**Taiwania 66**(4): 541–556, **2021**
*DOI: 10.6165/tai.2021.66.541*

# Diversity patterns of life forms and phenolic profiles of endemic *Nepeta* plants along an aridity gradient of a high-mountain zone in Central Asia

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(Manuscript received 7 June 2021; Accepted 3 November 2021; Online published 12 November 2021)

ABSTRACT: For the first time, the diversity of life forms and phenolic profiles of five high-altitude species of *Nepeta* endemic to Central Asia were studied by cluster analysis and principal component analysis. We revealed effects of climatic factors on the average number of short modules and the average length of long modules making up the perennial axis of the plant as well as on concentrations of cinnamic and chlorogenic acids, cynaroside, isoquercitrin, and total phenolics. The significance of edaphic factors was also shown. The species inhabiting stony and movable substrates form mainly long modules (*N. densiflora*, *N. kokamirica*). Under conditions of local moistening, life form with maximum length of long modules develops (*N. transiliensis*), whereas grass-covered soils promote the formation of short modules (*N. mariae* and *N. pulchella*). The findings made it possible to reconstruct the course of evolutionary transformations of life forms for the studied *Nepeta* species. The phenolic profiles were species-specific, but their geographic variation does not match the geographic variation of morphological traits. The highest concentrations of isoquercitrin and total phenolic compounds in *N. densiflora* (12.0 and 56.7 mg·g$^{-1}$ dry weight) - the species presumably closest to the ancestral form - indicate a decrease in the quercetin level during aridization in the course of evolution. The phenolic profile of this species, also characterized by high concentrations of chlorogenic and rosmarinic acids and of luteolin glycosides, is described for the first time in the genus and means that *N. densiflora* is a unique resource for therapeutic applications.

KEY WORDS: Adaptation, climate aridization, edaphic factors, geographic variation, *Nepeta*, ontogenesis.

## INTRODUCTION

The study of high-mountain plants is one of high-priority fields in botany and ecology because hotspots of biodiversity are concentrated in these territories, as confirmed by the richness of endemic species (Kamelin, 1998; Noroozi, 2020). The mountains of Central Asia are a part of an extensive tectonic system of uplifts, in which considerable diversity of flora is observed (Kamelin, 1973; Agakhanjanz and Breckle, 2002). According to C. Körner (2007), altitude gradients can be regarded as some of the most powerful natural experiments testing ecological and evolutionary responses of a biota to geophysical influences. At present, investigators are paying more and more attention to the study of plant life forms in alpine zones (Arila and Gupta, 2016; Das *et al*., 2020). The spectra of life forms of vegetation in different regions, in particular, depending on the altitude gradient (Wang *et al*., 2002; Das *et al*., 2020), are actively analyzed. The research on the biological diversity of the Central Asian highlands is becoming increasingly relevant due to climate change and increased anthropogenic pressure (Khan *et al*., 2012).

The diversity of plant life forms is an indicator of species richness, adaptation mechanisms (Körner, 2016; Xu *et al*., 2018), and the process of evolution (Xu *et al*., 2019). Because most of the traits that determine the form of growth are genetically determined and are the result of an ordered and coordinated sequence of morphogenetic events during which the plant grows and fills available space (Bell, 1986), the life form can be considered a taxonomic character that is effective at the level of species and higher taxa (Serebryakov, 1962).

The genus *Nepeta* L. (Lamiaceae) includes over 360 species. Most of these are concentrated in the mountainous regions of Central Asia, the Iranian Highlands, the Caucasus, and the Mediterranean (Budantsev, 1993). On the basis of morphological and geographical analyses, A. L. Budantsev (1993) made the assumption that the genus *Nepeta* arose in East Asia during differentiation of subtropical/tropical flora into northern and southern flora at the end of the Paleogene. High-mountain orogeny has played an enormous role in the development of the life forms. High-altitude regions have a complex geomorphological nature and are characterized by a heterogeneous environment. Different exposure of mountain slopes along with a specific microclimate - combined with a variety of substrates and soils and the availability of nutrients and water - give rise to a variety of microsites and have led to the separation of many groups of flowering plants (Körner, 2016). Representatives of the genus *Nepeta* that form local populations in subalpine and alpine mountain belts were no exception.

Representatives of the section Spicatae (16 species) in Central Asia show substantial form diversity (Budantsev, 1993). Due to the inaccessibility of mountainous areas, this group of plants has hardly been studied, although many of them are essential-oil plants with good prospects as sources of biologically active compounds (Formisano

**Table 1**. Environmental conditions at the sites of sampling of the five ***Nepeta*** species.

| Species | Sampling site | Environmental conditions |
|---|---|---|
| *N. mariae* | Zeravshan ridge, mountain gorge of Voru river, Republic of Tajikistan, 39°13'31.1"N., 67°56'16.4"E, 3016 m a.s.l. | Xerophytic conditions, alpine belt, dense sceletonless soil, sparse steppe vegetation |
| *N. pulchella* | Talassian ridge, mountain gorge of Ulken Kaindy river, Republic of Kazakhstan, 42°23'45.0"N, 70°37'43.8"E, 2300 m a.s.l. | Mesophytic conditions, subalpine belt, grass-covered soil, meadow vegetation |
| *N. transiliensis* | Karach ridge, valley of Turgen river, Republic of Kazakhstan, 46°16'43.6"N, 77°52'08.1"E, 2700 m a.s.l. | Mesophytic conditions, subalpine belt, meadow grass–covered stony river bank |
| *N. kokamirica* | Dzungarian Alatau ridge, mountain gorge of Burkhan river, Republic of Kazakhstan, 44°31'34.8"N, 80°02'04.7"E, 2890 m a.s.l. | Cryomesophytic conditions, upper boundary of subalpine belt, movable rocky scree |
| *N. densiflora* | Narym ridge, upper reaches of Zhaydak river, Republic of Kazakhstan, 49°03'47.6"N, 84°56'12.7"E, 2041 m a.s.l. | Mesophytic conditions, subalpine meadows, stony bed of temporary watercourse |

*et al*., 2011). In the recognized plant life form systems, herbs are classified according to the location of renewal buds relative to the substrate level (Raunkier, 1934) and according to specific features of the location of the underground parts of the shoots that perform the renewal function (Serebryakov, 1962). Taproot and rhizomatous forms have a special place in such systems. Life forms of herbaceous plants remain insufficiently studied, especially regarding the identification of the main structural units that lead to the formation of the habitus of the whole plant (Savinykh and Cheryomushkina, 2015).

In the structural analysis of plants, much attention is given to above-ground structural elements (Barthélémy and Caraglio, 2007). Some well-elaborated principles of tree architecture are applicable to grasses (Serebryakova, 1977, Bell and Tomlinson, 1980; Barthélémy and Caraglio, 2007; Chomicki, 2013). Furthermore, if general design of woody and semiwoody forms is based on the preservation of perennial above-ground (visible) axes, then the general design and form of perennial-herbaceous-plant individuals directly depend on the morphology, development, and arrangement of underground axes. As rightly noted by G. Chomicki (2013), the research on the shoot's underground proximal part - which is important for rhizome construction - remains neglected. The preserved proximal part of the shoot is usually called a residue (Nukhimovsky, 1997) or a rhizome module (Chomicki, 2013). The architecture of rhizomatous plants has been investigated in sufficient detail (Bell and Tomlinson, 1980; Chomicki, 2013). Nonetheless, the mechanisms of transformation of life forms and the factors affecting these processes are still not fully elucidated (Savinykh and Cheryomushkina, 2015). It is known that climatic and edaphic conditions largely determine the branching pattern and development of life forms in plants (Serebryakov, 1962; Stecconi *et al*., 2010; Charles-Dominique *et al*., 2012; Bruy *et al*., 2018). It would be worthwhile to identify the role and contribution of individual environmental factors to changes in structural elements and habitus of plants in the genus *Nepeta* under specific growth conditions.

Plant metabolism provides an adaptation process with protective components, including polymers that determine the shape, size and mechanical stability of a plant. Metabolites of *Nepeta* species are at the center of attention of researchers all over the world owing to the wide spectrum of biological activities (Formisano *et al*., 2011; Astashenkov *et al*., 2019; Reichert *et al*., 2018; Sarikurkcu *et al*., 2019). Phenolic compounds are a large group of secondary metabolites directly involved in the adaptation mechanism (Sharma *et al*., 2019). Their high physiological activity in plants correlates with medicinal properties in the human body. Phenolic compounds contribute to antitussive, diuretic, anti-asthmatic, antiseptic, antispasmodic, and antipyretic effects of *Nepeta* species (Tepe *et al*., 2007). Some species are used to treat of contusions, rheumatic pains, fever, cutaneous eruptions (Miceli *et al*., 2005). Antimicrobial (including anti-Candida), antitumor, and anti-inflammatory properties of some *Nepeta* species have been documented (Modnicki *et al*., 2007; Iscan *et al*., 2011; Köksal *et al*., 2017).

The genus *Nepeta* is characterized by high abundance of hydroxycinnamic acids (caffeic and cinnamic acids and their derivatives). It has been reported that rosmarinic acid (a dimer of caffeic acid) is the dominant compound in many species of *Nepeta* (Aras *et al*., 2016; Köksal *et al*., 2017). Some species contain ferulic, p-coumaric, chlorogenic and quinic acids (Formisano *et al*., 2011; Mišić *et al*., 2015). Malic, aconitic, gallic, protocatechuic, and tannic acids, vanillin, hesperidin, hyperoside, p-hydroxybenzoic acid, salicylic acid, myricetin, fisetin, coumarin, quercetin, naringenin, naringin, hesperetin, luteolin, kaempferol, apigenin, rhamnetin, and chrysin were also identified (Emre *et al*., 2011; Aras *et al*., 2016). Flavones are considered the most frequently occuring flavonoids in the genus. Luteolin and apigenin have been identified in many *Nepeta* species, and the same is true for such flavonols as galangin, kaempferol and its derivatives, and quercetin and its derivatives (including rutin) (Miceli *et al*., 2005; Proestos *et al*., 2006; Mišić *et al*., 2015; Kashchenko and Olennikov, 2016).

Even though *Nepeta* phylogeny within Central Asia has been researched in sufficient detail (Budantsev, 1993), data on the life forms and phenolic compounds of these species are virtually absent. Our work is aimed at assessing the diversity of life forms and leaf phenolic profiles of five strictly endemic poorly studied *Nepeta* species of Central Asia that constitute the Densiflora series of the Spicatae section as well as at identifying the environmental factors that determine these characteristics.

## MATERIALS AND METHODS

### Plant material and growth conditions

Samples of plants in a mature generative state for morphological and biochemical analyses were collected in their habitats in two mountainous regions of Central Asia during the flowering period (June to July 2014–2017; Table 1). Geographic ranges of these species are located in high-altitude areas on the ridges of the Pamir-Alai, Tien Shan, and Altai (Fig. 1s).

*N. mariae* Regel is an alpine endemic distributed on ridges of the latitudinal direction in the north-western part of the Pamir-Alai and the western part of the Tien Shan. Under the conditions of the Pamir-Alai, it grows in the belt of juniper forests and steppes; in the Tien Shan, it occurs from the upper border of the forest belt to the alpine belt. *N. pulchella* Pojark. is an endemic species found only in the western part of the Talas Alatau (Tien Shan). Individuals of this species occur from the upper strip of the forest belt to the subalpine belt in meadow communities of the axial part of the ridge. *N. transiliensis* Pojark. is an alpine endemic of the Tien Shan. The species grows in sparse plant groups on rocky mountain slopes and occurs along river banks in meadow communities of the subalpine and alpine belts. *N. kokamirica* Regel is endemic to the Tien Shan; it grows in the Kulja region, in the south east of Kazakhstan, and in the Dzungarian Alatau. The species grows on stony and gravelly mountain slopes, often on mobile talus substrates. It is a part of vegetation in the upper zone of the forest belt and alpine belt. *N. densiflora* Kar. & Kir. is endemic to southern spurs of the Altai. It is characteristic of sparse plant groups of screes and stony mountain slopes. The species is confined to the mesophytic highlands of the alpine belt of mountains (Budantsev, 1993).

### Morphological analysis

Ontogenetic and comparative morphological methods were used that are based on studying life form during its development in the ontogenesis of individuals (Serebryakov, 1962; Serebryakova, 1980).

In the analysis of ontogenesis, the concept of its discrete description was applied (Gatsuk *et al*., 1980). Characteristics of life form are given in accordance with the ecological and morphological approach, based on studying structural elements of life form and their arrangement (Serebryakov, 1962). The analyzed *Nepeta* species are geophytes (Raunkier, 1934) forming two types of perennial shoot structures: the caudex and rhizome. On their basis, three life forms are generated: rhizomatous, taproot-caudex, and taproot-rhizomatous forms. By caudiciform plants we mean the ones in which an underground perennial organ is formed that always has a connection with the tap root (Nukhimovsky, 1997). Depending on the length of basal parts, the caudex can be either compact or diffuse (extensive caudex). By the rhizomatous life form we mean plants in which a perennial structure with adventitious roots is formed that gradually dies off from the proximal end and loses its connection with the tap root. Depending on the length of internodes, the rhizome can be either short or long (Serebryakov, 1962). We categorize the plants in which the tap root is preserved for a long time in ontogenesis - and a perennial structure with perennial adventitious roots is formed - as the intermediate rhizomatous-taproot form.

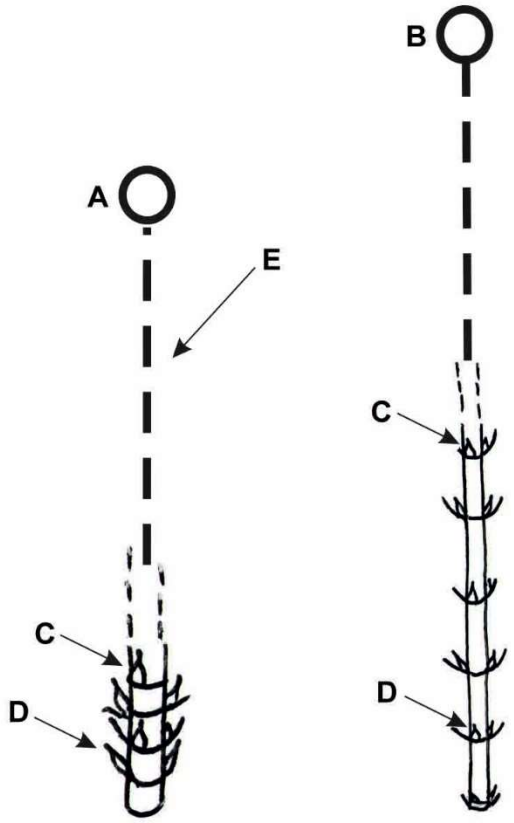


**Fig. 1**. Types of modules that constitute the perennial axis in representatives of ***Nepeta***. A. short module, consisting only of short metamers, B. long module, consisting of long metamers, C. renewal bud, D. dormant bud, E. the dead part of shoot.

We use the term module for a shoot's proximal underground part that constitutes the perennial underground structure of a herbaceous plant. This study covers long and short modules that make up the perennial axis (Fig. 1). By a short module we mean the one consisting only of short metamers, whereas a long module is formed by long metamers. In the long module, we deliberately excluded the first 1–2 short metamers with scale leaves because we believe that they have no impact on the structure and length of the module. We regard these metamers as a stage in the development of a long module.

We examined at least 10–15 individuals at each ontogenetic states for the description of plant ontogenesis. For the processing of morphological data, we evaluated 25 mature individuals with established life forms. The

analysis involved the following morphometric parameters: the number of short modules (n-Shm), the number of long modules (n-Lm) in the whole individual, the length of the short module (l-Shm), the length of the long module (l-Lm), the proportion of short modules in the overall structure of an individual (r)%, the length of short internodes (l-Shmet), the length of long internodes (l-Lmet), the number of short metamers in a module (n-Shmet), and the number of long metamers in a module (n-Lmet) per plant.

**HPLC analysis**

The leaves for chromatographic analysis were collected from 10 mature generative individuals of each species. Each sample combined 20 leaves gathered in the middle part of the shoots from a single individual. The leaves were healthy and free of necrosis or discoloration. For identification and quantification of phenolic compounds in the leaves of *Nepeta* species, air-dried herbarium samples were ground into a powder using a household mill. Precisely weighed samples of air-dried plant material (0.3 g) were exhaustively extracted with an ethanol: water mixture (70:30, v/v) in a water bath at 60–70°C. Dry-weight concentrations in the samples was calculated by the gravimetric method. Crude extract was filtered, diluted with the ethanol: water mixture (70:30, v/v) up to 25 mL volume in a graduated flask, and was subjected to the quantification of glycosides and free aglycons. A hydrolyzed extract was obtained by hydrolysis of the aqueous ethanol crude extract with 2N HCl for 2 h in a boiling water bath, followed by purification by means of a C16 Diapack cartridge and redissolution in ethanol. The hydrolyzed extract was utilized for aglycon quantification. Concentrations of individual compounds in the crude extract and aglycones in hydrolyzed extract were calculated and expressed in milligrams per gram of dry weight of the leaves.

The HPLC system for absolute quantification of phenolics consisted of an Agilent 1200 with a diode array detector (DAD) and the ChemStation software (Agilent Technologies, USA) for data processing. The chromatographic separation was conducted at 25°C on a Zorbax SB-C18 Column (4.6 x 150 mm, 5 mm i.d.) with the Agilent Guard Column Hardware Kit (p.n. 820888-901). The mobile phase consisted of MeOH (solvent A) and 0.1% orthophosphoric acid in water (solvent B). Separation of aglycones in hydrolyzed extracts with gradient 1 was described before (Chernonosov *et al*. 2017). Separation of glycosides and other derivatives of phenolic acids and flavonoids in crude extracts was performed with gradient 2 (Karpova *et al*. 2019). The flow rate was set to 1 $mL \cdot min^{-1}$. The sample injection volume was 10 µL, and the absorbance was measured at 210, 255, 270, 290, 325, 340, 360, and 370 nm.

The quantification of phenolic compounds was conducted by the external-standard method. Validation of the analytical procedures was performed in accordance with ICH guidelines (2005). Standard stock solutions were prepared at a concentration of 1 $mg.mL^{-1}$ in methanol. These were used for a serial dilution to construct calibration curves in a range 2–100 $\mu g.mL^{-1}$. Individual unidentified hydroxycinnamic acids were quantified as caffeic acid at 325 nm, flavonoid aglycones as luteolin at 350 nm, and flavonoid glycosides as cynaroside at 348 nm, with the corresponding compounds as external standards (Valkama *et al*., 2003).

All organic solvents were of analytical grade. Chemical reference standards of caffeic acid was purchased from Serva (Heidelberg, Germany). Ferulic acid, avicularin, hyperoside, and isoquercitrin were obtained from Fluka (Fluka, Sigma-Aldrich Chemie GmbH, Munich, Germany). Chlorogenic and rosmarinic acids, luteolin, vitexin, orientin, cynaroside, quercetin, kaempferol, rutin, and naringin were purchased from Sigma (St. Louis, MO, USA), and naringenin was acquired from LobaChemie (Fischamend, Austria).

Based on chromatographic data, values of paired affinity (PA) of native compounds in the extracts were calculated as follows (Baitha and Pandey, 2003):

PA = (Number of components common between species A and B/Total number of components in A and B)*100%.

***Data analysis***

All the data were processed in the Statistica 10.0 software (Statsoft Inc., Tulsa, OK, USA). The significance of difference between datasets was determined by one-way analysis of variance (ANOVA). The results of the morphological analysis were reported as mean ± standard errors (SE) of no less than 25 individual plants and were compared by Student's *t* test. Data from the quantification of phenolic compounds were expressed as mean ± SE of 15 (5 biological x 3 technical) replicates and were compared by Duncan's multiple range test. Differences between the means of any parameters were considered statistically significant at the 5% level ($p < 0.05$).

Similarity of morphological parameters or phenolic profiles among the species was determined by the cluster analysis. The morphological and biochemical parameters were processed separately. All characteristics of the profiles of aglycones and native compounds as well as the total concentration of phenolic compounds and percentage of a compound in total phenolics were employed in the cluster analysis.

To evaluate variations among the samples of the *Nepeta* species and to identify crucial climatic factors, principal components analysis (PCA) was performed. PCA was applied to climatic, morphological and biochemical parameters. Climatic factors for the data matrix were retrieved from WorldClim (Fick and Hijmans, 2017) and ENVIREM (Title and Bemmels, 2018) and were designated as follow: Bio1, average mean

**Table 2.** Morphometric parameters of the five ***Nepeta*** species.

| Species | n-Shm | n-Lm | l-Shm, cm | l-Lm, cm | r, % | n-Shmet | n-Lmet | l-Shmet, cm | l-Lmet, cm |
|---|---|---|---|---|---|---|---|---|---|
| *N. mariae* | 19.87 ± 2.05 | - | 1.13 ± 0.06 | - | 100 ± 0.00 | 3.0±1.1 | - | 0.2±0,01 | - |
| *N. kokamirica* | - | 8.14 ± 1.19 | - | 3.86 ± 0.28 | 0.00 ± 0.00 | - | 5.2±1.9 | - | 0.7±0.2 |
| *N. pulchella* | 13.10 ± 2.72 | 3.00 ± 0.79 | 0.82 ± 0.08 | 2.29 ± 0.44 | 83.46 ± 4.07 | 4.1±1.9 | 5.4±1.9 | 0.3±0.1 | 0.7±0.2 |
| *N. densiflora* | 2.22 ± 0.56 | 7.83 ± 1.02 | 0.46 ± 0.10 | 4.78 ± 0.32 | 21.61 ± 4.79 | 1.7±0.2 | 6.0±2.9 | 1.1±0.2 | 1.1±0.4 |
| *N. transiliensis* | 2.33 ± 1.31 | 9.60 ± 2.09 | 0.36 ± 0.16 | 5.85 ±0.54 | 12.95 ± 6.24 | 4.2±0.9 | 6.9±1.6 | 0.4±0.1 | 1.1±0.2 |

A dash indicates the absence of a module type.

temperature; Bio2, mean diurnal range = mean of monthly (max temp - min temp); Bio4, temperature seasonality (standard deviation ×100); Bio5, max temperature of warmest month; Bio6, min temperature of coldest month; Bio7, temperature annual range (Bio5–Bio6); Bio8, mean temperature of wettest quarter; Bio9, mean temperature of driest quarter; Bio10, mean temperature of warmest quarter; Bio11, mean temperature of coldest quarter; Bio12, annual precipitation; Bio13, precipitation of wettest month; Bio14, precipitation of driest month; and Arid, Aridity Index. We selected the climatic variables by following their importance for the development of high-mountainous perennial plants. The values for each site were determined at a very high spatial resolution of 30 seconds (~ 1 km$^2$).

The following biochemical parameters were analyzed: iQ, isoquercitrin; Cynar, cynaroside; Cin, cinnamic acid; Chl, chlorogenic acid; Ph, total phenolics.

## RESULTS

### Analysis of *Nepeta* life forms

The life forms of the studied species differed significantly. Individuals of *N. mariae* had a compact caudex life form with closely spaced flower-bearing shoots (Fig. 2s). Caudex structure consisted only of short modules formed by a small number of short metamers with short internodes (Table 2). During the entire ontogenesis, orthotropic monocyclic shoots are sequentially formed in *N. mariae* individuals. After vegetation, the entire above-ground photosynthesizing part of each shoot dies off down to the underground proximal part, which bears scale-like leaves with buds. Sympodial branching of closely located modules leads to the formation of a compact short branched axis (Fig. 2s). The tap root is preserved during the entire ontogenesis and takes part in the immersion of caudex in sceletonless soil.

*N. kokamirica* also has a caudex form. Nevertheless, during growth on a scree, the perennial axes of *N. kokamirica* get distributed in a fanlike manner, thereby giving rise to a spacious (diffuse) caudex, spread over the slope and consisting only of long modules (Table 2). Substrate mobility causes deep burial of the above-ground part of monocyclic shoots. The substrate-covered orthotropic part is composed of long metamers with etiolated and green leaves. After the substrate-free above-ground part of the shoots dies off, long underground modules are produced (Fig. 3s). From the renewal buds located in the upper part of the module, new monocyclic shoots successively develop, which are also covered with the substrate. During ontogenesis, with increasing plant age, the caudex grows and becomes multi-headed. Separate perennial branched underground systems become isolated, and ramets come into being, which have a connection with the seed (parent) individual and the tap root. Subsequently, complete longitudinal particulation of the individual takes place with the formation of unrejuvenated ramets. The ramets that separated exist for some time and then die off.

*N. pulchella* has a short-rhizomatous-taproot life form. The rhizome is composed mainly of short modules, the tap root persists for a long time and completely dies off at the end of the old generative state. Note that 93% of modules in the rhizome are short (Table 2). Different modules in the *N. pulchella* rhizome are generated by different shoots at different time points in ontogenesis. At the beginning of ontogenesis of individuals, the rhizome is erected by short modules, then long modules participate in the rhizome construction (Fig. 4s). Short modules arise as a result of the death of orthotropic shoots' above-ground part. These shoots consistently develop from buds of regular renewal. Long modules are produced by shoots that have developed from dormant buds located deeper in the soil layer. This position of the buds leads to lengthening of the proximal part of the shoot and to an increase in the number of long metamers. Then, the long module at the top branches out. In this way, orthotropic shoots with a short basal part develop from the buds. Over the course of several years, only orthotropic shoots with a short underground part unfold sequentially, which build on the rhizome. Ramets are formed on the basis of a long module. The outgrowth of underground structures from the center as a consequence of the appearance of long modules leads to parent bush loosening. At the base of the ramet, adventitious roots appear, some of which become perennial. The number of ramets associated with the parent individual can reach 10–15. Later, as a result of decomposition of one of the long modules, the individual undergoes particulation. The ramets that separated from the seed plant continue their individual ontogenesis.

Individuals of *N. densiflora* form long-rhizomatous-taproot extensive life form. The plant sequentially forms monocyclic shoots. The tap root persists for a long time

and dies off only by the end of the generative period. Both long and short modules take part in the construction of rhizome structure. The proportion of long modules is 78% (Table 2). In contrast to the above-mentioned species, in *N. densiflora* individuals, already at initial ontogenesis stages, the rhizome branches in dichasial mode (Fig. 5s). Growing among stones, monocyclic renewal shoots have long geophilic parts that erect a branched sympodial axis of the rhizome. Long parts of the rhizome produce modules from shoots that have arisen both from buds of regular renewal and from dormant buds. Short parts of the rhizome are formed {1} from shoots that have arisen in early spring and are dying off quickly (Fig. 5s A); {2} from sylleptic lateral shoots of the branching (Fig. 5s B); and {3} at the completion stage of the construction of the perennial rhizome axis (such modules determine the growth of the axis as a whole; Fig. 5s C).

On the basis of long modules, ramets with a short rhizome section come into being. Decay of long rhizome modules causes separation of ramets. Ramets undergo their ontogenesis and also disintegrate over time. This situation leads to the second senile particulation. This process is accompanied by the formation of branching old ramets of the next generation, incapable of a long independent life.

The life form of *N. transiliensis* individuals can be described as a long-rhizomatous form. This life form differs from that of *N. densiflora* by rhizome construction and by the death of the tap root at initial ontogenesis stages. The proportion of long modules in this form is 80% (Table 2). The long rhizome of *N. transiliensis* also results from sympodial branching of long and short modules. Increased local moistening causes the activation of dormant buds, whereas the matted and stony substrate leads to the elongation of the growing shoots' geophilic part. Shoots arising from dormant buds can be mono-, di-, tri-, or polycyclic (Fig. 6s). Shoot cyclicism depends on the depth of the dormant buds in the soil. The deeper the dormant bud is located, the greater is shoot cyclicism. It is worth noting that due to the deep location of dormant buds, the photosynthetic part of the shoots forms rarely. The apical bud of such shoots usually dies off, while the shoot itself persists and becomes a long module of the rhizome. The longest parts of the rhizome arise from dormant buds located at 10–12 cm depth as a consequence of monopodial growth of the shoot whose development has lasted more than 2–3 years. In this case, the module can reach a length of more than 20 cm. Furthermore, the branching of long rhizome modules can proceed from any bud of a long metamer. The short modules are generated just as they do in *N. densiflora* individuals. In its adult state, the plant is a clone consisting of rejuvenated and unrejuvenated ramets. The rejuvenated ramets go through their own ontogenesis and are also capable of the second particulation. The unrejuvenated ramets age and die.

According to the number and length of the modules leading to the construction of the life form, these five species separated into two clusters (Fig. 2). The first cluster is composed of only *N. transiliensis*, a species growing under mesophytic conditions on a matted stony substrate under increased local moistening. This species differs from the others by the sprawled form, long rhizome consisting mostly of long modules of maximum length generated by dormant buds, and by tap root death at the initial ontogenesis stages. The second cluster contains two subclusters. The first subcluster consists of species that retain the tap root and whose perennial structure is built mainly from long modules (*N. densiflora* and *N. kokamirica*). The second subcluster is composed of the species *N. pulchella* and *N. mariae*. The perennial foundation of these species is formed mostly by short modules (*N. pulchella*) or only by short modules (*N. mariae*). Their difference is tap root death in the middle of ontogenesis under mesophytic conditions in *N. pulchella* individuals and the appearance of a long module in a short rhizome as a consequence of dormant buds' functioning.

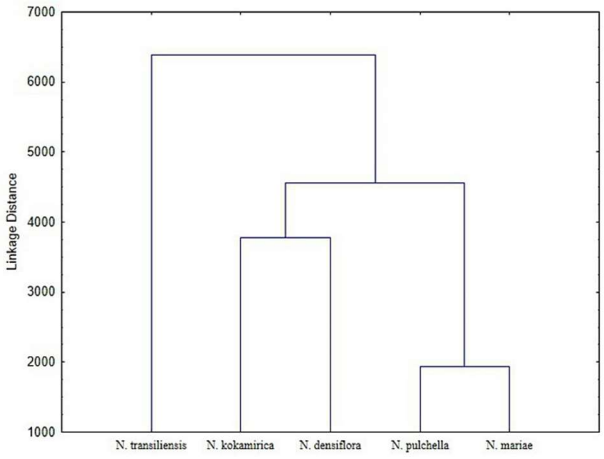


**Fig. 2**. A dendrogram of similarity of morphometric parameters of the five ***Nepeta*** species.

## The profiling of phenolic compounds in the leaves of *Nepeta* species

Hydroxycinnamic acids, flavones, flavanones and flavonols were found among the phenolic compounds in the leaves of *Nepeta* species, (Tables 3 and 1s, Fig. 7s). The maxima of UV spectra of compounds **9**, **22**, **24,** and **26** at 253 nm and 348–350 nm as well as the shoulder at 267 nm identified them as flavones (Fig. 8s), and the range of retention time of these compounds (between the retention time of phenolic acids and flavonoid aglycones) indicated that they were flavone O-glycosides. Naringin (**15**) was abundant in the leaves of *N. pulchella* and *N. densiflora*. Cynaroside (**14**) predominated in the leaves of *N. kokamirica* and *N. transiliensis*. A characteristic feature of *N. mariae* was naringin and cynaroside peaks

**Table 3.** Concentrations of major phenolic compounds in the leaves of the five ***Nepeta*** species.

| ID | T r* | Compound | Concentration, mg·g$^{-1}$ of dry weight | | | | |
|---|---|---|---|---|---|---|---|
| | | | *N. kokamirica* | *N. mariae* | *N. pulchella* | *N. transiliensis* | *N. densiflora* |
| 2 | 1.8 | Hydroxycinnamic acid | 2.1 ± 0.3b | 1.7 ± 0.3b | 1.1 ± 0.1a | 0.9 ± 0.1a | 4.8 ± 0.5c |
| 3 | 2.2 | Hydroxycinnamic acid | 1.9 ± 0.1c | 1.6 ± 0.3b | 1.3 ± 0.1b | 0.2 ± 0.0a | 4.8 ± 0.4d |
| 4 | 2.7 | Hydroxycinnamic acid | 0.7 ± 0.1a | 2.3 ± 0.4b | Nd | 0.8 ± 0.0a | Nd |
| 5 | 3.2 | Chlorogenic acid | 7.1 ± 0.9d | 3.8 ± 0.5b | 5.2 ± 0.4c | 0.7 ± 0.0a | 7.9 ± 0.9d |
| 8 | 7.2 | Hydroxycinnamic acid | 2.2 ± 0.3c | 0.6 ± 0.0b | 0.1 ± 0.0a | 0.5 ± 0.0b | 0.6 ± 0.0b |
| 10 | 7.9 | Orientin | 0.3 ± 0.0a | 3.0 ± 0.4b | 0.2 ± 0.0a | 0.3 ± 0.0a | Nd |
| 11 | 9.6 | Ferulic acid | 2.8 ± 0.3e | 0.6 ± 0.0c | 0.1 ± 0.0a | 0.4 ± 0.0b | 1.6 ± 0.1d |
| 13 | 12.0 | Vitexin | 0.4 ± 0.0a | 0.6 ± 0.0b | 0.5 ± 0.0b | 0.6 ± 0.0b | 0.6 ± 0.0b |
| 14 | 16.5 | Cynaroside | 3.7 ± 0.3c | 4.2 ± 0.5c | 0.3 ± 0.0a | 7.7 ± 0.8d | 1.3 ± 0.1b |
| 15 | 17.6 | Naringin | 0.8 ± 0.1a | 4.5 ± 0.5b | 12.3 ± 1.3d | Nd | 5.9 ± 0.6c |
| 17 | 19.4 | Isoquercitrin | 0.4 ± 0.0a | 0.6 ± 0.0a | Nd | 0.6 ± 0.1a | 12.0 ± 1.5b |
| 18 | 20.6 | Rutin | Nd | Nd | Nd | Nd | 2.2 ± 0.3a |
| 20 | 28.6 | Avicularin | 1.4 ± 0.1b | 0.3 ± 0.0a | Nd | 2.0 ± 0.1c | Nd |
| 21 | 30.2 | Hydroxycinnamic acid | 1.1 ± 0.2b | 2.4 ± 0.4c | 1.6 ± 0.1c | 0.8 ± 0.0a | 1.8 ± 0.2c |
| 23 | 35.7 | Cinnamic acid | 0.5 ± 0.0a | 0.4 ± 0.0a | 4.2 ± 0.3b | 0.3 ± 0.0a | Nd |
| 24 | 37.0 | Flavone glycoside | 1.5 ± 0.1b | 2.6 ± 0.3c | 0.3 ± 0.0a | 1.6 ± 0.2b | 2.3 ± 0.2c |
| Sum of phenolics **1**-**30** | | | 34.3 ± 3.2b | 37.0 ± 4.2b | 34.5 ± 2.9b | 28.3 ± 2.4a | 56.7 ± 6.1c |
| Percentage of total phenolic compounds | | | 89.2 ± 7.4a | 84.2 ± 6.9a | 77.8 ± 8.1a | 91.6 ± 9.8b | 98.5 ± 9.6c |

"*" Separation in gradient 2; Means in columns followed by the same letter do not differ significantly according to Duncan's test ($p < 0.05$).

**Table 4.** Concentrations of major phenolic aglycones in the leaves of the five ***Nepeta*** species.

| ID | T r* | Compound | Concentration, mg·g$^{-1}$ of dry weight | | | | |
|---|---|---|---|---|---|---|---|
| | | | *N. kokamirica* | *N. mariae* | *N. pulchella* | *N. transiliensis* | *N. densiflora* |
| 13 | 2.5 | Vitexin | 4.3 ± 0.4c | 4.8 ± 0.5d | 3.6 ± 0.4b | 2.8 ± 0.2a | 8.4 ± 0.6e |
| 31 | 2.7 | Flavone | 2.9 ± 0.2a | 7.9 ± 0.9c | 2.8 ± 0.2a | 3.9 ± 0.4b | 12.6 ± 1.5d |
| 32 | 3.1 | Rosmarinic acid | 2.4 ± 0.2c | 0.7 ± 0.0ab | 0.6 ± 0.0a | 0.8 ± 0.0b | 2.5 ± 0.2c |
| 34 | 3.9 | Flavone | 1.0 ± 0.1a | 2.1 ± 0.4c | 1.9 ± 0.1c | 1.1 ± 0.2a | 1.5 ± 0.1b |
| 37 | 6.6 | Quercetin | 0.2 ± 0.0c | 0.02 ± 0.00a | 0.05 ± 0.00b | 0.05 ± 0.00b | 1.6 ± 0.1d |
| 38 | 7.6 | Naringenin | 0.1 ± 0.0a | 0.6 ± 0.0c | 0.9 ± 0.1d | 0.3 ± 0.0b | 1.3 ± 0.1e |
| 29 | 8.4 | Luteolin | 0.4 ± 0.0a | 0.6 ± 0.0b | 0.4 ± 0.0a | 1.0 ± 0.1c | 0.4 ± 0.0a |
| The sum of 15 aglycones | | | 15.8 ± 1.2b | 18.3 ± 1.5c | 11.9 ± 0.6a | 12.6 ± 1.0a | 33.5 ± 3.2d |
| Percentage of total phenolic compounds | | | 87.5 ± 8.3a | 96.3 ± 9.9b | 94.8 ± 9.2ab | 93.7 ± 7.7ab | 93.4 ± 8.5ab |

"*" Separation in gradient 1.

of approximately equal areas.

Chlorogenic acid (**5**) was the third major phenolic compound well represented in the leaves of all the species in question except *N. transiliensis*. Other major phenolics differed from species to species. Ferulic acid (**11**) and hydroxycinnamic acid **8** were found in the leaves of *N. kokamirica* in substantial amounts. *N. mariae* was distinguished by a high concentration of orientin (**10**) and compounds **4**, **21** and **24**, whereas *N. pulchella* featured a large amount of cinnamic acid (**23**). Quercetin 3-α-L-arabinofuranoside (avicularin; **20**) was among the dominant phenolic compounds of *N. transiliensis*. *N. densiflora* differed from the other species by large amounts of isoquercitrin (**17**) and hydroxycinnamic acids **2** and **3**. The highest total concentration of phenolic compounds (57.2 mg·g $^{-1}$) was detected in the leaves of this species. *N. kokamirica*, *N. mariae* and *N. pulchella* did not differ significantly differences in this parameter (38–44 mg·g $^{-1}$), while *N. transiliensis* showed the lowest concentration (30.2 mg·g $^{-1}$).

Aglycone patterns of hydrolyzed aqueous ethanol extracts of the species, just as the extracts of native compounds, were characterized by abundant hydroxycinnamic acids and flavones (Tables 4 and 2s). Flavones were represented by vitexin (**13**), unidentified flavones **31** and **34**, luteolin (**29**), and apigenin (**40**), while flavonols by quercetin (**37**), and flavanones by naringenin (**38**). The pattern of aglycones retained the species specificity seen in the profile of native compounds.

Rosmarinic acid (**32**) was found in considerable quantities in the leaves of *N. kokamirica* and *N. densiflora*. The detection of rosmarinic acid only in the hydrolyzed extracts means that this compound is present in the leaves predominantly in the form of glycosides.

After hydrolysis, the level of vitexin (**13**) also significantly increased, indicating the prevalence of this phenolic compound's glycosylated form in the leaves. Along with flavone **31**, vitexin (**13)** was abundant in all the species under study. These phenolics, along with chlorogenic and rosmarinic acids, were dominant in the

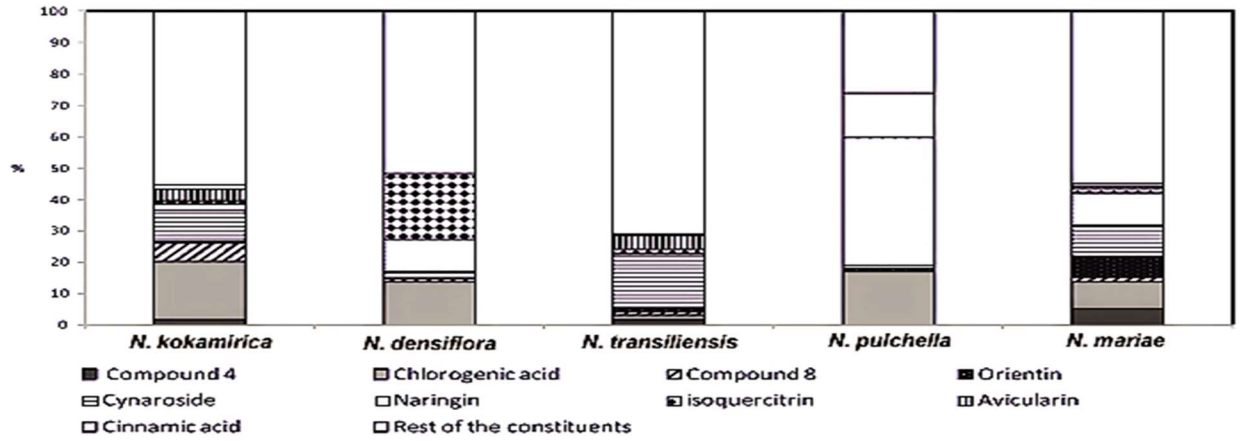


**Fig. 3**. Percentage of each major phenolic compounds in total phenolics in the leaves of the five ***Nepeta*** species.

**Table 5.** Paired affinity among the five ***Nepeta*** species, %.

| Species | *N. kokamirica* | *N. mariae* | *N. pulchella* | *N. transiliensis* | *N. densiflora* |
|---|---|---|---|---|---|
| *N. kokamirica* | 100 | | | | |
| *N. mariae* | 93.33 | 100 | | | |
| *N. pulchella* | 80.00 | 86.67 | 100 | | |
| *N. transiliensis* | 86.67 | 90.00 | 76.67 | 100 | |
| *N. densiflora* | 66.67 | 66.67 | 66.67 | 63.33 | 100 |

**Table 6.** Eigenvalues and cumulative contribution rates of principal components.

| Principal Components | Eigenvalues | Cumulative Eigenvalues | Contribution Rates (%) | Cumulative Contribution Rates (%) |
|---|---|---|---|---|
| PC1 | 10.36 | 10.37 | 45.07 | 45.07 |
| PC2 | 6.07 | 16.43 | 26.38 | 71.44 |
| PC3 | 3.23 | 19.67 | 14.06 | 85.50 |
| PC4 | 1.70 | 21.36 | 7.37 | 92.88 |

leaves of *N. kokamirica* and *N. densiflora*. The leaves of the latter species were also rich in quercetin. The high concentration of quercetin glycosydes was a distinctive feature of this species. The leaves of *N. mariae* and *N. pulchella* showed a substantial level of flavone **34**. *N. transiliensis* leaves contained a somewhat higher amount of luteolin (**29**) as compared to the other four species. Considerable amounts of naringenin (**27**) and luteolin (**29**) were an essential feature of *N. mariae*, *N. pulchella* and *N. densiflora*. Luteolin made a major contribution to the aglycone content of *N. transiliensis*.

Thus, the profiles of native and hydrolyzed leaf extracts of the five species revealed their major phenolic compounds to be chlorogenic acid, glycosides of vitexin, flavone **31**, and rosmarinic acid, along with cynaroside and naringin**.** HPLC data indicated that the phenolic profiles are highly similar among the analyzed species, but each species has its own specific features. Percentage of each major compounds in total phenolics varied substantially among the five species (Fig. 3).

A high concentration of chlorogenic acid unites all the species, except for *N. transiliensis*, which has another distinctive feature: a high concentration of avicularin. This trait, along with a high level of cynaroside, associates this species with *N. kokamirica*. The phenolic profile of *N. mariae* contains three major phenolics: chlorogenic acid, cymaroside, and naringin. In *N. pulchella* leaves, the latter compound had the highest concentration, while cinnamic acid was present in a considerable quantity. The distinct trait of *N. densiflora* is a high isoquercitrin concentration. This species is similar to *N. kokamirica* because of substantial levels of rosmarinic acid glycosides (Table 4), and to *N. pulchella* and *N. mariae* owing to a large amount of naringin (Table 3).

Cluster analysis of the phenolic profiles revealed *N. densiflora* separation from the other species, which did not form a single cluster (Fig. 4). The highest similarity was found between *N. mariae* and *N. kokamirica*. These species had the highest paired affinity in qualitative composition (93.33%) as calculated from the presence/ absence of phenolic compounds (Methods and Table 5). The lowest paired affinity was detected between *N. densiflora* and *N. transiliensis* (63.33%).

**Principal Component Analysis**

The results of this analysis indicated that the cumulative contribution rate of the eigenvalues of the first four principal components was 92.87%, and the individual

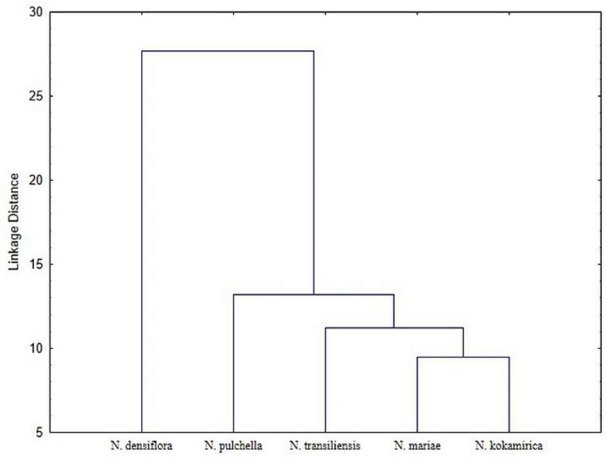


**Fig. 4.** A dendrogram of similarity of phenolic profiles of the five ***Nepeta*** species.

eigenvalues were greater than 1.0 (Table 6). Thus, these principal components can be analyzed to determine the load level of each factor and to build a matrix of components (Table 7).

The factors influencing the first principal component were Bio 6 (0.988), Bio 11 (0.960), Arid (0.951), Bio 9 (0.915), Bio 4 (-0.840), n-Shm (0.790), and l-Lm (-0.732) (Table 7, Fig. 5). The second principal component was affected by Bio 13 (0.924), Bio5 (0.899), and Cin (0.782). The third principal component were described by Cynar (-0.904), Ph (0.843), Chl (0.696), and iQ (0.731), whereas the fourth component by Bio14 (0.776).

Therefore, the morphometric parameters highly correlated with the first principal component, where the correlations with the number of short modules was positive, and the correlation with the length of long modules was negative. Phenolic compounds correlated with PC2 (cinnamic acid) and PC3 (cynaroside, total phenolics, chlorogenic acid, and isoquercitrin). Most of these correlations were positive and strong except for the correlation PC3 with cynaroside, which was negative and very strong (-0.904). The results suggested that the diversity of the samples is mainly determined by the variance of the number of short modules, variance of the length of long modules, and variance of concentrations of cinnamic acid, cynaroside, and total phenolics. The first two parameters correlated with PC1, which explained 45.07% of the variance. The rest of the parameters positively correlated with PC2 and PC3, whose cumulative contribution rate was 85.50%.

PCA revealed relations among the tested samples (Fig. 6). Samples of all the species grouped into separate clusters. The location of *N. pulchella* in the scatterplot of PC2 against PC1 (Fig. 6A) described this species as growing under the conditions of increased temperature and precipitation and as rich in cinnamic acid, unlike the other four species. On the other hand, *N. pulchella*, and *N. mariae* were characterized by high-temperature and an arid conditions and possessed a considerable number of

**Table 7.** Principal-component correlation matrix.

| Parameters | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Bio1* | 0.833 | 0.521 | -0.108 | 0.141 |
| Bio2 | -0.496 | 0.798 | -0.287 | 0.135 |
| Bio4 | -0.840 | 0.377 | 0.383 | -0.038 |
| Bio5 | 0.413 | 0.899 | 0.087 | 0.109 |
| Bio6 | 0.988 | -0.017 | 0.118 | -0.020 |
| Bio7 | -0.684 | 0.713 | -0.050 | 0.098 |
| Bio8 | -0.897 | 0.372 | -0.181 | 0.099 |
| Bio9 | 0.915 | 0.345 | 0.193 | -0.002 |
| Bio10 | 0.663 | 0.712 | 0.167 | 0.152 |
| Bio11 | 0.960 | 0.235 | -0.101 | 0.093 |
| Bio12 | 0.418 | 0.839 | 0.030 | -0.338 |
| Bio13 | -0.350 | 0.924 | 0.025 | -0.128 |
| Bio14 | 0.260 | -0.469 | -0.278 | 0.776 |
| Arid | 0.950 | -0.189 | 0.072 | -0.209 |
| l-Shm | 0.640 | -0.246 | 0.290 | -0.376 |
| l-Lm | -0.732 | 0.305 | -0.200 | -0.317 |
| n-Shm | 0.790 | -0.247 | 0.150 | -0.180 |
| n-Lm | -0.558 | 0.188 | -0.212 | -0.149 |
| iQ | -0.570 | -0.210 | 0.731 | -0.280 |
| Cynar | -0.045 | -0.294 | -0.904 | -0.276 |
| Cin | 0.589 | 0.782 | 0.191 | 0.031 |
| Chl | -0.369 | 0.024 | 0.696 | 0.590 |
| Ph | -0.377 | -0.271 | 0.843 | -0.127 |

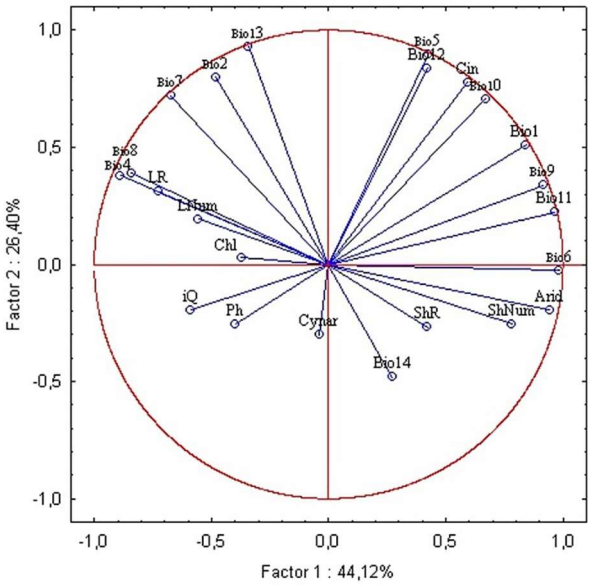


**Fig. 5**. Projections of the variables on the factor-plane of principal component analysis of climatic parameters and plant characteristics across 125 samples of the five Nepeta species.

short modules. *N. densiflora* was located in an area of increased seasonality of temperature (Fig. 6A). In the score plot of PC2 *versus* PC3, *N. densiflora* and *N. transiliensis* occupied opposite positions relative to the PC3 axis. The former was distinguished by the highest levels of isoquercitrin, total phenolics, and chlorogenic acid, whereas the latter by the highest concentration of cynaroside (Fig. 6B).

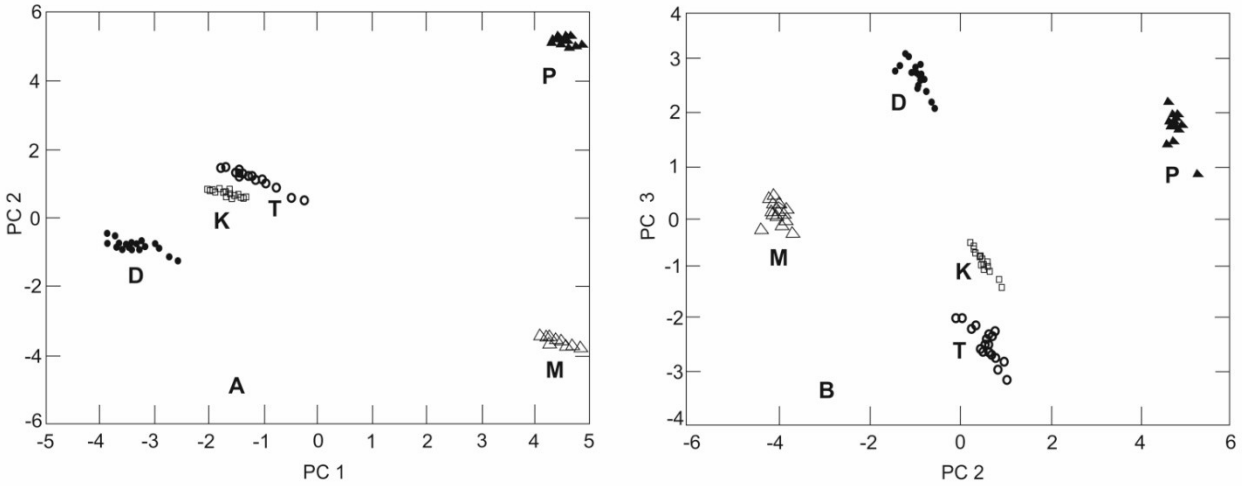


**Fig. 6**. Principal component analysis score plots of PC1 versus PC2 (**A**) and PC2 versus PC3 (**B**) from a PCA on the 23 variable matrix for the five Nepeta species (M: ***Nepeta mariae***, P: ***N. pulchella***, D: ***N. densiflora***, T: ***N. transiliensis***, K: ***N. kokamirica***). The first three components accounted for 45.07, 26.38, and 14.06% of the observed variance between individuals, respectively.

## DISCUSSION

It is known that the habitus of plants of the same species can change under different environmental conditions (Serebryakov, 1962; Khokhryakov, 1981; Barthélémy and Caraglio, 2007). The literature convincingly shows rearrangements of life forms of various plants depending on illumination (Charles-Dominique *et al*., 2012), soil composition, and substrate mobility (Talovskaya (Kolegova), 2015), height and steepness of the slope (Stecconi *et al*., 2010), features of the phytocoenotic environment (Cheryomushkina and Guseva, 2015), and temperature fluctuations (Norton and Schönenberg, 1984). Shading, substrate mobility, and excessive moistening were determined to contribute to shoot lodging and to the formation of elongated. These data are consistent with the formation of long modules and long rhizomes that we noted in *N. transiliensis* under conditions of increased moistening as well as in *N. kokamirica* and *N. densiflora* with high substrate mobility. According to the belt-zonal profile of the study areas, the habitats of the five studied *Nepeta* species [from 2300 m a.s.l. (Kazakhstan) to 3016 m a.s.l. (Tajikistan)] belong to the same alpine belt. All the analyzed species belong to the group of geophytes (cryptophytes) (Raunkier, 1934) that is dominant in the high-altitude zone of Central Asia (Klimeš, 2003; Arila and Gupta, 2016). In this context, the gradient of climate aridity in the meridional direction as well as different substrate mobility and turfness cause a rearrangement of module structure and of the life form of *Nepeta* overall.

The involvement of modules arising from dormant buds in rhizome structure has been revealed in some tropical plants (Bell and Tomlinson, 1980; Chomicki, 2013). This has also been noted in all the high-altitude *Nepeta* studies to date (Astashenkov and Cheryomushkina, 2019). Due to the functioning of the dormant buds, a sympodial axis is built, which, depending on climatic and edaphic circumstances, can be either short or long. Such patterns require further discussion and are of fundamental importance for understanding the interrelationships of forms and plant biology as a whole.

It should be pointed out that for the alpine *Nepeta* species, the long-module formation from a dormant bud and this module's necrosis are prerequisites for clonal reproduction. The abundance of clonal plants is promoted by high humidity, low temperatures, and a nutrient-poor soil (Lyubarsky, 1967; Klimeš, 2003). For the *Nepeta* species characterized here, with an increase in humidity and in temperature, there is a tendency toward stronger natural particulation in the series "*N. mariae* → *N. kokamirica* → *N. pulchella* → *N. densiflora*" until the appearance of rejuvenated ramets (*N. transiliensis*) capable of independent living.

The evolution of rhizome structure has been poorly investigated (Chomicki, 2013); however, our study fills this gap to some extent. According to A. Budantsev (1993), the evolution of life forms of perennial herbaceous representatives of *Nepeta* can be described as a series: taproot form → long-rhizomatous form → short-rhizomatous form. By contrast, our work suggests that the evolution of life forms of this genus could have gone in different directions. The ancestral morph of *Nepeta* presumably had a mesophilic appearance with a tap root (Budantsev, 1993). The results of the cluster analysis and the PCA imply that the hypothetical radiation of life forms from the ancestral morph (x-morph) proceeded along the following series: 1) x-morph → long-rhizomatous-taproot form → short-rhizomatous-taproot form → compact-caudex form; 2) x-morph → long-rhizomatous-taproot form → long-rhizomatous form; 3) x-morph → long-rhizomatous-taproot form → long-rhizomatous form → spacious-caudex form.

Our findings indicate that climate aridization and the

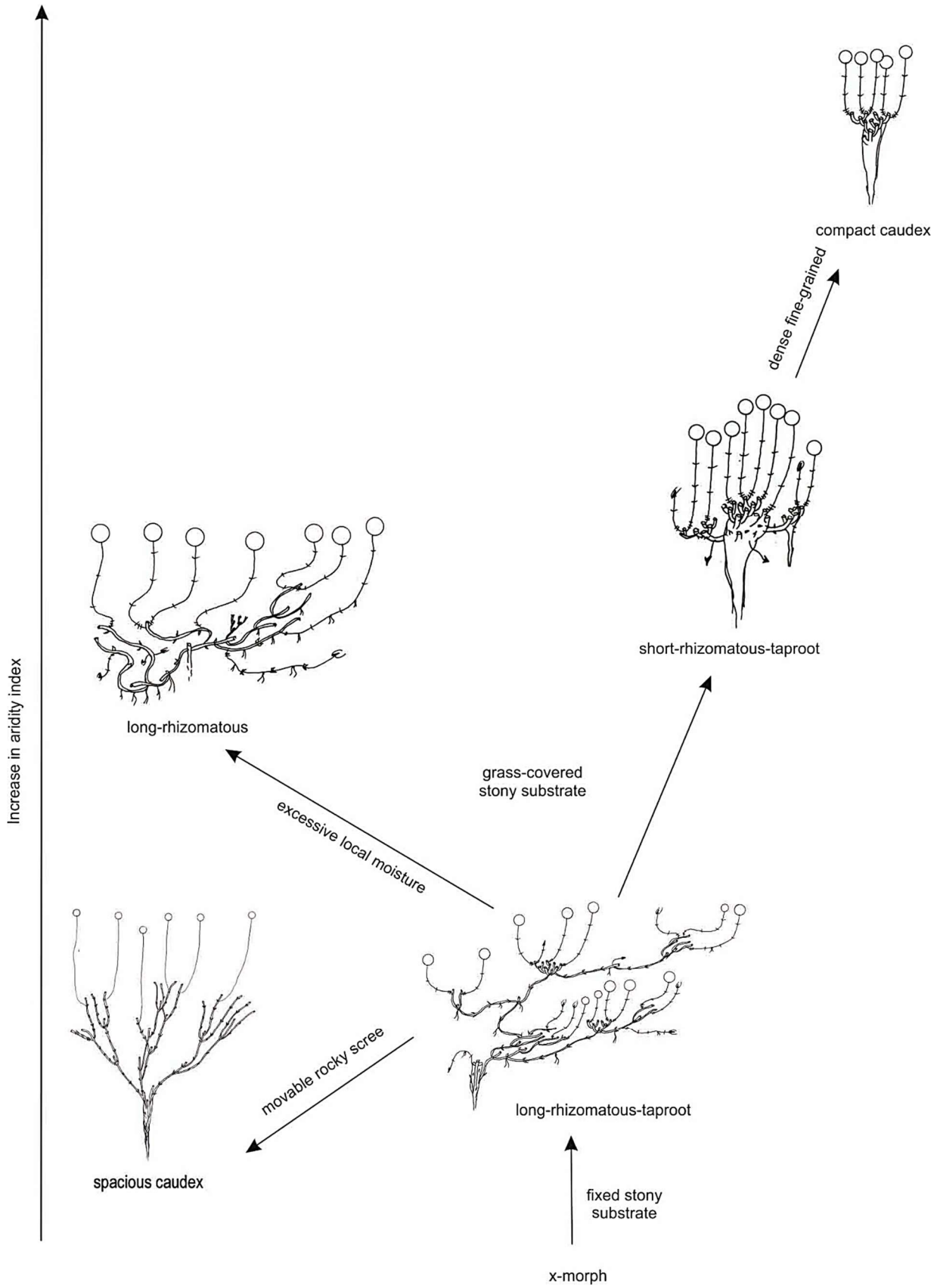


**Fig. 7**. A diagram detailing a hypothetical series of transformations of life forms in ***Nepeta*** species.

static nature of the substrate (skeletonless soil) result in the predominance of short modules in the general structure of plants and give rise to a compact form, whereas increased moistening, the grass-covered state, and a stony substrate lead to the predominance of long modules and the emergence of a sprawled long-rhizome form (Fig. 7).

The results of cluster analysis and PCA complement each other and clearly indicate that the divergence of life forms in the studied *Nepeta* species is linked with specific features of the substrate and local ecological conditions. Under the influence of various combinations of climatic and edaphic factors in the process of evolution, several separate species emerged that are well adapted in terms of whole-plant morphology and phenolic metabolism. Differentiation at the biochemical level led to species specificity of the profile of native phenolic compounds and aglycones. Nonetheless, there is a certain discrepancy between the grouping of the five species by morphological parameters and the grouping by phenolic profiles, as evidenced by the results of cluster analysis and indirectly by PCA. The cluster analysis of morphological parameters showed that species with long modules (*N. kokamirica* and *N. densiflora*) are distinct from species with short modules (*N. mariae* and *N. pulchella*), and *N. transiliensis* is isolated due to the greatest length of long modules. The cluster analysis of the phenolic profiles revealed that *N. densiflora* is isolated due to the highest level of quercetin glycosides and the greatest difference from other species in qualitative composition (the set of phenolic components), and that *N. mariae* and *N. kokamirica*, which have the highest similarity in qualitative composition, are grouped together. In addition, morphometric and biochemical parameters highly correlated with different principal components. Temperature and precipitation primarily influenced the morphological parameters and the concentration of cinnamic acid, correlating with PC1 and PC2, while for the phenolic compounds correlating with PC3, these influences were not significant (Table 7, Fig. 5).

A similar inconsistency in variation between morphological and biochemical parameters as well as subdivision of single morphological type into chemotypes have been repeatedly documented earlier (Petersen and Seberg, 1998; Heslop-Harrison, 2017; Mkindi *et al*., 2019). Genes and their products (especially hormones and enzymes), and corresponding metabolites are considered to influence morphological features (Sattler and Rutishauser, 1997). Phenolic compounds are involved in numerous adaptive reactions (Sharma *et al*., 2019), including those implemented through the synthesis of lignin and correlating with the plant form (Liu *et al*., 2018). Phenylpropanoids are intermediate products of lignin biosynthesis, whereas cinnamic acid is the first molecule in the phenylpropanoid pathway and a key compound in the synthesis of flavonoids and lignin (Ralph, 2010).

The highest level of cinnamic acid was registered in *N. pulchella* (Table 3), a species with a high percentage of short modules that grows under the conditions of relatively high climatic aridity and sufficiently high precipitation. Herewith, the concentration of this compound in *N. mariae* - a species forming exceptionally short modules - turned out to be low: at the level of species growing under mesophytic conditions. The low concentration of cinnamic acid probably has something to do with intensive synthesis of lignin for xeromorphic structure of *N. mariae* plants and for building the vascular system of long modules of mesophytes. *N. pulchella* forms primarily thick rhizome whose lignification is not very pronounced.

Chlorogenic acid is another phenylpropanoid taking part in lignin pathways (Sharma *et al*., 2019) and correlating with the principal components here. A high level of chlorogenic acid was revealed here in *N. densiflora*. A low concentration of this compound is a distinctive biochemical trait of *N. transiliensis*, which is separate from the other species judging by the cluster analysis of morphological parameters. *N. densiflora* and *N. transiliensis* have a similar habitus and grow under conditions comparable in air temperature and precipitation; these data mean that the amount of chlorogenic acid is determined by other factors.

The PCA highlighted a dissimilarity between the two phenylpropanoids, which is based on different roles in the pathways of lignin and flavonoids synthesis. Cinnamic acid is a common precursor in the synthesis of hydroxycinnamic acids, flavonoids and lignin (Dixon 1995), while chlorogenic acid is an intermediate constantly involved in responses to biotic and abiotic stressors (Geng *et al*., 2020). According to the PCA, cinnamic acid is part of the set of factors correlating with the second principal component, including temperature (Bio 2 and Bio 5) and precipitation (Bio 12 and Bio 13; Fig. 5), while chlorogenic acid does not have strong correlations with climatic parameters. The correlations of the chlorogenic acid level with the concentrations of cynaroside and isoquercitrin points to the interdependence of the metabolic pathways of all these compounds and to a role of chlorogenic acid in flavonoid synthesis. On the other hand, the structure of this principal component lacks environmental factors correlating with the aforementioned concentrations. Therefore, it can be concluded that this principal component is controlled by some other factors that are not taken into account in the system of parameters under study (for example, UV radiation, soil composition, or local moistening).

Of note, flavonoids correlating with PC3 have the highest concentration in *N. transiliensis* (cynaroside) and *N. densiflora* (isoquercetin; Table 3). That is, these

species have alternative metabolism of chlorogenic acid, cynaroside and isoquercitrin.

Judging by our data, in the studied species, cinnamic acid and chlorogenic acid, cynaroside, isoquercitrin, and the total phenolics are the main biochemical parameters describing variation among the species. The phenolic profiles of these species generally match those previously found in other *Nepeta* species, where hydroxycinnamic acids and luteolin glycosides dominate (Modnicki *et al*., 2007; Formisano *et al*., 2011; Mišić *et al*., 2015; Kashchenko and Olennikov, 2016). Rosmarinic and chlorogenic acids have been identified as major phenolics in many *Nepeta* species (Aras *et al*., 2016; Köksal *et al*., 2017). Meanwhile, these acids have been previously found in the leaves in free form, whereas in the leaves of the five *Nepeta* species, chlorogenic acid was detected in free form, while rosmarinic acid was registered as glycosides. In some previously examined species, rosmarinic acid has proven to be a minor phenolic compound of leaves (Kashchenko and Olennikov, 2016; Sarikurkcu *et al*., 2019). Ferulic acid, revealed in substantial quantities in *N. kokamirica*, is reported to be abundant in many species (Nasirkandi *et al*., 2019; Sarikurkcu *et al*., 2019). By contrast, quercetin and its glycosides have mainly been detected in minute amounts if at all (Aras *et al*., 2016; Köksal *et al*., 2017; Nestorović Živković, 2018). Some authors categorize rutin among major flavonoid compounds (Bošnjak-Neumüller *et al*., 2017; Nasirkandi *et al*., 2019), but its concentration was many times lower than that of hydroxycinnamic acids in those studies. In the literature, we did not find a phenolic profile similar to that of *N. densiflora*'s one. This uniqueness may be associated with the harsh climatic conditions along the eastern border of the natural geographic range of the genus, characterized by the lowest average annual air temperature (–3.7°C). Previously, we observed an increase in the total level of phenolic compounds and in the concentration of quercetin glycosides under low-temperature conditions in *Begonia* leaves (Karpova *et al*., 2016), and we subscribe to the opinion about the high adaptive value of quercetin glycosides (Hofmann *et al*., 2000; Fini *et al*., 2011). The combination of hydroxycinnamic acids with flavones, flavonols and terpenoids in the leaves of the five analyzed species indicates their potential usefulness for human health (Astashenkov *et al*., 2019).

Our results are in agreement with previous studies pointing to the effect of temperature and precipitation on concentrations of phenolic compounds in the leaves of some taxa (Liu *et al*., 2016; Yuan *et al*., 2020).

## CONCLUSION

This study reveals differentiation in life form traits and phenolic profiles among five alpine *Nepeta* species endemic to Central Asia. Effects of major climatic factors on morphological characteristics and phenolic profiles are documented too. PCA made it possible to reduce the system of climatic parameters to four parameters that most strongly correlate with the top principal components: Bio 6 (0.988), Bio 11 (0.960), Arid (0.950), and Bio 13 (0.924). The first two parameters describe temperature conditions of the coldest periods, the third one denotes water availability, and the last one characterizes wettest-month precipitation. The main morphological traits, according to PCA, are the number of short modules (n-Shm) (0.790) and the length of long modules (l-Lm) (-0.732); both correlate with the first principal component. Edaphic factors play an essential role too. The species that inhabit stony and movable substrates mainly form long modules, but grass-covered and static soil promotes the construction of short modules. Climate aridization and the static nature of the substrate (fine earth) lead to the formation of a compact life form, whereas increased moistening and a grass-covered state of the stony substrate give rise to a long-rhizome form.

The diversity of environments determines the differences in the levels of cinnamic acid, chlorogenic acid, cynaroside, isoquercitrin, and total phenolic compounds in the five studied species.

A lack of a match in geographical variation between morphological traits and phenolic profiles reflects adaptive evolution at different levels: whole-organism structure and phenolic metabolism. *N. densiflora*, distributed along the eastern border of the genus's natural range and possessing a unique profile of phenolic compounds - including hydroxycinnamic acids (chlorogenic and rosmarinic), luteolin glycosides, and quercetin glycosides - is a promising source of compounds for therapeutic use.

Our finding should expand the understanding of structural organization and diversification of life forms of alpine plants as well as clarify the accumulation of valuable compounds in representatives of *Nepeta*. This system of morphological and biochemical parameters can be considered as a model for analysis of evolutionary transformations of other herbaceous plant species.

## AUTHOR CONTRIBUTIONS

AA and VC collected the samples of high-altitude plants and designed the study and revised the manuscript. AA and EK drafted the manuscript and prepared tables and figures. AA and VC performed morphological analysis. KE performed HPLC analysis and PCA. All authors discussed the results and commented on the manuscript.

## ACKNOWLEDGMENTS

The work was financially supported by the publicly funded projects of the CSBG SB RAS # AAAA-A21-121011290025-2 and # AAAA-A21-121011290026-9 within the framework of a government contract.

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