

A new endemic species of the genus *Haloxylon* Bunge ex Fenzl. (Amaranthaceae s.l.) in the flora of Kazakhstan

Bektemir B. Osmonali

Al-Farabi Kazakh National University

Polina V. Vesselova

Gulmira M. Kudabayeva

Ussen Serikbay

Al-Farabi Kazakh National University

Abdildanov Sh. Daulet

Al-Farabi Kazakh National University

Friesen Nikolai

`nfriesen@uni-osnabrueck.de`

Osnabruck University

Research Article

Keywords: Chenopodiaceae, morphology, anatomy, molecular genetics, *Haloxylon*, *Arthrophytum*, hybrid, *Betpakdala*, endemic species

Posted Date: March 11th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4017910/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Plant Systematics and Evolution on February 10th, 2025. See the published version at <https://doi.org/10.1007/s00606-024-01926-x>.

Abstract

The article discusses the results of comparative analysis of morphological, anatomical and molecular-genetic structure of species *Arthrophytum longibracteatum*, *Arthrophytum balchaschense*, *Haloxylon aphyllum*). Morphological-anatomical and molecular-genetic methods were used during the study. Transverse sections were viewed using a Levenhuk Zoom&Joy microscope (China), images of transverse sections were taken with a Levenhuk D740T 5.1 camera, using the LevenhukLite programme. To clarify the correctness of species identification, the collected specimens were compared with the Type specimen of *Arthrophytum balchaschense* stored at the Institute of Botany and Phytointroduction (AA). Other herbarium specimens of this species stored in the AA Herbarium (Almaty, Kazakhstan) and herbarium collections: MW (Moscow, Russia), LE (St. Petersburg, Russia), TASH (Tashkent, Uzbekistan) were also reviewed. With the help of QGIS programme, a map of the location points of the studied specimens was made. The conducted studies allowed to form a proof base of clear belonging of *Arthrophytum balchaschense* to the genus *Haloxylon*. This circumstance became the reason for the necessity of nomenclatural changes consisting in assignment of *Arthrophytum balchaschense* to the genus *Haloxylon* under the name *Haloxylon balchaschense* (Iljin) Osmonali, Veselova & Kudab. This species has a central-eastern-North Turanian distribution, being endemic to the flora of Kazakhstan.

Introduction

The genus *Haloxylon* Bunge ex Fenzl of the family Chenopodiaceae (Amaranthaceae s.l.) according to the databases International Plant Names Index (IPNI) and Plants of the World Online (POWO) includes 11 species (*Haloxylon ammodendron* (C.A.Mey.) Bunge ex Fenzl, *H. gracile* (Aellen) Hedge, *H. griffithii* (Moq.) Boiss., *H. multiflorum* (Moq.) Bunge ex Boiss, *H. negevensis* (Iljin & Zohary) L.Boulos, *H. persicum* Bunge, *H. salicornicum* (Moq.) Bunge ex Boiss., *H. schmittianum* Pomel, *H. scoparium* Pomel, *H. tamariscifolium* (L.) Pau, *H. thomsonii* Bunge ex Boiss.) occurring from the Mediterranean to Central Asia inclusive (Zhu et al., 2004; Dong et al., 2016).

Many of these species were previously considered to be members of the genus *Arthrophytum* Schrenk, which, according to IPNI and POWO, currently comprises 8 species (*Arthrophytum balchaschense* (Iljin) Botsch, *A. betpakdalense* Korovin & Mironov, *A. iliense* Iljin, *A. korovinii* Botsch., *A. lehmannianum* Bunge, *A. longibracteatum* Korovin, *A. pulvinatum* Litv., *A. subulifolium* Schrenk).

In the flora of Kazakhstan (Goloskokov and Polyakov, 1960; Baitenov, 2001) and Central Asia (Pratov, 1972), there are 3 species of the genus *Haloxylon*: *Haloxylon ammodendron* (C.A.Mey.) Bunge, *Haloxylon aphyllum* (Minkw.) Iljin, *Haloxylon persicum* Bunge. However, according to recent literature sources, the first 2 species are now combined into one species *Haloxylon ammodendron* (C.A. Mey.) Bunge ex Fenzl. (<https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:941607-1>), which reduces, in turn, the number of representatives of the genus growing in Kazakhstan. From our point of view, the association of *Haloxylon aphyllum* with *Haloxylon ammodendron* is not quite unambiguous. Additional studies are needed here. Since only *Haloxylon aphyllum* is widespread in the area under study, we use this species name in the article. In the deserts of Central Asia, *Haloxylon ammodendron*, *Haloxylon aphyllum*, and *H. persicum* play an important role in human life, performing, among other things, a protective function (wind control and sand fixation) (Zhu et al., 2004; Dong et al., 2016). In Kazakhstan, species of the genus *Haloxylon* are distributed mainly in Turanian deserts, occurring in all southern and south-eastern regions of the republic (Goloskokov and Polyakov, 1960; Pratov, 1972).

The development of molecular genetic methods of analysis, now actively used in systematic studies, leads to numerous changes in the taxonomy of almost all groups of plants. Thus, some genera are divided into several genera, and others, on the contrary, are united into one, or species from one genus are considered by systematists within the scope of another genus, hybrid specimens are identified, etc. (Kechaykin et al., 2020; Sumbembayev et al., 2020; Sumbembayev et al. (Kechaykin et al., 2020; Sumbembayev et al., 2021, 2023a; 2023b; Sukhorukov et al., 2022, 2023a, 2023b; Friesen et al., 2022; Lazkov and Veselova, 2022; Terlets kaya et al., 2023; Osmonali et al., 2023 etc.).

Considering that morphologically some representatives of genera *Haloxylon*, *Arthrophytum* and *Anabasis* L., belonging to the same tribe Salsoleae of subfamily Salsoloideae, are very similar at certain stages of their development, the question of determination of their systematic belonging is actual. The present article is devoted to the results of one of such studies.

During expeditionary studies on the flora of the Eastern and Northeastern part of Betpakdala, in particular on the identification of rare and endemic species of this region, we noted a strong similarity between the species *Arthrophytum balchaschense* and species of the genus *Haloxylon* (*Haloxylon aphyllum*). The similar features that attracted our attention include: spatial arrangement of young green shoots, structure of bark, shape of fruits (in the latter case when examined in detail). Therefore, to clarify the systematic affiliation of the plants we were interested in, we collected their samples not only to replenish herbarium collections, but also as material for molecular genetic studies.

To clarify the correct identification of the species as *Arthrophytum balchaschense*, the collected specimens were compared (checked) with the Type specimen stored at the Institute of Botany and Phytointroduction (AA) under number AA0002446. It was once collected and described by M.G. Popov (Popov, 1936) as *Anabasis pauciflora* Popov. Almost in the same period, M.M. Ilyin described the species *Anabasis balchaschensis* Iljin (Ilyin, 1936a, 1936b, 1936c, 1936d), which was renamed *Arthrophytum balchaschense* (Iljin) Botsch (Botschancev, 1954) by V.P. Bochantsev in 1954 (Goloskokov and Polyakov, 1960). *Anabasis pauciflora* (Pratov, 1972) was also added to *Arthrophytum balchaschense* as a synonymy,

Type specimens of which were submitted to the V.L. Komarov Botanical Institute in Moscow. V.L. Komarov Botanical Institute in St. Petersburg (LE00054892, LE00054893) (<https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:163588-1>).

Thus, it was decided to make a molecular genetic analysis and to identify the relatedness of *Arthrophytum balchaschense* with species of the genus *Haloxylon*. One of the most important molecular methods in the study of kinship relations at the supra-species (genus) systematic level is the comparison and analysis of aligned DNA sequences of individual fragments of the genome and plastome of plants. In the study of genome fragments (nuclear DNA), nrITS (internal transcribed spacer) analysis of ribosomal DNA is the most popular, while in the plastome a large range of genes and introns is used (Friesen et al., 2006).

Material and methods

The objects of the study are species of genera *Haloxylon* and *Arthrophytum* of family Chenopodiaceae (Amaranthaceae s.l.), growing on the territory of Kazakhstan: *Arthrophytum longibracteatum* Korovin, *Arthrophytum balchaschense* (Iljin) Botsch., *Haloxylon ammodendron* (C.A.Mey.) Bunge ex Fenzl (*Haloxylon aphyllum* (Minkw.) Iljin). The species *Anabasis salsa* (C.A. Mey.) Benth. ex Volken was selected as a closely related species to the studied genera occurring directly in the same phytocenoses (Fig. 1). For greater reliability of the results, the studied specimens were collected at the same developmental phase. All collection points of the studied species are presented in Table 1.

Herbarium specimens of the species *Arthrophytum balchaschense* of the Institute of Botany and Phytointroduction (AA, Almaty, Kazakhstan), Lomonosov Moscow State University (MW, Moscow, Russia), V.L. Komarov Botanical Institute (LE, St. Petersburg, Russia), Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan (TASH, Tashkent, Uzbekistan) Fig. 3.

Images of herbarium specimens were taken on a Levenhuk DTX RC3 remote-controlled microscope (China).

Anatomic method

Materials of anatomical sections were fixed in 45% alcohol. The objects of transverse sections were frozen in histological paraffin in special moulds of 15x15 mm. Transverse sections of the specimens were made using a "Rotary semi-automatic microtome" (MEDITE M530) with a transverse section thickness of 40 µm. Transverse sections were viewed using a Levenhuk Zoom&Joy microscope (China), images of transverse sections were taken with a Levenhuk D740T 5.1 camera, using LevenhukLite software. Biometric data were also measured using this programme. The mean number and standard error of biometric data were calculated using Microsoft Excel using the data analysis function. Current literature data of closely related species were used to describe the anatomical structure of the studied specimens (Pyankov et al., 1997; Butnik et al., 2001, 2017; Voznesenskaya et al., 2013; Zhaglovskaya et al., 2015; Schüssler et al., 2016; Osmonali et al., 2020).

Table 1
– Origin, source, and GenBank accession numbers of sequences used for phylogenetic analyses

Accession	Name	Coordinates	Voucher	rITS	rps16 intron
B58	<i>Haloxylon balchaschense</i> (<i>Arthrophytum balchaschense</i>)	46.108889 N 73.254167 E	AA0003148	OR906301	OR913002
B59	<i>H. balchaschense</i>	46.356389 N 73.815278 E	AA0003149	OR906302	OR913003
B60	<i>Arthrophytum longibracteatum</i>	43.545556 N 78.591944 E	AA0003150	OR906303	OR913004
B61	<i>A. longibracteatum</i>	43.472778 N 79.010833 E	AA0003151	OR906304	OR913005
B62	<i>Arthrophytum sp</i>	43.472778 N 79.010833 E	AA0003152	OR906305	OR913006
B63	<i>Anabasis salsa</i>	45.366389 N 73.665833 E	AA0003153	OR906306	-
B64	<i>A. salsa</i>	46.186667 N 73.597778 E	AA0003154	OR906307	-
B66	<i>A. salsa</i>	46.041667 N 73.628056 E	AA0003155	OR906308	-
B67	<i>A. salsa</i>	46.319722 N 73.593889 E	AA0003156	OR906309	-
B70	<i>A. salsa</i>	44.629722 N 74.461944 E	AA0003157	OR906312	OR913008
B71	<i>A. salsa</i>	46.041667 N 73.628056 E	AA0003158	OR906313	-
B68	<i>Haloxylon aphyllum</i>	46.101667 N 73.276389 E	AA0003159	OR906310	OR913007
B69	<i>H. aphyllum</i>	44.629722 N 74.461944 E	AA0003160	OR906311	-

Molecular genetics methods

Extraction of DNA from leaves dried with silica gel was carried out using the NucleoSpin Plant II Mini kit (MACHEREY-NAGEL GmbH & Co. KG). The ITS fragment was amplified using ITS-A (Blattner, 1999) and ITS-4 (White et al., 1990) primers. The chloroplast fragments rps16 intron were amplified using the primers rps16f-rps16r2 described by Shaw et al. (2007).

Amplification and sequencing

To perform the PCR, the amplification protocol for Red Mix was used, involving a 20 µl reaction mixture with 2×HS Taq Mix Red (Biozym Scientific GmbH, Germany), where the mix was 1 µl each of direct primer and reverse primer, 10 µl Red Mix, and 8 µl H_2 . The amplified products were tested by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. The DNA fragments were visualized under UV light on a Gel I X20 Lmager (INTAS Science Imaging Instruments GmbH, Germany) and documented using a Mitsubishi P93D printer (Mitsubishi Elec. Corp., Japan). The PCR products were sent to Microsynth SeqLab (Göttingen, Germany; www.microsynth.seqlab.de) for sequencing. The sequences from all individuals were manually edited in Chromas Lite 2.1 (Technelysium Pty Ltd., South Brisbane, QLD, Australia) and aligned with ClustalX (Thompson et al., 1997); the alignment was manually corrected using MEGA7 (Kumar et al., 2016).

Phylogenetic analyses

Both datasets (nrITS and the cpDNA markers) were analyzed separately through Fitch parsimony with the heuristic search option in PAUP version 4.0 b10 (Swofford, 2002) with MULTREES, TBR branch swapping, and 100 replicates of random addition sequence. Gaps were treated as missing data. The consistency index (CI) was calculated to estimate the amount of homoplasy in the character set (Kluge and Farris, 1969). The most parsimonious trees returned by the analysis were summarized in one consensus tree using the strict consensus method. Bootstrap support (BS) – were performed using 1,000 pseudoreplicates to assess the support of the clades (Felsenstein, 1985). Bayesian phylogenetic analyses were also performed using MrBayes 3.1.23 (Ronquist and Huelsenbeck, 2002). The sequence evolution model was chosen by following the Akaike information criterion (AIC) obtained from jModelTest2. Two independent analyses with four Markov chains were run for 10 million generations, sampling trees every 100 generations. The first 25% of the trees were discarded as burn-in. The remaining 150,000 trees were combined into a single dataset, and a majority-rule consensus tree was obtained, along with posterior probabilities (PP).

Species from the subfamily Salicornioideae were selected as an outgroup: *Halostachys belangeriana* (Moq.) Botsch., *Halocnemum strobilaceum* (Pall.) M. Bieb., *Kalidium caspicum* (L.) Ung.-Sternb. and *K. foliatum* (Pall.) Moq.

Results

As a result of expeditionary research, samples of species *Arthrophytum longibracteatum* (2 populations), *Haloxylon balchaschense* (*Arthrophytum balchaschense*, 2 populations), *Haloxylon ammodendron* (*H. aphyllum*, 2 populations), *Arthrophytum* sp. (hybrid, 1 population), *Anabasis salsa* (6 populations) were attracted for study. The main points of the studied samples are located in the north-western part of Lake Balkhash, which corresponds to the north-eastern edge of the territory of the Betpakdala floristic district. With the help of QGIS programme, a map of the location points of the studied specimens was made (Fig. 2).

Typical materials of the studied species are in herbarium collections of Almaty (AA) and St. Petersburg (LE). A total of 70 herbarium specimens were analysed, on the basis of which a distribution map of *Haloxylon balchaschense* (*Arthrophytum balchaschense*) was made (Fig. 3). The map shows its locality points according to the data of specimen labels from 4 large herbarium collections (AA, MW, LE, TASH). The red ring on the map shows the point of the first description of the species (*locus classicus*), from where type materials were collected. The black ring marks the points of own collections of this species (Fig. 3).

Morphology

For visual assessment of similarity and difference of species of *Arthrophytum longibracteatum*, *Haloxylon balchaschense* (*Arthrophytum balchaschense*), *Haloxylon ammodendron* (*H. aphyllum*), their main morphological characters reflected on the photos of the viewed specimens were analysed. In particular, the following were considered: leaf arrangement, branching of young shoots and fruit wings (Fig. 4.).

Anatomy

Three species were selected for comparative anatomical analysis: *Arthrophytum longibracteatum*, *Haloxylon balchaschense* (*Arthrophytum balchaschense*), *Haloxylon ammodendron* (*H. aphyllum*). In *Arthrophytum longibracteatum*, anatomical slices of leaves and stems were studied, while in the other two species, slices of only young shoots with assimilation cells were studied, as they have no leaves.

The cross-sectional thickness for all specimens was 40 µm. Thinner slices could not be made due to the succulent origin of the studied species, the water-bearing cells of which rupture at smaller slice thicknesses (Fig. 5).

The following anatomical structures are indicated in transverse anatomical sections: epidermis, palisade mesophyll, Kranz cells, drusa, water-bearing cells, xylem, phloem, collenchyma, sclerenchyma, parenchyma cells, parenchyma of the core, vascular bundle. The biometric data are presented in Table 2.

Table 2
Biometric data on the anatomical structure of the studied species: 1 – *Haloxylon balchaschense*; *H. aphyllum*; 3 – *Arthrophytum longibracteatum* (leaves); 4 – *Arthrophytum longibracteatum* (stem).

Nº	Cross-sectional diameter, µm	Epidermis thickness, µm	Length of palisade mesophyll, µm	Kranz cell thickness, µm	Thickness of water-bearing cells layer, µm	Diameter of water-bearing cells, µm	Parenchyma µm	Sclerenchyma µm	Core diameter µm	Drill diameter µm
1.	2040,2 ± 33,5	31,88 ± 1,2	34,14 ± 0,96	15,88 ± 0,5	361,07 ± 16,8	74,42 ± 4,27	146,64 ± 3,6	45,18 ± 1,1	585,64 ± 6,8	31,98 ± 1,0
2.	1809,4 ± 11,5	30,66 ± 0,9	60,4 ± 1,45	17,89 ± 1,6	495,14 ± 12,9	68,88 ± 9,47	87,99 ± 4,7	51,98 ± 2,1	260,45 ± 4,1	22,24 ± 2,6
3.	1139,9 ± 4,2	21,3 ± 1,0	74,19 ± 7,42	19,26 ± 0,5	276,33 ± 21,8	99,73 ± 14,1	-	-	-	29,22 ± 1,4
4.	1581,9 ± 75,3	35,94 ± 1,5	59,8 ± 1,75	21,19 ± 0,7	226,88 ± 18,8	59,68 ± 7,3	145,13 ± 4,6	62,31 ± 1,5	732,7 ± 20,7	-

Molecular phylogeny

Sequencing of nrITS) and chloroplast (rps16 intron) fragments was performed for 5 species, one of which is of hybrid origin. The ITS sequencing tree is made using the studied samples and supplemented with samples from the NCBI database. In the tree, our samples are highlighted in bold. Colour highlighting shows the generic affiliation (*Haloxylon*, *Arthrophytum*, *Anabasis*) of the species (Fig. 6). It should be noted that the nrITS fragments in the genus *Haloxylon* are identical for all species of the genus, while the chloroplast fragments are more or less different according to the analyses. For detailed species distinction (scattering among species), a full genomic analysis of chloroplast fragments is needed. An attempt at amplification and sequencing of herbarium specimens of other species of the genus *Arthrophytum* was

unsuccessful because, unfortunately, the DNA in the succulent green parts of the plants was already severely degraded due to prolonged drying of the herbarium. For the chloroplast tree, rps16 intron sequences were used (Fig. 7). We also sequenced IGS trnQ-rpS16, but it did not work properly; the sequencing data were not suitable for analysis.

Discussion

Screening of literature data and available herbarium specimens revealed that *Haloxylon balchaschense* (*Arthrophytum balchaschense*) has a central-eastern-North Turanian range. In the north, it reaches the south-eastern part of the Kazakhstan Shallow Foothills; in the east (along the foothill deserts) it penetrates into the western part of the Dzungarian Alatau; in the west it is found in the Pribalkhashie and eastern spurs of Betpakdala; in the south it is confined to the desert-stony foothills of the Zailiyskiy Alatau (Iljin, 1936; Goloskokov and Polyakov, 1960; Pratov, 1972; Safronova, 2010; Kamelin, 2017) (Fig. 3). The species is endemic to Kazakhstan (Osmonali et al., 2019), prefers rubbly, clayey solonchak deserts, rarely enters sands (Iljin, 1936; Goloskokov and Polyakov, 1960; Pratov, 1972).

The main difference between the morphology of *Arthrophytum longibracteatum* and the other two leafless species is the presence of developed leaves. The similarity of morphological structure of *Arthrophytum balchaschense* and *Haloxylon aphyllum* (Figs. 1 and 4) is manifested in the structure of stems (young shoots), the spatial arrangement of which relative to the stems is 45°-50°, whereas in *Arthrophytum longibracteatum* young shoots, stems and leaves are arranged at an angle of 30°-40°. At the same time, young shoots of *Arthrophytum balchaschense* are larger ($2040.2 \pm 33.51 \mu\text{m}$) than those of *Haloxylon aphyllum* ($1809.4 \pm 11.51 \mu\text{m}$) (Table 2). In *Arthrophytum longibracteatum* these values are much smaller: in young shoots they are $1581.9 \pm 75.35 \mu\text{m}$ and the leaf diameter is $1139.9 \pm 4.22 \mu\text{m}$.

Besides, external morphological similarity of fruit wings (Fig. 4) is observed in *Arthrophytum balchaschense* and *Haloxylon aphyllum* species. However, while in *Haloxylon aphyllum* wings are located perpendicularly relative to the fruit base, in *Arthrophytum balchaschense* they bend inwards, thus covering the fruit itself. To some extent such arrangement of wings has external similarity with the similar character of such species of genus *Anabasis* as *A. truncata* (Schrenk) Bunge and *A. aphylla* L.

Comparative analysis of anatomical structure also showed similarity between *Arthrophytum balchaschense* and *Haloxylon aphyllum* species. Thus, the thickness of the epidermis in *Arthrophytum balchaschense* was $31.88 \pm 1.20 \mu\text{m}$, while in *Haloxylon aphyllum* it was $30.66 \pm 0.99 \mu\text{m}$. In *Arthrophytum longibracteatum* species, this layer was thinner in leaves $21.3 \pm 1.00 \mu\text{m}$, and on the contrary, it was thicker in stem $35.94 \pm 1.55 \mu\text{m}$. The thickness of palisade mesophyll in the first species is $34.14 \pm 0.96 \mu\text{m}$, in the second – $60.4 \pm 1.45 \mu\text{m}$, in the third – $74.19 \pm 7.42 \mu\text{m}$ (leaf), $59.8 \pm 1.75 \mu\text{m}$ (stem). According to this index, all species are well differentiated from each other (Fig. 5). The thickness of cranial cells of the species considered varies between 3–4 μm , with the lowest values observed in *Arthrophytum balchaschense* at $15.88 \pm 0.56 \mu\text{m}$ and the highest in *Arthrophytum longibracteatum* at $19.26 \pm 0.55 \mu\text{m}$ (leaf). The aquifer (water-bearing cells) is most well represented in the two compared species than in *Arthrophytum longibracteatum*. These data are also consistent with the smaller size of young shoots in this species. At the same time, despite the fact that *Arthrophytum balchaschense* exceeds the similar indicators of *Haloxylon aphyllum* in the size of the stem transverse section, they are smaller than those of *Haloxylon aphyllum* ($495.14 \pm 12.94 \mu\text{m}$) in the size of layers of some tissues, including the water-bearing layer ($361.07 \pm 16.81 \mu\text{m}$). As for other layers (except sclerenchyma), in *Arthrophytum balchaschense* they have a larger size than in *Haloxylon aphyllum*.

According to the results of molecular genetic analyses, *Arthrophytum balchaschense* is close to *Haloxylon aphyllum*, as well as other species of the genus *Haloxylon*. Given that all species lined up almost in the same lineage according to ITS sequences (Fig. 6), we can say that this fragment is the same for all species of the genus *Haloxylon*. At the same time, given the small number of phylogenetic works directly related to the studied specimens, it is difficult to judge the formation of this group (Pyankov et al., 2001; Sukhorukov, 2014; Dong et al., 2016; Schüssler et al. 2016; Sukhorukov et al, 2018; Wang et al, 2022). But the fact that the ITS sequencing fragment of *Arthrophytum balchaschense* appeared to be within *Haloxylon* species proves the generic affiliation of this species (Fig. 6). Chloroplast fragments also confirm this result (Fig. 7). In addition, the rps16 intron sequence of *Haloxylon aphyllum* B68 differs from that of *Haloxylon ammodendron* from Genbank (OR251181, NC_027668), which confirms the need for a more thorough analysis of the relationships between these species.

During the studies, other important points were noted in the structure of the phylogenetic trees obtained. According to ITS fragments, hybridogenic specimen B62 of *Arthrophytum* sp (Fig. 6) was located between species of genera *Arthrophytum* and *Haloxylon*. By chloroplast fragments the hybridogenic specimen was close to *Arthrophytum longibracteatum* (Fig. 7), while by morphological structure of leaves they differ. It is not possible to discuss the formation of the hybrid in question due to insufficient evidence. However, with a varying degree of probability it can be assumed that the parents of this specimen are *Arthrophytum longibracteatum* and *Haloxylon* sp. (possibly *Arthrophytum balchaschense*). The study of this specimen B62 *Arthrophytum* sp (hybrid) (coordinates 43.472778 N 79.010833 E) requires a full-fledged study within the whole population.

The next point worthy of attention, in our opinion, is the fact that *Salsola oppositifolia* Desf., currently understood as *Soda oppositifolia* (Desf.) Akhani (Rudov et al., 2020)), in the ITS tree showed relative closeness to the genera *Arthrophytum*, *Haloxylon*, and *Anabasis* than to *Salsola*

(Fig. 6). In terms of chloroplast tree, it appeared very close to the genus *Arthrophytum* (Fig. 7), as noted previously (Schüssler et al., 2016). In addition, comparing the presented photos of the species in the GBIF (Global Biodiversity Information Facility) database <https://www.gbif.org/species/11364737> and Plantarium <https://www.plantarium.ru/page/view/item/72511.html>, we can say that *Soda oppositifolia* is habitually very similar to species of the genus *Arthrophytum*.

Taxonomical treatment: *Haloxylon balchaschense* (Iljin) Osmonali, Veselova & Kudab. (comb. nova) - = *Anabasis balchaschensis* Iljin, in V.L.Komarov (ed.), Fl. URSS 6: 299 (1936), rossice; et in Acta Inst. Bot. Acad. Sc. URSS, Ser. I. Fasc. 2, 131 (1936), latine (1936).

= *Arthrophytum balchaschense* (Iljin) Botsch. Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 16: 85 (1954)

= *Anabasis pauciflora* Popov in V.L.Komarov (ed.), Fl. URSS 6: 879 (1936)

Lektotypus

Songoria, am Tschu. (LE 01248297). Figure 8.

Conclusion

The results of the conducted comparative analysis of morphological, anatomical and molecular-genetic structure of *Arthrophytum longibracteatum*, *Arthrophytum balchaschense*, *Haloxylon ammodendron* (H. aphyllum) species allowed to form a proof base of clear belonging of *Arthrophytum balchaschense* to the genus *Haloxylon*. This circumstance became the reason for the necessity of nomenclatural changes consisting in assignment of *Arthrophytum balchaschense* to the genus *Haloxylon* under the name *Haloxylon balchaschense* (Iljin) Osmonali, Veselova & Kudab. This species has a central-eastern-North Turanian distribution, being endemic to the flora of Kazakhstan.

Declarations

Author contribution statement

BBO molecular genetic studies, writing and coordination of the article for publication. **PVV** and **GMK** developed the concept and design of the research, organising an expedition trip, collecting material for subsequent work, and writing an article. **US** and **DSA** morpho-anatomical studies. **NF** conceptualisation, formal analysis, data processing, data curation, writing, reviewing and editing. All co-authors participated in interpreting the results and writing the final manuscript.

– BBO, <https://orcid.org/0000-0003-3060-8597>; PVV, <https://orcid.org/0000-0002-3903-6276>; GMK, <https://orcid.org/0000-0001-7315-4033>; US, <https://orcid.org/0000-0002-7485-6341>; DSA, <https://orcid.org/0000-0002-0681-5468>, NF, <https://orcid.org/0000-0003-3547-3257>.

Acknowledgements

This research has been funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (Grant No. AP19679078 - Studying the species diversity of ecotone territory of northeastern Betpakdala for preserving the relict gene pool of Kazakhstan arid flora).

References

1. Baitenov, M.S. (2001) The genus *Kalidium* Moq. Flora of Kazakhstan. Genus complex flora. Almaty. 2, p. 72. (in Russian)
2. Blattner F. R., (1999) Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. In.: Biotechniques 27. pp. 1180-1186
3. Botschancev V.P. (1954) *Arthrophytum balchaschense* (Iljin) Botsch Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 16:85.
4. Butnik A. A., Ashurmetov O. A., Nigmanova R. N., Payzieva S. A. (2001) Ecological anatomy of desert plants in Central Asia. (Half-shrubs, shrubs). Tashkent, – Fan 2. – P. 132
5. Butnik A. A., Duschanova G. M., Yusupova D. M., Abdullaeva A. T., Abdinazarov S. H. (2017) Types leaf mesophyll species of Chenopodiaceae Vent. Central Asia and their role in the monitoring of desertification // Journal of Novel Applied Sciences, – Vol. 6(1). – pp. 13–21
6. Dong W, Xu C, Li D, Jin X, Li R, Lu Q, Suo Z. (2016) Comparative analysis of the complete chloroplast genome sequences in psammophytic *Haloxylon* species (Amaranthaceae) PeerJ 4: e 2699. doi: 10.7717/peerj.2699
7. Edwards GE, V Franceschi, EV Voznesenskaya (2004) Single-cell C4 photosynthesis versus the dual-cell (Kranz) paradigm. Annu Rev Plant Biol 55 pp. 173–196
8. Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791. doi: 10.2307/2408678

9. Freitag H, W Stichler (2000) A remarkable new leaf type with unusual photosynthetic tissue in a central Asiatic genus of Chenopodiaceae. *Plant Biol* 2, pp. 154–160
10. Friesen; N. 2007. Molecular methods, used in Plant Systematic. Practical Approach. AsBuka-Press. Barnaul. 64 p. (In Russian).
11. Friesen, N., Grützmacher, L., Skaptsov, M., Vesselova, P., Dorofeyev, V., Luferov, A.N., Turdumatova, N., Lazkov, G., Smirnov, S.V., Shmakov, A.I. (2022) *Allium pallasii* and *A. caricifolium* – Surprisingly Diverse Old Steppe Species, Showing a Clear Geographical Barrier in the area of lake Zaysan. *Plants*, P. 11, 1465. doi: 10.3390/plants11111465
12. Goloskokov V.P. and Polyakov P.P. (1960) Chenopodiaceae. in *Flora of Kazakhstan*. – T. 3. Alma-Ata: Kasakh SSR Academy of Sciences. P.198-359.
13. Ilyin, M.M. (1936) The family Chenopodiaceae. In the *Flora of the URSS* VI. pp. 3-354. [in Russian]
14. Kamelin R.V. (2017) Flora of coloured clays of Middle Asia (brief analysis and problems of genesis) // *Turczaninowia* 20 (4): 125–151 doi: 10.14258/turczaninowia.20.4.14 [in Russian]
15. Kechaykin, A.A., Batkin, A.A., Sitpayeva, G.T., Vesselova P.V., Osmonali, B.B., Shmakov, A.I. (2020) New data on genus *Potentilla* L. (Rosaceae Juss.) in the flora of Kazakhstan // *Turczaninowia*, Vol. 23, № 1. P. 32-40 doi: 10.14258/turczaninowia
16. Kluge, A. G., and Farris J. S. (1969) Quantitative phyletics and the evolution of anurans. *Syst. Zool.* 18, 1–32. doi: 10.1093/sysbio/18.1.1
17. Kumar S., Stecher G., and Tamura K. (2016) Mega 7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, pp. 1870–1874. doi: <https://doi.org/10.1093/molbev/msw054>
18. Lazkov G.A., Vesselova P.V. (2022) On two species of *Dracocephalum* L. (Lamiaceae) // *Turczaninowia*, T. 25, № 4. pp. 52-57 doi:10.14258/turczaninowia
19. Osmonali B. B., Vesselova P.V., Kudabaeva G.M. (2019) Distribution peculiarities of endemic species from the family Chenopodiaceae Vent. of Kazakhstan flora // *Problems of botany of South Siberia and Mongolia*. pp. 343-346. doi: 10.14258/pbssm.2019068 [in Russian]
20. Osmonali B.B., Akhtaeva N.Z., Vesselova P.V., Kudabayeva G.M., Kurbatova N.V. (2020) The anatomical structure peculiarities of various species of the genus *Salsola* L. // *Problems of botany of South Siberia and Mongolia* – T. 19, № 2. pp. 146-154. Doi: 10.14258/pbssm.2020093 [in Russian].
21. Osmonali BB, Vesselova PV, Kudabayeva GM, Skaptsov MV, Shmakov AI, Friesen N. (2023) Phylogeny and Flow Cytometry of the genus *Kalidium* Moq. (Amaranthaceae s.l.) in Kazakhstan. *Plants* 12: 2619. Doi: 10.3390/plants12142619
22. Popov M.G. (1936) *Anabasis pauciflora* Popov. *Flora URSS* VI. P. 879. [in Russian]
23. Pratov U. (1972) Chenopodiaceae / *Plant Identifier of Plants of Central Asia*. – vol. III. - Tashkent: FAN Publishing House. – pp. 20–98. [in Russian]
24. Pyankov V.I., Voznesenskaya E.V., Kondratschuk A.V., Black C. C. (1997) A comparative anatomical and biochemical analysis in *salsola* (Chenopodiaceae) species with and without a kranz type leaf anatomy: a possible reversion of c4 to c3 photosynthesis. *American Journal of Botany*, 84(5), pp. 597–606. Doi: 10.2307/2445895
25. Pyankov, V.I., Artyusheva, E.G., Edwards, G.E., Black, C.C., Jr. and Soltis, P.S. (2001) Phylogenetic analysis of tribe Salsoleae (Chenopodiaceae) based on ribosomal ITS sequences: implications for the evolution of photosynthesis types. *Am. J. Bot.*, 88: pp.1189–1198. doi: 10.2307/3558329
26. Ronquist R., and Huelsenbeck J. P. (2003) Mr Bayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, pp. 1572–1574. doi: <https://doi.org/10.1093/bioinformatics/btg180>
27. Rudov A, Mashkour M, Djamali M, Akhani H (2020) A Review of C4 Plants in Southwest Asia: An Ecological, Geographical and Taxonomical Analysis of a Region with High Diversity of C4 Eudicots. *Front. Plant Sci.* 11:546518. Doi: 10.3389/fpls.2020.546518
28. Safronova I.N. (2010) Botanical and geographical zoning of Turan deserts as a basis for rational nature use // *Arid ecosystems*. – Vol. 16. – №2 (42). – pp. 47–53. [in Russian]
29. Schüssler C., Freitag H., Koteyeva N., Schmidt D., Edwards G., Voznesenskaya E., Kadereit G. (2016) Molecular phylogeny and forms of photosynthesis in tribe Salsoleae (Chenopodiaceae). *Journal of Experimental Botany*, 68(2), pp. 207–223. Doi:10.1093/jxb/erw432
30. Shaw J., Lickey E.B., Schilling E. E., Small R. L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III // *American Journal of Botany* 94(3): pp. 275–288
31. Sukhorukov AP (2014) The carpology of the Chenopodiaceae with reference to the phylogeny, systematics and diagnostics of its representatives. *Grif & Co., Tula*. p. 400
32. Sukhorukov AP, Nilova MV, Krinitsina AA, Zaika MA, Erst AS, Shepherd KA (2018) Molecular phylogenetic data and seed coat anatomy resolve the generic position of some critical Chenopodioideae (Chenopodiaceae – Amaranthaceae) with reduced perianth segments. *PhytoKeys* 109: pp. 103–128. Doi: 10.3897/phytokeys.109.28956
33. Sukhorukov AP, Singh N, Kushunina M, Zaika MA, Sennikov AN (2023) A new species of *Atriplex* (Amaranthaceae) from the Indian subcontinent. *Phyto Keys* 229: pp. 167-183. doi: 10.3897/phytokeys.229.105162

34. Sukhoruko A. P., Léger J.-F. & Chambouleyron M. Two new species aliens to the flora of Morocco: *Amaranthus spinosus* (Amaranthaceae) and *Cardamine occulta* (Brassicaceae). *Fl. Medit.* 33: pp. 31-38. 2023. Doi: 10.7320/FIMedit33.031.
35. Sukhorukov, P.; Kushunina, M.A.; Lomonosova, M.N. A new *Kalidium* species (Amaranthaceae s. l.) from northern Central Asia. *Turczaninowia* 2022, 25, pp. 24–33. doi: 10.14258/turczaninowia.
36. Sumbembayev A.A., Abugalieva S.I., Danilova A.N., Matveyeva E.V., Szlachetko D.L. Flower morphometry of members of the genus *Dactylorhiza* Necker ex Nevski (Orchidaceae) from the Altai Mountains of Kazakhstan // *Biodiversitas*, V. 22 (8), 2021. pp. 3545-3555. doi: 10.13057/biodiv/d220855.
37. Sumbembayev, A.A., Nowak, S.; Burzacka-Hinz, A., Kosiróg-Ceynowa, A., Szlachetko D.L. (2023) New and Noteworthy Taxa of the Genus *Dactylorhiza* Necker ex Nevski (Orchidaceae Juss.) in Kazakhstan Flora and Its Response to Global Warming. *Diversity* 2023, 15, P. 369. doi: 10.3390/d15030369
38. Sumbembayev, A.A.; Lagus O.A., Nowak S. (2023) Seed morphometry of *Rheum* L. (Polygonaceae) species from Kazakhstan and its implications in taxonomy and species identification // *Biodiversitas*, V. 24 (9), pp. 4677-4692. doi: 10.13057/biodiv/d240908
39. Swofford D. –L. (2002). PAUP*: Phylogenetic analysis using parsimony (* and other methods). version 4 (Sunderland, Massachusetts: Sinauer Associates).
40. Terletskaia N.V., Turzhanova A.S., Khapilina O.N., Zhumagul M.Z., Meduntseva N.D., Kudrina N.O., Korbozova N.K., Kubentayev S.A., Kalendar R. (2023) Genetic Diversity in Natural Populations of *Rhodiola* Species of Different Adaptation Strategies. *Genes*, 14, P. 794. doi: 10.3390/genes14040794
41. Thompson J. D., Gibson T. J., Plewniak F., Jeanmougin F., and Higgins D. G. (1997) The clustal X window interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25, pp. 4876–4882. doi: 10.1093/nar/25.24.4876.
42. Voznesenskaya E.V., Koteyeva N.K., Akhani H., Roalson E.H., Edwards G.E. (2013) Structural and physiological analyses in *Salsola* (Chenopodiaceae) indicate multiple transitions among C3, intermediate, and C4 photosynthesis // *Journal of Experimental Botany*, Vol. 64, No. 12, pp. 3583–3604, doi: 10.1093/jxb/ert19.
43. Voznesenskaya EV, VR Franceschi, O Kiirats, EG Artyusheva, H Freitag, GE Edwards (2002) Proof of C4 photosynthesis without Kranz anatomy in *Bienertia cycloptera* (Chenopodiaceae). *Plant J* 31. pp. 649–662.
44. Voznesenskaya EV, VR Franceschi, O Kiirats, H Freitag, GE Edwards (2001) Kranz anatomy is not essential for terrestrial C4 plant photosynthesis. *Nature* 414. pp 543–546
45. Wang M., Zhang L., Tong Sh., Jiang D., Fu Zh., Chromosome-level genome assembly of a xerophytic plant, *Haloxylon ammodendron*, *DNA Research*, Volume 29, Issue 2, April 2022, dsac006, doi: 10.1093/dnares/dsac006
46. White T. J, Bruns T., Lee S., Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Shinsky JJ, White TJ, editors. *PCR Protocols: A Guide to Methods and Applications*, pp. 315–322. Academic Press, San Diego
47. Zhaglovskaya A., Aidosova S., Akhtayeva N., Mamurova A., Yesimova D. (2015) Anatomical and Morphological Stem Features of two *Haloxylon* Species (Chenopodiaceae Vent.) of Drought Stress, Kazakhstan // *Biosci., Biotech. Res. Asia*, Vol. 12(3), pp. 1965-1974 doi: 10.13005/bbra/1863
48. Zhu GL, Mosyakin SL, Clemants SE. (2004) *Haloxylon* Bunge (Chenopodiaceae). In: *Flora of China* Editorial Committee, ed. *Flora of China*. Vol. 5. St. Louis: Science Press, Beijing/Missouri Botanic Garden Press, pp. 395–396

Figures

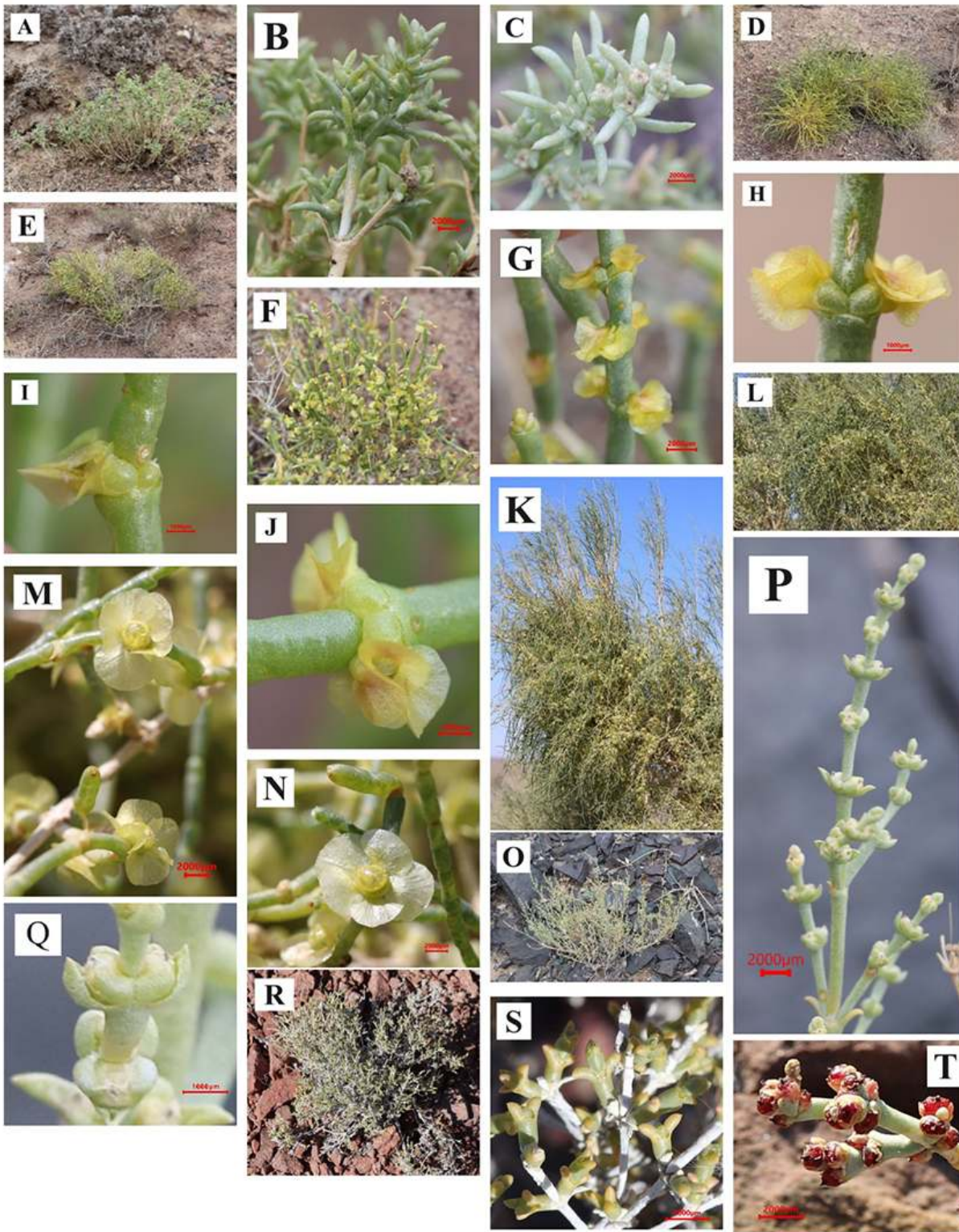


Figure 1

Objects of the study: A-C - *Arthrophytum longibracteatum*, D-J - *Haloxylon balchaschense* (*Arthrophytum balchaschense*), K-N - *Haloxylon ammodendron* (*H. aphyllum*), O-Q - *Arthrophytum* sp. (hybrid), R-T - *Anabasis salsa*

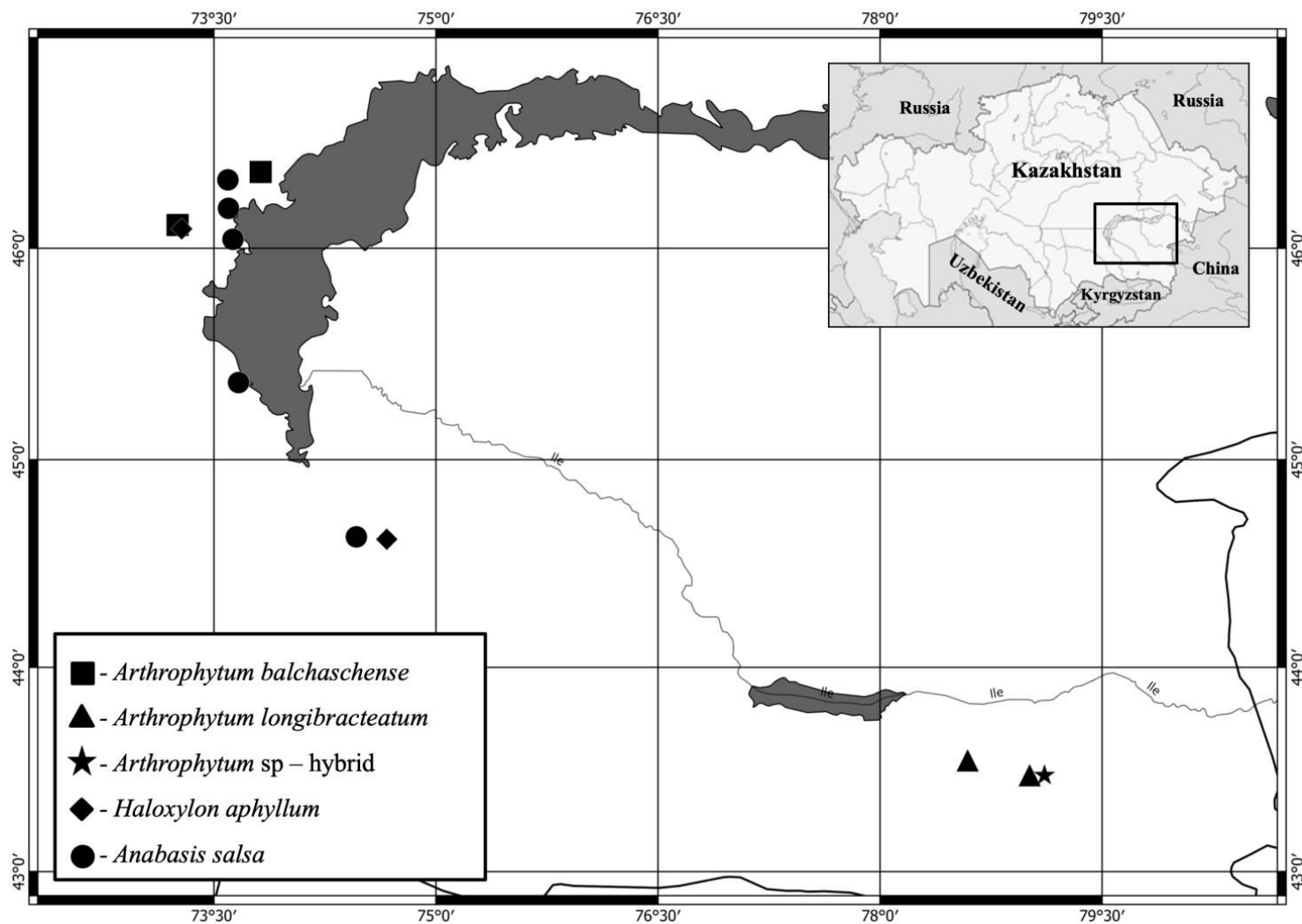


Figure 2

Distribution map of the samples studied

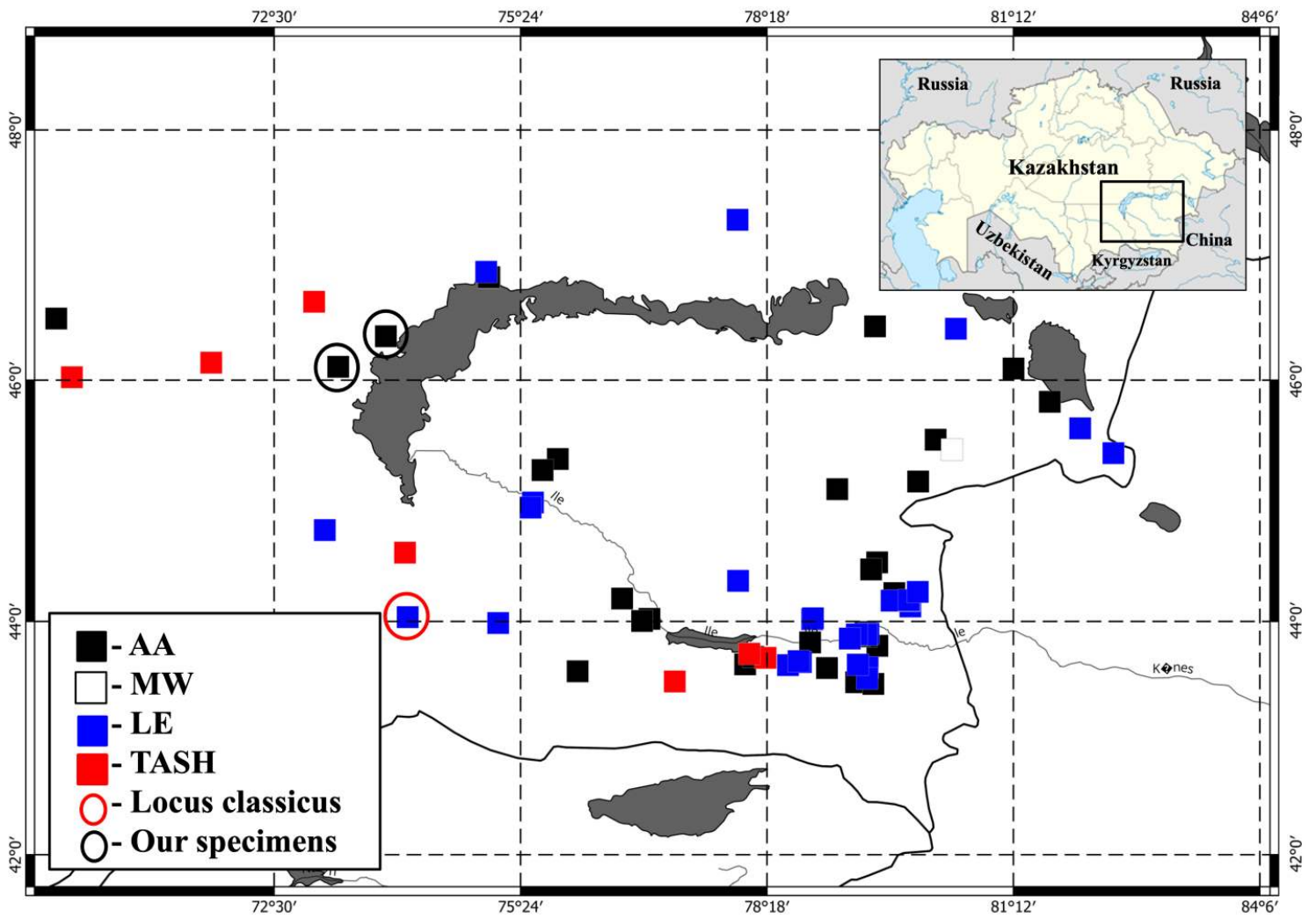


Figure 3

Map of *Haloxylon balchaschense* (*Arthrophytum balchaschense*) localities according to the studied herbarium specimens (Appendix 1)

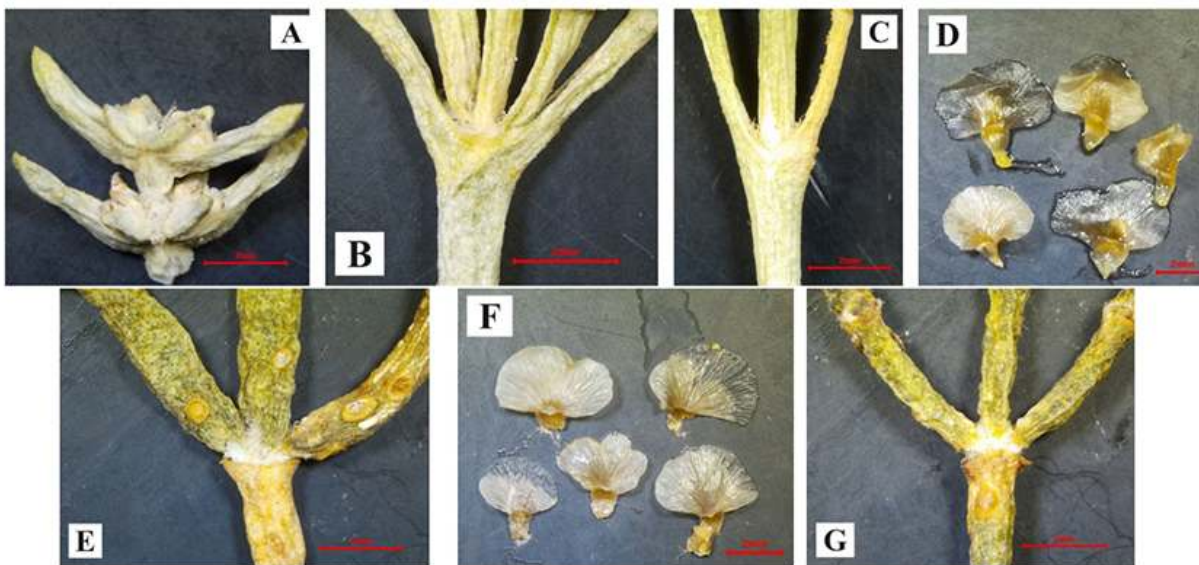


Figure 4

Morphological characters (herbarium specimens): A-C - *Arthrophytum longibracteatum*, D-E - *Haloxylon balchaschense*, F-G - *Haloxylon aphyllum*. A-C, E, G leaf arrangement; D, F fruit wings.

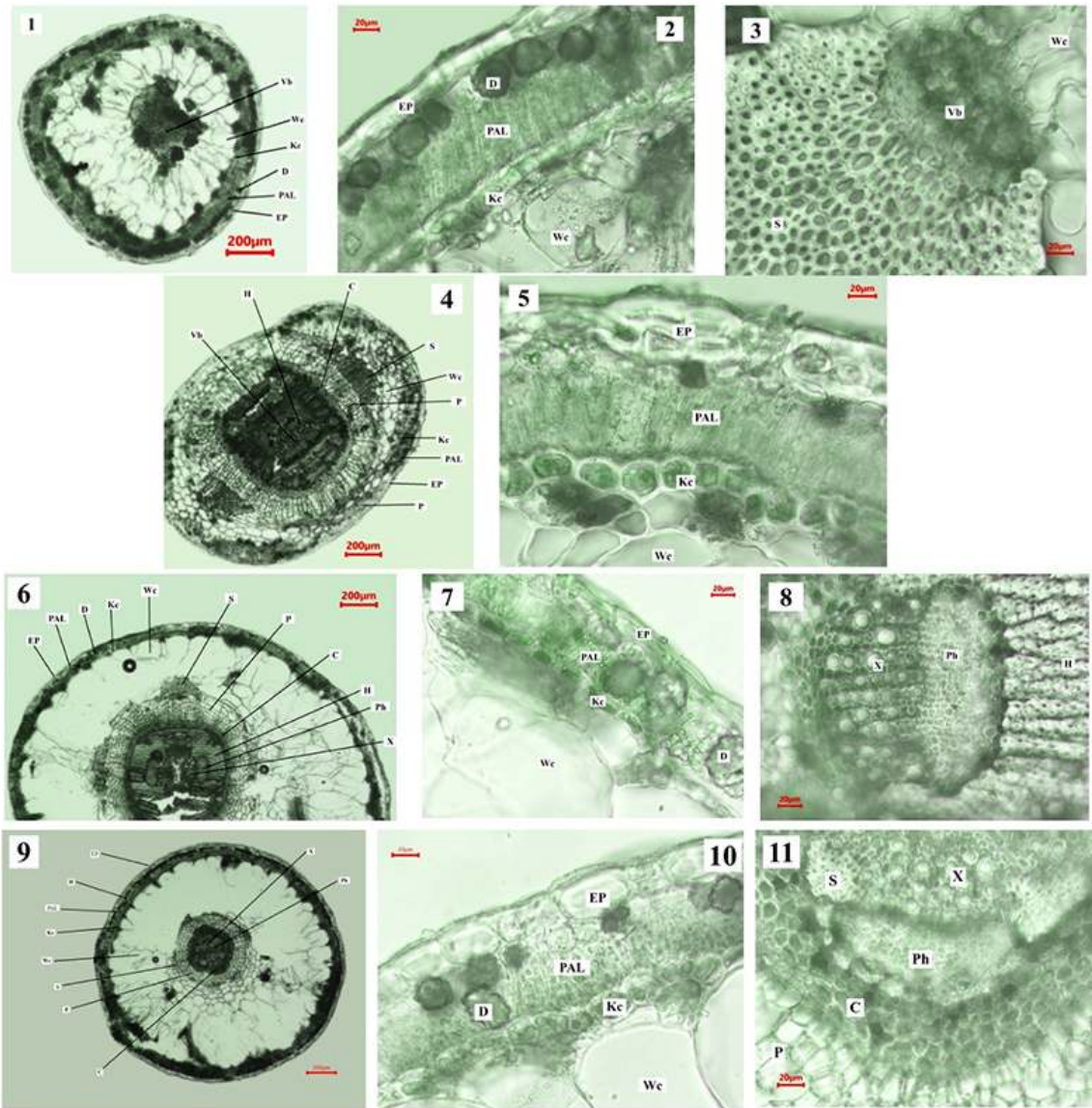


Figure 5

Comparative anatomical structure of the studied species: 1-5 - *Arthrophytum longibracteatum* (1-3 - leaf, 4-5 - stem), 6-8 - *Haloxylon balchaschense* (*Arthrophytum balchaschense*), 9-11 - *Haloxylon ammodendron* (*H. aphyllum*). EP - Epidermis, PAL - Palisade Chlorenchyma, Kc - Kranz cell, D - Druza, Wc - Water-bearing cell, X - Xylem, Ph - Phloem, C - Collenchyma, S - Sclerenchyma, P - Parenchyma cells, H - Core parenchyma, Vb - Vascular bundle

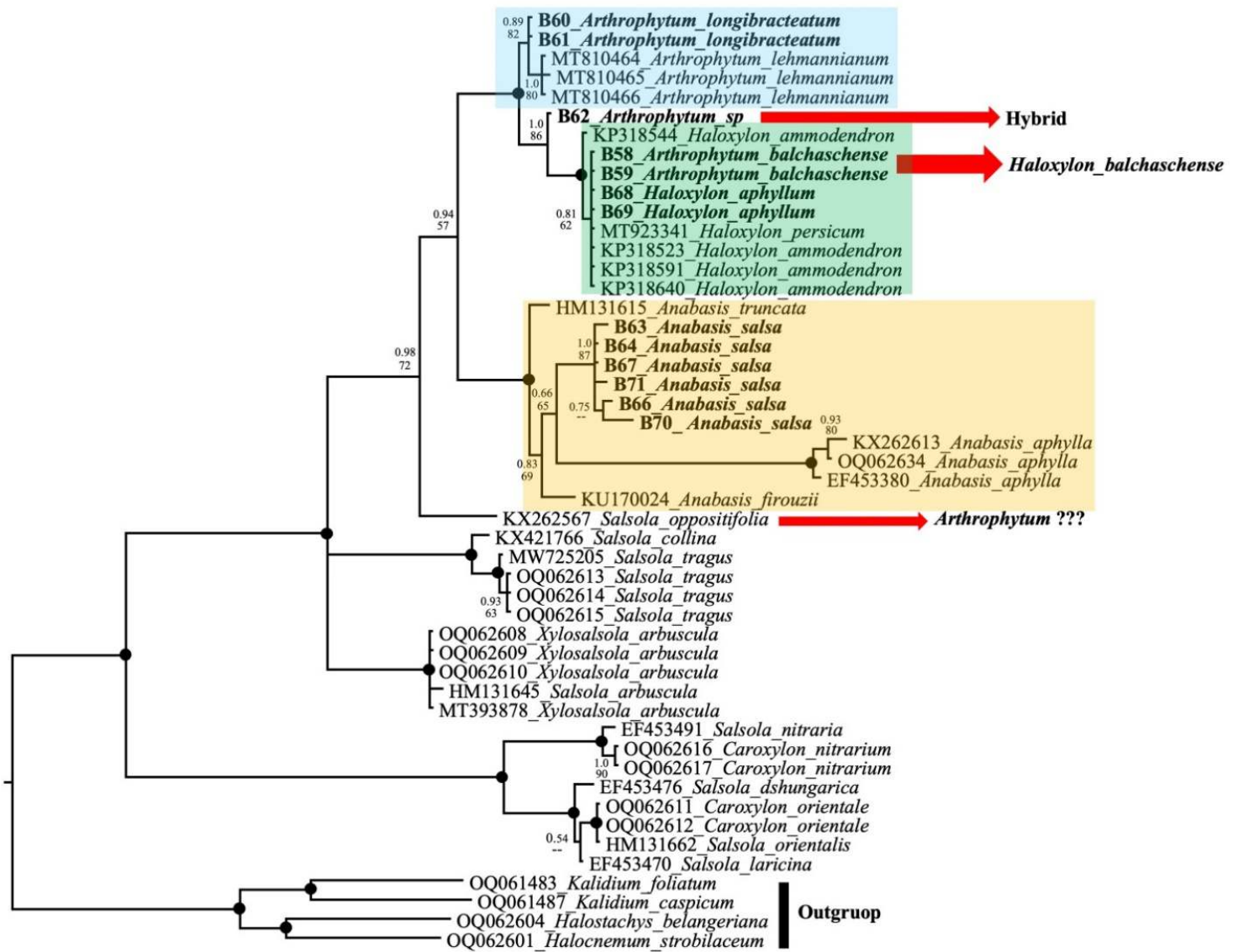


Figure 6

ITS tree of species of the genus *Haloxylon*, *Arthrophytum*, *Anabasis*. The joint presence of Bayesian with a probability greater than 0.98 and bootstrap support greater than 95% is indicated by a black dot. The samples we investigated are highlighted in bold. The following data were obtained by running the data through the JModeltest software: GTR+I+G, -lnL 2647.49996, AIC 5470.999920, weight 0.654477

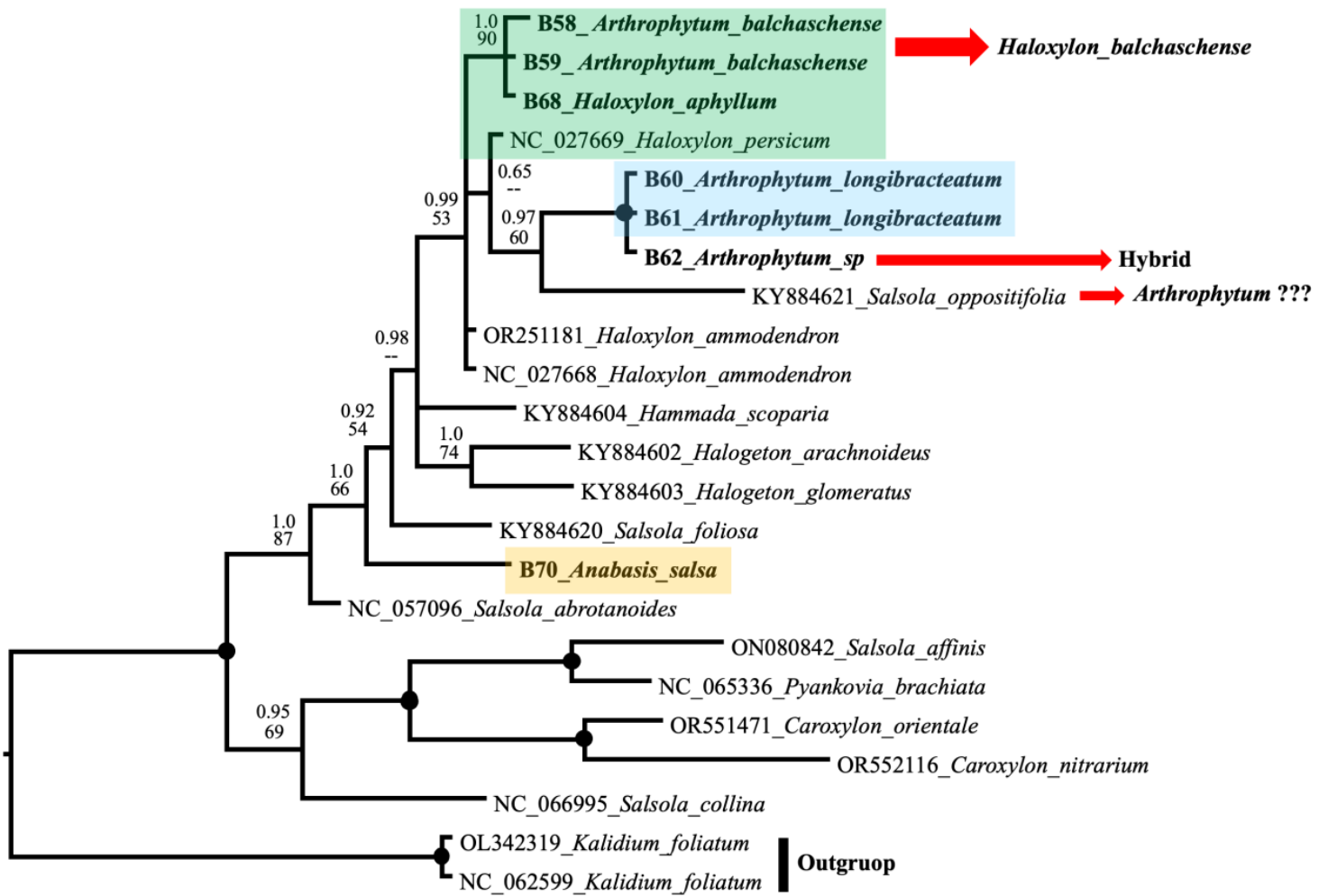


Figure 7

Plastiden tree (rps16 intron) species of the genus *Haloxylon*, *Arthrophytum*, *Anabasis*. The joint presence of Bayesian with probability greater than 0.98 and bootstrap support greater than 95% is indicated by a black dot. The samples we investigated are highlighted in bold. The following data were obtained by running the data through the JModeltest software: GTR+I, -lnL 2293.37211, AIC 4692.744220, weight 0.237090

