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Original Research Article

Morphological Characteristics and Systematics Analysis of a New Forma of *Opuntia monacantha* (Willd.) Haw. f. *jejuensis* J. K. Kim ex Y. S. Yang from Jeju Island, Korea

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Abstract - The taxonomic status and phylogenetic relationship of *Opuntia monacantha* Haw. f. *jejuensis* J. K. Kim ex Y. S. Yang (Jejubaiknyuncho), which is native to southern coast of Jeju Island, Korea was analyzed using DNA markers obtained from Korean *Opuntia*. *Opuntia stricta* Haw., *O. humifusa* Raf., and *O. humifusa* Raf. f. *jeollaensis* E. J. Kim and S. S. Whang, native or cultivated in Korea, have no stripes on the back of tepals and have a purple pulp, whereas *O. monacantha* f. *jejuensis* has purple stripes on the back of tepals and a greenish-yellow pulp color. *Opuntia monacantha* has purple stripes on both the front and back of its tepals, whereas stripes appear only on the back of tepals of *O. monacantha* f. *jejuensis*. *Opuntia monacantha* f. *jejuensis* was assigned to *Elatae* series in phylogenetic analysis and was found to be more closely related to *O. monacantha* subsp. *arechavaletae* (Speg.) Guiggi, compared with *O. monacantha* at a molecular level. Based on its phylogenetic and morphological differences from *O. monacantha* and *O. monacantha* subsp. *arechavaletae*, which are native or have been cultivated in Jeju areas, *O. monacantha* f. *jejuensis* was named as a new forma in this study.

Key words – Cacti of the genus *Opuntia*, DNA markers, Jejubaiknyuncho, *Opuntia monacantha* (Willd.) Haw. f. *jejuensis* J. K. Kim ex Y. S. Yang

Introduction

At the 1578 Compendium of Materia Medica, *Opuntia monacantha* f. *jejuensis* was introduced as a green cactus in the Encyclopedia of Oriental Herbal Medicine and was studied until 1975. Since its introduction in Jeju, it has been used medicinally in the region (Kwon *et al*., 2017; Koh *et al*., 2018). Currently, *O. humifusa* is grown on a large scale in the Chungcheong and Jeolla regions (Kim *et al.*, 2014). In particular, *O. stricta* is being cultivated on ~320 ㏊ of land (Yang *et al*., 2020). Cacti of the genus *Opuntia*, native to or grown in Korea, exhibit superior antibacterial, anti-glycosylation, antioxidant, anticancer, and cholesterol-lowering properties (Chung, 2000; Jung *et al*., 2012; Park *et al*., 2013; Choi, 2014; Jung *et al*., 2014). In addition to kimchi made with *Opuntia* powder, other foods, such as fermented milk, are also being made (Lee and Bae, 2009; Jung *et al*., 2016; Kim *et al*., 2016; Lee, 2017; Lee *et al*., 2018). Numerous studies have explored the potential of *Opuntia* as a cosmetic raw material (Lim and Hong, 2016; Kown *et al*., 2017).

Cacti is a collective name for succulent plants belonging to the Cactaceae family of Caryophyllaes. In dry areas, during periods of heavy rainfall, water is stored in the plant body and used for plant growth during the dry season. Cactus has a reduced surface area to minimize water loss in arid regions, converting leaves to spines, and shortening stems and roots. Among the 3,000 species of succulent plants found worldwide, ~1,800 are cacti, which originated in South America and evolved in a dry environment (Anderson, 2001). Cactaceae are classified into four subfamilies by the International Cactaceae Systematics Group: Pereskoideae, Maihuenioideae, Opuntioideae, and Cactoideae (Nyffeler and Eggli, 2010). Opuntioideae is classified into seven genera: *Brasiliopuntia, Tacinga*, *Consolea, Miqueliopuntia, Salmiopuntia, Opuntia*

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and *Tunilla* (Wallace and Dickie, 2002; Griffith and Poter, 2009; Hernandez-Hernandez *et al.*, 2011). In phylogenetic studies, it is classified into ten series: *Brasiliopuntia, Tacinga, Elatae, Macbridei, Scheerianae, Humifusa, Macrocentra, Nopalea*, *Basilares and Microdasys* (Table 1), (Majure *et al*., 2012b).

Opuntia s.s. is a large genus of Cactaceae with ~200 species (Anderson, 2001). In Korea, *O. humifusa* and *O. humifusa* f. *jeollaensis* are native and are cultivated in the regions of Chungcheong, Jeolla, and Gyeongsang (Park *et al*., 2013; Kim *et al.*, 2014). In the Jeju region, *O. stricta* Haw. also grows naturally and is cultivated (Yang *et al.,* 2020; Yang and Oh, 2021), and *O. monacantha* f. *jejuensis* (this study) grows naturally (Fig. 1), (Yang and Oh, 2021).

O. monacantha f. *jejuensis* is the largest indigenous cactus species in Korea and is found on Jeju Island in the Aewol, Taepyeong, and Bomok regions (Fig. 1). In previous studies, External morphological studies have differentiated *O. monacantha* f. *jejuensis* from *O. stricta* by renaming the misidentified *O. stricta*, cultivated in the Hallim area of Jeju, and examining the phylogenetic relationship between them (Yang *et al*., 2020; Yang and Oh, 2021). The taxonomic analysis of *O. monacantha* sensu lato and its synonymous species is shown in Table 2. *O. vulgaris* Mill. had been accepted as a synonym of *O. monacantha* or *O. humifusa* but was later accepted as *O. ficus-indica* (L.) Mill. (Leuenberger, 1993). *O. archavaletae* Speg. was classified as a subspecies based on its different characteristics (flower, fruit) of reproductive organs (*O. monacantha* subsp. *archavaletae* (Speg.) Guiggi.) (Guiggi, 2017). A variety of *O. monacantha* was named *O. monacantha* var. *gracilior* Salm-Dyck (Table 2).

Generally, phylogenetic analyses are conducted using the maximum likelihood (ML) and Bayesian inference (BI) methods which can be applied to a model of sequence evolution, and phylogenetic trees can easily be constructed using sequence data. Calculating the likelihood, ML maximizes the likelihood of the model for all data, whereas BI maximizes the posterior probability using likelihood. ML typically uses

Table 1. Classification of *Opuntia s.s.* based on phylogenetic studies (Majure *et al*., 2012b)

		Series
Sister clade of *Opuntia s.s.*		*Brasiliopuntia, Tacinga*
Opuntia s.s.	South American Clades	*Elatae, Macbridei*
	North American Clades	*Scheerianae, Humifusa, Macrocentra, Nopalea, Basilares, Microdasys*

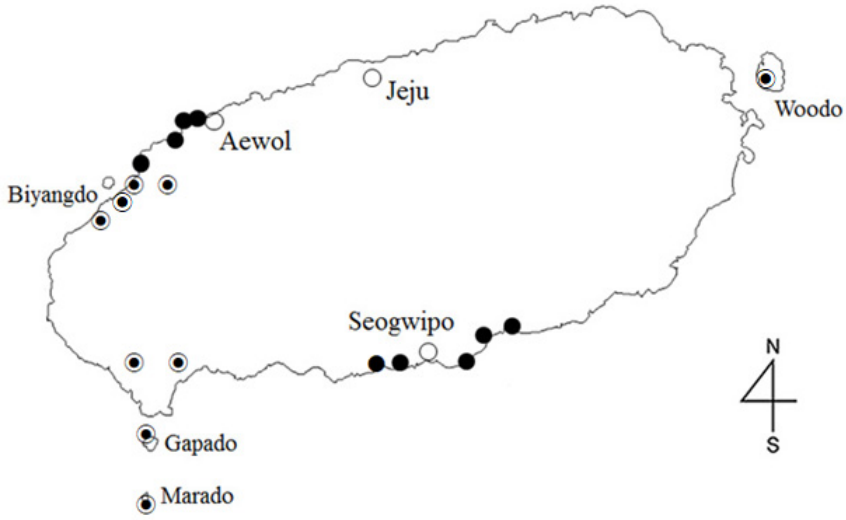


Fig. 1. Distribution map of *O. monacantha* f. *jejuensis* (●) and *O.* stricta (◉) in Jeju Island, Korea.

Table 2. The taxonomic history of *O. monacantha*

	Scientific Name	Reference
Synonym	Accepted Scientific Name:	
	O. monacantha (Willd.) Haw.	Haw. 1819. Suppl. pl. succ. 81.
	= *C. monacanthos* Willd.	Willd. 1814. Enum. Pl. Hort. Berol. Suppl.:33
	= *O. deflexa* Lem.	Lem. 1839. Cact. Gen. Sp. Nov.:68
	= *O. gracilior* Lem.	Lem. 1839. Cact. Gen. Sp. Nov.:68
	= *O. lemaireana* Console ex Bois	Bois. 1898. Dict. Hort. 2:894
	= *O. lemaireana* Console ex F.A.C.Weber	Weber, F.A.C.D. 1898. G. J. M. Bois, Dict. hort.2:894.
	= *O. monacantha* var. *deflexa* Salm-Dyck	Salm-Dyck. 1850. Cact. Hort. Dyck.:66
	= *O. monacantha* var. *gracilior* Lem.	Lem. 1839. Cact. Gen. Sp. Nov.:68
	= *O. monacantha* var. *variegata* Anon.	Anon. 1874. Gard. Chron., n.s. 2:49
	= *O. vulgaris* var. *lemaireana* (Console ex Bois) Backeb.	Backeb. 1958. Cactac.: Handb. Kakteenk. 1:400
Re-evaluation	*O. vulgaris* Mill.	Mill. 1768. Gard. Dict. ed. 8: n 1., nom. rej.
	(Accepted Scientific name: *O. ficus-indica* (L.) Mill.)	(Leuenberger, 1993; Plant List, 2013)
Subspecies	*O. arechavaletae* Speg.	Speg. 1905. Anal. Mus. Buenos Aires, 11: 520.
	Accepted Scientific Name: *O. monacantha* subsp. *arechavaletae* (Speg.) Guiggi.	Guiggi. 2017. Suppl. 5:1.
Variety	*O. monacantha* var. *gracilior* Salm-Dyck	Salm-Dyck. 1850. Cact. Hort. Dyck.(1849). 66, 235.

phylogenetic maximum likelihood (PhyML) or randomized accelerated maximum likelihood programs, whereas BI uses Bayesian inference of phylogeny (MrBayes) or Bayesian evolutionary analysis sampling tree (BEAST) programs to construct a phylogenetic tree. The obtained node support value for a phylogenetic tree written in ML is referred to as a bootstrap, and that in BI is called posterior probability. Majure *et al.* (2012a) used genetic ML and BI methods to conduct a phylogenetic analysis of the genus *Opuntia*. Kim *et al.* (2014) conducted genetic ML analysis and morphological classification by floral color for *O. humifusa* f. *jeollaensis*. Therefore, it was necessary to identify the position of *O. monacantha* f. *jejuensis* in the *Opuntia* via morphological comparisons and phylogenetic analysis using MI and BI methods.

Comparing the pulp color of *O. monacantha* f. *jejuensis* to that of *O. monacantha*, a distinct difference was observed in the characteristics of the two species (Koh *et al.,* 2018; Yang and Oh, 2021). Owing to the lack of studies confirming the exact taxonomic position of *O. monacantha* f. *jejuensis*, which exhibits a distinct difference in pulp color and characteristics from *O. monacantha*, species identification studies are required before considering it as a raw material for pharmaceuticals, health supplements, and cosmetics. Therefore, to determine the exact phylogenetic position of *O. monacantha* f. *jejuensis* within the *Elatae* series, we compared the morphological characteristics of the *O. monacantha* complex and comfirmed its taxonomic status using phylogenetic analysis. This study contributes to validating a new forma of the taxon and its potential use as a health-promoting material.

Materials and Methods

Plant materials

The *O. monacantha* sensu lato used in this study was collected from the Taepyeong-ro habitat in Seogwipo (33°14'40.6" N 126°32'38.0" E). We transferred the collected materials were transferred to the cultivation site of the Baiknyuncho Museum in Hogeun-dong, Seogwipo, Jeju Island, for morphological studies. An immersion specimen was created and stored in Baiknyuncho Museum along with the associated resource management number. For DNA sequencing, one-year-old leaves were collected considering the occurrence of bacteria

and contamination levels, and the obtained material was washed with distilled water, frozen in liquid nitrogen, and stored at -80℃ to extract the DNA.

DNA extraction and PCR amplification

Genomic DNA was isolated using the CTAB method (Doyle and Doyle, 1990). The primers and protocol were designed using the plastid intergenic spacers *atp*B-*rbc*L, *trn*L-F, and *psb*J-*pet*A, which are suitable for plant taxonomy phylogenetic classification studies, chloroplast gene *mat*K, and nuclear ribosomal gene ITS (Table 3). The polymerase chain reaction (PCR) cycling parameters involved initial denaturation was performed at 94℃ for 5 min. The thermal cycle consisted of denaturation at 94℃ for 30 s, annealing at 55℃ for 30 s, and extension at 72℃ for 1 min, and was repeated 35 times. We performed the final extension at 72℃ for 10 min.

DNA sequencing and alignment

For sequencing of the five genes (*atp*B-*rbc*L, *psb*J-*pet*A, *trn*L-F, *mat*K, and *nr*ITS) of the investigated taxon, 25 cycles of sequencing reactions were performed using the Big Dye Terminator Cycle Sequencing Kit v.3.1 and the automated DNA sequencing system, Applied BioSystems DNA 3730XL Analyzer (Applied BioSystems, Foster City, CA, USA). DNA was purified using the Montage SEQ96 PCR clean-up kit (Millipore Corporation, Billerica, MA, USA). The nucleotide sequence was aligned using the ClustalX program (Thompson *et al.*, 1997) and edited and rearranged using BioEdit ver. 7.2.6.2. program (Hall, 1999).

Phylogenetic analysis

After thoroughly confirming the DNA sequence of the target species, the sequences of the species were compared and analyzed from a molecular evolutionary perspective (Majure *et al.,* 2012a,b). Three previously studied (Kim *et al.,* 2014) cacti (*O. humifusa*, *O. humifusa* f. *jeollaensis*, and *O. stricta*) cultivated in Korea were sampled to obtain evolutionary data on *O. monacantha* f. *jejuensis*. Phylogenetic relationships were confirmed using the *Tacinga* series as outgroups using genetic information registered in the GenBank of the National Center for Biotechnology Information (Clegg, 1993; Soltis *et al.,* 1998; Park *et al*., 2010), (Appendix 1). Comparative analysis was used to establish the exact position of *O. monacantha* f. *jejuensis* within the *Elatae* series and the form and DNA sequence of *O. monacantha* complex, including *O. monacantha, O. monacantha* subsp. *arechavaletae*, and *O. monacantha* f. *jejuensis* species (Appendix 2). BI and ML analyses were used to confirm the phylogenetic relationships between the species, and the programs MrBayes-3.2.5 (Ronquist *et al.*, 2012) and PhyML 3.0 (Guindon *et al.,* 2010) were used. The GTR+I+G (nst = 6, rates = invgamma) model, based on the main parameters affecting the substitution ratio

Table 3. Genomic DNA regions and primer sequences used in this study

Gene	sequence or reference	Length amplified	No. pars. infor. characters	Model selected
*atp*B-*rbc*L	*atp*B Op : 5´-GTAAACTATGTCGAAATTCTTTGC-3 ´ *rbc*L. Op : 5´-ACAACAAAACAACAAGGTCTACTC-3 ´	861	20	HKY
*psb*J-*pet*A	*psb*J: (Shaw *et al*., 2007) *pet*A. Op : 5´-CAACATCAAGTTCGTAACAAG-3 ´	1169	72	K81uf+I
*trn*L-F	*trn*L: (Taberlet *et al*., 1991) *trn*F: (Taberlet *et al*., 1991)	441	14	K81uf
*mat*K	*mat*Kx: 5´-TAATTTACGATCAATTCATTC-3 ´ *mat*K5: 5´-GTTCTAGCACCAGAAAGTCG-3 ´	905	27	F81+I+G
*nr*ITS	ITS4: (White *et al*., 1990) ITS5: (White *et al*., 1990)	599	39	TVM+G
cpDNA combined	-	3407	115	-
Nuclear combined	-	568	57	-
All combined	-	3975	172	-

in the DNA sequence, was used for BI analysis. Each of the five genes was analyzed using a random phylogenetic tree of 1×106 generations and 100 generations of Markov chains. In ML analysis, random samples were selected to duplicate the same number of characters as in the existing data set, repeated 10,000 times to form the same tree using PhyML 3.0 program, a phylogenetic tree, and bootstrap.

We constructed a molecular phylogenetic tree to illustrate phylogenetic relationships between species using TRE and PhyML files generated by BI and ML analysis, respectively. The molecular phylogenetic tree was confirmed using the FigTree v1.4.3 program (Rambaut, 2016), which was designed to display the summarized contents and annotated phylogenetic tree produced by BEAST.

Comparison of morphological characters

Six morphological characteristics, i.e., tepal color, stigma lobe color, fruit color, pulp color, and morphological differences between the seed, stem, and fruit, were selected through a comparative morphological study of the investigated taxon, i.e., *O. monacantha* complex, and evolutionary differentiation patterns within the *O. monacantha* complex were compared using a phylogenetic tree.

Results

The results of morphological and phylogenetic analyses of the *Elatae* series, which includes the *O. monacantha* complex, *O. monacantha* f. *jejuensis*, and Korean *Opuntia* spp., showed different flower colors, cladode shapes, fruit shapes, fruit colors, and pulp colors. *O. monacantha* f. *jejuensis* was assigned to the *Elatae* series in the phylogenetic analysis and was more closely related to *O. monacantha* subsp. *arechavaletae* than to *O. monacantha* at a molecular level.

Morphological characters

The investigated taxon had different morphological characteristics than other *Opuntia* spp. in Korea (Table 4). The cladodes of mature specimens of *O. stricta, O. humifusa,* and *O. humifusa* f. *jeollaensis* are ovate, but those of *O. monacantha* f. *jejuensis* were oblong. Furthermore, plant size, flowering period, fruit shape, and pulp color were significantly different. *O. monacantha* f. *jejuensis* is a tree-like plant that grows to a height of > 2 m, while other species are shrub-like plants that grow to a height of < 1 m. *O. monacantha* f. *jejuensis* fruits are plum-shaped, whereas those of other species are elongated pear-shaped. The fruit of the investigated taxon is purple-green, whereas those of the others are reddish-purple (Fig. 2). Finally, the pulp of the investigated taxon was yellow-green, whereas that of the other species was purple.

O. monacantha f. *jejuensis* was observed to be morphologically similar to *O. monacantha*. However, *O. monacantha* had yellow tepals with abaxially red midveins, i.e., purple or red stripes on the front and back of tepals (Fig. 3), (Majure and Puente, 2014), whereas these stripes appear only on the back of *O. monacantha* f. *jejuensis* tepals. *O. monacantha* and *O. monacantha* subsp. *arechavaletae* had yellow or orange-yellow tepals, whereas *O. monacantha* f. *jejuensis* had bright yellow tepals (Fig. 3).

Stigma lobe color, fruit color, seeds, pulp color, and

Table 4. Morphological differences of *O. monacantha* f. *jejuensis* and *Opuntia* spp. in Korea

Taxa	*O. monacantha* f. jejuensis	*O. stricta*	*O. humifusa*	*O. humifusa* f. *jeollaensis*
Flower color	yellow-with out side purple stripes	yellow	yellow	yellow-with red in the red center
Cladode shape	Oblong (elongated)	Ovate or Obovate	Ovate or Obovate	Ovate or Obovate
Fruit shape	a plums	a long western pear	a long western pear	a long western pear
Fruit color	purple-green	reddish-purple	reddish-purple	reddish-purple
Pulp color	yellow-green	purple	purple	purple
Plant size	2-5 m erect, succulent tree-like plant	less than 0.8 m erect, succulent shrubby-like plant	less than 1 m shrubby-like succulent	less than 1 m shrubby-like succulent

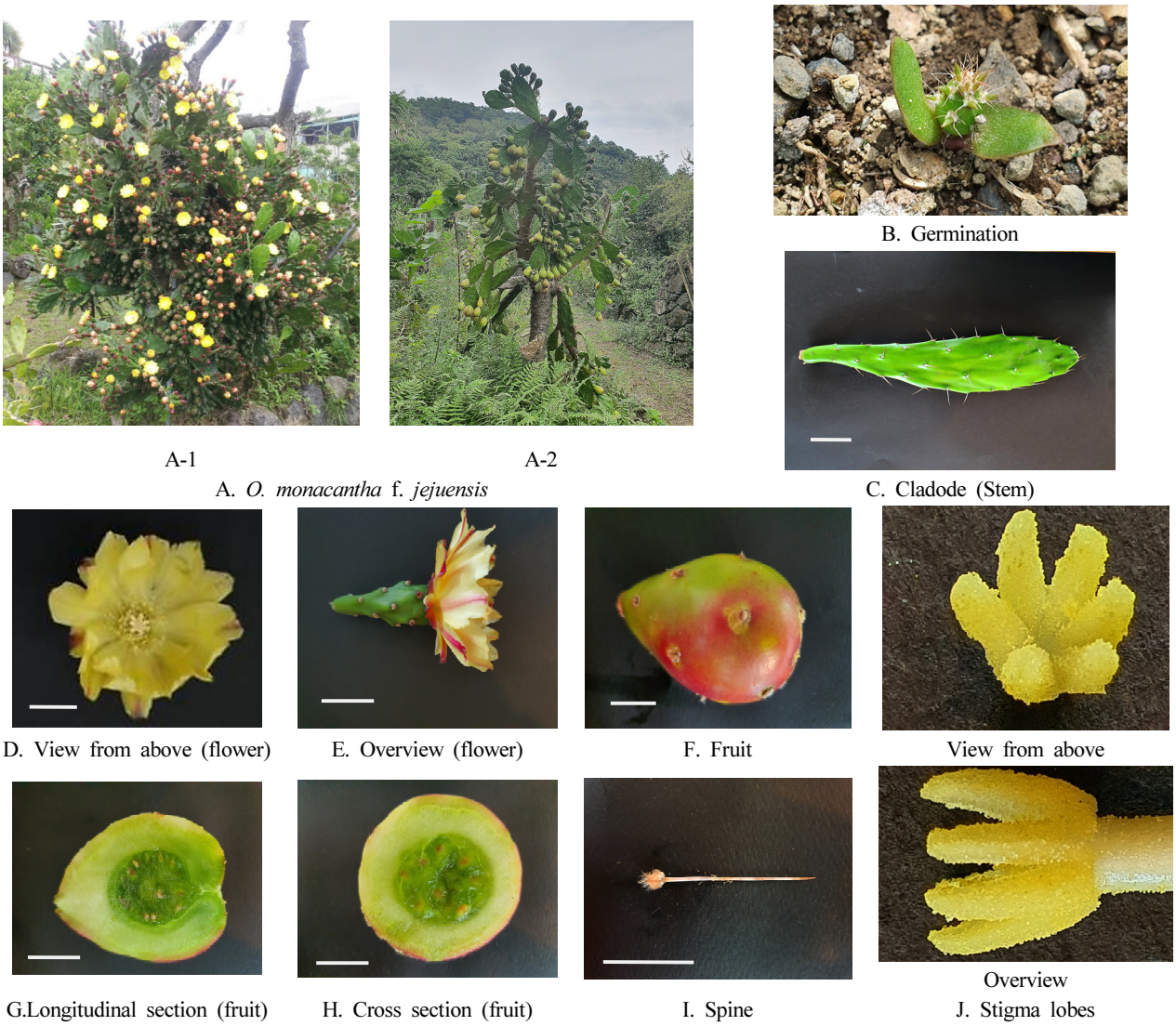


Fig. 2. Morphological characteristics of Cladode, flowers, fruit, and pulp of *O. monacantha* f. *jejuensis*, illustrating their morphological characteristics. The scale bar (white) across the bottom of each photograph is 2 ㎝. *O. monacantha* f. *jejuensis* (A). Germinated *O. monacantha* f. *jejuensis* (B). Oblong shape of a cladode (C). Flower with purple stripes on the outer surface of yellow petals (D,E). Plum-shaped fruit (F-H). Spine (4 ㎝ long) (I). Stigma lobes (×10) (J).

peculiar phenomenon were the traits used for morphological classification. *O. monacantha* has creamish stigma lobes, *O. monacantha* subsp. *arechavaletae* has greenish stigma lobes (Guiggi, 2017; Maria *et al.,* 2017), and *O. monacantha* f. *jejuensis* has creamish stigma lobes (Table 5, Fig. 3). The mature fruit of *O. monacantha and O. monacantha* f. *jejuensis* is purple-green, whereas those of *O. monacantha* subsp. *arechavaletae* is reddish-purple. The filaments of the stamens of *O. monacantha* were greenish in color, according to previous studies (Pardo and Alonso, 2017), but in this study they turned out to be yellow. Those of *O. monacantha* f. *jejuensis* were yellow, too. Sprouts of *O. monacantha* f. *jejuensis* were germinated (Fig. 2). The fruiting pattern on stems in the investigated taxon can be classified into two distinct types. One is that a stem grows from a fruit, with another fruit growing from this stem and the other is that three fruits grow sequentially at the end of a single stem, which is the most characteristic phenomenon that occurs only in *O. monacantha* f. *jejuensis* (Fig. 4).

A-1 B-1 C-1

A-2 B-2 C-2

A. *O. monacantha* B. *O. monacantha* subsp. *arechavaletae* C. *O. monacantha* f. *jejuensis*

Fig. 3. Flower and fruits of *O. monacantha* complex.

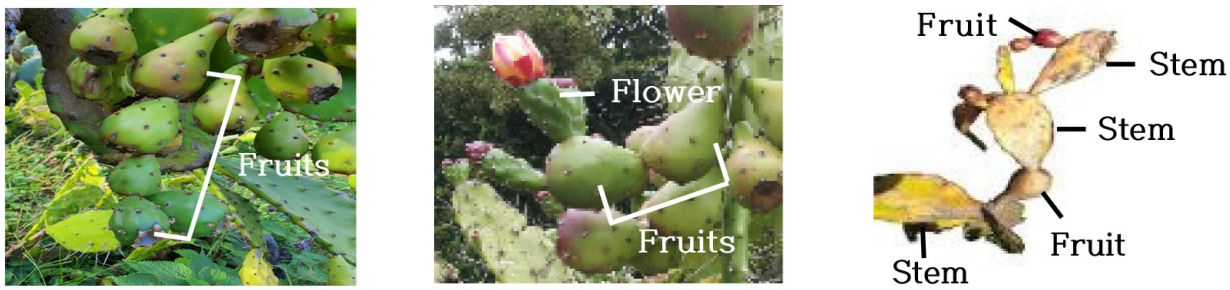
Flower of *O. monacantha* (A-1): https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:323260-2/images
Fruits of *O. monacantha* (A-2): https://www.gbif.org/occurrence/3859139935
Flower of *O. monacantha* subsp. *arechavaletae* (B-1) https://www.gbif.org/occurrence/3759979394
Fruits of *O. monacantha* subsp. *arechavaletae* (B-2) https://www.gbif.org/occurrence/3759979394

Table 5. Morphological differences in *O. monacantha* complex

Taxa	*O. monacantha*	*O. monacantha* subsp. *arechavaletae*	*O. monacantha* f. *jejuensis*
Flower color	Yellow or orange yellow tepal, Red stripes on the inside and outside of tepals	Yellow or orange yellow tepal, Red stripes on the outside of tepals	Yellow tepal, Red stripes on the outside of tepals
Stigma lobes color	Pale yellow to pale creamish (Haworth, 1819)	Greenish (Guiggi, 2017)	Pale yellow to pale creamish
Fruit color	The fruit maturing and reddish purple, greenish near base or green with red-purple shades (Haworth, 1819)	The fruit maturing and reddish-purple, greenish near base (club head part)	The fruit maturing and reddish purple, greenish near base or green with red-purple shades
Fruit shape	Narrowly turbinate to obovoid fruits, with well-developed loculus (Maria *et al*.,2017) Plum shape	Longer, elongate, with an apical and smaller loculus (Guiggi, 2017) Club shape	Narrowly turbinate to obovoid fruits, with well-developed loculus Plum shape
Seeds	3.5×4 mm (Haworth, 1819)	-	Seeds are light tan, Irregularly elliptic, ca. 2 ea 5×6 mm More than 50 ea 3×4 mm
Pulp color	Greenish (Maria *et al*., 2017)	Greenish (Maria *et al*., 2017)	Inside: Greenish Outside: Green-yellow
Peculiar phenomenon	-	-	Fruit+Stem+Fruit, Fruit+Fruit+Fruit

Phylogenetic analysis

Bayesian trees based on DNA sequence showed posterior probabilities summarized from a set of post-burn-in trees generated using the GTR+I+G model (Figs. 5 and 6). *Opuntia stricta* was included in *Scheerianae* series and *O. humifusa* and *O. humifusa* f. *jeollaensis* were included in *Humifusa* series (Fig. 5). These three species formed a part of North American clades. However, unlike other *Opuntia* spp. in Korea, *O. monacantha* f. *jejuensis* was included in *Elatae* series of South American clades (Fig. 6) as it shares numerous morphological characteristics with *O. monacantha*. The phylogenetic distinctions between *O. monacantha* f. *jejuensis*, *O. monacantha*, and *O. monacantha* subsp. *arechavaletae* (Fig. 6) were not distinguished. We conducted ML and BI analyses of the *O. monacantha* complex and other species in South American clades to identify the detailed molecular evolutionary aspects of *O. monacantha* f. *jejuensis.* The results of the analyses were highly relevant, because *O. monacantha* f. *jejuensis* branched with *O. monacantha* subsp. *arechavaletae* within *O. monacantha* complex.



A B C

Fig. 4. Characteristic phenomenon of *O. monacantha* f. *jejuensis*. (A) Consecutively generated fruits. (B) A flower growing from a fruit. (C) a stem growing from a fruit.

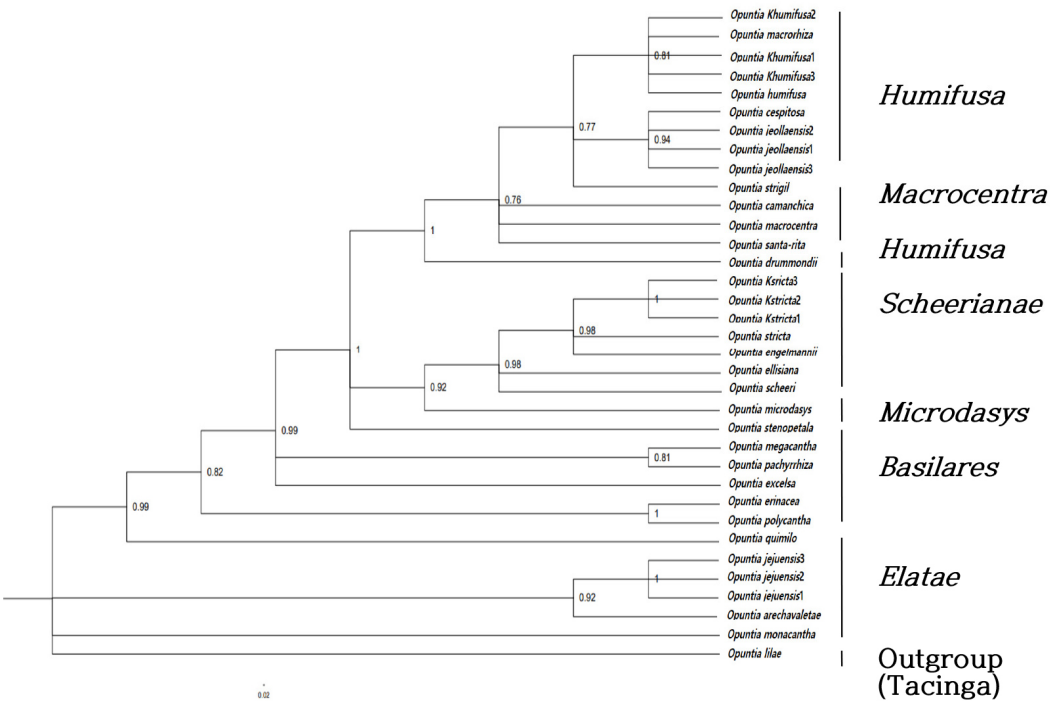


Fig. 5. Bayesian tree showing the phylogenetic relationship within *Opuntia* s.s., including Korean taxa (*trn*L-F, *mat*K and *nr*ITS sequences). Bayesian posterior probability values are shown above branch nodes. The BPP values < 0.76 are not provided.

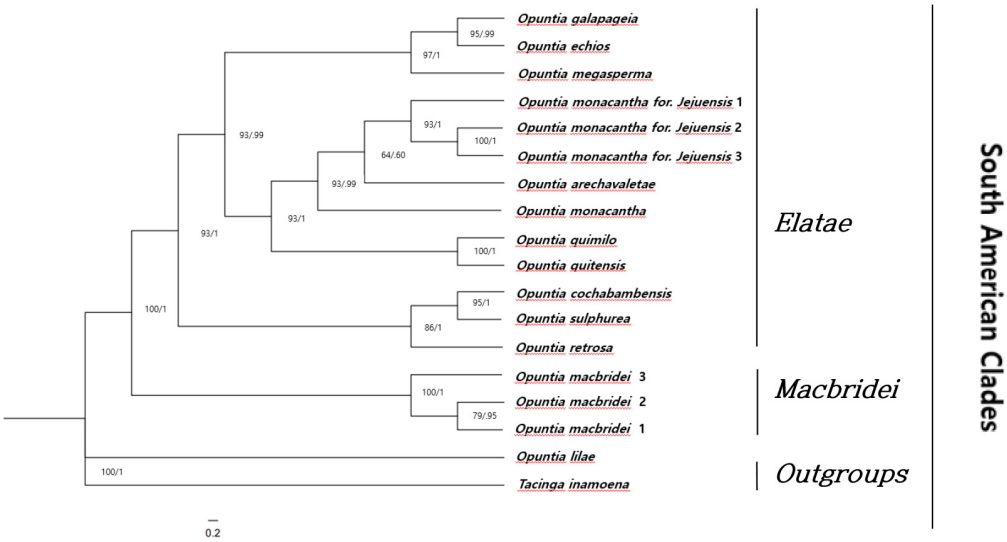


Fig. 6. Bayesian and ML trees of South American clades including *O. monacantha* complex taxa (using *atpB-rbcL*, *trn*L-F, *psb*J-*pet*A, *mat*K and *nr*ITS sequences). BPP (right) and Bootstrap values (left) are shown between branches. South American clades used the names of the series published by Britton and Rose (1920), Engelmann (1856), and Majure *et al*. (2012a) as a result of their research. The BPP values are > 0.6 and The Bootstrap values are ≥ 60%.

Discussion

Morphological characters

When compared to *O. monacantha* (Wagner *et al.,* 1999; Taylor and Zappi, 2004; Navie and Adkins, 2008) and *O. monacantha* subsp. *arechavaletae* (Speg, 1905; Guiggi, 2017), *O. monacantha* f. *jejuensis* (Yang and Oh, 2021) showed significant differences in flower color, stigma lobe color, fruit color, seed color, pulp color, and peculiar phenomenon (Fig. 4, Table 4). Based on the findings of this study, *O. monacantha* f. *jejuensis* has a pulp color that is distinct from that of *O. monacantha*. Yang and Oh (2021) reported that *O. monacantha* f. *jejuensis*, a species native to Jeju Island, was morphologically different from *O. monacantha* in fruit and stem growth characteristics. As the mature fruits of plants in *Opuntia* have the same color as pulp, fruit color indicates pulp color (Omweri *et al.*, 2016). However, the pulp of *O. monacantha* f. *jejuensis* is greenish-yellow, which differs from the color of its fruit (Table 5). The mature fruit of *O. monacantha* and *O. monacantha* f. *jejuensis* are purple-green, whereas that of and *O. monacantha* subsp. *arechavaletae* are reddish-purple. Moreover, *O. monacantha* f. *jejuensis* exhibited an unusual flowering phenomenon on the fruit. In other plants, it is common for the fruit to grow on the stem, but *O. monacantha* f. *jejuensis* also showed stem growth from in the fruit. Therefore, fruit areole has the same function as stem areole. This study showed that *O. monacantha* f. *jejuensis* is morphologically different from *O. monacantha* and thus can be classified as a new forma.

Phylogenetic analysis

Following Kim *et al*. (2014), wherein plastid intergenic spacer *trn*L-F, plastid gene *mat*K, and *nr*ITS, were used, this study included the three taxa, *O. stricta*, *O. humifusa*, and *O. humifusa* f. *jeollaensis*, in North American clades. *O. stricta* was included in *Scheerianae* series and *O. humifusa* and *O. humifusa* f. *jeollaensis* were included in *Humifusa* series (Majure *et al.,* 2012b). *O. monacantha* f. *jejuensis* was included in *Elatae* series of South American clades, which is consistent with the findings of previous studies (Yang and Oh, 2021) that it is evolutionarily closer to *O. monacantha* subsp. *arechavaletae* than to *O. monacantha*. Furthermore, according to the research findings of Majure *et al.* (2012a,b), Kim *et al.* (2014), and Yang and Oh (2021), alloploid species are a radial evolution type in North American clades. When comparing the *O. monacantha* complex with other species in *Elatae* series

using phylogenetic trees based on plastid and *nr*ITS sequences, the results were consistent with previous findings that *O. monacantha* f. *jejuensis* was evolutionarily closer to *O. monacantha* subsp. *arechavaletae* than *O. monacantha*.

Naming of a new forma

Recently, *O. monacantha* subsp. *arechavaletae* has been classified as a subspecies of *O. monacantha* (Guiggi, 2017). The stigma lobes are yellow in *O. monacantha* but green in *O. monacantha* subsp. *arechavaletae*. *O. monacantha* f. *jejuensis* differed morphologically from *O. monacantha* in several areas (Table 5). The fruit shape, size, stem, and spines of the investigated taxon were morphologically similar to those of *O. monacantha*, making it difficult to regard it as a new species. However, in light of the morphological (flower color, pulp color, and peculiar phenomenon) and phylogenetic (phylogenetic trees) analysis of *O. monacantha* f. *jejuensis,* it was deemed reasonable to classify it as a new forma. The species was named *O. monacantha* (Willd.) Haw. f. *jejuensis* J. K. Kim ex Y. S. Yang, based on the habitat characteristics, i.e., native or widely cultivated in Jeju, and the first discoverer and scientific name designator was Jekuk Kim, who is a strong advocate for the taxon's protection, cultivation, and breeding.

Taxonomic treatment

***Opuntia monacantha* (Willd.) Haw. f. *jejuensis* J. K. Kim. ex Y. S. Yang, for. nov. (Fig. 7)**

Korean name: Je-ju-baik-nyun-cho 제주백년초

Origin: Eastern coastal South America (Argentina, Brazil, Paraguay, and Uruguay)

A type of succulent tree (thorny shrub); Erect cylinder trunk shrub, trunk diameter 25-30 ㎝; Cladodes shiny green, 2-5 m height, oblong (elongated), 20-35 ㎝ long, 8-12 ㎝ wide, areoles arranged > 2 ㎝ wide; Leaves subulate; Petals always yellow with purple median stripes on the outer perianth segment, 16-25 mm long and 11-16 mm ; Bright yellow inner perianth parts, 22-40 mm long and 16-42 mm wide, with yellow to white staminal filaments (180-200 ea), the style yellow, 10-20 mm long, divided into 5-6 bright yellow stigma lobes and 8-10 mm long; Flowering May-January; Reddish flower buds; Spines, 4-5 ㎝ long, white-grey or yellowish to reddish-brown; Brownish glochids numerous; Seeds, irregularly elliptic, usually 6×5 mm 2 ea and 4×3 mm More than 40 ea, High germination rate (> 90%); Fleshy fruit, purple-green, yellow-green pulp color, plum-shaped, 5-7.5 ㎝ long and 4-5 ㎝ in diameter; Fruiting June to next year April.

Diagnostic characters: Red stripes on the outside of petals, yellow stigma lobes, purple-green fruit, plum-shaped fruit, green-yellow pulp, peculiar phenomenon (securely generated fruit, flower growing from fruit, stem growing from fruit).

Holotype: Taepyeong-ro 200, Seogwipo, Jeju Province, Korea. (33°14'40.6" N 126°32'38.0" E) Oct. 17, 2020. (YANG-202201) Herbarium of Jeju Baiknyuncho Museum. Donation to Herbarium of Jeju Baiknyuncho Museum (JBM).

Isotype: Taepyeong-ro 200, Seogwipo, Jeju Province, Korea. (33°14'40.6" N 126°32'38.0" E) Oct. 17, 2020. (YANG-202202, YANG-202203, YANG-202204) Herbarium of Jeju Baiknyuncho Museum (JBM).

Distribution: Jeju Island, Korea

Etymology: The specific epithet was derived from Jeju

Fig. 7. Holotype of *O. monacantha* (Willd.) Haw. f. *jejuensis* J. K. Kim. ex Y.S. Yang.

Island, where this new taxon is located.

Habitats: *O. monacantha* f. *jejuensis* grows on the coast of Seogwipo-si, Jeju-si, Jeju Province, Korea

Identification Key in *O. monacantha* complex

1a Yellow-orange or orange flowers ····· *Elatae* series

1b Yellow or Yellow-orange flowers ····· 2(*O. monacantha* complex)

2a Greenish stigma lobes ····· *O. monacantha* subsp. *arechavaletae*

2b Creamish stigma lobes ····· 3(*O. monacantha* subsp. *monacantha*)

3a Red stripes inside and outside tepals ····· *O. monacantha* f. *monacantha*

3b Red stripes on the outside of tepals ····· *O. monacantha* f. *jejuensis*

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Appendix 1. Genbank (NCBI) Accession number including *Opuntia* in Korea (*trn*L-F, *mat*K and *nr*ITS)

ID	Species	trnL	matK	nrITS
1	*Opuntia arechavaletae*	JF712714	JF786742	JF786906
2	*Opuntia camanchica*	JF712728	JF786756	JF786917
3	*Opuntia cespitosa*	JF712731	JF786759	JF786920
4	*Opuntia drummondii*	JF712742	JF786770	JF786930
5	*Opuntia ellisiana*	JF712747	JF786775	JF786935
6	*Opuntia engelmannii* var. *engelmannii*	JF712749	JF786777	JF786937
7	*Opuntia erinacea*	JF712753	JF786781	JF786941
8	*Opuntia excelsa*	JF712755	JF786783	JF786942
9	*Opuntia humifusa*	JF712762	JF786790	JF786949
10	*Opuntia lilae*	JF712769	JF786797	JF786955
11	*Opuntia macrocentra*	JF712773	JF786801	JF786959
12	*Opuntia macrorhiza*	JF712774	JF786802	JF786960
13	*Opuntia megacantha*	JF712778	JF786806	JF786963
14	*Opuntia microdasys*	JF712781	JF786809	JF786966
15	*Opuntia monacantha*	JF712782	JF786810	JF786967
16	*Opuntia pachyrrhiza*	JF712785	JF786813	JF786970
17	*Opuntia polyacantha*	JF712794	JF786822	JF786979
18	*Opuntia quimilo*	JF712804	JF786831	JF786988
19	*Opuntia santa-rita*	JF712818	JF786845	JF787001
20	*Opuntia scheeri*	JF712819	JF786847	JF787002
21	*Opuntia stenopetala*	JF712825	JF786852	JF787008
22	*Opuntia stricta*	JF712826	JF786853	JF787009
23	*Opuntia strigil*	JF712829	JF786856	JF787012
24	*Opuntia humifusa*3 (Korea)	KJ735938	KJ735947	KJ735929
25	*Opuntia humifusa*2 (Korea)	KJ735939	KJ735948	KJ735930
26	*Opuntia humifusa*1 (Korea)	KJ735940	KJ735949	KJ735931
27	*Opuntia humifusa* for. *jeollaensis*3 (Korea)	KJ735941	KJ735950	KJ735932
28	*Opuntia humifusa* for. *jeollaensis*2 (Korea)	KJ735942	KJ735951	KJ735933
29	*Opuntia humifusa* for. *jeollaensis*1 (Korea)	KJ735943	KJ735952	KJ735934
30	*Opuntia stricta*3 (Korea)	KJ735935	KJ735944	KJ735926
31	*Opuntia stricta*2 (Korea)	KJ735936	KJ735945	KJ735927
32	*Opuntia stricta*1 (Korea)	KJ735937	KJ735946	KJ735928
33	*Opuntia monacantha* f. *jejuensis*3 (Korea)	OP070934	OP070928	OP070940
34	*Opuntia monacantha* f. *jejuensis*2 (Korea)	OP070935	OP070929	OP070941
35	*Opuntia monacantha* f. *jejuensis*1 (Korea)	OP070936	OP070930	OP070942

Appendix 2. Genbank (NCBI) Accession number in *Elatae* series(*Psb*J-*pet*A, *atp*B-*rbc*L, *trn*L-F, *mat*K and *nr*ITS)

ID	Species	*Psb*J-*pet*A	*atp*B-*rbc*L	*trn*L-F	*mat*K	*nr*ITS
1	*Opuntia arechavaletae*	JF787496	JF787181	JF712714	JF786742	JF786906
2	*Opuntia cochabambensis*	JF787609	JF787202	JF712736	JF786764	JF787046
3	*Opuntia echios*	JF787522	JF787209	JF712744	JF786772	JF786932
4	*Opuntia galapageia*	JF787535	JF787225	JF712760	JF786788	JF786947
5	*Opuntia megasperma*	JF787550	JF787245	JF712780	JF786808	JF786965
6	*Opuntia monacantha*	JF787552	JF787247	JF712782	JF786810	JF786967
7	*Opuntia quimilo*	JF787569	JF787267	JF712804	JF786831	JF786988
8	*Opuntia quitensis*	JF787570	JF787268	JF712805	JF786832	JF786989
9	*Opuntia retrorsa*	JF787575	JF787274	JF712814	JF786839	JF786995
10	*Opuntia sulphurea*	JF787592	JF787294	JF712832	JF786859	JF787015
11	*Opuntia monacantha* for.*Jejuensis* 1	OP070931	OP070937	OP070934	OP070928	OP070940
12	*Opuntia monacantha* for.*Jejuensis* 2	OP070932	OP070938	OP070935	OP070929	OP070941
13	*Opuntia monacantha* for.*Jejuensis* 3	OP070933	OP070939	OP070936	OP070930	OP070942