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MORPHO-ANATOMICAL AND PALYNOLOGICAL STANDARDIZATION AND DNA BARCODING OF *FRITILLARIA CIRRHOSA* D. DON (SYN. *FRITILLARIA ROYLEI* HOOK.)

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Abstract

Fritillaria cirrhosa D. Don belonging to the family *Liliaceae* and is one of the important bulbous medicinal herbs from the Western Himalayan Region distributed between 2800 and 4000 m asl. It is a perennial herb having an underground bulb which is the main constituents of various Ayurvedic preparations. Due to natural habitat specificity and indiscriminate extraction due to high market demand, this plant is facing a survival threat in the wild. The problem of misidentification, adulteration, and substitution of this plant with other cheaper plants is widespread. The present study was conducted to study morphological, anatomical, and palynological characters of *F. cirrhosa* and to develop its DNA barcodes for its molecular authentication. The morphological characters of different plant organs such as bulb, root, stem, and leaf were studied. Transverse sections of bulb, root, stem, and leaf were examined in detail. Pollen grains were prolate shaped and monosulcate with the reticulate type of exine sculpturing. Genomic DNAs were extracted from the dried bulbs samples and high–quality marker sequences were obtained for one nuclear viz. ITS and one cytoplasmic molecular markers viz., rbcL of *F. cirrhosa*. The length of the ITS sequence of the three accessions ranged from 621 to 699 bp, while a sequence length range of 570 to 573bp was recorded for rbcL. The percentage GC content was 65.74, and 42.22 for ITS, and rbcL markers respectively.

***Keywords*:** *Fritillaria cirrhosa,* Morphoanatomy, Palynology, DNA Barcoding, Endangered

Introduction

Fritillaria L. genus belonging to family *Liliaceae* comprises of about 170 taxa and is mainly distributed in the temperate regions of the Northern hemisphere (Day *et al.,* 2014). Globally, it is represented by seven subgenera, two sections, and 165 taxa (Rix, 2001). From China, 24 species of this genus are reported, of which 15 are endemic (Cunningham *et al.,* 2018). In contrast, Turkey has 38 species and six subspecies of *Fritillaria* L., of which 27 species are endemic (Teksen and Aytac, 2008). In India, the genus *Fritillaria* L. is represented by six species, which grow in the Western Himalayan region between an altitude of 2400 and 4000 m asl (Anonymous, 1956). Traditionally, many species of *Fritillaria* (such as *F. cirrhosa*, *F. thunbergii,* and *F. verticillata*) are used to treat cough and related ailments (Da-Cheng *et al.,* 2013). In the traditional Chinese system of medicine, the *Fritillaria* species are used as medicine and are known as Bulbus Fritillariae Cirrhosae (BFC) (Wang *et al.,* 2017). Alkaloids are believed to be primary active constituents of BFC (Li *et al.,* 2013, Wang *et al.,* 2014), which are known to exhibit notable expectorant, antitussive, anti-inflammatory, hypotensive, and antitumor activities (Wang *et al.,* 2011, 2012, Kang *et al.,* 2004, Wang *et al.,* 2015).

Fritillaria cirrhosa D.Don (Syn. *Fritillaria roylei* Hook.), one of the most important medicinal plant species of this genera, is distributed between an altitudinal range of 2800 and 4000 m asl in the Western Himalayan region of India (Singh and Rawat, 2011; Chauhan *et al.,* 2011; Kala, 2005; Dad and Reshi, 2015). It is a perennial herb having an underground bulb, which are the main constituents of various Ayurvedic preparations such as *Astavarga*, *Chyavanprash*, *Mahatraiphala Ghritham*, *Jeevanthyadi Ghrutham*, and *Danwantharam Thailam* (Anonymous 1956, Kaul, 2010). In Ayurveda, the dried bulb of *F. cirrhosa* D. Don is used to treat asthma, bronchitis, and tuberculosis (Ambasta *et al.,* 1986). The indigenous communities of Jammu & Kashmir (J&K) use this plant for the treatment of rheumatism, asthma, tuberculosis, and as a tonic (Srivastava *et al.,* 1986).

Fritillaria cirrhosa has been categorized as critically endangered for Uttarakhand (UK) and endangered for J&K and Himachal Pradesh (HP) states of India by the International Union for Conservation of Nature (IUCN) (Anonymous, 2003). In recent times owing to its high medicinal value, demand for this plant species has increased drastically in the Indian market, which has led to the massive extraction of this species from the forests of Western Himalaya. Indiscriminate extraction of this species from the wild has posed a severe threat to its survival in the wild. During field and market surveys, authors of the present article have reported that the average price of bulbs of this plant in the black market is in the range of Rs. 15,000 to Rs. 20,000 (~US $ 210 to 280) per kg. The problem of misidentification, adulteration, and substitution of this plant with other cheaper plants is widespread. In the Ayurvedic Pharmacopeia of India, "*Kakoli*" is recognized as a Sanskrit language name of *F. cirrhosa* (APC, 2006), which is the same as a trading name of another plant species *i.e*., *Roscoea purpurea* Sm. Due to which both of these species are sold in a market under the same trade name as "*Kakoli,*" increasing the chances of intermixing or the wrong usage. Also, bulbs of few other unknown wild species are sold as *F. cirrhosa* in the market. Many times, forest officials and other law enforcing authorities confiscate dry plant material of this and other plants being smuggled out of the region illegally. However, due to a lack of standard reference monograph, it gets complicated to identify the dry material of *F. cirrhosa.* The safe usage of any herbal medicine is a critical affair (Yoo *et al.,* 2005; Chrubasik *et al.,* 2005) and the establishment of the botanical identity of the plant before usage is a must.

Many studies on the morphological, anatomical, and palynological characteristics of different species of genus *Fritillaria* have been conducted by various researchers (Pehlivan and Ozler, 2002; Ozler and Pehlivan, 2007; Alan, 2008; Advay and Sharifi-Tehrani, 2016; Schulze, 1980; Kosenko 1991a,b, 1992, 1999; Ozler and Pehlivan, 2007; Teksen and Aytac, 2004, 2008), but only a few studies have reported morphology, cytology, and distribution of *Fritillaria cirrhosa* (Koul and Wafai, 1980; Bisht *et al.,* 2016). Therefore, keeping in view the facts described above, the present study was conducted to standardize morphological, macroscopic, microscopic, and palynological characters of *F. cirrhosa*. Also, sequences of one nuclear viz. ITS and one cytoplasmic molecular markers viz., rbcL, of *F. cirrhosa* were also generated for future reference.

Material and Methods

Three accessions of *F. cirrhosa* D.Don were collected from three different localities of Jammu and Kashmir (J&K), a Union territory of India during 2018-2019 (Fig. 1 and Table 1). Morphological data of the plant and associated locational details like longitude, latitude, and altitude were recorded in the field itself. The collected specimens were brought to the internationally recognized Janaki Ammal Herbarium (RRLH) at CSIR-IIIM, Jammu. Herbarium sheets were prepared following standard taxonomic procedures (Rao and Sharma 1990), and duly identified herbarium specimens were submitted to the internationally recognized Janaki Ammal Herbarium (RRLH) at CSIR-IIIM, Jammu, India (Table 1). For anatomical studies, fresh specimens were collected from the field and stored in the FAA for 24 h and then transferred to 70% alcohol. The freehand sections of different plant parts were cut and stained with safranin, fast green, and fixed with Canada balsam. For pollen analysis, fully bloomed flowers were used. The pollen grains were acetolyzed, using Erdtman's acetolysis method (Erdtman, 1960) with modifications. The light microscopic (LM) pictures of the pollen grains, as well as the transverse cut sections of root, bulb, leaf, and stem, were taken using Leica light microscope DM750 (Germany). For scanning electron microscopy (SEM), the specimens were mounted on stubs of 12.5 mm diameter after acetolysis and then coated in a sputter with 25 nm of gold-palladium. Images were taken using JEOL JSM-IT 300 (JEOL, Ltd., Tokyo, Japan). The pollen terminology of Kosenko (1991a, 1991b), Pinar and Donmex (2000) and Hesse *et al.* (2009) were used.

For DNA extraction, leaf and rhizome samples were collected in airtight poly bags containing silica gel. Genomic DNA was extracted from dried bulbs using GenElute^{TM} Plant Genomic DNA Miniprep Kit (Sigma). Amplification of the marker regions *viz*., ITS (White *et al*. 1990), and rbcL (Levin *et al*., 2003) were done at a final volume of 20 µl in a thermal cycler (Applied Biosystems, Veriti Thermal Cycler). The reaction mixture consisted of 1 µL template g DNA (DNA concentration of 80–100ng), 0.5 µL forward and reverse primer each (10 pmols/microlitre), 10 µL Master Mix (Promega GoTaq Colourless, Master Mix, 2X) and 8 µL nuclease–free water. The PCR conditions were 35 cycles of denaturation at 94ºC for 4 min, annealing of ITS at 54ºC, rbcL at 52ºC, and a final extension of 8 min at 72ºC. The PCR products were analyzed on 1.2% Agarose gel (in Tris Acetate EDTA buffer) in the electrophoretic unit along with 100 bp ladder as a size marker. PCR bands were visualized in UVP EC3 Imaging System. Gel PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega, USA) and sequenced bidirectionally by Sanger sequencing.

Chromatograms of all the DNA marker sequences were analyzed by making use of Geospiza's Finch TV Version 1.4.0. All the nine newly generated sequences are under the process of submission to the NCBI database. The sequences were aligned by ClustalW initially and then manually modified. Intra and interspecific genetic distances among the sequences were calculated by Kimura 2-parameter (K2P) using MEGA 7 (Kumar *et al.,* 2016). For phylogenetic analysis and resolution of species, the primary sequences were complemented by the available sequences from the NCBI database retrieved for the *Fritillaria* species reported earlier from India (Table 5). The sequences were chosen based on their length and availability of voucher specimens in the database. Fifteen and twenty three sequences were analyzed for rbcL and ITS region. Standard phylogenetic approach viz., Maximum likelihood (ML) method based on Tamura 3-parameter model was employed for phylogenetic analysis (Tamura, 1992) using MEGA v.7 (Kumar *et al.,* 2016). A bootstrap of 1000 replicates was used for branch support. The gaps and the missing data were removed from the sequences. The generated phylogenetic tree results are shown in the Fig. 5 and 6. All the six newly generated sequences are under the process of submission in the NCBI GenBank database (http://www.ncbi.nim.nih.gov).

Results

Reported morphological characteristics of *F. cirrhosa* are given in Table 2 and shown in Fig. 2, whereas anatomical features of *F. cirrhosa* are presented in Table 3 and Fig. 3. Palynological characters of the studied samples of *F. cirrhosa* D.Don in comparison to some earlier reported *F.* species are given in Table 4. Polar and equatorial views of pollen grains of *F. cirrhosa* in LM, and Polar and equatorial views of pollen grains of *F. cirrhosa* in SEM showing exine ornamentation are presented in Fig. 4.

Morphological characters

The plant was a perennial herb having an average stem height of 35.10 cm (Fig. 2a and 2b). The underground bulb was globose to oval in shape with an average diameter of 1.33 cm (Fig. 2c and 2d). The stem was erect with sessile leaves arranged in whorls of 3–6 in the upper part of the plant and opposite on the lower part. The leaf blade was linear to lanceolate, with acute to an obtuse apex. Flower number per plant ranged between 1–2 and subtended by three bracts (leafy). Perigon was narrowly campanulate with dark green patches and heavily spotted with brownish–green along the inner side of tepals. Anthers were 6 in number with an average length of 1.9 cm. The anther filament was slender and papillose. The style was smooth or sparsely papillose and slender. The stigma was three–lobed.

Anatomical characters

Transverse Section (T.S.) of Bulb: T.S of the bulbs were of varying shape depending on the part of the bulb used for sectioning (Fig. 3a). A single–layered epidermis was present. A multilayered optical region with irregular shaped parenchymatous cells was observed. The size of the cortical region varied depending on the size of the bulb. Numerous starch grains were present in this region.

Transverse Section of root: A single–layered epidermis was present (Fig. 3b). The average size of the epidermal cell was

1071.68µm^2. Below the epidermis cortical region with irregularly shaped cells was observed. Multilayered endodermis was documented below the cortex region. Vascular bundles were arranged in a circular fashion and were 5 in number. The pith region was not observed.

Transverse Section of stem: The outermost single–layered epidermis with thick cuticle was observed, and the average cell size of the epidermal cells was 431.02 µm^2 (Fig. 3c). The hypodermis was 2–3 layered with an average cell size of 439.66 µm^2. Below the hypodermis region, a 114–140µm thick sclerenchymatous region composed of 5–7 layers with small intercellular spaces between the cells was present. The sclerenchyma cells were thick-walled, and the shape varied from oval, oblong to spherical with an average size of 587.59 µm^2. The cortex region was 37.162 µm to 49.209 µm in thickness and with an average cell size of 2683.3 µm^2. Vascular bundles were scattered throughout the cortex and were 27–36 in number.

Transverse Section of leaf: A single-layered epidermis with thick cuticle was present (Fig. 3f). The palisade and spongy parenchyma cells were not differentiated. Vascular bundles were observed at frequent intervals arranged in the center of mesophyll tissue.

Palynological characters

The present study reports morphological variation in shape, size, exine ornamentation, sulcus nature of pollen grains of the *F. cirrhosa* for the first time (Fig. 4). LA/SA ratio of 1.44 (1.12–2.28 µm) with the long axis (LA) 54.76 µm and short axis 39.25 µm was observed. As per the shape classification of Pinar and Donmez (2000), pollen grains were prolate in shape. Exine ornamentation observed was of reticulate type throughout and suprareticulate type near the sulcus region with few perforations. The average exine thickness was 1.42 µm (1.31–1.59 µm). Pollen grains were monad having a monosulcate aperture. Sulcus apex was round to sharp, which was different from the earlier studies carried out for other species of this genus *Fritillaria.* In those studies, the pollen apex was either round or sharp, but not both. The sulcus membrane was verrucate, and the sulcus length was 44.09–58.01 µm, which was similar to the range as recorded for other species of *Fritillaria* elsewhere (Teksen *et al.,* 2010).

DNA barcodes

For developing a DNA barcode, the success rate of sequence amplification is an important criterion (Kress and Erickson, 2007; Ford *et al.,* 2009; Hollingsworth *et al.,* 2009). Six barcode sequences (three each for ITS and rbcL) were generated for *Fritillaria cirrhosa*. The annealing temperature of 54ºC for ITS and 52ºC for rbcL, was found optimal to develop a high-intensity molecular size band in this study. From the collected samples, sequence lengths in the range of 621-699bp, 570-573bp were obtained for ITS and rbcL respectively. The phylogenetic analysis was based on the aligned sequences (including NCBI sequences) having 658 and 480 positions for ITS and rbcL respectively. Among the produced DNA barcodes, the mean interspecific distance was 0.088 for ITS and 0.0041 for rbcL whereas the intraspecific distance was 0.0469 and 0.0012 for ITS and rbcL respectively. For the nrITS region, the percentage of nucleotide composition was found to be T=15.75, C=33.09, A=17.63, and G=34.27. The cytoplasmic marker rbcL showed the nucleotide frequencies of T=28.59, C=21.30, A=27.24, G=22.87. The mean interspecific genetic distance using ITS was higher than samples amplified by rbcL indicating distinctness of the analysed species. Furthermore, the intraspecific distance was low compared to interspecific genetic distance. As per Hebert *et al.* (2004) and Mankga *et al.* (2013) a perfect barcode of DNA should possess significant variations to be used for species delineation and this can be achieved if a species has high genetic distance than the other intraspecific individual within the group.

From the Maximum likelihood tree using ITS, it can be seen that the samples of *F. cirrhosa* species got separated from the other *Fritillaria* species (Fig. 5). However, classification within clade was not clear especially for *F. raddeana* and *F. imperialis*, *F. delavayi* which is in consonance with the earlier phylogenetic work of Turktas *et al.* (2012). ITS2 region has been earlier used for differentiating Fritillariae Cirrhosae Bulbus from its adulterants by Luo *et al.* (2012) but it did not discriminate the original plants of Fritillariae Cirrhosae Bulbus. Similarly, using rbcL, *F. cirrhosa* was found genetically distinct from the other *Fritillaria* species as well as from the samples of *F. cirrhosa* retrieved from the database (Fig. 6). Many researchers have pointed out that it is very hard to find a barcode having universal applicability for the plant identification because of variations in the morphology and reticulate evolution (Mosa *et al.,* 2019).

Discussion

Although some studies on different species of *Fritillaria* are on record (Teksen *et al.,* 2010; Kosenko, 1999; Advay and Sharifi-Tehrani, 2016; Corneanu and Popescu, 1981; Teksen and Aytac, 2011) but the present study on the morphological, anatomical and palynological aspects of *F. cirrhosa* is first from India. The polar axis length of the pollens of *F. cirrhosa* was found to be closer to *F. imperilais*. A pollen grain of *F. cirrhosa* differs from *F. whittallii* in having monosulcate pollen instead of zonosulcate (Teksen *et al.,* 2010). Exine thickness was in the range as recorded for other *Fritillaria* species earlier (Kosenko, 1999; Teksen *et al.,* 2010). The presence of acute and obtuse sulcus apex in *F. cirrhosa* differentiated it from the rest of the *Fritillaria* species studied so far. This pollen character can be used to differentiate this species from other *Fritillaria* species. As already established in previous research studies about the occurrence of species–specific information in the DNA sequences. For the discrimination of *F. cirrhosa* from other species, the researchers have applied various molecular methods such as RAPD (Xin *et al.,* 2014), RPLF (Wang *et al.,* 2007), 5S-rRNA spacer region (Li *et al.,* 2003), ITS1 gene (Xu *et al.,* 2010), combination of trnH-psbA and rbcL (Zhang *et al.,* 2016). Therefore, for establishing a decisive and straightforward approach for the identification of *F. cirrhosa,* we generated three sequences each of nrITS, and rbcL for the easy identification of this important medicinal herb. The sequences generated in this study are first from India and are reliable.

The *F. cirrhosa* is an important medicinal plant resource, which has recently become the income source for the local inhabitants of the temperate regions of the Western Himalaya. Overexploitation of this plant from the wild has rung the alarm bell for the conservators. For devising proper conservation and enforcement of the conservation laws, there

is a need for the standards that could be referred in the time of need for correct identification of this plant. Besides, accurate methods like DNA Barcoding are required for proving the identity of the plant without any doubt. In conclusion, the two markers discriminate *F. cirrhosa* from the other reported Indian species and can be used for the rapid and reliable discrimination. However, combined marker approach and more genes are suggested to include for the validation of other *Fritllaria* species.

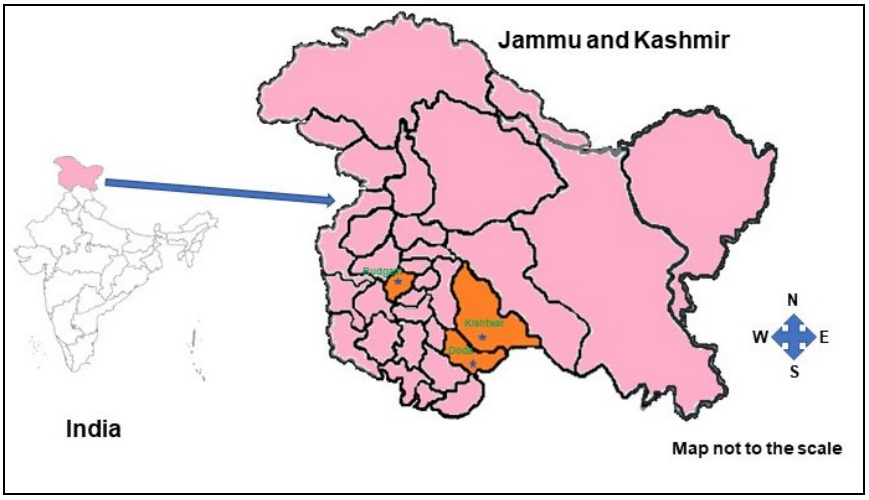

Fig. 1: Map of study area showing the locations of the collected accessions.

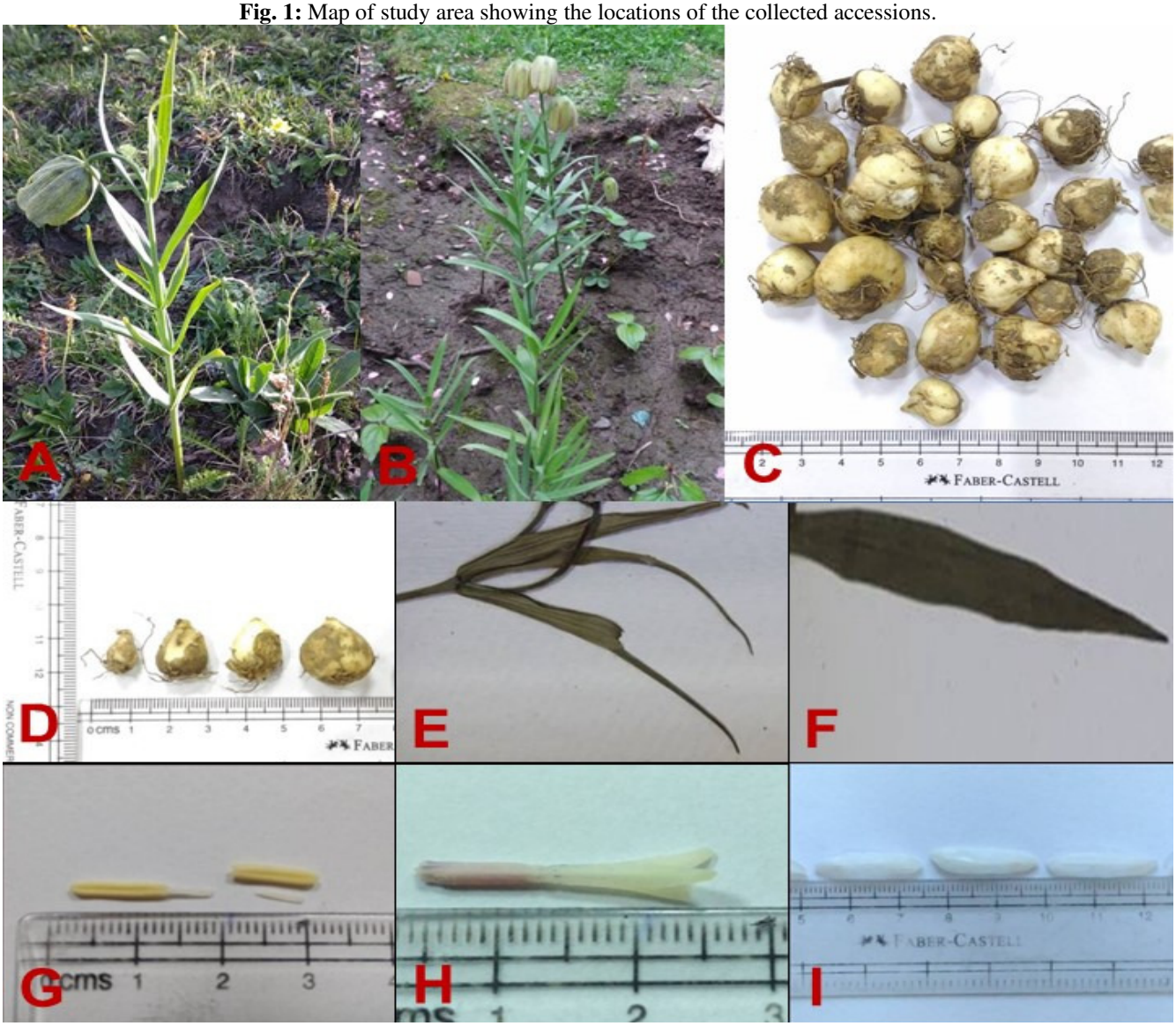

Fig. 2: Morphological characters of *Fritillaria cirrhosa* (A&B) Habit, (C&D) Fresh bulbs, (E) Leaf, (F) Bract, (G) Tepal, (H) Stamen, (I) Carpel.

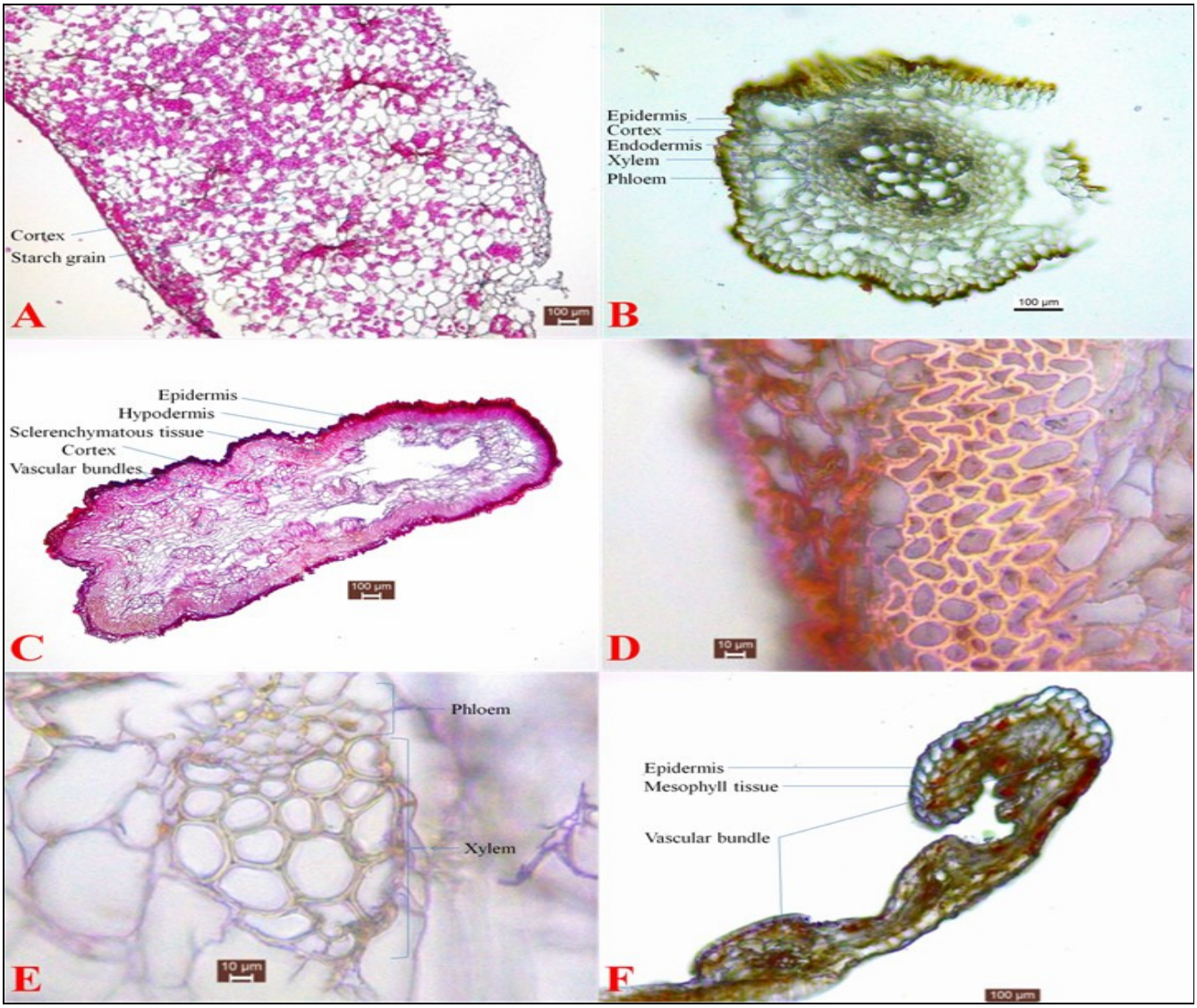

Fig. 3: Anatomical characters of *Fritillaria cirrhosa*. (A) T.S of Bulb, (B) T.S of the root, (C) T.S of the stem, (D) Sclerenchyma tissue, (E) Vascular tissue, (F) T.S of the leaf.

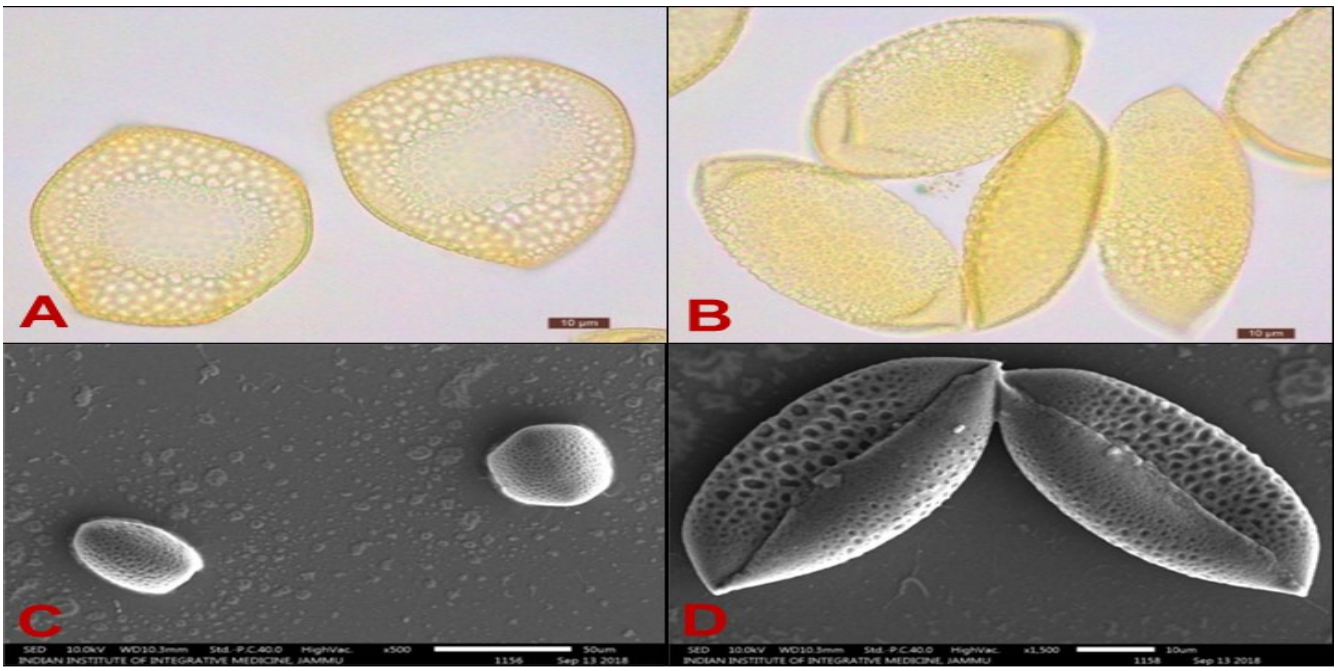

Fig. 4: Pollen grains of *Fritillaria cirrhosa*. (A&B) Polar and equatorial view in LM, (C&D) Polar and equatorial view in SEM showing exine ornamentation.

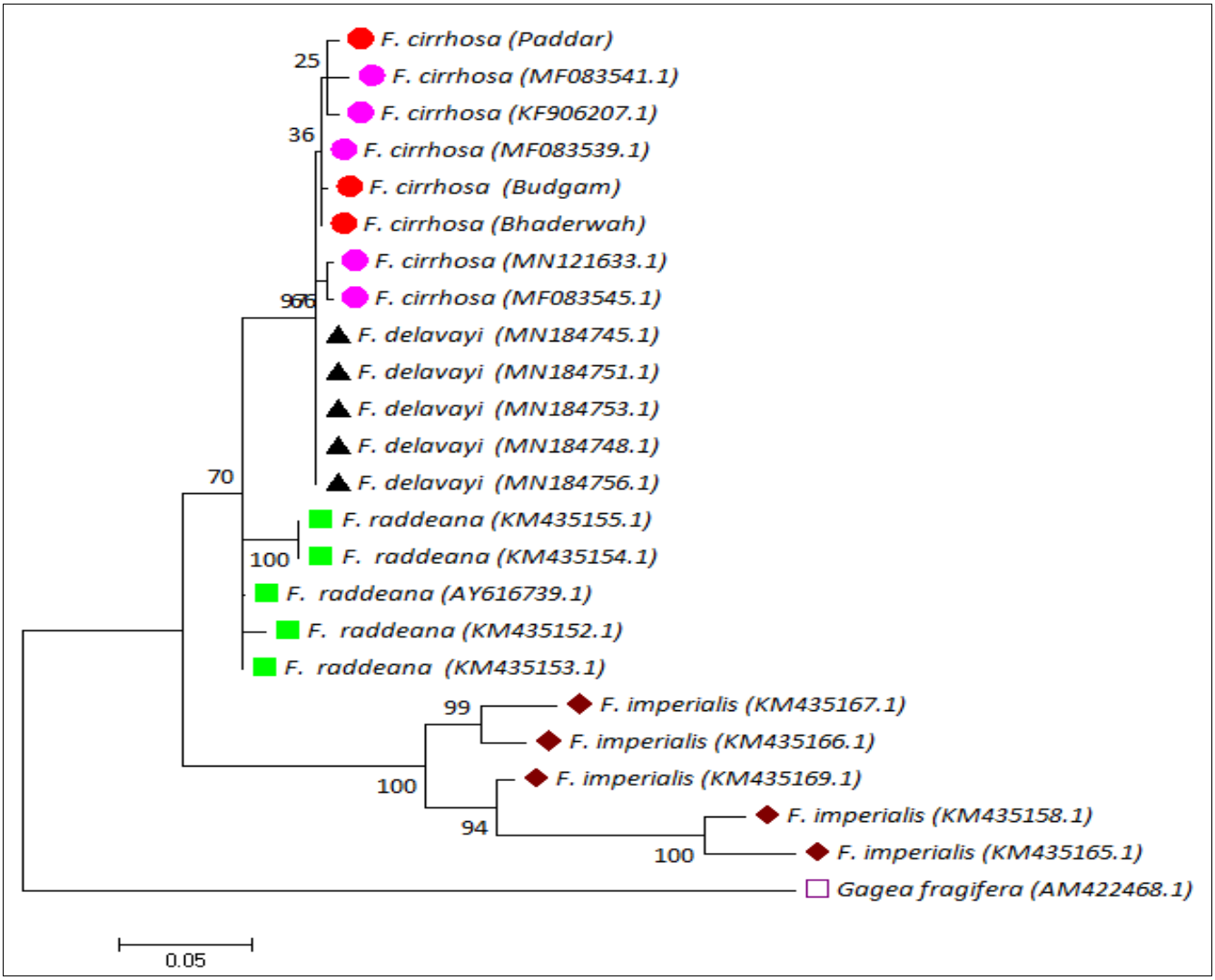

Fig. 5: Phylogenetic tree constructed using nucleotide sequences of ITS gene. Bootstrap support values (>50%) obtained from maximum likelihood (ML) analysis are shown at nodes. Value of 0.05 depicts the substitution rate per nucleotide site**.**

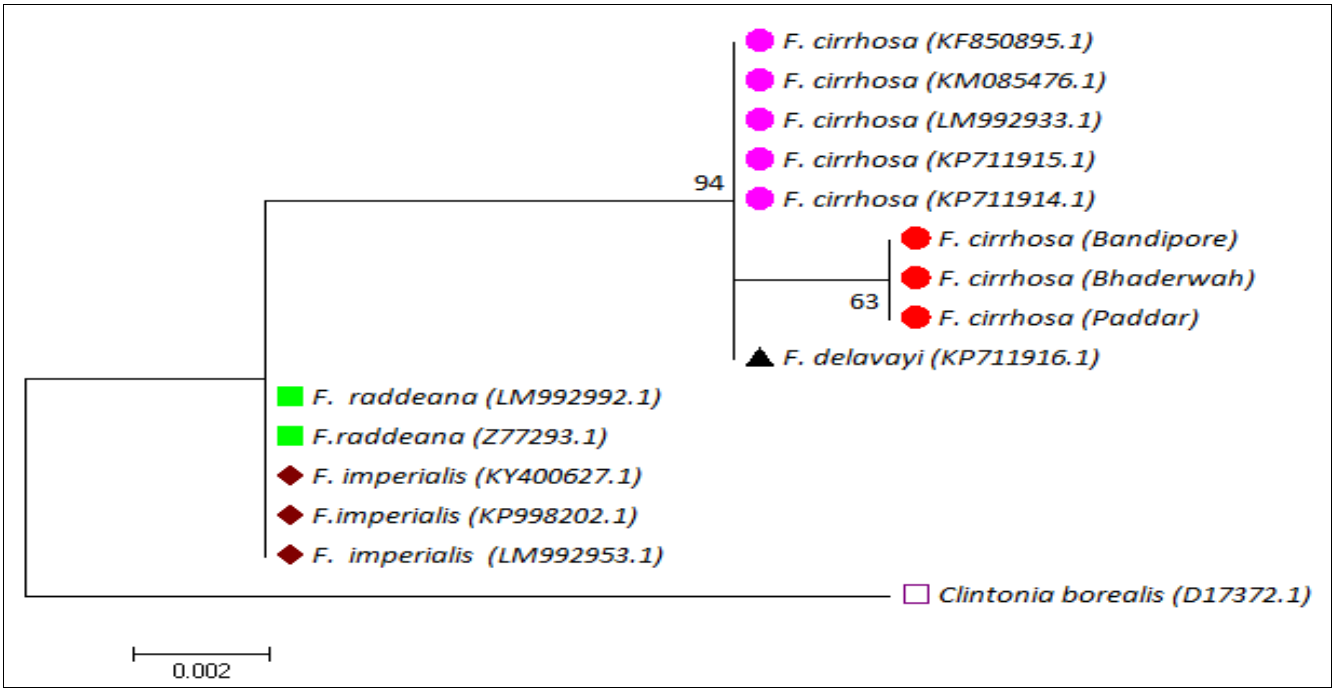

Fig. 6: Phylogenetic tree constructed using nucleotide sequences of rbcL gene. Bootstrap support values (>50%) obtained from maximum likelihood (ML) analysis are shown at nodes. Value of 0.002 depicts the substitution rate per nucleotide site.

Table 1: General locational details of the collected accessions.

District, Tehsil, Locality, State	Latitude	Longitude	Altitude (m asl)	Accession number (RRLH-)
Kishtwar, Paddar, Shroth Dhar, J&K	N 33˚13.20'	E 076˚07.61'	3074	23730
Doda, Bhaderwah, Chattergala, J&K	N 32˚52.89'	E 075˚42.94'	3489	23726
Budgam, Khag, Shopian, J&K	N 33˚58.16'	E 074˚29.22'	3641	23729

Table 2: Morphological characters of the studied samples of *Fritillaria cirrhosa* D.Don.

Characters	Min	Max	Mean±SD
Stem length (cm)	16.0	43.0	35.10±7.13
Bulb diameter (cm)	1.3	2.6	1.33±0.37
Lower leaf length (cm)	5.0	8.3	5.59±0.66
Lower leaf width (cm)	0.3	0.9	0.44±1.16
Length of filament (cm)	0.3	0.5	0.42±0.07
Length of anther (cm)	1.2	1.5	1.39±0.10
Length of style (cm)	1.9	2.2	1.97±0.11
Length of tepal (cm)	2.5	3.6	3.30±0.20
Width of tepal (cm)	0.8	1.0	0.98±0.05
Number of flowers/ plants	1.0	2.0	1.16±0.38
Number of leaves/ plants	13	20	16.50±2.46

Table 3: Anatomical characters of the studied samples of *Fritillaria cirrhosa* D.Don.

	Length (µm)			Breadth (µm)		
	Min	Max	Mean ±SD	Min	Max	Mean ±SD
Bulb						
Cortical cell	83.18	145.23	114.65±21.04	65.49	102.95	78.92±16.20
Starch grain	23.88	38.21	30.86±4.18	18.92	32.91	25.86±5.09
Root						
Epidermal cell	25.65	40.31	28.67±3.97	28.3	43.77	37.38±4.94
Cortical cell	30.51	61.51	45.31±10.68	25.41	52.59	37.11±10.04
Stem						
Epidermal cell	16.13	39.65	27.28±6.31	11.15	24.12	15.80±6.67
Hypodermal cell	18.33	44.82	29.33±7.32	6.21	28.72	14.99±5.35
Cortical cell	34.23	95.41	64.24±19.31	36	55.8	41.77±7.64
Sclerenchymatous cell	17.4	32.68	25.677±5.12	9.97	17.98	14.29±2.49
Leaf						
Epidermal cell size	39.96	61.25	48.55 ±7.1	16.71	29.45	23.10±4.54

Table 4: Palynological characters of the studied samples of *Fritillaria cirrhosa* D.Don in comparison to some earlier reported *Fritillaria* species.

Pollen characters	*F. cirrhosa* D.Don (Present study)			*F. imperialis* L. (Teksen *et al.* 2010)			*F. raddeana* Regel (Kosenko 1999)		
	Min	Max	Mean±SD	Min	Max	Mean	Min	Max	Mean
Long axis (LA)	44.74	62.95	52.72±5.05	54.7	59.9	57.9	40.3	44.1	NA
Short axis (SA)	26.11	48.52	36.77±7.59	38.2	41.7	40.4	63.3	67.2	NA
LA/SA	1.03	2.28	1.51±0.42	NA	NA	1.43	NA	NA	NA
Sulcus length	42.12	59.89	49.94±5.05	51.2	56.4	54.3	NA	NA	NA
Exine thickness	1.07	2.46	1.74±0.29	NA	NA	1.9	NA	NA	NA
Exine ornamentation	Reticulate			Regulate-reticulate			Macroreticulate		
Shape	Prolate			Prolate			NA		
Apex of sulcus	Round to sharp			Sharp			NA		
Aperture type	Monosulcate			Monosulcate			Monosulcate		
Sulcus membrane ornamentation	Verrucate			Psilate			Macrogranular		

Table 5: Accessions retrieved from the NCBI databse used in the current study

Species	Barcode Region	Accession number
F. cirrhosa D. Don	ITS	MF083541.1, KF906207.1, MF083539.1, MN121633.1, MF083545.1
	rbcL	KF850895.1, KM085476.1, LM992933.1, KP711915.1, KP711914.1
F. delavayi Franch.	ITS	MN184745.1, MN184751.1, MN184753.1, MN184748.1, MN184756.1
	rbcL	KP711916.1
F. raddeana Regel	ITS	KM435155.1, KM435154.1, AY616739.1, KM435152.1, KM435153.1
	rbcL	LM992992.1, Z77293.1
F. imperialis L.	ITS	KM435167.1, KM435166.1, KM435169.1, KM435158.1, KM435165.1
	rbcL	KY4006227.1, KP998202.1, LM992953.1

Table 6: Sequence characteristics of the barcoding regions

Barcode marker	No. of sequences	Aligned length (bp)	Mean intra specific distance ±SE	Mean inter specific distance ±SE
ITS	23	658	0.0469 ±0.015	0.088 ±0.0083
rbcL	14	480	0.0012 ±0.0004	0.0041 ±0.0019

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