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Molecular phylogeny elucidates the taxonomy of Indian Dipcadi Medik. (Asparagaceae) and reveal a new species from the bank of Hiranyakeshi River, Maharashtra, India

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Research Article

Keywords: cpDNA, Dipcadi, ITS, Monocots, Scilloideae, Taxonomy

Posted Date: March 14th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4058013/v1

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Abstract

Dipcadi (Scilloideae: Asparagaceae) is a genus of bulbous monocots with about 40 species. Species delimitation within the genus has been troublesome hindering a comprehensive phylogenetics. The most recent phylogeny (of Ornithogaloideae) by Martínez-Azorín et al. (2011) includes six species of *Dipcadi* but none is from India. Here, we reconstructed the phylogeny of Ornithogaloideae by including 23 accessions of Indian *Dipcadi* species. The phylogenetic analyses were based on nucleotide sequences of three plastid regions (*rbc*L, *mat*K and *trn*L-F spacer) and one nuclear region (ITS). *Pseudogaltonia clavata* exhibited sister relationship to *Dipcadi*. Our combined data phylogeny (covering 31 *Dipcadi* taxa) resolved *Dipcadi* into four clades, Clade I, II, III and Clade IV. Clade I, II and III included mainly Indian taxa whereas Clade IV mostly consisted of African species. Clade I included nine taxa including our newly described species *D. mukaianum*. The new species was phylogenetically placed along with *D. erythraeum*, *D. saxorum* and *D. ursulae*. Morphologically, the species resembled *D. montanum* and *D. ursulae* but differed in characters such as tepal cohesion (1/2), number of ovules per locule and foul-smelling flowers. Clade II and III included 11 and six taxa, respectively. *D. erythraeum* which has a native range from Egypt to western India was found to occupy Clade I and Clade IV. The accession sampled in the present study was placed in Clade I along with *D. saxorum* whereas the accession from Bahrain grouped with African species (*D. gracillimum* and *D. longifolium*) in Clade IV. Similarly, *D. serotinum* was found sister to the Indian Clade (Clade I, II and III) whereas *D. serotinum* var. *fulvum* was nested in the African Clade (Clade IV). Our studies suggest that additional molecular markers (plastid as well nuclear) should be tested for their taxonomy utility. A robust phylogeny can elucidate the biogeography of this small genus across three continents, Africa, Asia and Europe.

Introduction

Asparagaceae commonly known as the *Asparagus* family is placed in the largest order of the monocots, i.e. Asparagales (APG III 2009). The order comprises ca. 36,265 species (Birch and Kocyan 2021). Asparagaceae is divided into seven subfamilies, viz. Agavoideae, Aphyllanthoideae, Asparagoideae, Brodiaeoideae, Lomandroideae, Nolinoideae and Scilloideae (Chase et al. 2009). Chase et al. (2009) distinguished Scilloideae based on characters such as perennial bulbs, six tepals and stamens and a superior ovary. Scilloideae is further divided into four tribes, i.e. Hyacintheae, Ornithogaleae, Oziroëeae and Urgineeae (Chase et al. 2009). Scilloideae has also been treated as the family Hyacinthaceae (Manning et al. 2009; Martínez-Azorín et al. 2011). Following this taxonomic treatment, the aforementioned tribes can be upgraded as Hyacinthoideae, Ornithogaloideae, Oziroëoideae and Urgineoideae. Phylogenetic relationships within the subfamily Ornithogaloideae have been elucidated by Manning et al. (2009) and Martínez-Azorín et al. (2011). Manning et al. (2009) used four plastid regions (*mat*K, *trn*L intron, *trn*L-F spacer and *rbc*L) and included 70 species for their analysis. They recognized four genera: *Albuca* L., *Dipcadi* Medik., *Ornithogalum* L. and *Pseudogaltonia* (Kuntze) Engl. Martínez-Azorín et al. (2011) included the nuclear marker, intergenic transcribed spacer (ITS) region in addition to

the four plastid regions (*mat*K, *trn*L intron, *trn*L-F spacer and *rbc*L), sampled 80 species and recognized 19 genera.

Dipcadi, a bulbous scapigerous herbaceous genus has been placed in the subfamily Ornithogaloideae (Manning et al. 2009; Martínez-Azorín et al. 2011). The type species for the genus is *Dipcadi serotinum* (L.) Medik. which is based on the Linnaean binomial *Hyacinthus serotinus* L. The genus was separated from *Hyacinthus* L. based on flattened seeds and tubular flowers (Medikus 1790). *Dipcadi* is an Old World genus mainly confined to Africa, the Middle East, the Indian subcontinent, some parts of Europe and the islands of Socotra and Madagascar (POWO 2024) (Fig. 1). There are 44 species worldwide and 13 species occur in India (Deb and Dasgupta 1978; Rao et al. 2016; Ravichandran et al. 2020; Chandore et al. 2021). Most of the Indian species are distributed in the peninsular region with high concentration in the Western Ghats. Ten species, viz. *D. concanense, D. coimbatorensis, D. goaense, D. janae-shrirangii, D. krishnadevarayae, D. maharashtrense, D. minor, D. reidii*, *D. saxorum* and *D. ursulae* are endemic to the country (modified after Mao and Dash 2020).

The taxonomy of *Dipcadi* in India has not been revised since Deb and Dasgupta (1978). Also, the genus is poorly represented in Indian herbaria on account of its succulent habit and ephemeral flowering. Consequently, the taxonomy is confusing. Therefore, in the present investigation, we sampled 23 accessions covering all the Indian species of *Dipcadi.* DNA sequences of three plastid regions (*rbc*L, *mat*K and *trn*L-F spacer) and one nuclear region (ITS) were generated from the sampled taxa and combined with previously available sequences from NCBI to conduct the most comprehensive phylogenetic analysis of *Dipcadi* to date. The aims of the present study were: 1) to get an insight into the phylogenetic relationships of 23 accessions of Indian *Dipcadi* at subfamily level 2) to resolve the taxonomy of the genus in India and 3) to describe a new species of *Dipcadi* based on morphological and molecular data.

Materials and Methods

Sample collection and Taxonomy

The plant materials for the present study were collected from 23 different localities in India (Table 1). Vouchers were deposited in the Herbarium of Shivaji University, Kolhapur (SUK) (Table 1). Plants are being maintained in the Botanic Garden of the Department of Botany, Shivaji University, Kolhapur. The morphological analysis and description were based on field observations and examination of live specimens. The identity of specimens was ascertained by consulting protologues, type specimens and specimens of related species. We studied the specimens housed at major Indian herbaria such as BAMU, BLAT, BSD, BSI, BSID, BSJO, CAL, DD, MH, NGCPR and SKU. Types and voucher specimens available virtually in the herbaria K and C were also examined. Taxonomic description of the new species was made following the terminology of Hickey and King (2001).

Table 1
Collection localities and voucher details of *Dipcadi* accessions.

Sr. No.	Accessions (locality name for unidentified species)	Collection locality	GPS coordinates	Altitude (m)	Voucher Specimen
1.	*Dipcadi coimbatorensis*	Boluvampatti forest, Nilgiri Biosphere Reserve, Coimbatore district, Tamil Nadu	N 10º 59' 11.714" E 76º 42' 7.375"	635	PES018
2.	*Dipcadi concanense*	Sakhar, Rajapur, Ratnagiri district, Maharashtra	N 16° 33' 43.281" E 73° 24' 55.8"	105	PES031
3.	*Dipcadi erythraeum*	Near Amarsagar, Jaisalmer district, Rajasthan	N 26º 55'42.894" E 70º 52' 36.76"	200	PES017
4.	*Dipcadi goaense*	Kevona, Rivona, South Goa district, Goa	N 15° 08' 22.937" E 74° 07' 58.62"	52	PES030
5.	*Dipcadi janae-shrirangii*	Near Narvan, Guhagar, Ratnagiri, Maharashtra	N 17° 20' 59.308" E 73°13' 41.821"	13	PES020
6.	*Dipcadi krishnadevarayae*	Sri Krishnadevaraya University, Anantapur district, Andhra Pradesh	N 14º 36' 37.128" E 77º 38' 50.225"	400	PES025
7.	*Dipcadi montanum*	Near Varebua temple, Burondi, near Dapoli, Ratnagiri district, Maharashtra	N 17° 41' 24.2" E 73° 08' 12.7"	100	PES033
8.	*Dipcadi montanum* var. *madrasicum*	Bharathidasan University, Tiruchirappalli district, Tamil Nadu	N 10º 40' 32.376" E 78º 44' 31.253"	81	PES005

Sr. No.	Accessions (locality name for unidentified species)	Collection locality	GPS coordinates	Altitude (m)	Voucher Specimen
9.	*D. mukaianum*	Ramtirth waterfall, Ajara, Kolhapur district, Maharashtra	N 16° 6' 57.636" E 74° 12' 39.492"	683	PES036
10.	*Dipcadi reidii*	Pangla, Dharchula district, Uttrakhand	N 29º 59' 5.797" E 80º 41' 9.495"	1600	PES026
11.	*Dipcadi saxorum*	Kanheri Caves, Borivali, Mumbai Suburban district, Maharashtra	N 19º 13' 22.837" E 72º 54' 57.53"	200	PES013
12.	*Dipcadi ursulae*	Masai Plateau, Kolhapur district, Maharashtra	N 16° 49' 18.969" E 74° 02' 57.782"	964	PES032
13.	*Dipcadi ursulae* var. *longiracemosum*	Ralegaon Hills, Junnar, Pune district, Maharashtra	N 19º 12' 22.204" E 73º 45' 24.695"	800	PES015
14.	*Dipcadi* sp. (Ankai Fort)	Ankai Fort, Satmala range, Yeola, Nashik district, Maharashtra	N 20° 11' 17.094" E 74° 26' 35.688"	900	PES024
15.	*Dipcadi* sp. (Badami)	Badami, Bagalkot district, Karnataka	N 15º 55' 19.891" E 75º 41' 28.293"	613	PES034
16.	*Dipcadi* sp. (Belgaum)	Jadhavnagar ground, Belgaum, Belgavi district, Karnataka	N 16º 19' 59.98" E 74° 45' 00"	730	PES002
17.	*Dipcadi* sp. (Delhi)	Near ridges in Delhi University, North Campus, New Delhi	N 28º 41' 3.472" E 77º 13' 1.429"	207	PES007

Sr. No.	Accessions (locality name for unidentified species)	Collection locality	GPS coordinates	Altitude (m)	Voucher Specimen
18.	*Dipcadi* sp. (Dolkhamb)	Roadside near Dolkhamb, Shahapur, Thane district, Maharashtra	N 19º 29' 35.655" E 73º 34' 51.869"	200	PES027
19.	*Dipcadi* sp. (Gautala)	Near Sitakhori waterfall, Gautala Wildlife Sanctuary, Aurangabad district, Maharashtra	N 20º 22' 3.698" E 75º 11' 2.951"	630	PES022
20.	*Dipcadi* sp. (Halkarni)	Yashwantroa Chavan Mahavidyalay, Halkarni, Gadhinglaj, Kolhapur district, Maharashtra	N 15° 54' 53.086" E 074°17' 39.003"	748	PES029
21.	*Dipcadi* sp. (Mhaismal)	Mhaismal, Aurangabad district, Maharashtra	N 20º 05' 20.713" E 75º 11' 5.086"	800	PES028
22.	*Dipcadi* sp. (Rantale)	Rantale, Rajapur, Ratnagiri district, Maharashtra	N 16° 39' 23.2" E 73º 30' 0.519"	179	PES010
23.	*Dipcadi* sp. (Yercaud)	Yercaud, Tamil Nadu	N 11° 47' 23.2" E 78° 11' 32.416"	1200	PES006

Molecular data

Total genomic DNA was extracted from fresh leaf material of collected specimens using the modified CTAB method (Doyle and Doyle 1987). The modifications made are as follows: After phase separation with phenol:chloroform (24:1), to the aqueous phase 0.7 volumes of chilled *iso*-propanol was added. This was incubated overnight at -20℃ to precipitate out the DNA. The next day samples were transferred onto columns and treated with a solubilization buffer (Qiagen Buffer QG) and then centrifuged for 10 minutes at 14,000 X g. Columns were washed twice with a wash buffer (Qiagen Buffer QC). Columns were dried and DNA was eluted in 50 µl of nuclease-free water. Previously, available sequences of plastid regions (*rbc*L, *mat*K and *trn*L-F) and nrITS region (after Manning et al. 2009; Martínez-Azorín et al. 2011) were successfully utilized for phylogenetic study of the subfamily Ornithogaloideae (Hyacinthaceae). We used the same regions so as to include the published data. The *rbc*L was amplified using primer pair *rbc*La-F

(Levin et al. 2003, modified from Soltis et al. 1992) and *rbc*La-R (Kress 2009 modified from Fofana et al. 1997). The *mat*K region was amplified using KIM-3F (CGTACAGTACTTTTGTGTTTACGAG) and KIM-1R (ACCCAGTCCATCTGGAAATCTTGGTTC) primers (Kim, unpublished). The *trn*L-F region was amplified using primers c and f (Taberlet et al. 1991). Primers ITS1 and ITS4 from White et al. (1990) were used to amplify the nuclear ITS region. PCR reactions were performed in 30 µL reactions containing 15 µL nuclease-free water, 10 µL EmeraldAmp GT PCR Master Mix (TaKaRa, Japan) and 1 µL each forward and reverse primer (10 µM). PCR reactions were carried out in a Thermal cycler (TaKaRa, Japan). PCR protocol for *rbc*L, *trn*L-F and ITS regions was 94 ^{O}C for 4 min, 35 cycles (94 ^{O}C for 30 s, 55 ^{O}C for 1 min and 72 ^{O}C for 1 min). For *mat*K it was 94°C for 5 min, 35 cycles (94°C for 30 s, 52°C for 20 s and 72°C for 50 s), and for the all the regions final extension was carried out at 72°C for 10 min. The amplified PCR product was purified and sequenced at Macrogen Corporation (Seoul, Korea). All generated sequences were submitted to the NCBI GenBank database. Voucher ID and GenBank accession numbers for collected specimens are provided in Table 2.

Table 2
Taxa included in phylogenetic analysis.

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
A. abyssinica Jacq.	Manning 2687 (NBG), Chase 1784 (K)	AM902289	AM902391	AM902435	HQ615121
A. amboensis (Schinz) Oberm.	Beaumont 568 (NBG)	AM902290	AM902392	AM902436	HQ615122
A. aff. chaetopoda Chiov.	Chase 1845 (K)	HQ645917	-	HQ645887	HQ615123
A. cooperi Baker	Chase 24814 (K)	HQ645918	-	HQ645888	HQ615124
A. fragrans Jacq.	Manning 2184 (NBG)	HQ645919	-	HQ645889	HQ615125
A. glandulosa Baker	Goldblatt & Manning 11528 (NBG)	HQ645920	-	HQ645890	HQ615126
A. pendula B.Mathew	Chase 1776 (K)	HQ645921	-	HQ645891	HQ615127
A. rupestris Hilliard & B.L.Burtt	-	HQ645922	-	HQ645892	HQ615128
A. setosa Jacq.	Manning 2689 (NBG)	AM902292	AM902393	AM902438	HQ615129
A. shawii Baker	Chase 1012 (K)	AM902293	AM902394	AM902439	HQ615130
A. tortuosa Baker	Chase 1736 (K)	AM902294	AM902395	AM902440	HQ615131
A. wakefieldii Baker	Chase 1843 (K)	HQ645923	-	HQ645893	HQ615132
Avonsera					
A. convallarioides (H.Perrier) Speta	Lavranos s.n. (NBG)	AM902337	AM902431	AM902484	-
Battandiera					
B. amoena (Batt.) Maire	SALA 135875	HQ645916	-	HQ645886	HQ615116

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
B. pulchra (Schinz) Mart.-Azorín, M.B.Crespo & Juan	Mannheimer CM2506 (NBG, WIND)	AM902317	AM902417	AM902464	HQ615114
B. seineri (Engl. & Krause) Mart.-Azorín, M.B.Crespo & Juan	Snijman s.n. (no voucher)	AM902314	-	AM902366	AM902461
B. stapffii (Schinz) Mart.- Azorín, M.B.Crespo & Juan	Mannheimer 1747 (WIND)	AM902315	AM902415	AM902462	HQ615115
Cathissa					
C. concinna (Salisb.) Speta	ABH 47140	HQ645907	-	HQ645877	HQ615086
C. reverchonii (Lange ex Willk.) Speta	ABH 47138	HQ645908	-	HQ645878	HQ615087
C. reverchonii (Lange ex Willk.) Speta	Chase 1721 (K)	AM902331	AM902427	AM902478	-
C. unifolia (L.) Mart. Azorín, M.B.Crespo & Juan	ABH 50131	AM992656	AM992663	AM992634	HQ615085
Coilonox					
C. concordianum (Baker) Speta	Goldblatt, Manning & Savolainen 11527 (MO, NBG)	AM902296	AM902397	AM902442	HQ615118
C. secundum (Jacq.) Speta	Manning s.n. (no voucher)	AM992647	AM992674	AM992644	HQ615119
C. suaveolens (Jacq.) Speta	Goldblat, Manning & Savolainen 11493 (NBG)	AM902295	AM902396	AM902441	HQ615120
Dipcadi					

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
D. coimbatorensis V.Ravich., R.Kr.Singh & Murugan	PES018	PP340388[a]	PP340434[a]	PP340411[a]	PP335107[a]
D. concanense (Dalzell) Baker	PES009	PP340389[a]	PP340435[a]	PP340412[a]	PP335108[a]
D. concanense	GUH 5037	-	KP645194	-	KF871334
D. erythraeum Webb & Berthel.	PES017	PP340390[a]	PP340436[a]	PP340413[a]	PP335109[a]
D. erythraeum	BAHMP029311216R	MH133120	MH211039	-	-
D. erythraeum	EDNA15-0042943	KX282698	-	-	KX282110
D. erythraeum	EDNA15-0042845	KX282696	-	-	KX282109
D. glaucum (Burch. ex Ker Gawl.) Baker	Manning 2237	AM902301	AM902402	AM902447	HQ615134
D. goaense Prabhug., U.S.Yadav & Janarth.	PES014	PP340391[a]	PP340437[a]	PP340414[a]	PP335110[a]
D. goaense	GUH 5166	-	KP645195	-	KF871335
D. gracillimum Baker	Manning 2682	AM902298	AM902399	AM902444	HQ615135
D. janae-shrirangii Chandore, Borude, Bhalekar, Madhav & Gosavi	PES019	PP340392[a]	PP340438[a]	PP340415[a]	PP335111[a]
D. krishnadevarayae B.R.P.Rao	PES025	PP340393[a]	PP340439[a]	PP340416[a]	PP335112[a]
D. longifolium (Lindl.) Baker	MW Chase 16972	AM902302	AM902403	AM902448	HQ615136
D. minor Hook.f.	-	-	-	MK644172	-

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
D. montanum (Dalzell) Baker	PES033	PP340394[a]	PP340440[a]	PP340417[a]	PP335113[a]
D. montanum	DMONR	-	-	-	OL778873
D. montanum	DMONA	-	-	-	OL778851
D montanum var. *madrasicum* (E.Barnes & C.E.C.Fisch.) Deb & S.Dasgupta	PES005	PP340395[a]	PP340441[a]	PP340418[a]	PP335114[a]
D. mukaianum sp. nov. (Ajara)	PES004	PP340396[a]	PP431389[a]	PP340419[a]	PP335115[a]
D. reidii Deb & S.Dasgupta	PES026	PP340397[a]	PP431390[a]	PP340420[a]	PP335116[a]
D. saxorum Blatt.	PES013	PP340398[a]	PP340442[a]	PP340421[a]	PP335117[a]
Dipcadi sp. (Ankai Fort)	PES024	PP340399[a]	PP340443[a]	PP340422[a]	PP335118[a]
Dipcadi sp. (Delhi)	PES007	PP340400[a]	PP431391[a]	PP340423[a]	PP335119[a]
D. serotinum (L.) Medik.	MW Chase 1824 (K)	AM902299	AM902400	AM902445	HQ615137
D. serotinum var. *fulvum* (Cav.) Ball	-	-	-	AJ232602	-
Dipcadi sp. (Gautala)	PES022	PP340401[a]	PP340444[a]	PP340424[a]	PP335120[a]
Dipcadi sp. (Rantale)	PES010	PP340402[a]	PP340445[a]	PP340425[a]	PP335121[a]
Dipcadi sp. (Dolkhamb)	PES027	PP340403[a]	PP340446[a]	PP340426[a]	PP335122[a]
Dipcadi sp. (Halkarni)	PES003	PP340404[a]	PP340447[a]	PP340427[a]	PP335123[a]

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
Dipcadi sp. (Belgaum)	PES002	PP340405[a]	PP340448[a]	PP340428[a]	PP335124[a]
Dipcadi sp. (Badami)	PES012	PP340406[a]	PP340449[a]	PP340429[a]	PP335125[a]
Dipcadi sp. (Yercaud)	PES006	PP340407[a]	PP340450[a]	PP340430[a]	PP335126[a]
Dipcadi sp. (Mhaismal)	PES028	PP340408[a]	PP340451[a]	PP340431[a]	PP335127[a]
Dipcadi ursulae Blatt.	PES001	PP340409[a]	PP340452[a]	PP340432[a]	PP335128[a]
Dipcadi ursulae Blatt.	DURSU	-	-	-	OL778874
Dipcadi ursulae var. *longiracemosum* Deb & S.Dasgupta	PES015	PP340410[a]	PP340453[a]	PP340433[a]	PP335129[a]
Dipcadi viride (L.) Moench	Manning s.n.	AM902300	AM902401	AM902446	HQ615138
Dipcadi fillifolium Baker	MW Chase 1783 (K)	AM902303	AM902404	AM902449	HQ615133
Eliokarmos					
E. bicornutus (F.M.Leight.) Mart.-Azorín, M.B.Crespo & Juan	Goldblatt & Manning 11503 (NBG)	AM902320	AM902420	AM902467	HQ615105
E. ceresianus (F.M.Leight.) Mart.-Azorín, M.B.Crespo & Juan	ABH 49975	HQ645912	-	HQ645882	HQ615097
E. conicus (Jacq.) Mart.-Azorín, M.B.Crespo & Juan	Manning 2993 (NBG)	AM902324	AM902423	AM902471	HQ615095

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
E. constrictus (F.M.Leight.) Mart.-Azorín, M.B.Crespo & Juan	Manning s.n. (NBG)	AM902325	-	AM902472	HQ615104
E. corticatus (Mart.-Azorín) Mart.-Azorín, M.B.Crespo & Juan	ABH 51609; Martínez-Azorín & Manning 96 (K, MO, NBG)	HQ645910	AM992659	AM992630	HQ615099
E. dubius (Houtt.) Mart.-Azorín, M.B.Crespo & Juan sensu stricto	Martínez-Azorín & Manning 6 (NBG)	HQ645913	-	HQ645883	HQ615101
E. dubius (Houtt.) Mart.-Azorín, M.B.Crespo & Juan sensu stricto	Manning & Snijman 2722 (NBG)	AM902326	AM902424	AM902473	-
E. dubius (= Ornithogalum fimbrimarginatum F.M.Leight.)	Martínez-Azorín & Manning 8 (NBG)	HQ645914	-	HQ645884	HQ615102
E. neomaculatus Mart.-Azorín, M.B.Crespo & Juan	Manning s.n. (no voucher)	AM902340	AM902434	AM902487	HQ615107
E. pilosus (Jacq.) Mart.-Azorín, M.B.Crespo & Juan	Manning 2696 (NBG)	AM902328	-	AM902475	HQ615103
E. pruinosus (F.M.Leight.) Mart.-Azorín, M.B.Crespo & Juan	Goldblatt, Manning & Savolainen 11526 (NBG	AM902323	AM902422	AM902470	HQ615100
E. rupestris (L.f.) Mart.-Azorín, M.B.Crespo & Juan	Manning s.n. (no voucher)	AM902335	AM902429	AM902482	HQ615106

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
E. strictus (L.Bolus) Mart.-Azorín, M.B.Crespo & Juan	ABH 49977; Manning & MartínezAzorín 3 (NBG)	HQ645929	AM992662	AM992633	HQ615098
E. thyrsoides (Jacq.) Raf.	ABH 50035; Martínez-Azorín & Manning 13 (NBG)	HQ645911	-	HQ645881	HQ615096
Elsiea					
E. corymbosa F.M.Leight.	Helme 2850 (NBG)	AM902316	AM902416	AM902463	HQ615091
Ethesia					
E. haalenbergensis (U.Müll.- Doblies & D.Müll.-Doblies) Mart.-Azorín, M.B.Crespo & Juan	Mannheimer 2443 (WIND)	AM902334	AM902428	AM902481	HQ615111
E. haalenbergensis (U.Müll.- Doblies & D.Müll.-Doblies) Mart.- Azorín, M.B.Crespo & Juan	-	JX090463	JX090388	JX090570	-
E. xanthochlora (Baker) Mart.-Azorín, M.B.Crespo & Juan	Goldblatt, Manning & Savolainen 11491 (NBG)	AM902308	AM902409	AM902455	HQ615140
Galtonia					
G. candicans (Baker) Decne	Chase 2024 (K)	AM902305	AM902407	AM902452	HQ615141
G. saundersiae (Baker) Mart.-Azorín, M.B.Crespo & Juan	ABH 50132	HQ645924	-	HQ645894	HQ615139

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
G. saundersiae (Baker) Mart.-Azorín, M.B.Crespo & Juan	Chase 1871	AM902307	-	AM902454	-
G. viridiflora Verdoorn	Chase 17517 (K)	AM902306	AM902408	AM902453	HQ615142
Honorius					
H. nutans (L.) Gray	Martínez-Azorín s.n. (no voucher)	AM992658	AM992660	AM992631	HQ615083
Loncomelos					
L. pyrenaicus (L.) L.D.Hrouda ex Holub	ABH 50135	AM992655	AM992664	AM992635	HQ615081
Loncomelos pyrenaicus (L.) L.D.Hrouda	NMW7135	MK925306	MK925642	-	-
Melomphis					
M. arabica (L.) Raf.	ABH 50134	AM992653	AM992667	AM992638	HQ615084
Melomphis arabica (L.) Raf.	BM 2008/700	HM850214	HM850501	-	-
Neopatersonia					
N. namaquensis G.J.Lewis	Goldblatt & Manning 11369 (NBG)	AM902309	AM902410	AM902456	HQ615145
N. rotata (U.Müll.-Doblies & D.Müll.-Doblies) Mart.-Azorín, M.B.Crespo & Juan	Manning 2275 (NBG)	AM902313	AM902414	AM902460	HQ615143
N. uitenhagensis Schönland	Goldblatt & Manning 10723 (MO, NBG)	AM902310	AM902411	AM902457	HQ615144
Nicipe					

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
N. gracillima (R.E.Fr.) Mart.-Azorín, M.B.Crespo & Juan	Chase 1863 (K)	AM992651	-	AM992640	HQ615092
N. graminifolia (Thunb.) Mart.-Azorín, M.B.Crespo & Juan	ABH 49981; Martínez-Azorín & Manning 7 (NBG)	HQ645909	-	HQ645879	HQ615088
N. juncifolia (Jacq.) Mart.-Azorín, M.B.Crespo & Juan	Manning 2699 (NBG)	AM902322	AM902421	AM902469	HQ615089
N. paludosa (Baker) Mart.-Azorín, M.B. Crespo & Juan	Manning 3149 (NBG)	AM992649	AM992671	AM992642	HQ615090
N. sephtonii (Hilliard & B.L.Burtt) Mart.-Azorín, M.B.Crespo & Juan	Snijman 2036 (NBG)	AM902336	AM902430	AM902483	HQ615093
Ornithogalum					
O. armeniacum Baker	Chase 1682.B (K)	AM992654	AM992665	AM992636	HQ615073
O. baeticum Boiss.	ABH 47137	AM992652	AM992668	AM992639	HQ615068
O. bourgaeanum Jord. & Fourr.	ABH 47142	HQ645930	AM992666	AM992637	HQ615069
O. chionophilum Holmboe	K Mark Wayne Chase at Jodrell lab. MWC20637	AM992650	AM992670	AM992641	HQ615079
O. kochii Parl.	Peruzzi s.n. (no voucher)	HQ645899	-	HQ645869	HQ615070
O. lanceolatum Labill.	-	HQ645904	-	HQ645874	HQ615076

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
O. montanum Ten.	ABH 50139	HQ645906	-	HQ645876	HQ615080
O. monticolum Jord. & Fourr.	Peruzzi s.n. (no voucher)	HQ645900	-	HQ645870	HQ615071
O. oligophyllum E.D.Clarke	Chase 28108 (K)	HQ645902	-	HQ645872	HQ615074
O. orthophyllum Ten.	Peruzzi s.n. (no voucher)	HQ645901	-	HQ645871	HQ615072
O. orthophyllum Ten.	Chase 1823	JX090467	JX090391	JX090574	-
O. pedicellare Boiss. & Kotschy	K Mark Wayne Chase at Jodrell lab. MWC20634	AM992648	AM992672	AM992643	HQ615077
O. refractum Willd.	Chase 20632 (K)	HQ645903	-	HQ645873	HQ615075
O. sintenisii Freyn	Chase 20636 (K)	HQ645905	-	HQ645875	HQ615078
Pseudogaltonia					
P. clavata (Mast.) Phillips	Codd sub Chase 2255 (K)	AM902304	AM902406	AM902356	AM902451
Stellarioides					
S. longebracteata (Jacq.) Speta	Chase 1507 (K)	AM902312	AM902413	AM902459	HQ615108
S. longebracteata (Jacq.) Speta	MO: Carlsen 3246	OL536736	OL689867	-	OL536351
S. sordida (Baker) Speta	Chase 1846 (K)	HQ645915	-	HQ645885	HQ615109
S. tenuifolia (Redoute) Speta	Manning 2667 (NBG)	AM902318	AM902418	AM902465	HQ615110
Trimelopter					
T. etesiogaripense (U.Müll.- Doblies & D.Müll.-Doblies) Mart.-Azorín, M.B.Crespo & Juan	Goldblatt & Manning 11352 (NBG)	AM902311	AM902412	AM902458	HQ615113

[a]DNA sequences generated in this study

Taxa	Voucher ID	GenBank accession numbers			
		*rbc*L	*mat*K	*trnL-F*	ITS
Albuca					
T. unifolium (Retz.) Mart.-Azorín, M.B.Crespo & Juan	Manning 2270b (NBG)	AM902319	AM902419	AM902466	HQ615112
Outgroup					
Bowiea volubilis Harv. ex Hook.f	Chase 176 (NCU, K)	Z69237	AB088790	AJ232577	HQ615146

[a]DNA sequences generated in this study

DNA sequence alignment and phylogenetic analyses

DNA sequences were checked and edited using Sequencher v 5.1 (Gene codes Corporation, 2012). A total of 92 sequences of *rbc*L, *mat*K, *trn*L-F and ITS region were generated in this study (Table 2) and sequences of some *Dipcadi* taxa were retrieved from NCBI. Some unidentified *Dipcadi* taxa were also sampled to assess their relationship with other members of subfamily Ornithogaloideae. We used plastid and ITS sequences of the members of Ornithogaloideae from the study of Martinez-Azorin et al. (2011) for phylogenetic analyses. Multiple sequence alignments were carried out using MUSCLE (Edgar, 2004) implemented in MEGA X (Kumar et al. 2018). Alignments of each gene were then further refined using GBlocks v. 0.91b (Talavera & Castresana 2007). Three datasets cpDNA (Supplementary material 1), nrITS (Supplementary material 2) and combined cpDNA + ITS (Supplementary material 3) were used for phylogenetic analyses. For checking incongruence among cpDNA data set (*rbc*L + *mat*K + *trn*L-F) and nuclear ITS dataset, the incongruence length difference (ILD) test was performed (Farris et al. 1994) in PAUP 4.0a152 (Swofford 2002). Further, the combinability of the cpDNA data and ITS data was assessed by constructing phylogeny based on cpDNA data and ITS data and comparing topologies. The ILD test was performed with 1000 heuristic searches, by keeping simple taxon addition and Tree Bisection and Reconnection (TBR) as branch swapping algorithm. Both the dataset concatenated into a single matrix using SequenceMatrix (Vaidya et al. 2011). Phylogenetic analyses were carried out using Maximum Likelihood (ML) and Bayesian Inference (BI) methods based on the best-fit nucleotide substitution model. To root the phylogenetic tree, we used *Bowiea volubilis* Harv. ex T.Moore & Mast. as an outgroup. Each dataset was treated as single partition, model of nucleotide substitution for cpDNA, nrITS and cpDNA + nrITS datasets was selected under the Akaike Information Criterion (AIC) using jModelTest 2 (Darriba et al. 2012) on XSEDE on the CIPRES science gateway (http://www.phylo.org/) (Miller et al. 2010). The best-fit model for all the three datasets was GTR + I + G. The ML analysis including bootstrapping (BS) was conducted using IQ-TREE (Nguyen et al. 2015) via IQ tree web server (Trifinopoulos et al. 2016) by employing 1000 bootstrap replicates. BI implementing Markov Chain Monte Carlo (MCMC) was

conducted using MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003). MCMC consisted of four independent runs of 50,000,000 generations with trees sampled every 1000 generations. The first 10% trees were discarded as burn-in and the remaining were used to generate a bayesian tree with all compatible groups having posterior probabilities (PPs) for each node. The average standard deviation of split frequencies at the end of the run was below 0.01. In Tracer 1.7.1 (Rambaut et al. 2018), the effective sample size (ESS) presented values well above 200 for all statistics, which ensured that the selected settings were sufficient for the sampling.

Results

Morphology

Taxonomic Treatment

Dipcadi mukaianum Shelke, S.R.Yadav & Lekhak **sp. nov.** (Figs. 3 and 4)

Type: India, Maharashtra, Kolhapur district, Ajara, near Ramtirth waterfall, on a rocky slope, in loamy soil, 16° 6' 57.636"N 74° 12' 39.492"E, ± 683 m elevation, 3rd July 2021. P.E. Shelke, M.M. Lekhak & S.R. Yadav PES004 (holotype CAL; isotypes SUK, BSI)

Diagnosis

Dipcadi mukaianum is closely related to *D. montanum* and *D. ursulae*. The new species differs in its long and thin perianth tube and shorter bract (vs short and thick perianth tubes, longer bracts in *D. montanum* and *D. ursulae*). The flowers of *D. mukaianum* are foul smelling while *D. montanum* and *D. ursulae* flowers smell sweet. Other morphological differences are given in Table 3 and depicted in Fig. 5.

Table 3
Comparison of morphological attributes of *D. montanum, D. mukaianum* and *D. ursulae*

Attributes	*D. montanum*	*D. mukaianum* sp. nov.	*D. ursulae*
Leaf	3–6 per bulb, 150–400 x 2–8 mm long	3–6 per bulb, 150–500 x 5–20 mm long	2–8 per bulb, 200–500 x 2– 30 mm long
Scape	Mostly solitary, 200–600 mm long	Mostly solitary, rarely two per individual, 200–800 mm long	Mostly solitary, rarely two per individual, 200–500 mm long
Flowers	20–25 flowers per scape, 10–13 mm long, sweet-smelling	20–25 flowers per scape, 8–15 mm long, foul-smelling	12–20 flowers per scape, 10–15 mm long, sweet-smelling
Bract	10–18 x 2 – 5 mm	10–12 x 5–6 mm	10–25 x 2–6 mm
Pedicel	8–10 mm long	5–8 mm long	5–8 mm long
Cohesion of tepals	2/3	1/2	1/3
Tepal inner	16–17 x 30–33 mm	15–16 x 30–33 mm	15–16 x 30–33 mm
Tepal outer	17–18 x 30–35 mm	16–17 x 30–35 mm	17–18 x 30–35 mm
Gynoecium length	10–12 mm	10–15 mm	10–12 mm
Stipe length	1.5 mm	1 mm	1 mm
Ovules per locule	8–12	9–16	6–16

Bulbous, scapigerous herbs, 20–85 cm high. *Bulbs* ovoid 10–40 x 10–30 mm, rarely bulbiliferous producing 1–2 bulbils; outer scales, brown scarious; inner scales, fleshy, white coloured. *Leaves* 3–6 arising from the bulb, 15–50 x 0.5–2 cm, simple, linear, apex acute, margin entire, base sheathing, glabrous. *Scapes* solitary (rarely 2), 20–80 cm long, erect, terete, glabrous, whitish-green. *Inflorescences* a raceme, 20–25-flowered. *Flowers* greenish-white to brownish in colour, campanulate, pedicellate, bracteate, actinomorphic, bisexual, hypogynous; pedicels 5–8 mm long, ascending, green; bracts, ovate, apex acuminate, 10 – 12 x 5–6 mm long, thin, scarious at flower maturity. *Perianth tubes* 8–15 x 30–33 mm long; tepals 6, biseriate, 3 + 3, connate half of their length at the base; outer tepals 16–17 mm, apex rounded or obtuse with a minute hood, partly reflexed; inner tepals 15–16 mm long, apex acute, connate at the throat of the tube and reflexed 2/3rd of their length. *Stamens* 6, 10–12 mm long, epitepalous, tapering at apex, broader at base; anthers 2 mm long, bilobed, dithecous, dorsifixed, light green, introrse, dehiscence through longitudinal slits; filaments flat, ribbon-like, 10–12 mm long. *Pistil* 1, carpels 3, stipitate; stipes 1–1.2 mm long, terete; ovary, 4–5 x 2–3 mm, superior, trigonous, ellipsoid, green, ovules 8–10 per locule, placentation axile; styles 5–7 mm long, ridged, stout, white; stigma white, trilobed with

sub-lobes, papillose. *Capsules* 8–12 x 10–12 mm, trilocular, loculicidal, 9–16 seeds per locule; seeds, 4–6 x 3–5 mm, ovoid, discoid, flat, black with a glossy texture.

Phenology

Flowering in June to August and fruiting in August.

Distribution

It is only found near Ramtirth waterfall, Ajara, Kolhapur district, Maharashtra, India.

Ecology

Dipcadi mukaianum grows in sandy and gravelly soil, near the Hiranyakeshi River and grasslands in association with *Hypoxis aurea* Lour., *Iphigenia indica* (L.) A.Gray ex Kunth, *I. pallida* Baker, *Ledebouria revoluta* (L.f.) Jessop, *Curculigo orchioides* Gaertn., etc.

Etymology

The species is named after Prof. Yasuhiko Mukai, Osaka Kyoiku University, Osaka for his contributions in the field of molecular cytogenetics in general and wheat cytogenetics in particular.

Molecular phylogeny

For phylogenetic analysis we have created three datasets; plastid, nrITS and combined (cpDNA + ITS). cpDNA dataset included 102 taxa (117 accessions) with an aligned length of 1850 bp of which 1442 were conserved and 438 variable and 200 were parsimony informative sites. The ITS dataset comprised 109 accessions representing 97 taxa with an alignment length of 618 bp of which 293 conserved and 325 variable and 245 were parsimony informative sites. The combined dataset was made up of a total 103 taxa with 119 accessions with an aligned length of 2468 bp. There were 1710 conserved sites, 758 variable sites and 441 parsimony informative sites. A total of 39 accessions of *Dipcadi* were included in three phylogenetic analyses, viz. combined (Fig. 2), plastid (Fig. S1) and ITS (Fig. S2). Except for plastid phylogeny, combined and ITS phylogeny exhibited similar topology with four well-resolved clades supported by moderate and strong BI PP and ML BS values. Due to the lower resolution of plastid regions only three well-resolved clades were observed. However, the results of the analysis of the individual dataset revealed no significant topological conflicts between cpDNA and ITS data. Also, the incongruence length difference (ILD) test revealed no significant incongruence between the chloroplast and nrITS dataset (p-value = 0.0100); thus, we focused on the results of the combined dataset for further phylogenetic analysis.

Our combined data phylogeny (Fig. 2) reflected two main clades, namely Clade A + B (comprising *Dipcadi*, *Pseudogaltonia*, *Albuca*, *Coilonox*, *Stellerioides*, *Trimplopter* and *Battandiera*) and Clade C (comprising *Ornithogalum*, *Honorius*, *Lancomelos*, *Cathissa*, *Melomphis*, *Nicipe*, *Elsiea*, *Neopatersonia*, *Eliokarmos*,

Galtonia and *Ethesia*). *Dipcadi* was resolved as monophyletic with full support. Within *Dipcadi*, four well-supported clades, namely, Clade I, II, III and Clade IV mostly with low support were retrieved (Fig. 2). Clade I with BI PP = 0.58 and ML BS 73% comprised most of the unidentified *Dipcadi* species collected from Delhi, Belgaum, Halkarni, Yercaud and Badami regions in India. The known taxa which are placed in the same clade were *D. erythraeum* (PES017), *D. saxorum* (PES013) and *D. ursulae* (DURSU). The new species described in this study *D. mukaianum* (PES004) was also placed in the same clade and grouped with species collected from Belgaum, Halkarni and *D. ursulae* (DURSU) with BI PP = 0.78 and ML BS = 83% (Fig. 2). *D. erythraeum* (PES017) sampled in the present study grouped with Clade I whereas the accession from Bahrain (BAHMP029311216R) was placed in Clade IV.

Clade II consisted of *D. coimbatorensis* (PES018), *D. minor*, *D. montanum* (PES033), *D. montanum* var. *madrasicum* (PES005), *D. ursulae* var. *longiracemosum* (PES015), *D. ursulae* (PES001) as well as two unidentified species of *Dipcadi* collected from Rantale (Ratnagiri district) and Dolkhamb (Thane district) region both are confined to lower altitudes (coastal belt), i.e. upto 200 m msl. However, species collected from Ankai fort (Nashik district) and Guatala and Mhaismal (Aurangabad district) regions were confined to altitudes more than 600 m msl and grouped together with BI PP = 0.92 and ML BS = 100% and showed sister relationship to the rest of *Dipcadi* taxa placed in Clade II (Fig. 2). *D. concanense* (GUH5037), *D. montanum* (DMONR), *D. concanense* (PES009), *D. janae-shrirangii* (PES020), *D. goaense* (GUH5166), *D. goaense* (PES014), *D. krishnadevarayae* (PES025) and *D. reidii* (PES026) formed Clade III which has well supported (BI PP = 0.95, ML BS = 98). Nevertheless, our samples of *D. concanense* grouped with *D. janae-shrirangii* whereas the previously sequenced *D. concanense* (GUH5037) instead of grouping with its conspecific species clustered with *D. montanum* (DMONA, DMONR). Our sampled *D. goaense* grouped with its previously sequenced conspecific species *D. goaense* and *D. krishnadevarayae* with BI PP = 0.98 (Fig. 2). *Dipcadi reidii* was sister to all the taxa placed in Clade III (Fig. 2).

Clade IV with BI PP = 0.50 comprised most of the African species, namely *D. viride*, *D. glaucum*, *D. gracillium* and *D. longifolium, D. filifolium* and *D. serotinum* var. *fulvum* (Fig. 2). Contrasting phylogenetic placement of *D. erythraeum* (BAHMP0293111216R) sequenced previously has been observed in Clade IV; as it should have grouped with its conspecific species sampled by us and placed in Clade I.

In the cpDNA dataset, *Dipcadi* was also monophyletic with high support (BI PP = 1, ML BS = 100). However, three not well supported clades were observed instead of four. Clades I and II contained Indian *Dipcadi*, whereas Clade III comprised African *Dipcadi* (Fig. S1). In cpDNA data, *Pseudogaltonia clavata* was sister to *Dipcadi* with strong support (BI PP = 1, ML BS = 100).

In the nrITS dataset, *Dipcadi* was monophyletic without good support (BI PP = 0.66, ML BS = 80) (Fig. S2). However, in the ITS dataset, *Pseudogaltonia clavata* was not available and *Battandiera* showed a sister relationship with *Dipcadi* and this sister relationship was not well supported (BI PP = 0.93). The four clades I, II, III and IV were retreived as in the combined dataset.

Discussion

We have extended the previous comprehensive phylogeny of subfamily Ornithogaloideae provided by Martínez-Azorín et al. (2011) by adding Indian *Dipcadi* sampling into it. As our Indian *Dipcadi* sampling consisted of 10 unidentified taxa, therefore, in order to know their taxonomic status and to test the monophyly of *Dipcadi* and its relationship with members of the subfamily Ornithogaloideae, we reconstructed the subfamily level phylogeny based on plastid and nuclear ITS region. Our reconstructed combined data phylogeny showed grouping of all the genera of subfamily Ornithogaloideae into two clades, namely Clade A + B and Clade C as was observed in the combined data phylogeny of Martínez-Azorín et al. (2011). Only exception is the genus *Avonsera*, as it took the wrong phylogenetic position in plastid phylogeny (Fig. S1). Only *Avonsera* was excluded from the phylogeny because of high level of variation in ITS data leading to a very long branch. All this led us to decide not to consider it in combined phylogeny.

Dipcadi has widely been accepted as a member of Ornithogaloideae by most of the researchers (see Martínez-Azorín et al. 2011). The presence of a unilateral raceme and tepals curved outwards in the apical part are the two autapomorphies that define this genus (Martínez-Azorín et al. 2011). Other characteristic features include tepals fused into a tube about one-quarter to one-third (to two-thirds) of their length, stamens adnate to the tube and included, capsule quadrate in outline, as broad as or broader than long, truncate at the apex, and seeds discoid, flattened, horizontally stacked and uniseriate in each locule with five to seven angled polygonal testa cells (cf. Obermeyer 1964; Jessop 1975; Heywood 1980; Valdés 1987; Speta 1998; Manning et al. 2002, 2009). Previous phylogenetic studies (Pfosser and Speta 1999; Manning et al. 2004; Martínez-Azorín et al. 2011) show that *Dipcadi* forms a fully supported clade, sister to *Pseudogaltonia*. Similarly, our combined data phylogeny also revealed that genus *Dipcadi* forms a clade with BI PP = 1 and ML BS = 100% sister to *Pseudogaltonia* (Fig. 2).

Phylogeny of the genus Dipcadi and phylogenetic placement of the new species

In previous studies, *Dipcadi* has not been well represented and majority of the diversity occurs in India. Therefore, this study is of importance with reference to *Dipcadi*. However, only limited species have been included in family-level phylogeny previously (Manning et al. 2009; Martínez-Azorín et al. 2011). A few studies included some of the *Dipcadi* species in genus-level phylogenies (Deshpande et al. 2015; Abdulkareem and Mustapha 2019). In this study, we report, for the first time, the comprehensive phylogenetic analysis of the genus *Dipcadi* based on the plastid and nuclear ITS region, which includes 31 taxa (21 known and 10 unidentified). Therefore, more than 50% sampling (of the 43 taxa in *Dipcadi*) was used in this study.

The phylogeny resulted into four well-resolved clades. Clade I included nine taxa and our newly described species *D. mukaianum* has been phylogenetically placed in this clade along with *D. erythraeum* (PES017), *D. saxorum* (PES013) and *D. ursulae* (DURSU). The other five populations from Delhi, Belgaum, Halkarni, Yercaud and Badami could not be assigned any taxonomic status yet. All the populations bear less than 20 flowers except *D. mukaianum* (> 20 flowers). Also, flowers are longer and thinner. *D. mukaianum*

(collected from Ajara, Kolhapur) and populations of Badami showed 2*n* = 20 chromosomes (pers. obs.) whereas *D. saxorum* showed 2*n* = 12 (Naik 1974) and *D. erythraeum* 2*n* = 22 chromosomes (Samanta et al. 2023). *D. erythraeum* (PES017) sampled by us was placed in Clade I (Fig. 2). The previously sequenced three accessions of *D. erythraeum* were placed with African species (*D. viride*, *D. glaucum*, *D. gracillimum* and *D. longifolium*) in Clade IV (Fig. 2 and Fig. S2). However, in plastid phylogeny both (PES017 and BAHMP029311216R) the accessions occupied the same Clade III comprising the African species (Fig. S1). This could be on account of previous misidentification or availability of just ITS sequences for two specimens (EDNA15-0042943 and EDNA15-0042845) and only *rbc*L and *mat*K for one specimen (BAHMP029311216R). Missing data might have led to different *D. erythraeum* accessions occupying different positions in the phylogeny. As ITS is mostly available from these accessions except one (BAHMP029311216R) we obtained a similar relationship in ITS phylogeny wherein previous sampled accessions and our accession occupied the same Clade, i.e. Clade IV (Fig. S2). This indicates that misidentification of these specimens could have been the main reason for the discrepancy in their phylogenetic position. It is worth noting that *D. erythraeum* is a widespread species and one cannot completely exclude the possibility of existence of cryptic species in this taxon. Species complexes or cryptic species have been generally observed in case of widespread geophytic taxa such as *Spiranthes* Rich. (Orchidaceae) (Surveswaran et al. 2018) and *Aponogeton* L.f. (Aponogetonaceae) (Chougule et al. 2023). Both Surveswaran et al. (2018) and Chougule et al. (2023) have emphasized a wider geographical sampling of such taxa and adopting an integrated approach (high throughput sequencing, karyology and flow cytometry, etc.) to resolve their taxonomy. Another contrasting phylogenetic relationship of *D. serotinum* and *D. serotinum* var. *fulvum* has been observed as they did not group together. *D. serotinum* showed a close relationship with Indian species whereas *D. serotinum* var. *fulvum* was more related to African species. This may be due to the missing data for three regions (*rbc*L, *mat*K and ITS) for *D. serotinum* var. *fulvum* (Table 1) (Fig. 2, Fig. S1). The placement of *D. serotinum* with the Indian taxa (in Clade I, II and III) is also unusual on cytogenetical backgrounds. Three chromosome counts are on record for the Indian taxa, i.e. 2*n* = 12, 20 ad 22 (Samanta et al. 2023) whereas *D. serotinum* exhibits 2*n* = 8 chromosomes (RejÓN et al. 1981). Therefore, for phylogenetic analysis, extensive sampling of this widely distributed species should be a done. Clade II included 11 taxa of which six are known, namely *D. coimbatorensis*, *D. minor*, *D. montanum, D. montanum* var. *madrasicum*, *D. ursulae* and *D. ursulae* var. *longiracemosum.* The identity of rest of the taxa could not be deciphered. Two species of this clade, *D. montanum* and *D. ursulae* exhibit 2*n* = 20 chromosomes (Mahabale and Chennaveeraiah 1954; Samanta et al. 2023) and hence, are cytogenetically similar whereas for others chromosomal information is not on record. Also, all the members of this clade bear more than 15 flowers (per inflorescence). In case of *D. montanum, D. montanum* var. *madrasicum, D. ursulae, D. ursulae* var. *longiracemosum* the bracts are longer (10–18 mm long) whereas other members of the clade exhibit shorter bracts (6–10 mm long). Flowers are usually 14–16 mm long and thicker in all the members of the clade except *D. coimbatorensis* which has 15–18 mm long flowers. Our sampled *D. ursulae* (PES001) as well as *D. ursulae* var. *longiracemosum* were placed in Clade II whereas previously sequenced *D. ursulae* (DURSU) was placed in Clade I (Fig. 2). This could be because of the misidentification in previous studies or the lack of plastid region sequences for this specimen (DURSU) (Table 1). Clade III included, *D. concanense, D. goaense*

(GUH 5166, PES014), *D. montanum* (DMONA, DMONR), *D. janae-shrirangii, D. krishnadevarayae* and *D. reidii*. *D. concanense, D. goaense* and *D. janae-shrirangii* are characterized by $2n = 12$ chromosomes and their karyotypes are similar (Chandore et al. 2021; Samanta et al. 2023). Also, these species share morphological features such as large flowers (2.6–6.0 cm long) and lustrous white corolla and are confined to lower altitudes (13–105 m msl) in the coastal areas. Phylogenetic analysis based on the combined data of ITS and *mat*K also revealed that *D. concanense* and *D. goaense* are closely related species (Deshpande et al. 2015). Diploid chromosome counts of $2n = 10$, 12, 20, 21 and 22 are on record for *D. montanum* (Naik 1974; Vijayavalli and Matthew 1988). These variable numbers could be either because of species misidentification or because of the scoring of B chromosomes. Clade IV mostly consists of African *Dipcadi* species and the relationship within them is similar as observed in phylogeny given by Martínez-Azorín et al. (2011).

Future research in Dipcadi

This study provides the most comprehensive phylogeny of *Dipcadi* to date, which represents almost 50% of the known taxa. However, for some taxa (those sampled earlier and the unidentified ones we sampled in our study), we have observed contrasting phylogenetic placements. In order to resolve these questions and explore this genus in-depth, a robust phylogenetic approach including the samples of the remaining taxa (for all the plastid and nuclear genes used in the present phylogenetic analysis) is required. Furthermore, the utility of additional plastid regions that will provide better phylogenetic resolution should also be tested. A robust phylogeny would pave the way to understanding the biogeography of the genus.

Declarations

Acknowledgements

PES, SRY and MML are grateful to the Head, Department of Botany, Shivaji University, Kolhapur for providing the necessary research facilities. We are thankful to Ravikiran Kulloli (Research Associate, Arid Forest Research Institute Jodhpur, Rajasthan), Ashish Prabhugaonkar (Assistant Professor, Dhempe College of Arts and Science Panaji, Goa), Ravichandran Velliangiri and Kartik B. (BSI Southern region, Coimbatore, Tamil Nadu), Suchindra Dutta and Hensel Rodrigues (R.D. & S.H. National College, Bandra West, Mumbai, Maharashtra), Arun Chandore (Arts, Science and Commerce College, Mokhada, Palghar, Maharashtra) for the help rendered during plant collection.

Funding

This research work was financially supported by the Department of Science and Technology-Science and Engineering Research Board (DST-SERB), New Delhi vide sanction letter CRG/2020/000532-12 dated 25.03.2021. PES thanks DST-SERB for the Senior Research Fellowship (SRF). This research was also supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2016R1A6A1A05011910) and by the Institution of Eminence, University of Hyderabad (UoH-IoE-RC5-22-042) to SS.

Disclosure statement No potential conflict of interest was reported by the authors.

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Figures

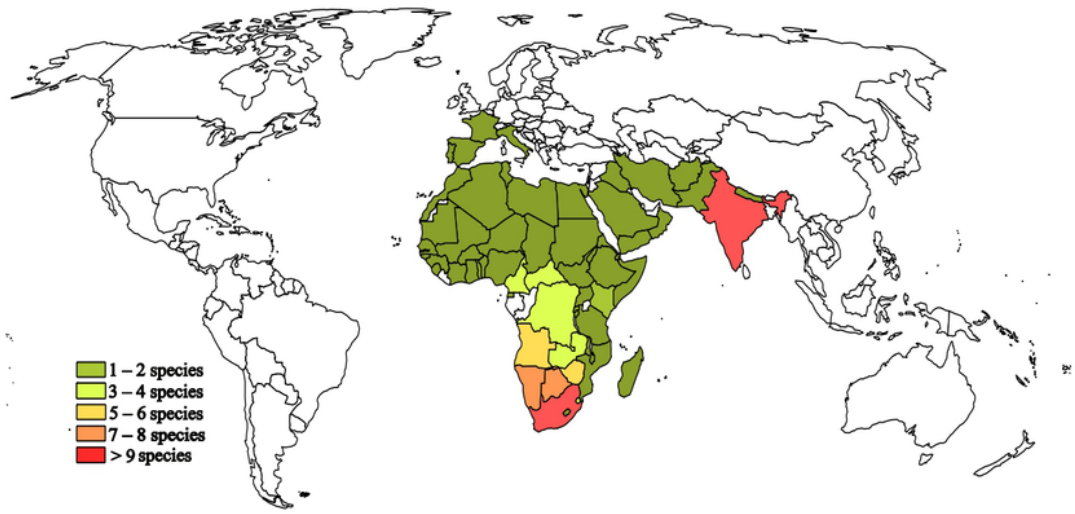


Figure 1

Distribution of genus *Dipcadi* (after POWO 2023)

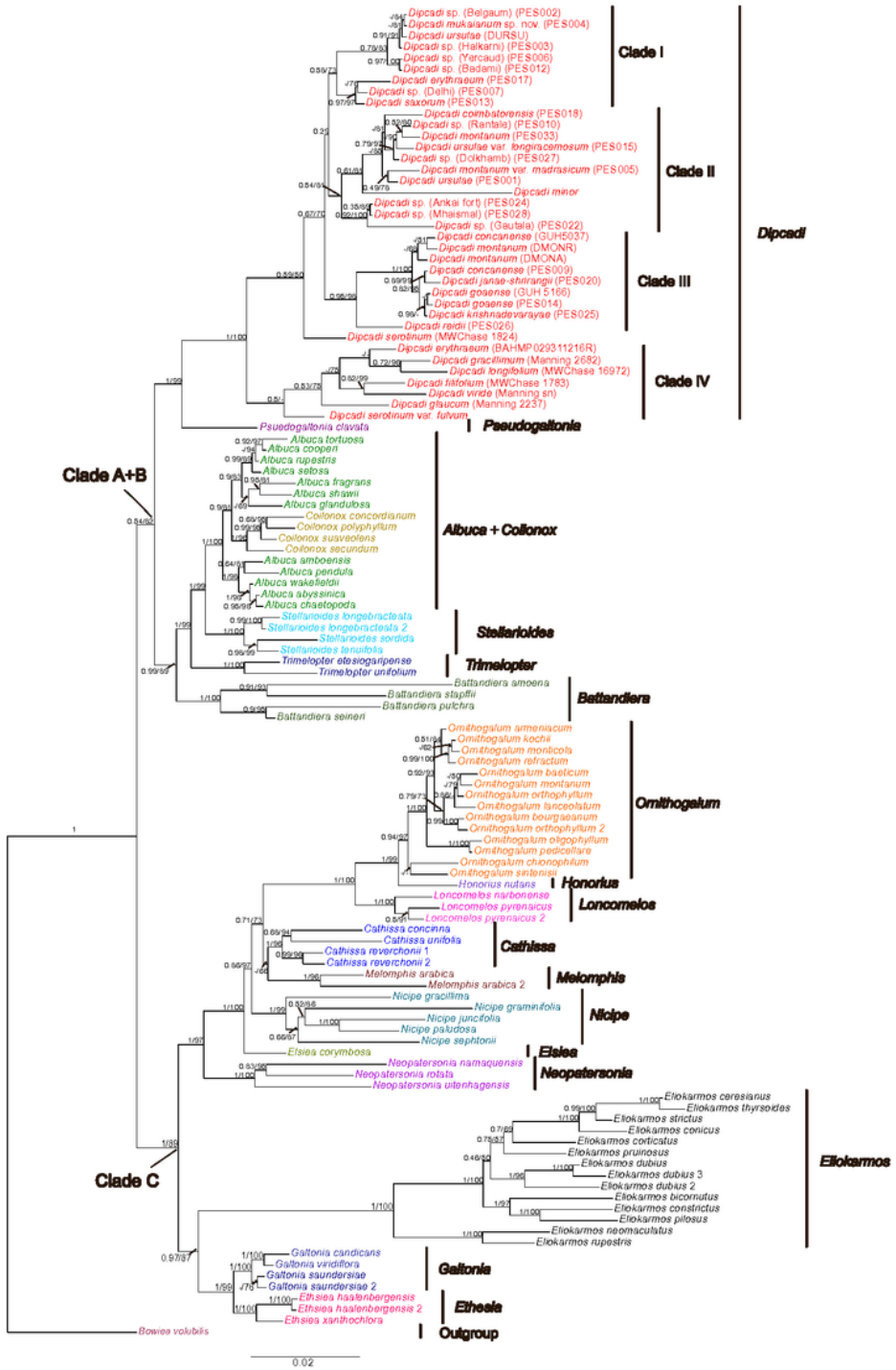


Figure 2

Bayesian analysis of combined ITS+*mat*K+*rbc*L+*trn*L-F sequences. Bayesian posterior probability values and maximum likelihood bootstrap values (BPP/ML BS) are provided above branches. Voucher ID of each *Dipcadi* taxa provided in parentheses. (-) indicates ML BS values < 50% and BI PP values < 0.5.

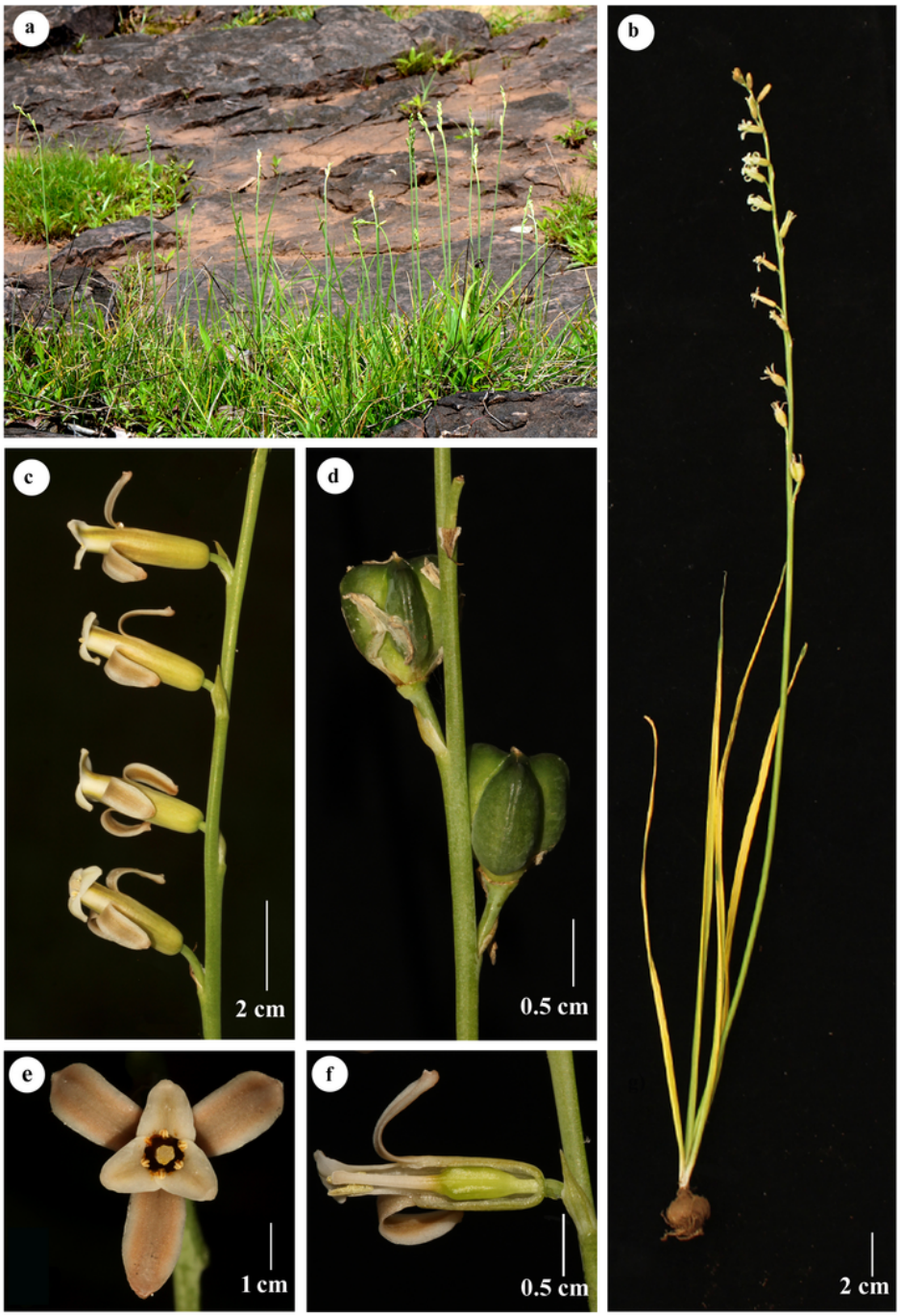


Figure 3

Dipcadi mukaianum. **a** Inflorescence; **b**Side view of the flower showing reflexed tepals; **c** Bract; **d** Inner tepal; **e** Outer tepal; **f** Flower split open and gynoecium removed; **g**Stamen (adaxial surface); **h** Stamen (abaxial surface); **i** Pistil; **j**Infructescence; **k** T.S. of ovary; **l** Seed **m** Bulb; **n**Leaf

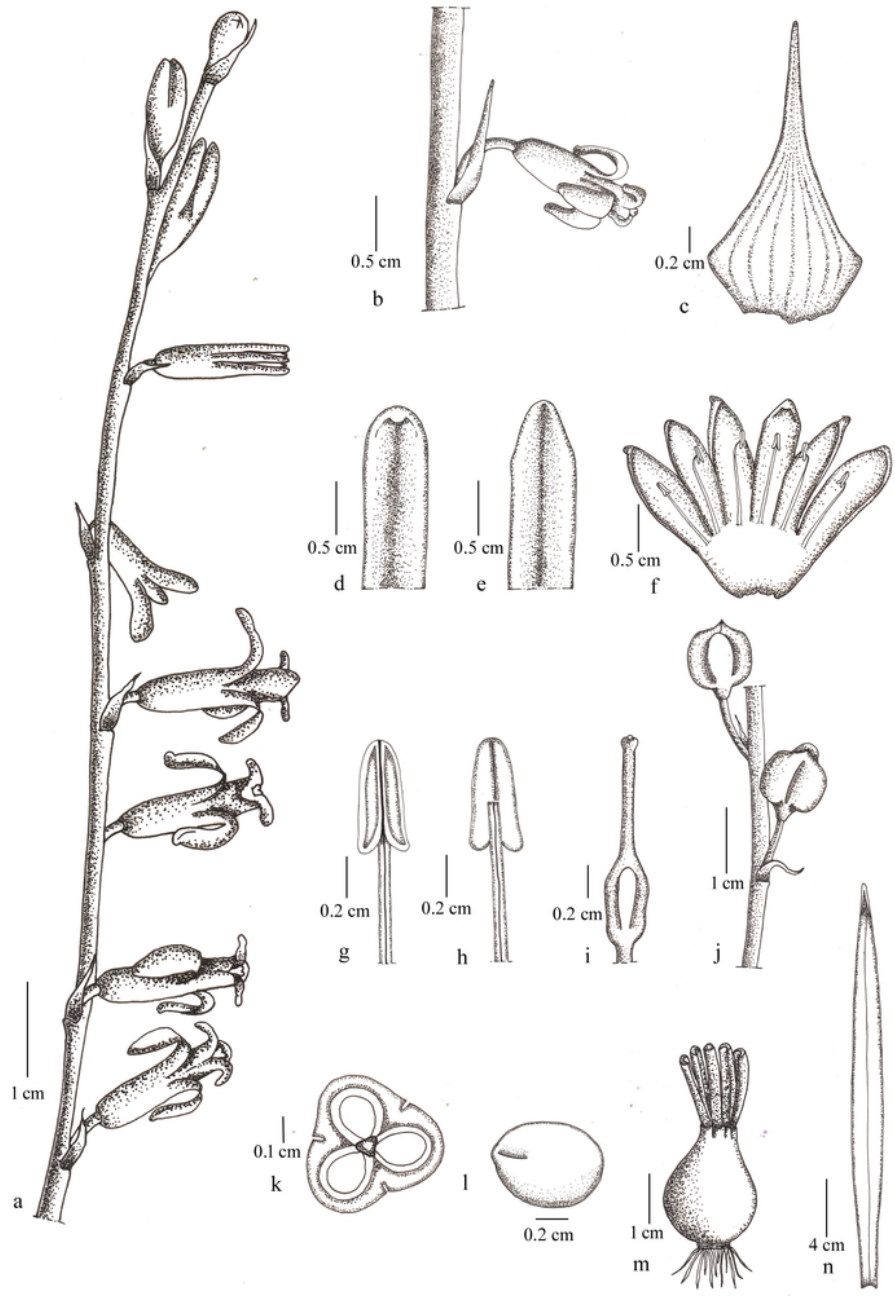


Figure 4

Dipcadi mukaiaum **a** Habitat; **b** Habit; **c** Inflorescence; **d** Infructescence; **e** Front view of the flower showing trilobed stigma and reflexed tepals; **f** L.S. of the flower showing pistil with small stipe



Figure 5

Comparative features of *Dipcadi* species. **a** – **c** inflorescence of *Dipcadi montanum, D. mukaianum*and *D. ursulae,* respectively; **d – f** side view of the flower showing attachment of tepals to each other; **g – i**. front view of the flower showing reflexed outer and inner tepals. (Scale bars a – c = 1 cm; d – f = 0.5 cm; g – i = 0.2 cm)

Supplementary Files

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