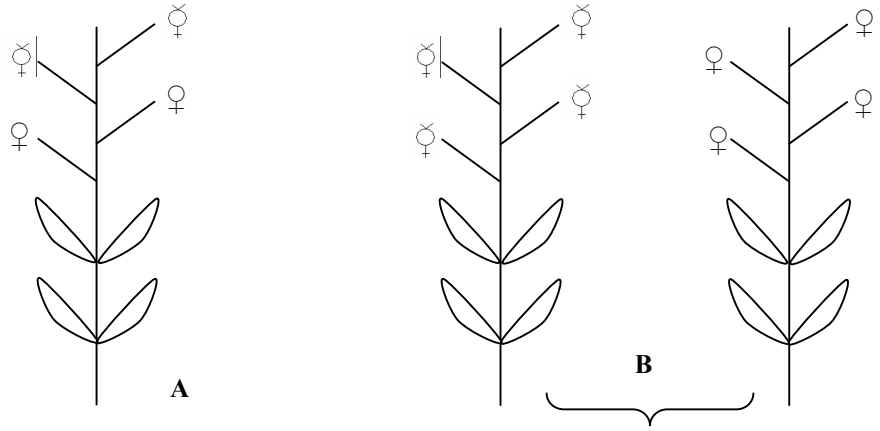


Fig. 1 - Polygamous-monoecious (gynomonoecious) and polygamous-dioecious (gynoecious) regarding the reproductive structures: **A-** *N. nuda*; **B-** *Oxynepeta* [3]

Table II - Morphological characteristics of flower and inflorescence with taxonomic significance in *Nepeta*: *N. cataria* (1)*, N. nuda* (2)*, N. parviflora* (3) and *N. ucranica* (4) [9].

Taxa	**Characteristics**	
	Flowers	Inflorescence
1	Corolla (C) dirty white, purple-spotted, C tube shorter than calyx (K), middle lobe of lower corolla lip broad-ovate and strongly denticulate, with involuted margin; bracteoles linear-subulate (fig. 2).	Flowers arranged in glomerular multi-flowered cymes at the end of stem ramifications, raceme shaped; upper cymes dense, few-flowered and subssesile; inferior cymes multi-flowered, with pedicelate flowers, ± distant, lax; bracts linear-lanceolate.
2	C pale-violet, sometimes pink tinted, rarely white, obvious violet-spotted, with middle lobe of lower corolla lip hemi-circular, with crenate-involute margins; bracteoles narrow-linear, shorter than K (fig. 3).	Numerous cymes, lax, upper ones with 2 to 5 pedicelate flowers, ± distant; inferior cymes multi-flowered, 1-2-dichotomic bifurcate; inferior cymes multi-flowered arranged in raceme, narrow to the top and axilar ramifications, pyramidal shaped; bracts linear-lanceolate, narrowed.
3	C blue-azure, equal with K, middle lobe of lower corolla lip hemi-circular, with crenate margin; K obconic, in fruition stage, ± globular, K teeth usually shorter than tube, rarely exserted, linear-	Cymes with 3-5 pairs of pedicelate flowers, 2-3-dihotomic branched, compact; median flower of dichazium short pedicelate or sessile; bracts sessile, narrow-lanceolate, long acuminate, with entire margins, longer than cyme axis (fig. 4).

	subulate; bracteoles sessile (lack in central flower of cyme), linear-lanceolate, shortly and appressed-hairy, longer than pedicels of lateral flowers.	
4	C blue-violet, longer tjan K, middle lobe of lower corolla lip reniform to hemi-circular, entire margins; K obconic or ± narrow-cilindrical to cylindrical at fructification; K teeth equal with K tube longer than calyx; bracteoles lanceolate, generally shorter than K, rarely equal with; bracteoles absent at central flower of cyme.	Dichazium 3-flowered, sometimes may noticed a terminal monochazium; pedicels of cymes 2-4 cm; median flower sessile; bracts lanceolate, long-acuminate, sessile, with entire margins, except the lower one (fig. 5).

The anatomical aspects of *Nepeta* flower are related with trichomes type (bglandular or non-glandular) in corolla and calyx; stomatal distribution in epidermis of bracts and calyx; midvein shape in calyx (in cross-section); the lamina type in bracts (number of layers in palisadic and spongy tissues) etc.

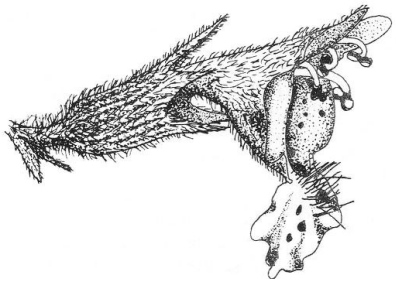


Fig. 2 - Flower of *N. cataria* L. [10]

Fig. 3 - Flower of *N. nuda* L. ssp. *nuda* [9]

Fig. 4 - Morphology of flower in *N. parviflora* M. Bieb. [9]

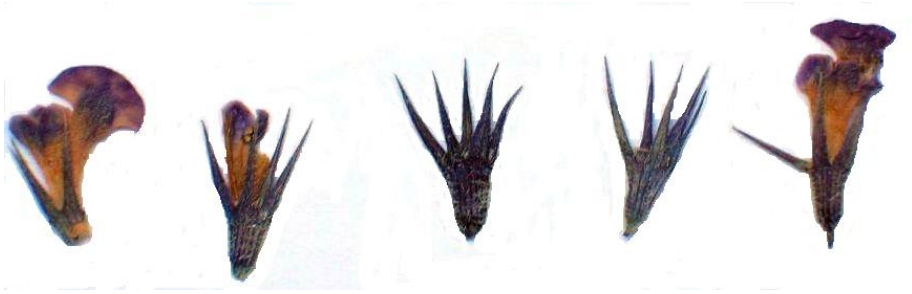


Fig. 5 - Morphology of flower in *N. ucranica* L. ssp. *ucranica* [9]

Regarding flower morphology in *Nepeta*, the anastomosis of vein in calyx represents an important feature in species delimitation (fig. 6). This aspect was noticed for the first time in *N. violacea* calyx [4] and has an important role in genera delimitation within *Lamiaceae* and between species.

The primary and secondary veins have different anastomosis patterns between the species. There are many similarities in *N. parviflora* and *N. ucranica* (*Oxynepeta)*, only the length of teeth differs. Veins anastomosis may be used like important taxonomic feature in species delimitation in *Nepeta* genus.

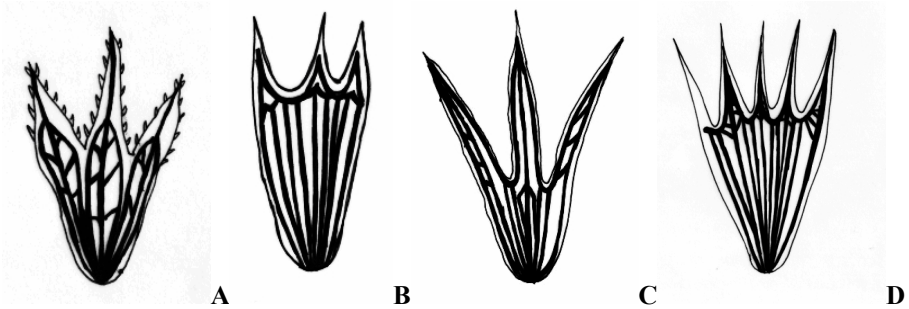


Fig. 6 - Calyx venation patterns in *Nepeta* L.: A - *N. cataria*; B - *N. nuda*; C - *N. parviflora*; D - *N. ucranica* [9]

Bracts morphology:

Aspects regarding bracts morphology were presented in tab. II. The bracts are herbaceous in *N. cataria* and *N. nuda* and weakly spiny at the top in sect. *Oxynepeta*; they have an important taxonomic role in species differentiation. The margins of upper floral bracts (bracteoles) are entire in all *Nepeta* species.

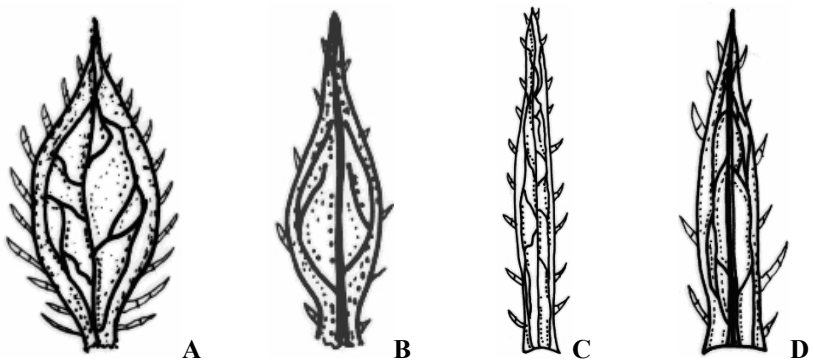


Fig. 7 - Morphology of bracts of *Nepeta*: A- *N. cataria*; B- *N. nuda*; C- *N. parviflora*; D- *N. ucranica* [9]

Anatomy of corolla, calyx and bracts:

The anatomical structure of corolla is very similar in all *Nepeta* species. The corolla with minutely spotted papillae and the trichomes types (non-glandular or protective and glandular ones or secretory trichomes- peltate) is shown in fig. 8 A, B for *N. parviflora*.

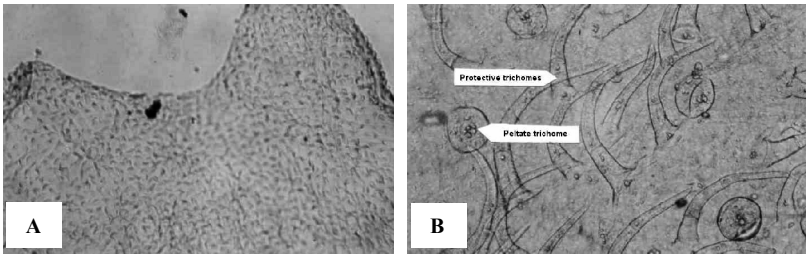


Fig. 8 - *N. parviflora*: corolla with minutely spotted papillae and trichomes types (orig.)

The anatomical structure of calyx, bracts (incl. bracteoles) are similar with leaf anatomy within *Nepeta*. The calyx in cross-section presents an undulate bifacial structure with palisade and spongy tissue. The primary calyx veins protrude at the abaxial epidermis; the prominence in cross-section are ± rounded (*N. cataria*), ± triangular in *N. nuda* and hemi-elliptical in *Oxynepeta* species, with long trichomes in *N. parviflora* (fig. 9 A-D).

The midvein is collateral closed with a mixt vascular bundle, sometimes encircled by a sclerenchyma cells ring. On outer epidermis of calyx may exist protective trichomes, long, lanate in *N. parviflora*, and short in another *Nepeta* species; glandular trichomes-peltate types are present in *N. cataria* and *N. ucranica*, and capitate type in *N. nuda*.

The bracts (generic name for bracts and floral leaves of inflorescence) in *Nepeta* are hypostomatic in *N. cataria* and amphistomatic in other species. There are many similarities with leaf anatomy regarding the stomata distribution on leaf epidermis in *Nepeta* genus [8]. Anatomically, the bracts present 1(2)-layered palisadic tissue and (4)5-layered spongy tissue. The epidermis is thin or weakly cutinized mostly on abaxial side of bracts.

Based on the characters of morphological and anatomical features of flower and inflorescence, an original identification keys are presented below [9].

1a Gynomonoecious species or plants with hermaphrodite flowers, axis of cymes shortly, erect inflorescence, bracteoles present ……..……. **2**

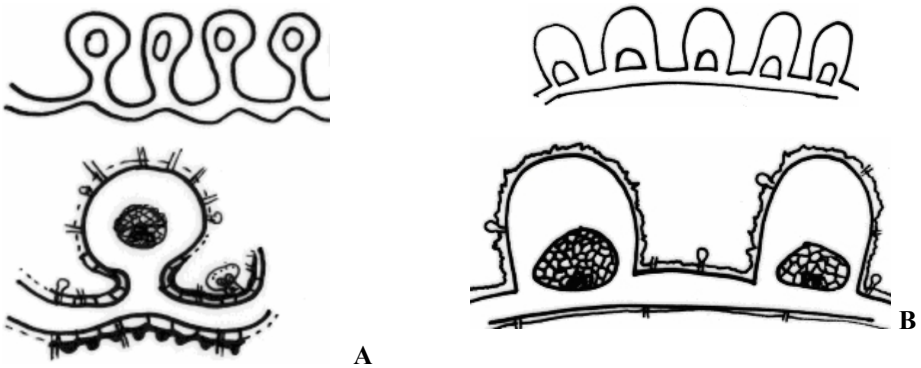
1b Gynoecious plants, axis of cymes longer, and few-flowered, 2-3 times dichotomic bifurcate, inflorescence patent, bracteoles may lack ……...…….... **3**

2a Hermaphrodite flowers with white corolla; calyx slightly oblique, 5-7,5 mm long; the middle lobe of lower corolla lip broadly-concave to trapezoidal, with dentate margin …….. *N. cataria*

2b Gynomonoecious plants (hermaphodite plus ♀), white-pink to pale violet flower, calyx (K) straight, 4-6 mm long, middle lobe of lower corolla lip shortly unguiculate, purple-spotted, margin crenate-denticulate ……..…….…… *N. nuda*

3a Flowers in ± compact cymes, corolla blue-azure shorter than calyx, 7,5-9 mm long; sessile bracteoles, linear-lanceolate, long-acuminate, entire; calyx teeth longer than throat (calyx tube) ……..……... *N. parviflora*

3b Flowers in lax cymes, corolla blue-violet, longer than K or equal, 9-12 mm; acute bracteoles, broadly-lanceolate, ± entire; K teeth shorter than throat ……... *N. ucranica*



A **B**

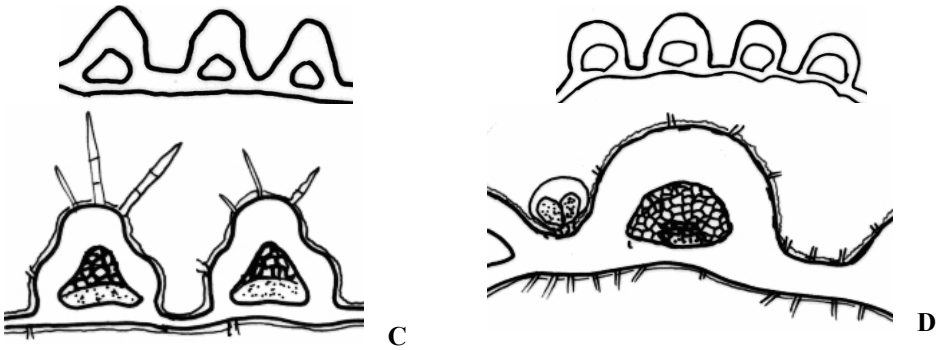


Fig. 9 - Cross-section in mature calyx of *Nepeta*: A- *N. cataria*; B- *N. nuda*; C- *N. parviflora*; D- *N. ucranica* [9]

Using the morpho-anatomical characters of bracts and bracteoles, a dichotomic key is presented [9].

1a Bracts in cross-section < 190 µm, colenchyma tissue lacks in abaxial part of median vein (MV), bracteoles ratio L/l less than 4:1 ……..…... **2**

1b Lamina bracts in cross-section > 190 µm, with colenchyma tissue abaxial (at MV level); bracteoles ratio L/l bigger than 7:1 ……... **3**

2a Hypostomatic bracts, mesophyl with 1-layered palisadic tissue (PT); spongy tissue (ST) 2-3-layered ……..... *N. cataria*

2b Amphistomatic bracts, mesophyl with 1(2)-layered palisadic tissue; spongy tissue 4-5-layered ……..……..…… *N. nuda*

3a PT/ST ratio 0,7:1; protective and secretory trichomes dense, abaxially; colenchyma tissue of MV < 60 µm …….....….... *N. parviflora*

3b PT/ST ratio 1,3:1; peltate trichomes numerous; colenchyma tissue of MV > 60 µm ……..... *N. ucranica*

Polytomic key based on morphological characters of flower and inflorescence in *Nepeta* genus [9]:

A Raceme with dense dichazial cymes arranged in glomerular inflorescence with ± sessile flowers

B Lax dichazial cymes, branches and cyme peduncles with different sizes ($\mathbf{B_S}$ lax cymes with *short* flower peduncules, inflorescence pyramidal shaped; $\mathbf{B_L}$ dichazial cymes few-flowered, 2(3)-dichotomic branched, patent, sometimes with a monochazium at the top of inflorescence, *long*-pedunculate; central flowers of cyme ± sessile)

C Bisexual flowers (hermaphrodite)

D Gynomonoecious plants (with hermaphrodite plus ♀ flowers)

E Gynoecious plants, one plant hermaphroditic (central flowers of cyme ♀, with staminodes, marginal flowers ♂, with rudimentary gynoceum), another plant with ♀ flowers

F Bracts 1-3 mm, linear-subulate

G Small bracts, ± obvious, shorter than calyx

H Bracts lanceolate, the lower one leaves-like

I Bracteoles subulate, not rigid, generally shorter than calyx

J Bracteoles 3-5 mm, linear to linear-lanceolate

K Bracteoles of lateral flowers of sessile cyme, narrow-lanceolate, entire; bracteoles of central flower lacks

L Bracteoles of lateral flowers of cymes 6-7 mm, linear, acute, shorter than calyx; bracteoles of central flower lacks

M Calyx cylindrical to ovoid, 5-6,5 mm long, often curved (patent), usually superior teeth longer than inferior ones

N Calyx straight, 4-6 mm long, narrow-tubular, to tubular-ovate at fructification, superior teeth are not longer than inferior teeth

O Calyx throat cylindrical, straight, with rare trichomes inside, at teeth base and between calyx teeth; superior teeth longer than inferior ones (**O_V** calyx 9-12 mm long, sometimes *violet* scented; **O_B** calyx 6-(7,5)8 mm long, sometimes *blue* scented)

P Calyx teeth linear-lanceolate, to 1,5-2,5 mm long, shorter than calyx throat

R Calyx teeth linear-lanceolate, to 1-2 mm long, green-violet, obviously shorter than calyx throat

S Calyx teeth acute, linear to linear-lanceolate, ± aristate, rigid (**S_L** teeth *longer* than calyx throat; **S_S** teeth *shorter* than calyx throat)

T Corolla white, slightly exserted, to 7-10 mm long, with middle lobe of lower corolla lip broad-concave to trapezoidal, white colored, with its margins evident dentate; anthers violet-blue

U Corolla violet-blue, with middle lobe of lower corolla lip with entire margins or crenate-dentate (**U_V** corolla pale-*violet* or pink, to 6(8)-10 mm long, median lobe short unguiculate, purplish scented, with crenate-dentate margin; anthers violet-blue; **U_{VA}** corolla blue-violet, . slightly exserted, to 9-12 mm long, median lobe reniform to hemi-circular, with entire margin; anthers violet; **U_{BA}** corolla blue-azure, equal with calyx, to 7,5-(8,5)9 mm long, median lobe hemi-circular, with crenate-dentate margin; anthers blue-azure)

A C F I M P T – *N. cataria* L.
B_S D G J N R U_V – *N. nuda* L.
B_L E H K O_B S_S U_{BA} – *N. parviflora* M. Bieb.
B_L E H L O_V S_L U_{VA} – *N. ucranica* L.

Conclusion

Numerous anatomical and morphological characters were found significant for an analysis of the relationship of *Nepeta* species. An original identification keys were developed based on used morpho-anatomical characteristics of *Nepeta* flower and inflorescence. The results concerning dichotomic and polytomic keys are original.

The characters recognized and used in the following taxonomic keys are summarized bellow. Only a part of them are used in elaboration of identification keys: **1.** the ratio of the length of corolla to the length of calyx throat; **2.** the ratio of the length of calyx to the length of calyx teeth; **3.** the color of corolla; **4.** the shape and margin of middle lobe of lower corolla lip; **5.** the plant type related with flower distribution and the reproductive structures; **6.** the absence of bracteole in central flowers of cymes; **7.** veins anastomisis pattern in calyx; **8.** calyx indumentum, etc.

Sometimes, the anatomical keys are more convincing than morphological ones, but it well known that they have low applicability in botany. The following anatomical characteristics of flowers and inflorescence can be used to differentiate *Nepeta* species successfully: **1.** type of lamina in bracts structure related with stomatal distribution; **2.** presence of colenchyma tissue in bracts; **3.** number of layers palisadic and spongy tissues in bracts structure; 4. the shape of the principal veins in calyx (in cross-section, fig. 9); **5.** type of trichomes in corolla epidermis, etc.

It is important to notice a similarity in leaf and bracts anatomical structures: the bracts and bracteoles are hypostomatic in *N. cataria* and amphistomatic in other species of *Nepeta*. The trichomes of bracts, calyx and corolla were studied for the first time.

In conclusion, the objective of this study was to evaluate the taxonomic value of flower and inflorescence characteristics in the Romanian *Nepeta* species, to see if they provide additional perspectives on the taxonomic problems elaborated upon above. We analyzed general flower and inflorescence morphology, anatomical characteristics of corolla, calyx and bracts. The final result was the elaboration of morpho-anatomical identification keys within *Nepeta*.

This study bring new information in the general knowledge on the morphology of flowers and inflorescence in *Nepeta* genus completing and providing new characteristics for a successfully separation between the species.

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