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Phylogenetic relationships among *Artemisia* species based on nuclear ITS and chloroplast *psbA-trnH* DNA markers

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Abstract: The taxonomic and phylogenetic relationships within the genus *Artemisia* s.l. (Asteraceae) are controversial, and it has been considered 1 to 8 different genera. This work re-investigated the phylogenetic relationships in *Artemisia* using nuclear ribosomal (*ITS*) and chloroplast *psbA-trnH* DNA sequences using three sections of *Artemisia, Dracunculus*, and *Serphidium*. Three phylogenetic trees were conducted separately on the basis of ITS, *psbA-trnH* and combined sequences using maximum parsimony. The results showed that the three sections were clearly separated from each other, and that the heterogamous *Dracunculus* and *Artemisia* are closely related to each other than either to homogamous *Serphidium*. This may suggest the taxonomic importance of capitulum morphology in *Artemisia* s.l. Our data also cast doubt on the use of cytogenetic similarity e.g., basic chromosome number in grouping *Serphidium* and *Artemisia* s.s. Furthermore, AMOVA analysis showed a higher level of ITS (55.29%) and combined ITS+cp*psbA-trnH* (55.63%) variations among sections. This provides further evidence for separation of these three sections and supports the phylogenetic results. The higher ITS nucleotide differences detected in *Artemisia* (30.4737) compared to very low value in *Dracunculus* (2.3333) and *Serphidium* (1.23077) may propose that the *Artemisia* comprises of several incipient sections. This supports the previous suggestion that *Artemisia* is a complex group.

Key words: *Artemisia*; chloroplast *psbA-trnH* DNA; *Dracunculus*; Nuclear ITS; *Serphidium*

Introduction

The genus *Artemisia* L. (Asteraceae) comprises of approximately 400 to 500 species (McArthur & Pope 1979; Ling 1991, 1995a,b; Bremer & Humphries 1993). The genus has been classified in 5 main groups, which differently considered as sections or subgenera (Torrell et al. 1999). The species of *Artemisia* are widely distributed in the Northern hemisphere and almost absent from the Southern hemisphere and are almost perennial species, with a few (< 10) annual species. The basic chromosome number in the genus is mostly 9 and the ploidy levels range from diploid to dodecaploid. In addition, basic chromosome number of 8 is less frequent in species having ploidy levels from diploid to hexaploid (Kawatani & Ohno 1964; Valles & Siljak-Yakovlev 1997; Valles & McArthur 2001). *Artemisia* includes many species of commercial uses e.g., *A. absinthium* L. (absinth), *A. genipi* G. Weber in Stechm. using for liquors, *A. dracunculus* L. (tarragon) as culinary herbs, *A. santonica* L. as antihelminthic, *A. annua* L. as antimalarial, *A. arborescens* L. as ornamentals, and A. *vulgaris* L. using for the landscape renovation. However, some species e.g., *A. verlotiorum* Lamotte, are invasive weeds causing damage to agriculture.

The taxonomy of the genus *Artemisia* s.l. is based on floral and capitular morphology (Watson et al. 2002). In *Serphidium* (Besser) the ray florets are reduced to a membranous vestige, consequently the capitulum lacks ray florets, and composes only of hermaphrodite disc florets. This capitulum is called homogamous. The capitulum in subgenera e.g., *Dracunculus* Besser, *Abrotanum* Besser, *Artemisia* and *Absinthium* Mill. has two sorts of florets: ray pistillate florets and hermaphrodite or staminate discs florets. This capitulum is called heterogamous (Bremer & Humphries 1993). The taxonomic relationships within the genus *Artemisia* s.l. is controversial, and over the past 50 years different taxonomic treats have been conducted ranging from a single large genus with over 500 species (Cronquist 1955; Kornkven et al. 1998; Kornkven et al. 1999; Torrell et al. 1999; Martin et al. 2001) to the recognition of six to eight genera (Bremer & Humphries 1993; Poljakov 1961; Ling 1994). In the

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Table 1. The sources and identification of *Artemisia* species used in phylogenetic study.

Code	Species	Location	Altitude (m)	Source/identification
fra300	*Artemisia fragrans* Willd.	Ajabshir to Azarshahr, East Azerbaijan	1490	Tabriz GenBank
fra302	*Artemisia fragrans* Willd.	Ahar–Meshkinshahr, East Azerbaijan	1450	Tabriz GenBank
fra761	*Artemisia fragrans* Willd.	Osku–Gonbarf, East Azerbaijan	2062	Tabriz GenBank
sco428	*Artemisia scoparia* Waldst. & Kit.	Tabriz, East Azerbaijan	1370	Tabriz GenBank
sco558	*Artemisia scoparia* Waldst. & Kit.	Arasbaran–Hasratan, East Azerbaijan	350	Tabriz GenBank
sco790	*Artemisia scoparia* Waldst. & Kit.	Jolfa to siyahrood, East Azerbaijan	750	Tabriz GenBank
inc309	*Artemisia incana* Druce.	Jazireye Islami, East Azerbaijan	1450	Tabriz GenBank
inc757	*Artemisia incana* Druce.	Osku–Gonbarf, East Azerbaijan	1950	Tabriz GenBank
inc788	*Artemisia incana* Druce.	Tabriz–Kahlik Bulaghi, East Azerbaijan	1550	Tabriz GenBank
spi301	*Artemisia spicigera* C. Koch.	Jolfa–Daredіz, East Azerbaijan	1100	Tabriz GenBank
spi318	*Artemisia spicigera* C. Koch.	Jolfa to siyahrood, East Azerbaijan	750	Tabriz GenBank
spi811	*Artemisia spicigera* C. Koch.	Maku, West Azerbaijan	1500	Urmiyeh GenBank
aus137	*Artemisia austriaca* Jacq.	Marand–Misho Daghi, East Azerbaijan	1950	Tabriz GenBank
aus764	*Artemisia austriaca* Jacq.	Tabriz–Zinjanab, East Azerbaijan	2200	Tabriz GenBank
aus778	*Artemisia austriaca* Jacq.	Bostanabad–Yousefabad, East Azerbaijan	1970	Tabriz GenBank
vul727	*Artemisia vulgaris* L.	Arasbaran–Arvin, East Azerbaijan	1360	Tabriz GenBank
vul812	*Artemisia vulgaris* L.	Khoy–Dare Gotur, West Azerbaijan	1700	Urmiyeh GenBank
Vul*3	*Artemisia vulgaris* L.	Kaleybar, 2 Km to Makidi, East Azerbaijan	1400	Tabriz GenBank
ann411	*Artemisia annua* L.	Arasbaran–Kalaleye sofla, East Azerbaijan	1000	Tabriz GenBank
ann806	*Artemisia annua* L.	Arasbaran–Ebrahimbayglo, East Azerbaijan	550	Tabriz GenBank
ann812	*Artemisia annua* L.	Tabriz, East Azerbaijan	1370	Tabriz GenBank
abs174	*Artemisia absinthium* L.	Arasbarn–Agdash–Marzgar, East Azerbaijan	2400	Tabriz GenBank
abs820	*Artemisia absinthium* L.	Chamaki, Golestan	65	Golestan GenBank
abs*6	*Artemisia absinthium* L.	Sharabad to Maraveh tape, Khorasan	700	Khorasan GenBank
sib821	*Artemisia sieberi* Besser.	Tilabad, Khorasan	105	Khorasan GenBank
sib*6	*Artemisia sieberi* Besser.	Gonabad, Khorasan	1200	Khorasan GenBank
bin178	*Artemisia biennis* Willd.	Khajeh–Garetape, East Azerbaijan	1400	Tabriz GenBank
bin*15	*Artemisia biennis* Willd.	Bajg–Khorasan, Bajg, Khorasan	1774	Khorasan GenBank
diff436	*Artemisia diffusa* Krasch. ex Poljakov	Bojnord–Baghlog, Khorasan	1350	Khorasan GenBank
diff*12	*Artemisia diffusa* Krasch. ex Poljakov	Bojnord–Khorkhor, Khorasan	900	Khorasan GenBank
diff*14	*Artemisia diffusa* Krasch. ex Poljakov	Bojnord–Khorkhor, Khorasan	800	Khorasan GenBank
cam679	*Artemisia campestris* L.	Ahar–Horand, East Azerbaijan	1200	Tabriz GenBank
cam507	*Artemisia campestris* L.	Aahar–Meshkinshahr, East Azerbaijan	1200	Tabriz GenBank
cam671	*Artemisia campestris* L.	Aahar–Meshkinshahr, East Azerbaijan	1300	Tabriz GenBank
cha23	*Artemisia chamaemelifolia* Vill.	Arasbaran–Marzgar, East Azerbaijan	2300	Tabriz GenBank
cha96	*Artemisia chamaemelifolia* Vill.	Arasbaran–Iylankesh, East Azerbaijan	2200	Tabriz GenBank
cha464	*Artemisia chamaemelifolia* Vill.	Arasbaran–Marzgar, East Azerbaijan	2500	Tabriz GenBank
kop*18	*Artemisia kopedaghensis* Krasch., M. Pop. & Lincz. ex Poljak.	Bojnord–gardoneye bio, Khorasan	1820	Khorasan GenBank
auc*1	*Artemisia aucheri* Boiss.	Kordyane Sofan, Khorasan	1600	Khorasan GenBank

past, the genus *Artemisia* had been divided into three genera of *Artemisia, Absinthium,* and *Abrotanum*, but later all these three genera were considered as a single genus by Linnaeus. Moreover, De Candolle (Valles & McArthur 2001) recognized four sections within the genus primarily based on the presence or absence of ray florets and the fertility and sterility of disc florets: (1) *Abrotanum* Besser having ray fertile pistillate florets; disc perfect fertile florets situating on glabrous receptacle, (2) *Absinthium* (Mill.) DC with ray pistillate fertile florets and disc perfect fertile florets on the hairy receptacle, (3) *Serphidium* (Besser) Besser lacking ray florets, but having disc perfect fertile florets sitting on glabrous receptacle, and (4) *Dracunculus* Besser having ray pistillate fertile florets with disc functionally staminate florets situating on the glabrous receptacle. The phylogenetic relationship among the subgenera has been also controversial. The first phylogenetic investigation based on the evolutionary concept of loss of fertility in the disc florets and loss of ray florets confirmed these four sections and proposed section *Artemisia* as the progenitor to the others (Hall & Clements1923). However, section *Artemisia* and *Absinthium* were later united and all sections were raised to the level of subgenus (Rydberg 1916). Moreover, the subgenus *Serphidium* has been taxonomically treated differently. Many taxonomist separated *Serphidium* as a distinct genus from *Artemisia* (e.g., Kornkven et al. 1998, 1999; Torrell et al. 1999), while Torrell et al. (2001) latter disagreed with this separation, and included *Serphidium* within *Artemisia*.

The current work is aimed to re-investigate the phylogenetic relationships in *Artemisia* using nuclear ribosomal DNA (ITS) and chloroplast *psbA-trnH* sequences.

Material and methods

Species study

15 species were included in the study from the three sections of *Artemisia*, *Dracunculus*, and *Serphidium*, by enclosing a minimum of 2–3 genotypes of each species making overall 39 taxa (Table 1). The haplotype of each species was used in the analysis in order to avoid any individual variations. The

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Table 2. ITS and *psbA–trnH* sequences of *Anthemis arvensis* used as outgroup for rooting the phylogenetic trees of the sections *Artemisia*, *Serphidium* and *Dracunculus*.

Locus	Species	Submission date	Genome	Date
gi|171190837|gb| EU547792.1|	*Anthemis arvensis* L. PsbA (psbA) gene, partial cds; *psbA-trnH* intergenic spacer, complete sequence; and tRNA–His (trnH) gene, partial quence;	Submitted (06-MAR-2008) Federal Institute for Drugs and Medical Devices, Kurt-Georg-Kiesinger–Allee 3, Bonn D 53175, Germany	cpDNA	PLN 02-APR-2008
gi|158266469|gb| EU179214.1|	*Anthemis arvensis* L. 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	Submitted (26-SEP-2007) Federal Institute for Drugs and Medical Devices, Kurt-Georg-Kiesinger–Allee 3, Bonn D 53175, Germany, Location/Qualifiers	nrDNA	PLN 20-OCT-2007

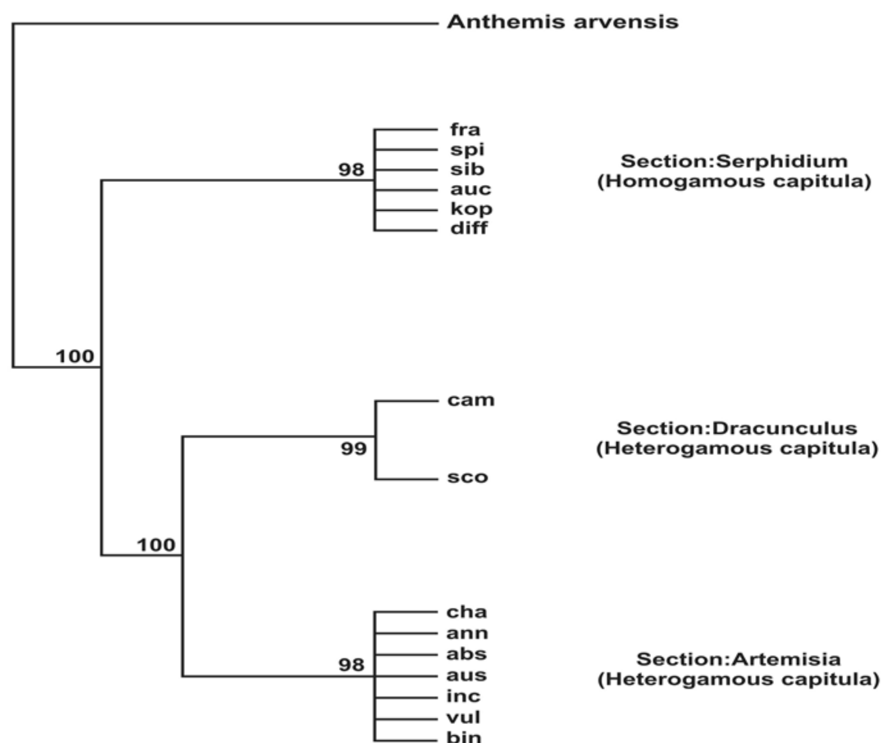

Fig. 1. Majority-rule consensus tree obtained from Maximum Parsimony analysis based on nuclear rDNA ITS sequences data of *Artemisia* species and the outgroup *Anthemis arvensis*. Bootstrap values above branches are based upon 100 replicate (Consistency Index = 0.9118, Retention Index = 0.967, Rescaled Consistency Index = 0.8817)

haplotypes recognition and analysis were carried out using DNASp Ver. 5. *Anthemis arvensis* was included in the study as an out-group using its ITS and *psbA-trnH* sequence data presented in NCBI (Table 2).

DNA extraction and PCR profile

After disinfecting the seeds by sodium hypochloride (20%) for 10 min, total genomic DNA was extracted from the seeds following Ziegenhagen et al. (2003). PCR reaction was made using 27 μL of master mix (Fermantas kit), 1 μL of 50–100 ng of DNA, 1 μL of a mixed-primers (10 pmol), and 21 μL deionized water, making overall PCR reaction 50 μl in a Quanta Biotech thermo-cycle (Quanta Co., England). The PCR profile conducted for amplification of nuclear ITS region was as follow: pre-denaturation at 94℃ for 2 min, followed by 35 cycles of denaturation at 94℃ for 50 s, annealing at 56℃ for 40 s, and extensions at 72℃ for 45 s, with final extension at 72℃ for 5 min. This profile for chloroplast *psbA-trnH* was as follow: pre-denaturation at 94℃ for 2 min, followed by 35 cycles of denaturation at 94℃ for 30 s, annealing at 50℃ for 30 s, extension at 72℃ for 1 min, with final extension at 72℃ for 10 min. The electrophoresis of PCR products were carried out at 85 voltages for 30 min in 1% Agarose gels, and consequently visualized under UV light. The size of the amplified region was determined on the gel using 100 bp DNA standard size markers (Fermentas Company). After purification, the amplified PCR products were sequenced (Macrogen Company) using only PCR products. The primer sequences used for amplifying ITS and *psbA-trnH* regions (White et al. 1990; Kress et al. 2005) were

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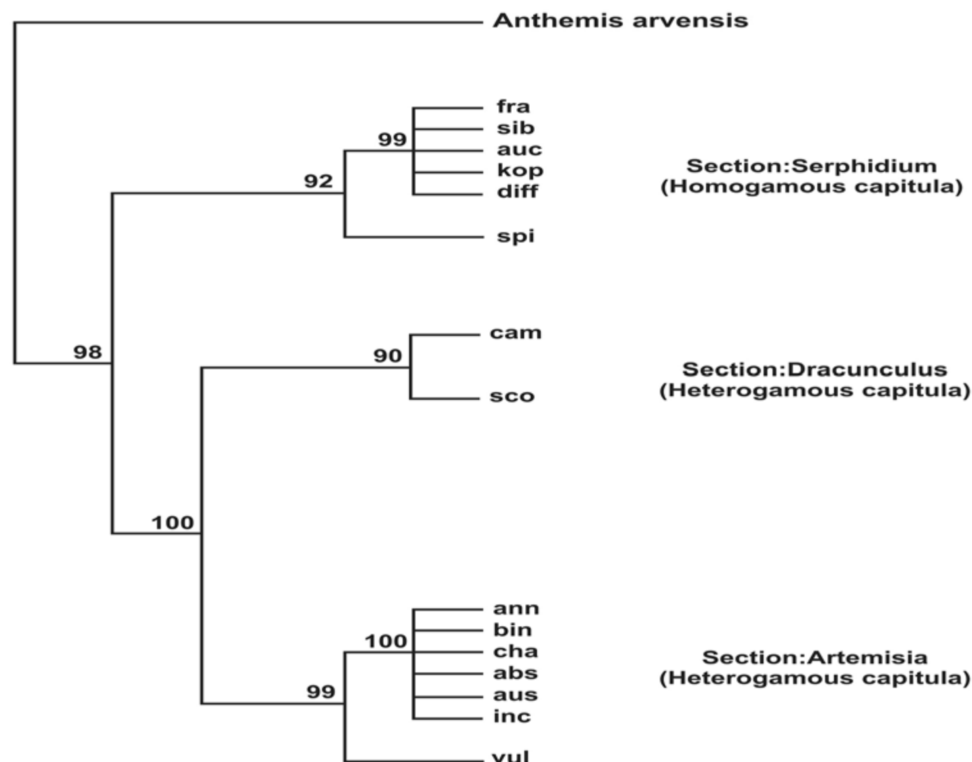

Fig. 2. Majority-rule consensus tree from parsimony analysis of *psbA-trnH* data of *Artemisia* species and the outgroup *Anthemis arvensis*. Bootstrap values above branches are based upon 100 replicate (Consistency Index = 0.9211, Retention Index = 0.965, Rescaled Consistency Index = 0.888)

as follow:
ITS4 (5'-TCCTCCGCTTATTGATATGC-3')
ITS5 (5'-GGAAGGAGAAGTCGTAACAAGG-3')
Chloroplast
F (5'-CGCGCATGGTGGATTCACAATCC-3')
R (5'-GTTATGCATGAACGTAATGCTC-3')

Statistical Analysis
The sequence alignment was performed using MAFFT online multiple alignment tools. To infer the phylogenetic relationships among species studied, Modeltest 3.7 and PAUP 4.0b10 (Posada & Crandall 2005; Felsenstein 1985; Swofford 2002) were used for cluster analysis of sequences.

Analysis of molecular variance (AMOVA) was used to estimate within- and among-sections differentiation using Arlequin 3.11 (Excoffier et al. 1992, 2005), and the significance level for F_{st} was determined using 1023 permutation tests. Average number of nucleotide differences, nucleotide diversity and number of polymorphic sites in the examined taxa were calculated using DNASp Ver5 (Librado & Rozas 2009). The nucleotide compositions in all species were calculated using a MEGA5 package (Tamura et al. 2011).

Results

The length of ITS sequence (including ITS1, 5.8S and ITS2 regions) in the species studied ranged from 672 base pairs in *Artemisia vulgaris* to 707 in *A. biennis*, while this range for cpDNA *Psba-Trnh* sequence was 420–465 bp in *A. kopetdaghensis* and *A. scoparia*, respectively. The best suitable evolutionary model for ITS sequences was found to be SYM+G, while it was TVM+I model for *psbA-trnH* sequences, and TIM+I+G model for combined ITS+*psbA-trnH* sequence data. The three phylogenetic analyses conducted separately for ITS, *psbA-trnH* and combined sequences using maximum parsimony showed that the all three sections of *Serphidium*, *Artemisia* and *Dracunculus* were clearly separated from each other (Figs 1–3). However, all three dendrograms showed that the sections *Artemisia* and *Dracunculus* are more closely related to each other than either to section *Serphidium*.

In the tree based on rDNA ITS sequence, the species belonging to both *Serphidium* and *Artemisia* were not separated from each other (Fig. 1), while in *psbA-trnH* and combined ITS+*psbA-trnH* trees, only species of *Artemisia spicigera* from section *Serphidium* and *Artemisia vulgaris* from section *Artemisia* were isolated from the rest species (Figs 2, 3).

The AMOVA test showed that 55.29% ITS variation belongs to among the sections, while this variation was 41.52% on the basis of *psbA-trnH* and 55.63% based on combined ITS+*psbA-trnH* (Table 3). The average numbers of nucleotide difference, nucleotide diversity and number of polymorphism sites of ITS and *psbA-trnH* regions are shown in (Table 4). The section *Artemisia* showed higher levels of these indices for all three genomic regions studied while the section *Serphidium* indicated the lowest amount, and the section *Dracunculus* had intermediate level of the indices.

Discussion

The all three trees based on ITS, *psbA-trnH* and com-

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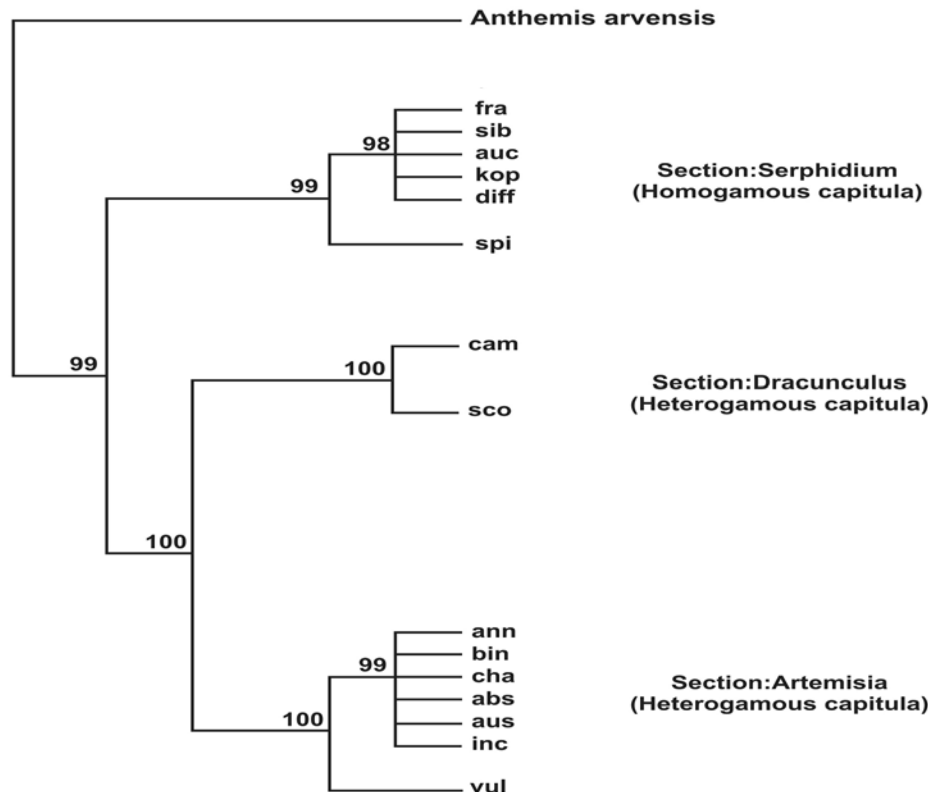

Fig. 3. Majority-rule consensus tree obtained based on Maximum Parsimony analysis of combined nuclear rDNA ITS and chloroplast *psbA-trnH* sequences of *Artemisia* species and the outgroup *Anthemis arvensis.* Bootstrap values above branches are based upon 100 replicate (Consistency Index = 0.9229, Retention Index = 0.969, Rescaled consistency Index = 0.894).

Table 3. AMOVA analysis for sequence variation among 39 genotypes belonging to 15 species of the three sections of *Artemisia*, *Serphidium* and *Dracunculus.*

Source of variation	d. f.	Sequence variation (%)			Significance tests *
		ITS	*psbA-trnH*	*ITS* + *psbA-trnH*	
Among sections	2	55.29	41.52	55.63	0.00000
Within sections	36	44.71	58.48	44.37	0.00000

*1023 permutations

Table 4. Sequence specifications in the sections of *Artemisia, Serphidium* and *Dracunculus.*

Varicodes	Combined: 1270bp			*ITS*: 742 bp			*psbA-trnH*: 538 bp		
	Artemisia	*Dracunculus*	*Seriphidium*	*Artemisia*	*Dracunculus*	*Serphidium*	*Artemisia*	*Dracunculus*	*Serphidium*
1	20	6	13	20	6	13	20	6	13
2	34.67368	3.867	4.69231	30.47368	2.33333	1.23077	2.88947	1.20000	0.38462
3	0.03241	0.00361	0.00439	0.04617	0.00354	0.00186	0.00705	0.00293	0.00094
4	108	17	10	91	9	8	11	2	1

Variable codes: 1 – No. of sequences, 2 – Average No. of nucleotide differences, 3 – Nucleotide diversity, 4 – No. of polymorphic sites.

bined data showed that the heterogamous *Dracunculus* and *Artemisia* s.s. are closely related to each other than either to homogamous *Serphidium.* This may suggest that the capitulum morphology and lack of ray florets have the taxonomic importance in the classification of *Artemisia* s.l. The results of the current study also indicate that the use of cytogenetic similarity such as basic chromosome number in grouping *Serphidium* and *Artemisia* (Torrell et al. 2003) is not a reliable character.

Our results are also consistent with Bremer & Humphries (1993) who treated the section *Serphidium* as separate genus from *Artemisia* s.l., and disagree with other taxonomists such as Kornkven et al. (1998, 1999), Torrell et al. (1999), who classified *Serphidium* within *Artemisia.*

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The very low genetic distances among species of the section *Artemisia* s.s. show that they were very recently isolated from the common ancestor. This is also the case in species of the section *Serphidium*. This may suggest that speciation in these two groups is a recent event.

Higher level of ITS (55.29%) and combined ITS+cp*psbA-trnH* (55.63%) variations detected among sections by AMOVA analysis provides further evidence for separation of these sections. However, lower level of cp*psbA-trnH* variation (41.52%) provides weak support for this separation, indicating lower evolutionary rate of this genome in *Artemisia*. The higher average number of nucleotide differences in ITS region revealed in *Artemisia* (30.4737) compared to very lower amount in *Dracunculus* (2.3333) and *Serphidium* (1.23077) may suggest that the subgenus *Artemisia* comprises of several incipient sections, and supports the idea that *Artemisia* is a complex group (Persson 1974; Valles-Xirau & Seoane-Camba 1987).

This study showed the capability of ITS and cp*psbA-trnH* sequence data in systematic revision of problematic taxa at intra-genus level in plants.

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References

Bremer K.R. & Humphries C.J. 1993. Generic monograph of the Asteraceae-Anthemideae. Bull. Nat. Hist. Mus. Bot. Ser. **23:** 71–177.

Cronquist A. 1955. Phylogeny and taxonomy of the Compositae. Amer. Midland Natural. **53:** 478–511.

Excoffier L., Laval G. & Schneider S. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinform. Online **1:** 47–50.

Excoffier L., Smouse P.E. & Quattro J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131:** 479–491.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:** 783–791.

Hall H.M. & Clements F.E. 1923. The phylogenetic method in taxonomy: the North American species of *Artemisia*, *Chrysothamnus*, and *Atriplex*. Carnegie Institute of Washington Publication No. 326.

Kawatani T. & Ohno T. 1964. Chromosome numbers in *Artemisia*. Bulletin of the National Institute of Hygienic Sciences **82:** 183–193.

Kornkven A.B., Watson L.E. & Estes J.R. 1998. Phylogenetic analysis of *Artemisia* section Tridentatae (Asteraceae) based on sequences from the internal transcribed spacers (ITS) of nuclear ribosomal DNA. Amer. J. Bot. **85:** 1787–1795.

Kornkven A.B., Watson L.E. & Estes J.R. 1999. Molecular phylogeny of *Artemisia* section Tridentatae (Asteraceae) based on chloroplast DNA restriction site variation. System. Bot. **24:** 69–84.

Kress W.J., Wurdack K.J., Zimmer E.A., Weigt L.A. & Janzen D.H. 2005. Use of DNA barcodes to identify flowering plants. Proc. Natl. Acad. Sci. U.S.A. **102:** 8369–8374.

Librado P. & Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinform. **25:** 1451–1452.

Ling Y. 1991. A review of the classification, distribution and application of *Artemisia* L. and Seriphidium (Bess.) Poljak.(Compositae) in China. Guihaia **11:** 19–35.

Ling Y. 1994. The genera *Artemisia* L. and *Seriphidium* (Bess.) Poljak. in the world. Compositae Newsl. **25:** 39–45.

Ling Y. 1995a. The new world *Artemisia L.* Advances in Compositae systematics. Kew: Royal Botanic Gardens, Kew, pp. 255–281.

Ling Y. 1995b. The New World Seriphidium (Besser) Fourr. Advances in Compositae systematics. Kew: Royal Botanic Gardens, Kew, pp. 283–291.

Martin J., Torrell M. & Valles J. 2001. Palynological features as a systematic marker in *Artemisia* L. and related genera (Asteraceae, Anthemideae). Plant Biol. **3:** 372–378.

McArthur E.D. & Pope C.L. 1979. Karyotypes of four *Artemisia* species: *A. carruthii*, *A. filifolia, A. frigida,* and *A. spinescens*. West. North Amer. Natural. **39:** 419–426.

Persson K. 1974. Biosystematic studies in the *Artemisia maritima* complex in Europe. Op. Bot. **35:** 1–188.

Poljakov P. 1961. Materials and systematics, the genus *Artemisia* L. Trudy Inst. Bot. Alma–Ata **11:** 134–177.

Posada D. & Crandall K. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics **14**: 817–818.

Rydberg P. 1916. *Artemisia* and Artemisiastrum. North American Flora **34:** 244–285.

Swofford D. 2002: PAUP 4.0 b10: Phylogenetic analysis using parsimony. Sinauer Associates, Sunderland, MA, USA.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. **28:** 2731–2739.

Torrell M., Cerbah M., Siljak-Yakovlev S. & Vallčs J. 2003. Molecular cytogenetics of the genus *Artemisia* (Asteraceae, Anthemideae): fluorochrome banding and fluorescence in situ hybridization. I. Subgenus *Seriphidium* and related taxa. Plant Syst. Evol. **239:** 141–153.

Torrell M., Garcia-Jacas N., Susanna A. & Vallčs J. 1999. Phylogeny in *Artemisia* (Asteraceae, Anthemideae) inferred from nuclear ribosomal DNA (ITS) sequences. Taxon **48:** 721–736.

Torrell M., Vallčs J., Garcia-Jacas N., Mozaffarian V. & Gabrieliane E. 2001. New or rare chromosome counts in the genus *Artemisia* L. (Asteraceae, Anthemideae) from Armenia and Iran. Bot. J. Linn. Soc. **135:** 51–60.

Vallčs J. & Siljak-Yakovlev S. 1997. Cytogenetic studies in the genus *Artemisia* L.: fluorochrome banded karyotypes of five taxa, including the Iberian endemic species *Artemisia barrelieri* Besser. Can. J. Bot **75:** 595–606.

Vallčs J. & McArthur E.D. 2001: *Artemisia* systematics and phylogeny: cytogenetic and molecular insights. USDA Forest Service Proceedings, pp. 67–74.

Watson L., Bates P., Evans T., Unwin M. & Estes J. 2002. Molecular phylogeny of subtribe Artemisiinae (Asteraceae), including Artemisia and its allied and segregate genera. BMC Evol. Biol. **2:** 17.

White T.J., Bruns T., Lee S. & Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315–322. In: Innis M., Gelfand D., Sninsky J. & White T., (eds), PCR Protocols: a Guide to Methods and Applications, Acad. Press, Orlando, Florida

Ziegenhagen B., Liepelt S., Kuhlenkamp V. & Fladung M. 2003. Molecular identification of individual oak and fir trees from maternal tissues of their fruits or seeds. Trees **17:** 345–350.

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