Intraspecific genetic variation in *Corynandra chelidonii* (Angiosperms: Cleomaceae) as revealed by SCoT, ISSR and RAPD analyses

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Abstract The genetic diversity of two subpopulations of *Corynandra chelidonii*, one of terrestrial and the other of aquatic environments, was measured with molecular markers, such as start codon targeted (SCoT), inter simple sequence repeats (ISSR), and random amplification of polymorphic DNA (RAPD). The traditional morphological traits such as habitat, habit, leaf morphology, the colour of the sepals and petals, number of stamens, and seed morphology formed the base for their realization as two varieties, *C. chelidonii* var. *pallae* and *C. chelidonii* var. *chelidonii*. The polymorphism between the two variants was 100% with the primers SCoT-2 and OPA-1 and 4, while maximum polymorphism was detected with ISSR-2, SCoT-3, and OPA-3. The study used, for the first time, more than one molecular marker to assess the genetic variation underscoring the morphological variation in *Corynandra chelidonii* (L.f.) Cochrane & Iltis. The study justifies the recognition of the two subpopulations of *Corynandra chelidonii* from aquatic and terrestrial environments as two distinct varieties, *C. chelidonii* var. *pallae* (Reddy & Raju) V.S.Raju and *C. chelidonii* var. *chelidonii*, respectively, based on the traditional taxonomic evidence.

Keywords *Cleome chelidonii*, varieties, genetic variation analyses, molecular markers, taxonomic status assessment

Introduction

Cleomaceae Bercht. & J. Presl is a now a well-recognized

family under Brassicales, placed in between Capparaceae and Brassicaceae (APG IV 2016). *Corynandra* Schard. ex Spreng is a segregate genus of *Cleome* (Cleomaceae), which was reinstated recently (Cochrane and Iltis 2014). The commonly available species of *Cleome* in India, such as *Cleome chelidonii* L.f., *C. feline* L.f., *C. flava* Banks ex DC., and *C. viscosa* L. belong to *Corynandra* and, therefore, were transferred to it.

Cleome chelidonii var. *pallae* C.S.Reddy & V.S.Raju is a new variety described from the littoral waters of Pakhal reservoir in Pakhal Reserve Forest, Mahabubabad district, Telangana state, India (Reddy and Raju 2001). It is a taxon growing in the fringes of lentic and/or lotic water bodies as tall plants with 3–6 foliolate proximal and linear distal leaves, pink flowers, more number of stamens, and somewhat different seeds. While transferring *Cleome chelidonii* to *Corynandra*, Cochrane and Iltis (2014) have not transferred this variety but merely listed it as a synonym, mentioning that they have not seen the type material. Recently, Subash and Ragan (2017) studied the seed dormancy and *in vitro* germination of the seeds in *Corynandra chelidonii* var. *pallae* (Reddy & Raju) V.S.Raju. Somkumar et al. (2018) reported this variety as a new record from Nagpur for Maharashtra state, India. Conversely, there is a need to assess whether there is any other evidence that corroborates its distinctiveness as an intraspecific taxon or not?

The seeds of *Cleome chelidonii* are used as a condiment by the local people. It contains medicinal compounds like glucocapparin and glucocleomin and the suspension cultures of it produced glucosinolates (Songsak and Lockwood 2002). Parimalakrishnan et al. (2007) studied the anti-inflammatory, antinociceptive and antipyretic effects of methanol extract of *Cleome chelidonii*. Anti-inflammatory and hepatoprotective activities of different extracts of *Cleome chelidonii* root was evaluated in albino rats (Ethadi et al. 2013). Sridhar et al. (2014) screened *in vitro* the methanolic extracts of *Cleome chelidonii* and *C. gynandra* for antimicrobial activity. Re-

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cently, Sumitha and Gurulakshmi (2015) studied antioxidant and free radical scavenging activity of leaf extracts of *C. chelidonii* while Minh et al. (2015) isolated two new flavonol glycosides from the leaves of *C. chelidonii*.

The spatial distribution of genetic variation within plant populations is influenced by many factors such as gene flow, micro-environmental selection, spatial distribution pattern, life history, and human activities (Ying et al. 2007). The study of genetic diversity with molecular markers among the plant species has become crucial for understanding the genetic variation (Frascaroli et al. 2013). Screening of genetic variations among different species is essential in conservation genetics and molecular breeding techniques (Kalia et al. 2011; Shekhawat et al. 2018). Different molecular markers like Start Codon Targeted (SCoT), Inter-Simple Sequence Repeat (ISSR), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR), Amplified Fragment Length Polymorphism (AFLP), and Random Amplified Polymorphic DNA (RAPD) are important tools for evaluating genetic variation among various genotypes (Frascaroli et al. 2013; Kudikala et al. 2020; Shekhawat et al. 2018). K'Opondo et al. (2009) assessed the genetic variation in the morphotypes of *Cleome gynandra* in Kenya. So, an attempt was made here to evaluate the extent of genetic difference between the subpopulations of *Cleome chelidonii* in Telangana state, India, using the molecular markers such as SCoT, ISSR, and RAPD. It was to assess their bearing on the taxonomy of the species.

Materials and Methods

The plant material

The plant material of *Corynandra chelidonii* var. *chelidonii* and *C. chelidonii* var. *pallae* were collected from the districts of Warangal Rural and Mahabubabad (Pakhal RF, Pakhal tank), northern Telangana, India (17.9526°N; 80.0060°E). The taxa were identified and authenticated by Professor Dr. Vatsavaya S. Raju, Plant Systematics Laboratory, Kakatiya University, Warangal. The voucher specimens were deposited in KUW (Kakatiya University Herbarium, Warangal, India).

DNA isolation

The total DNA was isolated from the plants of the two varieties of *Corynandra chelidonii* (Taxon A: 'var. *pallae*' and Taxon B: 'var. *chelidonii*') using modified Cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1987). The quality of DNA was checked on 0.8% agarose gel with 1X TAE buffer adjusted to 50 ng/μl with TE buffer and stored at −20°C.

Analysis of genetic variation using SCoT, ISSR, and RAPD markers

Three sets of markers were used for genetic variation studies of *Corynandra chelidonii*. The PCR analysis was performed with 4 primers, each of SCOT, ISSR, and RAPD (Table 1). The PCR amplification was performed using a

Table 1 List of SCoT, ISSR, and RAPD markers used in genetic study

S. No.	Primer Code	Primer Sequence (5–3)	Total number of alleles	Polymorphic alleles	Percentage polymorphism	PIC
1	SCoT-1	CAACAATGGCTACCAACC	6	2	33	0.193
2	SCoT-2	CAACAATGGCTACCAACCG	1	1	100	0.032
3	SCoT-3	ACGACATGGCGACCAACG	7	5	71	0.225
4	SCoT-4	CCATGGCTACCAACCGCAG	4	2	50	0.129
5	ISSR-1	GAGAGAGAGAGAGAGAG	12	8	66	0.387
6	ISSR-2	GAGAGAGAGAGAGAGAT	14	12	85	0.483
7	ISSR-3	AGAGAGAGAGAGAGATC	14	10	71	0.451
8	ISSR-4	TGTGTGTGTGTGTGTGRA	15	11	73	0.483
9	OPA-1	CAGGCCCTTC	1	1	100	0.032
10	OPA-2	TGCCGAGCTG	15	11	73	0.483
11	OPA-3	AGTCAGCCAC	12	8	66	0.387
12	OPA-4	AATCGGGCTG	1	1	100	0.032
Total			102	72		
Average			8.5	6	74	0.296

total reaction volume of 50 µl containing 50 ng/µl DNA, 10 p mole of primer, and 2X PCR master mix (GCC Biotech, India) and amplification reaction was carried out in a thermal cycler (Eppendorf Germany) for 35 cycles with an initial denaturation of DNA at 94°C for 5 min, followed by 30 sec denaturation at 94°C. The annealing done was 45 sec each at 50°C for SCoT, ISSR, and 37°C for RAPD with 2 min extension at 72°C, the final extension of 10 min at 72°C, and cool down holding to 4°C. All the PCR products were subjected to electrophoresis on 1% agarose gel using 1X TAE buffer. The size of amplicons was estimated using a 100 bp DNA ladder (Himedia), and the gels were photographed in a gel documentation system (Bio-Rad, USA). All amplification reactions were repeated thrice to check the reproducibility.

Each amplified product was scored either as 1 or 0, corresponding to the presence or absence of a band. The frequency of microsatellite polymorphism was calculated based on the presence or absence of common bands. The polymorphism information content (PIC) value = $\sum (1-P_i^2)/n$ where 'n' is the number of band positions analyzed, and P_i is the frequency of the i^{th} pattern band positions analyzed. The ability of the primers to distinguish the two variants was assessed by calculating their resolving power (Rp) = $\sum I_b$, where I_b is band informativeness, $I_b = 1 - [2 \times (0.5 - p_i)]$ and p_i is the proportion of variants containing band I (Prévost and Wilkinson 1999). The genetic associations among the two varieties were evaluated manually by counting the total number of bands/alleles (similar and dissimilar) using 4 primers each of SCoT, ISSR, and RAPD (Table 1). The PCR amplification was performed in triplicate, and the results obtained produced identical electrophoretic patterns. The molecular weight of all bands was calculated, and the binary matrix was prepared by scoring as a present (1) or absent (0). For each of the markers, PIC was calculated using Molecular Kinships version 3.0. The observed number of alleles (Na), the effective number of alleles (Ne), gene diversity (H), Shannon's information index (I), relative differentiation (GST), the estimate of gene flow from [GST (Nm)], total heterozygosity (Ht), within the subpopulation heterozygosity (Hs), genetic identity and genetic distance within population and group, number of polymorphic bands (NPB), and percentage of polymorphic bands (PPB) were calculated using Popgene version 1.32. The proportion of total genetic diversity residing among populations (GST) was calculated as $GST = (HT - HS)/HT$, as described by Nei (1973), whereas the measures of genetic identity and genetic distance were after Nei and Roychoudhury (1972). The phenogram was constructed for

the two variants based on UPGMA and modified NEIGHBOR producer of PHYLIP version 3.5.

Results and Discussion

Taxonomy of *Cleome* L.

The genus *Cleome* L. (1753), once placed in Capparaceae (Capparales), is now removed to Cleomaceae (Brassicales) of Rosids – Malvids – Eudicots of Angiosperms. Within the Cleomaceae, the genus *Cleome* L. was shown to be grossly paraphyletic (APG IV 2016). Several investigations were carried out about its naturalness in terms of macromolecules and biogeography (Barrett et al. 2017; Cochrane and Iltis 2014; Roalson et al. 2015; Zang and Tucker 2008). Of the several segregates of *Cleome*, *Corynandra* is the taxon to which the present investigation confines.

Genus *Corynandra*

The genus *Corynandra* Schard. ex Spreng. (1827) was reinstated as the earlier name for *Arevila* Raf. (1838), which was recently taken up as a distinct genus by Zhang and Tucker (2008). Cochrane and Iltis (2014), who reviewed this genus recently, justified its segregation from *Cleome* and transferred the taxa, which naturally belongs to it. Accordingly, the common weedy species like *Cleome viscosa* L. and *Cleome chelidonii* L.f. belong to *Corynandra*. The genus *Corynandra* is circumscribed by the closed aestivation of the flowers, the high number of stamens, adaxial filaments more or less apophysate, capsules sessile and erect with persistent valves that dehisce from the apex, and seed with an open cleft (Rao et al. 2018). The solitary flowers borne in the axils of upper leaves and an obsolete disk are the additional features associated with *Corynandra* against *Cleome* (Cochrane and Iltis 2014).

Corynandra chelidonii (L.f.) Cochrane & Iltis in Novon 23: 22. 2014. *Cleome chelidonii* L.f. Suppl. Pl. 300. 1782. *Polanisia chelidonii* (L.f.) DC., Prodr. 1: 242. 1824. *Aubion chelidonii* (L.f.) Raf. Sylva Tellur. 110. 1838 (as "chelidoni").

Distribution: India (throughout the country as a weed of fallow fields), Myanmar, and Malaysia.

The presence of two morphotypes of *Cleome chelidonii*, one on the land, and the other along the marginal of tanks in the district of Warangal, Andhra Pradesh (now Telangana

Table 2 Morphological distinction between the two varieties of *Corynandra chelidonii*

Character	var. <i>chelidonii</i>	var. <i>pallae</i>
Habit	Annual erect herbs to <1 m tall	Annual erect herbs to 1.5 m tall
Habitat	Terrestrial; fallow fields, dried-up ponds	Aquatic to semi-aquatic
Leaflets:		
(i) Basal (proximal)	7~9-foliolate, obovate, 3.5~12.2 cm long, entire	Same as in typical form, but bigger; 4-7 0.3~0.7 cm; crenate to crenate-serrate
(ii) Middle (mesial)	5~7-foliolate, elliptic to oblanceolate	3~5 foliolate, elliptic lanceolate
(iii) Upper (distal)	1~5-foliolate, oblanceolate to lanceolate	1~3; foliolate; often linear
Internodes	Up to 5 cm	Up to 15 cm
Pedicle length	4 cm	4.5 cm
Flowers	1.5~2.0 cm across	2.5~3.5 cm across
Petals	4, close-by, elliptic, ovate to obovate; 1~1.4 × 0.3~0.8 cm; pink	4, somewhat distant, oblong to ovate, 1.5~1.8 cm; pink
Stamens (no/filaments)	ca. 35~60; 0.8~1.2 cm long	ca. 60~100 or more; 1.6~1.9 cm
Capsule (beak length)	2~4 mm	5~10 mm
Seeds	Ovoidal, 1.3~1.8 mm across; cleft more closed; testa tuberculate; appendages shorter, lesser, more or less pointed; with no waxy coating.	Rounded, 1.5~2.5 mm across; cleft more open; testa tuberculate with blunt tubercles of different heights; appendages with waxy coating.

Fig. 1 The two varieties of *Corynandra chelidonii*. A–C: Habitat/habit, flowers, and seed image (Stereo binocular microscope Nikon SMZ 18 captured) of var. *chelidonii*; D–F: Habitat/habit, flowers, and seed of var. *pallae*

state) was noted by Vijayakumar (1980) in his light and SEM study of the seeds. Consequently, Reddy and Raju (2001) were the first to describe the aquatic form as a new variety, *Cleome chelidonii* var. *pallai* (Table 2; Fig. 1). Technically speaking (Article 60.8(a) of the ICN), the varietal epithet '*pallai*' has to be "*var. pallae*". The taxon was named after the late Dr. Palla Vijayakumar, Reader in Botany, Kakatiya University, Warangal, who worked on the seed morphology of *Cleomaceae* for his doctoral thesis.

Fig. 2 Genetic variation in *Corynandra chelidonii* as disclosed by SCOT. Lane 1, Lane 10: 100 bp DNA ladder; Lane 2: var. *pallae* and Lane 3: var. *chelidonii*, with SCOT-1; Lane 4: var. *pallae* and Lane 5: var. *chelidonii*, with SCOT-2; Lane 6: var. *pallae* and Lane 7: var. *chelidonii*, with SCOT-3; Lane 8: var. *pallae* and Lane 9: var. *chelidonii*, with SCOT-4 primer. SCOT: Start codon targeted

Genetic diversity according to primers

Three sets of markers (SCoT, ISSR, and RAPD) were used for genetic variation studies. The PCR analysis was performed with four primers each. Taxon-A (var. *pallae* DNA) with SCoT1 primer showed no band whereas Taxon-B (var. *chelidonii*) DNA showed the band in between 1000 and 1500 bp with SCoT1 (Fig. 2). Taxon-A DNA with SCoT3

Fig. 3 Genetic variation in *Corynandra chelidonii* as per ISSR markers. Lane 1, Lane 10: 100bp DNA ladder; Lane 2: var. *pallae* and Lane 3: var. *chelidonii*, with ISSR-1; Lane 4: var. *pallae* and Lane 5: var. *chelidonii*, with ISSR-2; Lane 6: var. *pallae* and Lane 7: var. *chelidonii*, with ISSR-3; Lane 8: var. *pallae* and Lane 9: var. *chelidonii*, with ISSR-4 primer. ISSR: Inter simple sequence repeat

showed a band at 700 bp, whereas the Taxon-B DNA showed band around 1000 bp with SCoT3 (Fig. 2). In the case of ISSR analysis, ISSR3 primer showed band variation with the same primer: Taxon-A DNA showed a band at 600 bp whereas Taxon-B DNA developed a band at 1000 bp (Fig. 3). In RAPD analysis, OPA-1 primer evinced no bands in Taxon-A, whereas the DNA of Taxon-B amplified showed a band at 1000 bp. The OPA-4 primer amplified evinced a band at 400 bp in Taxon-A DNA whereas Taxon-B DNA produced no such band with the same primer (Fig. 4).

Percentage of polymorphism

The percentage of polymorphism was calculated according to the presence (1) or absence (0) of bands for SCoT, ISSR, and RAPD markers. The twelve primers used for genetic variation and diversity study generated 102 bands, an average 4.25 bands of SCoT, 14 bands of ISSR, and 7 bands of RAPD.

Polymorphic Information Content (PIC)

The genetic variation and differences were clearly seen between the two subpopulations of *Corynandra chelidonii*. The number of bands for Taxon-A in terms of markers was 9 (SCoT), 31 (ISSR), and 12 (RAPD). For Taxon-B, they were 8 (SCoT), 25 (ISSR), and 16 (RAPD). Among all primers, between two variants, the mean average PIC

Fig. 4 Genetic variation in DNA of *Corynandra chelidonii*, as revealed by RAPD analysis. Lane 1, Lane 10: 100bp DNA ladder. Lane 2: var. *pallae* and Lane 3: var. *chelidonii*, with OPA-1; Lane 4: var. *pallae* and Lane 5: var. *chelidonii*, with OPA-2; Lane 6: var. *pallae* and Lane 7: var. *chelidonii* with OPA-3; Lane 8: var. *pallae* DNA and Lane 9: var. *chelidonii*, with OPA-4 primer. RAPD: Random amplified polymorphic DNA

observed was 0.296. However, the highest mean (PIC 0.483) was shown with ISSR-2, ISSR-4, and OPA-2 (RAPD). On the other, the lowest mean (PIC 0.032) was observed with SCoT-2 and OPA-1 and OPA-4 (RAPD) (Table 1).

Genetic variation

The observed number of alleles (N_a), effective number of alleles (N_e), gene diversity (H), Shannon's information index (I), total heterozygosity (H_t), within-population heterozygosity (H_s), number of polymorphic bands (NPB) and Percentage of polymorphic bands (PPB) were calculated for the estimation of genetic variation between the two (A and B) subpopulations (Table 3).

Genetic variation between two taxa

(i) The Primers SCoT exhibited the genetic variation of N_a (1.225 ± 0.425) N_e (1.161 ± 0.315), N_p (07 (22.58)) h (0.092 ± 0.176) I (0.135 ± 0.256) for Taxon-A and N_a (1.258 ± 0.444), N_e (1.167 ± 0.297), N_p (08 (25.81)), h (0.100 ± 0.175), I (0.149 ± 0.258) for Taxon-B. (ii) The ISSR primers revealed the genetic variation as of N_a (1.548 ± 0.50), N_e (5 1.432 ± 0.426), N_p (17 (54.84)) h (0.237 ± 0.224) I (0.342 ± 0.319) in Taxon-A and N_a (1.580 ± 0.501), N_e (1.438 ± 0.408), N_p (18 (58.06)), h (0.246 ± 0.217), I (0.356 ± 0.311) for Taxon-B, and (iii) The RAPD primes have shown the genetic variation N_a (1.354 ± 0.486), N_e (1.238 ± 0.340), N_p (11 (35.48)), h

Table 3 Genetic diversity and differentiation parameters for Taxon A and B subpopulations of *Corynandra chelidonii*

Marker	Population	Diversity indices											
		na (± SD)	ne (± SD)	Np (PI %)	h (± SD)	I (± SD)	Gd	Gi	Ht	Hs	Gst	Nm	
SCOT	Taxon-A	1.225±0.425	1.161 ± 0.315	07 (22.58)	0.092 ± 0.176	0.135 ± 0.256	0.0135	0.986	0.102 ± 0.026	0.096 ± 0.025	0.058	8.000	
	Taxon-B	1.258±0.444	1.167± 0.297	08 (25.81)	0.100 ± 0.175	0.149 ± 0.258							
ISSR	Taxon-A	1.548±0.505	1.432 ± 0.426	17 (54.84)	0.237 ± 0.224	0.342 ± 0.319	0.0435	0.957	0.258 ± 0.049	0.241 ± 0.044	0.062	7.500	
	Taxon-B	1.580 0.501	1.438± 0.408	18 (58.06)	0.246 ± 0.217	0.356 ± 0.311							
RAPD	Taxon-A	1.354±0.486	1.238 ± 0.340	11 (35.48)	0.141 ± 0.195	0.208 ± 0.286	0.0611	0.940	0.176 ± 0.026	0.151±0.020	0.142	3.000	
	Taxon-B	1.387±0.495	1.283 ± 0.382	12 (38.71)	0.161 ± 0.209	0.234 ± 0.302							
ALL (primers)	Taxon-A	1.612± 0.495	1.323±0.341	19 (61.29)	0.198 ± 0.187	0.303 ± 0.271	0.0133	0.986	0.208 ± 0.028	0.202 ± 0.026	0.025	19.255	
	Taxon-B	1.741± 0.444	1.314± 0.278	23 (74.19)	0.207 ± 0.159	0.328 ± 0.231							

value (mean) Taxon A: *C. chelidonii* var. *pallae*; Taxon B: *C. chelidonii* var. *chelidonii*.

Gd = Genetic distance; Gi = Genetic identity; G_{st} = Degree of genetic differentiation; h = Nei's (1973) gene diversity; Hs = Average heterozygosity; Ht = Heterozygosity at the polymorphic loci; I = Shannon's Information index (Lewontin 1972); na = Observed number of alleles; ne = Expected number of alleles/Effective number of alleles (Kimura and Crow 1964); Nm = Estimate of gene flow from Gst or Gcs. e.g., $Nm = 0.5(1 - G_{st})/G_{st}$; Np = Number of polymorphic; PI = Percentage of polymorphic loci; see McDermott and McDonald 1993).

Fig. 5 Phenogram: *Corynandra chelidonii* varieties

(0.141 $\pm$ 0.195) I (0.208 $\pm$ 0.286) for Taxon-A in contrast to Taxon-B of Na (1.387 $\pm$ 0.495), Ne (1.283 $\pm$ 0.382) Np (12 (38.71)) h (0.161 $\pm$ 0.209) I (0.234 $\pm$ 0.302) (Table 3).

All the primers produced individual bands together for clear observation of genetic distance and variation between the two taxa as Na (1.612 $\pm$ 0.495), Ne (1.323 $\pm$ 0.341), Np (19 (61.29)), h (0.198 $\pm$ 0.187), I (0.303 $\pm$ 0.271) of Taxon-A and Na (1.741 $\pm$ 0.444), Ne (1.314 $\pm$ 0.278), Np (23 (74.19)), h (0.207 $\pm$ 0.159), h (0.328 $\pm$ 0.231) for Taxon-B, and together have shown a genetic distance Gd (0.0133), Gi (0.986), Ht (0.208 $\pm$ 0.028), Hs (0.202 $\pm$ 0.026), G_{st} (0.025) and Nm (19.255). Nei's genetic similarity between the subpopulations (A-var. *pallae* and B-var. *chelidonii*) was 0.9868. The phenogram (Fig. 5) was constructed using UPGMA after cluster analysis based on random data using the cophenetic correlation coefficient (Rohlf and Fisher 1968; Rohlf 2000).

Relative differentiation and estimate of gene flow

Assessment of gene flow from one population to another is an essential parameter for the study of genetic diversity.

Gene flow is a collective term that includes all the mechanisms resulting in the movement of genes from one species to another. In this study, lower relative differences between these two taxa 0.025 (G_{st}) indicates a high variation at a genetic level for these plants while the gene flow was showing high Nm value between them.

These results, as per SCoT, ISSR, and RAPD analyses, suggest that there is apparent variation within the species *Corynandra chelidonii*. The SCoT, RAPD, and ISSR markers were demonstrated to be not only effective techniques to screen genetic differences but also quick, very consistent, cost-effective and one can investigate the whole genome of the plant randomly (Jogam et al. 2020; Pendli et al. 2019; Rohela et al. 2020). The use of more than one molecular marker to detect the genetic dissimilarity is always useful for better results because every molecular marker has its benefits and drawbacks. Besides the genetic diversity studies, SCoT, ISSR, and RAPD molecular markers were also used in genetic fidelity analysis of micropropagated plants (Sadhu et al. 2020; Sirikonda et al. 2020; Thakur et al. 2016). There were similar assessments of genetic variation among various taxonomic groups and ranks of

angiospermous taxa. For example, SCoT based genetic polymorphism studies were done in a wild population of *Maytenus emarginata* (Shekhawat et al. 2018). The SCoT markers were effectively applied for the genetic diversity determination of *Echinacea* varieties (Jedrzejczyk et al. 2020). The ISSR markers were used to study the molecular phylogeny and genetic diversity for the detection of inter- and intraspecific variation in *Capparis* (Tamboli et al. 2018). The ISSR primers were used to study the genetic variation in 56 *Amaranthus* accessions of three species (Stefunova et al. 2014). Sixty-two taxa of *Vigna*, with a focus on the species *V. unguiculata*, were studied to distinguish the taxa at the specific and infraspecific levels (Ajibade et al. 2000). Twenty-four SSR markers were utilized to evaluate the genetic variation across thirty-six guava varieties, including wild species (Kherwar et al. 2018). The ISSR markers were employed successfully to know the genetic polymorphism in two morphological variants of the exotic invasive *Hyptis suaveolens* in India (Gadidasu et al. 2011). Using RAPD and ISSR markers, the genetic variation was assessed among the populations of the medicinal herb *Bacopa monnieri* in central India (Tripathi et al. 2012).

Taxonomic status

The molecular data gathered in the present study of *Corynandra chelidonii* provides a genetic basis for the distinction of *C. chelidonii* var. *pallae* from the type. Since the phenetic similarity between the two taxa (subpopulations/varieties) compared is 66% (Fig. 5), the present authors inclined to accept them as varieties rather than subspecies. It is also because the subpopulations are not geographically isolated (allopatric), but ranged between adjacent terrestrial and aquatic environments. *Corynandra elegans*, which was recently described as a new species from the aquatic habitats in the Konkan region of Maharashtra, India (Chandore et al. 2016), was not realized as a species in the present study after comparison with its closely allied *C. chelidonii* var. *pallae*. In all probability, *C. elegans* does not stand as a distinct species but only proves to be this variety. The decision to merge it with *C. chelidonii* var. *pallae* is kept pending, awaiting the examination of the type material.

The presence of the aquatic form of *Cleome chelidonii* was reported in the past from India and elsewhere (Cooke 1996; Hewetson 1954; Raipur, now in Chhattisgarh; Jacobs 1960; Sundararaghavan 1993). But, Mirashi (1956) noted, in this form (which is now called *C. chelidonii* var. *pallae*), the mainly terrestrial race, showing hydrophytic nature and marked adaptation to watery habitat. Since the present

study clearly establishes the genetic (molecular) and morphological (Table 2) variation between the two varieties of *Corynandra chelidonii*, there is a need to maintain them as distinct taxa. It is in contradiction to Cochrane and Iltis (2014) and Barrett et al. (2017) who merged them on the taxonomic grounds without examining those specimens in the field and herbaria. Therefore, we have in India and, perhaps elsewhere (e.g., Sri Lanka, Java), there exist two varieties under *Corynandra chelidonii* (L.f) Cochrane & Iltis: one on terrestrial land (var. *chelidonii*) and another in freshwater marshes (var. *pallae*).

Corynandra chelidonii (L.f.) Cochrane & Iltis var. *pallae* (C.S.Reddy & V.S.Raju) V.S.Raju in Int. J. Res. Pharm. Biol. Sci. 7(2): 240. (April–June) 2017. *Corynandra chelidonii* var. *pallae* (C.S.Reddy & V.S.Raju) Kottaim. in Int. J. Curr. Res. Biosci. Pl. Biol. 6(10): 38. Oct. 2019 (*isonym*) *Cleome chelidonii* L.f. var. *pallae* C.S.Reddy & V.S.Raju in J. Econ. Tax. Bot. 25(1): 217.2001 ("pallai"). Type: INDIA, Telangana state, Mahabubabad distr., Pakhal Lake margins: 8.08.1961, K.M. Sebastine 13106 (MH).

Distribution : India (Maharashtra, Madhya Pradesh, Chhattisgarh, Telangana, Andhra Pradesh and Tamil Nadu [Chengalput distr., Vandanthal Waterbirds Sanctuary: 26.09.1974, A.N. Henry 45447 at MH: V.S. Raju, per. obs.], Sri Lanka, Java, and Malaysia.

Key to the varieties of *Corynandra chelidonii*

- 1a. Plants terrestrial, of fallow fields; annual; erect herbs to 0.5 m height; leaflets elliptic to lanceolate; stamens about 60; distal leaves usually trifoliate; seeds light brown, ovoidal, smaller (1.3 ~ 1.8 mm across); testa with tubercles pointed to curved, sparse; cleft somewhat closed var. *chelidonii*
- 1b. Plants aquatic to semi-aquatic, of littoral zones of water bodies; largely annual; erect to clump-forming herbs to 1.5 m height; stamens 60 ~ 100 or more; distal leaves linear; seeds dark brown, globose, bigger (1.5 ~ 2.5 mm across); testa with tubercles straight and blunt, denser; cleft fairly open var. *pallae*

Conclusion

Of the three kinds of molecular markers used to screen the intraspecific genetic variation in *Corynandra chelidonii*, SCoT was found to be the best indicator in bringing out the genetic diversity. The genetic variation in the two plant subpopulation (terrestrial and aquatic) of *Corynandra chelidonii*

was analyzed through several parameters like the number of alleles (Na), Number of polymorphic (Np), polymorphic loci percentage (pl), gene diversity (H) and Shannon's information index (I) revealed higher values for 'var. *chelidonii*' (Taxon-B) than 'var. *pallae*' (Taxon-A) in regard to Na, H, and I. Similarly, the observed and expected heterozygosity between var. *chelidonii* and var. *pallae* was high (Ht [0.208 ± 0.028]; Hs [0.202 ± 0.026]). So, for the infraspecific morphological and molecular diversity, there is obvious ground to realize two varieties in *Corynandra chelidonii*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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