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Conflicting Phylogenetic Signals between the Nuclear Ribosomal and Plastome DNA as Evidence for Hybrid Origin of the Tetraploid Member of *Salicornia* (Amaranthaceae s.l.)

Tahir H. Samigullin[1,a]*, Maria D. Logacheva[1,2], Galina V. Degtjareva[1,3], Dmitry D. Sokoloff[4], Svetlana S. Beer[5], and Carmen M. Valiejo-Roman[1]

[1]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119992 Moscow, Russia*

[2]*Center for Molecular and Cellular Biology, Skolkovo Institute of Science and Technology, 121205 Moscow, Russia*

[3]*Research and Educational Center – Botanical Garden of Peter I, Faculty of Biology, Lomonosov Moscow State University, 119991 Moscow, Russia*

[4]*School of Plant Sciences and Food Security, Faculty of Life Sciences, Tel Aviv University, 6997820 Tel Aviv, Israel*

[5]*Private School "Obninsk Free School", 249038 Obninsk, Russia*

[a]*e-mail: samigul@belozersky.msu.ru*

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Abstract—Species of the genus *Salicornia* (Amaranthaceae s.l.) are globally distributed and highly salt-tolerant. They are used as food and for biofuel production. Formation of pure lines through self-pollination, combined with sporadic cross-pollination, polyploidy, high phenotypic plasticity, and a limited number of diagnostic characters, significantly complicates taxonomy of the genus. *Salicornia* (glassworts) is an evolutionarily young group, where the number of informative substitutions in the traditionally analyzed regions of nuclear and plastid DNA is insufficient to establish relationships between the species. The very concept of a species in this genus remains a subject of debate. To clarify relationships among the Eastern European species, we used high-throughput sequencing to determine sequences and perform phylogenetic analysis of the plastomes of 11 samples representing all major morphotypes of Eastern European glassworts. We also analyzed variability of the nuclear rDNA external transcribed spacer (nrETS). The sizes of the assembled plastomes ranged from 153,290 bp to 153,504 bp and exhibited a typical architecture with a large single-copy region (84,625-84,797 bp), a small single-copy region (18,818-18,870 bp), and two inverted repeats (24,898-24,908 bp). Comparison of phylogenetic trees reconstructed from all currently available plastome data and nrETS alignments of the same glasswort accessions revealed a discrepancy in the placement of the tetraploid *S. procumbens* subsp. *pojarkovae* and *S. brachiata* accessions, which show affinities to different lineages depending on the use of either plastid or nuclear (nrETS) data. Our results highlight the role of reticulate evolution in the genus *Salicornia*.

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Keywords: plastome, nrETS DNA, phylogenetic signal conflict, polyploidy, hybridization, *Salicornia*

INTRODUCTION

Hybridization, followed by potential loss of genetic material, is one of the primary mechanisms of angiosperm evolution. In addition to formation of auto- or allopolyploids, mitochondrial or plastid introgression is frequently observed [1-7]. These processes collectively lead to the emergence of groups where organismal relationships do not fit the standard model of dichotomous evolution and can be poorly

* To whom correspondence should be addressed.

illuminated by classical methods. Additional challenges arise from the often-insufficient informativeness of the analyzed genome regions of organelles and nucleus, especially in the context of recent evolutionary events. This situation is observed in glassworts (*Salicornia* L., Amaranthaceae s.l.) – an evolutionarily young group where the very concept of a species is a subject of debate [8-13].

Salicornia is a genus of hygrohalophytic succulent annual plants widely distributed in Eurasia, Africa, North and South America, Australia, and New Zealand [14-19]. In Europe and America, *Salicornia* species are cultivated and consumed as food; oil extracted from their seeds is similar in fatty acid composition to safflower oil [20, 21]. Glassworts are also used for biofuel production on coastal saline lands where traditional crops do not thrive and are considered promising for phytoremediation [22].

Taxonomic boundaries of the genus *Salicornia* are highly controversial due to the high phenotypic plasticity, scarcity of diagnostic morphological traits, simplified morphology, polyploidy, and formation of inbred lines with minor but presumably fixed phenotypic differences [10, 23]. It is no coincidence that the traditional division of European *Salicornia* species into sections was based on chromosome number [24]. Typically, *Salicornia* species are either diploid (2n = 18) or tetraploid (2n = 36) [25-30], although a triploid form of *Salicornia veneta* Pign. et Lausi was found in Italy [31], and a decaploid species, *Salicornia altaica* Lomon., was discovered in the southeastern Altai and northwestern Mongolia [32]. Tetraploids generally differ from diploids by having larger anthers, a greater ratio of the length of the central flower in the dichasium to the lateral flower, longer and thicker inflorescences, and fewer sterile metamers on the main shoot [24, 33]. However, the ranges of morphological traits in diploids and tetraploids overlap, making delineation of the species within the diploid and tetraploid lineages an extremely challenging task [10].

In delineation of the *Salicornia* species, there is a growing interest in molecular markers. Using various non-coding regions of nuclear and plastid DNA, as well as microsatellite loci, it has been shown that reticulate evolution is common among the Western European species, tetraploids have an allopolyploid origin, and there is insufficient information on both diploids and tetraploids to understand their interrelationships [8-13, 33-37].

A comprehensive phylogenetic study of *Salicornia*, covering all major regions of its geographic range based on nucleotide sequences of the nuclear ribosomal external transcribed spacer (nrETS), revealed widespread parallel evolution of morphological traits (combined with strong phenotypic plasticity) [10]. Some species of the genus have been proposed to be considered cryptic, as they cannot be distinguished using morphological traits [23].

In this study, we focused on Eastern European representatives of the genus *Salicornia*. Compared to the Western Europe, a relatively small number of species occur here, simplifying the study of reticulate evolution. Tzvelev [38] distinguished four *Salicornia* species in Eastern Europe: *S. perennans* Willd., *S. europaea* L., *S. borysthenica* Tzvel., and *S. pojarkovae* N. Semen. Later, Beer and Demina [39] described the species *S. heterantha* Beer et Demina from the Rostov Region (Kuma-Manych Depression), which differs from all other species of the genus by the complete fusion of the perianth tube of the central flowers of the dichasium and the main axis of inflorescence. According to Tzvelev, two diploid species, *S. perennans* and *S. europaea*, grow in the southern and eastern regions of Eastern Europe (*S. perennans*) and on the coasts of the White and Baltic Seas (*S. europaea*); the tetraploid species *S. pojarkovae* is found on the coast of the White Sea, and *S. borysthenica* is morphologically similar to the Western European tetraploid *S. dolichostachya* Moss, but Tzvelev had no data on the chromosome number for this species. Analysis of nrETS sequences [10, 23] placed the diploid species *S. heterantha* and *S. borysthenica* in one clade with the tetraploid *S. pojarkovae* and some other tetraploids (including *S. dolichostachya*). Sukhorukov and Akopyan [40] placed *S. heterantha* among the synonyms of *S. perennans* Willd. Sukhorukov [41], using the treatment suggested by Kadereit et al. [23] regarding the species boundaries, synonymized *S. heterantha* with *S. procumbens* subsp. *procumbens*. Chatrenoor and Akhani [33], using new materials, support the species status of *S. heterantha*; however, their work does not use all available GenBank data.

Based on the molecular data, the boundaries of European *Salicornia* species have been revised, and new nomenclatural combinations have been proposed [23]. Most species have been transferred to the rank of subspecies, while others have been synonymized. According to the system of Kadereit et al. [23], four taxa occur in Eastern Europe: *Salicornia perennans* Willd. subsp. *perennans*, *S. procumbens* Sm. subsp. *procumbens* (incl. *S. borysthenica* Tzvel.), *S. procumbens* subsp. *heterantha* (S. S. Beer & Demina) G. Kadereit & Piirainen, and *S. procumbens* subsp. *pojarkovae* (Semenova) G. Kadereit & Piirainen. It should be noted that molecular data correlate well with geographic distribution but often do not correspond to any morphological traits. Therefore, *S. perennans* and *S. europaea* should be considered cryptic species in the new concept [23, 42]. Molecular data suggest that material from the north and northwest of European Russia, previously identified as *S. europaea*

(e.g., in Tzvelev's work [38]), should be attributed to *S. perennans* [23].

The aim of this article is to clarify relationships of the Eastern European species using an expanded sample and additional molecular data. For the first time, we used high-throughput sequencing to determine the plastome sequences of 11 *Salicornia* samples from Eastern Europe. We analyzed these data in comparison with the nrETS data. We studied material that, according to the system of Kadereit et al. [23], corresponds to *S. perennans*, *S. procumbens* subsp. *pojarkovae*, *S. procumbens* subsp. *procumbens*, and *S. procumbens* subsp. *heterantha*. For the description and discussion of the results, we followed the system of Kadereit et al. [23]. Our results allow us to discuss the possibility of hybridization among the representatives of the genus *Salicornia*.

MATERIALS AND METHODS

Plant material. Samples were collected from natural habitats in the southern (Kuma-Manych Depression, Black Sea and Caspian Sea lowlands) and northern (White Sea coast) parts of Eastern Europe. Detailed information on the studied samples, GenBank identifiers, and references to the samples already sequenced in previous studies are provided in the Online Resource 1.

DNA extraction. DNA was extracted using a NucleoSpin Plant II kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. Quantity and quality of DNA were assessed using a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and electrophoresis in 0.8% agarose gel.

Sequencing, plastome assembly, annotation, and alignment. DNA from each sample was fragmented using a Covaris S220 ultrasonicator (Covaris, USA). End repair, adenylation, and adapter ligation followed by PCR were performed using an Illumina TruSeq DNA Sample Prep Kit (Illumina, USA) according to the manufacturer's instructions. Libraries were sequenced using an Illumina HiSeq 2000 sequencer with a read length of 101 bp from each end of the fragment. Read trimming was performed using Trimmomatic [43]. *De novo* plastome assembly was carried out using the CLC Genomics Workbench v5.5 software package. Contigs with extensive similarity to the known plastid genomes were joined at overlapping ends. Correctness of the assembly was verified by mapping trimmed reads to the assembled plastomes. Automatic annotation was performed using the online CpGAVAS program [44], followed by manual correction. All tRNAs were verified using tRNAscan-SE v2.0 [45] and ARAGORN [46]. The plastome map was visualized using OGDRAW [47].

Plastome sequences were aligned using MAFFT v7.017 [48] and then manually corrected in BioEdit [49].

Amplification, sequencing, assembly from GenBank raw data, and alignment of nrETS. Amplification of the 3′ end of nrETS was performed using combination of primers ETS-Salicornia-5′-GTCCCTATTGTGTAGATTTCAT-3′ and 18S-II rev. 5′-CTCTAACTGATTTAATGAGCCATTCGCA-3′ [10, 50]. PCR products were purified using a Cleanup Mini Kit for PCR product purification (Evrogen, Russia). Direct sequencing of both strands of the spacer was performed at the Genome Center for Collective Use (Russian Academy of Sciences).

To correctly compare topologies of the plastid and ETS trees, we assembled genomes from GenBank sequence read archive data for six glasswort samples with known plastid genomes (as described above, except for PacBio HiFi sequencing data, for which we used the Canu assembler [51]) and obtained nrETS sequences (Online Resource 1), which were also used for analysis. DNA sequences were aligned using MUSCLE [52] and then manually corrected in BioEdit [49].

Intragenomic polymorphism of nrETS. To detect intragenomic polymorphism, the trimmed reads were mapped to nrETS sequences. Polymorphism in eleven *Salicornia* samples was assessed visually using Tablet (version 1.17.08.17) [53].

Phylogenetic analysis. A set of ten completely assembled and one partially assembled plastomes was supplemented with six sequences from the GenBank database (Online Resource 1). As an outgroup, we used plastomes of *Suaeda malacosperma* Hara (NC_039180), *Bienertia sinuspersici* Akhani (MT316307), *Kalidium gracile* Fenzl (ON149858), and *Salicornia fruticosa* (L.) L. (NC_066030). Phylogenetic analysis included 20 plastome sequences in total; one inverted repeat was removed. Gap-rich positions (more than 50%) were excluded from the analysis. Phylogenetic analysis was performed using the Bayesian approach in MrBayes v3.2.6 [54, 55] with the GTR+Γ model, which was selected as the best according to the Akaike Information Criterion (AIC). For 16 Markov chains (4 parallel runs of 4 chains each), 20,000,000 steps were set, and every thousandth tree was sampled. The first thousand trees were discarded, and the remaining trees were used to construct a majority-rule consensus tree and estimate posterior probabilities of the internal branches.

The matrix of aligned nrETS sequences was also analyzed using the Bayesian approach with the same settings as for the plastome data; in polymorphic positions, the predominant (major) base was indicated. As an outgroup, we used sequences of *Microcnemum coralloides* (Loscos & J. Pardo) Buen (EF433589), *Arthrocnemum macrostachyum* (Moric.) K. Koch (EF433587), and *S. fruticosa*.

RESULTS AND DISCUSSION

Characteristics of *Salicornia* plastomes. We performed shallow genomic sequencing of 11 glasswort samples representing all major morphotypes of the genus *Salicornia* found in Eastern Europe. Complete plastome sequences were assembled for ten samples: one sample of *S. procumbens* subsp. *procumbens* (1209); two samples of *S. procumbens* subsp. *pojarkovae* (1196 and 1194); three samples of *S. procumbens* subsp. *heterantha* (1213, 1214, and 1219); and four samples of *S. perennans* (1197, 1195, 1211, and 1226). The assembled and annotated sequences were deposited in the GenBank database; accession numbers are provided in the Online Resource 1. For sample 1208, approximately 98% of the plastome length was assembled, so we considered it acceptable to use this incomplete plastome in phylogenetic analysis. Sizes of the fully assembled plastomes ranged from 153,290 bp to 153,504 bp and exhibited

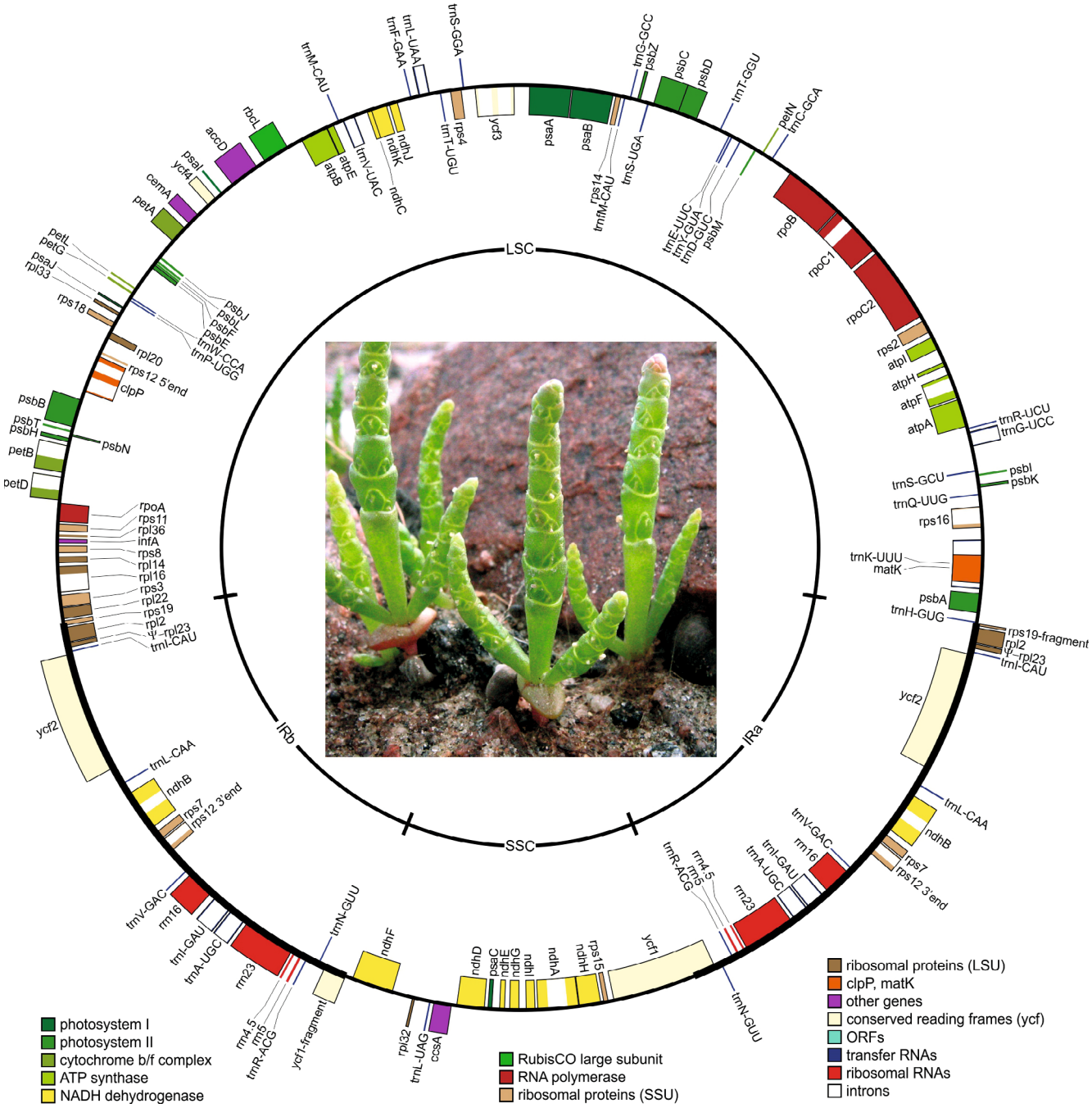

Fig. 1. Circular map of the *Salicornia* plastid genome. Genes located inside and outside the circle are transcribed clockwise and counterclockwise, respectively. Pseudogenes are marked with "ψ." LSC, large single-copy region; SSC, small single-copy region; IRa and IRb, inverted repeats. Photo: *S. procumbens* subsp. *pojarkovae*, by G. V. Degtjareva.

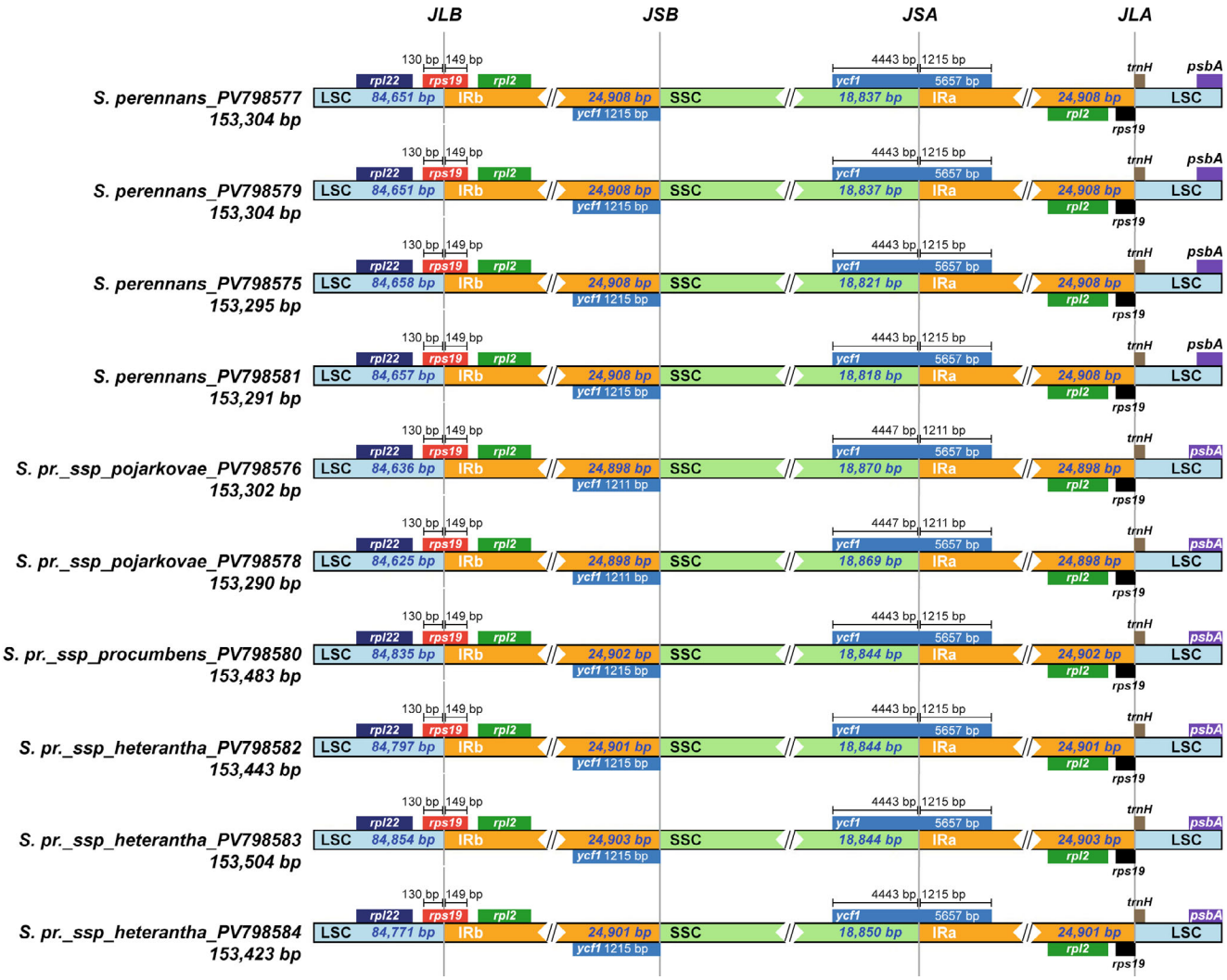

Fig. 2. Position of the boundaries of inverted repeats in the plastomes of glassworts. *S. pr.* – *S. procumbens*; JLB and JSB – boundaries of IRb with LSC and SSC, respectively; JSA and JLA – boundaries of IRa with SSC and LSC, respectively.

a typical architecture with a large single-copy region (LSC; 84,625-84,797 bp), a small single-copy region (SSC; 18,818-18,870 bp), and two inverted repeats (IR; 24,898-24,908 bp) (Fig. 1).

After annotation, 113 unique genes were identified, including 30 tRNA genes, 4 rRNA genes, and 79 protein-coding genes (listed in the Online Resource 2). The plastomes contained three pseudogenes: *rpl23*, *ycf1*, and *rps19*, the latter two being truncated fragments of the corresponding genes at the boundaries of the inverted repeats. Pseudogenization of *rpl23* is observed in all representatives of the Amaranthaceae family; multiple cases of independent pseudogenization of this gene in plastomes have also been noted in other families of the order Caryophyllales [56, 57]. Composition and order of the genes in the newly assembled and previously known *Salicornia* plastomes do not differ from each other, and no any differences were found in the arrangement of homologous genes (synteny) compared to other autotrophic representatives of the order Caryophyllales. In the plastid genomes of glassworts, as in the overwhelming majority of the species in the core Caryophyllales, the intron of the *rpl2* gene is absent [57, 58]. Overall, it can be stated that the plastomes of glassworts are highly conserved in structure, and the differences in genome sizes of *Salicornia* samples are mainly determined by the variability of intergenic regions: indels of various lengths are present in many spacer sequences, with the most variable being the *psbA*–*trnH* and *rpl33*–*rps18* spacer regions.

Boundaries of the inverted repeats with the large single-copy region in all glassworts intersected the *rps19* (LSC/IRb) and *trnH*-GUG (LSC/IRa) genes and were located identically, while positions of the SSC/IRb (between the *ycf1* gene fragment and the *ndhF* gene) and SSC/IRa (within the *ycf1* gene) boundaries differed slightly (Fig. 2).

Table 1. Intragenomic polymorphism of nrETS sequences in glassworts

Samples		Alignment positions					
		171	274	376	414	456	469
S. perennans subsp. *perennans* 2n = 18	1211	C	A	T	C	A	G
	1226	C	A	T	C	A	G
	1195	C	A	T	C	A	G
	1197	C	A	T	C	A	G
S. procumbens subsp. *pojarkovae* 2n = 36	1194	C	A	T	C	A	T
	1196	C	A	T	C	A	T
S. procumbens subsp. *heterantha* 2n = 18	1219	A/C	A	T	C	A	T
	1213	C/A	G/A	T	C	A	T
	1214	C/A	G/A	T	C	A	T
S. procumbens subsp. *procumbens* 2n = 18	1209	C/A	G/A	T	C	A	T
	1208	A/C	A/G	T	C	A	T
S. europaea 1		C	A	T	C	R	G
Salicornia sp. variant 2		C	A	T	C	A	G
Salicornia sp. variant 1		C	A	A	T	A	T
S. sinus-persica MW679260		C	A	A	T	A	T
S. persica subsp. *iranica* EF433645		C	A	A	T	A	T
**S. persica* subsp. *persica* MW679241		C	A	W	Y	A	K
**S. persica* subsp. *iranica* MW679243		C	A	W	Y	A	K

Note. In polymorphic positions, the predominant (major) variant is listed first. R = G or A, W = A or T, Y = C or T, K = G or T.
* Sequences not included in phylogenetic analysis.

Intragenomic polymorphism and phylogenetic analysis of nrETS. Mapping of the reads obtained directly by us using high-throughput sequencing to the nrETS sequence revealed intragenomic polymorphism at two positions (171 and 274) in five diploid southern samples: *S. procumbens* subsp. *heterantha* (samples 1219, 1213, 1214) and *S. procumbens* subsp. *procumbens* (samples 1208 and 1209). Polymorphic positions are presented in Table 1 along with other characteristic substitutions.

Notably, in the assembled contigs of the *Salicornia* sp. sample (data from the GenBank sequence read archive, PacBio HiFi sequencing, SRR24425728) we identified two variants of ETS sequences differing at three alignment positions: 376, 414, and 469 (Table 1). This sample was deposited in the database as *S. europaea*, but the detected intragenomic polymorphism differs from that observed in *S. europaea* and is more consistent with the sequences of *S. persica* subsp. *iranica* (GenBank ID: MW679243) or *S. persica* subsp. *persica* (GenBank ID: MW679241), which calls into question the species identification of this sample (see also Jamdade et al. [59]).

No intragenomic polymorphism was detected in the diploid northern *S. perennans* (samples 1195 and 1197), southern *S. perennans* (samples 1211 and 1226), northern tetraploids *S. procumbens* subsp. *pojarkovae* (samples 1194 and 1196), or the tetraploid *S. bigelovii*. Concerted evolution of the nuclear ribosomal operon sequences has long been well known [60], so heterogeneity of the ribosomal operon sequences is generally considered characteristic of the recent hybrids. Since there have been no suggestions about the hybrid nature of these *S. procumbens* subsp. *heterantha* and *S. procumbens* subsp. *procumbens* samples, it would be premature

to explain the detected polymorphism by recent hybridization, especially since potential second parental form is clearly not represented in our analysis. Nevertheless, such explanation cannot be ruled out at this stage, nor can be the alternative hypothesis of incomplete unification of inherited ancestral polymorphism (see also the discussion in Yurtseva et al. [61]).

Phylogenetic analysis. Phylogenetic tree obtained from the Bayesian analysis of 48 nrETS sequences is presented in Fig. 3a. The tree shows three main groups of Eastern European *Salicornia*, with samples grouped mainly by ribotypes (nrETS haplotypes). *S. procumbens* forms a well-supported monophyletic group (posterior probability PP = 1), within which two clades of southern diploid *S. procumbens* subsp. *heterantha* with *S. procumbens* subsp. *procumbens* are distinguished. One clade consists of six sequences with ribotype 20 (here and below, ribotypes are indicated according to Kadereit et al. [23]), and the other consists of four sequences of two ribotypes differing by a single nucleotide substitution, with both clades containing representatives of two subspecies. In the unresolved part of this monophyletic group, eight northern tetraploids of *S. procumbens* subsp. *pojarkovae* (ribotype 21) are located. Sequences of the *S. perennans* representatives do not form a monophyletic group: samples from the southern regions with ribotype 9 formed a weakly supported (PP = 0.87) unresolved clade, while representatives of the northern populations of *S. perennans* with ribotype 2 are located in the group combining sequences of *S. brachiata*, *S. europaea*, and *S. aff. europaea* with variant 2 ETS of *Salicornia* sp. (PP = 0.98). Details of relationships within this group remain unclear, as does the position of the *S. perennans* 484 sample with ribotype 11 and the trio of *S. bigelovii* representatives. In a separate clade, also of unclear position relative to other glasswort species, the sequences of *S. sinus-persica*, *S. persica*, and variant 1 ETS of *Salicornia* sp. are combined (PP = 0.99). Overall, the arrangement of glasswort samples does not contradict the previously presented results obtained from the analysis of a similar set of samples [10, 12, 23, 62].

In the consensus tree obtained from the Bayesian analysis of plastid data, the plastomes of glasswort assembled by us were distributed into several well-supported clades (Fig. 3b). The plastomes of five samples of diploid southern *S. procumbens* subsp. *heterantha* and *S. procumbens* subsp. *procumbens* (1208, 1209, 1213, 1214, and 1219) formed a well-supported but poorly resolved monophyletic group (as in the nrETS tree). The plastome sequences of the northern diploid *S. perennans* (1195 and 1197) and *S. europaea* 1 formed a group sister to the southern diploid *S. perennans* (1211 and 1226) plus *S. brachiata*. Two samples of the tetraploid *S. procumbens* subsp. *pojarkovae* (1196 and 1194) cluster with the plastome sequence of *Salicornia* sp. with low support (PP = 0.96) and are more closely related to the plastomes of *S. perennans* than to other plastomes of *S. procumbens*. The plastome with the identifier OL449699, deposited in GenBank as *S. europaea* [63], turned out to be a sequence of *S. bigelovii* or a very closely related taxon, which is also confirmed by phylogenetic analysis of the ETS sequence of this sample.

Notably, the plastid tree shows a good resolution in the relationships of *S. perennans* samples that remain unclear in the nrETS tree, and at the same time, within the species *S. procumbens*, it shows slightly worse resolution, revealing only closer relationship between the samples *S. procumbens* subsp. *procumbens* 1208 and *S. procumbens* subsp. *heterantha* 1219. However, if the lack of resolution in the nrETS tree is due to the lack of variability (for example, nrETS sequences of samples 1213, 1214, and 1209 are identical and belong to the same ribotype), then the resolution in the plastid tree is associated with the lack of informative substitutions, since all analyzed plastomes differ (both in length and sequence), including those of samples with nrETS of the same ribotype. The plastome variability we identified is an example of the widespread non-strict conservation of plastome sequences in the plants within a species [64], the scale of which remains to be assessed, but it is already clear that the plastome data may be promising in resolving questions of relationships at least for some glasswort species.

Comparison of phylogenetic trees reconstructed from the plastid and nrETS alignments reveals discrepancy in the placement of a pair of tetraploid *S. procumbens* subsp. *pojarkovae* samples (1194 and 1196), which show closer relationship with the diploid *S. procumbens* in the nrETS tree but cluster with the diploid *S. perennans*, *S. brachiata*, and *S. europaea* in the plastid tree. It can be definitively stated that the tetraploid glasswort lineage studied in this work should be interpreted as a hybrid, and hybridization occurred between the lineage represented in our study by the southern diploids of *S. procumbens* and the lineage combining *S. perennans*, *S. europaea*, *S. brachiata*, and possibly *S. persica*. Further research using expanded sampling with involvement of plastome data is required for more accurate determination of the parental forms of these hybrids. Differences in the revealed relationships for *S. brachiata* in the plastid (representatives of the southern populations of *S. perennans*) and ETS (*S. europaea* and representatives of the northern populations of *S. perennans*) trees also raise suspicions about the hybrid nature of this taxon, but additional research is required to confirm this assumption and identify the parental forms.

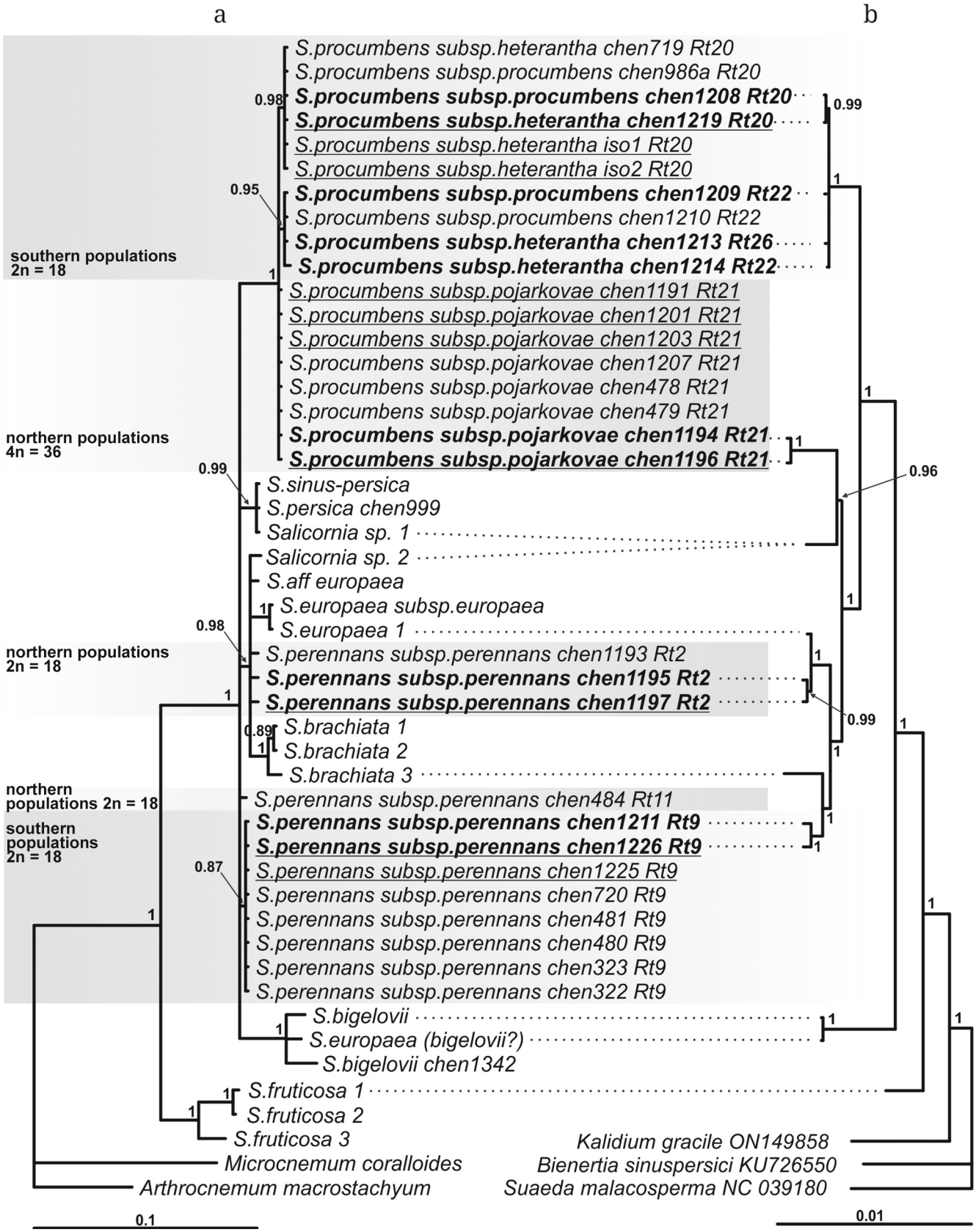

Fig. 3. Phylogenetic tree reconstructed using the Bayesian approach for nrETS (a) and plastid (b) sequences. Posterior probabilities exceeding 0.85 are indicated. Branch lengths are proportional to the number of substitutions per site, according to the corresponding scale. Samples with known chromosome numbers are underlined; samples with plastomes sequenced by us are in bold. Ribotypes are indicated according to the numbering proposed by Kadereit et al. [23].

CONCLUSION

Our data indicate the need for further study of microevolution of the genus *Salicornia* with broader use of plastome data and further improvement of glasswort systematics. In particular, the revealed position of the *S. procumbens* subsp. *procumbens* samples relative to *S. procumbens* subsp. *heterantha* samples calls into question the criteria for distinguishing subspecies within *S. procumbens*. In addition to solving purely taxonomic issues (which will remain a subject of debate in such complex group), the study of genetic regulation and adaptive value of the main trait underlying the distinction of *S. heterantha* – fusion of the perianth of the central flower of the dichasium with the inflorescence axis [65] – is of interest. The fact that samples possessing this trait do not form a single clade suggests a relatively simple genetic regulation of this trait. With the fascinating progress in plant evo-devo and considering fast life cycle of glassworts, it is possible to identify the genes associated with this trait, which will contribute to understanding the patterns of organ fusion regulation in angiosperm flowers [66].

Abbreviations

nrETS	nuclear ribosomal external transcribed spacer

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1134/S0006297925602072.

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Contributions

M. D. Logacheva, C. M. Valiejo-Roman, and S. S. Beer – collection of material and experiments; T. H. Samigullin, D. D. Sokoloff, G. V. Degtjareva, and M. D. Logacheva – processing and discussion of experimental results; C. M. Valiejo-Roman, T. H. Samigullin, and D. D. Sokoloff – writing the text; M. D. Logacheva, C. M. Valiejo-Roman, G. V. Degtjareva, D. D. Sokoloff, S. S. Beer, and T. H. Samigullin – editing the article text.

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Ethics approval and consent to participate

This work does not contain any studies involving human and animal subjects performed by any of the authors.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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