**UDC (UDK): 635.74**

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# THE APPLICABILITY OF MORPHOLOGICAL CHARACTERS IN TAXONOMY OF *ARTEMISIA* (ASTERACEAE)

## SUMMARY

Many different morphological characteristics were widely used by several Flora in preparing keys for taxonomic identification of the taxa in the genus *Artemisia* (Asteraceae). In the current work an intensive review was carried out on different Flora and published papers and literatures on applications of morphological qualitative characteristics in taxonomy of *Artemisia*, of which 17 widely used characters were investigated for their importance in taxonomy of the genus. Multivariate analyses were performed using several statistical package i.e. Principal Component Analysis (PCA) for detecting the most important characters differentiating the species, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) for cluster analysis, Canonical Discriminant Function Analysis (CDFA) in order for confirmation of the clustering results. All these analyses showed that out of 17 morphological characters investigated; only 7 were useful for identification of the species and grouping the subgenera, and clearly separated homogamous species from heterogamous ones, indicating the importance of capitulum morphology in taxonomy of *Artemisia* at subgenera level. Our data showed that the four characters of cauline leaf type, leaf colour adaxial, leaf colour abaxial and capitalism attachment accounted for majority of variability. Phylogenetic analysis based on morphology indicated that the subgenera *Artemisia* and *Dracunculus* were more closely related to each other than either to the subgenus *Serphidium*. This work highlights the morphology importance in the taxonomic application and taxa identification.

**Keywords:** *Artemisia*, *Dracunculus*, morphological characters, Multivariate Statistical Analysis, phylogeny, *Serphidium*

## INTRODUCTION

The genus *Artemisia* L. (Asteraceae) includes more than 500 species (Ling, 1991, 1995a,b, Bremer and Humphries 1993), almost all of which are perennial with less than 10 annual species. The ploidy levels in *Artemisia* are

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diverse and include diploid, tetraploid, hexaploid, octaploid, decaploid and dodecaploid (Kawatani and Ohno 1964,Vallas and Siljak-Yakovlev 1997,Vallas and McArthur 2001). The basic chromosome number in the genus is mostly 9 (Kawatani and Ohno 1964) but also less frequently (x=8). *Artemisia* includes many species of commercial uses including culinary herbs (e.g. tarragon, *A. dracunculus* L. and absinth *, A. absinthium* L.), antihelminthic (e.g. *A. santonica* L.), ornamentals (e.g. *A. annua* L.), antimalarial (e.g. *A. arborescens*) and renovation of landscapes (e.g. *A. vulgaris* L.), Nazar and Mahmood (2010). The taxonomic and phylogenetic relationships within the genus are controversial.The genus *Artemisia* is differently treated as a single and large genus by many taxonomists (e.g. Cronquist, 1955, Kornkven *et al*. 1998, 1999, Torrell *et al*. 1999, Martin *et al*. 2001) or differently divided into five to eight genera (Bremer and Humphries 1993, Poljakov, 1961, Torrell *et al*. 1999). The most widely characteristics used in the taxonomy of the genus are floral and capitular morphology and gender (Watson *et al*. 2002). For example, the capitulum in subgenus *Seriphidium* Besser lacks ray florets, and composes only of perfect-bisexual disc florets, so the capitulum called homogamous, while in the subgenera *Dracunculus* Besser, *Abrotanum* Besser and *Absinthium* Mill. there are two sort of florets on the capitulum, in which ray florets are pistillate along side perfect or staminate discs florets, therefore, the capitulum is called heterogamous (Bremer and Humphries 1993).The taxonomy of the genus *Artemisia* has been also investigated using different molecular markers. Based on nuclear ribosomal DNA (ITS) and chloroplast psbA-trnH sequences data, the genus was divided into five subgenera (Torrell *et al*. 1999).The current work re-investigated the applicability of the 17 qualitative morphological characteristics in taxonomy and phylogeny in some *Artemisia* species.

## MATERIALS AND METHOD

The total umber of 39 genotypes belonging to 15 species were included in the study from the three subgenera of *Artemisia*, *Dracunculus,* and *Seriphidium* by enclosing a minimum of 2-3 genotypes of each species (Table 1).More than 30 morphological qualitative characteristics were examined for their ability in differentiating the *Artemisia* taxa based on the intensive review of different flora including Flora Iranica (Podlech *et al*.1986), Flora of Turkey (Wagentiz and Davis 1975),as well as published papers(e.g.Ghafoor,2002),and consequently 17 of these morphological characters were selected for investigation (Table 2).Multivariate analyses were performed using several statistical package such as Mega version 5(Tamura *et al*. 2011),MVSP version3.1(Kovach,1999)and SPSS version9 packages.Principal Component Analysis (PCA) was used to determine the most important characters recognizing the species,Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was conducted for cluster analyzing of the taxa under study using 100 bootstrap replications,and Canonical Discriminant Function Analysis (CDFA)was used for confirmation of the grouping the taxa.

Table 1: The sources and identification of plant materials used in the current study

| Code | Species | Location | Altitude (m) | Source/identification |
|---|---|---|---|---|
| fra300 | *Artemisia fragrans* | Ajabshir to Azarshahr, East Azerbaijan | 1490 | Tabriz GenBank |
| fra302 | *Artemisia fragrans* | Ahar - Meshkinshahr, East Azerbaijan | 1450 | Tabriz GenBank |
| fra761 | *Artemisia fragrans* | Osku - Gonbarf, East Azerbaijan | 2062 | Tabriz GenBank |
| sco428 | *Artemisia scoparia* | Tabriz, East Azerbaijan | 1370 | Tabriz GenBank |
| sco558 | *Artemisia scoparia* | Arasbaran-Hasratan, East Azerbaijan | 350 | Tabriz GenBank |
| sco790 | *Artemisia scoparia* | Jolfa to siyahrood, East Azerbaijan | 750 | Tabriz GenBank |
| inc309 | *Artemisia incana* | Jazireye Islami, East Azerbaijan | 1450 | Tabriz GenBank |
| inc757 | *Artemisia incana* | Osku - Gonbarf, East Azerbaijan | 1950 | Tabriz GenBank |
| inc788 | *Artemisia incana* | Tabriz – Kahlik Bulaghi, East Azerbaijan | 1550 | Tabriz GenBank |
| spi301 | *Artemisia spicigera* | Jolfa – Darediz, East Azerbaijan | 1100 | Tabriz GenBank |
| spi318 | *Artemisia spicigera* | Jolfa to siyahrood, East Azerbaijan | 750 | Tabriz GenBank |
| spi811 | *Artemisia spicigera* | Maku, West Azerbaijan | 1500 | Urmiyeh GenBank |
| aus137 | *Artemisia austriaca* | Marand-Misho Daghi, East Azerbaijan | 1950 | Tabriz GenBank |
| aus764 | *Artemisia austriaca* | Tabriz-Zinjanab, East Azerbaijan | 2200 | Tabriz GenBank |
| aus778 | *Artemisia austriaca* | Bostanabad-Yousefabad, East Azerbaijan | 1970 | Tabriz GenBank |
| vul727 | *Artemisia vulgaris* | Arasbaran-Arvin, East Azerbaijan | 1360 | Tabriz GenBank |
| vul812 | *Artemisia vulgaris* | Khoy – Dare Gotur, West Azerbaijan | 1700 | Urmiyeh GenBank |
| Vul*3 | *Artemisia vulgaris* | Kaleybar ,2 Km to Makidi, East Azerbaijan | 1400 | Tabriz GenBank |
| ann411 | *Artemisia annua* | Arasbaran-Kalaleye sofla, East Azerbaijan | 1000 | Tabriz GenBank |
| ann806 | *Artemisia annua* | Arasbaran-Ebrahimbayglo, East Azerbaijan | 550 | Tabriz GenBank |
| ann812 | *Artemisia annua* | Tabriz, East Azerbaijan | 1370 | Tabriz GenBank |
| abs174 | *Artemisia absinthium* | Arasbarn–Agdash-Marzgar, East Azerbaijan | 2400 | Tabriz GenBank |
| abs820 | *Artemisia absinthium* | Chamaki, Golestan | 65 | Golestan GenBank |
| abs*6 | *Artemisia absinthium* | Sharabad to Maraveh tape, Khorasan | 700 | Khorasan GenBank |
| sib821 | *Artemisia sieberi* | Tilabad, Khorasan | 105 | Khorasan GenBank |
| sib*6 | *Artemisia sieberi* | Gonabad, Khorasan | 1200 | Khorasan GenBank |
| bin178 | *Artemisia biennis* | Khajeh – Garetape, East Azerbaijan | 1400 | Tabriz GenBank |
| bin*15 | *Artemisia biennis* | Bajg –Khorasan, Bajg, Khorasan | 1774 | Khorasan GenBank |
| dif436 | *Artemisia diffusa* | Bojnord–Baghlog, Khorasan | 1350 | Khorasan GenBank |
| dif*12 | *Artemisia diffusa* | Bojnord–Khorkhor, Khorasan | 900 | Khorasan GenBank |
| dif*14 | *Artemisia diffusa* | Bojnord–Khorkhor, Khorasan | 800 | Khorasan GenBank |
| cam679 | *Artemisia campestris* | Ahar –Horand, East Azerbaijan | 1200 | Tabriz GenBank |
| cam507 | *Artemisia campestris* | Aahar –Meshkinshahr, East Azerbaijan | 1200 | Tabriz GenBank |
| cam671 | *Artemisia campestris* | Aahar –Meshkinshahr, East Azerbaijan | 1300 | Tabriz GenBank |
| cha23 | *Artemisia chamaemelifolia* | Arasbaran–Marzgar, East Azerbaijan | 2300 | Tabriz GenBank |
| cha96 | *Artemisia chamaemelifolia* | Arasbaran–Iylankesh, East Azerbaijan | 2200 | Tabriz GenBank |
| cha464 | *Artemisia chamaemelifolia* | Arasbaran–Marzgar, East Azerbaijan | 2500 | Tabriz GenBank |
| kop*18 | *Artemisia kopedaghensis* | Bojnord–gardoneye bio, Khorasan | 1820 | Khorasan GenBank |
| auc*1 | *Artemisia aucheri* | Kordyane Sofan, Khorasan | 1600 | Khorasan GenBank |

## RESULTS

The 17 morphological characters, which found to be capable of differentiating the *Artemisia* species were listed in Table 3. The principal components analysis of these 17 morphological characters showed that the first two components contributed 93.8% of the total variance (Table 4, Fig. 1). In the biplot ordination diagram (Figure 1) the vector X5 representing Leaf color ad axial was longest and completely overlapping with the X axis, and therefore, had strongest impact on differentiating species. At next step, the vectors X4, X14 and X3 had relatively the longest length indicating their importance in species separation. PCA clearly separated homogamous species from heterogamous ones as the former was situated in the first area, while the later species were nested in the second area of the plot. This analysis also showed that the majority of the variability (88 %) was related to the first main component, which was mainly caused by the four characters of cauline leaf type, leaf color adaxial, leaf color abaxial and capitalism attachment.

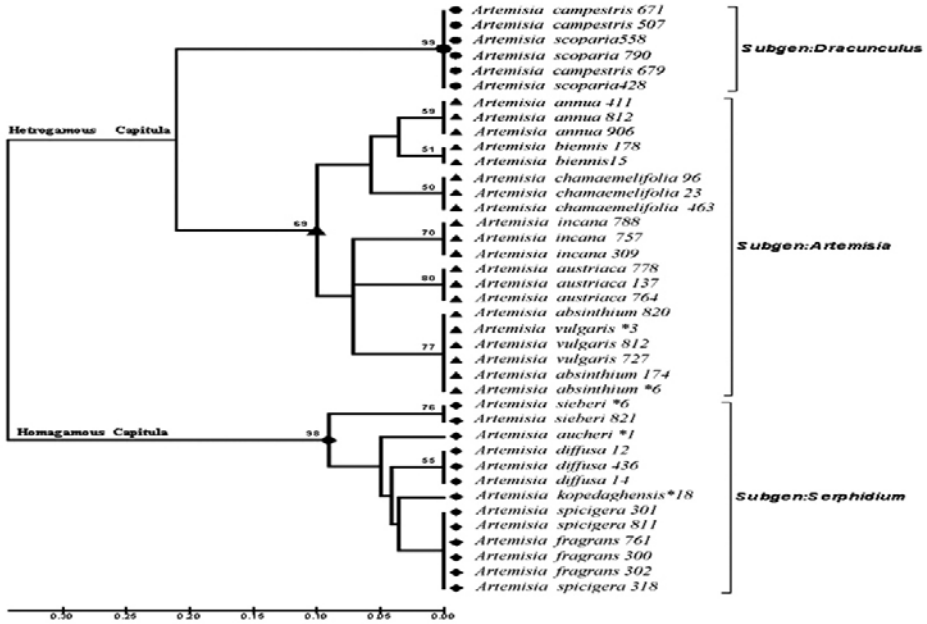


Figure 1. Taxonomic character dendrogram created by the UPGMA method based on p-distance of cluster analysis (Bootstrap values are shown when >50%

Table 2. The morphological qualitative characters used in the multivariate analyses

| Variable | Traits | Character description |
|---|---|---|
| $X_1$ | Stem color | Brown to Red (1) Green to light Brown (2) Gray (3) Yellow (4) |
| $X_2$ | Stem ornamentation | Absent (0) Present (1) |
| $X_3$ | Stem surface hair | Absent (0) Present (1) |
| $X_4$ | Cauline leaf type | Palmatifid (1) Deepl Palmatisect (2) |
| $X_5$ | Leaf color ad axial | Gray (1) Green (2) |
| $X_6$ | Leaf color ab axial | Gray (1) Green (2) |
| $X_7$ | Leaf surface ad axial hair | Absent (0) Present (1) |
| $X_8$ | Leaf surface ab axial hair | Absent (0) Present (1) |
| $X_9$ | Leaf attachment (upper leaves) | Absent (0) Present (1) |
| $X_{10}$ | Leaf attachment (lower leaves) | Absent (0) Present (1) |
| $X_{11}$ | Leaf pri-segments | Linear (1) oblong (2) lanceolate (3) acute (4) |
| $X_{12}$ | Auricles | Absent (0) Present (1) |
| $X_{13}$ | Inflorences | Wide panicle (0) Narrow panicle ( 1) |
| $X_{14}$ | Capitulum attachment | Sessile (1) Present peduncle (2) |

| | | |
|---|---|---|
| $X_{15}$ | Phyllaries surface glabrous | Absent (0) Present (1) |
| $X_{16}$ | Florets color | Yellow (1) Red (2) |
| $X_{17}$ | Capitulum shape | Ovate (1) Oblong (2) Linear (3) Globose (4) Broadly Ovate to Globose (5) Elliptic (6) |

Table 3. Eigen vector of a 2-Dimensional for 17 characters of 39 Artemisia Taxon

| Variable | PCA variable loadings | Axis 1 | Axis 2 |
|---|---|---|---|
| $X_1$ | Stem color | 0.209 | 0.148 |
| $X_2$ | Stem ornamentation | 0.135 | -0.342* |
| $X_3$ | Stem surface hair | 0.106 | 0.3 |
| $X_4$ | Cauline leaf type | 0.475* | 0.316 |
| $X_5$ | Leaf color ad axial | 0.518* | 0.003 |
| $X_6$ | Leaf color ab axial | 0.329* | -0.254 |
| $X_7$ | Leaf surface ad axial hair | 0.103 | 0.334* |
| $X_8$ | Leaf surface ab axial hair | 0.061 | 0.356* |
| $X_9$ | Leaf attachment (upper leaves) | 0.103 | -0.188 |
| $X_{10}$ | Leaf attachment (lower leaves) | 0.1 | -0.163 |
| $X_{11}$ | Leaf pri-segments | 0.161 | -0.103 |
| $X_{12}$ | Auricles | 0.08 | 0.28 |
| $X_{13}$ | Inflorences | 0.161 | -0.261 |
| $X_{14}$ | Capitulum attachment | 0.332* | -0.252 |
| $X_{15}$ | Phyllaries surface glabrous | 0.122 | 0.286 |
| $X_{16}$ | Florets color | 0.292 | -0.07 |
| $X_{17}$ | Capitulum shape | 0.147 | 0 |

Table 4. Percentage of variance of variables accounted for by first two components for 17 characters of 39 Artemisia Taxon

| Factors | Axis 1 | Axis 2 |
|---|---|---|
| Eigen values | 117.55 | 7.24 |
| Percentage of variance | 88.41 | 5.44 |
| Cumulative percentage of variance | 88.41 | 93.85 |

Table 5. Canonical Correlation, Eigen values, Percentage of Variance, Percentage of Cumulative Variance for 17 characters of 39 Artemisia Taxon

| Function | Eigenvalue | % of Variance | Cumulative % | Canonical Correlation |
|---|---|---|---|---|
| 1 | 45,062[a] | 82,7 | 82,7 | ,989 |
| 2 | 9,455[a] | 17,3 | 100,0 | ,951 |

a. First two canonical discriminant functions were used in the analysis

Table 6. Wilks' Lambda calculated for 2 function of three subgenus *Artemisia*

| Test of Function(s) | Wilks' Lambda | Chi-square | df | Sig. |
|---|---|---|---|---|
| 1 through 2 | ,002 | 197,665 | 18 | ,000 |
| 2 | ,096 | 75,105 | 8 | ,000 |

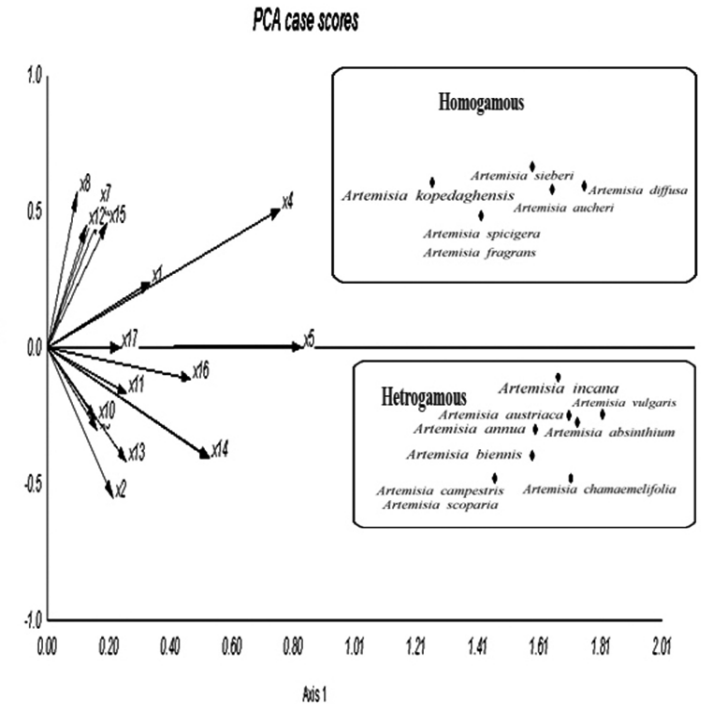


Figure 2. Principal components analysis (bi-plot ordination) of 39 taxon (15 species) of *Artemisia*

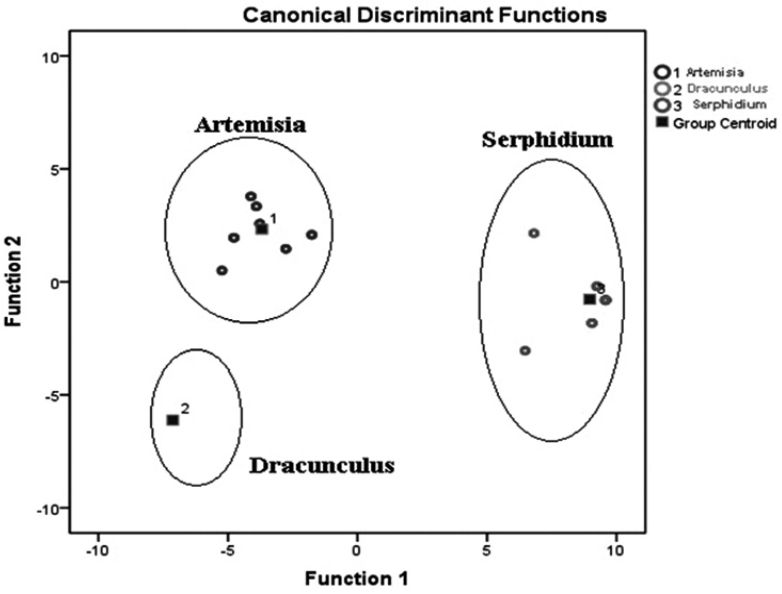


Figure 3. Canonical Discriminant function analysis of 39 taxon (15 species) of *Artemisia*

However, the second principal component indicated small amount of the variation of traits (5%), and this variation was produced by the three traits of stem ornamentation,leaf surface adaxial hair and leaf surface ab-axial hair. This clearly highlights the importance of the first component in recognizing the species under study. Cluster analysis (UPGMA method)of 17 morphological characters clearly separated 39 genotypes belonging of 15 specie into three distinct clusters,which completely corresponded to the subgenera classification (Figure 2).

In this tree, the subgenera *Artemisia* and *Dracunculus* were found to be more closely related to each other than either to the subgenus *Serphidium*.The *Dracunculus* cluster included six genotypes belonging to the two species *A. campestris* and *A. scoparia*.The subgenus *Artemisia* cluster included 20 genotypes of seven species(*A. annua, A. biennis, A. chamemelifolia, A. incana, A. austriaca, A. absinthium* and *A. vulgaris*),and the third cluster comprises of 13 genotypes of six species of *Serphidium* subgenus (*A.sieberi, A.aucheri, A. diffusa, A. kopedaghensis, A. spicigera* and *A fragrans*).

CDFA analysis confirmed the UPGMA clustering of the subgenera by the first function at 82%, and by 100% overall function (Tables 5),and further analysis of this classification of subgenera by Wilks' Lambda confirmed that the separation of the species into three subgenera was significant $P < 0.001$. (Table 6).

## DISCUSSION

This study showed that only 7 out of 17 morphological characters widely used by many flora in designing keys for recognizing of *Artemisia* species are useful for taxonomic purposes, since these characters were revealed to be capable of recognizing the species and grouping them in their own subgenus group.However, anatomical characters were reported not to be useful in the taxonomy of the genus at subgenera levels (Noorbakhsh *et al*. 2008). Our results indicate that morphological characters are useful enough to separate taxa at species levels. In addition, Ovstedal and Mjaavatten(1992) have shown that application of leaf and floral morphological characters were able to recognize variation among populations of *Artemisia norvegica* by differentiating Scottish populations from Norway ones. Furthermore, foliar epidermal anatomical characteristics have been shown to be valuable in resolving taxonomic issues in the genus *Artemisia* (Ashraf *et al*. 2010). Nazar and Mahmood (2010) have used the 26 morphological characters including those of characters used in the current study for investigating their taxonomic power in populations of three *Artemisia* species.However, they did not apply PCA analysis to determine the most important characteristics separating the species. Our results obtained from morphological application to taxonomy of *Artemisia* are consistent with those results previously reported on the basis of morphology (Rydberg, 1916, Beetle, 1960), anatomy (Moss, 1940, Carlquist, 1966, Shultz, 1983), cytology (Ward,

1953, McArthur and Plummer, 1978, McArthur *et al*. 1981), and chemistry (Irwin 1971, Hanks *et al*. 1973, Geissman and Irwin, 1974; Kelsey, 1974). Moreover, the results obtained in the current work are consistent with those data we previously obtained from nuclear ITS and chloroplast PSba-*trn*H sequences using the same plant materials (unpublished data).This consistency between morphological and DNA sequencing data provides further supports for the reliability of our morphological application in resolving taxonomic issues in the genus *Artemisia*,and suggest that these characters can be used in revision of taxonomic keys for the genus.

## CONCLUSION

This study strongly suggests that the traditional taxonomy based on descriptive morphological characteristics widely used in almost all Flora must be re-viewed based on morphological analysis using different multivariate analyses e.g. PCA (Principal Component Analysis), Cluster Analysis and CDFA (Canonical Discriminant Function Analysis) in order to determine the validity and importance of morphological characters in plant taxonomy.

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## PRIMJENJIVOST MORFOLOŠKIH KARAKTERISTIKA U TAKSONOMIJI *ARTEMISIA* (ASTERACEAE)

### SAŽETAK

Velik broj različitih morfoloških karakteristika korišćen je u mnogim radovima u pripremi ključeva za taksonomsku identifikaciju taksona u rodu *Artemisia* (Asteraceae). U ovom radu, izvršen je temeljan pregled različitih radova i objavljenih radova i literature o primjeni kvalitativnih morfoloških karakteristika u taksonomiji *Artemisia*, od čega je 17 najviše korišćenih karaktera ispitivano po njihovom značaju u taksonomiji roda. Izvršena je multivarijantna analiza pomoću nekoliko statističkih paketa, a to su Principal Component Analysis (PCA) za detekciju najvažnijih karaktera koje diferenciraju vrste, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) za analizu klastera, Canonical Discriminant Function Analysis (CDFA) kao potvrda rezultata formiranja klastera. Sve ove analize su pokazale da je od 17 ispitivanih morfoloških karaktera, samo 7 bilo korisno za identifikaciju vrste i grupisanje podrodova, i jasno je razdvajalo homogamne vrste od heterogamnih, ukazujući na značaj morfologije kapituluma u taksonomiji *Artemsia* na nivou podroda. Naši podaci su pokazali da su četiri karaktera: tip lista sa peteljkom, boja adaksijalne strane lista, boja abaksijalne strane lista i postavljenost kapituluma uzrokovali najveći dio varijabilnosti. Filogenetska analiza zasnovana na morfologiji pokazala je da su podrodovi *Artemisia* i *Dracunculus* međusobno mnogo bliži nego sa podrodom *Serphidium.* Ovaj rad naglašava značaj morfologije u primjeni taksonomije i identifikaciji taksona.

**Keywords:** *Artemisia*, *Dracunculus*, morfološke odlike, multivarijantna statistička analiza, filogenija, *Serphidium*