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Molecular analysis of the section *Sphagnum* (Sphagnaceae): first study on Turkish bryophytes using nucleotide sequences of the *trnL* intron region

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Abstract: The genus *Sphagnum* is one of the richest taxa among the Turkish bryophytes. Up to now, it has been represented by 27 taxa in six sections (*Sphagnum*, *Acutifolia*, *Squarrosa*, *Subsecunda*, *Cuspidata*, and *Rigida*). In this study, an identification key for the section *Sphagnum* was prepared, and the section was investigated using phylogenetic analyses, with a main focus on Turkish bryophytes. This study provides the first sequence data of the *trnL* intron region for five species of the section *Sphagnum* from Turkey (namely *S. centrale*, *S. divinum*, *S. medium*, *S. palustre*, and *S. papillosum*) to be used in future studies and gives valuable information about their phylogenetic relationships. However, further studies on Turkish members of the section *Sphagnum* using more specimens and molecular markers should be performed to obtain more detailed information regarding the phylogenetic relationships among them.

Key words: Bryophyta, molecular taxonomy, peatland, bog

1. Introduction

Eight hundred eighty-seven bryophytes (three species of hornworts, 163 species and intraspecific taxa of liverworts and 721 species and intraspecific taxa of mosses) from Turkey according to the checklist prepared in 2005 by Erdağ and Kürschner have been reported. Based on 2015 data, this number was updated to 1030 (835 mosses; 759 species, 76 varieties, 191 liverworts; 179 species, 2 subspecies, 10 varieties and 4 hornworts) by the same researchers (Erdağ and Kürschner, 2017). This increase from 2005 to 2015 reveals the potential of Turkish bryophytes. The genus *Sphagnum* is one of the richest taxa among the Turkish bryophytes. In the last decade, several new taxa have been added to the list of Turkish Sphagnas (Tonguç Yayıntaş, 2013; Kırmacı and Kürschner, 2013; Abay and Keçeli, 2014; Kırmacı and Kürschner, 2017; Ören et al., 2017; Kürschner et al., 2019a, 2019b; Kırmacı et al., 2019; Erata and Batan, 2020). Recently, the first author finished a revision project supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) on this genus in Turkey. Up to now, it has been represented by 27 taxa in six sections, i.e. *Sphagnum*, *Acutifolia, Squarrosa, Subsecunda, Cuspidata*, and *Rigida*. The section *Sphagnum* is easily identified with spiral fibrils on cortical cells of stems. Also, the members of the section are normally large with distinct capitula. Stem leaves are relatively large lingulate or spatulate and branch leaves distinctly concave and often strongly overlapping to give branches a turgid appearance, the apex strongly hooded (Daniels and Eddy, 1985). Until now, five taxa, i.e. *S. centrale* C.E.O. Jensen, *S. palustre* L., *S. papillosum* Lindb., *S. divinum* Flatberg & K.Hassel and *S. medium* Limpr, have been given from the section *Sphagnum* in Turkey. The last two taxa known as *S. magellanicum* previously were newly added to Turkish bryoflora, and it was reported that *S. divinum* was the most frequent member of the section, while *S. medium* was rarer (Kürschner et al., 2019a, 2019b). Hassel et al. (2018) and Kyrkjeeide et al. (2016) showed *S. magellanicum* relationship with *S. divinum* and *S. medium* with their study. And also, Hassel et al. (2018) stated that *S. magellanicum* was limited to South America and it could not be found in Europe and the Old World. After these papers, all taxa in the Herbarium of Adnan Menderes University (Aydın) that have already been identified as *S. magellanicum* were rechecked and revised to *S. medium* or *S. divinum* based on this work. *S. papillosum* was collected during the revisional project and identified as new record for Turkey. Later, it was reported from Anzer Valley (Rize) by Erata et al. (2021) as second distributional locality.

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As the classical morphology-based taxonomy studies are sometimes insufficient to identify closely related taxa, molecular methods, which depend on nucleotide sequences of RNA and DNA and sequences of amino acids of a protein (Patwardhan et al., 2014), have been increasingly used in the solution of systematic problems since the 1990s and applied to all organism groups with the development of new techniques (i.e. Shaw et al., 2019; Mishra et al., 2022; Sari, 2022). Compared with flowering plants, there are fewer molecular analysis-based studies on bryophytes; however, in Turkey, there is no study on this subject. Therefore, this study aimed to analyse the phylogenetic relationships of some species from the section *Sphagnum*, with a main focus on the species collected from Turkey, and to test the adequacy of existing keys for identifying these specimens to species level. The importance of the study is that molecular methods were used on Turkish bryophytes for the first time. In addition, a section key based on morphological characters was given in the present study.

2. Materials and methods

2.1. Sampling methods

All specimens were collected during a revision project on the genus *Sphagnum* of Turkey supported by TÜBİTAK between 2013 and 2017. Information belonging to taxa including elevation, geographic coordinates, and general vegetation were noted during the field trips, and all taxa were photographed in their habitats. Taxa were identified using relevant studies, floras, and revision studies and kept at the Herbarium of Adnan Menderes University (Herbarium code: AYDN).

2.2. Locality details

***Sphagnum centrale* C.E.O. Jensen**

1. Artvin, Arhavi, Sazak Bog (to Arhavi 21 km), 1650 m; 41°13′14.2″N 41°20′00.5″E, 28.08.2016; MKIR 7414, coll. and det. M: Kırmacı.

2. Artvin, between Arhavi and Sırt Plateau, Sazak Bog, 1590 m; 41°13′41.8″N 41°19′38.2″ E, 28.08.2016; MKIR 7388, coll. and det. M: Kırmacı.

3. Artvin, Murgul, Şevval Mountain, 2200 m; 41°09′59″N 41°29′56.5″ E, 29.08.2016; MKIR 7456, coll. and det. M: Kırmacı.

4. Artvin, Borçka, Klaskur Plateau, 2340 m; 41°21′07.2″ N 41°21′07.2″ E, 30.08.2016; MKIR 7470, coll. and det. M: Kırmacı.

5. Giresun, grassland, 2280 m; 40°49′31.1″ N 39°02′06.9″ E, 2016; MKIR 7542, coll. and det. M: Kırmacı.

6. Gümüşhane, Kürtün, Kabayalık Plateau,2016; MKIR 7516, coll. and det. M: Kırmacı.

7. Gümüşhane-Giresun, Kızılali Plateau, grassland, 1650 m; 40°49′15.4″ N 39°02′33.9″ E, 2016; MKIR 7539, coll. and det. M: Kırmacı.

8. Rize, İkizdere, Anzer Plateau, Öküz Yatağı, 2650 m; 40°31′55.0″ N 40°30′18.8″E, 2012; MKIR 6290, coll. and det. M: Kırmacı.

9. Rize, İkizdere, Çimil Plateau, 2110 m; 40°43′47.9″N 40°48′14.5″ E, 2012; MKIR 6305b, coll. and det. M: Kırmacı.

10. Rize, Çamlıhemşin, Aşağı Kavrun, 950 m; 40°54′29.9″ N 41°08′20.9″ E, 2012; MKIR 6321 coll. and det. M: Kırmacı.

11. Rize, Fındıklı, between Aslandere and Koçdüzü Plateau, 380 m; 41°13′36.8″ N 28°22′16.01″ E, 27.08.2016; MKIR 7365, coll. and det. M: Kırmacı.

12. Rize, Çamlıhemşin, Koçdüzü Plateau, 2340 m; 41°00′12.4″N 41°10′47.1″E, 31.08.2016; MKIR 7473, coll. and det. M: Kırmacı.

13. Rize, Çamlıhemşin, Elevit Plateau, 2280 m; 40°51′35.5″ N 41°02′17.4″E, 2016; MKIR 7547, coll. and det. M: Kırmacı.

14. Rize, Çamlıhemşin, between Elevit and Trovit Plateau, 2400 m; 40°51′44.2″N 41°02′46″E, 2016; MKIR 7557, coll. and det. M: Kırmacı.

15. Rize, Çamlıhemşin Trovit Plateau, 2440 m; 40°51′43.8″N 41°02′46″E, 2016; MKIR 7561, coll. and det. M: Kırmacı.

16. Trabzon, Sürmene-Küçükdere, Ağaçbaşı Plateau, 2000 m; 40°42′24.2″ N 40°05′40.6″E, 2012; MKIR 6070, coll. and det. M: Kırmacı.

17. Trabzon, Tonya, between Karakısrak and Sazalan Plateau, 1700–1800 m; 40°47′28.8″N 39°04′18.8E, 2012; MKIR 6157, coll. and det. M: Kırmacı.

18. Trabzon, Sürmene-Köprübaşı, Ağaçbaşı, Ayı yatağı, 25.08.2016; MKIR 7280, coll. and det. M: Kırmacı.

19. Trabzon, Çaykara, between Sultanmurat and Barma Plateau, 1870 m; 40°41′ 7.2″N 40°09′03.6″ E, 25.08.2016; MKIR 7326, coll. and det. M: Kırmacı.

20. Trabzon, Çaykara, Barma Plateau, 1860 m; 40°42′11.2″ N 40°08′57.7″E, 26.08.2016; MKIR 7336, coll. and det. M: Kırmacı.

***Sphagnum divinum* Flatberg & Hassel**

1. Artvin, between Arhavi and Sırt Plateau, Sazak Bog, 1590 m; 41°13′41.8″ N 41°19′38.2″E, 28.08.2016; MKIR 7415, coll. and det. M: Kırmacı.

2. Artvin, Arhavi, Sazak Bog (to Arhavi 21 km), 1650 m; 41°13′14.2″ N 41°20′00.5″E, 28.08.2016; MKIR 7383, coll. and det. M: Kırmacı.

3. Artvin, Borçka, Beyaz su Plateau, 2290 m; 41°21′07.1″ N 41°56′59.4″E, 30.08.2016 MKIR 7464, coll. and det. M: Kırmacı.

4. Rize, İkizdere, between Aşağı Çağıran and Yukarı Çağırankaya, 2190 m; 40°49′46.4″N 40°39′31.8″E, 2012; MKIR 6260, coll. and det. M: Kırmacı.

5. Rize, Fındıklı, between Aslandere and Koçdüzü Plateau, 380 m; 41° 13′ 36,8″ N 28° 22′ 16,01″ E, 27.08.2016; MKIR 7367, coll. and det. M: Kırmacı.

6. Trabzon, Tonya, between Karakısrak and Sazalan Plateau, 1700–1800 m; 40°47′28.8″ N 39°04′18.8 E, 2012; MKIR 6156c, MKIR 6147, coll. and det. M: Kırmacı.

***Sphagnum medium* Limpr.**

1. Trabzon, Sürmene-Köprübaşı, Ağaçbaşı Plateau, Ayı yatağı, 25.08.2016; MKIR 7265, coll. and det. M: Kırmacı.

2. Trabzon, Sürmene-Köprübaşı, Ağaçbaşı Plateau, 1940 m; 40°41′41.5″N 40°04′59.6″E, 2012; MKIR 6121, coll. and det. M: Kırmacı.

***Sphagnum palustre* L.**

1. Çanakkale, Çan, Söğütalan Village, Ciğer lake bog, 650 m, 39°52′37″N 26°55′40″E, MKIR 7577, coll. and det. M: Kırmacı.

2. Giresun, Yeşilpınar Village, Maden neighbourhood, 210 m; 40°54′33.7″N 38°53′17.5″E, 29.10.2015; MKIR 6989, coll. and det. M: Kırmacı.

3. Gümüşhane, Kürtün, Kabayalık Plateau, 2016; MKIR 7517, coll. and det. M: Kırmacı.

4. Rize, Fındıklı, between Aslandere and Koçdüzü Plateau, 380 m; 41°13′36.8″N 28°22′16.01″E, 27.08.2016; MKIR 7367, coll. and det. M: Kırmacı.

5. Rize, Çamlıhemşin, Elevit Plateau, 2280 m; 40°51′35.5″N 41°02′17.4″E, 2016; MKIR 7549, coll. and det. M: Kırmacı.

6. Trabzon, Sürmene-Köprübaşı, Ağaçbaşı Plateau, 1950 m; 40°41′48.8″N 40°05′01.6″E, 2012; MKIR 6085b, coll. and det. M: Kırmacı.

7. Trabzon, Sürmene-Köprübaşı, Ağaçbaşı, Ayı yatağı, 25.08.2016; MKIR 7257, coll. and det. M: Kırmacı.

***Sphagnum papillosum* Lindb.**

1. Gümüşhane, Kürtün, Kabayalık Plateau, 15.09.2016; MKIR 7424, coll. and det. M: Kırmacı.

2. Artvin, between Arhavi and Sırt Plateau, Sazak Bog, 1590 m; 41°13′41.8″N 41°19′38.2″E, 28.08.2016; MKIR 7414h, coll. and det. M: Kırmacı.

2.3. Molecular methods

Sphagnum specimens were transported from sampling sites to the Biochemistry and Molecular Toxicology Laboratory in Pamukkale University, Denizli. All the samples were stored dry until use.

2.3.1. DNA isolation

All specimens which were taxonomically identified based on morphological and anatomical characters and bookmarked for molecular analyses were rinsed with distilled water to remove dirt and biological contaminants. DNA was isolated from dried gametophytic tissue of seven specimens from five species (Table) using the DNeasy Plant Mini Kit (Qiagen, Redwood City, CA, USA) following the manufacturer's protocol. The extracted DNA was quantified spectrophotometrically at 260/280 nm and DNA integrity was analysed using 1% agarose gel (90 V, 40 min).

2.3.2. PCR amplification

PCR reactions were performed in a single tube with gene primers specific for the intron region of the chloroplast tRNA gene (*trnL*): forward 5′-CGAAATCGGTAGACGCTACG-3′, reverse 5′-ATTTGAACTGGTGACACGAG-3′ (Taberlet et al., 1991). The DNA was amplified in a 25 µL reaction mixture containing 0.4 mM forward and reverse primers, 0.3 mM dNTPs, 2 mM $MgCl_2$ and 2.5 units of hot start *Taq* DNA polymerase (ABM, Canada) in Reaction Buffer.

The PCR conditions consisted of incubation for 5 min at 95 °C, followed by 30 cycles at 94 °C for 1 min, 60 °C for 1 min, 72 °C for 3 min and final extension at 72 °C for 6 min. All analyses were done at least three times. The PCR products were visualized on a 1% agarose gel (90 V, 40 min), and successful amplifications were purified with the PureLink PCR Purification Kit (Invitrogen, Carlsbad, CA, USA) according to the instructions provided by the manufacturer. Sequence analyses of the purified DNAs were performed by GATC Biotech (Ebersberg, Germany).

2.3.3. Sequence alignment and phylogenetic analyses

In this study, seven specimens representing five species of the section *Sphagnum*, were sequenced and these sequences were deposited in GenBank (accession numbers MW547007-MW547013). In addition, sequences of 30 specimens representing 21 species of the section *Sphagnum* as well as *S. compactum* (from the section *Rigida*), *S. fallax* (from the section *Cuspidata*), and *S. subsecundum* (from the section *Subsecunda*) selected as outgroup to root generated phylogenetic trees were obtained from GenBank (Table). Nucleotide sequences of the *trnL* intron region of seven specimens were checked by eye and edited using MEGA 6 (Tamura et al., 2013), and the sequences of 40 specimens (including outgroup) were aligned by ClustalW using MEGA X version 10.0.5 (Kumar et al., 2018). Nucleotide saturation was determined by plotting the absolute number of transitions and transversions against genetic distance values with a TN93 distance model using DAMBE software (Xia and Xie, 2001), and the quality of the phylogenetic information was evaluated. Evolutionary distances were calculated using the Tamura–Nei method (Tamura and Nei, 1993). Neighbour-joining (NJ) tree of the calculated distances were created in PAUP *4.0b10 (License code; ADU B418788) (Swofford, 2002) and MEGA X version 10.0.5 with 10,000 bootstrap replicates. Maximum likelihood (ML) analysis was performed in RAXMLGUI 1.0 (Silvestro and Michalak, 2012) and MEGA X version 10.0.5. Data analyses were performed using GTRGAMMA model. Branch reliability of ML trees was calculated with 10,000 bootstrap replicates. Bayesian inference (BI) analysis was performed in MrBayes v.3.2.5 (Ronquist et al., 2012) under the general time reversible model with a gamma distribution and proportion of

Table. List of species used in the phylogenetic analyses and their GenBank accession numbers.

Genus	Section	Species	Isolate no.	Collection no.	Accession no.	Reference
Sphagnum	*Cuspidata*	*Sphagnum fallax*	SB356	99959	AF192595	Shaw, 2000
	Rigida	*Sphagnum compactum*	comp1196	9712	AY298049	Shaw et al., 2003
	Sphagnum	*Sphagnum affine*	aff1210	26521	AY297995	Shaw et al., 2003
			aff1211	28884	AY297996	Shaw et al., 2003
		Sphagnum alaskense	SB4569	13960	MF362353	Shaw et al., 2019
		Sphagnum amoenoides	SB4519	2697	MF362354	Shaw et al., 2019
		Sphagnum austinii	SB536	9730	AF192576	Shaw, 2000
			aust1005	6669	AY298017	Shaw et al., 2003
		Sphagnum australe	EK193	0511-1013	KF864545	Karlin, 2014
			EK194	0511-0719	KF864548	Karlin, 2014
		Sphagnum billbuckii	bilb876	26642	AY298027	Shaw et al., 2003
		Sphagnum brevirameum	SB621	26844	AF192627	Shaw, 2000
		Sphagnum buckianum	buck879	20513	AY298030	Shaw et al., 2003
		Sphagnum centrale	TR045	MKIR 7326	MW547007	This study
			cen1220	VIT 21149	AY298045	Shaw et al., 2003
			cent1062	7686	AY298046	Shaw et al., 2003
		Sphagnum cristatum	SB140	47127	MF362359	Shaw et al., 2019
		Sphagnum cuculliforme	cucu846	53745B	AY298053	Shaw et al., 2003
		Sphagnum divinum	TR004	MKIR 6156c	MW547009	This study
			TR073	MKIR 7415	MW547010	This study
		Sphagnum henryense	SB657	65925	AF192637	Shaw, 2000
		Sphagnum imbricatum	imbr1344	40308	AY298137	Shaw et al., 2003
		Sphagnum magellanicum	mag1351	2011	AY298165	Shaw et al., 2003
			mag666	1505	AY298166	Shaw et al., 2003
			mag690	5858	AY298167	Shaw et al., 2003
		Sphagnum medium	TR062	MKIR 7265	MW547011	This study
		Sphagnum monzonense	monz1054	1277	AY298183	Shaw et al., 2003
		Sphagnum palustre	TR019	MKIR 6989	MW547008	This study
			pal1206	28678	AY298204	Shaw et al., 2003
			pal1209	28667	AY298205	Shaw et al., 2003
			MR1473	160	MF362362	Shaw et al., 2019
		Sphagnum papillosum	TR036	MKIR 7414h	MW547012	This study
			TR035	MKIR 7424	MW547013	This study
			pap1063	27582	AY298206	Shaw et al., 2003
			pap1348	2005	AY298207	Shaw et al., 2003
		Sphagnum patens	pat1020	7474	AY298208	Shaw et al., 2003
		Sphagnum perichaetiale	SB74	9213	AF192575	Shaw, 2000
		Sphagnum reclinatum	recli854	12751	AY298246	Shaw et al., 2003
		Sphagnum steerei	SB367	8574	AF192574	Shaw, 2000
	Subsecunda	*Sphagnum subsecundum*	SB1554	12312	EU431653	Shaw et al., 2008

invariable sites. Two simultaneous runs of four chains were performed for one million generations. Convergence of the BI analysis was maximised by ensuring that the average standard deviation of split frequencies fell below 0.01 and that potential scale reduction factors of all parameters approached 1.0. Produced trees were visualized and edited using FigTree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Besides, SplitsTree4 v4.14.2 (Huson and Bryant, 2006) was used to reconstruct a neighbour-net phylogenetic network for *Sphagnum* species using the default settings in the software with 1000 bootstrap replicates.

3. Results and discussion

3.1. Morphological analysis

The key of Turkish *Sphagnum* section which was rearranged using the Hölzer, 2010; Kırmacı and Kürschner, 2013 and Hassel et al., 2018 is presented below.

1. Exterior walls of the green cells with papillae; green cells in section urn-shaped with oval lumina and thick adaxial walls, ombrogenic raised peat bogs ... ***S. papillosum***

1*. Exterior walls of the green cells smooth throughout **2**

2. Green cells in section elliptic, central, enclosed; plants often reddish **3**

2*. Green cells in section triangular, trapeziform, oval; plants never red **4**

3. Divergent branch leaf hyalocysts at proximal end convex surface with large pores, many to most filling half or more of cell breadth; ombrotrophic mires (bogs) .... ***S. medium***

3*. Divergent branch leaf hyalocysts at proximal end convex surface with rather small pores, mostly filling out less than half of cell breadth. Divergent branch leaves broadly ovate to circularovate; stem leaves ca. 1.2–1.8 mm, wide rectangular to somewhat spathulate; chlorocyst cell walls of divergent branch leaves in transverse section thin walled; stem leaf hyalocysts with scattered to sometimes abundant membrane gaps and perfect pores in distal leaf-half; ombrotrophic to oligotrophic (poor fen) and sometimes mesotrophic (intermediate

fen), hummock-dominated vegetation ..... ***S. divinum***

4. Green cells in section oval-triangular to trapeziform, thin-walled all around; hyaline cells of stem leaves commonly with fibres, variously green, brownish or yellowish; in mesotrophic marshes, streamsides and wet woodland ..... ***S. palustre***

4*. Green cells in section urn-shaped, oval to nearly triangular, sometimes lenticular to narrowly elliptical, strongly thickened walls at the concave surface; hyaline cells of stem leaves rarely with fibres; plants robust, pale to yellowish-green or yellowish-brown, branches cuspidate; plants in mesotrophic peatlands and swamps. .... ***S. centrale***

S. papillosum which was newly added to Turkish bryophyte flora is clearly separated from other members of the section using anatomical characters. The most prominent feature of the taxon is the papillose thickening in the cell walls, which can be easily seen in the lateral crosssection. It is also generally easy to recognize with its short thick branches and characteristic soil yellow colour. However, shadow forms are partly similar to *S. palustre* and are not always distinguishable in the field. The well-developed species of *S. palustre* are distinguished from *S. papillosum* by having 3–4 pendant branches per fascicle. However, compact forms that are likely to be confused with *S. papillosum* have only (1–) 2 pendant branches, so this difference in practice is not very valid. While the compact forms of both species are similar in appearance to *S. compactum* DC., they differ in having much larger stem leaves and more hooded leaves.

In the last decade, several new records have been added to the genus *Sphagnum* in Turkey. Today, it is the third richest genus with 27 taxa (including *S. papillosum*) after the genus *Grimmia* (32) and *Orthotrichum* (30) (Kürschner and Erdağ, 2020). Members of the genus *Sphagnum* which are known as peat or bog mosses have quite specific habitat preferences. Generally, they are found in ombrotrophic bogs which are acidic and poor in mineral salts. Turkey has very limited blanket bogs (Ağaçbaşı Peatland, Barma Yaylası Peatland, Yılanlıtaş Yaylası Peatland/Trabzon, Kabaca–Petek Yaylası Peatland, Sazak Peatland/Artvin, and Ciğer Gölü Peatland/Çanakkale) (Kırmacı et al., 2019). Active living bog is a priority habitat as listed on Annex I of the EU Habitats Directive. Although Ağaçbaşı Peatland and Barma Yaylası Peatland are sensitive areas to be precisely protected, unfortunately any raised bogs have not been designated as Special Areas of Conservation and Natura 2000 sites in Turkey up to now. These areas, which are limited in number, need to gain emergency protection status.

3.2. Molecular analysis

In phylogenetic analyses, the *trnL* intron region of seven specimens representing five species of the section *Sphagnum* were sequenced after species identifications were made. For an aligned dataset of 661-bp nucleotide sequence of specimens from the section *Sphagnum*, 629 characters were conserved, and 21 were variable. Of the variable characters, 4 were singleton, and 17 were parsimony informative. Mean base frequencies for A, C, G, and T were found to be 0.3601, 0.1638, 0.1543, and 0.3218, respectively. Moreover, GC and GT percentages were detected to be 31.8128 and 47.6172, respectively. According to the pairwise distance analysis, the percentage of genetic distances of 37 specimens representing 23 species of the section *Sphagnum* varied from 0.0000 to 0.0209, with an overall mean distance of 0.0059. The

transition/transversion bias was estimated to be 6.350. The transition/transversion ratio was 21.254 for purines and 18.760 for pyrimidines, with an overall transition/transversion bias of 8.713. It was determined in the substitution saturation analysis that both transitions and transversions linearly increased with increasing genetic distance and transitions outnumbered transversions. The index of substitution saturation (0.1111) was significantly lower than the critical index of substitution saturation for symmetrical trees (0.7415) as well as for asymmetrical trees (0.4891). The findings of the substitution saturation analysis indicated that the sequences of this gene did not undergo substantial saturation, had sufficient signal for phylogenetic tree reconstruction, and hence could be used for phylogenetic analyses (Xia et al., 2003).

In this study, *trnL* sequences of 40 specimens were used as a phylogenetic marker to identify phylogenetic relationships among species in the section *Sphagnum*. All the three methods (NJ, ML, and BI) resulted in similar topologies with resultant bootstrap values varying from poor to strong supports at nodes of the trees; however, the BI method was computationally more robust than the NJ and ML methods (Figures 1 and 2). In all tree topologies, three main clusters of species from the section *Sphagnum* were revealed, which have high bootstrap support (BS) and posterior probability (PP) values. All the constructed trees placed Cluster I containing *S. australe* as basal to the studied species of the section (BS = 85 for NJ, BS = 91 for ML, and PP = 1.000 for BI). Well-supported Cluster II consisting of *S. steerei*, *S. austinii*, *S. affine*, and *S. imbricatum* (BS = 88 for NJ, BS = 92 for ML, and PP = 0.997 for BI) was sister to well-supported Cluster III consisting of the remaining species of the section (BS = 90 for NJ, BS = 92 for ML, and PP = 0.978 for BI). In Cluster III, two *S. papillosum* specimens from Turkey were placed in a group with two *S. papillosum* specimens from Japan and USA, which formed a separate clade in the cluster (BS = 63 for NJ and ML and PP = 0.867 for BI). *S. billbuckii* and *S. brevirameum* specimens were placed in a group (BS = 62 for NJ, BS = 64 for ML, and PP = 0.978 for BI), and this group was clustered together with *S. buckianum*, *S. perichaetiale*, *S. reclinatum*, *S. patens*, *S. cuculliforme*, and *S. amoenoides* specimens and *S. palustre* specimen from Brazil (BS = 63 for NJ, BS = 64 for ML, and PP = 0.835 for BI). *S. palustre*, *S. divinum*, *S. centrale*, and *S. medium* specimens from Turkey were placed in a group with *S. palustre* specimens from Bhutan and *S. magellanicum*, *S. centrale*, *S. alaskense*, *S. henryense*, *S. monzonense*, and *S. cristatum* specimens; however, this group lacked high BS and PP values and was not well resolved by the three phylogenetic analyses.

Further, for better understanding and visualising the relationships among the species from the section *Sphagnum*, a neighbour-net phylogenetic network was constructed in the study using SplitsTree4 software that makes a phylogenetic network with reticulations rather than constructing a bifurcating topology in a conventional single phylogenetic tree. Compared to the routinely bifurcating phylogeny, a phylogenetic network is known to be more useful in describing and visually presenting complicated relationships. As seen in Figure 3 demonstrating the relationships among the studied species, the centre of the constructed network was slightly netted. This pattern implied some conflicting deep splits. Nevertheless, three main clusters were recognised in the constructed network (Figure 3) as revealed by the topologies of the NJ, ML, and BI methods (Figures 1 and 2).

Sphagnum is one of the largest genera of bryophytes with a worldwide distribution (Shaw et al., 2003). It is known that *Sphagnum* species are excellent ecosystem engineers, and they dominate peatland habitats where they occur by creating the environmental conditions that contribute to their dominance (e.g., Turetsky et al., 2008). In this study, we analysed the phylogenetic relationships of some of the species from the section *Sphagnum* of this genus using nucleotide sequence of the *trnL* intron region, with a main focus on the species collected from Turkey and used in the phylogenetic analyses for the first time. It is possible to consider this study as a preliminary study on this subject, since the number of analysed specimens is limited, and the number of used markers is few. Although this was the case, the nucleotide saturation analyses provided compelling evidence that the selected sequences did not undergo substantial saturation and could be used for phylogenetic studies. In addition, it was reported in a comprehensive study on the genus *Sphagnum* that nucleotide sequences from the plastid genome alone provided highly resolved, well-supported, and compatible estimates of phylogenetic relationships (Shaw et al., 2016). The selected marker in the study (the *trnL* intron region) is a locus from the plastid genome, and hence the results of the phylogenetic analyses in this study can be considered as accurate and valid.

Two specimens identified in this study as *S. papillosum* —which is a species newly added to Turkish bryophyte flora— were genetically clustered within a group together with two others from the same species from Japan and USA in our phylogenetic analyses. When the sequences of these two *S. papillosum* specimens were assessed by submitting to BLAST search in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to verify the sequence similarity, it was found that the sequences of the specimens from Turkey showed 100% similarity with the sequence of the specimen from Japan and 99.69% similarity with the sequence of the specimen from USA. These findings are informative with respect to confirmation of species identification and strongly support the accuracy of identification of the specimens

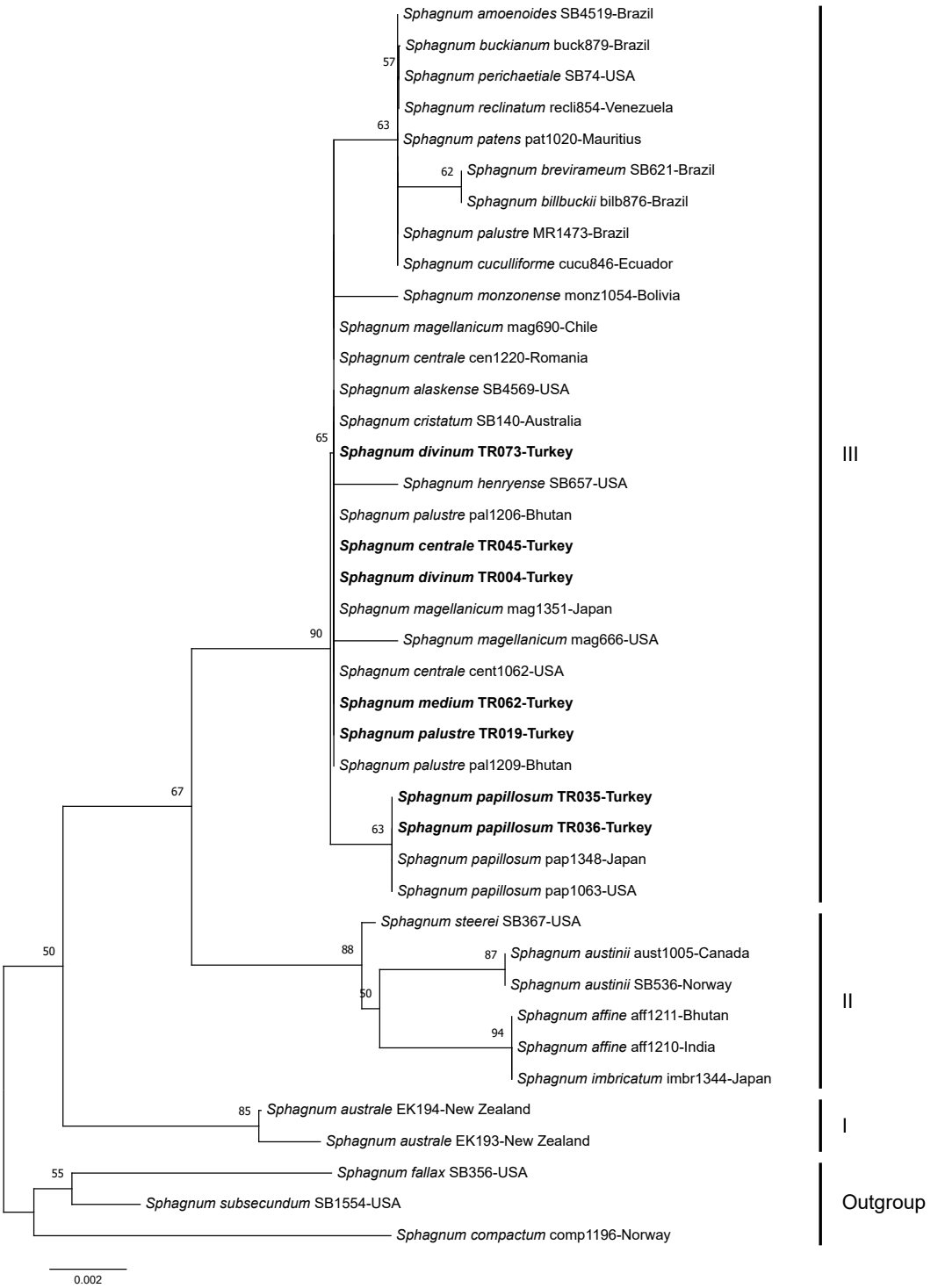

Figure 1. Neighbour-joining (NJ) reconstruction of the phylogenetic relationships of some species from the section *Sphagnum* based on *trnL* intron region sequences. NJ bootstrap values of >50% after 10,000 replicates are shown above branches. Specimens collected and sequenced in this study are demonstrated in bold.

based on morphological and anatomical characters. The phylogenetic analyses in the present study implied that *S. palustre* from Turkey was more closely related to *S. palustre* from Bhutan than the same species from Brazil but did not provide detailed information regarding the phylogenetic relationships of *S. palustre*, *S. centrale*, *S. divinum*, and *S.*

Figure 2. Maximum likelihood (ML) reconstruction of the phylogenetic relationships of some species from the section *Sphagnum* based on *trnL* intron region sequences. ML bootstrap values of >50% after 10,000 replicates (in black) and BI posterior probabilities (in red) are shown above branches. Specimens collected and sequenced in this study are demonstrated in bold.

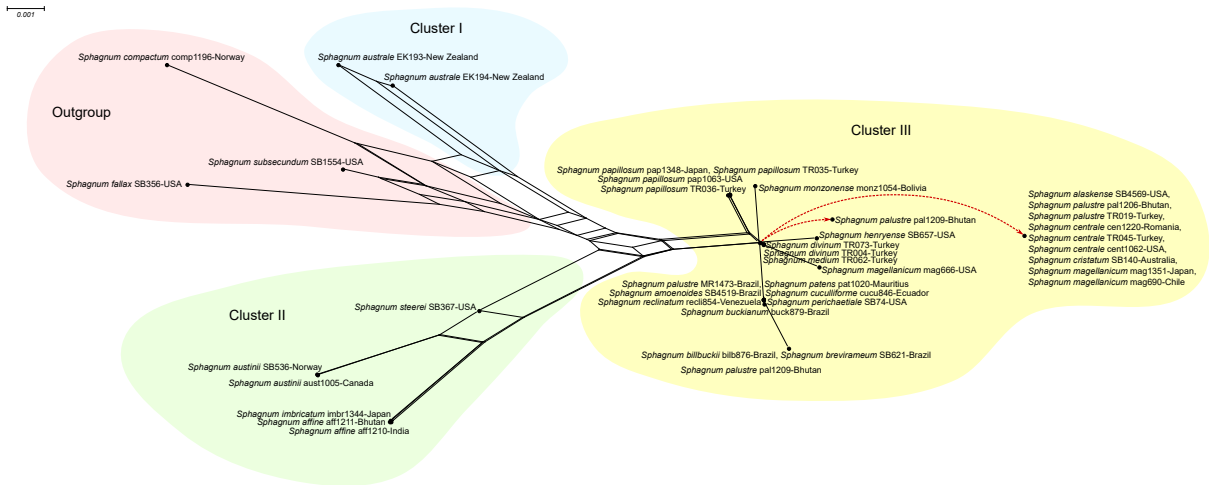

Figure 3. A neighbour-net phylogenetic network constructed using *trnL* intron region sequences of studied species. Each labelled node is marked with a circle. For the clarity of the network, bootstrap support values of the nodes are not demonstrated.

medium specimens collected from Turkey and sequenced to be used in the phylogenetic analyses for the first time. As a matter of fact, these four species were not resolved as distinct species by nucleotide sequences obtained from the *trnL* intron region in the analyses. This may be regarded as a weakness of this study and attributed to relatively small sample size and/or only one molecular marker used in the analyses. Therefore, further phylogenetic studies with a greater sampling of *Sphagnum* diversity of Turkey and the surrounding region and with the inclusion of more sequence data from additional molecular markers are highly recommended for more detailed information regarding the phylogenetic relationships of *Sphagnum* species.

4. Conclusion

Taken together, this study provides the first sequence data of the *trnL* intron region for five species of the section *Sphagnum* from Turkey (namely *S. centrale*, *S. divinum*, *S. medium*, *S. palustre*, and *S. papillosum*) to be used in future studies and gives valuable information about their phylogenetic relationships, albeit with small specimen size and only one molecular marker. However, further studies on Turkish members of the section *Sphagnum* using more specimens and molecular markers should be performed to obtain more detailed information regarding the phylogenetic relationships among them.

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