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Research Article

The Systematic Position of Some Species of Boraginaceae Family as Inferred Using *Rbc*l Barcode Marker of Chloroplast Genome

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Abstract

The Boraginaceae family is a large group of flowering plants that includes over 2,000 species in approximately 140 genera. The family has long been known to have a complex taxonomy, with many genera and species that are difficult to distinguish from one another. This is due in part to the wide range of morphological diversity within the family, as well as the lack of clear, consistent diagnostic features. To address these problems, a combination of morphological, molecular, and ecological data is often used to resolve taxonomic issues and improve our understanding of these plant groups. Two plastid gene Ribulose 1, 5 bisphosphate (rbcL), maturase kinase (matK), and one nuclear region inter transcribed spacer (ITS) markers were selected for the study though rbcL gave the desired results. A total of 66 sequences were examined for the analysis, 13 of which were newly produced from samples taken from various locations in Maharashtra (GenBank accession number [submitted]). This is the first study to examine any Boraginaceae family plants from India.

Keywords: *Boraginaceae, Genbank, Molecular, rbcL, matK, IT.*

Introduction

The Boraginaceae family is a large group of flowering plants that includes over 2,000 species in approximately 140 genera. The family is widely distributed throughout the world, with the greatest diversity found in Mediterranean and arid regions. The most well-known genera in the family include Borago (borage), Echium (vipers bugloss), and Myosotis (forget-me-nots). The plants in this family are characterized by their five-petaled flowers and nut-like fruits. Many species in the Boraginaceae family are important forage plants for livestock and wildlife, and some are also cultivated as ornamental plants.

The Boraginaceae family has long been known to have a complex taxonomy, with many genera and species that are difficult to distinguish from one another. This is due in part to the wide range of morphological diversity within the family, as well as the lack of clear, consistent diagnostic features. Additionally, many species have been misclassified or wrongly placed in the wrong genus. The taxonomy of the family is also complex due to its large number of species, many of which have a limited geographic distribution and are not well known.

Another problem with the family Boraginaceae is that the taxonomy is based mainly on morphological characters, which can be unreliable for certain species. Some studies have suggested that the use of molecular data may be more useful for resolving the taxonomy of thefamily.

In recent years, some researchers have attempted to revise the classification of the Boraginaceae family and create a more stable and consistent system. But the classification of the family is still a work in progress and more research is needed to fully understand the relationships among the different genera and species

Ehretia is a genus of flowering plants in the

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Boraginaceae family, which is known for its small trees and shrubs. The genus is widely distributed in tropical and subtropical regions of the world, particularly in Asia and Africa. *Ehretia* species are known for their small, five-petaled flowers and small, hard fruits. The leaves are typically simple, opposite and have an oval shape. They have a wide range of ecological adaptability and can be found in different habitats such as rocky hills, deserts, and tropical forests. Some species are used in traditional medicine, and others have potential as ornamental plant.

The taxonomy of the *Ehretia* genus within the Boraginaceae family has long been a source of confusion and debate. One of the main problems is the lack of clear and consistent diagnostic characters that can be used to distinguish between different species of *Ehretia*. Additionally, many species have been misclassified or wrongly placed in the wrong genus.

The morphological variation within the genus is wide and some species are very similar in appearance, making it difficult to identify and classify them correctly. This is further complicated by the presence of intermediates, hybrids, and polyploids.

Another issue is that Ehretia species are mostly found in tropical and subtropical climates, where the majority of species are little understood and many are still unrecorded. This lack of knowledge about the genus makes it difficult to understand its diversity and to propose an accurate classification.

Molecular studies have been used in recent years to help understand the relationships among species within the genus and to propose a more stable and consistent classification. But the classification of the genus *Ehretia* is still a work in progress and more research is needed to fully understand the relationships among the different species and to resolve the taxonomic problems in the genus.

In India, the taxonomy of the *Ehretia* genus within the Boraginaceae family has been a source of confusion and debate. A major problem is that many species have been misidentified or misclassified, which has resulted in a lack of accurate information about their distribution, ecology, and conservation status. This is due in part to the lack of clear and consistent diagnostic characters that can be used to distinguish between different species of Ehretia, particularly in the Indian context.

The morphological variation within the genus is wide and some species are very similar in appearance, making it difficult to identify and classify them correctly. This is further complicated by the presence of intermediates, hybrids, and polyploids. Additionally, many species of Ehretia found in India are poorly known, and many remain undescribed, which makes it difficult to understand the diversity and distribution of the genus in the country.

Another problem is that traditional taxonomic methods such as morphological analysis are becoming less reliable, as they are not able to detect the genetic variations among the species. This has led to the use of molecular tools to help understand the relationships among species within the genus and to propose a more stable and consistent classification.

In recent years, some research studies have been done on the genus Ehretia in India. But the +classification of the genus Ehretia in India is still a work in progress and more research is needed to fully understand the relationships among the different species and to resolve the taxonomic problems in the genus in India

Cordia is a genus of flowering plants in the Boraginaceae family, which is known for its trees, shrubs, and woody vines. The genus is widely distributed in tropical and subtropical regions of the world, particularly in Central and South America, Africa, and Asia. Cordia species have simple, alternate or opposite leaves and they are known for their large, showy, and colorful flowers. They produce a variety of fruit types, including capsules, berries, and drupes. Some species of Cordia have medicinal properties and have been traditionally used to treat a variety of ailments. Some species are used for ornamental purposes and for timber and fruit production. They are also known for their tolerance to drought, heat,

and salt making them adaptable to different environments.

The Cordia genus of the Boraginaceae family is known to have a taxonomic problem in India. This is because the genus is highly diverse and contains many species that are difficult to distinguish from one another based on morphological characteristics alone. This has led to confusion and disagreements among taxonomists about the number of species that should be recognized in the genus, as well as the classification of individual species. Additionally, there are many unresolved synonyms and misidentifications that have further complicated the taxonomy of the genus. To resolve these issues, molecular techniques such as DNA sequencing are being used to help clarify the relationships among the different species within the genus. Taxonomic problems:

1. Hybridization and polyploidy: Interbreeding and polyploidization events can create taxonomic confusion by producing hybrids or polyploids with intermediate morphological characters.
2. Morphological similarity: Species within these genera may show morphological similarity, making it difficult to distinguish between them based on physical characteristics alone.
3. Limited taxonomic descriptions: Many species in these genera have not been thoroughly described, leading to confusion and inconsistencies in their classification.
4. Geographical and ecological overlap: Overlapping geographical ranges and similar ecological preferences can also lead to taxonomic difficulties in separating species.

To address these problems, a combination of morphological, molecular, and ecological data is often used to resolve taxonomic issues and improve our understanding of these plant groups.

Phylogenetic problems in the genera *Trichodesma, Cynoglosum*, and *Heliotropium* of the Boraginaceae family include:

1. Lack of genetic data: Limited genetic data from these genera makes it difficult to resolve the evolutionary relationships among species and genera.
2. Limited taxonomic sampling: Phylogenetic studies that include limited taxonomic sampling can lead to incomplete or biased evolutionary reconstructions.
3. Ambiguous character states: Species within these genera may show ambiguous or convergent morphological characters, making it difficult to infer evolutionary relationships.
4. Homoplasy: Homoplasy, or convergent evolution, can result in similar morphological traits evolving independently in different lineages, making it difficult to reconstruct evolutionary relationships.

To address these problems, phylogenetic studies on these genera often use multiple genes and taxa to ensure comprehensive and accurate evolutionary reconstructions. Additionally, using a combination of molecular and morphological data can also help to resolve these challenges.

Euploca is a genus of flowering plants in the Boraginaceae family, which is known for its small shrubs and herbs. The genus is primarily found in tropical and subtropical regions of Africa and Asia. *Euploca* species are known for their small, five-petaled flowers and small, nut-like fruits. The leaves are typically simple, opposite, and have an oval shape. They are known for their tolerance to drought and heat, making them adaptable to different environments. They are not commonly found in cultivation and are not well known in terms of their medicinal or ornamental uses. Some species have been used in traditional medicine in Africa and Asia.

EUPLOCA genus of the Boraginaceae family in India, as it is not a genus found in India. *EUPLOCA* is a genus of flowering plants in the Boraginaceae family, which is known to have a wide distribution in Africa, Asia, and Europe.

Heliotropium is a genus of flowering plants in the borage family (Boraginaceae), with ap-

proximately 300 species widely distributed in tropical and subtropical regions. In India, species of *Heliotropium* are found in various habitats including coastal regions and dry areas. Taxonomic research on *Heliotropium* has been carried out globally, with a focus on classifying and describing the species, their distribution, and relationships. Several species have been reclassified and renamed based on molecular and morphological data.

In India, several species of *Heliotropium* have been documented and described, with taxonomic work ongoing. Some of the species found in India include *Heliotropium indicum, Heliotropium bacciferum*, and *Heliotropium curassavicum*.

Taxonomic research on *Heliotropium* continues to play an important role in improving our understanding of the genus and its diversity, as well as providing a basis for conservation efforts and potential medicinal uses.

Phylogenetic studies are a type of research that aim to infer the evolutionary relationships between different species within a genus, such as *Heliotropium*. These studies use molecular data, such as DNA sequences, to construct evolutionary trees that show the relationships between species.

In the case of *Heliotropium*, several phylogenetic studies have been conducted to explore the evolutionary relationships within the genus and with other genera in the Boraginaceae family. These studies have provided valuable insights into the origin and diversification of the genus, and have helped to clarify the classification of species within the genus.

One example of a phylogenetic study on *Heliotropium* is a molecular analysis that used DNA sequences from the chloroplast trnL-F region to infer the relationships between species of *Heliotropium* and other genera in the Boraginaceae. This study provided evidence for the monophyly of the genus *Heliotropium* and revealed several distinct clades within the genus, each with a distinct geographic distribution.

Another study used both molecular and morphological data to infer the relationships between species of *Heliotropium* and related genera. This study confirmed the monophyly of *Heliotropium* and provided evidence for the presence of two major clades within the genus, one of which was well-supported and consisted of species from the Americas.

Overall, phylogenetic studies have contributed to a better understanding of the evolutionary relationships within the genus *Heliotropium* and have provided valuable information for futuretaxonomic and conservation efforts.

Cynoglosum is a genus of flowering plants in the Boraginaceae family, native to Europe, Asia, and Africa. Taxonomic research on Cynoglosum in India has focused on identifying and describing new species and understanding their distribution and relationships with other species.

Phylogenetic studies on the genus have aimed to reconstruct the evolutionary history and relationships among species using molecular data. These studies have shown that Cynoglosum is closely related to other genera in the Boraginaceae family, and have provided insights into the origin and diversification of the genus.

Overall, research on Cynoglosum has advanced our understanding of the taxonomy, evolution,and ecology of this group of plants.

Trichodesma is a genus of flowering plants in the Boraginaceae family, widely distributed in tropical and subtropical regions of the world, including India. Taxonomic research on *Trichodesma* in India has involved revision and documentation of species diversity, distribution, and identification of new species.

Phylogenetic studies on the genus *Trichodesma* have aimed to reconstruct the evolutionary relationships among species and to understand the origin and diversification of the genus. These studies have used molecular data, such as DNA sequencing, to analyze the genetic relationships among species.

Overall, research on *Trichodesma* has improved our understanding of the taxonomy, evolution, and ecology of this group of plants

and has helped to identify and conserve its biodiversity.

The study was conducted with an aim to study the systematic position of some species of Boraginaceae family and to develop bar-code for easy and quick identification of lesser known species.

Material and Methods

1. Literature Survey

The angiosperm family Boraginaceae includes 142 genera & 2450 species; 43 genera, 209 species, 7 subsp. & 30 var. are found in India (Punekar & Lakshminarsimhan 2011); of which 13 genera & 45 species occur in Maharashtra, 4 of these species are endemic to India and are found in Maharashtra, (Almeida 2001; N. P. Singh & S. Karthikeyan 2001). However, recent studies by various workers (R. R. Mill 2010) have resulted in merger of some species, giving a total of 11 genera &37 species in Maharashtra. The family is represented by 10 genera & 31 species in North West Maharashtra.

All the old literature was referred at libraries of Blatter Herbarium (BLAT), Bombay Natural History Society (BNHS), Botanical Survey of India – Pune (BSI) and Agharkar Research Institute (ARI), Pune. The digital literature was referred on the following URLs such as www.biodiversitylibrary.org, www.jstor.org, https://scholar.google.co.in/, www.researchgate.net and www.academia.edu.

Research papers published in journals like Rheedea, Journal of Economic and Taxonomic Botany and Journal of Threatened Taxa (JoTT) were referred. Some literature was sought from Kew and National History Museum London.

2. Field Survey

Various areas under North – West Maharashtra were selected for the study purpose. These areas were surveyed regularly during all seasons in order to collect and document the detailed information of the plant species. The areas covered are noted in table no.

3. Herbarium Study

Study of previous Herbarium specimens collected by various authors from Maharashtra state, deposited at Blatter Herbarium, Botanical Survey of India (BSI) Pune was conducted.

Collected plants were pressed, dried and preserved for the preparation of Herbarium sheets by standard methods (Bridson & Forman, 1999). Collected plants were identified with the help of Flora, Herbarium and other published literature.

4. Lab Work

Taxon Sampling: The taxon sampling for the molecular data includes at least 2 representatives of each species from the region. Fresh material was collected using silica gel pouches. Collected species were tabulated, digitally photographed and all relevant information were annotated. Tissue was subsampled for laboratory processing. Primary species assignment was done and specimen was curated so as to deposit them as a voucher specimen to museum with unique code.

5. Molecular Analysis

Extraction of DNA from the fresh leaf tissues for each species was carried out by modifying the standard protocol of CTAB. After the purification and optimization the quantification and visualization DNA was carried out following the protocol mentioned in chapter II. Two plastid gene Ribulose 1, 5 bisphosphate (rbcL), maturase kinase (matK), and one nuclear region inter transcribed spacer (ITS) were amplified using the primers mentioned in table 6. For all samples, the amplified products were cleaned with the Qiagen Gel Extraction kit to remove excess template, primer-dimers, and oligonucleotides. The sequencing processes were then carried out using the Sanger sequencing technique (Excelris). ITS and matK did not show the result up to mark and hence were dropped from the further studies. rbcL gave the proper results as selected for the further studies. Sequencing, alignment, and data analysis Sequencing was carried out on a Sanger sequencer. Using the FinchTV

application, the sequence chromatograms were modified and put together. Using BLAST, the sequences amplified in this work were compared to the nucleotide database at NCBI.. The sequences for each sample were generated by using Beckman Coulter sequencer. Results obtained were uploaded on the NCBI portal.

Phylogenetic Analyses

After establishing the models of evolution chosen in jModelTest v.2.1.5 (Dariba, *et al*., 2012) by the Akaike test (option AIC), maximum likelihood (ML) and Bayesian analyses (BI) were carried out at CIPRESS Science gateway (Miller, *et al*., 2010). On the gateway, maximum likelihood analyses were carried out using RAxML-HPC2 running on XSEDE version 8.2.12 (Stamatakis 2014). The model chosen from the jModel test was used to run RAxML with 20 heuristic searches from various random stepwise addition sequence beginning trees, after which the best scoring tree was chosen. 1000 replications were used to determine the RAxML bootstrap (BS) values. The outgroups were chosen from earlier research (Chandler, *et al*., 2007).

Table No: 1 List of plants selected for the study

Sr. No.	Taxon
1	*Adelocaryum coelestinum* (Lind.) Brand
2	*Carmona retusa* (Vahl) Masam.
3	*Cordia alba* (Jacq.) Roem. & Schult.
4	*Cordia dichotoma* G. Forst
5	*Cordia domestica* Roth
6	*Cordia macleodii* Hook. f. & Thomson
7	*Cordia sebestena* L
8	*Cordia sinensis* Lamk.
9	*Cordia subcordata* Lam
10	*Cynoglossum glochodiatum* Wall. Ex Benth.
11	*Ehretia laevis* Roxb.
12	*Heliotropium ovalifolium* Forsk.
13	*Hiloiotropium indicum* L.
14	*Heliotropium strigossum* Wild.
15	*Rotula aquatica* Lour.
16	*Trichodesma inaequale* Edgew.
17	*Trichodesma indicum* (Linn.) Lehm.
18	*Trichodesma zeylanicum* (Burm. f.) R. Br.

Table No 2: Composition of PCR mixture

Components	Volume in µl
Taq buffer	5
MgCl2	5
dNTP's	1
Forward primer	4.4
Reverse primer	4
Taq polymerase	1
Template	1
Nucleolus free water	28.6
Total	50

Table No 3: Cyclic condition for amplification of rbcL gene

Conditions	Temperature °C	Time	
Initial denaturation	96	5 mins	
Denaturation	95	30 secs	35 Cycles
Annealing	58	30 secs	
Extension	72	30 secs	
Final extension	72	5 mins	

Table No 4: List of primers and their sequence utilized for DNA amplification

Primer Name	Primer Sequence	Amplicon length in base pairs	Tm in degree	Cyclic Conditions
RbcL	Forward primer ATGTCACCACAAACA GAGACTAAAGC Reverse primer GTAAAATCAAGTCCACCRCG	613	58°C	95°C/5, 94°C/1, 58°C/1, 35 cycles 72°C/1, °C/10, 4°∞
ITS	ITS – 1 Forward primer ATGTCACCACAAACA GAGACTAAAGC ITS – 2 Reverse primer TCCTCCGCTTATTGATATGC	700	58°C	
Matk	390F- Forward primer CGATCTATTCATTCAATATTTC 1326R- Reverse primer TCTAGCACACGAAAGTCGAAGT	90	54°C	95°C/5, 94°C/1, 54°C/1, 35 cycles 72°C/1, °C/10, 4°∞

Results and Discussion Based on ML Analyses of Ribulose-1, 5- Bisphosphate Carboxylase/ Oxygenase (Rubisco, *RBCL*)

For the study, each sample was taken in triplicates. During the study it was found that ***Adelocaryum coelstinum*** (Lind.) Brand**,** ***Cordia domestica*** Roth, ***Cordia macleodii*** Hook. f. & Thomson, ***Heiloiotropium indicum*** L, ***Heliotropium strigossum*** Wild., ***Rotula aquatica*** Lour. did not show the results up to the mark hence the sequencing for the above-mentioned plants was not performed. The sequences obtained were submitted to the NCBI. **Table no. 5** enlists the plants sequenced and submitted to GenBank with their accession No.

Sequence Analyses

A total of 66 sequences were examined for the analysis, of which 13 accessions (GenBank accession number [submitted]) were newly created from samples taken from various locations throughout Maharashtra. This is the first investigation of any Boraginaceae species from India.

Multiple Sequence Alignment

The alignments of the rbcL regions consists of 1198 characters; Of which are no gaps; GC content is 61.6 %; 935 identical sites in the entire alignment; parsimonious informative sites are 99. Final alignment will be submitted to TreeBASE.

Evolutionary Model Estimation

The best fit model according to BIC criterion is K3Pu+F+I+G4 as evaluated in jModeltest (Dariba, *et al*., 2012). Model selected implemented as -f parameter for subsequent phylogenetic analyses. The rate parameters were - A-C: 1.00000 A-G: 2.45584 A-T: 0.50450 C-G: 0.50450 C-T: 2.45584 G-T: 1.00000 Base frequencies:
A: 0.268 C: 0.201 G: 0.241 T: 0.291 Proportion of invariable sites: 0.613.

ML Phylogenetic Analyses

The tree topology retrieved (Figure 1) has similar topology to that from the previous studies (see Chandler, *et al*., 2007). The log-likelihood of the tree is -5605.384. The total tree length i.e. sum of all branch length is 1.5651. Sum of internal branch length is 0.6297.

Discussion

In the analyses thirteen sequences generated and the phylogenetic relationship is as follows: (Figure1).

For the study undertaken only one species of Euploca has been sequenced viz. *Euploca ovalifolia.* It is grouping with the outgroup selected *Solanum dulcamara* (Bootstrap 97) while in the supplementary tree it can be seen grouping with the remaining *Euploca* spp. the species relationship is not clear as the limitation of the marker *rbc*L. (Figure 1 and Suppl figure 1).

1. Four species of *Trichodesma* have been sequenced *viz. T. zeylanicum, T. inaequale, T, indicum* and *T. scotia*. All the four species group together in polytomy. the relationship between them cannot be deciphered as the variability of the marker is very less. (Figure 1).

2. Two species of *Ehretia* have been sequenced viz: *E. laevis* and *E. microphylla*. in the phylogram it is clear that all the species Ehretia are clubbing together (Bootstrap 100) and distinction among species is not statistically significant (Bootstrap value less than 75). However, it is evident that *E. acuminata* and *E. thrysiliflora* are sister species (Bootstrap 78) and rest other species are in polytomy.

3. Three species of *cordia* have been sequenced viz. *C. gharaf*, *C. sebestena* and two accessions of *C. subcordata*. There is certain amount of genetic variability but still the species cannot be distinguished with good support. All the Cordia species form a monophyletic group (Bootstrap 100) with certain standard support for polytomy of C. myxa, *C. sinensis* and *C. gharaf* (Bootstrap 87).

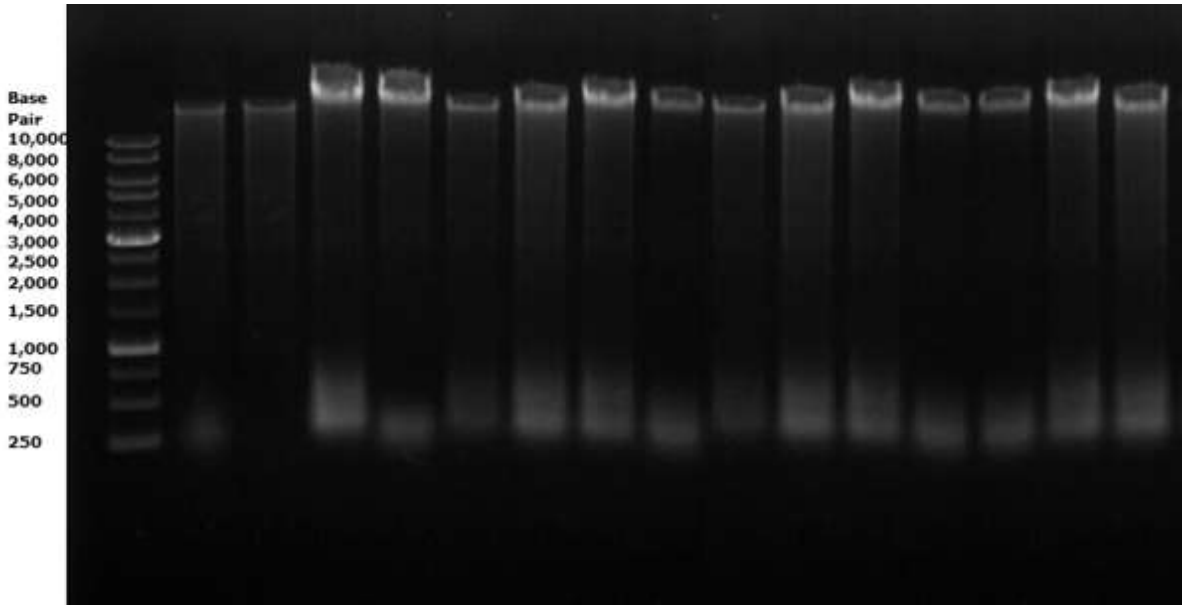
4. There is one miss-identification of C. dentata which is being placed with *Lithospermum incisium.*

5. Only one species of *Cynoglossum wallichii* var. *glochidiatum* sequenced which is placed along with *Cynoglossum officianalis* and one accession of *Paracaryum lithospermifolium*. All the species placed in polytomy.

Table No 5: Accession number for the sequences submitted to GenBank (NCBI)

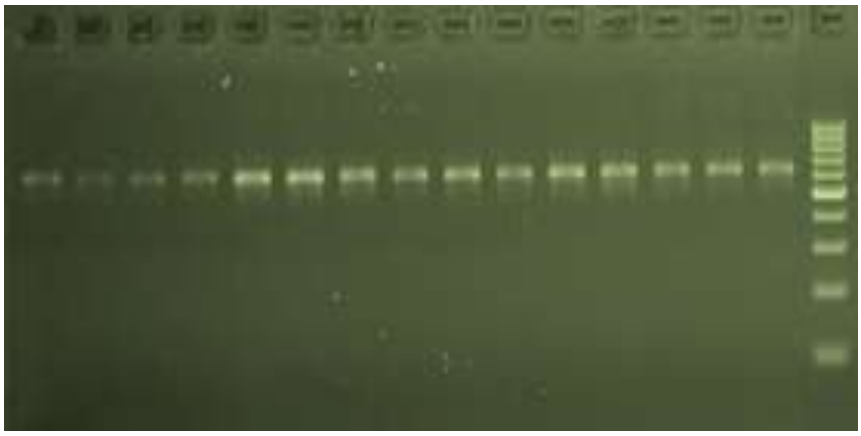
Sr. No.	Plant Name	Accession No. (GenBank)
1	*Euploca ovalifolia*	MW711860
2	*Cynoglossum wallichii var. glochidiatum*	MW711861
3	*Cordia subcordata*	MW711862
4	*Cordia dichotoma*	MW711863
5	*Trichodesma zeylanicum*	MW711864
6	*Trichodesma inaequale*	MW711865
7	*Trichodesma indicum*	MW711866
8	*Ehretia microphylla*	MW711868
9	*Ehretia laevis*	MW711869
10	*Cordia sebestena*	MW711870
11	*Cordia gharaf*	MW711871
12	*Cordia subcordata*	MW711872
13	*Cordia dentata*	Cordia

Figure 1: Best Maximum Likelihood (ML) tree of 66 species belonging to genera offamily Boraginaceae



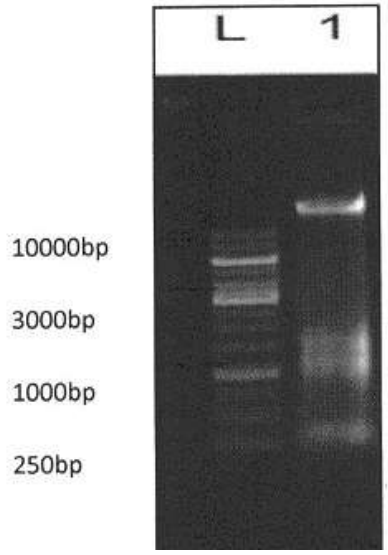
Extracted genomic DNA were analyzed on 0.8 % agarose gel stained with 0.1 microgram/mL of Ethidium bromide.

From left well 1 ladder, Well 2 *Heliotropium ovalifoium* (1), well 3 *Heliotropium ovalifolium* (2), Well 4 *Cynoglossum glochodiatum*, Well 5 *Cordia subcordata*, Well 6 *Cordia dichotoma*, Well 7 *Trichodesma zeylanica*, Well 8 *Trichodesma inequale*, Well 9 *Trichodesma indicum* (1), Well 10 *Trichodesma indicum* (2), Well 11 *Carmona retusa*, Well 12 *Ehretia laevis*, Well 13 *Cordia sebestena*, Well 14 *Cordia sinensis*, Well 15 Cordia *subcordata*, Well 16 *Cordia sebestena*

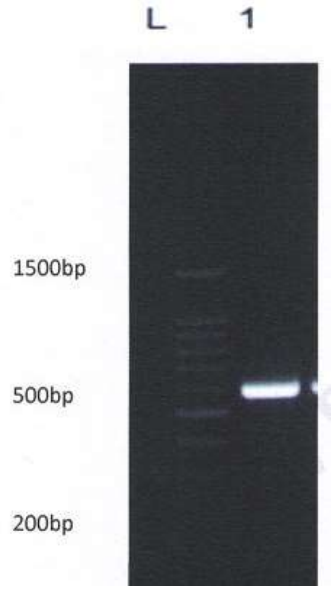


PCR Amplified product of RBCL gene analyzed on 1.5 % agarose gel stained with 0.1 microgram/mL of Ethidium bromide

From left well 1 *Heliotropium ovalifoium* (1), well 2 *Heliotropium ovalifolium* (2), Well 3 *Cynoglossum glochodiatum*, Well 4 *Cordia subcordata*, Well 5 *Cordia dichotoma*, Well 6 *Trichodesma zeylanica*, Well 7 *Trichodesma inequale*, Well 8 *Trichodesma indicum* (1), Well 9 *Trichodesma indicum* (2), Well 10 *Carmona retusa*, Well 11 *Ehretia laevis*, Well 12 *Cordia sebestena* (1), Well 13 *Cordia sinensis,* Well 14 *Cordia subcordata*, Well 15 *Cordia sebestena* (2), Well 16 Ladder



L is the ladder and 1 is *Cordia alba* (Extracted DNA)



L is the ladder and 1 is *Cordia alba* (PCR amplified product)

Conclusion

Among plant genes, *rbcL* was the first to be sequenced (Zurawski, *et al*., 1981). It encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), a critical enzyme in photosynthesis. This is the best characterized gene and its sequence has been widely used in deciphering plant phylogenies. This could be the reason for the availability of more than 10,000 *rbcL* sequences in GenBank (Newmaster, *et al*., 2006; Chase, *et al*., 2007). In most of the phylogenetic studies, this locus has proved to be useful for reconstructing phylogenies down to generic level, but was less effective at the species level (Soltis & Soltis, 1998). Furthermore, the length of *rbcL* (~ 1430 bp) as that of *matK*, poses difficulty in single-pass sequencing (Chase, *et al*., 2007). Moreover, in order to obtain enough variation at the species level entire length of the locus has to be sequenced (Chase, *et al*., 2007). As a solution to this problem, primers for PCR amplification for sequencing of a short segment of *rbcL* containing adequate amount of variability, have been developed for most of the taxa (Fay, *et al*., 1997; Kress & Erickson, 2007). As one of the most effective DNA barcodes for land plants, rbcL has been suggested by the CBOL Plant Working Group, (2009) due to the ease with which its amplicons may be recovered in a wide variety of plants and the availability of sequence data in many plant groups (Vijayan & Tsou, 2010). Nonetheless, most research groups have recommended using rbcL as a DNA barcode in conjunction with other loci due to its weak ability to discriminate across species (Kress & Erickson, 2007; CBOL Plant Working Group 2009; Hollingsworth, *et al*., 2009; Kress, *et al*., 2009; Ebihara, *et al*., 2010; Kress, *et al*., 2010; Jeanson, *et al*., 2011). The data have been extrapolated and discussed based on information found in the literature.

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References

1. Ahn, Y.M. and Sang, T.L. "A palynotaxonomic study of the Korean Boraginaceae."

Korean Journal of Plant Taxonomy 16.3 (1986): 199-215.
2. Almeida, M. R. "Flora of Maharashtra." Orient Press, Mumbai 3A (2001).
3. Al-Nowaihi, A.S., Khalifa, S.F. and K. Hamed. "A contribution to the taxonomy of Boraginaceae." *Phytologia* 62(1987): 107-125.
4. Al-Shehbaz, I.A. "The genera of Boraginaceae in the Southeastern United States." *Journal of the Arnold Arboretum, Supplementary Series* 1(1991): 1-169.
5. Anerao, J., Vikas, J. and Nishaat, S, *et al.* "DNA barcoding of important fruit tree species of agronomic interest in the genus Garcinia L. from the Western Ghats." *Genetic Resources and Crop Evolution* 68.8 (2021): 3161-3177.
6. Angiosperm Phylogeny Group (APG). "An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG 3." *Bot. J. Linn. Soc*. 161(2009): 105– 121.
7. Buys, M.M. and Hilger, H.H. "Boraginaceae cymes are exclusively scorpioid and not helicoid." *Taxon* 52(2003): 719–724.
8. Cohen, J.I. "A case to which no parallel exists": the influence of Darwin's different forms of flowers." *Am. J. Bot*. 97 (2010): 701–716.
9. Cohen, J.I. "A phylogenetic analysis of morphological and molecular characters of Lithospermum L. (Boraginaceae) and related taxa: evolutionary relationships and character evolution." *Cladistics* 27(2011): 559–580.
10. Cohen, J.I. "A revision of the Mexican species of Lithospermum L. (Boraginaceae)." in review. *Ann. Mo. Bot. Gard. in press*.
11. Cohen, J.I. and Davis, J.I. "Nomenclatural changes in Lithospermum (Boraginaceae) and related taxa following a reassessment of phylogenetic relationships." Brittonia 61(2009): 101–111.
12. Cohen, J.I. and Davis, J.I. "Molecular phylogenetics, molecular evolution, and patterns of clade support in Lithospermum (Boraginaceae) and related taxa." *Syst. Bot.* 37(2012): 490–506.
13. Cooke, T. "The Flora of the Presidency of Bombay, 2." *Government of India. London* (1904).
14. Doyle, J.J. and Doyle, J.L. "Isolation of plant DNA from fresh tissue." *Focus* 12(1990): 13–15.
15. Edgar, R.C. "MUSCLE: multiple sequenced alignment with high accuracy and high throughput." *Nucleic Acids Res*. 32(2004): 1792– 1797.
16. Farris, J.S., Victor, A. A., Mari, K., Diana, L. and Arnold, G. K. "Parsimony jackknifing outperforms neighbor-joining." Cladistics 12.2(1996): 99–124.
17. Fitch, W.M. "Toward defining the course of evolution: minimum change for a specific tree topology." *Syst. Zool*. 20(1971): 406–416.
18. Hilger, H.H., Marc, G., Federico, S., Massimo, B., Elisabeth, L., Elke, Z., Nadja, D. and Maximilian, W. "The Euro+ Med treatment of Boraginaceae in Willdenowia 34—a response." *Willdenowia* 35.1 (2005): 43-48.
19. Hilger, H.H. and Elke, Z. "Studies in the Boraginaceae" – an index to the publications of Ivan M. Johnston dealing with the borage family." *Haussknechtia Beiheft* 11(2001): 1–151.
20. Hooker, J.D. "Flora of British India." *L. Reeve & Co. Ltd., London* 4 (1885): 148-153.
21. Kazmi, S.M.A. "A revision of the Boraginaceae of West Pakistan and Kashmir." *Journal of the Arnold Arboretum* 51.2 (1970): 133-184.
22. Mabberley, D.J. "Mabberley's Plant Book, 3rd edn." *Cambridge University Press, Cambridge* (2008).
23. Miller, M.A., Wayne, P. and Terri, S. "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" *in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA* (2010): 1 – 8.
24. Miller, J.S. "Classification of Boraginaceae subfam. Ehretioideae: resurrection of the genus Hilsenbergia Tausch ex Meisn." *Adansonia, ser*. 3(2003): 151–189.
25. Shinde, R.D. and Neha, G. "A Brief Account of the Genus Cordia (Boraginaceae)

in Maharashtra." *Annals of Plant Sciences* 11.10 (2022): 5455-5464.
26. Singh, N. P., S. Karthikeyan., P. Lakshminarsimhan. and Prasanna, P. V. "Flora of Maharashtra state, Dicotyledons." *BSI, Calcutta* 2(2001).
27. Smith, S. A. and Michael, J. D. "Rates of molecular evolution are linked to life history in flowering plants." *Science* 322 (2008): 86–89.
28. Stamatakis, A. "RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies." *Bioinformatics* 30.9 (2014): 1312-1313.
29. Darriba, D., Guillermo, L. T., Ramón, D. and David, P. "jModelTest 2: more models, new heuristics and parallel computing." *Nature methods* 9.8 (2012): 772-772.
30. National Center for Biotechnology Information (NCBI) [Internet]. "Bethesda (MD): National Library of Medicine (US)." *National Center for Biotechnology Information* [1988] – [2017 Apr 06, 2018, Aug 11]. https://www.ncbi.nlm.nih.gov/

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