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Artemisia herba-alba complex: morphological diversity and molecular uniformity

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Abstract

A study aiming to clarify the taxonomic status of the species in Iran known as *A. herba-alba* (or *A. sieberi*) and *A. kermanensis*, and to illustrate their relationships to *A. aucheri* was performed using morphological and molecular ITS (internal transcribed spacer) and ETS (external transcribed spacer) data. Our results showed that despite their differences in gross morphology, very little differences were observed in their floral morphology and ITS and ETS sequences. All populations belonging to the species named *A. herba-alba*, *A. sieberi*, *A. kermanensis* and *A. aucheri* form a morphologically variable species complex which can not be segregated as different taxonomic units using the ITS and ETS sequencing data. It is also concluded that the morphological diversity in populations of the *A. herba-alba* complex could be related to the phenotypic plasticity associated with climate differences of their habitats.

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Introduction

Artemisia L. is the largest genus of *Asteraceae* tribe *Anthemideae* and comprises ca. 500 species and subspecies worldwide (Valles and McArthur, 2001; Sanz *et al*., 2008; Mahmood *et al*., 2011). The genus includes annual, biennial and perennial species (Valles *et al*., 2003). Three main centers of biodiversity have been reported for the genus; these are central Asia, Mediterranean region and Northwest America (McArthur and Plummer, 1978; Valles and McArthur, 2001; Sanz *et al*., 2008). Based on fossil and phytogeographical data, the genus *Artemisia* is originated in the arid or semi-arid regions of temperate Asia in the mid-Tertiary (Wang, 2004). *Artemisia* species are distributed in diverse ecosystems and altitudes, from dry to moisture environments, and from sea level to high altitudes, at almost 4000 m. They are mostly recognized as prevailing type of vegetations in some localized plant communities, and formed associations with other taxa in steppe regions (Valles and McArthur, 2001). *Artemisia* is also recognized as indicator of steppe region (Erdtman, 1952). *Artemisia* species are widely distributed in the temperate and subtropical areas of the Northern hemisphere, and only a few species can be found on the southern hemisphere (Ling, 1982; Valles and McArthur, 2001). In many species of *Artemisia* flowering occurs from July to November. Many species are clearly wind pollinated (Valles and McArthur, 2001).

Majority of *Artemisia* species found to have a high economical and medicinal importance. Some species are used for food, forage, ornamentals and soil stabilizers in desert or semi-desert areas (Pareto, 1985; Tan *et al*., 1998; Hayat *et al*., 2009). Also some species are used for the treatment of diabetes, high blood pressure and gastrointestinal ailments (Mossa, 1985; Al-Shamaony *et al*., 1994).

Taxonomy of the genus has been controversial. Different authors have divided the genus into different number of taxa below the rank of genus (Valles and McArthur, 2001). For example, Podlech (1986) divided the genus *Artemisia* into three subgenera *Artemisia*, *Seriphidium* Besser and *Dracunculus* Besser. McArthur *et al*. (1981) introduced a new group *Tridentatae* (Rydb.) McArthur, which is only restricted to North America. The genus *Artemisia* is traditionally divided into three subgenera by some authors such as Podlech (1986): 1) *A*. subg. *Artemisia* with heterogamous capitula, glabrous or hairy receptacle, all flowers fertile, marginal ones female, and central ones hermaphrodite; 2) *A*. subg. *Dracunculus* with heterogamous capitula, glabrous receptacle, marginal flowers female, central flowers hermaphrodite, all or inner ones fertile; and 3) *A*. subg. *Seriphidium* with homogamous capitula, glabrous receptacle, all flowers hermaphrodite.

According to Valles and McArthur (2001) *Artemisia* was divided into following five main groups, based mainly on the capitula type and florets fertility: *Absinthium* DC., *Artemisia, Dracunculus, Seriphidium* and *Tridentatae* (Rydb.) McArthur. Ling (1991a, 1995b) Separated *Seriphidium* from *Artemisia* as new genus. Bremer (1994) accepted this separation but Watson *et al*., (2002) again united *Seriphidium* with *Artemisia*. In addition, a number of authors (McArthur and Plummer, 1978; McArthur *et al*., 1981; Kornkven *et al*., 1998, 1999; Rydberg, 1916) suggested that American woody sagebrushes to have an independent origin from the woody Asian species (subg. *Seriphidium*), and recognized them as group *Tridentatae* (Watson *et al*., 2002). Poljakov (1961) and others (Bremer and Humphries., 1993; Bremer, 1994) segregated subg. *Seriphidium* as a independent genus along with several small genera from within the boundaries of *Artemisia*. Pollen morphological data (Martin *et al*., 2001, 2003) confirm the existence of two pollen morphological forms in subtribe *Artemisiinae*: one with long spines (*Anthemis* type) and the other with short spinules (*Artemisia* type). It seems that different species of *Artemisia* gradually degenerated pollen spines to spinules during their evolutionary development (Jiang *et al*., 2005).

Molecular studies based on chloroplast DNA (cpDNA) restriction site variation and internal transcribed

spacers (ITS) of nuclear ribosomal DNA (Kornkven *et al.*, 1998; Torrell *et al.*, 1999; Watson *et al.*, 2002; Valles *et al.*, 2003) have traversed this separation (Pellicer *et al.*, 2007a). However the classification of *Artemisia* and relationships among its different sections still has been very controversial. In the cladogram made by Tkach *et al.*, (2007) based on ITS and ETS sequences, topology does not support the traditional classification in several details. For example, subgenus *Seriphidium* was shown to consist of two segregate groups and three of the four section analyzed within subgenus *Artemisia* were polyphyletic. Sanz *et al.*, (2008) based on the analyses of ETS and ITS sequences showed that combined analysis of *Artemisia* and allies highly supports the monophyly of the genus *Artemisia*.

Boissier (1875) in his Flora Orientalis reported 19 species of *Artemisia* from Iran. Podlech (1986) in Flora Iranica reported 64 species of *Artemisia,* 31 species of them from Iran. In addition, he introduced *A. kermanensis* Podl. as an endemic species to SE Iran. Mozaffarian (1988) reported 34 species of *Artemisia* from Iran. *Artemisia sieberi* and *A. aucheri* forms the principal vegetation of steppes and semi steppes in the main part of Irano-Turanin region (Mozaffarian, 1988). Once accounted as *A. herba-alba* Asso, *A. sieberi* Besser (subg. *Seriphidium*) is the best-known and most distributed species of the genus in Iran (Podlech, 1986). It is widely distributed in desert and semi desert parts of Iran, having stems with ± right-angled branches, and mostly growing in altitudes below 1500 m. Its vegetative form is highly variable. Towards the highlands of central Iran and Afghanistan, where the altitudes soars higher than 2000, it mainly substituted by *A. aucheri* Boiss., having stems with acute-angled branches (Podlech, 1986). *Artemisia kermanensis* Podlech, having intermediate branching angles, is described from SE of Iran. All three species are suffruticose, with a thick woody rootstock, developing numerous stems. Stems are usually canescence-arachnoid, grey colored due to a dense indumentums. Lower leaves are withering at or before anthesis, after ward deciduous, 1-2 pinnatisect, with oblong-linear lobes. Heads have 4-6 seriate phyllaries. Phyllaries are ovate to oblong.

There are different viewpoint about the true name of the *Artemisia* species distributed in steppes of SW Asia. Boissier (1875) named these populations *A. herba-alba*, distributed from Canary Islands in west to Afghanistan in east. He distinguished three varieties in Flora Orientalis area: *A. herba-alba* var. *densiflora* Boiss., var. *laxiflora* Boiss. and var. *tenuiflora* Boiss. Boissier (1875) synonymed *A. sieberi* to *A. herba-alba* var. *laxiflora*. Parsa (1943) in Flore de l'Iran accepted Boissier's viewpoint about *A. herba-alba* and its three varieties. Poljakov (1961b) in Flora of the USSR introduced *A. sieberi* for Irano-Turanian steppes of central Asia. Cullen (1975) in Flora of Turkey called *Artemisia* species in Irano-Turanian steppes of Turkey, *A. herba-alba* Asso. Podlech (1986) in Flora Iranica segregated the Iranian (and SW Asian) populations of *A. herba-alba* and named them as *A. sieberi* with two subspecies: *A. sieberi* subsp. *sieberi* distributed in Palestine, Syria, Iraq, Afghanistan, Pakistan, Central Asia and Iran, and subsp. *deserticola* Podl. endemic to Afghanistan. *Artemisia herba-alba* and *A. sieberi* in N Africa and SW Asia have been subject of different studies (for example Mohsen and Ali, 2008; Nazar and Mahmoud, 2011; Rabie, 2008).

In this study, we mainly aim to clarify the taxonomic status of the species known as *A. herba-alba* or *A. sieberi*, and *A. kermanensis*, and to illustrate their relationships to *A. aucheri* and the rest of *A.* subg. *Seriphidium* using morphological and molecular ITS and ETS data.

Material and methods

Plant material of three species *A. sieberi*, *A. kermanensis* and *A. aucheri* were collected from 25 different localities across Iran (Table1). In addition, one specimen of *Artemisia sieberi* was collected from its type locality in Palestine. *Ajania fastigiata* (C. winkler) Poljakov. was selected as outgroup according to a previous study of the *Artemisia* group by Sanz *et al.* (2008). The angle between the stems and their branches was measured for all plant material collected by us.

Table 1. List of taxa sequenced in this study and/or used for SEM microscopy, and included in the molecular analysis, with voucher information and GenBank accession numbers.

***Artemisia aucheri* Boiss.**
Iran: Kerman: Sarduiyeh, 20 km before of Sarduiyeh, 2460 m 57 ° 26.057' E, 29° 21.442' N, Salari & Mehregan 000013470 (IAUH).

***Artemisia kermanensis* Podlech**
Iran: Kerman: Golbaf, 100 km from Kerman to Bam, Salari 000013508 (IAUH).
Iran: Esfahan: Naeen, 26 Km from Naeen to Ardakan, 1332 m, 53 ° 17.8125' E, 32° 41.862' N, Salari 000013494 (IAUH).
Iran: Kerman: Kerman, 45 km from Kerman to Bam, 2180 m, 57 ° 22.379' E, 29° 57.652' N, Salari & Mehregan 000013467 (IAUH).
Iran: Kerman: Sirjan, 46 Km NE Sirjan, 2010 m, Salari 000013515 (IAUH).
Iran: Kerman: Rayen, 70 km from Kerman to Rayen , 2501 m, 57 ° 34.393' E, 29° 46.757' N, Salari & Mehregan 000013468 (IAUH).
Iran: Kerman: Golbaf, 87 Km from Kerman To Bam, 2308 m, Salari 000013507 (IAUH).

***Artemisia sieberi* Besser**
Iran: Kerman: Rabor, 115 Km from Jiroft to Rabor, 2340 m, 57 ° 4.942' E, 29° 17.378' N, Salari & Mehregan 000013472 (IAUH).
Iran: Fars: Safa Shahr, 15 Km from Safa Shahr to Abadeh, 2220 m, 53 ° 7.67' E, 30° 50.422' N, Salari & Mehregan 000013481 (IAUH).
Iran: Esfahan: Ardestan, 32 Km from Ardestan to Naeen, 1957 m, 52 ° 33.3543' E, 33° 7.429' N, Salari 000013493 (IAUH).
Iran: Kerman: Sirjan, 50 Km before Shahr-e-Babak of Sirjsn, 1860 m, 55 ° 28.105' E, 29° 49.993' N, Salari & Mehregan 000013476 (IAUH).
Iran: Kerman: Bardsir, 50 Km from Bardsir to Sirjan, 2475 m, Salari 000013512 (IAUH).
Iran: Kerman: Shahr-e-Babak, 6 Km from Shahr-e-Babak to Harat, 1850 m, 55 ° 2.12' E, 30° 7.742' N, Salari & Mehregan 000013477 (IAUH).
Iran: Qom: Qom, 71 Km from Tehran to Qom, 1063 m, 50 ° 56.308' E, 35° 6.881' N, Salari 000013490 (IAUH).
Iran: Fars: Izadkhast, 72 Km from Abadeh to Izadkhast, 2125 m, 52 ° 5.742' E, 31° 37.57' N, Salari & Mehregan 000013483 (IAUH).
Iran: Esfahan: Esfahan, 74 Km from Esfahan to Kashan, 2090 m, 51 ° 49.773' E, 33° 12.649' N , Salari & Mehregan 000013485 (IAUH).
Iran: Kerman: Zarand, Babtangel, 2035 m, Salari 000013505 (IAUH).
Iran: Khorassan: Bejestan, Bajestan, 1272 m, 58 ° 11.625' E, 34° 30.443' N, Salari 000013495 (IAUH).
Iran: Fars: 30 km Shiraz to Jahrom, before Akbar-Abad, 1450 m, Mozaffarian 66126 (TARI).
Iran: Markazi: between Parandak and Anjilvand, 1350 m, Mozaffarian 67867 (TARI).
Iran: Baluchestan: E slpes of Mt. Taftan, 1600-2300 m, Mozaffarian 58778 (TARI).
Iran: Alborz: Karaj, Mehrshahr, Halgheroude, 1100 m, Mozaffarian 68968 (TARI).
Iran: Yazd: 203 km from Tabas to Yazd, before Robat-e Posht-e Badam, 1300 m, Assadi & Abouhamzeh 40242 (TARI).
Iran: Khorassan: 40 km from Ferdous to Boshrouyeh, 1250 m, Assadi & Abouhamzeh 40191 (TARI).
Iran: Semnan: 22 km from Semnan to Damghan, 1700 m, Assadi & Abouhamzeh 40044 (TARI).
Palestine: Arafeh s.n.

Scanning Electron Microscopy (SEM)

Capitula, florets and achenes (ventral and dorsal surface) were taken from samples belonging to three species *A. sieberi*, *A. kermanensis* and *A. aucheri* (Table 1). Three to five samples for each species were taken from herbarium material, washed and dried. Samples were directly mounted on aluminum stubs and then coated with a tiny layer of gold using a SCDOOS sputter coater (BAL-TEC, Switzerland). Observations were performed using a XL30 Scanning Electron Microscope (Philips, the Neterlands).

Molecular analyses

Total genomic DNA was extracted from either silica-gel dried leaves directly collected from plants in wild or herbarium specimens following a modified CTAB protocol of Doyle and Doyle (1990). The DNA extraction was performed using the NucleoSpin® Plant II Kit (Macherey-Nagel GmbH & Co. KG, Dŭren, Germany) after the manufacturer's protocol. The entire ribosomal ITS region (ITS1 + 5.8s + ITS2) was amplified using the primer pairs AB 101 (forward, 5'- ACG AAT TCA TGG TCC GGT GAA GTG TTC G - 3') and AB 102 (reverse, 5' - TAG AAT TCC CCG GTT CGC TCG CCG TTA C - 3') (Douzery *et al.*, 1999) using the following PCR protocol: a pretreatment of 5 min at 95° C, 35 cycles of 30 sec at 95° C, 30 sec at 50° C, and 1 min 30 sec at 72° C, and a final extension of 7 min at 72° C.

The entire ribosomal ETS region was amplified using the primer pairs ETS-1f (forward, 5' - CTT TTT GTG CAT AAT GTA TAT ATA TAG GGG G – 3') and 18S-2L (reverse, 5' –TGA CTA CTG GCA GGA TCA ACC AG – 3') (Linder *et al.*, 2000) using the following PCR protocol: a pretreatment of 5 min at 95° C, 7 min at 74°, 30 cycles of 45 sec at 94° C, 45 sec at 50° C, and 40 sec at 72° C, and a final extension of 7 min at 72° C. The quality of PCR products were checked by electrophoresis on a 1.0 % agarose gel and then visualized under UV light.

Forward and reverse sequences were visually compared and edited, and then initially aligned using Sequencher 4 software (Gene Codes Corporation, Ann Arbor, MI USA). In addition to our sequences, 197 ITS and ETS sequences from other taxa were taken from Genbank (Table 2). All ITS and ETS sequences were assembled and aligned using MacClade 4 (Maddison and Maddison, 2002).

Table 2. List of taxa included in the molecular analysis, with region of origin and GenBank accession numbers taken from the GenBank.

***Ajania fastigiata* (C. winkler) Poljakov.,** Kazakhstan: AF504169, AF504142, EF055420.

***Artemisia santolina* Schrenk,** Uzbekistan: AF504181, AF504154, DQ028873.

***Artemisia afra* Jacq. ex Willd.,** South Africa: AF045392, AF140484, EF055390.

Artemisia absinthium L.
Spain: EF055405, DQ02881, DQ028850.

Artemisia annua L.
Spain: AF045383, AF079935, EF055431, DQ028879.

Artemisia araxina Takht.
Armenia: AF045408, AF079959, EF055422, DQ028870.

Artemisia arenaria DC.
Russia: EF063639, EF063640, EF055443, DQ028897.

Artemisia aschurbajewii C. Wink.
Kazakhstan: AF504170, AF504143, EF055393, DQ028838.

Artemisia austriaca Jacq.
Armenia: AF504171, AF504144, EF055399, DQ028844.

Artemisia baryelieri Besser
Spain: AF045410, AF079961, DQ028875.

Artemisia caerulescens L.
Portugal: AF045409, AF079960, EF055424, DQ028872.

Artemisia campestris L.
Spain: AF045398, AF079950, EF055408, DQ028854.

Artemisia cana Pursh.
U.S.A: AF045413, AF079965, AF055433, DQ028882.

Artemisia canariensis Less.
Spain: DQ028920, DQ028907, EF055407, DQ028852.

Artemisia crithmifolia L.
Portugal: AF045399, AF079962, EF055410, DQ028856.

Artemisi diversifolia (*Syn.: Sphoeromeria diversifolia* Rydb.)
U.S.A: HQ013071, AH012436, EF055436, DQ028885.

Artemisia dracunculoides Pursh.
U.S.A: AF504145, AF504172, EF055413, DQ028860.

Artemisia dracunculus L.
Spain: AF045401, AF079952, EF055412, DQ028859.

Artemisia eriantha Ten.
Spain: DQ028919, DQ028906, EF055397, DQ028842.

Artemisia filifolia Tar.
U.S.A: DQ028922, DQ028909, EF055414, DQ028862.

Artemisia fragrans Willd.
Armenia: AF045406, AF079957, EF055423, DQ028871.

Artemisia granatensis Boiss.
Spain: AF045397, AF079949, EF055396, DQ028841.

Artemisia glaciais L.
Italy: DQ028921, DQ028908, EF055395, DQ028840.

Artemisia haussknechtii Boiss.
Iran: AF504173, AF504146, EF055392, DQ028837.

Artemisia herba-alba Asso
Spain: AF045403, AF079954, EF055426, DQ028874.
Morocoo: AF045404, AF079955, EF055429, DQ028877.

Artemisia incana L.
Armenia: EF055394, DQ028839.

Artemisia inculta Delile
Egypt: AF045405, AF079956, AF055430, DQ028878.

Artemisia judaica L.
Egypt: AF504175, AF504148, EF055403, DQ028848.

Artemisia lagocephala Fisch. ex Besser
Russia: DQ028917, DQ028904, EF055444, DQ028898.

Artemisia lucentica O.
Spain: AF045390, AF079943, EF055401, DQ028846.

Artemisia mexicana Willd.
Mexico: AF045414, AF079966, EF055388, DQ028892.

Artemisia nova Nelson
U.S.A: AF045412, AF079964, EF055434, DQ028883.

Artemisia persica Boiss.
Uzbekistan: AF504179, AF504152, EF055432, DQ02880.

Artemisia reptans C. Sm. ex Link
Morocco: AF045391, AF079944, EF055402, DQ028847.

Artemisia rutifolia Steph. ex Spreng
Kazakhstan: AF504153, AF504180, EF055404, DQ028849.

Artemisia santolina Schrenk
Uzbekistan: AF504181, AF504154, EF055425, DQ028873.

Artemisia santolinifolia Turcz. ex Besser
Kazakhstan: AF504182, AF504155, EF055391, DQ028836.

Artemisia scoparia Waldst. & Kit.
Armenia: AF045402, AF079953, EF055411, DQ028857.

Artemisia sieberi Besser
Iran: AF045407, AF079958, EF055428, DQ028879.

Artemisia sieberica L.
(syn.: *Filifolium sibiricum* (L.) Kitam.)
Russia: AH012433, EF079958, EF055440, DQ028894.
Spain: AF504187, AF504160.

Artemisia sieversiana Ehrh. ex Willd.
Uzbekistan: AF504183, AF504157, EF055406, DQ028851.

Artemisia splendens Willd.
Iran : AF045396, AF079948, EF055400, DQ028845.

Artemisia stelleriana Besser
Russia: DQ028918, DQ028905, EF055442, DQ028896.

Artemisia tridentata Nutt.
U.S.A: AF045411, AF079963, EF055435, DQ028884.

Artemisia umbelliformis L.
Spain: AF045395, AF079947, EF055398, DQ028843.

Artemisia verlotiorum Lamotte
Spain: AF045387, AF079939, EF055439, DQ028891.

Artemisia vulgaris L.
Japan: EF055438, DQ028890.

Kaschgaria brachanthemoides (C. Winkler) Pojakov.
Kazakhstan: AF504189, AF504162, EF055417, DQ028865.

Kaschgaria komarovii (Krasch & Rubtzav) Poljakov
Mongolia: DQ028925; DQ028912; EF055447; DQ028902.

Picrothamnus desertorum Mutt.
U.S.A: AM774424, HQ019066, EF055437, DQ028887.

Maximum parsimony analyses (MP)

MP analyses of the ITS, ETS and ITS + ETS datasets were performed with PAUP* (Swofford 2002) software using following criteria: 100 heuristic search replicates, random stepwise addition of taxa, and tree-bisection reconnection (TBR) branch swapping. Bootstrap support (BS) for clades was calculated using PAUP* (Swofford 2002) with 100 replicates of heuristic searches, and randomly stepwise addition of taxa. Clades with a bootstrap value of 70% or more were considered as robustly supported nodes.

Bayesian analysis (BA)

The BA analyses of the ITS, ETS and combined ITS + ETS datasets were performed using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001). In order to find the appropriate model of DNA substitution, the Maximum Likelihood criteria for three datasets were determined by the Akaike Information Criterion (AIC; Akaike, 1974) as implemented in the software ModelTest v3.7 (Posada and Crandall, 1998). For the ITS dataset, the GTR (general time-reversible) + G model was chosen with gamma distribution set to 0.3300. The substitution rates were set to A-C = 1.6947, A-G = 6.7091, A-T = 2.0197, C-G = 0.6789, C-T = 9.5632, and G-T = 1.000. For the ETS dataset, the TrN + I + G model was chosen with proportion of invariable site set to 0.3568 and gamma distribution set to 0.6767. The substitution rates were set to A-C = 1.0000, A-G = 2.6421, A-T = 1.0000, C-G = 1.0000, C-T = 5.1477, and G-T = 1.000. For the combined ITS + ETS dataset, the GTR + I + G model was chosen with proportion of invariable site set to 0.3407 and gamma distribution set to 0.6123. The substitution rates were set to A-C = 1.4364, A-G = 4.1984, A-T = 1.4704, C-G = 0.8432, C-T = 6.8176, and G-T = 1.000.

Results and discussion

The angle between the stems and their branches for all herbarium material of *A. sieberi*, *A. aucheri* and *A. kermanensis* was measured. It continuously varies from 20° to 90° with no distinct intervals (data not shown). There was a statistically significant correlation between the branching angle and altitude of collecting locality (p = 0.05). The minimum branching angles were observed in the material collected from the areas higher than 2500 m. In the lowland with altitude lower than 1500 m, the branching angle gradually soars to 90°.

SEM microscopy

There were no diagnostic differences between capitula of all three species *A. aucheri*, *A. sieberi* and *A. kermanensis*. They have small ovate to oblong capitula, 3 – 4 mm long. Outer involucral bractes in all three species were ovate and inner bracts were oblong (Fig. 1). Achenes were narrow obovate to fusiform, with no papus. Despite variation in size (data not shown), no diagnostic differences were observed in morphology of achenes and florets of those three species.

Molecular phylogeny

Numerical results of the analysis of the ITS, ETS, and combined ITS + ETS datasets are given in Table 3. Of 428 total aligned characters of the ITS region 93 (21.7 %) were parsimony informative. Of 606 total aligned characters of the ETS region 106 (17.5 %) were parsimony informative. In comparison to the ITS dataset, ETS dataset was resulted in a consensus tree with higher CI (0.627 vs. 0.555) but slightly lower RI (0.842 vs. 0.842).

Table 3. Numerical results of the analysis of the ITS, ETS, and combined ITS + ETS datasets.

	ITS dataset	ETS dataset	Combined ITS+ETS dataset
Total characters	428	606	1034
Constant characters	278	429	707
Parsimony uninformative characters	57	71	128
Parsimony informative characters	93 (21.7 %)	106 (17.5 %)	199 (19.2 %)
Tree Length (consensus tree)	328	330	621
CI (consensus tree)	0.555	0.627	0.594
RI (consensus tree)	0.844	0.842	0.824

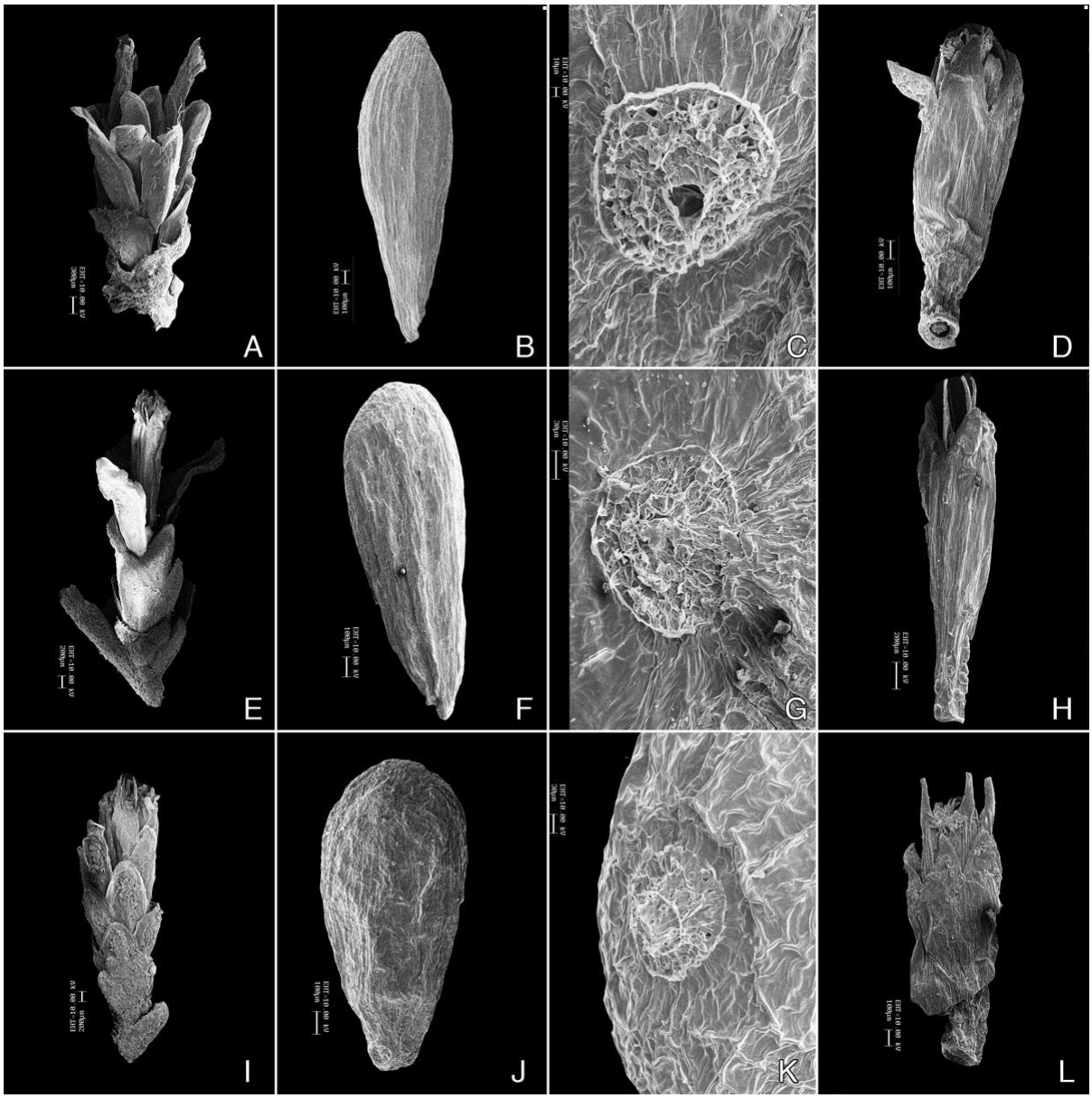

Fig. 1. Floral morphology of *A. sieberi* (A. capitulum, B. achene, C. nectary, D. tubular floret); *A. kermanensis* (E. capitulum, F. achene, G. nectary, H. tubular floret); *A. aucheri* (I. capitulum, J. achene, K. nectary, L. tubular floret).

The 50 % majority rule tree resulted from the Bayesian analysis of the ITS dataset is shown in Fig. 2. As illustrated in Fig. 2, species belonging to the subgenus *Dracunculus* and subgenera *Artemisia* + *Seriphidium* form monophyletic clades with the genus *Kaschgaria* as sister group. The clade including *A.* subg. *Dracunculus* shows a low posterior probability of 0.6 in the Bayesian analysis and a low bootstrap support of 50 in the maximum parsimony analysis. *Artemisia* subg. *Artemisia* seems to be paraphyletic with species belonging to *A.* subg. *Seriphidium* as a nested monophyletic subclade with the posterior probability value of 0.83 and robust bootstrap value of 100 % (Fig. 2). Phylogenetic relationships within the subgenus *Seriphidium* is essentially unresolved and its species including *A. aucheri*, *A. kermanensis*, *A. sieberi* form a large polytomy. Our specimens from Iran and from the type locality of *A. sieberi* in Palestine showed no variability in the ITS nucleotides.

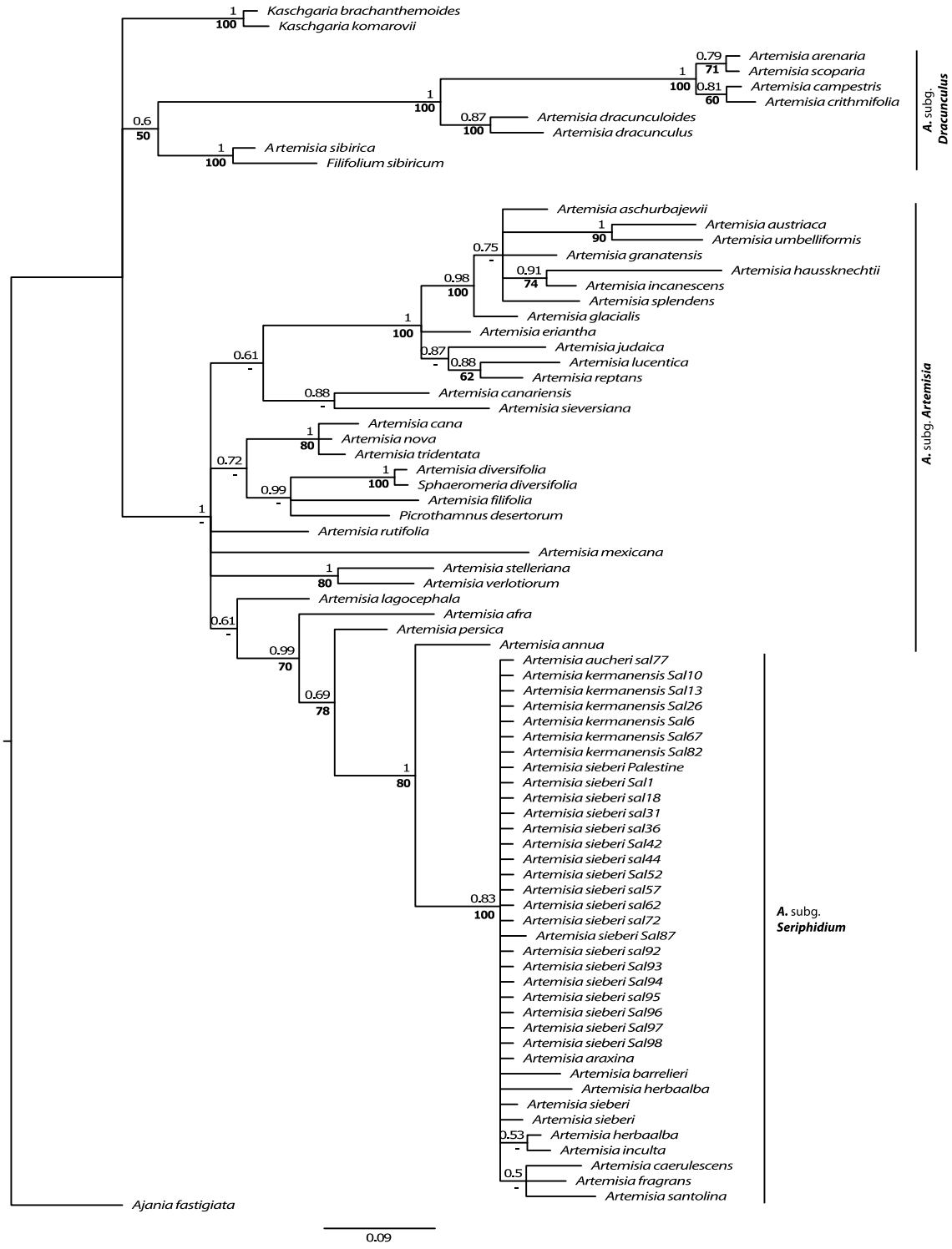

Fig. 2. 50% majority-rule consensus tree obtained from the Bayesian analysis of the ITS dataset. Numbers on branches are posterior probabilities and those below branches are bootstrap (BS) values of those clades retrieved in the maximum parsimony analysis.

As shown in Fig. 3, the Bayesian analysis of ETS datasets resulted in a phylogenetic tree with better resolution. The genus *Kaschgaria* is seen as sister to the rest of group. Species of the subgenus *Dracunculus* form a monophyletic clade with posterior probability (PP) of 0.91 in the Bayesian analysis and bootstrap value of 70 % in maximum parsimony analysis. Similar to the ITS analysis, *A.* subg. *Artemisia* showed to be paraphyletic in the analysis of ETS dataset. All species of the subgenus *Seriphidium* plus *A. annua* (subg. *Artemisia*) form a nested monophyletic group with posterior probability value of 0.79 in the Bayesian analysis and a bootstrap support value of 84 %. Phylogenetic relationships of species belonging to the subgenus *Seriphidium* is not essentially resolved and they form a large polytomy.

Analysis of the combined ITS + ETS datasets with a lower number of taxa yielded phylogenies with better resolutions (Fig. 4). As seen in the 50 % majority-rule consensus tree obtained from the analysis of the combined dataset, the *Artemisia* group form a monophyletic clade with PP = 0.82 and BS = 71 %, with *Kaschgaria* as sister. The *Artemisia* group is divided into two main clades: 1) subg. *Dracunculus* clade with PP = 1 and BS = 100 %, and 2) subgenera *Artemisia* + *Seriphidium* clade with PP = 1 and no significant BS support. The subgenus *Artemisia* is paraphyletic and consists of some clades. Within the *Artemisia* + *Seriphidium* clade some taxonomic groups form monophyletic clades with robust support. Species of the section *Tridentatae* form a monophylet with robust PP = 1 and BS = 96 %. *Artemisia vulgaris* complex form a monophyletic clade with PP = 1 and BS = 100 %. Species of the subgenus *Seriphidium* including all our samples form a monophyletic nested subclade with PP = 0.99 and BS = 86 % within the subgenus *Artemisia* (Fig. 4). Phylogenetic relationships within the subgenus *Seriphidium* is not essentially resolved and it includes a polytomy of single taxa and low supported subclades. All our specimens of *A. aucheri*, *A. sieberi* and *A. kermanensis* from Iran form a monophyletic clade with no significant support.

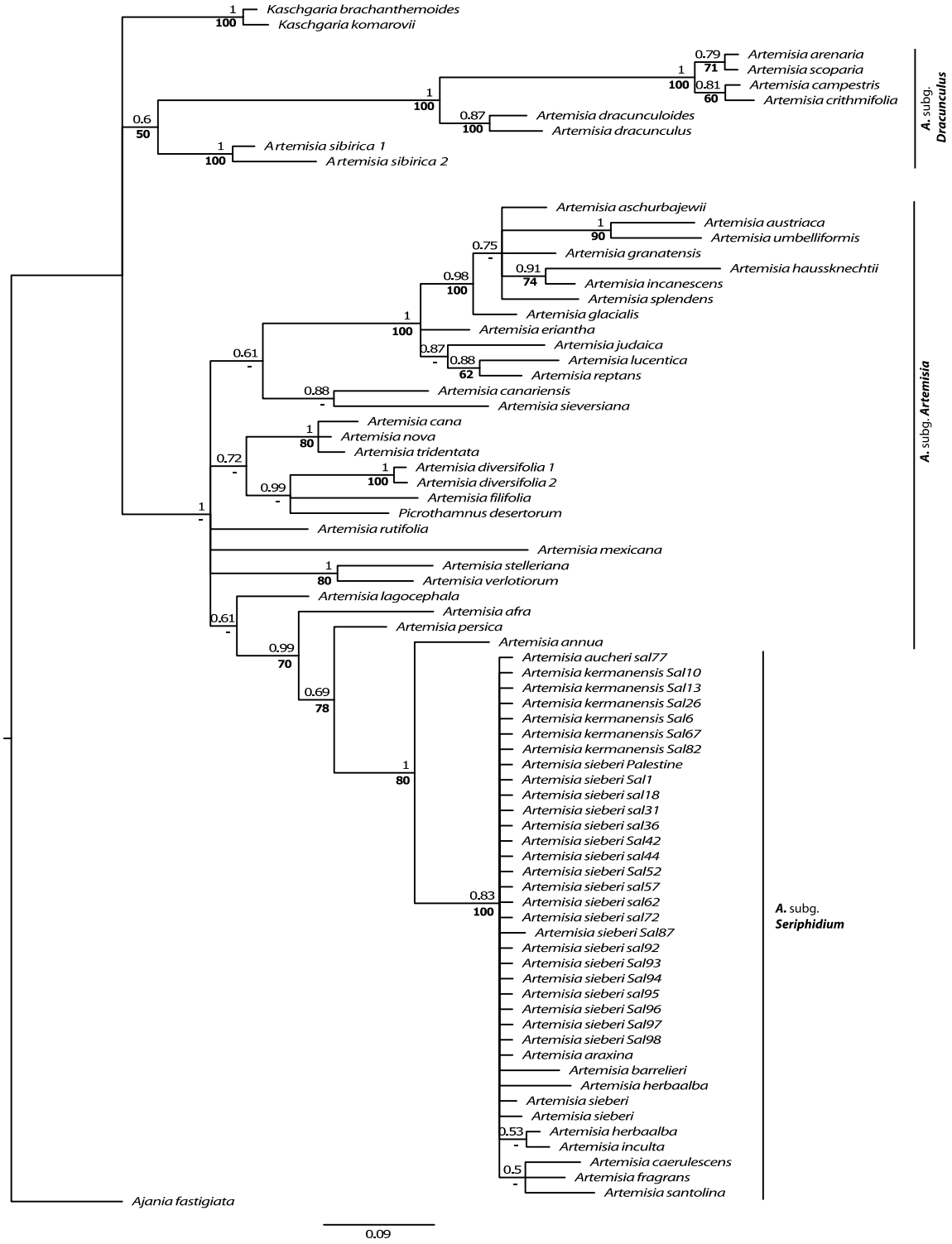

Fig. 3. 50% majority-rule consensus tree obtained from the Bayesian analysis of the ETS dataset. Numbers on branches are posterior probabilities and those below branches are bootstrap (BS) values of those clades retrieved in the maximum parsimony analysis.

Fig. 4. 50% majority-rule consensus tree obtained from the Bayesian analysis of the ITS dataset. Numbers on branches are posterior probabilities and those below branches are bootstrap (BS) values of those clades retrieved in the maximum parsimony analysis.

Discussion

In the phylogenetic analyses based on the ITS, ETS and combined ITS + ETS datasets some groups are well positioned and resolved. Species of the subgenus *Dracunculus* formed monophyletic clades in analysis of all three datasets. This group is characterized by the having heterogamous capitula with female-sterile (functionally male) central florets as synapomorphy (Sanz *et al.*, 2008). This group was consisted of four closely related genera: *Filifolium*, *Mausolea*, *Neopallasia* and *Turaniphytum*. They are now classified in this subgenus (Sanz *et al.* 2008). Within the group, *Artemisia sibrica* (syn.: *Filifolium sibiricum*) positioned as a sister taxon to the remaining taxa (Figs. 2 – 4).

There has been no complete agreement on the taxonomy of the subgenus *Artemisia* (Valles and Mc Arthur, 2001). As one of the more recent taxonomic treatments, Podlech (1986) divided the genus into three subgenera *Artemisia*, *Dracunculus* and *Seriphidium*. Mc Arthur *et al.* (1981) segregated the new world members of the subgenus *Seriphidium* as subgenus *Tridentatae*. Recent molecular studies support this segregation and suggest definition of new subgeneric taxa. According to recent molecular phylogenies, some well-defined groups appear in the *Artemisia* group (Watson *et al.*, 2002; Valles *et al.*, 2003; Sanz *et al.*, 2008). They are not fully in agreement with the classic subgenera, but some of them agree totally or partially with the currently used infrageneric classification.

Phylogenetic studies could not resolve the phylogenetic relationships within the subgenus *Seriphidium*. The subgenus is seen as a nested subclade within the subgenus *Artemisia*. The former new world members of the subgenus *Seriphidium* are now treated as subgenus *Tridentatae*. Monophyly of the subgenus *Tridentatae* is well documented in different phylogenetic studies (Watson *et al.*, 2002; Valles *et al.*, 2003; Sanz *et al.*, 2008). According to Sanz *et al.* (2008), the ancestor of the *Artemisia*/*Kaschgaria* group had disciform-heterogamous capitula with central hermaphroditic and outer female, non radiate florets, and during the evolution of the *Artemisia* group, the ancestral disciform capitula evolved as a reversal into discoid at least twice, in subgenera *Seriphidium* and *Tridentatae*.

Artemisia annua and *A. persica* (subgenus *Artemisia*) are the successive sister taxa of the *Seriphidium* group. It is suggested that subgenus *Seriphidium* has evolved from members of the subgenus *Artemisia* (Ling, 1994; Sanz *et al.* 2008). The group might be originated in C Asia and migrated westward the Mediterranean region (Ling, 1994). The ITS and ETS data do not show sufficient variation and insufficient divergence of the sequences in the *Seriphidium* group indicate a rapid radiation of the group, which is also supported by the morphological and cytological homogeneity of this group (Torrell *et al.*, 2003; Sanz *et al.* 2008).

Our analysis could not effectively segregate specimens of *Artemisia herba-alba*, *A. sieberi*, *A. kermanensis* and *A. aucheri* from each other. They form a species complex with no significant difference in the ITS sequences and very low differences in ETS sequences. Previous studies showed a very low variability in the ITS and ETS sequences in the *Seriphidium* group. There was no significant differences in the floral morphology of all material examined in this research. The main morphological differences between *Artemisia sieberi*, *A. kermanensis* and *A. aucheri* are those of gross morphology, specially the angle between the stems and their branches. We observed a significant correlation between the branching angle and locality altitude of the material collected from different parts of Iran. Therefore we here assume that the branching angle could be highly affected by the habitat and its ecological condition. Members of the *A. herba-alba* complex are widely distributed in the Mediterranean regions in Europe and N Africa, and SW and Central Asia, from lowland deserts with altitude below 1000 m to highlands with altitude higher than 3000 m. Ecological conditions in these

regions are highly variable. We here suggest that the morphological diversity in the *A. herba-alba* complex could be related to the phenotypic plasticity. Many studies have recorded phenotypic changes in natural populations and attributed them to climate change and it is concluded that plasticity often makes a strong contribution to phenotypic trends associated with contemporary climate change (Merila and Hendry, 2014; Franks *et al.*, 2014).

Conclusion

All populations belonging to the species named *A. herba-alba*, *A. sieberi*, *A. kermanensis* and *A. aucheri* form a morphologically variable species complex which can not be segregated as different taxonomic units using the ITS and ETS sequencing data. Regarding the presence of a high correlation between the branching angle and altitude as the most visible morphological character, it can be concluded that the morphological diversity in the *A. herba-alba* complex could be related to the phenotypic plasticity associated with climate differences. In order to clarify the taxonomic boundaries within the complex, it is necessary to use other markers such as AFLP finger printing techniques.

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