Ribosomal DNA marker as a molecular barcode tool for species delimitation of some IranianJuncus L. (Juncaceae) species

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Abstract

The genus Juncus L. belongs to the family Juncaceae, with over 220 species worldwide. These plants are found in diverse habitats and occupy areas of every continent except Antarctica. In this study we investigated phylogenetic relationships among 12 Juncus species distributed in Iranby using the nuclear ribosomal ITS region. With respect to the outgroup species, the Juncus species are grouped together in a clade. Due to the sufficient molecular variability among the species, it seems that the ITS marker may be offered as a DNA barcode candidate at the interspecific level of the genus Juncus.

Keywords: Juncaceae, Ribosomal DNA ITS, Phylogenetic analysis, Iran.

1. Introduction

Juncus L. (Juncaceae)with about 220 species worldwide is a widely distributed genus of rushes [1]. These plants are found in diverse habitats and occupy areas of every continent except Antarctica [2]. Most diversity of the genus *Juncus* is in mesophytic and boreal regions of the word [3]. After Linnaeus, several authors [3, 4] have studied this genus based on morphological characters and tried to provide a system to divide the genus into subgenera, sections and subsections. Due to the occurrence of morphological ambiguities among the species of the genus, the use of only morphological characters seems to be confusing the taxonomic boundaries. In the present study we used molecular data from the nuclear ribosomal internal transcribed spacer (ITS) region to evaluate its discriminative potential as taxonomic characters.

2. Materials and Methods

Leaf materials of some species of the genus *Juncus* were field collected from various areas of Iran and voucher specimens of the samples were deposited in Ferdowsi University of Mashhad Herbarium (FUMH) (Table 1). One individual of five different genera of Juncaceae were included as outgroup in the molecular study. The dried leaf material was frozen in liquid nitrogen and ground to powder. Total DNA was extracted using the CTAB method [5]. The nuclear ribosomal internal transcribed spacer (ITS1-5.8S-ITS2) was used as a molecular marker (Fig. 1). This region was sequenced for one individual as a representative of each species. Primer sequences (ITS4 and ITS5) for polymerase chain reaction (PCR) amplification was obtained from White et al. [6]. The PCR reactions were performed in 25-μL volumes on a Perkin Elmer 9600 Thermal Cycler, using *Taq* DNA polymerase. Each reaction mixture included 1 μL of the total DNA template, 2 μL of a dNTP mixture (2.5

mM each), 2.5 µL of 10X Ex *Taq* Buffer, 0.25 µL of bovine serum albumin (20 mg/mL), 1.25 µL of each primer (2pmol/µL), and 0.5 µL of *Taq* (1.25 U/µL). The PCR profile consisted of an initial denaturation at 94 ºC for two minutes; 30 cycles at 94 ºC, 50 ºC, and 72 ºC for 15s, 30s, and 1.5 min, respectively; and followed by a final extension step at 72 ºC for 5 min. Direct sequencing was conducted using Macrogen's sequencing service (Macrogen Inc., Korea). Sequences were assembled with BioEdit Alignment Editor [7]. Bayesian phylogenetic inference was carried out in MrBayes 3.1.2 [8] for the data set. Tree visualization was carried out using TreeView version 1.6.6 [9].

Figure 1. Schematic representation of the nuclear ribosomal internal transcribed spacer (ITS) structure used in the current study.

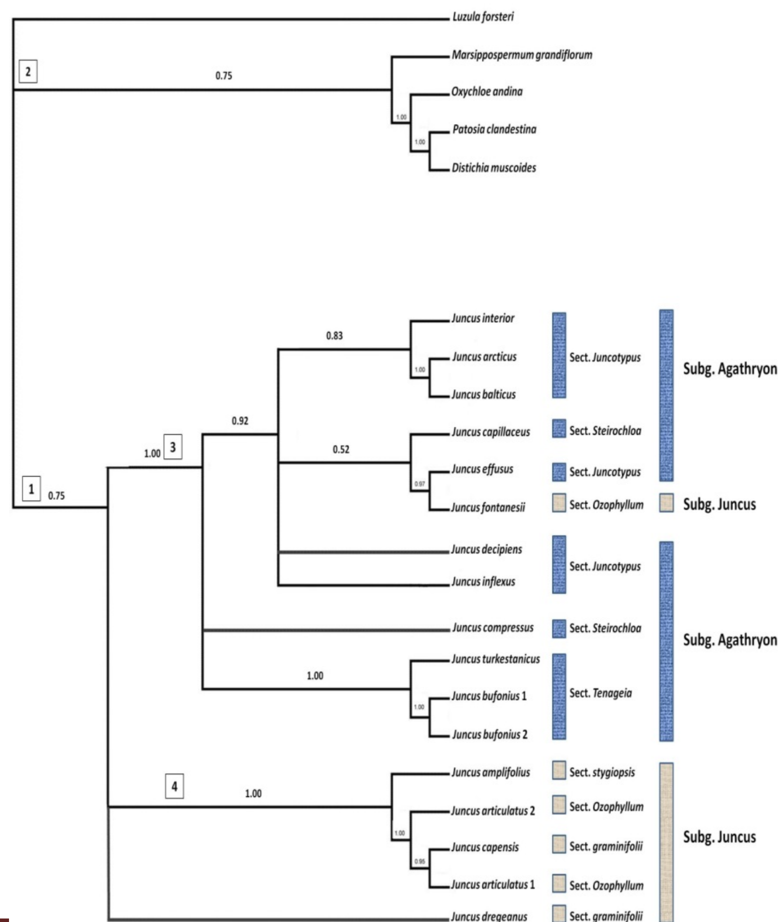

Figure 2. Phylogenetic relationships among the *Juncus* species of Iran based on Bayesian analysis of ribosomal DNA of the internal transcribed spacer marker. Bayesian posterior probabilities are indicated above branches.

3. Results and Discussion

The ITS data compiled for our analysis consisted of 19 alleles. The 50% majority rule consensus tree (Fig. 2) resulting from the Bayesian analysis show that all the *Juncus* species are grouped in a single clade (posterior probability=PP=0.75) with respect to the outgroup. Furthermore, no polytomy was met in the clade *Juncus*. Results of this study comfirms that the genus *Oxychloe* belongs to the Juncaceae family sister to the genera *Distichia* and *Patosia* rather than a member of the family Cyperaceae as reported by Drábková et al. [10]. Kirschner et al. [1] divided the genus *Juncus* into two subgenera and 10 sections based on morphological investigation. Results of this study (Fig. 2) confirm that the genus is split in two subgenera including subg. *Agathryon* and subg. *Juncus*. The subg. *Juncus* forms a paraphyletic group with the subg. *Agathryon* where the species *J. fontanesii* is grouped within the latter subgenus. The members of *Agathryon* could be distinguished from the subg. *Juncus* by having the cyme inflorescence and floral bract. In overall, it seems that the nDNA ITS marker could be useful to phylogenetically differentiate the species of the genus *Juncus*. Thus, The ITS nuclear region would be more eligible as a barcoding tool for the genus *Juncus* but further research is required to find loci with sufficient genetic variability.

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