



Hybridization between the linear-leaved *Potamogeton* species in Turkey



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ABSTRACT

Hybridization is an important source of generating diversity in *Potamogeton*. However, the recorded frequency and distribution of hybrids in the world is highly uneven, and so is our knowledge of this diversity. So far, no hybrid between linear-leaved species has been recorded in Turkey. Morphologically intermediate and taxonomically unclear forms collected during an extensive field survey in wetlands throughout Turkey were subjected to detailed morphological and molecular analyses. Direct sequencing and cloning of the ITS region revealed two hybrids previously unknown in Turkey: *P. obtusifolius* × *P. bertholdii* and *P. trichoides* × *P. bertholdii*. Sequences of the chloroplast *trnL-trnF* intergenic spacer region confirmed *P. obtusifolius* and *P. trichoides* as maternal parents of these hybrids. One of the maternal species, *P. obtusifolius*, is not known to currently occur in Turkey. Brief morphological descriptions of the new hybrids are provided, as well as a comparison of diagnostic characters with those of all parental and similar linear-leaved species. These results demonstrate that hybridization studies and appropriate approaches bring important information about both past and present biodiversity.

1. Introduction

Potamogeton L. (pondweeds; Potamogetonaceae) is one of the most diverse and taxonomically difficult plant genera in the aquatic environment (Wiegleb and Kaplan, 1998; Kaplan, 2002a). The members of this genus are found in freshwater and brackish lakes, marshes, ponds, rivers and streams (Uotila, 1984; Preston, 1995a). The genus includes about 72 species and 99 hybrids, and the highest diversity is found in temperate regions of the Northern Hemisphere (Kaplan et al., 2013).

The genus exhibits a huge taxonomic complexity due to the occurrence of many different hybrids (Preston, 1995a; Wiegleb and Kaplan, 1998; Kaplan and Fehrer, 2007; Kaplan and Fehrer, 2009). The occurrence of hybrids in the genus *Potamogeton* has been generally accepted for more than a century (see the reviews in Wiegleb et al., 2008; Kaplan et al., 2009). The first detailed descriptions of a higher number of *Potamogeton* hybrids were provided by Hagström (1916). More recently, pondweed hybrids were revised in several countries (e.g., Dandy, 1975; Zalewska-Galosz, 2002; Wiegleb et al., 2008; Kaplan, 2010). The most comprehensive treatments are available for the British Isles (Preston, 1995a, 2015).

In the genus *Potamogeton*, two morphological groups have been recognized, broad-leaved species and linear-leaved species (Hagström, 1916; Preston, 1995b; Iida et al., 2004; Lindqvist et al., 2006). Hybrids are known mainly between broad-leaved species of sect. *Potamogeton* (Preston, 1995b). The linear-leaved species, sometimes classified as sect. *Graminifolii* Fries (Dandy, 1980) are more difficult to identify because of their reduced morphology, which limits the number of characters that can be examined (Kaplan and Štěpánek, 2003). Furthermore, this section is anatomically and genetically less variable than the broad-leaved species (Hagström, 1916; Preston, 1995a; Wiegleb and Kaplan, 1998; Lindqvist et al., 2006; Kaplan et al., 2013). Possible hybridization between the linear-leaved pondweeds is especially difficult to detect, and consequently, very few hybrids are recognized nowadays (Wiegleb and Kaplan, 1998; Zalewska-Galosz and Ronikier, 2010; Preston, 2015). However, a targeted field survey using a combination of appropriate morphological and molecular identification tools showed that hybridization among linear-leaved pondweeds may be more frequent than previously assumed (Les et al., 2009).

The known centres of *Potamogeton* hybridization are Great Britain, Denmark, Sweden, and Japan (Wiegleb, 1988; Kaplan and Fehrer,

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2013). High hybrid diversity was recently identified also in the north-eastern USA (Kaplan et al., 2009). However, the importance of hybridization in the genus does not rest upon the number of hybrids recorded, but upon the capacity of hybrids to persist and spread vegetatively once they are established (Dandy, 1975). Many putative hybrids have been successfully confirmed or detected with the aid of isozyme and molecular techniques (Preston, 1995b; Preston et al., 1998a,b; Fant et al., 2001, 2003; Fant and Preston, 2004; Kaplan and Fehrer, 2004, 2006, 2007; Whittall et al., 2004; Iida et al., 2007; Zalewska-Gałosz et al., 2010). Nuclear ribosomal DNA (nrDNA), especially the internal transcribed spacer (ITS) region, is frequently used for the identification of hybrids because ITS sequences of hybrids often show additive patterns of both parental species (Whittall et al., 2000, 2004; Kaplan and Fehrer, 2007; Les et al., 2009). Most angiosperms have maternal transmission of chloroplast DNA (cpDNA), which was confirmed also in *Potamogeton* by Kaplan and Fehrer (2006) based on the only artificial *Potamogeton* hybrid that has ever been produced.

As far as known until recently, the genus *Potamogeton* was represented by 13 species and one hybrid in Turkey (Uotila, 1984; Secmen, 2001; Aykurt et al., 2016). The sect. *Graminifolii* included four species, namely *P. pusillus* L. (syn.: *P. panormitanus*), *P. berchtoldii* Fieber, *P. trichoides* Cham. & Schltdl. and *P. acutifolius* Link. There have been no detailed taxonomic studies carried out on *Potamogeton* in Turkey since the “Flora of Turkey and the East Aegean Islands”. In this Flora, Uotila (1984) hypothesized that hybrids might occur in Turkey.

The aims of this study are: 1) to examine the taxonomic status of the samples recently collected in Turkey that were taxonomically unclear or morphologically intermediate between linear-leaved *Potamogeton* species; 2) to collate evidence from molecular and morphological data; 3) to provide morphological descriptions of the hybrids; 4) to prepare a revised identification key to the linear-leaved *Potamogeton* species occurring in Turkey; and 5) based on these analyses, to draw more attention to hybridization between *Potamogeton* species in Turkey.

2. Materials and methods

2.1. Plant material used for morphological and molecular studies

Numerous linear-leaved *Potamogeton* specimens were collected within the project “Revision of pondweeds (Potamogetonaceae) in Turkey” from different types of wetlands in all regions of the country. These specimens were observed during field studies, and more detailed morphological evaluations have been carried out in the laboratory. Some of the samples were taxonomically unclear or morphologically intermediate between the linear-leaved species. The specimens collected from both Abant Lake, Örencik Plateau, Bolu Province (samples 4068b and 4070) and Gök Lake, Denizli Province (sample 4107) were morphologically not fairly identified. In order to clarify their identity, molecular analyses were carried out. Specimens 4068b and 4070 represented a hybrid of which one parent could not be readily identified by the first molecular analyses conducted in Antalya, Turkey. The sample 4070 was (under the reference number 3050) analyzed in Průhonice, the Czech Republic, and it turned out that the missing parent was *P. obtusifolius*, a species not yet known from Turkey. Material of this species from the Czech Republic and Denmark was therefore included in the study for comparison. Apart from these, 1–3 specimens of the four above-mentioned linear-leaved species from different localities in Turkey were included in the molecular analyses. The analyzed specimens are listed in Table 1. Voucher specimens are mostly preserved in the herbarium of the Akdeniz University (acronym AKDU), with a few duplicates in the herbarium of the Institute of Botany, Průhonice (acronym PRA).

Table 1
Locality data of linear-leaved *Potamogeton* taxa used in molecular analyses for the current study and GenBank accession numbers.

Taxon	Reference number	Locality and voucher collection data	Genbank number	
			ITS	trnL-trnF spacer
<i>P. acutifolius</i>	4079	Turkey: Sakarya, Karasu, Acarlar Freshwater Swamp Forest, canals, 12 m, 1 VIII 2014, coll. C. Aykurt 4079, i. G. Deniz (AKDU).	KX273116	KX273129
<i>P. berchtoldii</i>	3440b	Turkey: Afyonkarahisar, Eldere village, wetlands, 1015 m, 15 VIII 2014, coll. C. Aykurt 3440b, i. G. Deniz (AKDU).	KX273115	KX273128
	4258	Turkey: Afyon, Çay, 1 km to Çayırığı village, Karamik Swamp, 1008 m, 29 V 2015, coll. C. Aykurt 4258, i. G. Deniz (AKDU).	KX273113	KX273126
	4174	Turkey: Artvin, Şayşat, Yalnızçam Mountains, above Hanlı plateau, Karağaç Plateau, Akgöl, glacial lake, 2340 m, 26 VIII 2014, coll. C. Aykurt 4174, i. G. Deniz (AKDU).	KX273111	KX273124
<i>P. berchtoldii</i> × <i>P. trichoides</i>	4107	Turkey: Denizli, Çivril, Gököl, wetlands, 836 m, 04 VII 2014, coll. C. Aykurt 4107, i. G. Deniz (AKDU).	KX273114	KX273127
<i>P. obtusifolius</i> × <i>P. berchtoldii</i>	4068b	Turkey: Bolu, Gerede, 1. km on the road of Örencik Plateau, grazing marshes, 1383 m, 30 VI 2014, coll. C. Aykurt 4068b, i. G. Deniz (AKDU).	KX273107	KX273122
	3350, clone 1	Turkey: Bolu, Mudurnu, Abant Lake, 1339 m, 30 VI 2014, coll. C. Aykurt 4070 (PRA).	KY513710	KY513713
	3350, clone 16		KY513711	
<i>P. obtusifolius</i>	1834	Czech Republic: distr. Česká Budějovice, Třebeč, Stropnice stream, 3 IX 2006, coll. Z. Kaplan 06/418 (PRA).	KY513712	KY513714
	2567	Denmark: Jylland, Syddanmark, Ribe, pool at Ribeå River, 31 VII 2012, coll. Z. Kaplan & J. Prantl 12/217 (PRA).	KF270911	KY513715
<i>P. pusillus</i>	4274	Turkey: Çanakkale, Ezine, between Ezine and Çanakkale, around Sarımsakçı Bridges, river, 44 m, 21 VI 2015, coll. C. Aykurt 4274, i. G. Deniz (AKDU).	KX273112	KX273125
	4123	Turkey: Burdur, Bucak, Alkaya village, around Oraç Dam, wetlands, 15 VII 2014, coll. C. Aykurt 4123, i. G. Deniz (AKDU).	KX273110	
<i>P. trichoides</i>	4071	Turkey: Sakarya, Adapazarı, Poyrazlar Lake, 28 m, 30 VI 2014, coll. C. Aykurt 4071, i. G. Deniz (AKDU).	KX273108	KX273123
	4263	Turkey: Denizli, Buldan, Yayla Lake, 1155 m, 7 VI 2015, coll. C. Aykurt 4263, i. G. Deniz (AKDU).	KX273109	

2.2. DNA extraction, PCR, and sequencing

Fresh leaves were stored at -20 °C until DNA extraction. Total DNA was extracted by the CTAB method of Doyle and Doyle (1987). DNA concentrations were estimated on 1% agarose gels stained with ethidium bromide. The internal transcribed spacer region (ITS1, 5.8S rDNA and ITS2) was amplified using primers ITS 4 and ITS 5 (White et al., 1990). The intergenic spacer region between the *trnL* (UAA) 3' exon and *trnF* (GAA) was amplified using primers e and f of Taberlet et al., (1991). The PCR analysis was carried out with 1 U of *Taq* DNA polymerase (Fermentas Life Sciences, Burlington, Canada) in the supplied reaction buffer, 2 mM MgCl₂, 0.2 mM of each dNTP, 0.2 μM of each primer and 40 ng of template DNA, and Milli-Q water to a final volume of 15 μL. PCR amplification conditions for the ITS region were as follows: 94 °C for 1 min, 35 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C, and a final extension step of 10 min at 72 °C. PCR amplification conditions for the chloroplast region were the same as for the ITS region except that the annealing temperature was 68 °C. Amplifications were performed on a Bionerr thermocycler (MyGenie™). PCR products were separated on 1.5% agarose gels run at 75 V in 1 × TAE buffer and visualized under UV light after staining with ethidium bromide. The products of both ITS and chloroplast region of each sample were purified using the GeneJET Gel Extraction Kit (Thermo Scientific Fermentas, Vilnius, Lithuania). Sequencing was carried out at Macrogen Inc., Europe via BM Laboratories Ltd., as direct sequencing in both directions using the amplification primers. All sequences were trimmed, BLASTed against GenBank, and aligned using the software Geneious 6.1.2 (<http://www.geneious.com/>, Biomatters). Sequences were submitted to GenBank; accession numbers of all sequences used in this study are included in Table 1.

2.3. Hybrid identification

Hybrids were recognized by double peaks and/or shifts in direct sequencing reads of the ITS region. Double peaks were represented by IUPAC ambiguity codes. Comparison with putative parents showed additivity of the respective character states that differed between them. One hybrid sample with shifts caused by length differences of the parental sequences was cloned to confirm the additivity patterns. Cloning was done as described in Fehrer et al., (2009), and 16 clones were sequenced with the forward primer at GATC Biotech (Konstanz, Germany). The maternal parents were identified by chloroplast DNA.

3. Results

3.1. Variation in the nuclear ITS region

No intraspecific variation was found in any of the parental sequences analyzed. A total of 13 ITS sequences belonging to the linear-leaved *Potamogeton* taxa studied were obtained to determine differences between the parents and for comparison with their hybrids (Table 2). The hybrid plants presented a rigorously additive sequence pattern of the respective parental species and displayed no polymorphisms in other sites of their sequences. Two cloned sequences of 4070, one for *P. bertholdii* (x1) and one for *P. obtusifolius* (x16), as well as the direct reads for 4070 and 4068b are included in Table 2. In these hybrids, parental sequences were present in strongly unequal amounts according to direct sequencing so that it was also possible to infer one of the parents, *P. bertholdii*, by dismissing the minor peaks of the other parental sequence. These unequal proportions are reflected by clones of sample 4070: six clones corresponded to *P. bertholdii*, but only two showed the *P. obtusifolius* sequence. The remaining eight were different recombinants (unavoidable PCR artifacts) between the parental copies. Two individuals of the 4070 hybrid from different locations had identical sequences. They displayed an additive pattern at 11 sites and also a shift in both sequences that corresponds to an indel in the

Table 2
Nucleotide sites in nuclear ribosomal ITS sequences differing between the parents and additivity patterns of hybrids.

Species (accession)	Position in alignment																						
	49	61	145	149	193	228	233	234	247	254	258	448	469	478	490	528	551	558	585	595	601	603–617	663
<i>P. acutifolius</i> (4079)	G	T	G	C	T	G	G	T	G	C	-	C	T	G	T	C	A	C	G	C	C	TGGGCATCTTCGTCC	T
<i>P. trichoides</i> (4071)	A	T	A	C	T	G	A	C	G	T	-	C	T	G	A	-	A	T	G	C	C	TGGGCATCTTCGTCC	T
<i>P. trichoides</i> (4263)	A	T	A	C	T	G	A	C	G	T	-	C	T	G	A	-	A	T	G	C	C	TGGGCATCTTCGTCC	T
<i>P. bertholdii</i> × <i>P. trichoides</i> (4107)	A	Y	A	C	T	G	A	Y	G	T	A/-	C	T	K	A	-	A	Y	K	C	C	TGGGCATCTTCGTCC	Y
<i>P. bertholdii</i> (3440b)	A	C	A	C	T	G	A	T	G	T	A	C	T	A	-	A	C	C	T	C	C	TGGGCATCTTCGTCC	C
<i>P. bertholdii</i> (4258)	A	C	A	C	T	G	A	T	G	T	A	C	T	A	-	A	C	C	T	C	C	TGGGCATCTTCGTCC	C
<i>P. bertholdii</i> (4174)	A	C	A	C	T	G	A	T	G	T	A	C	T	A	-	A	C	C	T	C	C	TGGGCATCTTCGTCC	C
<i>P. obtusifolius</i> × <i>P. bertholdii</i> (4070/3350), clone 1	A	C	A	C	T	G	A	T	G	T	A	C	T	A	-	A	C	C	T	C	C	TGGGCATCTTCGTCC	C
<i>P. obtusifolius</i> × <i>P. bertholdii</i> (4070/3350)	A	Y	A	M	W	K	A	T	G	T	A/-	C	Y	T	A	-	A	C	K	Y	Y	TGGGCATCTTCGTCC/-	Y
<i>P. obtusifolius</i> × <i>P. bertholdii</i> (4068b)	A	Y	A	M	W	K	A	T	G	T	A/-	C	Y	T	A	-	A	C	K	Y	Y	TGGGCATCTTCGTCC/-	Y
<i>P. obtusifolius</i> × <i>P. bertholdii</i> (4070/3350), clone 16	A	T	A	A	A	T	A	T	G	T	-	T	Y	T	A	-	T	C	G	T	T	-	T
<i>P. obtusifolius</i> (1834)	A	T	A	A	A	T	A	T	G	T	-	T	Y	T	A	-	T	C	G	T	T	-	T
<i>P. obtusifolius</i> (2567)	A	T	A	A	A	T	A	T	G	T	-	T	Y	T	A	-	T	C	G	T	T	-	T
<i>P. pusillus</i> (4123)	A	T	A	A	A	T	A	T	G	T	-	T	Y	T	A	-	T	C	G	T	T	-	T
<i>P. pusillus</i> (4274)	A	T	A	A	A	T	A	T	G	T	-	T	Y	T	A	-	T	C	G	T	T	-	T

Note: Additive sites in hybrid sequences are shown as IUPAC ambiguity codes (Y = T/C, K = G/T, W = A/T, M = C/A); indels (shifts in sequence reads) are indicated as ‘-’/‘-’. In addition, two clones of sample 4070/3350 corresponding to the parents are included.

Table 3
Variable nucleotide sites in the *trnL-trnF* chloroplast region differing between the parents and maternal origin of hybrids.

Species (accession)	Position in alignment									
	4–8	40	182	238	247	261	262	263	279	342–349
<i>P. pusillus</i> (4274)	–	T	T	G	G	–	–	–	C	–
<i>P. berchtoldii</i> (3440)	TAAAA	T	T	T	A	T	–	–	C	–
<i>P. berchtoldii</i> (4174)	TAAAA	T	T	T	A	T	–	–	C	–
<i>P. berchtoldii</i> (4258)	TAAAA	T	T	T	A	T	–	–	C	–
<i>P. berchtoldii</i> × <i>P. trichoides</i> (4107)	TAAAA	T	T	T	A	T	–	–	C	–
<i>P. obtusifolius</i> (1834)	TAAAA	T	T	T	G	T	T	–	A	TGAATATT
<i>P. obtusifolius</i> (2567)	TAAAA	T	T	T	G	T	T	–	A	TGAATATT
<i>P. obtusifolius</i> × <i>P. berchtoldii</i> (4068b)	TAAAA	T	T	T	G	T	T	–	A	TGAATATT
<i>P. obtusifolius</i> × <i>P. berchtoldii</i> (4070/3350)	TAAAA	T	T	T	G	T	T	–	A	TGAATATT
<i>P. acutifolius</i> (4079)	TAAAA	T	T	T	G	T	T	T	A	–
<i>P. trichoides</i> (4071)	TAAAA	C	G	T	G	T	T	–	A	–

alignment between the two parental species. Sample 4107 was a different hybrid, namely between *P. berchtoldii* and *P. trichoides*. It was readily identified by additive sites only, because these parental ITS sequences do not show length differences (Table 2). All other linear-leaved species can be excluded as parents based on these patterns.

3.2. Variation in the chloroplast region

The intergenic spacer region between the *trnL* (UAA) 3' exon and *trnF* (GAA) was amplified for altogether eleven samples. Only one sample of *P. trichoides* was studied, because this region did not amplify from another individual. No intra- or inter-population variation was detected within the parental species. In total, one nucleotide substitution and two indels including one single base and a 8 bp indel were detected in the sequence alignment (Table 3). The hybrid 4068b matched its maternal parent, *P. obtusifolius*. Hybrid 4107 had the same sequence with its maternal parent, *P. berchtoldii*. Other putative parental taxa can be excluded by these patterns.

4. Discussion

4.1. Patterns of variation and distribution of *Potamogeton* hybrids

In Turkey, the occurrence of hybrids between the species of *Potamogeton* sect. *Graminifolii* was determined for the first time. During the fieldwork, numerous linear-leaved *Potamogeton* specimens collected from approximately 45 different locations in all regions of Turkey were morphologically evaluated, and 11 of them were included into the molecular studies. Only two hybrids from three different localities were recorded, which indicates that the hybridization frequency between the linear-leaved *Potamogeton* species in Turkey is apparently low.

Potamogeton hybrids are generally intermediate morphologically between the parental species (e.g., Hagström, 1916; Preston, 1995a,b; Wiegleb and Kaplan 1998; Kaplan et al., 2011). Several previous molecular studies have demonstrated that for most *Potamogeton* hybrids, either the parental species themselves or at least their respective species group can be reliably identified morphologically, as long as adequate expert inspection of a large set of key features is adopted (Kaplan et al., 2011). However, discovery of hybrids between linear-leaved species is difficult, particularly because of the reduced morphology and high similarity and phenotypic plasticity of its members (Kaplan and Štěpánek, 2003; Les et al., 2009).

Potamogeton hybrids mostly co-occur at their sites together with their parents, but there are exceptions (Kaplan and Fehrer, 2009, 2011). The occurrence of a *Potamogetonaceae* hybrid in the absence of one or both parents has been documented from several countries (Dandy and Taylor, 1946; Hollingsworth et al., 1996; Preston et al., 1998a,b, 1999; Kaplan and Fehrer, 2004; Kaplan et al., 2009). Similarly, one of the

parental species of the hybrids detected in this study, *P. obtusifolius*, is not known to currently occur in Turkey. Although *Potamogeton* hybrids are almost always sterile (Hagström, 1916; Dandy, 1975; Preston, 1995a; Wiegleb and Kaplan, 1998; Kaplan and Fehrer, 2007), hybrid clones can persist at a locality for a considerable period, even for hundreds or thousands of years (Hollingsworth et al., 1996; Preston et al., 1998b; Kaplan and Fehrer, 2007, 2011, 2013). Thus, hybrids with old histories are mostly found in ecologically stable habitats (Kaplan and Fehrer, 2009). However, some hybrids can be at least partially fertile and produce viable seed, and can potentially spread to other sites and establish new populations (Kaplan, 2010). Although some enlarged carpels were observed in *P. obtusifolius* × *P. berchtoldii*, it is unclear if they contain a viable embryo. Carpels in flowers of *P. berchtoldii* × *P. trichoides* remain small and appear to be abortive. As no fully developed fruits have been observed in these hybrids, it can be assumed that they are sterile and persist at their localities vegetatively, like the majority of *Potamogeton* populations (Kaplan et al., 2002; Kaplan and Štěpánek, 2003).

The occurrences of *P. berchtoldii* × *P. trichoides* and *P. obtusifolius* × *P. berchtoldii* were previously recorded from a few European countries (Fischer, 1907; Hagström, 1916). However, examination of the voucher herbarium specimens revealed that at least some of them may represent extreme forms of the species rather than true hybrids. The records provided here are the first convincing evidence for the existence of these hybrids.

4.2. Morphological description of the new hybrids

Potamogeton obtusifolius × *P. berchtoldii* (Fig. 1).

Stem sparingly branched, slender, slightly compressed, 0.4–0.6 mm wide, annual. Submerged leaves sessile, linear, 27–60 × 1.2–3 mm, bright green to dark green, entire at margins, narrowly cuneate at base, obtuse or very shortly and indistinctly mucronate at apex, 3-veined, with up to 3 rows of lacunae on each side of the midrib; nodal glands present on most nodes, well developed. Floating leaves absent. Stipules axillary, open and convolute, 7–12 mm long, translucent, decaying with age. Inflorescences terminal; peduncle 18–22 mm long, 2–2.5 times longer than the spike, as thick as the stem; spike cylindrical, 4–9 mm long. Flowers 3–6, with 4 carpels; carpels sometimes abortive. Fruits not fully developed, flat, 2–2.2 × 1.1–1.3 mm, presumably sterile.

The hybrid is more or less intermediate between the parental species. It resembles slender forms of *P. obtusifolius*, but differs by its longer peduncles, which are 2–2.5 times longer than the spike in the hybrid, whereas usually only 0.4–1.2 times as long as the fruiting spike in *P. obtusifolius*. The hybrid may be difficult to distinguish morphologically from extreme forms of the parental species, and it is advisable to have molecular proof of the identity of questionable plants.

Specimens examined: Turkey: Bolu, Mudurnu, Abant Yolu, Abant Lake, 1339 m, 30.06.2014, C.Aykurt 4068b & İ.G.Deniz (AKDU);

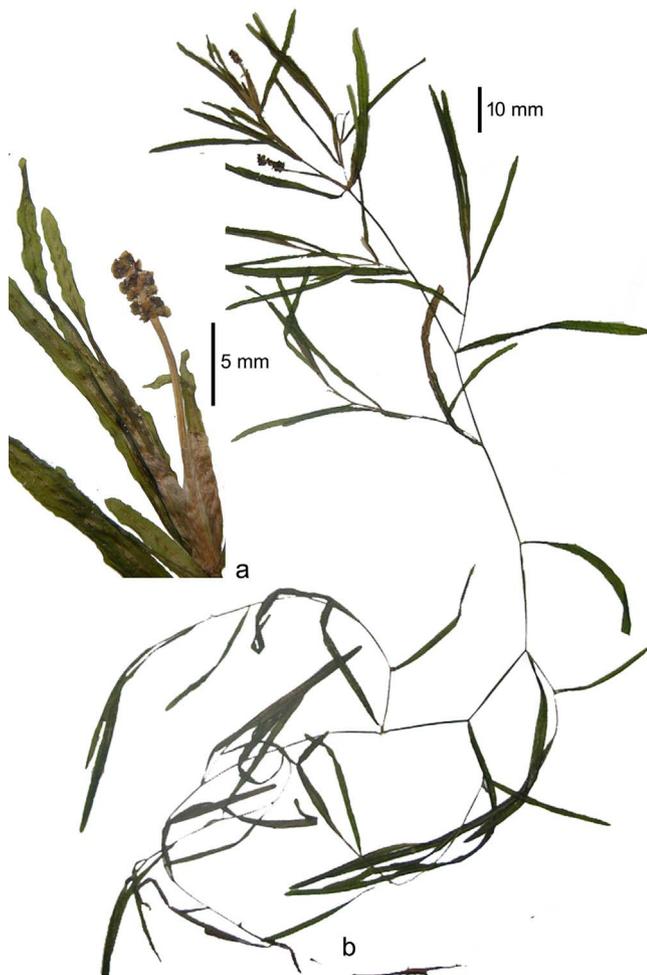


Fig. 1. *Potamogeton obtusifolius* × *P. berchtoldii* (collection C. Aykurt 4068b): a) inflorescence, b) general appearance.

27.06.2016, C.Aykurt 4439 & İ.G.Deniz (AKDU). Bolu: Gerede, Örencik Plateau, grazing marshes, 1383 m, 30.06.2014, C.Aykurt 4070 & İ.G.Deniz (AKDU, PRA); 27.06.2016, C.Aykurt 4437 & İ.G.Deniz (AKDU).

Distribution in Turkey: The hybrid is known only from the Abant Lake and the Örencik Plateau located nearby this lake, both in the Western Black Sea Region. The hybrid individuals were collected from shallow edges of the lake and from grazing marshes in the plateau.

Potamogeton berchtoldii × *P. trichoides* (Fig. 2).

Stem sparingly to richly branched, slender, terete to slightly compressed, 0.4–0.6 mm wide, annual. Submerged leaves sessile, linear, 35–50 × 1–1.8 mm, green, entire at margins, narrowly cuneate at base, acute to mucronate at apex, 3-veined, midrib slightly thickened toward the leaf base, lateral veins distinct, some leaves with up to 2 rows of lacunae on each side of the midrib; nodal glands present on most nodes, well developed. Floating leaves absent. Stipules axillary, open and convolute, 12–18 mm long, translucent, fibrous, persistent or disintegrating to fibres. Inflorescences (a single one seen) terminal; peduncle 2.5 mm long; spike almost globose, 1.5 mm long. Flowers 2, with 2–3 carpels; carpels abortive. Fruits not produced (plants sterile).

The hybrid shows a combination of parental characters. It is more similar to *P. berchtoldii*, but its leaves show a midrib slightly thickened toward the leaf base, which is a character inherited from *P. trichoides*. The stipules in the hybrid are more fibrous than in *P. berchtoldii*. Finally, the only spike available shows flowers with the number of carpels that is intermediate between those of the parental species, being 4 in *P. berchtoldii* and 1(–2) in *P. trichoides*, respectively.

Specimens examined: Turkey: Denizli Çivril, Gök Lake, wetlands, 836 m, 04.07.2014, C.Aykurt 4107 & İ.G.Deniz (AKDU, PRA); 27.08.2016, C.Aykurt 4503 & İ.G.Deniz (AKDU).

Distribution in Turkey: The hybrid is known only from the Gök Lake, near Çivril, Denizli, in the Aegean Region. The plants were collected from shallow edges of the lake.

4.3. Morphological identification of the linear-leaved *Potamogeton* taxa in Turkey

With the two new hybrids included, there are now six linear-leaved *Potamogeton* taxa known in Turkey: *P. acutifolius*, *P. berchtoldii*, *P. pusillus*, *P. trichoides*, *P. obtusifolius* × *P. berchtoldii* and *P. berchtoldii* × *P. trichoides*. Among these, *P. acutifolius* can be clearly



Fig. 2. *Potamogeton berchtoldii* × *P. trichoides* (collection C. Aykurt 4107): a) upper part of the stem, b) general appearance.

Table 4
Distinguishing characters of the linear-leaved *Potamogeton* taxa occurring in Turkey.

Morphological characters	<i>P. acutifolius</i>	<i>P. obtusifolius</i>	<i>P. obtusifolius</i> × <i>P. berchtoldii</i>	<i>P. berchtoldii</i>	<i>P. trichoides</i> × <i>P. berchtoldii</i>	<i>P. trichoides</i>	<i>P. pusillus</i>
Stem	Strongly compressed to flattened	Slightly compressed	Slightly compressed	Terete to slightly compressed	Terete to slightly compressed	Terete	Terete
Sclerenchymatous strands in leaf lamina	Present (12–22)	Absent	Absent	Absent	Absent	Absent	Absent
Nodal glands	Absent or sometimes present but poorly developed	Present, well developed	Present, well developed	Present, well developed	Present, well developed	Absent or present	Absent, rarely present but poorly developed
Leaf apex	Acute	Rounded, often very shortly and rather obscurely mucronate, rarely obtuse	Obtuse to shortly mucronate	Acute to obtuse, or mucronate	Acute to mucronate	Acuminate	Acute to subobtusely
Stipule	Open and convolute	Open and convolute	Open and convolute	Open and convolute	Open and convolute	Open and convolute	Closed and tubular
Length of peduncle (mm)	5–8	18–22	18–22	6–32	2.5	10–24	8.5–40
Spike shape and length (mm)	Globose, 3–8 mm	Cylindrical, 7–13 mm	Cylindrical, 4–9 mm	Cylindrical to globose, 4–7 mm	Almost globose, 1.5 mm	Cylindrical, 3–5 mm	Cylindrical, 5–9 mm
Number of carpels (rare exceptions omitted)	1	4	4	4	2–3	1	4

distinguished from the others by its strongly compressed to flattened stems and leaves with numerous sclerenchymatous strands. It is also characteristic by short peduncles, globose spikes and flowers with one carpel.

Although *P. berchtoldii*, *P. pusillus* and *P. trichoides* are very similar to each other in respect to their general appearance, they differ in several characters such as the structure of stipules, occurrence of nodal glands, details in venation and number of carpels. *Potamogeton trichoides* differs from the other two by its flowers with 1(–2) carpels, bigger and laterally compressed fruits, generally narrower and stiffer submerged leaves that are acuminate at apex, and the midrib usually distinctly thickened toward the leaf base. The last two, *P. berchtoldii* and *P. pusillus*, are the most difficult to distinguish morphologically, although they are relatively distant phylogenetically (Kaplan and Štěpánek, 2003; Les et al., 2009; Kaplan et al., 2013). They consistently differ in the structure of stipules and by a couple of less reliable characters such as the occurrence of glands and some details of leaf anatomy (Hagström, 1916; Preston, 1995b; Kaplan and Štěpánek, 2003). Morphological features of the two hybrids are described above.

To facilitate comparison, the most important diagnostic characters of the linear-leaved *Potamogeton* taxa occurring in Turkey and of *P. obtusifolius* as a parental species are summarized in Table 4.

The following identification key does not include the hybrids, because they are usually intermediate between their parental species and difficult to be integrated in dichotomic keys. However, because the hybrids are rare, the key facilitates the identification of the great majority of the Turkish diversity. In case of doubts or if an intermediate or transitional character state is encountered, Table 4 should be consulted. In any case, it should be understood that hybrids may be easily confused with parental species because of the overall similarity and enormous phenotypic plasticity (see Kaplan, 2002b; Kaplan and Zalewska-Gałosz, 2004) and sometimes even misidentified as an unrelated species (Kaplan, 2005). Any morphological identification of linear-leaved hybrids should be considered as only tentative and confirmed by DNA analysis.

Identification key to linear-leaved *Potamogeton* species occurring in Turkey.

1a. Leaves with 12–22 faint sclerenchymatous strands in addition to 3 vascular veins; stem strongly compressed to flattened (particularly in its upper part), 0.6–2.4 mm wide.....*P. acutifolius*

1b. Leaves lacking sclerenchymatous strands, with only 3(–5) vascular veins; stem terete to only slightly compressed, 0.2–0.8 mm wide..... 2

2a. Peduncle 0.4–1.2 times as long as the fruiting spike; leaf apex usually rounded; leaves mostly 2.1–3.5 mm wide *P. obtusifolius*

2b. Peduncle 1.2–8 times as long as the fruiting spike; leaf apex acute, acuminate, obtuse or mucronate; leaves 0.2–2.5(–3.0) mm wide..... 3

3a. Flowers with 1(–2) carpels; fruits laterally compressed, 2.5–3.3 mm long; midrib distinctly thickened toward the leaf base, occupying 1/4–2/3 of the leaf width; lateral veins mostly indistinct; leaves narrowly linear, 0.2–1.0 mm wide, apex acuminate.....*P. trichoides*

3b. Flowers usually with 4 carpels; fruits laterally convex, not compressed, 1.8–2.5 mm long; midrib not thickened or thickened only indistinctly toward the leaf base, occupying 1/20–1/4 of the leaf width; lateral veins distinct; leaves linear, mostly 0.5–2.5(–3.0) mm wide, apex usually acute to obtuse or mucronate.....4

4a. 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.....*P. pusillus*

4b. 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.....*P. berchtoldii*

5. Conclusion

Two hybrids between linear-leaved *Potamogeton* species have been discovered in Turkey as new taxa for the country. *Potamogeton obtusifolius*, a parental species of one of the hybrids, is not known to currently occur in Turkey. These results demonstrate that hybridization studies are an important source of information about the past and present flora. Intensive field studies and appropriate approaches involving molecular analyses can bring new insights on the biodiversity of Turkish wetlands. No detailed taxonomic investigation on *Potamogeton* has been conducted since the period of writing of the respective volume of the Flora of Turkey in late 1970s. The present study is a first step towards a modern revision of Turkish *Potamogeton*.

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