Morphological variability and differentiation of selected Achillea species (sections Achillea sensu lato and Anthemoideae) from Serbia

Abstract:

In the present study, morphological variability and differentiation of 13 Achillea species, including 11 from the Achillea section Achillea sensu lato (A. chrysocoma, A. clypeolata, A. coarctata, A. collina, A. crithmifolia, A. distans, A. grandifolia, A. millefolium, A. nobilis, A. pseudopectinata and A. vandasii) and two species from the Achillea section Anthemoideae (A. ageratifolia and A. lingulata) from Serbia were analyzed. Morphometric analysis included a total of 9 quantitative characteristics from the vegetative and reproductive plant parts. Multivariate statistical analyses reflected almost clear differentiation between species from two studied sections whereby ligulate floret length and stem leaf segment length had the greatest influence for their distinction. Thus, the section Achillea sensu lato was characterized by significantly shorter ligulate florets and longer segments of stem leaf compared to the section Anthemoideae. Furthermore, A. grandifolia featured the longest segments of stem leaf and showed considerable separation from all other species within the section Achillea sensu lato. It could be concluded that the selected set of morphological characteristics in the present study mainly positioned the studied species in accordance with the current infrageneric classification of Achillea.

Key words:

Achillea, morphometry, multivariate statistical analysis, Balkan Peninsula

Apstrakt.

Morfološka varijabilnost i diferencijacija odabranih vrsta roda Achillea (sekcija Achillea sensu lato i Anthemoideae) iz Srbije

U ovom istraživanju analizirana je morfološka varijabilnost i diferencijacija 13 vrsta roda Achillea, uključujući 11 iz sekcije Achillea sensu lato (A. chrysocoma, A. clypeolata, A. coarctata, A. collina, A. crithmifolia, A. distans, A. grandifolia, A. millefolium, A. nobilis, A. pseudopectinata i A. vandasii) i dve vrste iz sekcije Anthemoideae (A. ageratifolia i A. lingulata) iz Srbije. Morfometrijskom analizom obuhvaćeno je ukupno 9 kvantitativnih karaktera iz vegetativnih i reproduktivnih delova biljaka. Multivarijantne statističke analize pokazale su gotovo jasnu diferencijaciju izmeđju vrsta iz dve istraživane sekcije, pri čemu su dužina jezičastog cveta i dužina režnja lista stabla imale najveći uticaj na njihovo razlikovanje. Tako se sekcija Achillea sensu lato odlikovala znatno kraćim jezičastim cvetovima i dužim režnjevima lista stabla u odnosu na sekciju Anthemoideae. Osim toga, A. grandifolia je imala najduže režnjeve lista stabla i pokazala značajno odvajanje od svih ostalih vrsta u okviru pomenute sekcije. Može se zaključiti da je odabrani skup morfoloških karaktera u ovoj studiji pozicionirao istraživane vrste uglavnom u skladu sa aktuelnom infrageneričkom klasifikacijom roda Achillea.

Ključne reči:

Achillea, morfometrija, multivarijantne statističke analize, Balkansko poluostrvo

Introduction

Genus Achillea L. (yarrows) is one of the most polymorphic and taxonomically complicated genera in the family Asteraceae, including more than 100

species, distributed in Europe, temperate climate Asia and few species in North America (Saukel et al., 2003; Ehrendorfer & Guo, 2006; Guo et al., 2006). In the flora of Serbia, 27 *Achillea* species have been recognized (Euro+Med, 2006).

Original Article

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Received: September 26, 2023 Revised: October 22, 2023 Accepted: October 27, 2023



The genus is characterized by the ability to adapt in different ecological amplitudes and ranges in extreme environmental conditions, such as deserts to water-logged habitats and from sea coastal areas to the high mountains (Ehrendorfer & Guo, 2006). Consequently, several widespread Achillea species show extremely high morphological variability and complexity. In addition, the presence of spontaneous hybrids, allo- and autopolyploids, aneuploids and phenocopies results in a great cytological, morphological and phytochemical variability both at inter- and at intraspecific level. Therefore, taxonomic evaluation, identification of species and classification has been a difficult task for decades (Inotai et al., 2016). Taxonomical grouping within the genus has been rearranged several times in the past and intensively researched even today (Kidlovits & Nemeth, 2012). Based on previous morpho-anatomical and ecogeographical analyses, many classification schemes of the genus Achillea have been proposed (Afanasyev & Bochantsev, 1961; Huber-Morath, 1975). However, the most recent multidisciplinary studies recognized five sections within this genus: Achillea sensu lato, Anthemoideae (DC.) Heimerl, Babounya (DC.) O. Hoffm., Ptarmica (Mill.) W. D. J. Koch and Otanthus (Hoffmanns, & Link) Ehrend, & Y. P. Guo (Saukel et al., 2003; Guo et al., 2004; Ehrendorfer & Guo, 2005).

Certain morphological characteristics, including characteristics of leaves, florets and pollen grains may be useful for morphological characterization and classification of *Achillea* taxa (Saukel & Länger, 1992; Valant-Vetschera & Kastner, 2000; Rauchensteiner et al., 2002; Nedelcheva, 2008; Azani et al., 2009; Akyalcin et al., 2011). The main aims of the present study were: 1) determination of highly variable morphological characteristics as well as those of low variability in two sections, *Achillea sensu lato* and *Anthemoideae*, and 2) assessment of the degree of differentiation of the studied species based on selected set of morphological characteristics.

Materials and Methods

Plant material

Plant material (whole plants) of 13 Achillea species, including 11 from the section Achillea sensu lato (A. chrysocoma Friv., A. clypeolata Sm., A. coarctata Poir., A. collina (Becker ex Rchb. f.) Heimerl, A. crithmifolia Waldst. & Kit., A. distans Waldst. & Kit. ex Willd., A. grandifolia Friv., A. millefolium L., A. nobilis L., A. pseudopectinata Janka and A. vandasii Velen.) and two species from the section Anthemoideae (A. ageratifolia (Sm.) Benth. & Hook.

f. and A. lingulata Waldst. & Kit.) were collected in their natural habitats in Serbia. Additionally, plant material of one population of A. pseudopectinata was collected in North Macedonia. The voucher specimens of each population were deposited in the "Herbarium Moesiacum Niš" (HMN) of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš. The map of the studied area, with locations of the selected populations, is shown in Fig. 1. Furthermore, the original data on position, habitat and voucher information for each population are listed in Tab. 1. Plant material was identified by Dr. B. K. Zlatković, Dr. Z. S. Mitić and M.Sc. J. P. Stojković.

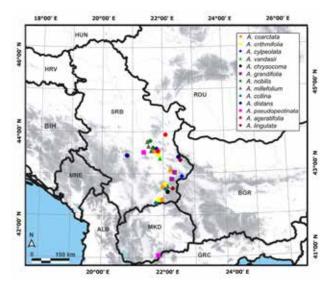


Fig. 1. Geographical position of studied populations. For the description of the location and habitat conditions of the populations, *cf.* **Tab. 1**

Morphometric analysis

A total number of 384 individuals from 26 populations and 13 species of Achillea, from two different sections, were compared at the level of 9 quantitative morphological characteristics. Ouantitative traits such as rosette leaf segment length (RLSL) / rosette leaf tooth length (RLTL), stem leaf segment length (SLSL) / stem leaf tooth length (SLTL), ligulate floret length (LFL), tubular floret length (TFL) and involucral bract length (IBL) of each examined individual were mounted as permanent microscope slides and photographed after preparation with a Leica MZ-16A binocular Microsystems©, magnifier (Leica Wetzlar, Germany). As a background, graph paper was used in the photography process. Each characteristic was measured with three consecutive measurement repetitions. All measurements of the mentioned properties were performed using Digimizer Image Analysis Software (MedCalcSoftware©, Belgium),

Table 1. Position and habitat description of studied populations of Achillea species

| | | | | | Altitude | | Number of | | Number of |
|------------|-----------------|--|--------------|---------------|---------------|----------------------|-------------------------|-----------------------|------------------|
| Section | Species | Locality | Latitude (N) | Longitude (E) | (m a.s.l.) | Substratum | individuals analyzed | Date of collection | vaucher (HMN) |
| | A. chrysocoma | Serbia: Mt. Besna Kobila | 42°31'05" | 22°13'05" | 1923 | Silicate | 15 | July 2020 | 14300 |
| | A. clypeolata | Serbia: Sićevačka gorge | 43°18'36" | 22°10'49" | 276 | Limestone | 15 | May 2019 | 13957 |
| | A. clypeolata | Serbia: Vlkovija | 43°05'02" | 22°54'24" | 1020 | Limestone | 15 | June 2021 | 14310 |
| | A. coarctata | Serbia: Pčinja river valley, Budovija | 42°20'23" | 21°53'26" | 440 | Silicate | 15 | June 2018 | 13681 |
| | A. coarctata | Serbia: Sićevačka gorge | 43°33'42" | 22°06'95" | 365 | Limestone | 15 | June 2018 | 13685 |
| | A. coarctata | Serbia: Temska | 43°17'06" | 22°35'06" | 440 | Silicate | 15 | July 2020 | 14303 |
| | A. collina | Serbia: Pčinja river valley, Budovija | 42°20'23" | 21°53'26" | 089 | Silicate | 15 | June 2019 | 13952 |
| Achillea | A. crithmifolia | Serbia: Pčinja river valley, Budovija | 42°20'23" | 21°53'26" | 440 | Silicate | 22 | June 2018 | 13684 |
| sensu lato | A. crithmifolia | Serbia: Kunovica | 43°17'30" | 22°06'34" | 467 | Limestone | 15 | June 2018 | 13680 |
| | A. distans | Serbia: Mt. Stara planina, Babin zub | 43°23'04" | 22°35'35" | 1600 | Silicate | 15 | July 2020 | 14305 |
| | A. distans | Serbia: Kopaonik | 43°18'51" | 20°51'01" | 1370 | Silicate | 15 | July 2020 | 14306 |
| | A. grandifolia | Serbia: Jerma gorge | 42°58'18" | 22°37'23" | 548 | Limestone | 15 | June 2020 | 14302 |
| | A. grandifolia | Serbia: Temska | 43°16'56" | 22°35'04" | 440 | Silicate | S | July 2020 | 14614 |
| | A. millefolium | Serbia: Sićevačka gorge | 43°20'04" | 22°04'09" | 240 | Limestone | 15 | June 2018 | 13682 |
| | A. millefolium | Serbia: Niš | 43°18'19" | 21°53'50" | 260 | Neogene sediments | 15 | June 2019 | 13955 |
| | A. millefolium | Serbia: Lalinačka salt marsh | 43°20'42" | 21°44'45" | 200 | Neogene sediments | 15 | May 2019 | 14400 |
| | A. millefolium | Serbia: Pčinja river valley, Brnjare | 42°23'30" | 21°55′01" | 507 | Neogene sediments | 15 | June 2019 | 13961 |
| | | | | | | | | | |

| | 1 . 1 . 1 | Serbia: Sićevačka | 1200000 | 10000 | 1)6 | | | 0.00 | 6006 |
|--------------|--------------------|---|-----------|-----------|------|----------------------|----|-----------|-------|
| | A. nobilis | gorge | 43~20.04" | | 303 | Limestone | SI | June 2018 | 13683 |
| Achillea | A. nobilis | Serbia: Lalinačka salt marsh | 43°20'42" | 21°44'45" | 200 | Neogene sediments | 15 | June 2020 | 14298 |
| sensu lato | A. pseudopectinata | North Macedonia: Kajmakčalan | 40°59'12" | 21°40'06" | 718 | Silicate | 12 | June 2020 | 14309 |
| | A. pseudopectinata | Serbia: Oblačina | 43°18'35" | 21°40'15" | 333 | Silicate | 15 | June 2020 | 14301 |
| | A. vandasii | Serbia: Vrandol | 43°16'41" | 22°15'31" | 426 | Carbonate | 15 | June 2020 | 14304 |
| | A. ageratifolia | Serbia: Bor, Mt. Veliki Krš | 44°10'47" | 22°04'30" | 995 | Carbonate | 15 | June 2019 | 13954 |
| Anthemoideae | A. ageratifolia | Serbia: Jelašnička gorge | 43°16'37" | 22°04'09" | 360 | Limestone | 15 | May 2021 | 14542 |
| | A. lingulata | Serbia: Mt. Stara planina, Babin zub | 43°22'06" | 22°35'42" | 1600 | Silicate | 15 | June 2019 | 13953 |
| | A. lingulata | Serbia: Mt. Besna Kobila | 42°31'45" | 22°13'40" | 1850 | Silicate | 15 | July 2020 | 14299 |

on previously taken photographs. Characteristics of large plant parts, such as rosette leaf length (RLL), and stem leaf length (SLL), were determined using a ruler. In contrast, rounded structures, capitulum length (CL) and capitulum width (CW), were measured with a high-precision digital meter (Mahr Federal 410710716U, Esslingen, Germany).

Statistical analysis

The statistical analysis of the obtained data was performed using the STATISTICA 8 software (StatSoft, Inc., Tulsa, OK, USA). Statistical data processing included descriptive statistics and multivariate statistical analyses: canonical discriminant analysis (CDA), and agglomerative hierarchical clustering (AHC). Unweighted pair group average (UPGMA) was used as a criterion for the clusters development, and Euclidean distances as diversity assessment criteria in AHC analysis.

Results and discussion Variability of morphological characteristics of the studied Achillea species

Results of descriptive statistics for 9 quantitative morphological characteristics of 13 Achillea species were presented in **Tab. 2**. According to the obtained results, most of the studied characteristics were within a moderate degree of variability (10<CV%<50). Nevertheless, in almost all species, some of the characteristics of the reproductive plant parts stood out as low variable (CV%<10): involucral bract length (A. chrysocoma, A. clypeolata, A. coarctata, A. collina, A. distans, A. grandifolia, A. millefolium, A. nobilis, A. pseudopectinata and A. vandasii), tubular floret length (A. ageratifolia, A. chrysocoma, A. collina, A. crithmifolia, A. distans, A. grandifolia, A. millefolium and A. vandasii), ligulate floret length (A. ageratifolia, A. collina, A. distans, A. grandifolia and A. pseudopectinata), capitulum length (A. collina, A. distans and A. pseudopectnata) and capitulum width (A. collina and A. grandifolia).

There is a relatively limited amount of literature data which is related to the degree of variability of morphological characteristics of *Achillea* species. Morphological characteristics such as the height of plant, length, width and shape of leaf, shape and color of involucrum, as well as length and color of flowers were mainly commented in a general sense (Gajić, 1975; Saukel et al., 2003; Guo et al., 2004, 2006). In agreement with our data, Mottaghi et al. (2015) reported that involucrum length and width and ligulate florets length were the most stable characteristics, while leaf width, capitulum number and seed weight were the most variable characteristics of six Iranian *Achillea* species.

Table 2. Results of descriptive statistics for studied quantitative morphological characteristics of studied Achillea species

| | | | | | Char | Characteristics | | | | |
|------------------|-------------------------------|------------------------------------|--|---------------------------------|---|-----------------------------|----------------------------|---------------------------------------|--------------------------------------|--|
| Species | Descriptive statistics | Rosette leaf length (RLL) | Rosette leaf segment length/ rosette leaf tooth length (RLSL/RLTL) | Stem leaf length (SLL) | Stem leaf segment length/ stem leaf tooth length (SLSL/ SLTL) | Capitulum length (CL) | Capitulum width (CW) | Ligulate floret length (LFL) | Tubular floret length (TFL) | Involucral bract length (IBL) |
| | \mathbf{M}^{1} | 47.7 | 3.7 | 21.8 | 2.8 | 4.2 | 3.1 | 5.7 | 4.2 | 4.9 |
| A Lines comments | Min^2 | 37.2 | 2.5 | 16.0 | 2.2 | 3.6 | 2.3 | 4.5 | 3.6 | 4.1 |
| A. chrysocoma | Max ³ | 61.7 | 4.7 | 29.2 | 3.6 | 4.9 | 3.7 | 6.4 | 4.7 | 5.6 |
| | CV4 | 13.2 | 16.4 | 18.1 | 14.4 | 10.1 | 12.6 | 11.7 | 7.2 | 9.0 |
| | M | 217.0 | 9.7 | 8.09 | 5.0 | 3.6 | 2.3 | 2.7 | 2.5 | 2.9 |
| A olimpoolista | Min | 153.9 | 6.7 | 40.4 | 2.2 | 2.8 | 1.8 | 2.1 | 2.0 | 2.5 |
| A. ciypeoidid | Max | 288.6 | 12.9 | 87.9 | 9.1 | 4.4 | 2.8 | 3.6 | 3.1 | 3.3 |
| | CV | 15.6 | 14.6 | 19.2 | 37.1 | 12.6 | 10.0 | 16.8 | 12.6 | 6.3 |
| | M | 142.2 | 4.5 | 52.1 | 3.1 | 3.2 | 2.3 | 2.0 | 2.2 | 2.6 |
| A connectator | Min | 63.4 | 3.0 | 25.3 | 2.0 | 2.4 | 1.8 | 1.4 | 1.7 | 2.3 |
| A. Courciana | Max | 216.4 | 8.9 | 95.7 | 4.9 | 4.7 | 3.3 | 3.7 | 3.3 | 3.3 |
| | CV | 24.6 | 20.3 | 30.6 | 20.7 | 12.7 | 16.5 | 24.2 | 14.7 | 7.7 |
| | M | 140.4 | 4.3 | 33.0 | 2.4 | 3.3 | 1.8 | 3.4 | 2.6 | 3.8 |
| A colling | Min | 103.4 | 3.2 | 21.3 | 1.8 | 2.8 | 1.6 | 2.8 | 2.2 | 3.3 |
| A. Couina | Max | 172.8 | 5.4 | 45.7 | 3.7 | 3.7 | 2.1 | 3.6 | 2.9 | 4.3 |
| | CV | 18.1 | 13.4 | 18.7 | 19.6 | 7.3 | 7.8 | 6.9 | 9.7 | 7.1 |
| | M | 75.7 | 5.3 | 29.7 | 3.9 | 2.9 | 1.8 | 3.4 | 2.6 | 3.3 |
| A cuithmitfolia | Min | 34.6 | 2.9 | 14.5 | 2.1 | 1.6 | 1.2 | 2.8 | 2.1 | 2.5 |
| A. Crummyoum | Max | 122.5 | 7.5 | 48.7 | 6.4 | 3.2 | 2.2 | 4.4 | 3.0 | 4.3 |
| | CV | 30.5 | 22.7 | 28.7 | 30.3 | 11.5 | 11.8 | 11.1 | 7.5 | 13.0 |
| | M | 222.8 | 12.9 | 8.79 | 9.6 | 4.4 | 2.4 | 3.9 | 3.1 | 3.8 |
| A. distans | Min | 139.8 | 8.0 | 51.6 | 6.1 | 3.6 | 1.6 | 3.1 | 2.5 | 3.0 |
| | Max | 304.9 | 18.7 | 95.1 | 12.7 | 5.0 | 3.0 | 4.5 | 3.5 | 4.2 |
| | | | | | | | | | | |

| A. distans | CV | 19.2 | 18.5 | 17.5 | 17.0 | 8.5 | 11.5 | 8.0 | 7.9 | 7.0 |
|---------------------|-----|-------|------|-------|------|------|------|------|------|------|
| | M | 134.4 | 24.7 | 111.0 | 25.5 | 3.6 | 2.4 | 3.8 | 2.8 | 3.4 |
| - Vilotina V | Min | 105.8 | 16.7 | 86.1 | 18.2 | 3.0 | 2.1 | 3.3 | 2.4 | 3.0 |
| A. grandijona — | Max | 191.3 | 30.1 | 138.2 | 33.7 | 4.5 | 2.8 | 4.5 | 3.1 | 3.8 |
| I | CV | 20.2 | 17.2 | 14.6 | 16.8 | 10.9 | 9.3 | 9.3 | 8.9 | 7.3 |
| | M | 171.9 | 6.5 | 36.3 | 3.8 | 3.1 | 1.9 | 3.2 | 2.5 | 3.3 |
| | Min | 62.7 | 4.2 | 21.3 | 2.0 | 2.2 | 1.3 | 2.3 | 2.0 | 2.6 |
| A. munejouum – | Max | 282.4 | 10.5 | 50.8 | 5.4 | 3.8 | 2.4 | 4.0 | 3.0 | 4.1 |
| I | CV | 32.1 | 20.0 | 20.4 | 16.9 | 12.1 | 12.2 | 12.1 | 6.7 | 8.9 |
| | M | 6.92 | 8.9 | 30.7 | 6.3 | 2.2 | 1.7 | 1.9 | 1.8 | 2.1 |
| l sobilis | Min | 39.0 | 4.8 | 18.9 | 3.7 | 1.8 | 1.3 | 1.3 | 1.3 | 1.8 |
| A. nobuts – | Max | 130.2 | 12.9 | 42.6 | 10.4 | 3.3 | 2.3 | 2.6 | 2.3 | 2.4 |
| I | CV | 27.7 | 23.6 | 20.3 | 25.8 | 15.4 | 16.1 | 19.0 | 16.3 | 6.3 |
| | M | 25.7 | 2.5 | 33.0 | 3.6 | 3.1 | 2.2 | 2.8 | 2.9 | 3.0 |
| - A meandomandum to | Min | 18.3 | 1.7 | 24.5 | 2.4 | 2.6 | 1.7 | 2.3 | 2.4 | 2.6 |
| A. pseudopecundud — | Max | 34.9 | 4.0 | 42.3 | 5.0 | 3.7 | 2.7 | 3.2 | 3.5 | 3.4 |
| I | CV | 13.6 | 24.3 | 13.4 | 19.4 | 9.2 | 16.1 | 7.3 | 12.0 | 6.9 |
| | M | 156.6 | 4.5 | 56.6 | 3.3 | 3.1 | 2.3 | 2.3 | 2.5 | 2.9 |
| - A wandacii | Min | 124.1 | 3.0 | 37.4 | 2.6 | 2.6 | 1.8 | 1.8 | 2.3 | 2.4 |
| A. Vanadsti | Max | 208.3 | 0.9 | 75.1 | 4.8 | 3.7 | 2.7 | 2.8 | 2.8 | 3.2 |
| I | CV | 17.0 | 20.9 | 16.9 | 18.0 | 11.0 | 11.5 | 10.5 | 6.1 | 7.5 |
| | M | 39.3 | 0.3 | 14.4 | 0.2 | 5.5 | 5.2 | 8.8 | 3.7 | 4.2 |
| A agovatifolia | Min | 22.9 | 0.2 | 9.7 | 0.0 | 4.3 | 3.8 | 7.3 | 2.9 | 2.8 |
| A. ugerunjouu | Max | 54.8 | 9.0 | 24.1 | 0.4 | 9.9 | 9.9 | 10.2 | 4.5 | 5.6 |
| I | CV | 19.2 | 23.0 | 22.9 | 32.5 | 13.1 | 13.3 | 9.3 | 8.4 | 16.0 |
| | M | 122.1 | 6.0 | 41.3 | 9.0 | 5.3 | 4.2 | 6.5 | 3.6 | 4.5 |
| A linearlata | Min | 83.7 | 0.3 | 29.9 | 0.2 | 4.3 | 3.3 | 5.2 | 2.9 | 3.7 |
| - mgama | Max | 182.0 | 6.0 | 56.7 | 0.7 | 8.9 | 5.5 | 9.1 | 4.2 | 5.8 |
| | CV | 26.0 | 28.5 | 18.1 | 33.9 | 14.0 | 12.4 | 14.9 | 10.8 | 11.4 |
| | | | | | | | | | | |

¹M: mean (mm). ²Min: minimum (mm). ³Max: maximum (mm). ⁴CV: coefficient of variation (%).

Nevertheless, differences in leaf shapes among *Achillea* taxa are stable and often used in taxonomy as diagnostic characteristics (Sha et al., 2018).

Multivariate analyses (CDA and AHC) of morphological characteristics of the studied Achillea species

The CDA based on 9 morphological characteristics of 13 species of Achillea showed that the first two canonical axes participated in 80.26% of the total discrimination, of which the first axis (CA1) accounted for 53.23% (Fig. 2, Tab. 3). The obtained scatter plot showed the tendency of differentiation of species from two studied sections along the CA1 that explained the highest percentage of discrimination (Fig. 2). Namely, individuals of most species from the section Achillea sensu lato were positioned at the negative part of CA1, while individuals of two studied species from the section Anthemoideae formed the group on the positive part of this axis. Despite the positioning of individuals of species from the section Achillea sensu lato at the negative part of CA1, they formed two groups clearly separated along the CA2. The first group, with positive values for CA2, included only individuals of A. grandifolia, while individuals of other species from this section formed the second group showing mostly negative

values for CA2. Besides that, individuals of one species from the section *Achillea sensu lato* (*A. chrysocoma*) were positioned at the positive part of CA1 approaching the section *Anthemoideae*.

Table 3. Standardized coefficients for the first two canonical axes (CAs) of variation in quantitative morphological characteristics from the discriminant functional analysis of studied *Achillea* species. Significant coefficients are in boldface

| Variables | CA1 | CA2 |
|-----------------------------------|--------|--------|
| RLL | -0.004 | -0.332 |
| RLSL | -0.393 | 0.359 |
| SLL | -0.074 | 0.068 |
| SLSL | -0.41 | 0.514 |
| CL | 0.042 | 0.195 |
| CW | 0.354 | 0.175 |
| LFL | 0.822 | 0.819 |
| TFL | -0.386 | -0.406 |
| IBL | -0.058 | -0.245 |
| Eigenvalue | 28.038 | 14.236 |
| Cumulative percentage of variance | 0.532 | 0.803 |

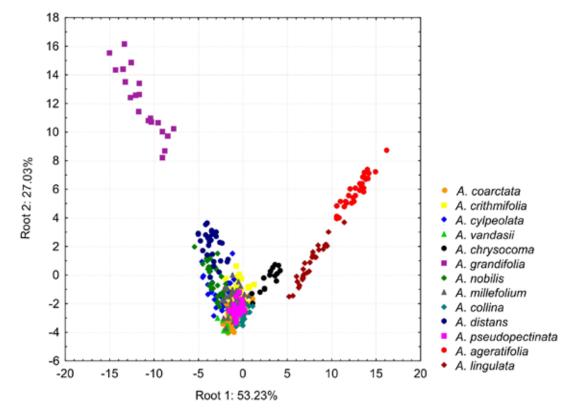


Fig. 2. Canonical discriminant analysis (CDA) based on morphological characteristics of studied *Achillea* species

Characteristic ligulate floret length (LFL) had significant impact on both axes, while stem leaf segment length (SLSL) significantly affected only the CA2 (Tab. 3). Thus, the basic differences between observed groups were related to fact that the section Achillea sensu lato was characterized by significantly shorter ligulate florets and longer segments of stem leaf compared to the section Anthemoideae (Tab. 2). In addition, A. grandifolia featured the longest segments of stem leaf and showed considerable separation from all other species of the section Achillea sensu lato. On the other hand, intermediate position of A. chrysocoma between the studied sections was probably the consequence of the fact that this species had the longest ligulate florets within the section Achillea sensu lato, i.e., almost like A. lingulata from the section Anthemoideae. Finally, if we compare differentiation of Achillea species on the scatter plot (Fig. 2) and the geographical position of their populations included in the analysis (Fig. 1), it is obvious that there is no correlation between them. This result suggested a stronger differentiation between morphologically identified species than between geographical regions.

The dendrogram obtained by AHC has also shown a tendency to divide analyzed *Achillea* species in a similar way but indicating the closer position of *A. chrysocoma* to *A. lingulata* than to other species of the section *Achillea sensu lato* (Fig. 3). Specifically, the dendrogram suggested the division of the studied species into four main groups: 1) *A. grandifolia*; 2) *A. ageratifolia*; 3) *A. lingulata* and *A. chrysocoma*; and 4) other studied species. Thus, *A. grandifolia* again showed considerable separation from all other species of the section *Achillea sensu lato*, and *A. chrysocoma* was in the same cluster with *A. lingulata* from the section *Anthemoideae*.

Both multivariate analyses were used to determine the level of morphological differentiation of the studied *Achillea* species and sections. Apparently, characteristics ligulate floret length (LFL) and stem leaf segment length (SLSL) had the greatest influence on the differentiation of the two investigated sections. Moreover, within the section *Achillea sensu lato* some separation of *A. grandifolia* and *A. chrysocoma* was evident. In agreement with our findings, Saukel & Länger (1992) reported that the characteristics of leaf segment and ligulate

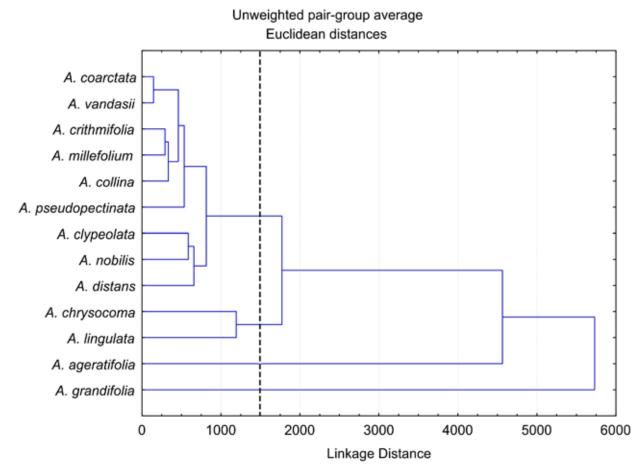


Fig. 3. Dendrogram obtained by agglomerative hierarchical clustering (AHC) of studied *Achillea* species based on morphological characteristics

floret were the most significant features for the morphological characterization of taxa within *A. millefolium* aggregate from Central Europe. However, Azani et al. (2009) showed that some quantitative and qualitative morphological characteristics did not position Iranian *Achillea* species in agreement with the infrageneric classification.

Conclusion

Most of the studied morphological characteristics of 13 species of Achillea from the central Balkans showed moderate level of variability. Nevertheless, in almost all species, some of the characteristics of the flowering region stood out as low variable. Multivariate statistical analyses reflected almost clear morphological differentiation between species from two studied sections whereby ligulate floret length and stem leaf segment length had the greatest influence for their distinction. Thus, the section Achillea sensu lato was characterized by significantly shorter ligulate florets and longer segments of stem leaf compared to the section Anthemoideae. Furthermore, A. grandifolia featured the longest segments of stem leaf and showed considerable separation from all other species of the section Achillea sensu lato. On the other hand, A. chrysocoma was characterized by the longest ligulate florets within the section Achillea sensu lato and thus showed some tendency to approach the section Anthemoideae. Nevertheless, it could be concluded that the selected set of morphological characteristics in the present study mainly positioned the studied species in accordance with the current infrageneric classification of Achillea.

Acknowledgements. This research was supported by Grant No 451-03-47/2023-01/200124 by the Ministry of Education, Science and Technological Development of the Republic of Serbia. The authors would like to thank Ivan Gnjatović for kindly producing the distribution map.

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