

A SYNOPSIS OF ZANNICHELLIA L. (POTAMOGETONACEAE) IN IRAN

Sh. Abbasi, S. Afsharzadeh & M. Dinarvand

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Zannichellia L. (Potamogetonaceae) is a cosmopolitan genus widely distributed in aquatic ecosystems of Iran. The last taxonomic treatment of this genus dates back to 1971 in Flora Iranica, with some recent modifications in Flora of Iran. This research aimed to provide a new taxonomic treatment of *Zannichellia* in Iran based on distributional, morphological, and molecular studies (using ITS, PHYB, trnH-psbA and rpl32-trnL molecular markers). In this research, one species (*Zannichellia palustris*) with two varieties is distinguished. An identification key to varieties is presented and descriptions of them are provided. A distribution map of the genus in Iran is compiled. The distribution is concentrated in northern, central and southwestern parts of Iran.

Shabnam Abbasi, Saeed Afsharzadeh (correspondence <s.afshar@sci.ui.ac.ir>), Department of Plant and Animal Biology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran. -Mehri Dinarvand, Forests and Rangelands Research Division, Khuzestan Agricultural and Natural Resources Research and Education Center, Ahvaz, Agricultural Research Education and Extension Organization (AREEO), Iran.

Key words: Aquatic plants; *Zannichellia*, varieties; taxonomy; ITS; cpDNA

بازنگری جنس *Zannichellia* L. (Potamogetonaceae) در ایران

شبنم عباسی: محقق پسادکتری دانشکده علوم و فناوری‌های زیستی، گروه زیست‌شناسی گیاهی و جانوری دانشگاه اصفهان، اصفهان، ایران

سعید افشارزاده: دانشیار گروه زیست‌شناسی گیاهی و جانوری دانشگاه اصفهان، اصفهان، ایران

مهری دیناروند: استادیار پژوهشی، بخش تحقیقات جنگل‌ها و مراتع، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان خوزستان، اهواز، سازمان تحقیقات، آموزش و ترویج کشاورزی، ایران

گیاه *Zannichellia* L. (Potamogetonaceae) یک جنس جهان وطن است که در اکوسیستم‌های آبی ایران به طور گسترده پراکنش دارد. آخرین بررسی تاکسونومیکی این جنس مربوط به فلورا ایرانیکا در سال ۱۹۷۱ به همراه بعضی تغییرات اخیر صورت گرفته در فلور ایران است. هدف از این پژوهش، بررسی تاکسونومیکی جدید این جنس در ایران براساس مطالعات پراکنش جغرافیایی، ریخت‌شناسی و مطالعات مولکولی (با استفاده از نشانگرهای ITS، PHYB، trnH-psbA و rpl32-trnL) است. در این تحقیق، گونه *Z. palustris* با دو وارسته تشخیص داده شد و یک کلید شناسایی برای وارسته‌ها به همراه توصیف آنها ارائه گردیده است، همچنین نقشه پراکنش جغرافیایی این جنس در ایران نیز تهیه شده است. این جنس در بخش‌های شمالی، مرکزی و جنوب‌غربی ایران پراکنش دارد.

INTRODUCTION

Zannichellia L. is either regarded as a member of a larger family Potamogetonaceae (The Angiosperm

Phylogeny Group IV 2016; Les & Tippery 2013) or a small separate family Zannichelliaceae (Tomlinson & Posluszny 1976). Plants are growing completely

submerged. They are rhizomatous with thread-like stems bearing linear leaves. They are found in different types of water bodies, such as fresh waters, brackish waters, and marine intertidal habitats (Ito & al. 2016).

The genus has a cosmopolitan distribution (IUCN 2010; Ito & al. 2016). There are considerable disagreements about the number of species for *Zannichellia* among taxonomists in the world Dandy (1971) pointed out one species and many synonyms for *Zannichellia* (*Zannichellia palustris* L.) in Flora Iranica. Uotila (1984) studied the morphology and taxonomy of *Zannichellia* with morphological and cytogenetic data in Turkey. Ito & al (2016) studied the phylogenetic relationship of *Zannichellia* and closely related genera with chloroplast and nuclear markers.

The first treatment of *Zannichellia* in Iran including neighboring countries, i.e. Pakistan, Afghanistan and Turkey were carried out by Dandy (1971) in the framework of the Flora Iranica project. According to the Flora Iranica, *Z. palustris* is distributed in almost all parts of Iran. Talavera et al (1986) treated the *Zannichellia* group as three separate species. They identified Iranian specimens of *Zannichellia* as *Z. pedunculata*. These works were published many years ago and needed a revision. Dinarvand (2017) reported

Z. palustris from Iran based on morphological evidence in the framework of the aquatic Flora of Iran project.

The aims of this study are:

To provide a new taxonomic treatment of the genus *Zannichellia* in Iran based on morphological and molecular data.

To give a comprehensive overview of the current distribution of *Zannichellia* taxa in Iran.

MATERIALS AND METHODS

For this study, 24 accessions of *Zannichellia palustris* (table 1) were collected during spring and summer from 2012 to 2018 in rivers, wetlands, and other aquatic ecosystems of Iran. Specimens are deposited in the Herbarium of the University of Isfahan (HUI) and Herbarium of the Research Center of Agriculture & Natural Resources of Khuzestan. Specimens of this genus in other herbaria such as Herbarium of the Research Center of Agriculture & Natural Resources of Isfahan, National Herbarium of Iran in Research Institute of Forests and Rangelands (TARI) were examined.

Morphological characters used were length of leaf, width of leaf, length of fruit, fruit stalk, length of beak and number of fruits per node.

Table 1. The information of the studied *Zannichellia* accessions and outgroups GenBank accession numbers in Iran.

Accession code	GenBank code <i>ITS, PHYB, trnH-psbA, rpl32-rpL</i>	Locality/ Voucher no.	Habitat
1	LC479815/LC479839/LC479863/LC479887	East Azerbaijan, Sarab to Ardebil/8321	River
2	LC479814/LC479838/LC479862/LC479886	Fars, between khanezenian and chehelcheshmeh/8693	Wetland
3	LC479813/LC479837/LC479861/LC479885	Kordistan, Marivan/8304	Dam
4	LC479812/LC479836/LC479860/LC479884	Ahwaz to Shush, Alhaei/8840	River
5	LC479811/LC479835/LC479859/LC479883	Khuzestan, Bostan/8054	River
6	LC479810/LC479834/LC479858/LC479882	Khuzestan, Hamidieh/8070	Channel
7	LC479809/LC479833/LC479857/LC479881	Khuzestan, Andica/8549	Wetland
8	LC479808/LC479832/LC479856/LC479880	Khuzestan, Dezful/8603	River
9	LC479807/LC479831/LC479855/LC479879	Fars, Dashte Arjan/8697	River
10	LC479806/LC479830/LC479854/LC479878	Khuzestan, Shushmazrae/8433	Channel
11	LC479805/LC479829/LC479853/LC479877	Fars, Haftbarm/8687	Wetland
12	LC479804/LC479828/LC479852/LC479876	Hamedan, Shirinsoo/8326	Wetland
13	LC479803/LC479827/LC479851/LC479875	Khuzestan, Karoon/8355	River
14	LC479802/LC479826/LC479850/LC479874	Yasouj, 15 km to Yasouj/8661	River
15	LC479801/LC479825/LC479849/LC479873	Bakhtiari, Dehno village/8241	River
16	LC479800/LC479824/LC479848/LC479872	Gilan, Astaneh/8155	River
17	LC479793/LC479817/LC479841/LC479865	17 km to Delijan, Neizar/22666	River
18	LC479796/LC479820/LC479844/LC479868	Borujen, Gandoman/22667	Wetland

Table 1. Continued.

19	LC479795/LC479819/LC479843/LC479867	West Azerbaijan, Miandoab/22668	Wetland
20	LC479794/LC479818/LC479842/LC479866	Kordistan, Ghorveh/22669	River
21	LC479799/LC479823/LC479847/LC479871	Isfahan, Falavarjan/12799	River
22	LC479798/LC479822/LC479846/LC479870	Isfahan, Lenjan, Chamasehan/15498	Channel
23	LC479797/LC479821/LC479845/LC479869	Chahar mahal and Bakhtiari, Shalamzar/20212	River
24	LC479816/LC479840/LC479864/LC479888	Khorasan, SW of Mashhad, Binalood, Kordineh/22670	Wetland
25*	AF102273.1, DQ786565.1, FJ883620.1, KX059500.1	-	-
26*	DQ84029.1, KX059507.1, AB488460.1, KT1528.1	-	-

* are outgroup specimens taken from GeneBank

DNA extraction, PCR amplification, and sequencing

The leaves of *Zannichellia* were dried on silica gel, and genomic DNA was extracted from leaf tissue using CTAB (Abbasi & Afsharzadeh 2016). For phylogenetic study of the genus in Iran, we used four different markers, ITS (Nuclear ribosomal internal transcribed spacer), PHYB (Nuclear gene molecular marker), trnH-psbA (Plastid intergenic spacer), and rpl32-trnL (Plastid intergenic spacer). The primer pairs used for amplifying each locus were as follows: ITS1 (forward) 5' TCCGTAGGTGAACCTGCGG 3' and ITS4 (reverse) 5' TCCTCCGCTTATTGATATGC 3' (White & al. 1990), PHYB (forward) 5' ATGTGACACAGTTGTGGACCA 3' and PHYB (reverse) 5' CATCATCCTTGTCTTCAGGGT 3' (Yang & al. 2016), trnHR (CGCGCATGGTGATTACAAATC) and psbAF (GTTATGCATGAACGTAATGCTC) (Sang & al. 1997) and rpl32 (forward) 5' CAGTTCCAAAAAACGTACTTC 3' and trnL (reverse) 5' CTGCTTCCTAAGAGCAGCGT 3' (Shaw & al. 2007). The PCR amplification for ITS was performed in a 30 µl reaction mixture containing 3 µl DNA (50 ng), 17.8 µl water, 6 µl PCR buffer 5 mM, 0.6 µl dNTP 10mM, 1.8 µl MgCl₂ 25 mM, 0.06 µl forward primer 0.1 mM, 0.06 µl reverse primer 0.1 mM, 0.6 µl BSA (10mg/ml) and 0.2 µl Taq (5u/ µl). The PCR amplification for PHYB was performed in 50 µL, containing 50 ng total DNA, 2.5 mM dNTP, 1.5 mM MgCl₂, 50 mM KCl, 5 µM forward and reverse primers, and 2 units Taq DNA polymerase. The PCR amplification for trnH-psbA and rpl32-trnL was performed in a 25 µl reaction mixture, containing 5 µl water, 12.5 µl MasterMix, 2.5 µl Primer Mix, and 5 µl DNA. The information of PCR amplification

conditions is shown in table 2. Amplification products were resolved on 1.5 % agarose gel, visualized by ethidium bromide staining under ultraviolet light. The products were sent to Genomin Company for sequencing.

Data analysis

A total of six characters were examined for each of the specimen. For cluster analysis of morphological data, we used NTSYS-Pc software (version 2.02e; Rohlf 2000) with the Jaccard coefficient and UPGMA method for statistical analyses. Also, we used PCoA for the ordination of accessions with PC-ORD software (McCune & Mefford 1997).

The sequences were cleaned using ChromasPro version 1.7.7 and were aligned using CLUSTAL X (Thompson & al. 1994) and Muscle (Edgar 2004). Sequences produced in this research have been deposited in GenBank (table 1). The alignments were then checked manually. The nucleotide substitution model was selected using MrModeltest version 2.3 (Nylander 2008) and based on the Akaike information criterion (AIC) (Posada and Buckley 2004). On the basis of the MrModeltest results, the best substitutions models were the GTR+G model for nrDNA and GTR+I+G for cpDNA. After checking the congruency of both datasets (nrDNA v. cpDNA), the combined sequences were analyzed as a single partition with the GTR+I+G model. The program MrBayes version 3.1 software (Ronquist & Huelsenbeck 2003) was used for the Bayesian Phylogenetic Analyses. We ran four chains (Markov Chain Monte Carlo), one cold and three heated. 50 million generations were performed with trees sampled every 100 generations. The first 25% trees were excluded as burn in after convergence of the chains, which was evaluated by the average standard

deviation of splitting frequencies reaching near to 0.0001. The remaining trees were assumed to represent

the posterior probability (PP) distribution.

Table 2. The information of PCR amplification conditions of used markers.

Step	ITS (34 x)	PHYB (35 x)	trnH-psbA (30 x)	rpl32-trnL (30 x)
Initial denaturation	95 °C (4 min)	94 °C (5 min)	94 °C (4 min)	80 °C (5 min)
Denaturation	95 °C (1 min)	94°C (1 min)	94°C (1 min)	95°C (1 min)
Annealing	54 °C (1 min)	55°C (1 min)	57°C (1 min)	50°C (1 min) ramp of 0.3 °C/s
Extension	72 °C (1 min)	72 °C (1 min)	72°C (1 min)	65°C (4 min)
Final extension	72 °C (10 min)	72 °C (10 min)	72°C (30 min)	65°C (5 min)

The Maximum Likelihood (Felsenstein 1981) was performed on both unpartitioned and partitioned datasets based on RAxML (Stamatakis 2006) using the RAxML GUI version 1.2 (Silvestro and Michalak 2011). We performed 1000 bootstrap searches and the GTR GAMMA substitution model was chosen for each dataset and combined datasets. All trees were viewed with the program Tree View version 1.5 (Page 1996). Clades were supported by ML and Bayesian posterior probability (PP). We selected *Potamogeton nodosus* Poir. and *Stuckenia pectinata* L. taxa for outgrouping specimens similar to the work of Lindqvist & al (2006).

RESULTS

Distribution

The geographical distribution of *Zannichellia* in Iran is shown in fig. 1, based on older specimens (black dots) and recent own findings (red and blue dots). Twenty-four new sites of *Zannichellia* are reported. The distribution of the genus in Iran is only restricted to these locations. The highest frequency of *Zannichellia* was found in the western and northern parts of Iran. No specimen of *Althenia filiformis* Petit, a species close to *Zannichellia* previously reported in Flora Iranica, was found in Iran.

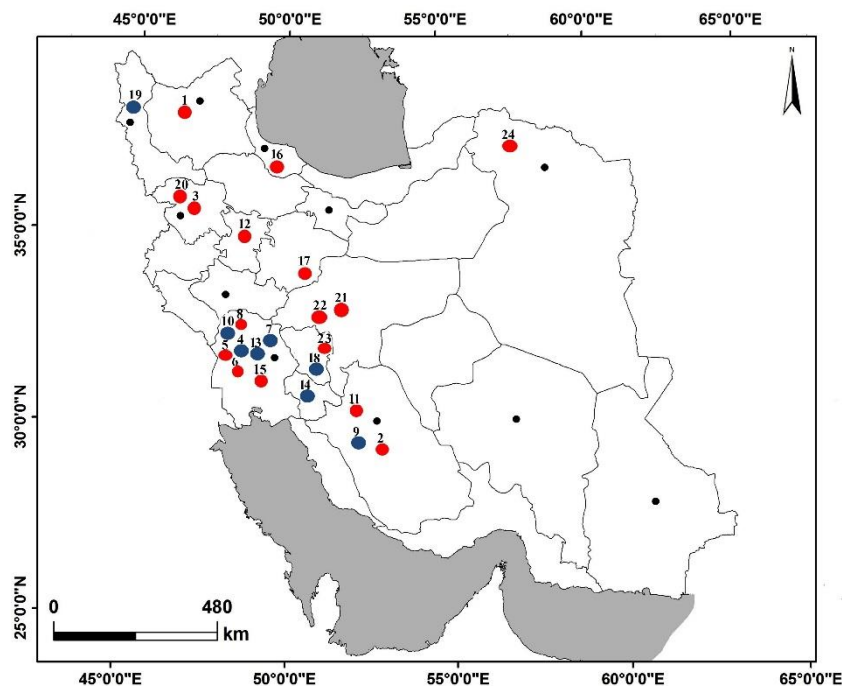


Fig. 1. Distribution map of Iranian *Zannichellia*.

(Red dots: newly observed locations for *Z. palustris* var. *palustris*, blue dots: newly observed locations for *Z. palustris* var. *pedicellata*, black dots: previously observed locations).

Morphological analysis

Zannichellia palustris specimens can be divided into two groups (I and II, fig. 2). The accession codes 4, 7, 9, 10, 13, 14, 18 and 19 (group II) are assigned to *Z. palustris* var. *pedicellata* (Wahlenb. & Rosen) Hook.f. (1892: 568) because of the pedicel length of ca. 2 mm. The other accession codes (group I) are

assigned to *Z. palustris* var. *palustris* as they have no recognizable pedicel. The diagnostic characters of the varieties are shown in table 3.

Cluster Analysis and PCoA analysis (fig. 2 and fig. 3) indicate the separation of these taxa. The grouping of accessions in PCoA analysis is corresponding to the Cluster Analysis.

Table 3. Diagnostic morphological characters of two varieties.

No. of characters	Character	<i>Z. palustris</i> var. <i>palustris</i>	<i>Z. palustris</i> var. <i>pedicellata</i>
1	Length of leaf	10-30 mm	40-80 mm
2	Width of leaf	1 mm	0.2-0.8 mm
3	Length of fruit	2.7-3 mm	1-2.5 mm
4	Fruit stalk	0-0.5 mm	1-2 mm
5	Length of beak	0-0.8 mm	1-2.5 mm
6	Number of fruits per node	5-6	1-4

Phylogenetic relationships

The concatenated sequence dataset (ITS:735 bp, PHYB: 749 bp, trnH-psbA: 342 bp and rpl32-trnL: 619 bp) for 24 taxa analyzed includes 2854 nucleotide sites, of which 1014 (35.52 %) are variable and 462 (16.18 %) are parsimoniously informative (251 nucleotides for nuclear regions and 211 nucleotides for cpDNA regions).

According to our molecular data (combination of ITS, PHYB, trnH-psbA and rpl32-trnL) (fig. 4), *Z. palustris* var. *pedicellata* (the accession codes 4, 7, 9, 10, 13, 14, 18 and 19) are separated from other codes.

Taxonomic treatment

***Zannichellia palustris* L.**

Plants submerged, growing in fresh and brackish water; stems thin and articulate; leaves are acute, opposite with parallel leaf venation, margin entire, membranous sheath without petiole; roots unbranched; globose pollen grains; male and female flower near to each other at the base of leaves, male flower with one stamen without perianth, carpels asymmetric and jar shaped; fruit a serrated nut of sickle shape.

-Key to the varieties

- 1. Fruits without pedicel or rarely with very short pedicel (0-0.1-0.5 mm long), leaf width 1 mm *Z. palustris* var. *palustris*
- Fruits with distinct pedicel (1-2 mm long), leaf width 0.2-0.5-0.8 mm *Z. palustris* var. *pedicellata*

Z. palustris* L. var. *palustris

Stems are articulated; the leaves are acute and opposite with parallel leaf venation, entire margin and membranous sheath without petiole, leaf 10-30 mm long and 1 mm wide; unbranched roots and globose pollen grains; male flower and female flower are near to each other in the base of leaves, male flower consists of one stamen without perianth, anther 0.5 mm long, filament 2 mm long; fruit 2.7-3 mm long; pedicel 0-0.5 mm long; number of fruits per node 5-6 (fig. 5).

Total distribution: Europe, Asia, Australia and Africa, typus: Switzerland.

Distribution in Iran: North, Northwest, West, Center, Northeast, South, Southeast

Flowering time: Late winter, fruiting time: Spring and summer.

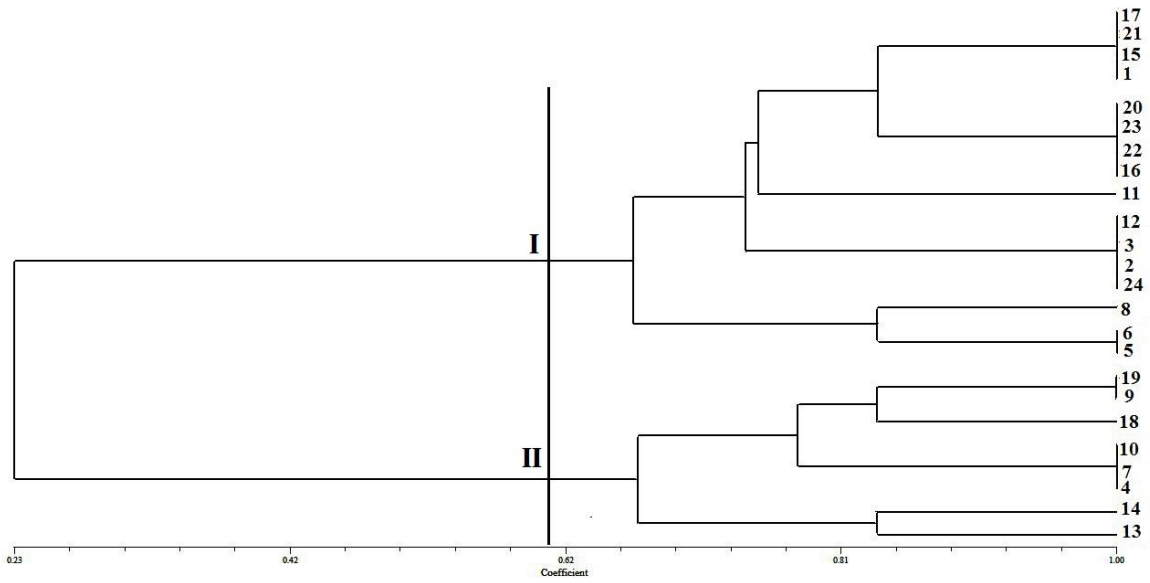


Fig. 2. Similarity dendrogram of 24 Iranian *Zannichellia* accessions based on morphological traits

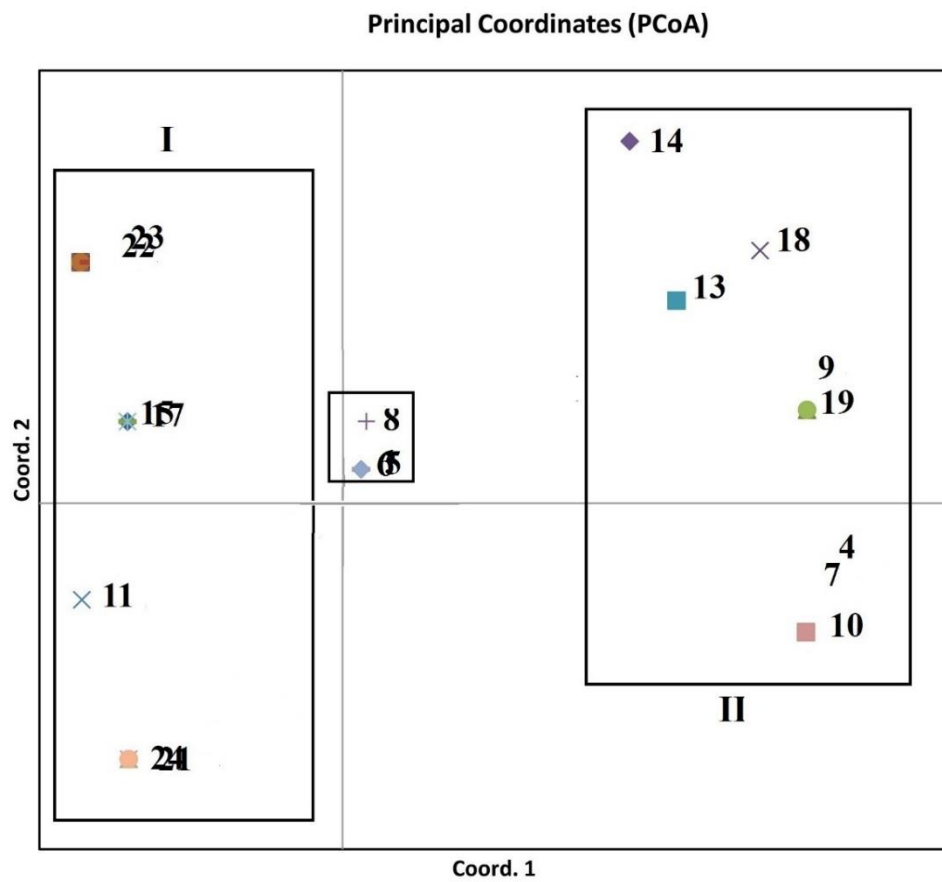


Fig. 3. PCoA grouping of Iranian *Zannichellia* showing two groups of *Zannichellia*.

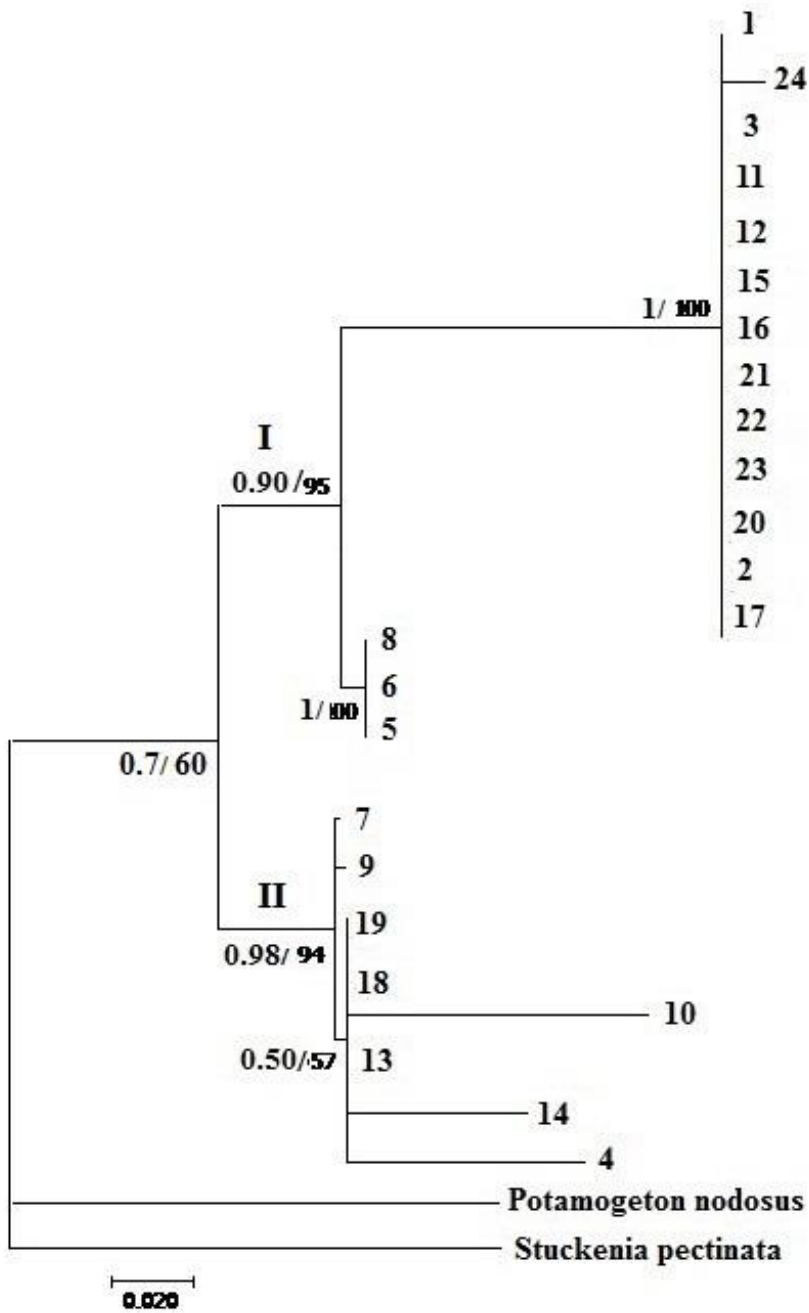


Fig. 4. Species relationships of Iranian *Zannichellia* based on (ITS, PHYB, trnH-psbA and rpl32-trnL) resulting from merging Bayesian and maximum likelihood. Numbers at nodes are Bayesian Posterior Probabilities and bootstrap values.

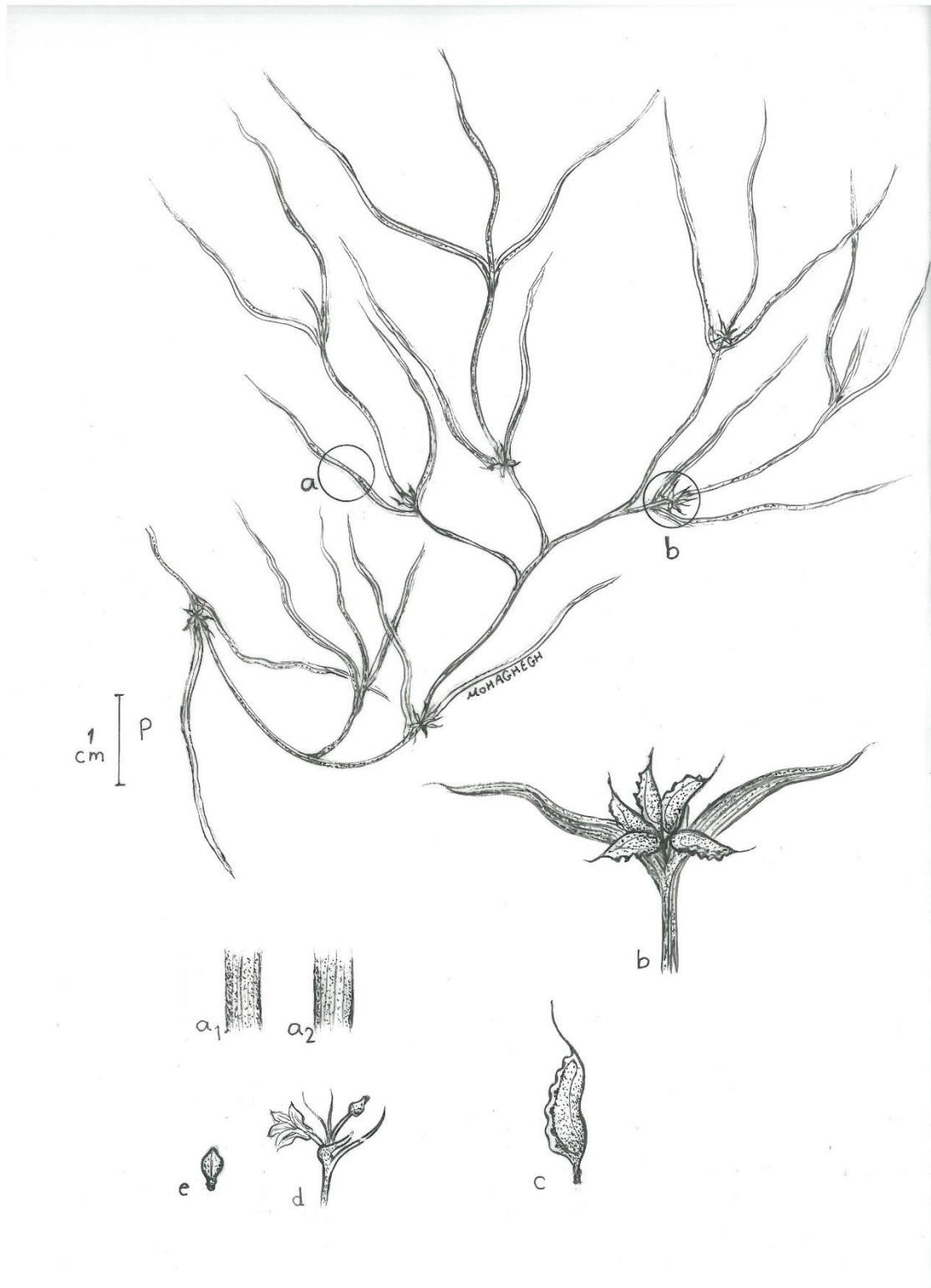


Fig. 5. Line drawing of Iranian *Z. palustris* var. *palustris*. P: main plant $\times 2$, a1,a2: back and front of leaf $\times 10$, b: fruit structure $\times 10$, c: fruit $\times 20$, d: inflorescence $\times 10$, e: female flower $\times 20$.

Specimens seen: IRAN. Gilan: Astaneh, under Sefidrood bridge, 94 m, 12 July 2009 Dinarvand 8155, Herbarium of the Research Center of Agriculture & Natural Resources of Khuzestan (HRCANRK); Azerbaijan: Sarab to Ardabil, 1 km after Mijmir village, 5 July 2010 Dinarvand & Mohammadi 8321, HRCANRK, Kordistan: Marivan to Chenareh, Garan Dam, 1400 m, 5 July 2010 Dinarvand & Mohammadi 8304, HRCANRK, Ghorveh, 5 August 2015 Abbasi & Afsharzadeh 22669 Herbarium of the University of Isfahan (HUI); Hamedan: Zanjan to Hamedan, after Khodabandeh, 120 km to Hamedan, Shirinsoo wetland, 1838 m, 5 July 2010 Dinarvand & Mohammadi 8326, HRCANRK; Bakhtiari: 80 km to Shahrekord, Dehno village, 2120 m, 5 July 2010 Dinarvand & Mohammadi 8241 HRCANRK; Fars: between Khanehzenian and Chehelcheshmeh, 20 km after Haftbarm wetland, 2180 m, 5 July 2010 Dinarvand & Mohammadi 8693 HRCANRK, Haftbarm wetland, 2180 m, 5 July 2010 Dinarvand & Mohammadi 8687 HRCANRK; Khuzestan: Dezful, Hamidabad village, Dez River, 80 m, 5 July 2010 Dinarvand & Mohammadi 8603 HRCANRK, Hamidieh, 80 m, 5 July 2010 Dinarvand & Mohammadi 8070 HRCANRK, 25 km to Bostan, 838 m, 5 July 2010 Dinarvand 8054 HRCANRK; Khorasan: SW of Mashhad, Binalood, Kordineh, 8 July 2017, Abbasi & Afsharzadeh 22670 HUI, Markazi: 17 km to Delijan, Neizar, 838 m, 5 July 2015 Abbasi & Afsharzadeh 22666 HUI, Isfahan: Falavarjan, 1580 m, 5 July 1999 Shams 12799 Herbarium of the Research Center of Agriculture & Natural Resources of Isfahan (HRCANRI), Lenjan, Chamaseman, 1750 m, 5 July 2011 Akkafi 15498 HRCANRI; Chaharmahal va Bakhtiari: Shalamzar wetland, 2052 m, 27 Aug 2014, Abbasi & Afsharzadeh 20212 HUI.

Other locations are referred to Flora Iranica (Dandy 1971).

Z. palustris var. pedicellata (Wahlenb. & Rosén) Hook.f.

Stems are very thin and articulated; the leaves are acute and opposite with parallel leaf venation, entire margin and membranous sheath without petiole, leaf 40-80 mm long, and 0.2-0.8 mm wide; unbranched roots, and globose pollen grains; male flower and female flower are near to each other in the base of leaves; male flower consists of one stamen without perianth, anthers 0.5 mm long, filaments 2 mm long; fruits 1-2.5 mm long; pedicels 1-2 mm long; the number of fruits per node 1-4 (fig. 6).

Distribution in Iran: Northwest, Center, South

Total distribution: Europe, Asia, Australia and Africa.

Flowering time: Late winter, fruiting time: Spring and summer.

Specimens seen: IRAN. West Azerbaijan: Miandoab wetland, 8 Aug 2015, Abbasi & Afsharzadeh 22668 HUI, Kohgiluyeh and Boyer-Ahmad: Yasouj, 15 km to Yasouj, 2120 m, 5 July 2010 Dinarvand & Mohammadi 8661 HRCANRK; Fars: Dasht-e Arzhan, Ghareaj river, 1990 m, 5 July 2010 Dinarvand & Mohammadi 8697 HRCANRK, Chaharmahal va Bakhtiari: Borujen, Gandoman wetland, 27 Aug 2017, Abbasi & Afsharzadeh 22667 HUI, Shush: Shush Mazrae, 80 m, 5 July 2010 Dinarvand & Mohammadi 8433 HRCANRK, Shush, Alhaei, 80 m, 5 July 2010 Dinarvand & Mohammadi 8840 HRCANRK, Ahwaz, Karoon river, 80 m, 5 July 2010 Dinarvand & Mohammadi 8355 HRCANRK, Andica, Ghalekhaje, Abzaloo wetland, 838 m, 5 July 2010 Dinarvand & Mohammadi 8549 HRCANRK.

Other locations are referred to Flora Iranica (Dandy 1971).

DISCUSSION

In Flora Iranica, Dandy (1971) has reported *Zannichellia palustris* and *Althenia filiformis*, belonging to Zannichelliaceae from Iran. He reported *Althenia filiformis* from Neiriz Lake (Fars Province, Iran) that is now called "Bakhtegan Lake". Dinarvand (2017) did not observe this species in this location and among older specimens in the herbaria of Iran and the University of Shiraz. According to the morphological research of Dinarvand (2017), most specimens of *Zannichellia palustris*, collected from the south of country, look like *Althenia filiformis*; therefore, *A. filiformis* is probably misidentified in Flora Iranica. Furthermore, *Althenia filiformis* is a west-Mediterranean endemic (Ito 2013), distributed over a long distance from Iran in Europe and Africa (Dandy 1971).

According to our more comprehensive sampling from the south of the country and further observations of herbarium specimens as well as morphological and molecular investigations, we conclude that these specimens are *Z. palustris* var. *pedicellata*, and they located in the same cluster with other specimens of *Z. palustris* var. *pedicellata* from other sides of country (fig. 4). Therefore, we confirm the results of Dinarvand (2017), who reported that the occurrence of *A. filiformis* in Iran was based on misidentification.



Fig. 6. Line drawing of Iranian *Z. palustris* var. *pedicellata*. P: main plant $\times 2$, a1,a2: back and frond of leaf $\times 10$, b: fruit structure with pedicels $\times 10$, c: fruit $\times 20$, d: inflorescence $\times 10$, e: female flower $\times 20$.

In our study, although morphological differences were found between var. *palustris* and var. *pedicellata* corresponding to observed genetic differences within the genus, it seems these specimens have not been isolated geographically. Therefore we treat the morphological differences in *Zannichellia* at the variety level.

Dandy (1971) identified *Z. palustris* for Iran. Talavera & al (1986) recognized Iranian specimen of *Zannichellia* as *Z. pedunculata*, and according to the “The Plant List website”, it is now synonym of *Z. palustris* subsp. *pedicellata*. Talavera & al (1986) reported a leaf width of 0.8 mm for *Z. pedunculata*. We also saw a leaf width of 0.2-0.8 mm for *Z. palustris* var. *pedicellata* (table 3).

We propose the division of *Z. palustris* into two varieties (*Z. palustris* var. *palustris*) and (*Z. palustris* var. *pedicellata*). The morphological separation of the two taxa was confirmed by molecular data (ITS, trnH-psbA, PHYB and rpl32-trnL). In other studies, these markers have been reported as robust phylogenetic markers for revealing the intraspecific relationships in *Potamogeton* L. (Yang & al., 2016; Bobrov & al., 2018). We used them to understand the species relationships of *Zannichellia* for the first time.

Some accessions were from a separate sub-cluster in fig. 2-4, including 5 (Bostan, leaf width 0.5 mm and fruit size 2.5 mm), 6 (Hamidieh, leaf width 0.3 mm and fruit size 2.7 mm) and 8 (Dezful, leaf width 0.3 mm and fruit size 3 mm). These accessions have a larger fruit size (ca. 3 mm) and narrower leaves of ca. 0.5 mm width. We regard these morphotypes as variations of *Z. palustris* var. *palustris*.

None of our specimens is matching *Z. major* Boenn. ex Reichenb., as *Z. major* is a robust and perennial plant with the leaves of 1-2 mm wide. The fruits of this species are large (2.5-4.5 mm). All of our specimens have narrower leaves and a fruit size of only ca. 3 mm.

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