SYSTEMATICS AND PHYLOGENY

Taxonomic treatment and phylogenetic analysis of the family Potamogetonaceae in Turkey

Candan Aykurt,[1] Judith Fehrer,[2] Duygu Sarı Yol,[3] Zdenek Kaplan,[2,4] Veronika Bambasová,[2] İ. Gökhan Deniz,[5] Esra Aydemir[1] & Nilüfer İmir[5]

1 *Department of Biology, Faculty of Science, Akdeniz University, Antalya, Turkey*
2 *Czech Academy of Sciences, Institute of Botany, Průhonice, Czech Republic*
3 *Department of Field Crops, Faculty of Agriculture, Akdeniz University, Antalya, Turkey*
4 *Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic*
5 *Department of Biology Education, Faculty of Education, Akdeniz University, Antalya, Turkey*
Address for correspondence: *Candan Aykurt*, *candan@akdeniz.edu.tr*

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Abstract The family Potamogetonaceae has been taxonomically re-evaluated in Turkey, employing traditional morphological as well as molecular approaches (*rbcL*, ITS). Our fieldwork, herbarium studies and molecular analyses proved the existence of 22 taxa, of which 18 belong to *Potamogeton* (including 4 interspecific hybrids), 3 to *Stuckenia* and 1 to monotypic *Groenlandia*. Morphological re-descriptions of the recognized taxa were prepared, and the information concerning their distribution in Turkey was refined, based on plant material from extensive fieldwork and on specimens stored in herbaria that were previously not examined. Additionally, new identification keys to genera and species, and distribution maps of the species were prepared. Phylogenetic relationships and intraspecific variations were assessed by including samples from other regions. The status of the Zannichelliaceae was investigated using nuclear and chloroplast DNA markers. Our results from ITS sequence divergence corroborate the separation of the two families noted by some authors, and are in accordance with the substantial morphological differences between them. Relatively large genetic distance and non-monophyly indicate that two genotypes of *P. gramineus* constitute cryptic species, for which the Turkish localities expand upon the previously known distribution areas. We identified *P. schweinfurthii* as a new species for Turkey and report *P. ×angustifolius* for the first time for this country. Weak morphological differentiation and high sequence similarity did not permit reliable differentiation between the closely related *Stuckenia amblyphylla* and *S. filiformis.*

Keywords *Groenlandia*; molecular phylogeny; *Potamogeton*; *Stuckenia*; taxonomic revision; Turkey

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

Potamogetonaceae, known as the pondweed family, is notable amongst water plants as it harbours the highest number of taxa in the world. The Potamogetonaceae (excluding Zannichelliaceae) include about 80 species and 105 hybrids classified into three genera, namely *Potamogeton* with about 72 species and 99 hybrids, monotypic *Groenlandia*, and *Stuckenia* with 7 species and 6 hybrids (Kaplan, 2008, 2010a,c; Kaplan & al., 2013). The latter genus superseded the previously recognized *Potamogeton* subg. *Coleogeton* (Holub, 1997; Haynes & al., 1998; Kaplan, 2008). *Stuckenia* includes solely hexaploids, whereas *Groenlandia densa* is diploid, and *Potamogeton* includes mainly diploids and tetraploids (Kaplan & al., 2013).

Over a period of more than a hundred years, there have been readjustments concerning the family rank of Potamogetonaceae, Zannichelliaceae and Ruppiaceae. Ascherson & Graebner (1907) assessed *Ruppia* in Potamogetonaceae, located in tribe Potamogetoneae. However, Hutchinson (1959) later separated the monogeneric Ruppiaceae from Potamogetonaceae, and this distinction was generally adopted by subsequent researchers (Cronquist, 1968; Takhtajan, 1969; Aston, 1973; Cook & al., 1974). In a study involving a revision of Australian *Ruppia* species by Jacobs & Brock (1982), this genus was again classified in Potamogetonaceae, principally due to the similarity of leaves to *Potamogeton* subg. *Coleogeton*. However, Potamogetonaceae and Ruppiaceae differ in a number of characters in both vegetative and generative morphology (Haynes & al., 1998). Today, the Ruppiaceae family is regarded as separate from Potamogetonaceae (Lindqvist & al., 2006; Angiosperm Phylogeny Group, 2009; Petersen & al., 2016). Zannichelliaceae were for a long time recognized as a separate family (e.g., Cronquist, 1968; Takhtajan, 1969; Cook & al., 1974), but were included in Potamogetonaceae according to the APG II (Angiosperm Phylogeny Group, 2003), Stevens (2004) and APG III (Angiosperm Phylogeny Group, 2009), based on molecular data. Nowadays, Zannichelliaceae still have a controversial position at the family level. The phylogenetic relationships among the individual

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genera in a clade consisting of Potamogetonaceae and Zannichelliaceae vary among the studies (e.g., Les & al., 1997; Iida & al., 2004; Lindqvist & al., 2006; Les & Tippery, 2013; Ito & al., 2016a; Ross & al., 2016), and the evidence is inconclusive. Some authors favour the inclusion of Zannichelliaceae into Potamogetonaceae, while others have treated them as different families because of distinctive morphology (Les & al., 1997; Lindqvist & al., 2006; Li & Zhou, 2009). Les & Tippery (2013) prefer the recognition of larger, more diverse families rather than numerous depauperate ones, especially when there is strong evidence indicating close relationships amongst them. However, in spite of the considerable morphological reduction, Potamogetonaceae and Zannichelliaceae differ in many substantial characters (Posluszny & Tomlinson, 1977; Haynes & al., 1998) that are traditionally used for separation at the family level. Watson & Dallwitz (1992) pointed out that these families differ "in 45 characters, representing conspicuous features of vegetative and floral morphology as well as embryology and cytology". This indicates an enormous amount of differentiation between these families. The evolutionary classification should reflect not only the branching pattern of phylogenetic trees, but also the level of differentiation and occurrence of significant evolutionary novelties within the lineages (Stuessy, 1997; Brummitt, 2003, 2008; Grant, 2003; Hörandl, 2007, 2010; Stuessy & König, 2009). We therefore recognize Zannichelliaceae as a phenotypically clearly defined family, distinct from the traditionally delimited Potamogetonaceae. Zannichelliaceae, with four genera, has been recently investigated by Ito & al. (2016a) in a study combining molecular and morphological characters. Based on phylogenetic analyses of one nuclear and five plastid markers, the authors include *Lepilaena* in *Althenia* s.l., which was well separated from the sister taxa *Pseudalthenia* and *Zannichellia*. Both Zannichelliaceae and Ruppiaceae were evaluated as separate families in the *Flora of Turkey* (Uotila, 1984). However, the *Resimli Türkiye Florası* ("Illustrated Flora of Turkey") is being re-written, and the plant families are arranged according to APG III. The second volume, containing the family Potamogetonaceae (including Zannichelliaceae), was recently published (Bayındır, 2018). We focus here on Potamogetonaceae excluding Zannichelliaceae (represented by two species in Turkey); the latter are, however, used as outgroup in phylogenetic analyses.

The family Potamogetonaceae is one of the most diverse and taxonomically difficult families of aquatic plants (Wiegleb & Kaplan, 1998; Kaplan & al., 2013), mainly because of high species diversity, reduced morphology, extensive phenotypic plasticity and frequent hybridization. *Potamogeton* can be divided morphologically into broad-leaved species and linear-leaved species (Hagström, 1916; Preston, 1995). The identification of the linear-leaved species is particularly difficult because of the limited number of taxonomic characters that can be used to discriminate species (Kaplan & Štěpánek, 2003). In addition, this group is much less variable anatomically and genetically when compared to the broad-leaved species (Hagström, 1916; Preston, 1995; Wiegleb & Kaplan, 1998; Lindqvist & al., 2006; Kaplan & al., 2013). All Potamogetonaceae species show a fairly wide morphological variation. Many characters such as the general appearance, length and width of leaves, shape of leaf apex and effective fruit production depend on environmental conditions (Kaplan, 2002, 2008). Markedly different phenotypes are produced, particularly in running water (Kaplan & Zalewska-Gałosz, 2004; Kaplan, 2005). Fruits are often not produced in running water, which can sometimes be incorrectly interpreted as evidence of sterility (Kaplan, 2008). Although hybrids in Potamogetonaceae were first recognized already in the late 19th century (see Kaplan & al., 2009 for a brief review), molecular evidence became available only during the past decades by using isozyme electrophoresis (e.g., Hollingsworth & al., 1995; Fant & al., 2001; Iida & Kadono, 2002; Kaplan & al., 2002; Kaplan & Wolff, 2004; Kaplan, 2007; Bobrov & Sinjushin, 2008) and DNA-based techniques (e.g., King & al., 2001; Fant & al., 2003; Kaplan & Fehrer, 2004, 2009, 2011, 2013; Ito & al., 2007, 2014; Du & al., 2009, 2010; Les & al., 2009; Zalewska-Gałosz & al., 2009, 2010, 2018; Kaplan & al., 2011, 2018; Aykurt & al., 2017; Iida & al., 2018). While these studies focused on hybrids, a few of them performed also phylogenetic analyses (e.g., Les & al., 2009; Kaplan & Fehrer, 2011; Ito & al., 2014; Kaplan & al., 2018; Zalewska-Gałosz & al., 2018), but these include only taxa relevant for the identification of hybrids. Several studies focused on the phylogeny of Potamogetonaceae (Iida & al., 2004; Lindqvist & al., 2006; Wang & al., 2007; Zhang & al., 2008; Ito & Tanaka, 2013). The study of Iida & al. (2004) included relatively few species and was based on a single chloroplast marker. Others suffered from obvious misidentifications of plant material and/or the use of misleading species concepts (Wang & al., 2007; Zhang & al., 2008; Ito & Tanaka, 2013) or the failure to correctly identify hybrid individuals that were mistaken for pure species (Lindqvist & al., 2006; see Kaplan & al., 2018). The most comprehensive phylogenies so far available were provided by Kaplan & al. (2013) and Ito & al. (2016b); the latter was largely based on GenBank sequences, including misidentified ones, and contained only few new samples. The different molecular markers and datasets used in various studies consistently showed *Potamogeton*, *Stuckenia*, *Groenlandia* and *Zannichellia* as different genera, but tended to produce different tree topologies within *Potamogeton*. Intrageneric relationships of *Potamogeton* are difficult to assess due to the largely non-overlapping datasets and the diversity of the markers used, but a well-supported split into the traditionally recognized broad-leaved and narrow-leaved species based on 5S-NTS sequences was found (Lindqvist & al., 2006; Kaplan & al., 2013; Ito & al., 2016b). Material of Turkish origin has scarcely been investigated at all by molecular methods, and no phylogenetic analysis is available as yet. Applying two widely used markers, the chloroplast *rbcL* gene and the nuclear ITS region, we assess species relationships of Turkish Potamogetonaceae and their correspondence to samples from other parts of the world and identify hybrid plants.

The first revision of Turkish Potamogetonaceae appeared in the *Flora of Turkey* (Uotila, 1984) and dealt with 14 species and one hybrid. Another species (*P. coloratus*) was reported for the country later (Wiegleb, 1990; Seçmen, 2000). An additional species (*P. acutifolius*) and two hybrids (*P. obtusifolius* × *P. berchtoldii*, *P. berchtoldii* × *P. trichoides*) were discovered in Turkey recently (Aykurt & al., 2016, 2017). The mainly African species *P. schweinfurthii* was added by Bayındır (2018) in the illustrated Turkish Flora. However, recent discoveries of taxa new for Turkey, which is rich in wetlands, show that the diversity of species and hybrids of this family and their detailed distributions are still insufficiently known; additionally, some species groups include taxa with blurred taxonomic boundaries in the current treatment. Investigation of this diversity using molecular methods has started only recently and has covered only a small fraction of the morphological diversity. The main objectives of this study are therefore: (1) taxonomic re-evaluation of the diversity of the family in Turkey, employing traditional morphological as well as molecular approaches; (2) re-description of the recognized taxa in Turkey and identification of their distribution based on plant material from extensive fieldwork and many specimens stored in herbaria that were previously not examined; (3) preparation of new identification keys including new data; (4) investigation of phylogenetic relationships and assessment of intraspecific variation with nuclear and chloroplast DNA markers, compared with samples from other regions and (5) identification of putative hybrids using molecular markers.

■ MATERIALS AND METHODS

Morphological studies. — Field trips were mainly conducted in 2014–2017. Approximately 1500 Potamogetonaceae samples were collected from different aquatic habitats throughout Turkey. Voucher specimens are preserved in the herbarium of the Akdeniz University (AKDU), with a few duplicates in the herbarium of the Institute of Botany, Průhonice (PRA). In addition to observations made on specimens collected in the field, extensive herbarium studies were carried out in major herbaria of Turkey: ANK, GAZI, HUB, ISTE and ISTF. Additionally, herbarium specimens from Turkey were examined in foreign herbaria such as B, BM, BP, BRNM, C, E, FR, G, GOET, H, K, L, LD, LE, M, P, PR, PRA, PRC, ROZ, S, U, W, WAG, WU and Z (for a complete list of herbaria studied, see Kaplan, 2008, 2010a,c; Kaplan & Marhold, 2012). Morphological observations and measurements were carried out in the field as well as in the laboratory. All characters commonly used in the Potamogetonaceae literature were used (e.g., Wiegleb & Kaplan, 1998; Kaplan, 2008). Distribution maps of all the species and hybrids were prepared based on records from both our fieldwork and the localities from examined herbarium specimens. The records used for producing the maps are listed and sorted according to Davis's (1965) geographical divisions of Turkey (Fig. 1).

Molecular studies. — All taxa of Potamogetonaceae occurring in Turkey, representing about 28% of the total species diversity of the family, were included in the molecular analyses. For widespread species, e.g., *Potamogeton nodosus*, *P. lucens*, and *Stuckenia pectinata*, specimens collected from various habitats throughout Turkey were chosen for the molecular analyses. For taxa that have only local or limited distribution, e.g., *P. acutifolius*, *P.* ×*angustifolius*, *P. alpinus*, *P. coloratus*, *P. praelongus*, *P.* ×*salicifolius*, almost all collected specimens were included in the molecular studies. The analyzed specimens are listed in Appendix 1.

For this study, the nuclear ribosomal internal transcribed spacer region (including ITS1, 5.8S and ITS2) and part of the chloroplast gene coding for the ribulose bisphosphate carboxylase/oxygenase large subunit (*rbcL*) were chosen to evaluate the relationships between species. Both markers have previously been used in phylogenetic studies of Potamogetonaceae (Wang & al., 2007; Iida & al., 2007; Li & Zhou, 2009). As they are often used for DNA barcoding in plants, large amounts of data from other studies are available for comparison. Total genomic DNA was extracted from fresh leaves and dried herbarium material using the CTAB protocol described by Doyle & Doyle (1990). DNA concentrations were estimated by electrophoresis on 1% agarose gels. The ITS region was amplified as described in Kaplan & Fehrer (2004) and Aykurt & al. (2017). The *rbcL* region was amplified with the primer pairs 1F and 724R (Fay & al., 1997) or rbcL26F and rbcL1375R (Iida & al., 2007). PCRs were carried out with 1 U of *Taq* DNA polymerase (Fermentas Life Sciences, Burlington, Canada) in the supplied reaction buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each primer and 40 ng of template DNA, and ddH_2O to a final volume of 15 μl; for difficult (partially degraded) samples also with the Combi PPP MasterMix (Top-Bio, Vestec, Czech Republic). PCR amplification conditions were as follows: an initial pre-denaturation step at 94°C or 95°C for 5 min; 30 cycles of 30 s at 94°C or 95°C, 30 s at 45°C or 55°C, 1 min at 72°C; and a final extension step of 10 min at 72°C. PCR products were purified using the GeneJET Gel Extraction Kit (Thermo Scientific Fermentas, Vilnius, Lithuania) or the QIAquick PCR purification kit (Qiagen, Hilden, Germany). Sequencing was carried out at Macrogen, Europe via BM Laboratories or at GATC Biotech (Cologne, Germany) as direct sequencing in two directions using the amplification primers. All sequences were manually edited using Chromas v.1.45 (McCarthy, 1996–1998) and aligned in Bioedit v.7.0.9.0 and v.7.1.8 (Hall, 1999). Sequences were submitted to GenBank; accession numbers are included in Appendix 1.

Data treatment and phylogenetic analyses. — ITS sequencing revealed several *Potamogeton* accessions with double peaks and/or shifts caused by indel polymorphisms. The respective samples were identified as hybrids based on character additivity in comparison with sequences of particular parental taxa (Table 1).

For phylogenetic analyses using the *rbcL* gene, a large range of outgroup taxa was chosen based on results of previous studies of the Alismatales (Les & al., 1997; Petersen & al., 2016) including representatives of Hydrocharitaceae (including Najadaceae), Aponogetonaceae, Scheuchzeriaceae, Juncaginaceae, Liliaceae, Posidoniaceae, Ruppiaceae, Cymodoceaceae and Zosteraceae (altogether 16 accessions). For Zannichelliaceae, a representative sampling of genera and species based on Ito & al. (2016a) was included (nine accessions). For all Turkish Potamogetonaceae and outgroup species (69 accessions, see Appendix 1), 22 available sequences from GenBank were retrieved for comparison. *Potamogeton* hybrids identified by ITS sequence additivity were included in the *rbcL* phylogeny to determine the direction of the cross, i.e., the maternal parent. Linear-leaved species of *Potamogeton* did not produce sufficiently good amplification products or sequences with this marker and were excluded from the *rbcL* analysis. Multiple samples of the same species were usually represented only once if they had identical sequence, but samples of *Stuckenia filiformis* and *S. amblyphylla* as well as *Potamogeton* hybrids and different haplotypes of the same species were included individually. The final alignment (suppl. Appendix S1) consisted of 75 sequences and 703 characters of which 26 variable ones were uninformative and 123 characters were parsimony informative.

The ITS region is far more variable than *rbcL* and was not alignable for outgroup taxa. Therefore, for phylogenetic analyses with this marker, only Turkish Potamogetonaceae (including linear-leaved species, but no hybrids) and *Zannichellia* were included along with one *Groenlandia densa* accession from Switzerland for comparison. The ITS dataset consisted of 73 sequences and 769 aligned characters. It contained indels of 1–32 bp length, most of which were phylogenetically informative. Indels were therefore coded using FastGap v.1.2 (Borchsenius, 2009) producing a matrix of 41 additional characters that were concatenated to the sequences. The final dataset comprised 810 aligned characters of which two variable ones were parsimony-uninformative and 315 characters were parsimony informative. In addition, we compared the similarity of ITS sequences of Turkish Potamogetonaceae with samples from other parts of the world. One ITS sequence of each species was subjected to BLAST searches to assess similarity

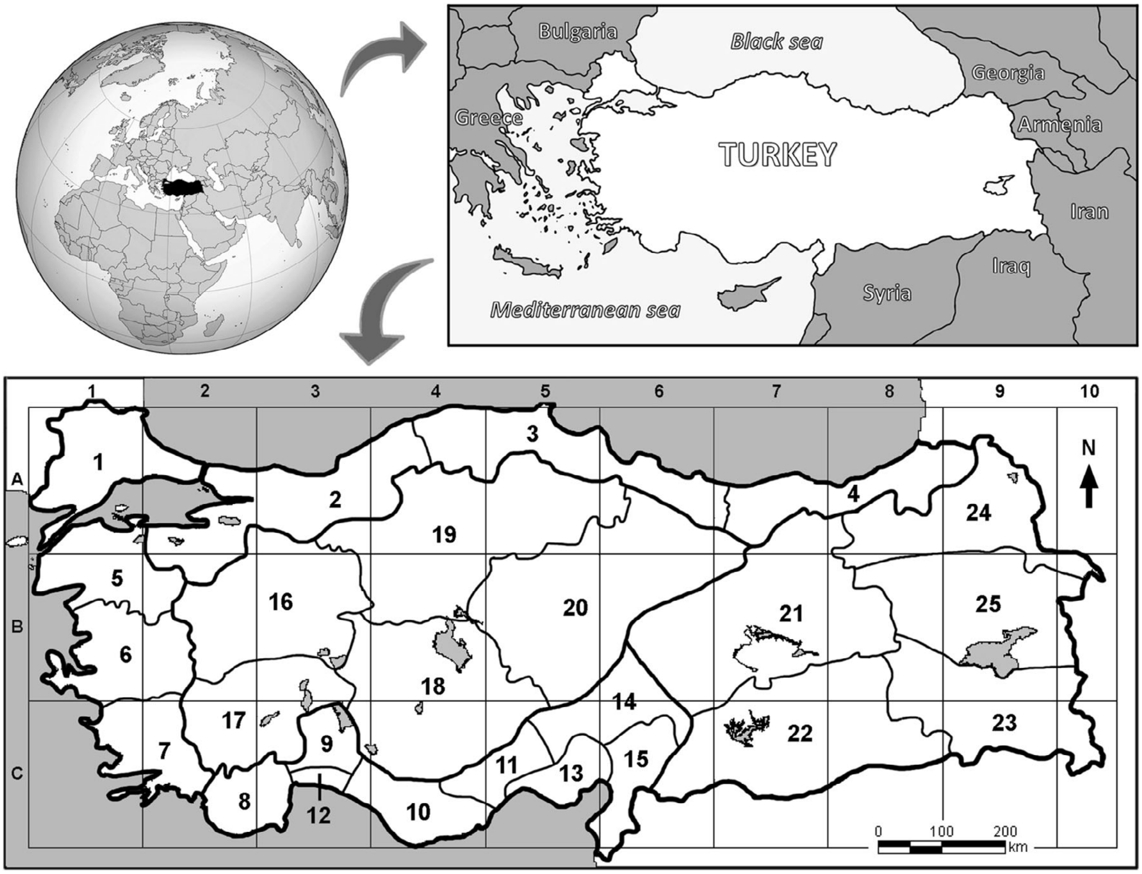

Fig. 1. Geographical position of Turkey in the world and geographical divisions within Turkey according to Davis (1965: map 2). 1: Turkey in Europe; 2–4: North Anatolia; 5–7: West Anatolia; 8–15: South Anatolia; 16–20: Central Anatolia; 21–25: East Anatolia. The following terms are also used: Outer Anatolia: areas 2–15; Inner Anatolia: areas 16–25; North-West Turkey: areas 1, 2, 5, together with vilayet Bilecik; North-East Anatolia: areas 4, 24, together with vilayet Gümüşhane in area 21; South-West Anatolia: areas 7– 9, 12, 17; South-East Anatolia: area 23, together with those parts of vilayets Van and Bitlis in area 25; North Turkey: North Anatolia and Turkey in Europe; West Turkey: West Anatolia and Turkey in Europe.

Table 1. Species- and genotype-specific diagnostic differences in ITS sequences and character additivity in hybrids.

Species / hybrid, no. of accession (genotype, gt)	Position in alignment																									
	44	67	70	81	99	106	116	168	188	196	197	205	213	247	259	433	439	443	449	452	496	532	535	562	588	651	670
P. perfoliatus 4089	T	T	C	T	T	T	C	T	T	G	G	A	T	T	A	T	C	A	T	–	G	G	T	G	T	T	T
P. perfoliatus 4189	T	T	C	T	T	T	C	T	T	G	G	A	T	T	A	T	C	A	T	–	G	G	T	G	T	T	T
P. perfoliatus 4312	T	T	C	T	T	T	C	T	T	G	G	A	T	T	A	T	C	A	T	–	G	G	T	G	T	T	T
P. perfoliatus 4392	T	T	C	T	T	T	C	T	T	G	G	A	T	T	A	T	C	A	T	–	G	G	T	G	T	T	T
P. perfoliatus 4498	T	T	C	T	T	T	C	T	T	G	G	A	T	T	A	T	C	A	T	–	G	G	T	G	T	T	T
P. ×salicifolius 4088	**Y**	**W**	**S**	**K**	**W**	**Y**	**Y**	**Y**	**K**	G	G	A	T	**Y**	**R**	**K**	**M**	**W**	**Y**	**c/–**							
P. ×salicifolius 4143	**Y**	**W**	**S**	**K**	**W**	**Y**	**Y**	**Y**	**K**	G	G	A	T	**Y**	**R**	**K**	**M**	**W**	**Y**	**c/–**							
P. ×salicifolius 4348	**Y**	**W**	**S**	**K**	**W**	**Y**	**Y**	**Y**	**K**	G	G	A	T	**Y**	**R**	**K**	**M**	**W**	**Y**	**c/–**							
P. lucens 3993	C	A	G	G	A	C	T	C	G	G	G	A	T	C	G	G	A	T	C	C	G	G	T	T	C	C	A
P. lucens 4087	C	A	G	G	A	C	T	C	G	G	G	A	T	C	G	G	A	T	C	C	G	G	T	T	C	C	A
P. lucens 4105	C	A	G	G	A	C	T	C	G	G	G	A	T	C	G	G	A	T	C	C	G	G	T	T	C	C	A
P. lucens 4141	C	A	G	G	A	C	T	C	G	G	G	A	T	C	G	G	A	T	C	C	G	G	T	T	C	C	A
P. lucens 4196	C	A	G	G	A	C	T	C	G	G	G	A	T	C	G	G	A	T	C	C	G	G	T	T	C	C	A
P. lucens 4259	C	A	G	G	A	C	T	C	G	G	G	A	T	C	G	G	A	T	C	C	G	G	T	T	C	C	A
P. lucens 4447	C	A	G	G	A	C	T	C	G	G	G	A	T	C	G	G	A	T	C	C	G	G	T	T	C	C	A
P. lucens 4457	C	A	G	G	A	C	T	C	G	G	G	A	T	C	G	G	A	T	C	C	G	G	T	T	C	C	A
P. lucens 4470	C	A	G	G	A	C	T	C	G	G	G	A	T	C	G	G	A	T	C	C	G	G	T	T	C	C	A
P. ×angustifolius 4323	C	A	G	G	A	C	T	C	G	**S**	G	**R**	**Y**	C	G	G	A	T	C	C	G	**r**	T	**k**	**y**	C	**M**
P. ×angustifolius 4440	C	A	G	G	A	C	T	C	G	**S**	G	**R**	**Y**	C	G	G	A	T	C	C	G	**R**	T	**K**	**y**	C	**m**
P. ×angustifolius 4535	C	A	G	G	A	C	T	C	G	**S**	G	**R**	**Y**	C	G	G	A	T	C	C	k	**R**	T	**K**	**Y**	C	**M**
P. ×angustifolius 4537	C	A	G	G	A	C	T	C	G	**S**	G	**R**	**Y**	C	G	G	A	T	C	C	k	**R**	T	**K**	**Y**	C	**M**
P. gramineus 4393 (gt 2)	C	A	G	G	A	C	T	C	G	C	G	G	C	C	G	G	A	T	C	C	G	A	T	G	T	C	C
P. gramineus 4394 (gt 2)	C	A	G	G	A	C	T	C	G	C	G	G	C	C	G	G	A	T	C	C	G	A	T	G	T	C	C
P. gramineus 4441 (gt 2)	C	A	G	G	A	C	T	C	G	C	G	G	C	C	G	G	A	T	C	C	G	A	T	G	T	C	C
P. gramineus 4446 (gt 2)	C	A	G	G	A	C	T	C	G	C	G	G	C	C	G	G	A	T	C	C	G	A	T	G	T	C	C
P. gramineus 4449 (gt 2)	C	A	G	G	A	C	T	C	G	C	G	G	C	C	G	G	A	T	C	C	G	A	T	G	T	C	C
P. gramineus 4182 (gt 1)	C	A	G	G	A	C	T	C	G	G	T	A	T	C	G	G	A	T	C	C	G	G	C	G	T	T	C

Notes: Additive characters (double peaks in direct sequences) are represented by IUPAC codes in bold; lower case letters indicate unequal proportions of the respective bases; k at position 496 is a polymorphic site occurring in two *P. ×angustifolius* hybrids from the same locality. The hybrid sequence of *P. ×salicifolius* is only readable until the first diagnostic indel position. Genotype 1 of *P. gramineus*, which is not involved in any of the *P. ×angustifolius* hybrids, is shown for comparison.

with samples from other areas represented in GenBank. Sequences with 100% identity were chosen from the broadest geographical range available, often comprising samples spanning the entire Northern Hemisphere, depending on species distribution. If several identical sequences from similar areas were available, only representative examples are shown. In some cases, nearly identical sequences were included, and the differences to the ITS sequences from Turkey were commented. A list of species, accession numbers, countries and ITS sequence features is provided in Table 2.

For both datasets, the model best fitting the presumed molecular evolution was determined using Modeltest v.3.5 (Posada & Crandall, 1998). For *rbcL*, a HKY + I + Γ model and for ITS, a GTR + Γ model were found most suitable according to the Akaike information criterion. Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) analyses were performed using MrBayes v. 3.2.6 (Ronquist & al., 2012), MEGA v.7 (Kumar & al., 2016) and PAUP v.4.0b10 (Swofford, 2002). For BI, the basic model parameters – two substitution rates and gamma distribution of rates among sites with a proportion of invariant sites (*rbcL*) or six substitution rates and gamma distribution of rates among sites (ITS) – were used with the default settings, sampling every 1000th tree. According to all statistical parameters, for *rbcL* 1.7 million generations and for ITS 1 million generations were needed to achieve convergence. The first 25% of the sampled trees of each analysis were discarded as burn-in, and the rest of the trees were summarized. For ML, the Hasegawa-Kishino-Yano model was used for *rbcL* and a general time reversible model for ITS according to the Modeltest results. For both datasets, a discrete gamma distribution was used to model evolutionary rate differences among sites (6 categories). Extensive subtree-pruning-regrafting, a very strong branch swap filter and 1000 bootstrap replicates were applied using all sites. MP analyses were done as heuristic searches with 100 random addition sequence replicates and tree bisection-reconnection branch swapping, saving no more than 100 trees with length ≥ 1 per replicate, automatically increasing the maximum number of trees saved. Bootstrapping was performed using the same settings and 1000 replicates, but without branch swapping.

■ RESULTS

Identification of *Potamogeton* hybrids. — Several accessions with mixed ITS sequences were detected (Table 1). They formed two groups, and comparisons with ITS sequences of other species showed that they belonged to two hybrid taxa, *P.* ×*salicifolius* (*P. lucens* × *P. perfoliatus*) and *P.* ×*angustifolius* (*P. gramineus* × *P. lucens*). The latter is a new record for Turkey, while the former was previously recorded based only on morphological examination. Each of them was found in three different localities during our fieldwork (Appendix 1). Both hybrids originated from reciprocal crosses according to their chloroplast DNA sequences (Fig. 2). Two *P.* ×*salicifolius* accessions had *P. lucens* as maternal parent (4088, 4348), whereas one had maternal *P. perfoliatus* (4143). Similarly, three *P.* ×*angustifolius* accessions, of which two were from the same locality (4440, 4535, 4537), had maternal *P. gramineus*, whereas accession 4323 had *P. lucens* as its maternal parent. Two different genotypes of *P. gramineus*, genotype 1 and genotype 2, were found, as in previous studies based on non-Turkish material (Kaplan & Fehrer, 2006, 2007, 2011); they are indistinguishable morphologically. Both *P. gramineus* genotypes occur also in Turkey, but genotype 1 was found only once (4182), whereas genotype 2 occurred more frequently (five samples from three localities were investigated), and this genotype was involved in all *P.* ×*angustifolius* hybrids analyzed. The *rbcL* sequence of *P. gramineus* genotype 1 is not distinguished from *P. lucens*; however, ITS character additivity shows that genotype 2 of *P. gramineus* has contributed to the hybrid and that *P. gramineus* genotype 1 can be excluded so that the maternal parent of sample 4323 must be *P. lucens*. Not only reciprocal crosses indicate independent origins of the respective hybrids, but also *P.* ×*angustifolius* accessions show ITS sequence polymorphisms that distinguish samples from Girdev Lake (4535, 4537) from all others (Table 1, position 496), indicating that these may have originated independently from those of other localities and also that the two samples from Girdev Lake probably belong to the same vegetative clone (for distribution areas of the taxa, see Fig. 1 and suppl. Figs. S1–S4).

Phylogenetic analysis based on the *rbcL* gene. — Potamogetonaceae and Zannichelliaceae are forming a monophyletic clade (Fig. 2). Zosteraceae are sister to Potamogetonaceae/Zannichelliaceae. All other outgroup taxa are much more distantly related. Relationships among the three genera of Potamogetonaceae and Zannichelliaceae remain unresolved (given that posterior probabilities <0.95 are not significant). All genera appear as monophyletic groups; however, only *Groenlandia* and *Potamogeton* are well supported, whereas *Stuckenia* and Zannichelliaceae lack significant branch support. The *rbcL* marker also does not distinguish between some species within the genera *Stuckenia* and *Potamogeton*. In *Stuckenia*, *S. filiformis* and *S. amblyphylla* sequences are identical or nearly so. Several Turkish samples potentially belong to *S. amblyphylla* (4160, 4184, 164095), especially accession 164095, originating from Eastern Turkey, an area for which the occurrence of *S. amblyphylla* has been described. Unfortunately, the respective specimens were not well developed or were poorly preserved and did not allow to distinguish between these two species, and available sequences of *S. filiformis* and *S. amblyphylla* did not show any diagnostic substitutions. In *Potamogeton*, three species that are very distinct in their morphology and rather unrelated according to other molecular markers (Kaplan & al., 2013, see also below) were not distinguished by *rbcL* (*P. coloratus*, *P. alpinus*, *P. crispus*). In contrast, several other species were well delimited and showed sister relationships. These are *P. praelongus*–*P. natans*, *P. perfoliatus*–*P. nodosus* and *P. lucens*–*P. gramineus*; of the latter, even two different genotypes were distinguishable. These

Table 2. ITS sequence similarity of Turkish samples compared to other areas.

Species	GenBank accession number	Country	Percent sequence identity (remarks)
Zannichellia palustris	AB906895	Japan	100%
	EF526374	U.S.A.	99.32% (unreliable sequence, contains two Ns and two 1 bp-indels in the 5.8S region)
Groenlandia densa	KF270900	Switzerland	100%
	KF270901	Austria	100%
	KY214165	Poland	100%
Stuckenia amblyphylla (identical with *S. filiformis* from different areas)	FJ956802	China	100% (as "*S. amblyophylla*")
	KX062123	China	100% (as "*S. amblyophylla*")
S. filiformis	KT175311	China	100%
	KY407966	Russia	100%
	KF270924	Switzerland	100%
	HE613429	United Kingdom	100%
	KF270925	U.S.A.	99.87% (one polymorphic site)
S. pectinata	MH427628	Russia	100%
	FJ151216	Switzerland	100%
	MH427635	U.S.A.	100%
P. acutifolius	KF270902	Czech Republic	100%
	GU593252	Poland	100%
P. alpinus	FJ151201	Czech Republic	99.86% (one transition)
	HQ263485	Finland	99.86% (one transition)
	HQ263486	Russia	99.72% (one transition, one polymorphism)
P. berchtoldii	KF270903	Czech Republic	100% (plus several Chinese samples under different names)
P. coloratus	MN337270	Czech Republic	100%
	HQ263487	Austria	99.47% (three transitions)
	HQ263488	Germany	99.47% (three transitions)
P. crispus	EF526369	U.S.A.	100%
	GU814242	Czech Republic	100%
	JQ241258	Denmark	100% (hybrid with *P. praelongus*)
	KF733792	Russia	100% (hybrid with *P. natans*)
P. gramineus genotype 1	HQ263492	Czech Republic	100%
	HQ263490	Sweden	100%
	HQ263500	U.S.A.	100%
P. gramineus genotype 2	HQ263501	Montenegro	100%
	HQ263503	Slovakia	100%
	DQ468861	France	100%
P. lucens	HQ263510	Finland	100%
	HQ263511	Russia	100%
	JF977897	China	100%
	HQ263508	Japan	100%

(Continues)

Table 2. Continued.

Species	GenBank accession number	Country	Percent sequence identity (remarks)
P. natans	AB744006	Japan	100%
	HQ263548	Russia	100% (hybrid with *P. praelongus*)
	FJ151208	Germany	100%
	FJ151209	U.S.A.	99.86% (one transition)
P. nodosus	FJ151210	France	99.86% (one polymorphic site)
	HQ263539	Sweden	100% (hybrid with *P. gramineus*)
	HQ263518	U.S.A.	99.86% (one transition)
P. perfoliatus	AY529525	Germany	100%
	HQ263521	Bosnia and Herzegovina	100%
	JF977914	China	100%
P. praelongus	AB744005	Japan	100%
	HQ263546	Russia	100% (hybrid with *P. natans*)
	JX012091	Estonia	100%
	HQ263529	Italy	100%
P. pusillus	LC128691	Japan	100%
	JF977917	China	100%
	MF315059	Russia	100%
P. trichoides	MF070550	Iran	100%
	KF270919	India	100%

interspecific differences were also useful to determine the direction of the cross in hybrid accessions. Sequences of the same species retrieved from GenBank were identical with the Turkish samples confirming reliable species identification and low intraspecific variation across relatively large geographic distances. Even for *P. perfoliatus*, of which we found two haplotypes among Turkish accessions, both variants were present in GenBank also from other regions. All 13 Turkish accessions of *Stuckenia pectinata* were identical with an American sample (as "*Coleogeton pectinatus*"). Identical sequences were also found for *Groenlandia densa* and *Zannichellia palustris*, respectively. *Najas marina* sequences differed slightly, but Turkish and Czech accessions were identical and formed a well-supported clade with an American sample.

Phylogenetic analysis based on the ITS region. — ITS sequence divergence between Potamogetonaceae-Zannichelliaceae and the outgroup was by far too large to allow meaningful alignments of the spacer regions. Therefore, for phylogenetic analysis with this marker, only *Zannichellia* was used as outgroup (Fig. 3). Genetic distances between the four genera were still very high (ca 15%–25%, Table 3); alignments between the genera and especially with *Zannichellia* were in parts ambiguous due to the high variation. Based on ITS sequence similarity, *Zannichellia* was more divergent from each of the Potamogetonaceae genera than they were among each other, which is also evident from the long branch in Fig. 3. While *Groenlandia* was sister to *Potamogeton* in the phylogenetic analyses (Fig. 3, but with significant branch support only in maximum likelihood and Bayesian analyses), genetic similarities between *Groenlandia* and *Potamogeton* were higher than those between each of these genera and *Stuckenia* (Table 3). Thus, relationships among Potamogetonaceae genera remained unclear also with ITS because they depended on the method of inference and probably on other parameters (alignment, taxon sampling, choice of outgroup) as well. Despite the high variability of this marker, intraspecific variation was absent or extremely low (*S. pectinata*). The only exception were the different genotypes of *P. gramineus*, which made *P. gramineus* as a taxon not monophyletic. Further, ITS showed that three species with high morphological divergence that shared a *rbcL* haplotype – *P. coloratus*, *P. crispus* and *P. alpinus* – formed independent lineages with high sequence divergence and no particularly close relationship to any other *Potamogeton* species analyzed. Of these, *P. coloratus* appeared as sister to all others. The same closely related species pairs were found as with *rbcL*: *P. perfoliatus*–*P. nodosus*, *P. lucens*–*P. gramineus* and *P. natans*–*P. praelongus*. Of the linear-leaved species, which were not included in the *rbcL* analyses, three formed a monophyletic group (*P. berchtoldii*, *P. trichoides*, *P. acutifolius*), whereas *P. pusillus* occurred in a basal position among

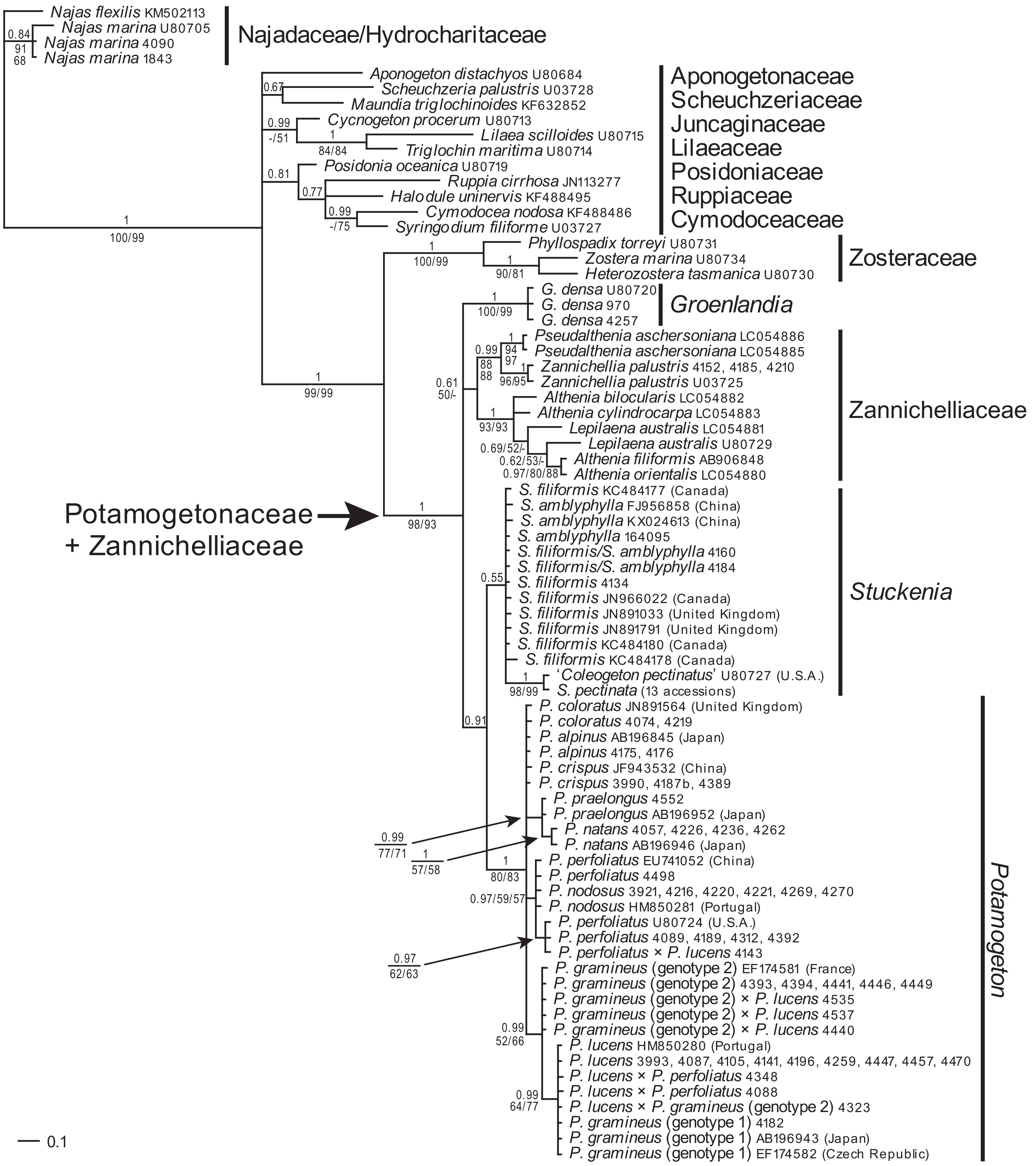

Fig. 2. Phylogenetic analysis of the Turkish Potamogetonaceae based on *rbcL*. A Bayesian consensus tree is shown with posterior probabilities above branches and bootstrap support percentages for MP and ML analyses below branches. Additional sequences from GenBank are indicated by their accession numbers; sequences newly produced in this study (Appendix 1) are shown with their collection number. Multiple collection numbers indicate identical sequences of the respective species. All accessions of *S. pectinata* have identical *rbcL* sequences. For *P. gramineus*, two genotypes that may represent cryptic species are distinguished.

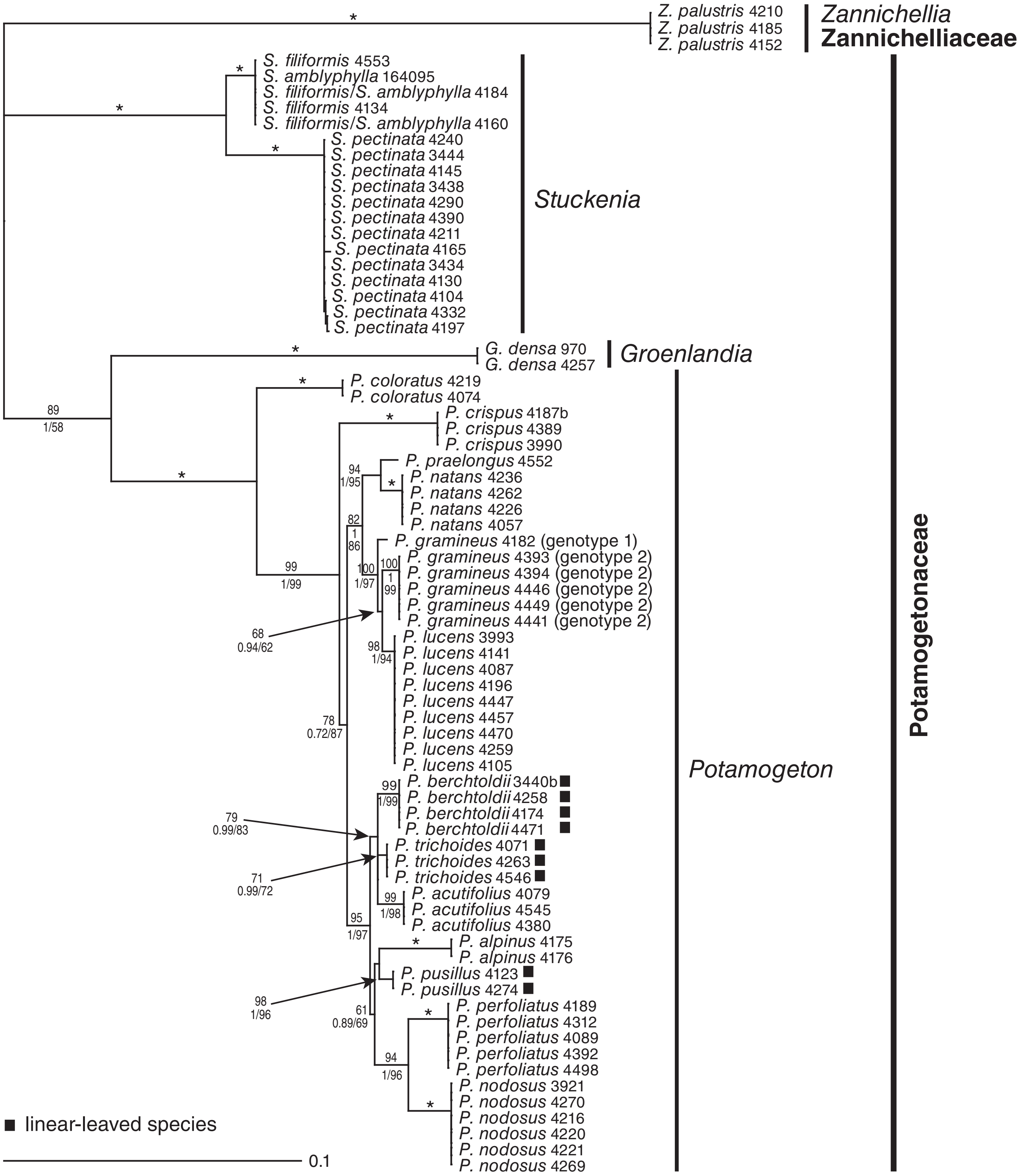

Fig. 3. Phylogenetic analysis of the Turkish Potamogetonaceae based on ITS. The ML tree with the highest log likelihood (−3568.02) is shown with bootstrap support above branches and posterior probabilities from BI and bootstrap support percentages from MP analysis below branches. Branches with values 100% or 1.00 are marked by an asterisk (*). For *P. gramineus*, two genotypes that may represent cryptic species are distinguished. Details about accessions are provided in Appendix 1.

Table 3. Pairwise ITS sequence identities between Potamogetonaceae and Zannichelliaceae.

Genus	Percent identity
Zannichellia–Groenlandia	75.8
Zannichellia–Stuckenia	77.1–78.2
Zannichellia–Potamogeton	77.4–78.4
Groenlandia–Stuckenia	80.0–81.7
Groenlandia–Potamogeton	83.6–84.3
Stuckenia–Potamogeton	80.9–83.8

Notes: Uncorrected P-distances were used; indels were calculated as a single change independent of length. Calculations for *Potamogeton* are based on comparisons with *P. coloratus*, *P. trichoides* and *P. nodosus*.

other lineages. All linear-leaved species grouped together with *P. alpinus*, *P. perfoliatus* and *P. nodosus*. Another clade was formed by *P. praelongus*, *P. natans*, *P. gramineus* and *P. lucens*. *Potamogeton crispus* constituted a distinct lineage that was moderately supported as sister to these two major groups.

Sequences of 18 species (including two genotypes of *P. gramineus*) represented in the ITS tree (Fig. 3) were investigated for their similarity with samples from other regions. For almost all species, one or several accessions from Europe, Asia and sometimes North America were found in GenBank that had 100% identity with the Turkish samples (Table 2); some of these originated from cloned sequences of hybrids with other species. Samples of *S. filiformis*, *P. nodosus*, and *P. natans* from North America differed in a single substitution or polymorphic site, whereas Eurasian samples were identical with the Turkish material. Only for *P. alpinus*, no identical sequences were available in GenBank. Turkish samples differed by at least one substitution from other material, corresponding to 99.86% sequence similarity.

Taxonomic diversity and distribution of taxa in Turkey. — Our fieldwork, herbarium studies and molecular analyses proved the occurrence of 22 taxa of Potamogetonaceae in Turkey. Of these, 14 species and 4 hybrids belong to *Potamogeton*, 3 species to *Stuckenia* and 1 species to *Groenlandia* (see suppl. Appendix S2 for details).

We failed to find unequivocal specimens of *S. amblyphylla* during our fieldwork. This species was reported to occur in eastern Turkey based on previously collected herbarium specimens (Uotila, 1984; Kaplan, 2008). We examined these vouchers and they are indeed very similar to plants from the type collection, which originates from Mount Kazbek, located about 200 km from the north-eastern border of Turkey. We collected vegetative specimens morphologically resembling this species in north-eastern and eastern Anatolia, and tentatively identified them as *S. amblyphylla* due to their linear leaves (up to 2.2 mm wide) with obtuse apex and their more robust and branched stems. Unfortunately, inflorescences and ripe fruits, which are essential for reliable identification, have not been observed on these individuals. The DNA markers used did not distinguish any of these individuals from *S. filiformis*. Because their taxonomic assignment is equivocal, they are annotated as "*S. filiformis*/*S. amblyphylla*" in the trees. Taxonomically, we include *S. amblyphylla* as a member of the Turkish flora based on earlier herbarium specimens that correspond to the variation pattern of specimens of this species from more eastern areas.

We identified two *Potamogeton* taxa as new for Turkey. We discovered *P.* ×*angustifolius* at three sites during our fieldwork. At each of them, this hybrid co-occurred with its parental species. Our herbarium studies indicate that it had apparently never been collected previously in Turkey. In contrast, we detected the Turkish occurrence of *P. schweinfurthii* only in herbaria. It was collected from Side, Antalya, in 1972, but remained unidentified in herbarium collections until we discovered it and recognized its identity in 2011. Our finding has been only recently published by Bayındır (2018) based on the same herbarium specimen. We attempted to re-discover *P. schweinfurthii* in the vicinity of Side during our fieldwork. Unfortunately, this is an attractive seaside area that has been exploited by the tourism industry in recent years and all suitable habitats were damaged.

The knowledge on the distribution of several species has been considerably improved. For example, *P. coloratus* was previously recorded in only two sites in Turkey, located in Antalya and Muğla Provinces (Wiegleb, 1990; Seçmen, 2000; Bayındır, 2018). We confirmed the occurrence of this species at these two localities and also found it in the Black Sea Region. Another rare species, *P. praelongus*, was reported from a single site in the province of Kars in the Eastern Anatolia Region (Uotila, 1984), based on an old literature record. However, no herbarium voucher supporting this occurrence is known. We collected *P. praelongus* in the Mediterranean Region during our fieldwork, which definitely confirms the occurrence of this species in Turkey; its *rbcL* sequence is identical with that of a Japanese accession (Fig. 2). The hybrid *P.* ×*salicifolius* was previously recorded from only one site in the province of Kocaeli (Uotila, 1984; Bayındır, 2018). We confirmed this occurrence and found it in three other lakes, in South-West Anatolia and the western part of North Anatolia. Also *P. alpinus* was previously known from only a single site and we found another. Distribution maps of all species are provided in supplementary Figs. S1–S4.

Based on the frequency of distribution, the recorded species of Potamogetonaceae can be sorted into three groups. Seven species (*Groenlandia densa*, *Stuckenia pectinata*, *Potamogeton crispus*, *P. lucens*, *P. nodosus*, *P. perfoliatus*, *P. pusillus*) are widespread, being recorded in all or almost all provinces of Turkey. Four species (*Potamogeton berchtoldii*, *P. gramineus*, *P. natans*, *P. trichoides*) can be classified as infrequent, occurring only in some regions of Turkey, and even within these areas they are only scattered. The last group includes rare taxa that are confined to small geographical areas and known from a single site or a small number of sites. Seven species (*Stuckenia filiformis*, *S. amblyphylla*, *Potamogeton* *acutifolius*, *P. alpinus*, *P. coloratus*, *P. praelongus*, *P. schweinfurthii*) and all the hybrids belong to this category. Some notable variation was observed in one of the species: *Potamogeton natans* is remarkable for its very dissimilar submerged and floating leaves. During our fieldwork, individuals with intermediate leaves were observed in running water in canals in Döşemealtı (Antalya). The floating leaves were extremely small (33–50 × 10–15 mm) when compared to the other Turkish populations, and the discoloured section between the petiole and the lamina of floating leaves was indistinct. Besides, their phyllodes were extremely long, up to 80 cm. While plants in standing waters produce fruits regularly, this running-water form produced very small and few-flowered spikes and no fruit. One sample (4236) from this location was included in the molecular analyses but proved to be identical with normal *P. natans*. The specimens collected from mesotrophic Yayla Lake (Buldan, Denizli) had numerous submerged phyllodes rather than floating leaves. Their phyllodes were much shorter and narrower than the phyllodes of forms from running water.

Comparing the diversity of Potamogetonaceae with bordering regions, Turkey includes almost all species known for the adjacent countries of south-eastern Europe and south-western Asia. In the global classification of distribution types defined by Wiegleb (1988), a majority (11 of 18) of the Turkish representatives belong among Northern species, with the Circumboreal and European subtypes being most represented. Widespread species include *P. crispus*, *P. nodosus*, *P. perfoliatus*, *P. pusillus* and *S. pectinata*. The African species are represented by *P. schweinfurthii*, which, however, is now known also from the Mediterranean part of Europe (Kaplan, 2005; Lastrucci & al., 2010; Aymerich & al., 2012) and from Iran (Abbasi & al., 2017). The only exclusively Asian species is *S. amblyphylla*, distributed in western and central Asia (Kaplan, 2008).

The Potamogetonaceae species recorded in Turkey can be identified using the following keys. The detailed taxonomic treatment of this family in Turkey, including an account of all genera, species and hybrids, morphological descriptions from family to species levels, and distributions and lists of examined herbarium specimens, is given in supplementary Appendix S2.

Identification key to genera

1. Leaves all opposite or rarely some of them in whorls of 3, sessile; spike capitate, with 2 opposite flowers, peduncle strongly recurved at fruiting period .... ***Groenlandia* (*densa*)**
1. Leaves alternate, only involucral ones sometimes subopposite, sessile or petiolate; spike mostly cylindrical, with at least 3 flowers, peduncles straight or slightly curved at fruiting period 2
2. Leaves with a sheathing base, lamina arising from the top of the sheath, ligule at the junction of the sheath and the lamina; leaf lamina elliptical in section, with inconspicuous midrib .... ***Stuckenia***
2. Leaves without a sheathing base, arising directly from the node, stipule free from the leaf base; leaf lamina flat in section, with a conspicuous midrib, or leaves reduced to phyllodes.. ***Potamogeton***

Identification key to *Potamogeton* species

1. Leaf margins serrate, with teeth easily visible to the naked eye, leaves of mature plants usually strongly undulate; fruits adnate at base, beak at least half as long as the rest of the fruit; all leaves submerged .... ***P. crispus***
1. Leaf margins entire or denticulate, with teeth not or scarcely visible to the naked eye, leaves flat or only slightly undulate; fruits free at base; beak absent or mostly much less than half as long as the rest of the fruit; floating leaves absent or present 2
2. All leaves filiform to linear, 0.4–4 mm wide, sessile, entire at margins 3
2. Some or all leaves narrowly lanceolate to orbicular (sometimes some or all submerged leaves reduced to linear phyllodes), generally more than 4 mm wide, sessile or petiolate, entire or denticulate at margins 6
3. Leaves with 12–22 faint sclerenchymatous strands in addition to 3 vascular veins; stem strongly compressed to flattened (particularly in its upper part), 0.6–3.0 mm wide .... ***P. acutifolius***
3. Leaves lacking sclerenchymatous strands, with only 3(–5) vascular veins; stem terete to only slightly compressed, 0.2–2.0 mm wide 4
4. Flowers with 1(–2) carpels; fruits laterally compressed, dentate at dorsal keel, 2.6–3.5 mm long; leaves filiform to narrowly linear, 0.2–1.0 mm wide, acuminate at apex .... ***P. trichoides***
4. Flowers usually with 4 carpels; fruits laterally convex, not compressed, smooth at dorsal keel, 2.0–2.8 mm long; leaves linear, mostly 0.6–2.5(–3.0) mm wide, usually acute to obtuse or mucronate at apex 5
5. Young stipules (in apical parts of the stem and branches) closed and tubular, appearing as a closed ellipse when transversally dissected; nodal glands absent or inconspicuous; leaves without rows of lacunae bordering midrib or rarely with 1 row of lacunae on each side of the midrib; turions axillary .... ***P. pusillus***
5. All stipules open and convolute, appearing as an open ellipse or a short spiral when transversally dissected; nodal glands present at least on some nodes, often well developed; leaves mostly with 1–3 rows of lacunae on each side of the midrib; turions terminal .... ***P. berchtoldii***
6. Mature plants with both floating and submerged leaves 7
6. Mature plants with only submerged leaves 11
7. All submerged leaves reduced to linear opaque phyllodes; petiole of floating leaves usually with a discoloured section at the junction with the lamina .... ***P. natans***
7. Submerged leaves with narrowly lanceolate to broadly elliptical lamina, sometimes only some of them reduced

to phyllodes; petiole of floating leaves without a discoloured section at the junction with the lamina 8

8. Floating leaves with translucent lamina, with conspicuous secondary veins; petioles of floating leaves shorter than the lamina; fruits 1.2–1.6 mm long.............. ***P. coloratus***
8. Floating leaves with opaque lamina, with inconspicuous secondary veins; petioles of floating leaves mostly as long as or longer than the lamina; fruits 1.8–3.5 mm long.....9
9. Submerged leaves petiolate, lamina 10–40 mm wide....... .... ***P. nodosus***
9. Submerged leaves sessile, 3–13 mm wide....................10
10. Submerged leaves 15–90 × 3–6 mm, (5–)7-veined, acute to mucronate at apex, denticulate and slightly undulate at margins; leaves green or rarely only with a slight brownish tinge when dry, stem branched.... ***P. gramineus***
10. Submerged leaves 60–140 × 4–13 mm, 9–15-veined, obtuse to rounded at apex, entire and flat at margins; both submerged and floating leaves with a strong reddish to brownish tinge when dry; stem unbranched...... ***P. alpinus***
11. Leaves petiolate, petioles at least 2 mm long................12
11. Leaves sessile...15
12. Most or all petioles 2–20 mm long; lamina 7–11-veined, acute to mucronate at apex...13
12. Petioles 20–140 mm long; lamina 9–17-veined, obtuse to slightly acute at apex..14
13. Lamina usually lanceolate to broadly elliptical, 11–46 mm wide, 9–11-veined; petioles mostly 2–7 mm long, usually of almost the same length along the stem .... ***P. lucens***
13. Lamina narrowly lanceolate to oblong, 9–19 mm wide, 7(–9)-veined; lower leaves usually sessile or with a short petiole, upper leaves with a petiole up to 21 mm long .... ***P. schweinfurthii***
14. Lamina narrowly lanceolate to broadly elliptical, 25–105 mm long; petiole 20–40 mm long .... ***P. coloratus***
14. Lamina lanceolate to oblong, 65–180 mm long; petiole 30–140 mm long.. ***P. nodosus***
15. Leaves narrowly lanceolate, 3–6 mm wide, (5–)7-veined .... ***P. gramineus***
15. Leaves lanceolate to elliptical or orbicular, 9–46 mm wide, 7–33-veined..16
16. Leaves mostly ovate to orbicular, 9–65 mm long, amplexicaul at base; stipules 4–20 mm long, decaying early....... .... ***P. perfoliatus***
16. Leaves lanceolate to elliptical, 24–120 mm long, mostly attenuate at base, rarely rounded and semi-amplexicaul; stipules 13–62, mostly persistent 17
17. Leaves 7(–9)-veined, acute to mucronate at apex .... ***P. schweinfurthii***
17. Leaves 9–19-veined, obtuse to rounded at apex 18
18. Leaves with a strong reddish to brownish tinge when dry; stem straight; leaves only slightly cucullate at apex; fruits 2–3.1 mm long, brown; stipules semi-translucent, decaying early .... ***P. alpinus***
18. Leaves green, sometimes only slightly brownish when dry; stem flexuous at least in the upper part; leaves distinctly cucullate at apex, often splitting when pressed; fruits 4–4.5 mm long, dark green; stipules opaque when dry, persistent .... ***P. praelongus***

Identification key to *Stuckenia* species

1. Leaf sheaths open at base even when young, often with shortly overlapping edges, appearing as a short spiral when transversally sectioned; mature fruits 3.4–4.7 mm long .... ***S. pectinata***
1. Leaf sheaths closed and tubular at base at least when young, appearing as a closed ellipse when transversally sectioned; mature fruits mostly 1.8–2 mm long, rarely up to 3.3 mm long...2
2. Fruits 1.8–2 mm long; leaves mostly filiform, sometimes linear, 0.2–2.2 mm wide; flower whorls mostly distinctly remote at least near the base of the spike .... ***S. filiformis***
2. Fruits (2.7–)2.9–3.3 mm long; leaves narrowly linear to linear, 0.7–2.4 mm wide; flower whorls mostly contiguous to shortly remote............................... ***S. amblyphylla***

■ DISCUSSION

The "Illustrated Flora of Turkey" has recently been rewritten, and Zannichelliaceae is included in the family Potamogetonaceae in the second volume of the Illustrated Flora of Turkey because the APG III system was adopted (Ekim & Güner, 2014). However, in addition to the phenotypic differentiation of the families (e.g., Watson & Dallwitz, 1992; Les & al., 1997; Lindqvist & al., 2006; Li & Zhou, 2009), our results of ITS sequence similarity show that *Zannichellia* is more divergent from each of the Potamogetonaceae genera than the latter are among each other (Table 3), which corroborates the separation of the two families by some authors and is in keeping with the large morphological differences between them.

Among the Turkish *Potamogeton* species, *P. coloratus* is genetically most distinct and appeared as sister to all others in the ITS analyses. This species belongs to the *P. polygonifolius* group, which differs from all other true broad-leaved species in consisting only of diploids with a chromosome number of 28, while the rest includes mainly tetraploids and octoploids (Kaplan & al., 2013). The most remarkable morphological differences of *P. coloratus* from the rest of the broad-leaved species in Turkey are (1) its small and beakless fruits and (2) its translucent floating-leaves.

Although *P. praelongus*–*P. perfoliatus* and *P. natans*–*P. nodosus* are very similar to each other morphologically, they are not closely related. In contrast, both ITS and *rbcL* trees show that *P. praelongus*–*P. natans* and *P. perfoliatus*–*P. nodosus* have sister relationships (Figs. 2, 3), although they are phenotypically well differentiated. Similar results have been shown previously by different researchers (e.g., Iida & al., 2004; Kaplan & Fehrer, 2011; Kaplan & al., 2013). The species within the pairs of *P. praelongus*–*P. natans* and *P. perfoliatus*–*P. nodosus* have similar stem anatomical characters: (1) The endodermal cells show "U" type thickening in *P. praelongus* and *P. natans*, whereas they are of "O" type in *P. nodosus* and *P. perfoliatus*. Further, interlacunar and subepidermal bundles are present in the stems of *P. natans* and *P. praelongus*, whereas *P. nodosus* and *P. perfoliatus* generally lack them (e.g., Wiegleb & Kaplan, 1998).

Potamogeton pusillus belongs to a highly polymorphic and taxonomically difficult species complex with almost cosmopolitan distribution. All forms of this group were formerly included in a single, broadly circumscribed *P. pusillus*. However, molecular studies support the taxonomic position of *P. berchtoldii* as a species distinct from *P. pusillus* s.str. (Hettiarachchi & Triest, 1991; Kaplan & Štěpánek, 2003; Les & al., 2009; Kaplan & al., 2013). These two species are highly similar morphologically. The most important morphological characters separating *P. berchtoldii* from *P. pusillus* are the structure of stipules (open and convolute in the former, closed and tubular when young in the latter) and the shape and position of turions (more robust and terminal in the former, slender and axillary in the latter). In addition, well-developed nodal glands are often present at least on some nodes in *P. berchtoldii* whereas they are usually absent or only inconspicuous in *P. pusillus*. Other characters often claimed in the literature are less reliable (Kaplan & Štěpánek, 2003; Kaplan, 2010b; Kaplan & Reveal, 2013).

In Turkey, two *P. gramineus* genotypes occur that were also found previously in other areas (Kaplan & Fehrer, 2006, 2007, 2011). In the broadest sampling of accessions of this species covering large areas of Europe and parts of North America (Kaplan & Fehrer, 2011), genotype 1 was found most frequently and also occurs in parts of Asia according to GenBank entries, whereas genotype 2 was restricted to central and southern Europe. In Turkey, genotype 1 was found only once, whereas genotype 2 occurred in five samples from three localities and was also involved in all *P. ×angustifolius* hybrids analyzed. Thus, with respect to *P. gramineus* genotypes, Turkish samples conform to the observation of a more restricted and southerly distribution of genotype 2, whereas the single occurrence of genotype 1 extends the south-eastern range of its previously known distribution. According to nuclear and chloroplast markers applied here and also with the nuclear 5S-NTS region (Kaplan & al., 2013), these *P. gramineus* genotypes are not monophyletic and can therefore be considered as different cryptic species rather than as mere intraspecific varieties. Also, the genetic distance between them is larger than that of a number of closely related *Potamogeton* species.

Apart from these cryptic species within *P. gramineus*, chloroplast as well as nuclear DNA markers show that intraspecific variation in the Potamogetonaceae studied is very low or even absent. Only *P. perfoliatus* showed a single substitution with *rbcL*; both haplotypes occur also in China or America and therefore represent true variation (Fig. 2). The ITS region did not show any variation among the Turkish samples apart from occasional polymorphic sites (Fig. 3). A comparison with material from Europe, the Far East or America confirms this picture: except for *P. alpinus* (one substitution), all ribotypes were found in other areas with 100% identity along the entire sequence (Table 2). This confirms our previous results (Kaplan & Fehrer, 2011; Kaplan & al., 2013), but is in stark contrast to some publications (Wang & al., 2007; Yang & al., 2017) that claimed to have found large intraspecific or even intra-individual variation in the ITS region of Potamogetonaceae. Apart from known cases of different taxonomic treatments such as *P. pusillus*–*P. berchtoldii*, comparison with other material in GenBank and the phylogenies included in these papers show, however, that both studies suffer from a considerable amount of misidentified samples (some even ending up in the wrong genus) and that they included hybrids between distantly related species that were not recognized as such.

Only one *Potamogeton* hybrid, *P. ×salicifolius* from Sapanca Lake, has been indicated by Bayındır (2018). Uotila (1984) suggested that more records of *Potamogeton* hybrids were expected to occur in Turkey. Indeed, *P. ×angustifolius*, a hybrid between broad-leaved species (in addition to *P. ×salicifolius*) and two hybrids between linear-leaved species (Aykurt & al., 2017) were recently discovered in this country within our studies. *Potamogeton ×angustifolius* (*P. gramineus* × *P. lucens*) is one of the most common hybrids in the genus. It is intermediate between its parental species. Its similarity to slender forms of *P. lucens* makes its identification by morphology alone difficult. Besides the intermediate size and shape, the occurrence of both sessile and shortly petiolate leaves (like in *P. lucens*) may indicate this hybrid. It is one of the very few Potamogetonaceae hybrids that are capable of producing well-developed fruit (Preston, 1995; Bobrov & Chemeris, 2009) and the only one that sets fruit regularly (Kaplan, 2010a). *Potamogeton ×angustifolius* was collected from only three localities, where it always co-occurred with both its parents. Other specimens that were in the field tentatively suspected to be this hybrid proved to be extreme forms of the parental species when examined more closely. Based on the ITS marker, the hybrid can be determined unequivocally.

Potamogeton ×salicifolius also co-occurred with its parents and is morphologically intermediate between them. The shape of the leaves is similar to *P. lucens*, but the leaves are sessile and semi-amplexicaul at base, which is a character inherited from *P. perfoliatus*. Besides, the upper part of the stem is distinctly flexuous. Some of the leaves are falcate. So far, *P. ×salicifolius* was recorded from only one location in Turkey, which was Sapanca Lake (Uotila, 1984; Bayındır, 2018). During our fieldwork we found it in three other lakes, in Eğirdir Lake and Beyşehir Lake in South-West Anatolia and in Terkos Lake in European Turkey. No generative organs were observed in these specimens.

Two hybrids between linear-leaved *Potamogeton* species, *P. berchtoldii* × *P. trichoides* and *P. obtusifolius* × *P. berchtoldii*, have recently been discovered in Turkey (Aykurt & al., 2017). *Potamogeton berchtoldii* × *P. trichoides* shows a combination of parental characters and may therefore be easily confused with these similar species. Its identity was unequivocally identified only by DNA analysis. *Potamogeton obtusifolius* × *P. berchtoldii* was also identified using DNA analysis. One of its parental species, *P. obtusifolius*, is not known to currently occur in Turkey (for details see Aykurt & al., 2017), and this hybrid may represent an old hybrid clone. Previous studies showed that hybrid clones can persist at a locality for a considerable period, even for hundreds or thousands of years (Hollingsworth & al., 1996; Preston & al., 1998; Bobrov, 2007; Kaplan & Fehrer, 2007, 2011, 2013) thanks to their perennial structures such as rhizomes or turions.

Taxonomic problems emerged in the classification of some *Stuckenia* samples. *Stuckenia filiformis* was previously recorded from two different high-mountain lakes in the Mediterranean Region, while *S. amblyphylla* was recorded from a few locations in north-eastern and eastern Anatolia (Uotila, 1984; Kaplan, 2008). We collected unequivocal *S. filiformis* with flowering and fruiting spikes in the area of its reported occurrence. However, the specimens collected in the area where *S. amblyphylla* was expected had only vegetative parts. Their reliable identification was not possible because, in the absence of fruit, *S. amblyphylla* may be indistinguishable from broad-leaved forms of *S. filiformis*, which are mainly found in running water (Kaplan, 2008). According to results of our molecular studies, these problematic specimens cannot be distinguished from *S. filiformis*. The lack of genetic differentiation may not be surprising given that even the morphologically well-distinguishable *S. filiformis* and *S. vaginata* (the latter not occurring in Turkey) have almost identical ITS sequences (McMullan & al., 2011; Kaplan & al., 2013). The latter paper also included two sequences of *S. amblyphylla* from Tajikistan that are distinguished by three unique substitutions from both, *S. filiformis* and *S. vaginata*. Evidently, this group requires taxonomic re-evaluation based on well-developed specimens from its entire range and the use of additional, highly variable molecular markers that would clearly distinguish between these species. On the other hand, it is possible that morphological and genetic divergence are not corresponding well in this species complex independently of the markers used, but may reflect a history characterized by relatively recent speciation and affected by incomplete lineage sorting.

■ CONCLUSIONS

We present the most comprehensive revision of the family Potamogetonaceae in Turkey to date, based on morphological and molecular analyses of an unrivalled number of specimens collected all around Turkey as well as material of 35 national and international herbaria. We evaluated the status of the family Zannichelliaceae and phylogenetic relationships between Potamogetonaceae species as well as their intraspecific variation at a large geographic scale. Hybrid taxa and the occurrence of cryptic species were inferred from molecular markers. The results of our study contribute to the literature of the family Potamogetonaceae on a global scale.

■ AUTHOR CONTRIBUTIONS

CA, İGD: fieldwork; CA, ZK: herbarium and morphological studies; JF, DSY, VB, EA, Nİ: molecular studies; JF: phylogenetic analyses; ZK, CA: taxonomy; CA, JF, ZK, DSY: writing of manuscript. — CA, https://orcid.org/0000-0001-8700-3500; JF, https://orcid.org/0000-0002-0337-5444; DSY, https://orcid.org/0000-0002-7909-2627; ZK, https://orcid.org/0000-0003-1707-7461; İGD, https://orcid.org/0000-0003-2190-372X; EA, https://orcid.org/0000-0002-5206-7333; Nİ, https://orcid.org/0000-0002-5508-8666

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Appendix 1. Specimens used for molecular studies.

Taxon (given only once for multiple accessions of the same taxon), **reference number**, locality, collector and (herbarium code) for voucher specimen, and GenBank accession numbers in order ITS, *rbcL*, downloaded sequences indicated with an asterisk. A dash (–) indicates missing data.

Groenlandia densa (L.) Fourr., **4257**, Turkey: Afyon: Çay, 1 km to Çayırağzı Village, Karamık Swamp, springs, 1008 m, 29 May 2015, coll. *C. Aykurt 4257, İ.G. Deniz* (AKDU), MH171033, MH170960. **970**, Switzerland: canton Graubünden, Silvaplana, Champfèrer See, coll. *Z. Kaplan 98/70* (PRA), KF270900*, MH170961. ***Najas marina*** L., **4090**, Turkey: Sakarya: Sapanca Lake, near Seka Camping, 36 m, 1 Jul 2014, coll. *C. Aykurt 4090, İ.G. Deniz* (AKDU), MH171037, MH170965. **1843**, Czech Republic: distr. České Budějovice, Zliv, Mydlovarský rybník fishpond, coll. *Z. Kaplan & K. Šumberová 06/464* (PRA), MH171038, MH170966. ***Potamogeton acutifolius*** Link, **4079**, Turkey: Sakarya: Karasu, Acarlar Freshwater Swamp Forest, canals, 12 m, 1 Jul 2014, coll. *C. Aykurt 4079, İ.G. Deniz* (AKDU), KX273116*, –. **4380**, Turkey: Samsun: Terme, near Akgöl, canals, sea level, 22 Jul 2015, coll. *C. Aykurt 4380, İ.G. Deniz* (AKDU), KX273117*, –. **4545**, Turkey: Konya: Seydişehir, Dikilitaş Village, Gavur Lake, 1848 m, 18 Aug 2017, coll. *C. Aykurt 4545, İ.G. Deniz* (AKDU), MH171014, –. ***P. alpinus*** Balb., **4175**, Turkey: Artvin: Şavşat, Yalnızçam Mountains, above Hanlı Plateau, Akgöl, glacial lakes, 2340 m, 26 Aug 2014, coll. *C. Aykurt 4175, İ.G. Deniz* (AKDU), MH171009, MH170940. **4176**, Turkey: Artvin: Şavşat, Yalnızçam Dağları, Karagöl, glacial lakes, 2850 m, 26 Aug 2014, coll. *C. Aykurt 4176, İ.G. Deniz* (AKDU), MH171010, MH170941. ***P. berchtoldii*** Fieber, **3440b**, Turkey: Afyon: Eldere Village, wetlands, 1015 m, 15 Aug 2014, coll. *C. Aykurt 3440b*, KX273115*, –. **4174**, Turkey: Artvin: Şavşat, Yalnızçam Mountains, above Hanlı Plateau, Akgöl, glacial lakes, 2340 m, 26 Aug 2014, coll. *C. Aykurt 4174, İ.G. Deniz* (AKDU), KX273111*, –. **4258**, Turkey: Afyon: Çay, 1 km to Çayırağzı village, Karamık Swamp, 1008 m, 29 May 2015, coll. *C. Aykurt 4258, İ.G. Deniz* (AKDU), KX273113*, –. **4471**, Turkey: Ordu: Aybastı, Aybastı Plateau, meanders, 1446 m, 23 Aug 2016, coll. *C. Aykurt 4471, İ.G. Deniz* (AKDU), MH171013, –. ***P. coloratus*** Hornem., **2252**, Czech Republic: distr. Nymburk, Lysá nad Labem, coll. *Z. Kaplan 10/171* (PRA), MN337270, –. **4074**, Turkey: Sakarya: Karasu, Acarlar Freshwater Swamp Forest, fens, 1 Jul 2014, coll. *C. Aykurt 4074, İ.G. Deniz* (AKDU), MH170977, MH170908. **4219**, Turkey: Antalya Döşemealtı, 3 km to Kırkgöz, canals, 292 m, 3 May 2015, coll. *C. Aykurt 4219, İ.G. Deniz* (AKDU), MH170978, MH170909. ***P. crispus*** L., **3990**, Turkey: Osmaniye: Kadirli, Bahçe Village, Castabala Valley, Yeniköy Bird of Paradise, 54 m, small ponds, 31 May 2014, coll. *C. Aykurt 3990, İ.G. Deniz* (AKDU), MH171006, MH170937. **4187b**, Turkey: Ardahan: Çıldır, in Çıldır River, 1900 m, 27 Aug 2014, coll. *C. Aykurt 4187b, İ.G. Deniz* (AKDU), MH171007, MH170938. **4389**, Turkey: Konya: Seydişehir, near Gökhüyük Village, canals, 1095 m, 4 Aug 2015, coll. *C. Aykurt 4389, İ.G. Deniz* (AKDU), MH171008, MH170939. ***P. gramineus*** L., **4182**, Turkey: Ardahan: between Şavşat and Ardahan, pools, 2406 m, 27 Aug 2014, coll. *C. Aykurt 4182, İ.G. Deniz* (AKDU), MH170996, MH170927. **4393**, Turkey: Konya: Seydişehir, on the road of Gökhüyük Village, Suğla Lake, 1095 m, 4 Aug 2015, coll. *C. Aykurt 4393, İ.G. Deniz* (AKDU), MH170997, MH170928. **4394**, Turkey: Konya: Seydişehir, on the road of Gölyüzü Village, Suğla Lake, 1095 m, 4 Aug 2015, coll. *C. Aykurt 4394, İ.G. Deniz* (AKDU), MH170998, MH170929. **4441**, Turkey: Bolu: Tepebaşı, Sünnet Lake, 1068 m, 27 Jun 2016, coll. *C. Aykurt 4441, İ.G. Deniz* (AKDU), MH170999, MH170930. **4446**, Turkey: Bolu: Tepebaşı, Sünnet Lake, under the bridge, in the lake, 1061 m, 27 Jun 2016, coll. *C. Aykurt 4446, İ.G. Deniz* (AKDU), MH171000, MH170931. **4449**, Turkey: Bolu: Tepebaşı, Çubuk Lake Natural Park, 1025 m, 27 Jun 2016, coll. *C. Aykurt 4449, İ.G. Deniz* (AKDU), MH171001, MH170932. ***P. lucens*** L., **3993**, Turkey: Osmaniye: Castabala Valley, wetlands, 56 m, 31 May 2014, coll. *C. Aykurt 3993, İ.G. Deniz* (AKDU), MH170979, MH170910. **4087**, Turkey: Sakarya: near Old Eşme, Sapanca Lake, 30 m, 1 Jul 2014, coll. *C. Aykurt 4087, İ.G. Deniz* (AKDU), MH170980, MH170911. **4105**, Turkey: Denizli: Çivril, near Beydilli Village, Işıklı Lake, 850 m, 4 Jul 2014, coll. *C. Aykurt 4105, İ.G. Deniz* (AKDU), MH170981, MH170912. **4141**, Turkey: Konya: Beyşehir, Beyşehir Lake, canals, 19 Jul 2014, coll. *C. Aykurt 4141, İ.G. Deniz* (AKDU), MH170982, MH170913. **4196**, Turkey: Kars: Susuz, Gölbaşı Village, Aygır Lake, 2130 m, 28 Aug 2014, coll. *C. Aykurt 4196, İ.G. Deniz* (AKDU), MH170983, MH170914. **4259/3349** Turkey: Afyon: Çay, 1 km to Çayırağzı Village, Karamık Swamp, 1008 m, 29 May 2015, coll. *C. Aykurt 4259, İ.G. Deniz* (AKDU, PRA), MH170984, MH170915. **4447**, Turkey: Bolu: Tepebaşı, Çubuk Lake, 1025 m, 27 Jun 2016, coll. *C. Aykurt 4447, İ.G. Deniz* (AKDU), MH170985, MH170916. **4457**, Turkey: Kayseri: İncesu, in Tohma stream, 1603 m, 8 Jul 2016, coll. *C. Aykurt 4457, İ.G. Deniz* (AKDU), MH170986, MH170917. **4470**, Turkey: Tokat: between Niksar and Perşembe Plateau, small lakes, 618 m, 23 Aug 2016, coll. *C. Aykurt 4470, İ.G. Deniz* (AKDU), MH170987, MH170918. ***P. natans*** L., **4057**, Turkey: Bolu: Yedigöller, Sazlı Lake, 870 m, 29 Jun 2014, coll. *C. Aykurt 4057, İ.G. Deniz* (AKDU), MH170973, MH170904. **4226**, Turkey: Burdur: Ağlasun, near Kibrit Village, wetlands, 1019 m, 21 May 2015, coll. *C. Aykurt 4226, İ.G. Deniz* (AKDU),

Appendix 1. Continued.

MH170974, MH170905. **4236**, Turkey: Antalya: Döşemealtı, near Kırkgöz Caravanserai, canals, 306 m, 22 May 2015, coll. *C. Aykurt 4236, İ.G. Deniz* (AKDU), MH170975, MH170906. **4262**, Turkey: Denizli: Buldan, Yayla Lake, 1155 m, 7 Jun 2015, coll. *C. Aykurt 4262, İ.G. Deniz* (AKDU), MH170976, MH170907. ***P. nodosus*** Poir., **3921**, Turkey: Antalya: Döşemealtı, Kırkgöz Water Basin, small ponds, c. 300 m, 5 May 2014, coll. *C. Aykurt 3921, İ.G. Deniz* (AKDU), MH170967, MH170898. **4216**, Turkey: Antalya: Döşemealtı, Kırkgöz Water Basin, natural canals, c. 320 m, 3 May 2015, coll. *C. Aykurt 4216, İ.G. Deniz* (AKDU), MH170968, MH170899. **4220**, Turkey: Antalya: Döşemealtı, Kırkgöz Water Basin, fishponds, c. 320 m, 3 May 2015, coll. *C. Aykurt 4220, İ.G. Deniz* (AKDU), MH170969, MH170900. **4221**, Turkey: Antalya: Döşemealtı, Kırkgöz Water Basin, fishponds, c. 320 m, 3 May 2015, coll. *C. Aykurt 4218, İ.G. Deniz* (AKDU), MH170970, MH170901. **4269**, Turkey: Antalya: Alanya, Dim River, semi-saline river mouth, 17 Jun 2015, coll. *C. Aykurt 4269, İ.G. Deniz* (AKDU), MH170971, MH170902. **4270**, Turkey: Antalya: Manavgat, Taşkesiği ponds, 600 m, 17 Jun 2015, coll. *C. Aykurt 4270, İ.G. Deniz* (AKDU), MH170972, MH170903. ***P. perfoliatus*** L., **4089**, Turkey: Sakarya: Sapanca, near Old Eşme, Sapanca Lake, 30 m, 1 Jul 2014, coll. *C. Aykurt 4089, İ.G. Deniz* (AKDU), MH170991, MH170922. **4189**, Turkey: Kars: Çıldır Lake, 1976 m, 27 Aug 2104, coll. *C. Aykurt 4189, İ.G. Deniz* (AKDU), MH170992, MH170923. **4312**, Turkey: İstanbul: Terkos, Balaban, Balaban Burun, Terkos Lake, 6 m, 24 Jun 2015, coll. *C. Aykurt 4312, İ.G. Deniz* (AKDU), MH170993, MH170924. **4392**, Turkey: Konya: Seydişehir, on the road of Gökhüyük Village, Suğla Lake, 1095 m, 4 Aug 2015, coll. *C. Aykurt 4392, İ.G. Deniz* (AKDU), MH170994, MH170925. **4498**, Turkey: Kars: between Doğruyol and Kars, canals, 1723 m, 26 Aug 2016, coll. *C. Aykurt 4498, İ.G. Deniz* (AKDU), MH170995, MH170926. ***P. praelongus*** Wulfen, **4552**, Turkey: Niğde: Ulukışla, Bolkar Mountains, Karagöl, glacial lake, 2600 m, 19 Aug 2017, coll. *C. Aykurt 4552, İ.G. Deniz* (AKDU), MH171011, MH170942. ***P. pusillus*** L., **4123**, Turkey: Burdur, Bucak, Alkaya village, around Oraç Dam, wetlands, 15 Jul 2014, coll. *C. Aykurt 4123, İ.G. Deniz* (AKDU), KX273110*, –. **4274**, Turkey: Çanakkale: Ezine, between Ezine and Çanakkale, near Sarımsakçı bridge, in the river, 44 m, 21 Jun 2015, *C. Aykurt 4274, İ.G. Deniz* (AKDU), KX273112*, –. ***P. trichoides*** Cham. & Schltdl., **4071**, Turkey: Sakarya: Adapazarı, Poyrazlar pool, 28 m, 30 Jun 2014, coll. *C. Aykurt 4071, İ.G. Deniz* (AKDU), KX273108*, –. **4263**, Turkey: Denizli: Buldan, Yayla Lake, 1155 m, 7 Jun 2015, coll. *C. Aykurt 4263, İ.G. Deniz* (AKDU), KX273109*, –. **4546**, Turkey: Konya: Seydişehir, near Dikilitaş Village, Gavur Lake, 1848 m, 18 Aug 2017, coll. *C. Aykurt 4546, İ.G. Deniz* (AKDU), MH171012, –. ***P. ×angustifolius*** J.Presl (*P. lucens* × *P. gramineus*), **4323**, Turkey: İstanbul: Eyüp, Belgrad Forests, Ayvat Dam, 112 m, 25 Jun 2015, coll. *C. Aykurt 4323, İ.G. Deniz* (AKDU), MH171002, MH170933. **4440**, Turkey: Bolu: Tepebaşı, Sünnet Lake, 1068 m, 27 Jun 2016, coll. *C. Aykurt 4440, İ.G. Deniz* (AKDU), MH171003, MH170934. **4535**, Turkey: Muğla: Fethiye, Girdev Lake, 1730 m, 13 Aug 2017, coll. *C. Aykurt 4535, İ.G. Deniz (*AKDU), MH171004, MH170935. **4537**, Turkey: Muğla: Fethiye, Girdev Lake, 1730 m, 13 Aug 2017, coll. *C. Aykurt 4537, İ.G. Deniz* (AKDU), MH171005, MH170936. ***P. ×salicifolius*** Wolfg. (*P. lucens* × *P. perfoliatus*), **4088**, Turkey: Sakarya: near Old Eşme, Sapanca Lake, 30 m, 1 Jul 2014, coll. *C. Aykurt 4088, İ.G. Deniz* (AKDU), MH170988, MH170919. **4143**, Turkey: Konya: Beyşehir, Beyşehir Lake, 1130 m, 19 Jul 2014, coll. *C. Aykurt 4143, İ.G. Deniz* (AKDU), MH170989, MH170920. **4348**, Turkey: Isparta: Yalvaç, beach of Taşevi Village, Eğirdir Lake, 930 m, 12 Jul 2015, coll. *C. Aykurt 4348, İ.G. Deniz* (AKDU), MH170990, MH170921. ***Stuckenia filiformis*** (Pers.) Börner, **4134**, Turkey: Antalya: Gündoğmuş, Geyik Mountain, Eğrigöl, 2069 m, 17 Jul 2014, coll. *C. Aykurt 4134, İ.G. Deniz* (AKDU), MH171028, MH170956. **4553**, Turkey: Niğde: Ulukışla, Bolkar Mountains, Karagöl, glacial lake, 2600 m, 19 Aug 2017, coll. *C. Aykurt 4553, İ.G. Deniz* (AKDU), MH171031, –. ***S. filiformis / S. amblyphylla*, 4160**, Turkey: Erzincan: Çayırlı, between Ozanlı and Yeşilyaka, streams, 1600 m, 24 Aug 2014, coll. *C. Aykurt 4160, İ.G. Deniz* (AKDU), MH171029, MH170957. **4184**, Turkey: Kars: 3 km from Ardahan to Çıldır, Ölçek river, 1744 m, 27 Aug 2014, coll. *C. Aykurt 4184, İ.G. Deniz* (AKDU), MH171030, MH170958. **164095**, Turkey: Van: Çaldıran, Kaz Lake, 2053 m, 17 Aug 2017, coll. *S. İşler* Herb. No.164095 (VANF), MH171032, MH170959. ***S. pectinata*** (L.) Börner, **3434**, Turkey: Isparta: entrance of Kovada Natural Park, Kovada Lake, 12 Aug 2012, coll. *C. Aykurt 3434, İ.G. Deniz* (AKDU), MH171015, MH170943. **3438**, Turkey: Isparta: entrance of Kovada Natural Park, Kovada Lake, 12 Aug 2012, coll. *C. Aykurt 3438, İ.G. Deniz* (AKDU), MH171016, MH170944. **3444**, Turkey: Denizli: Çivril, Işıklı Lake, 15 Aug 2012, coll. *C. Aykurt 3444, İ.G. Deniz* (AKDU), MH171017, MH170945. **4104**, Turkey: Denizli: Çivril, Işıklı Lake, 850 m, 4 Jul 2014, coll. *C. Aykurt 4104, İ.G. Deniz* (AKDU), MH171018, MH170946. **4130**, Turkey: Antalya: Gündoğmuş, Geyik Mountains, Eğrigöl, 2071 m, 17 Jul 2014, coll. *C. Aykurt 4130, İ.G. Deniz* (AKDU), MH171019, MH170947. **4145**, Turkey: Konya: Beyşehir, Beyşehir Lake, 1130 m, 19 Jul 2014, coll. *C. Aykurt 4145, İ.G. Deniz* (AKDU), MH171020, MH170948. **4165**, Turkey: Erzurum: Uzundere, Tortum Lake, 1018 m, 25 Aug 2014, coll. *C. Aykurt 4165, İ.G. Deniz* (AKDU), MH171021, MH170949. **4197**, Turkey: Kars: Susuz, Gölbaşı Village, Aygır Lake, 2130 m, 28 Aug 2014, coll. *C. Aykurt 4197, İ.G. Deniz* (AKDU), MH171022, MH170950. **4211**, Turkey: Antalya: Demre, Beymelek, small lakes and canals, 3 m, 28 Apr 2015, coll. *C. Aykurt 4211, İ.G. Deniz* (AKDU), MH171023, MH170951. **4240**, Turkey: Muğla: Köyceğiz, Köyceğiz Lake, 3 m, 26 May 2015, coll. *C. Aykurt 4240, İ.G. Deniz* (AKDU), MH171024, MH170952. **4290**, Turkey: Edirne: İpsala, Yeni Karpuzlu Dam, 15 m, 22 Jun 2015, coll. *C. Aykurt 4290, İ.G. Deniz* (AKDU), MH171025, MH170953. **4332**, Turkey: Sakarya: Ferizli, Akgöl, Konakcık Village, Donmaz District, in the lake, 9 m, 26 Jun 2015, coll. *C. Aykurt 4332, İ.G. Deniz* (AKDU), MH171026, MH170954. **4390**, Turkey: Konya: Seydişehir, near Gökhüyük Village, canals, 1095 m, 4 Aug 2015, coll. *C. Aykurt 4390, İ.G. Deniz* (AKDU), MH171027, MH170955. ***Zannichellia palustris*** L., **4152**, Turkey: Erzincan: Çayırlı, near Turnaçayırı Village, small stream, 2167 m, 24 Aug 2014, coll. *C. Aykurt 4152, İ.G. Deniz* (AKDU), MH171034, MH170962. **4185**, Turkey: Kars: Kars: 3 km from Ardahan to Çıldır, Ölçek River, 1744 m, 27 Aug 2014, coll. *C. Aykurt 4185, İ.G. Deniz* (AKDU), MH171035, MH170963. **4210**, Turkey: Antalya: Demre, near dalyan, sea level, 28 Apr 2015, coll. *C. Aykurt 4210, İ.G. Deniz* (AKDU), MH171036, MH170964.