DNA barcode developement based on chloroplast and ITS genes for species identification of endangered and threated species of

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Abstract

Accurate identification is crucial for conserving species, especially in regions such as the Western Ghats, where trade poses a significant threat to endangered and threatened forest species. Traditional morphology-based identification can be challenging and time-consuming, leading to inaccuracies, especially with similar-looking species or dried specimens. Therefore, DNA barcoding offers a potent solution for precise species identification to address illicit trade and address impactful conservation measures. DNA barcoding is a taxonomic technique that uses standardized short DNA sequences to differentiate and classify species. This approach is especially valuable when morphological characteristics alone are insufficient for accurate species identification. In this study, we focused on the development of a DNA barcoding system for the efficient and accurate identification of threatened and endangered important forest species of Western Ghats Karnataka. To develop the DNA barcoding system, a multilocus approach utilizing sixteen standard DNA barcoding markers was used. A total of 47 threatened and endangered forest species from the Western Ghats were selected for this study. Using a larger number of markers to develop DNA barcodes led to the most precise species identification rates. Moreover, the wide availability of DNA barcode databases allows for quick and accurate species identification. In our study, we observed the highest amplification rates for rbcL1 (40 species), psbtrnH2 (36 species), and PsbA-trnH1 (33 species). DNA amplification varied from 11.76–94.11%. Notably, the highest DNA amplification rates were detected for *A. wightii* (94.11%) and *A. hondala (*92.34%), both of which belong to the Arecaceae and Passifloraceae families, respectively. Sequencing success rates ranged from 37.5–100%. This study will aid in the development of a database of available threatened forest species in western Ghats Karnataka and other regions.

Introduction

The identification of forestry species plays a crucial role in biodiversity conservation, particularly in regions such as the Western Ghats Karnataka, where plant biodiversity hotspots face threats from habitat fragmentation, overexploitation, and human activities. The preservation of threatened and endangered plant species is essential for biodiversity conservation efforts (Kress et al. 2005). However, traditional methods of plant identification are facing challenges due to declining taxonomic expertise and the lack of precise tools to distinguish between various plant forms, such as seeds, plant parts, seedlings, and herbal adulterants (Saddhe and Kumar 2018). Recognizing commercial plant materials can be particularly difficult because they are often traded in dry or processed forms and lack the necessary morphological features for accurate identification by retailers and customers (Ghorbani et al 2017). Taxonomic identification using macro- and micromorphological techniques is laborious, prone to errors, and requires expertise and reliable resources. DNA barcoding has emerged as a promising method for accurately identifying plant species, offering distinct advantages over traditional morphological identification techniques (Saddhe and Kumar 2018). This molecular marker-based approach allows for rapid screening of DNA variation, resulting in increased taxonomic resolution (Fatima et al 2019). It has proven particularly effective for species with ambiguous morphological features (Ghorbani 2017). Despite extensive research on DNA barcoding for trees and plant species, a significant number of important species, particularly those found in the Western Ghats, still lack DNA barcode sequences for molecular identification. In the literature, it has been noted that barcoding and DNA sequences are accessible for certain species. However, a significant portion of species currently lack available DNA barcodes, highlighting a gap in our knowledge and resources within this domain. Our study aimed to address this gap by providing DNA barcodes for threatened forest species, thereby supporting conservation efforts in western Ghats Karnataka. To ensure accuracy, researchers often employ a multilocus approach utilizing various DNA markers (Saddhe and Kumar 2018; Abubakar et al 2017; Li et al 2011). We evaluated the efficacy of sixteen sets of primers targeting different regions in the nuclear and plastid genomes of plants, including commonly used markers such as matK, rbcL, trnH-psbA, Ar matk (Cuenoud et al. 2002), rpoB, rpoC (Kurian et al 2020, Sass et al 2007), rpoB-trnCGAR (Anerao et al 2021), psbZ-trnfM (Kurian et al 2020; Scarcelli et al 2011), trnHpsbA (Kurian et al 2020; Kress et al 2005), psbK (Kurian et al 2020; Lee et al 2007), rbcL (Fatima et al. 2019), PRK (Lewis and Doyle 2002), RPB2, ITS2, ATP (Kurian et al 2020), ndhF-rpl32 (Sass et al. 2007), MatK2 (Cuenoud P et al 2002), psbA-trnH (Chandrasekara et al 2021, Sang et al 1997), Ar rbcL (Jeanson M L et al.2011), and DI ITS (Fatima et al 2019)

In our study, our goal was to create a robust DNA barcoding system capable of accurately identifying 47 endangered and threatened forest species from 21 different families, *namely*, Fabaceae, Lauraceae, Santalaceae, Balsaminaceae, Myristicaceae, Ebenaceae, Annonaceae, Rubiaceae, Arecaceae, Dipterocarpaceae, Cluciaceae, Calophyllaceae, Myrtaceae, Malvaceae, Apiaceae, Apocynaceae, Asteraceae, Passifloraceae, Sapotaceae, Chrysobalanaceae, Cycadaceae and Anacardiaceae, providing important insights into their conservation and management (Table 1) of forest species in western Ghats Karnataka. The results of this study contribute to the development of a molecular database containing information on threatened forest species not only in western Ghats but also across other regions. Such a database can significantly aid conservation efforts by enabling accurate identification and monitoring of endangered species and threatened forest species.

Table 1
Description of the sample collection of Threatened and Endangered forestr Species of Karnataka

Sl No.	Plant species/Tree type/IUCN status	Location	DNA Barcoding status	Sample voucher No.	Coordinates	Reference
(1) **Apocynaceae**						
1.	*Rauvolfia serpentina*/**H**/**En**	FRLHTB	1. (trnL-trnF) 2.(ITS2,matK, rbcL and trnL)	rs11frlhtbk	N12.722387/E77.569387	1. Eurlings et al (2013); 2. Cahyaningsih et al (2022) Ved et al (2016); Gowthami et al (2021)
2.	*Tabernaemontana heyneana*/**T**/**NT**	FRLHTB	NA	tah06frlhtbk	N13.1234777/E77.5478629	World Conservation Monitoring Centre (1998); Bhavana et al (2020)
(2) **Anacardiaceae**						
3.	*Holigarna arnottiana/* **T**/**E**	PFRK	NA	hr07pumk	N12.6629086/E75.6849674	Mane et al (2020)
4.	*Semecarpus auriculata/***T**/**NT**	PFRK	NA	sesa12pfrk	N12.6628086/E75.6849432	Rao et al (2019)
(3) **Arecaceae**						
5.	*Calamus nagbettai/***C**/**E**	RMSK	rbcL, matK, psbA-trnH, rpoC, rpoB, psbKpsbI, atpF-atpH and RPB2	can09rkmb	N12.722387/E77.569387	Kurian et al (2020); Dev et al (2019)
6.	*Arenga wightii*/**T**/**V**	PFRK	NA	arw29puk	N12.6628086/E75.6849432	Rao et al (2019)
(4) **Balsaminaceae**						
7.	*Impatiens mysorensis*/**H**/ **E**	Baluru forest (Mudigere), Karnataka	NA	im17bfmk	N13.1321413/E77.4943489	Babu et al (2007)
8.	*Impatiens pulcherrima*/**H**/**V**	PFRK	NA	ip09pfrk	N12.5460548/E75.7019173	Rao et al (2019)
(5) **Bignoniaceae**						
9.	*Oroxylum indicum/***T**/**V**	IIHR Bangalore, Karnataka	ITS2, psbA-trnH	oi21iihrk	N13.1321413/E77.4943487	Gowthami et al (2021); Rajasekharan et al (2017); Cui et al (2020)
(6) **Calophyllaceae**						
10.	*Calophyllum apetalum/***T**/**V**	FRLHTB	NA	cpa06frlbk	N13.1234156/E77.5461281	Prakash and Nigam (1984); Ved et al (2016)
(7) **Clusiaceae**						
11.	*Garcinia indica/***T**/**V**	RMSK	rbcL, ITS, psbA-trnHf, RpoB-trnCGAR, Matk	gc06rmsk	N12.97194/E77.59369	Seethapathy et al (2018); Anerao et al (2021); Gowthami et al (2021)
12.	*Garcinia wightii/***T**/**V**	PFRK	NA	gw16pfrk	N12.6628086/E75.6849432	Rao et al (2019)
(8) **Cycadaceae**						
13.	*Cycas circinalis*/**T**/**En**	GKVKB	ITS2	cc03gkvkb	N13.0859021/E77.5539838	Gao et al (2010); GBIF Secretariat (2022).
(9) **Dipterocarpaceae**						

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Sl No.	Plant species/Tree type/IUCN status	Location	DNA Barcoding status	Sample voucher No.	Coordinates	Reference
(1) Apocynaceae						
14.	*Vateria indica*/T/V	Doresanipalya Forest Campus Bangalore, Karnataka	NA	vi01dfcb	N12.89654/E77.592095	Dhyani and Barstow (2020)
15.	*Shorea roxburghii*/T/V	DFCB	ITS1 and ITS2; psbM, rbcL, psbH, rpoC2	sro06dfcb	N12.89654/E77.592094	Osathanunkul et al (2021); Yu et al (2021); Raju et al (2011)
16.	*Hopea ponga*/T/LC	RMSK	NA	hp07rkmsb	N12.722387/E77.569387	Rao et al (2019)
17.	*Dipterocarpus indicus*/T/T	PFRK	NA	di18pfrk	N12.5460548/E75.7019177	Shivaprasad et al (2014)
18.	*Hopea parviflora*/T/NT	GKVKB	MatK, rbcL (data unpublished)	hp02gkvkb	N13.0859021/E77.5539838	Rao et al (2019)
19.	*Vatica chinensis*/T/En	Agumbe range, Karnataka	rbcL and matk (data unpublished)	vc05ark	N13.1321413/E77.4943489	Sanilkumar et al (2022)
20.	*Vateria macrocarpa*/T/CR	PFRK	maturase K, tRNA-Leu (trnL) trnL-trnF intergenic spacer (data unpublised)	vm04pfrk	N12.5460548/E75.7019172	Keshavanarayan et al (2015)
21.	*Dipterocarpus bourdiollon*/T/CR	PFRK	NA	db02pfrk	N12.6628086/E75.6849432	Page et al (2022)
(10) Ebenaceae						
22.	*Diospyros paniculata*/T/V	PFRK	NA	dp16pfrk	N12.5460548/E75.7019174	Ved et al (2015)
23.	*Diospyros candolleana*/T/V	PFRK	NA	dc23mk	N12.6628075/E75.6849443	Gopalakrishna (2014)
24.	*Diospyros crassiflora*/T/V	RMSK	trnK matK, trnH-psbA	dc13rkmb	N12.722387/E77.569387	Mascarello et al (2021); Schatz et al (2019); Geeraerts et al (2009)
(11) Fabaceae						
25.	*Dalbergia latifolia*/T/V	DFCB	ITS and rbcL	dl12dbk	N12.896542/E77.592093	Manikandan et al (2017); Fatima et al (2019); Hartvig et al (2015)
26.	*Pterocarpus marsupium*/T/NT	GKVKB	ITS, MatK, rbcL, ndhF-rpl32	pm02gkvkb	N12.985211/E77.5188456	Ghosh, et al (2021); Jio et al (2018)
27.	*Saraca asoca*/T/V	GKVKB	rbcL and psbA-trnH	sa06gkvkbk	N13.0705725/E77.5781428	Saini et al (2018); Urumarudappa et al (2016)
28.	*Kingiodendron pinnatum*/T/En	GKVKB	NA	kp14gkvkbk	N13.0859021/E77.5539838	Jose et al (2018)
29.	*Pterocarpus santalinus*/T/E	GKVKB	ITS2, matK, ndhF-rpl32 and rbcL	ps13gkvkb	N13.0859021/E77.5539838	Arunkumar and Joshi (2014); He et al (2019)
(12) Flacourtiaceae						
30.	*Hydnocarpus macrocarpa*/T/V	PFRK	NA	hm05pfrk	N12.5460548/E75.7019176	Mariyaraj et al (2019)
(13) Lauraceae						
31.	*Cinnamomum riparium*/T/V	PFRK	NA	ca15pfrk	N12.6628086/E75.6849432	Rao et al (2019); Chandrasekara et al (2021); de Kok (2020)
(14) Primulaceae						
32.	*Embelia ribes*/C/V	IIHR Bangalore, Karnataka	ITS, matK, rbcL, and psbA-trnH	er15iihrb	N13.1321413/E77.4943489	Kumari and Misra (2020)

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Sl No.	Plant species/Tree type/IUCN status	Location	DNA Barcoding status	Sample voucher No.	Coordinates	Reference
(1) Apocynaceae						
						Bajpe et al (2022)
(15) Malvaceae						
33.	*Pterospermum reticulatum*/T/V	GKVKB	NA	pm10gkvkb	N12.985212 E77.5188456	Keshavanarayan et al. (2015).
34.	*Syzygium occidentale*/T/E	PFRK	NA	so04pfrk	N12.5460548/E75.7019175	Varghese and Sreekala (2017)
35.	*Syzygium travancoricum*/T/ CR	FRLHT Bangalore, Karnataka	Data unpublished	stmf13frlhtbk	N13.1234156/E77.5461282	Sreekumar et al (2020)
(16) Meliaceae						
36.	*Dysoxylum malabaricum*/T/En	PFRK	NA	dm24pfrk	N12.5460548/E75.7019173	Ved et al (2015)
(17) Myristicaceae						
37.	*Gymnacranthera canarica*/T/V	PFRK	Available (data unpublished)	gca11pfrk	N12.5460548/E75.7019173	Anusha et al (2023)
38.	*Myristica malabarica*/T/V	GKVKB	rbcL, matK, psbA-trnH (data unpublished)	mma15gkvkbk	N13.0705724/E77.5781423	Jose and Pillai (2016)
39.	*Myristica beddomei*/T/E	PFRK	rbcL, matK, psbA-trnH, ITS	mbe15pfrk	N12.6628086/E75.6849432	Govind and Dan (2022); Swetha et al (2019)
40.	*Knema attenuata*/T/LC	Sirsi, Karnataka	rbcL, matK, psbA-trnH, ITS	ka12srk	N14.6187728/E74.8021528	Hegde et al (2015); Swetha et al (2019)
(18) Sapotaceae						
41.	*Madhuca bourdillonii*/T/CR	PFRK	Data unpublished	mb26pfrk	N12.5460548/E75.7019174	Chandran et al (2008)
42.	*Madhuca insignis*/T/CR	FRLHTB	NA	mi28frlhtb	N13.1234777/E77.5478629	Dhyani et al (2020)
(19) Santalaceae						
43.	*Santalum album*/T/V	DFCB	rbcL matK, trnH-psbA, trnK, trnL	sa03dfcbk	N12.89654/E77.592092	Kumar et al (2019); Suma et al (2014); Jiao et al (2018)
(20) Rubiaceae						
44.	*Ochreinauclea missionis*/T/V	BFK	NA	om21bfmk	N13.100953/E75.482093	Chandrika and Ravishankar (2009)
45.	*Psydrax dicoccos*/T/V	RMSK	NA	pds09rkmsb	N12.722387/E77.569387	World Conservation Monitoring Centre (1998)
46.	*Tarenna agumbensis*/S/En	BFK	NA	ta23bfmb	N13.100952/E75.482092	Puyravaud et al (2003)
(21) Passifloraceae						
47.	*Adenia hondala*/C/En	FRLHTB	NA (data unpublished)	ah09frlbk	N13.1234156/E77.5461281	Sivakamasundari et al (2015)

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Methodology

Study area

The sampling site for this study consisted of various locations across Karnataka. The samples of selected species were collected from the regions of Bengaluru, Sirsi, Madikeri, Agumbe, and Chikkamagaluru. These locations were selected based on their availability and relevance to the study of threatened

and endangered plant species in the region. Samples from a total of 47 different threatened and endangered species were collected, each of which was carefully chosen to represent the overall biodiversity of the area. To maintain the accuracy of the study, the GPS coordinates of each sampling site were recorded during the fieldwork. This information was used to map the distribution of the selected species across the various locations in Karnataka (Fig. 1).

Details about the selected plant species, sample locations, GPS coordinates, and altitudes were recorded (Table 1), providing a comprehensive overview of the environmental conditions present at each location. This information can be used to better understand the factors that contribute to the survival of these threatened and endangered species in the region.

The data collected from these locations can provide valuable insights into the conservation of these species and their habitats.

2. Sample collection: The sample collection for this study involved the collection of leaf samples from 47 selected threatened and endangered species from various parts and forests of the western Ghats. To ensure a representative sample, five to ten samples (five to ten leaves) of each species were selected.

Leaf sampling was carried out by carefully selecting healthy leaves from each plant, taking care not to damage the plant. The leaf samples were then placed in a plastic cover to prevent any damage or contamination during transport.

To prepare the samples for further analysis, the collected samples were cleaned and left to dry for a period of at least seven to fourteen days, allowing the moisture content of the samples to be eliminated. This process helps to preserve the samples for further analysis and helps to prevent the growth of fungi and other microorganisms that can degrade the samples.

In addition to sample preservation, herbarium sheets were prepared for each species. The process of preparing the herbarium sheets involved pressing and drying the collected plant specimens onto acid-free herbarium sheets. The specimens were then labeled with information such as the species name, collection date, location, and collector's name. This ensures that the samples can be identified and referenced in the future, even after the plant material has deteriorated.

3. Genomic DNA isolation and quantification

Total genomic DNA was isolated from the dried leaves by using a modified CTAB protocol (Fatima et al. 2018, Fatima et al. 2019). The extracted DNA was quantified, and to confirm the DNA's suitability for sequencing and barcoding, the samples were run on a 1% agarose gel stained with 4 µL of 0.3% ethidium bromide (Fatima et al. 2019).

4. PCR amplification and purification of amplified PCR products

Sixteen sets of primers were selected from the literature for the current DNA barcoding study. The details of the primer sequences are shown in Table 2. A reaction volume of 13 µL was used for DNA amplification, containing 1.5 µL of genomic DNA (30 ng/µL), 2 µL of each forward and reverse primer (10 mM), 1.5 µL of PCR buffer, 1.5 µL of dNTPs (10 mM), 1.5 µL of $MgCl_2$, 0.3 µL of Taq polymerase (3 U/µL) (Bangalore genus), and 4.2 µL of double-distilled water. The amplification cycle consisted of an initial denaturation step for 3 min at 94°C, followed by 40 cycles of 1 min at 60–61°C and 1 min at 72°C and a final extension step for 10 min at 72°C (Williams et al. 1990).

Table 2
Primers used for DNA barcoding

Sl. No.	Primer Name	Primer Sequence 5' to 3'	No. of Bases	Reference
1.	**MatK1**	F:CGTACAGTACTTTTGTGTTTACGAG	25	Cuenoud et al (2002)
		R:ACCCAGTCCATCTGGAAATCTTGGTTC	27	
3.	**rpoC**	F:GGCAAAGAGGGAAGATTTCG	20	Kurian et al.(2020); Sass et al. 2007)
		R:CCATAAGCATATCTTGAGTTGG	22	
5.	**rpoB**	F:AAGTGCATTGTTGGAACTGG	20	Kurian et al.(2020)
		R:GATCCCAGCATCACAATTCC	20	
7.	**rpoB-trnCGAR**	F:CKACAAAAYCCYTCRAATTG	20	Anerao et al (2021)
		R:CACCCRGATTYGAACTGGGG	20	
9.	**psbZ-trnfM**	F:GGTACMTCATTATGGATTGG	20	Kurian et al. (2020); Scarcelli et al. 2011)
		R:GCGGAGTAGAGCAGTTTGGT	20	
11.	**trnHpsbA1**	GTWATGCAYGAACGTAATGCTC	20	Kurian et al. (2020); Kress et al. (2005)
		CGCGCATGGTGGATTCACAATCC	20	
13.	**psbK**	F:TTAGCCTTTGTTTGGCAAG	20	Kurian et al. (2020)
		R:AGAGTTTGAGAGTAAGCAT	20	
15.	**RbcL1**	F:GCTGCCGAATCTTCTACTGG	20	Fatima et al. (2019)
		R:GGATTCGCAAATCTTCCAGA	20	
17.	**PRK**	F:GTGATATGGAAGAACGTGG	19	Kurian et al. (2020); Lewis and Doyle (2002)
		R:ATTCCAGGGTATGAGCAGC	19	
19.	**RPB2**	F:CAACTTATTGAGTGCATCATGG	22	Kurian et al. (2020)
		R:CCACGCATCTGATATCCAC	19	
21.	**ITS1**	F:ATGCGATACTTGGTGTGAAT	20	Kurian et al. (2020)
		R:GACGCTTCTCCAGACTACAAT	21	
23.	**Atp**	F:ACTCGCACACACTCCCTTTCC	21	Kurian et al. (2020)
		R:GCTTTTATGGAAGCTTTAACCAAT	24	
			20	
13.	**MatK2**	F:CGATCTATTCATTCAATATTTC	22	Cueenoud et al. (2002)
		R:TCTAGCCACGAAAGTCGAAGT	21	
14.	**PsbA-trnH2**	F:GTTATGCATGAACGTAATGCTC	22	Chandrasekara et al. (2021); Sang et al. (1997)
		R:CGCGCATGGTGGATTCACAATC	22	
15.	**RbcL2**	F:ATGTCACCACAAACAGAGACTAAAGC	26	Jeanson et al. (2011)
		R:ATGTCACCACAAACAGAGACTAAAGC	26	
16.	**ITS2**	F:AGGAAGCAACAACCGAACAG	20	Fatima et al. (2019)
		R:TTGCGTTCAAAGACTCGATG	20	

5. DNA sequencing and DNA barcode generation: The purified PCR products were subjected to Sanger sequencing at Eurofins Genomics India Pvt. The primers and barcodes for each sequence were developed by using Bio-Rad online software. The developed sequences were submitted to the NCBI Sequence Data Submission Bank (http://www.ncbi.nlm.nih.gov/gen-bank/) to obtain accession numbers. The nucleotide database of the US National Centre for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov) was searched for homologs of related genes.

Testing DNA barcode accuracy:

Before evaluating the identification success of the selected DNA barcode regions, we used the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare our sequences with those submitted to GenBank, which will be accessed on February 10, 2025 (https://www.ncbi.nlm.nih.gov/genbank/), with the aim of confirming the molecular level of identification of selected species.

Results and Discussion

The 47 endangered, vulnerable, and threatened forest species selected for the project represent diverse plant families. Among these families, Dipterocarpaceae had the greatest number of species, totaling eight. Fabaceae had five species, and Myristicaceae had four species (Fig. 2; Table 1).

Frequency of selected marker amplification

In this study, a total of sixteen DNA barcoding markers were selected from various literature references (Table 2). When examining these markers individually, some were notably more effective at identifying species than others were. For instance, the RbcL1 marker emerged prominently with a high success rate of 37% in species identification, closely followed by PsbA-trnH2 and PsbK, which achieved success rates ranging from 36–32%. However, some Barcoding markers, such as MatK2 and ITS2, exhibited lower amplification rates (Fig. 3).

DNA barcoding marker amplification and sequencing across selected families and their respective species: The present investigation listed 21 families according to the chosen species (Table 1). All of these samples were amplified using selected DNA barcoding markers. While amplification was detected in all species, it was limited to only a few markers in certain instances. DNA amplification ranged from 11.76–94.11%. The highest DNA amplification rates were observed for *A. wightii* (94.11%) and *A. hondala* (92.34%), which belong to the Arecaceae and Passifloraceae families, respectively. Conversely, the lowest amplification success rate (30.76%) was noted for four species, namely, *T. heyneana, V. indica, P. marsupium* and *M. insignis*. The sequencing success rates ranged from 37.5–100%, with twenty out of the 47 selected species achieving a 100% sequencing success rate (Fig. 4). *The* Apocynaceae family includes species such as *R. serpentina* and *T. heyneana*. Both species were analyzed using DNA amplification techniques, with common markers such as RpoB, RbcL1 and Atp. Additional markers, such as PsbZ-trnfM, trnHpsbA1, and PsbA-trnH2, were detected in *R. serpentina*, while *T. heyneana* was amplified solely with the PsbK marker. The collective amplification rate was 46.15 ± 30.76%, with a subsequent sequencing rate of 100% observed across the respective species within the family. The Anacardiaceae family comprises species such as *H. arnottiana* and *S. auriculata*. DNA amplification techniques were applied to both species utilizing common markers such as PsbK, PsbA-trnH2, and RbcL1. *H. arnottiana* was amplified with the trnHpsbA1, RPB2, and Atp markers, while *S. auriculata* was amplified with the MatK1, RpoC, and RpoB-trnCGAR markers. The collective amplification and sequencing rates were as follows: *H. arnottiana* (76.92 ± 100%) and *S. auriculata* (52.94 ± 88.9%). In the Arecaceae family, the analysis of two species, *C. nagbettai* and *A. wightii*, revealed intriguing findings. Both species were amplified using common markers such as RpoB, PsbZ-trnfM, and RbcL1. Notably, *A. wightii* exhibited additional amplification with markers such as MatK2, RpoC, and RbcL2, revealing a broader genetic profile. The amplification rates for each species were distinct: *C. nagbettai* displayed amplification and sequencing rates of 87.65% ± 100%, while *A. wightii* showed a robust amplification rate of 94.11% ± 87.5%. In the context of the Balsaminaceae, the investigation included *I. mysorensis* and *I. pulcherrima*. Both species were amplified using common markers such as trnHpsb1, PsbA-trnH2, and PsbK. Notably, *I. mysorensis* displayed additional amplification with RpoB, while *I. pulcherrima* exhibited amplification with markers such as RbcL2 and MatK1 (Table 3). The amplification and sequencing rates for each species were as follows: *I. mysorensis* (46.15 ± 100) and *I. pulcherrima* (64.7 ± 90.9). Within the Bignoniaceae family, investigations have focused on *O. indicum*. This species exhibited amplification with multiple barcoding markers, including MatK1, RpoC, and RpoB (Table 3). The amplification and sequencing rates observed for *O. indicum* were 64.7 ± 100%, respectively. Within the Calophyllaceae family, *C. apetalum* is a solitary member. Notably, *C. apetalum* demonstrated amplification and sequencing rates of 61.5 ± 87.5%. This species shows amplification and sequencing patterns involving markers such as RpoB, PsbZ-trnfM, and trnHpsbA1. In the Clusiaceae family, DNA barcoding analysis was conducted on two species, namely, *G. indica* and *G. wightii*. While *G. indica* exhibited amplification with only two specific barcode markers, namely, PsbZ-trnfM and ITS2, Garcinia wightii displayed a more extensive amplification profile. Twelve barcoding markers, namely, MatK1, RpoC, RpoB, RpoB-trnCGAR, PsbZ-trnfM, trnHpsbA1, PsbK, RbcL2, RPB2, PsbA-trnH2, RbcL1, and Atp, were successfully captured from G. wightii. The amplification and sequencing rates observed for *G. indica* and *G. wightii* were 38.46 ± 40.0% and 76.47 ± 92.3%, respectively. In the Cycadaceae family, DNA barcoding analysis was performed on *C. circinalis*, which revealed the amplification of four distinct DNA barcode markers. These markers, namely, RpoB-trnCGAR, PsbZ-trnfM, PRK, and Atp, contributed to the generation of unique DNA barcodes. The observed amplification and sequencing rates for *C. circinalis* were 53.84 ± 57.1%. A comprehensive investigation revealed various species of the Dipterocarpaceae family, including *V. indica, S. roxburghii, H. ponga, D. indicus, H. parviflora, V. chinensis, V. macrocarpa* and *D. bourdiollon*. Each species exhibited specific amplification patterns during genetic analysis. *V. indica* was amplified with four barcoding markers: trnHpsbA1, PsbK, PsbA-trnH2, and RbcL1. The amplification of the RpoB, trnHpsbA1, PsbK, RPB2, PsbA-trnH2, RbcL1, and ITS2 markers in S. roxburghii is shown. *H. ponga* displayed amplification of RpoB-trnCGAR, trnHpsbA1, PsbK, RPB2, PsbA-trnH2, RbcL1, and ITS2. *D. indicus* was amplified with the markers MatK1, RpoC, RpoB, PsbK, RbcL2, RbcL1, and ITS2. *H. parviflora* was characterized by amplification of three DNA barcoding markers, namely, trnHpsbA1, PsbA-trnH2, and PsbK. The MatK1, RpoC, PsbK, RbcL2, RbcL1, and ITS2 markers of V. chinensis were amplified. V. macrocarpa was amplified by seven DNA barcoding markers: MatK1, RpoC, PsbZ-trnfM, trnHpsbA1, PsbK, PsbA-trnH2, and RbcL1. Finally, *D. bourdiollon* was amplified with ten DNA barcoding markers, generating barcodes with MatK1, RpoC, RpoB, RpoB-trnCGAR, trnHpsbA1, PsbK, RbcL2, ITS1, PsbA-trnH2, and RbcL1. The observed amplification and sequencing rates for each species were as follows: *V. indica* (30.76 ± 100), S. roxburghii (46.15 ± 100), *H. ponga* (69.23 ± 77.8), *D. indicus* (41.17 ± 100), *H. parviflora* (23.07 ± 100), *V. chinensis* (35.29 ± 100), *V. macrocarpa* (41.17 ± 100), and *D. bourdiollon* (70.58 ± 83.3). In the Ebenaceae family, three species were examined: *D. paniculata, D. crassiflora*, and *D. candolleana*. *D. paniculata* exhibited amplification with RpoC and RbcL2 DNA barcoding markers, while *D. crassiflora* showed amplification with eleven DNA barcoding markers, including RpoB, RpoB-trnCGAR, PsbZ-trnfM, trnHpsbA1, PsbK, RPB2, MatK2, PsbA-trnH2, RbcL1, Atp, and ITS2. D. candolleana, on the other hand, was amplified by the RpoC, trnHpsbA1, PsbK, RbcL2, and RbcL1 DNA barcoding markers (Table 3). The amplification and sequencing rates were 11.76 ± 100 for D. paniculata, 84.61 ± 100 for *D.* crassiflora and 29.41 ± 100 for D. candolleana. The Fabaceae family was represented by five species: *D. latifolia, P. marsupium, S. asoca, K. pinnatum*, and *P. santalinus*. *D. latifolia* was amplified and sequenced with nine DNA barcoding markers, namely, RpoB, RpoB-trnCGAR, PsbZ-trnfM, trnHpsbA1, RPB2, PsbA-trnH2, RbcL1, Atp and ITS2. Four DNA barcoding markers were used to amplify P. marsupium: RpoB, trnHpsbA1, PsbA-trnH2 and RbcL1. *S. asoca* was amplified with RpoB, trnHpsbA1, RPB2, PsbA-trnH2 and RbcL1. *K. pinnatum* was amplified and sequenced with RpoB, RpoB-trnCGAR, PsbA-trnH2, and RbcL1. Finally, *P. santalinus* was amplified with five barcoding markers: RpoB, TrnHpsbA1, PsbA-trnH2, RbcL1, and Atp (Table 3). The amplification and sequencing rates *were* 76.92 ± 90.8 *for D. latifolia*, 30.76 ± 100.0 *for P. marsupium*, 46.15 ± 83.3 *for S. asoca*, 61.53 ± 37.5 for K. pinnatum, and 38.46 ± 100% *for P. santalinus* (Fig*).* Within the Flacourtiaceae family, *H. macrocarpa* was the focal species subjected to amplification and sequencing utilizing two markers, RpoC and RbcL1, with an amplification and sequencing rate of 11.76 ± 100%. In the Lauraceae family, *C. riparium* emerged as the prominent species, displaying the highest

amplification success in this investigation. Among the sixteen markers analyzed, fourteen were effectively amplified. These markers included MatK1, RpoC, RpoB, PsbZ-trnfM, trnHpsbA1, PsbK, RbcL2, PRK, RPB2, MatK2, PsbA-trnH2, RbcL1, Atp, and ITS2, with an amplification and sequencing rate of 88.23 ± 86.7%. *E. ribes* represents the Primulaceae family and was successfully amplified by twelve markers: MatK1, RpoC, RpoB, RpoB-trnCGAR, PsbZ-trnfM, trnHpsbA1, PsbK, RbcL2, PsbA-trnH2, RbcL1, Atp, and ITS2. The amplification and sequencing rates for E. ribes were 70.58 ± 91.7%. Within the Malvaceae family, three species have been identified: *P. reticulatum, S. occidentale* and *S. travancoricum*. Three DNA barcoding markers, RpoB, PsbK, and RbcL1, were successfully amplified from P. reticulatum. On the other hand, S. occidentale displayed amplification and sequencing of thirteen markers, namely, MatK1, RpoC, RpoB, RpoB-trnCGAR, PsbZ-trnfM, trnHpsbA1, PsbK, RbcL2, PRK, RPB2, PsbA-trnH2, RbcL1, and Atp. Similarly, three DNA barcoding markers were used to amplify and sequence S. travancoricum: RpoB, PsbZ-trnfM, and RbcL1. The amplification and sequencing rates for each species were recorded as follows: *P. reticulatum* (76.92 ± 100), *S. occidentale* (70.58 ± 100), and *S. travancoricum* (46.15 ± 57.1). In this study, *D. malabaricum*, which is a member of the Meliaceae family, was effectively amplified through four markers: MatK1, PsbK, RbcL1, and Atp. The recorded rate of amplification and sequencing for *D. malabaricum* was 46.15 ± 57.1%. Four species from the Myristicaceae family were examined in this study: *G. canarica, M. malabarica, M. beddomei* and *K. attenuata*. Commonly amplified markers across all species included trnHpsbA1, PsbA-trnH2, PsbZ-trnfM, and PsbK. The seven barcode markers of G. canarica were amplified and sequenced: MatK1, RpoC, RpoB, and PsbA-trnH2. *M. malabarica* was amplified by RpoB, RpoB-trnCGAR, RPB2, MatK2, RbcL1, and Atp. *M. beddomei* exhibited amplification by RpoC, RbcL2, and Atp. K. attenuata showed amplification of RbcL1 and Atp. The observed amplification and sequencing rates were as follows: *G. canarica* (52.94 ± 77.8), *M. malabarica* (76.92 ± 100), *M. beddomei* (52.94 ± 77.8) and *K. attenuata* (69.23 ± 66.7). In the Sapotaceae family, two species were examined: *M. bourdillonii* and *M. insignis*. M. bourdillonii was subjected to amplification and sequencing using the RpoC, PsbZ-trnfM, trnHpsbA1, PsbK, RbcL2, PsbA-trnH2, and Atp primers. *M. insignis* was amplified with RpoB, RpoB-trnCGAR, PsbZ-trnfM, trnHpsbA1, RPB2, PsbA-trnH2, RbcL1, and Atp. The amplification and sequencing rates were 52.94 ± 88.9 for *M.* bourdillonii and 30.76 ± 88.9 for M. insignis (Fig.; Table).

Table 3
Amplified DNA Barcoding markers in selected species belongs to 21 family

Sl. No	Species	Species amplification status using specific barcoding markers													
		MatK1	RpoC	RpoB	RpoB-trnCGAR	PsbZ-trnfM	trnHpsbA1	PsbK	RbcL2	PRK	RPB2	ITS1	MatK2	PsbA-trnH2	RbcL
Apocynaceae															
1.	*R. serpentina*														
2.	*T. heyneana*														
Anacardiaceae															
3.	*H. arnottiana*														
4.	*S. auriculata*														
Arecaceae															
5.	*C. nagbettai*														
6.	*A. wightii*														
Balsaminaceae															
7.	*I. mysorensis*														
8.	*I. pulcherrima*														
Bignoniaceae															
9.	*O. indicum*														
Calophyllaceae															
10.	*C. apetalum*														
Clusiaceae															
11.	*G. indica*														
12.	*G. wightii*														
Cycadaceae															
13.	*C. circinalis*														
		MatK1	RpoC	RpoB	RpoB-trnCGAR	PsbZ-trnfM	trnHpsbA1	PsbK	RbcL2	PRK	RPB2	ITS1	MatK2	PsbA-trnH2	RbcL
Dipterocarpaceae															
14.	*V. indica*														
15.	*S. roxburghii*														
16.	*H. ponga*														
17.	*D. indicus*														
18.	*H. parviflora*														
19.	*V. chinensis*														
20.	V. *macrocarpa*														
21.	*D. bourdiollon*														
Ebenaceae															
22.	*D. paniculata*														
23.	*D. crassiflora*														
24.	*D. candolleana*														
		MatK1	rpoC	rpoB	rpoB-trnCGAR	psbZ-trnfM	trnHpsbA1	psbK	RbcL2	PRK	RPB2	ITS1	MatK2	PsbA-trnH2	RbcL
Fabaceae															

Sl. No	Species	Species amplification status using specific barcoding markers													
25.	*D. latifolia*														
26.	*P. marsupium*														
27.	*S. asoca*														
28.	*K. pinnatum*														
29.	*P. santalinus*														
Flacourtiaceae															
30.	*H. macrocarpa*														
Lauraceae															
31.	*C. riparium*														
Primulaceae															
32.	*E. ribes*														
Malvaceae															
33.	*P. reticulatum*														
34.	*S. occidentale*														
35.	*S. travancoricum*														
		MatK1	**RpoC**	**RpoB**	**RpoB-trnCGAR**	**PsbZ-trnfM**	**trnHpsbA1**	**PsbK**	**RbcL2**	**PRK**	**RPB2**	**ITS1**	**MatK2**	**PsbA-trnH2**	**RbcL**
Meliaceae															
36.	*D. malabaricum*														
Myristicaceae															
37.	*G. canarica*														
38.	*M. malabarica*														
39.	*M. beddomei*														
40.	*K. attenuata*														
Sapotaceae															
41.	*M. bourdillonii*														
42.	*M. insignis*														
Santalaceae															
43.	*S. album*														
Rubiaceae															
44.	*O. missionis*														
45.	*P. dicoccos*														
46.	*T. agumbensis*														
		MatK1	**RpoC**	**RpoB**	**RpoB-trnCGAR**	**PsbZ-trnfM**	**TrnHpsbA1**	**PsbK**	**RbcL2**	**PRK**	**RPB2**	**ITS1**	**MatK2**	**PsbA-trnH2**	**RbcL**
Passifloraceae															
47.	*A. hondala*														

Within the Santalaceae family, the species *S. album* was included in this investigation. The gene was amplified using eight DNA barcoding markers: RpoB, RpoB-trnCGAR, PsbZ-trnfM, trnHpsbA1, PsbK, PsbA-trnH2, RbcL1, and Atp. The observed amplification rate for *S. album* was 61.53 ± 100%. In the Rubiaceae family, three species were examined: *O. missionis, P. dicoccos*, and *T. agumbensis*. These genes shared commonly amplified markers, including RpoB, PsbK, RPB2, PsbA-trnH2, RbcL1, and Atp. Additionally, RpoB-trnCGAR was amplified in *O. missionis* and *P. dicoccos*, while trnHpsbA1 was amplified in *O. missionis* and *T. agumbensis*. PsbZ-trnfM was specifically amplified in *T. agumbensis*. The amplification and sequencing rates were as follows: *O. missionis* (61.53 ± 100), *P. dicoccos* (53.84 ± 85.7), and *T. agumbensis* (61.53 ± 100). In this study, *A. hondala* was representative of the Passifloraceae family. The species were

amplified and sequenced using eight DNA barcoding markers: RpoB, RpoB-trnCGAR, PsbZ-trnfM, trnHpsbA1, PsbK, PsbA-trnH2, RbcL1, and Atp. The amplification and sequencing rates were 92.34 ± 100 (Fig. 4; Table 3).

Molecular analysis of species identification using DNA barcodes: Among the 47 species examined, 28 lacked DNA barcodes or any associated sequences, as outlined in Table 4. In this study, species without existing DNA barcodes underwent amplification and sequencing utilizing selected DNA barcoding markers, resulting in a diverse array of sequences. These sequences were subsequently compiled and collectively subjected to BLAST analysis to identify the top hits specific to each species. By constraining the database and refining it based on a pairwise identity (PI) threshold of more than 95%, several significant matches were identified. For instance, *T. heyneana* exhibited a high similarity with *Tabernaemontana bovina* (NC079611.1; PI = 98.86%). Similarly, *H. arnottiana* and *S. auriculata*, which belong to the same family, were matched with *Semecarpus reticulatus* (NC069617.1; PI = 98.82% and 98.94%, respectively). *A. wightii* was matched with *Wallichia disticha* (ON248714.1; PI = 97.72%). Additionally, *I. mysorensis* and *I. pulcherrima*, from the same family, were matched with *I. hawkeri* (NC048520.1; PI = 98.02%) and *D. mespiliformis* (MZ274088.1; PI = 98.73%), respectively. Furthermore, *C. apetalum* was matched with *Calophyllum soulattri* (NC_068749.1; PI = 96.13%), and *G. wightii* was matched with *Kingiodendron pinnatum* (EU361987.1; PI = 98.96%). In the Dipterocarpaceae family, *V. indica, H. ponga, D. indicus*, and *D. bourdiollon* (with PIs ranging from 95.36–98.50%) were matched with *Vatica bantamensis, Hopea reticulata, Holigarna arnottiana*, and *Vatica bantamensis* (NC_071231.1; NC_052744.1; MZ648043.1; NC071231.1), respectively. Moreover, *D. paniculata* and *D. candolleana* from the Ebenaceae family were matched with *Diospyros mespiliformis* and *Dipterocarpus littoralis* (MZ274088.1; PI = 99.17% and NC_081465.1; 99.49%). *K. pinnatum* was matched with *P. oxyphylla* (MZ274107.1; PI = 95.63%), and *H. macrocarpa* was matched with *H. hainanensis* (NC042720.1; PI = 98.98%). Furthermore, *C. riparium* was matched with *Cinnamomum verum* (MG280937.1; PI = 99.54%). In the Malvaceae family, *P. reticulatum, S. occidentale*, and *S. travancoricum* were matched with *Pterospermum heterophyllum, Semecarpus australiensis*, and *Syzygium grijsii* (ON100918.1; PI = 98.83%, AY594479.1; 97.74%, and NC_065156.1; 99.23%, respectively). Additionally, *D. malabaricum* was matched with *Tabernaemontana divaricata* (MZ_07333.1; PI = 99.07%), and *G. canarica* was matched with *M. teysmannii* (NC_079584.1; PI = 100%). Furthermore, *M. bourdillonii* and *M. insignis* from the Sapotaceae family were matched with *Impatiens hawkeri* and *Madhuca hainanensis* (NC_048520.1; PI = 98.64% and NC_053619.1; PI = 99.23%, respectively). *O. missionis, P. dicoccos*, and *T. agumbensis* were matched with *Atalantia ceylanica, Psydrax obovata*, and *P. rubra* (NC_065396.1; PI = 99.77%, KY378666.1; 98.66%, and MZ958829.1; 99.85%, respectively). Finally, *A. hondala* was matched with *Adenia mannii* (KF724302.1; PI = 98.97%) (Table 4; Supplementary file 1). The obtained nucleotide sequences were submitted to the DNA databank (https://www.ncbi.nlm.nih.gov/guide/dna-rna/) (Supplementary file 2).

Table 4
BLAST results of the amplified sequences of 28 species of western ghats

Sl No.	Species	Barcoding markers	PI%	Species matching	Accession number
Family					
Apocynaceae					
1.	*T. heyneana*	RbcL1 + Atp + PsbK + RpoB	98.86	*Tabernaemontana bovina*	NC079611.1
Anacardiaceae					
2.	*H. arnottiana*	RbcL1 + psbA_trnH1 + Atp + PsbK + psbZ_trnfM + RPB2 + RpoB + trnHpsbA2 + RpoB_trnCGAR + ITS2	98.82	*Semecarpus reticulatus*	NC069617.1
3.	*S. auriculata*	MatK2 + RpoC + RbcL1 + psbA-trnH1 + ITS2 + PsbK + RpoB + RpoB-trnCGAR	98.94	*S. reticulatus*	NC069617.1
Arecaceae					
4.	*A. wightii*	MatK2 + RbcL1 + MatK2 + psbAtrnH2 + Atp + PsbK + psbZtrnfM + RPB2 + RpoB + RpoC + trnHpsbA1 + RbcL2 + RpoBtrnCGAR	97.72	*Wallichia disticha*	ON248714.1
Balsaminaceae					
5.	*I. mysorensis*	RbcL1+ trnHpsbA1 + PsbK + RpoB + psbA-trnH1	98.02	*I. hawkeri*	NC048520.1
6.	*I. pulcherrima*	MatK2 + RbcL1+ trnHpsbA1 + Atp + PsbK+ psbZtrnfM + RpoC + RpoBtrnCGAR+ psbAtrnH2 + RbcL2	98.73	*D. mespiliformis (ebenaceae)*	MZ274088.1
Calophyllaceae					
7.	*C. apetalum*	RbcL1 + Atp + PsbK+ trnHpsbA1 + RpoB+ psbAtrnH2	96.13	*Calophyllum soulattri*	NC_068749.1
Clusiaceae					
8.	*G. wightii*	MatK2 + RbcL1+ psbAtrnH2+ trnHpsbA1 + RpoB + RpoC + RpoBtrnCGAR + Atp + PsbK + RPB2 + RbcL2	98.96	*Kingiodendron pinnatum*	EU361987.1
Dipterocarpaceae					
9.	*V. indica*	RbcL1 + PsbA-trnH2 + trnHpsbA1 + PsbK	98.50	*Vatica bantamensis*	NC_071231.1
10.	*H. ponga*	RPB2 + trnHpsbA1 + RpoB_trnCGAR + ITS2	96.97	*Hopea reticulata*	NC_052744.1
11.	*D. indicus*	MatK2 + RbcL1 + PsbK + RpoB + RpoC + ITS2 + RbcL2	98.48	*Holigarna arnottiana*	MZ648043.1
12.	*D. bourdiollon*	MatK2 + RbcL1+ trnHpsbA1 + PsbK + RpoB + RpoC RpoB-trnCGAR+ psbAtrnH2 + RbcL2	95.36	*Vatica bantamensis)*	NC071231.1
Ebenaceae					
13.	*D. paniculata*	RpoC + RbcL1	99.17	*Diospyros mespiliformis*	MZ274088.1
14.	*D. candolleana*	RbcL1+ psbAtrnH2+ + PsbK + RpoC + RbcL2	99.49	*Dipterocarpus littoralis*	NC_081465.1
Fabaceae					
15.	*K. pinnatum*	trnHpsbA1 + RpoB + RpoB_trnCGAR	95.63	*Prioria oxyphylla*	MZ274107.1
Flacourtiaceae					
16.	*H. macrocarpa*	RbcL1 + RpoC	98.98	*H. hainanensis*	NC042720.1
Lauraceae					
17.	*C. riparium*	MatK2 + RbcL1 + Atp + PsbK + RpoB + RpoC + RPB2 + PsbZ-trnfM + trnHpsbA1 + ITS2	99.54	*Cinnamomum verum*	MG280937.1
Malvaceae					
18.	*P. reticulatum*	RbcL1 + PsbK + RpoB	98.83	*Pterospermum heterophyllum*	ON100918.1
19.	*S. occidentale*	MatK2 + RbcL1+ psbAtrnH1 + Atp + PsbK + PsbZ-trnfM + RpoB + RpoC + RpoB-trnCGAR + RPB2+ psbAtrnH2 + RbcL2	97.74	*Semecarpus australiensis*	AY594479.1
20.	*S. travancoricum*	RbcL1+ PsbZ-trnfM + RpoB+ psbAtrnH1	99.23	*Syzygium grijsii*	NC_065156.1
Meliaceae					
21.	*D. malabaricum*	MatK2 + Atp + RpoC + RbcL1 + PsbK + RpoB+ rbcL2 + ITS2	99.07	*Tabernaemontana divaricata*	MZ_07333.1

Sl No.	Species	Barcoding markers	PI%	Species matching	Accession number
Family					
Apocynaceae					
Myristicaceae					
22.	*G. canarica*	MatK2+ trnHpsbA1 + PsbK + PsbZ-trnfM + RpoC + trnHpsbA_2 + RpoB	100	*M. teysmannii*	NC_079584.1
Sapotaceae					
23.	*M. bourdillonii*	RbcL1+ trnHpsbA1 + RpoC+ trnHpsbA_2 + Atp + PsbK + rbcL2	98.64	*Impatiens hawkeri*	NC_048520.1
24.	*M. insignis*	RbcL1+ trnHpsbA1 + Atp + PsbZ-trnfM + RpoB + RpoB-trnCGAR + trnHpsbA_2 + RPB2	99.23	*M. hainanensis*	NC_053619.1
Rubiaceae					
25.	*O. missionis*	Cn_RbcL + Atp + PsbK + RpoB + RpoB-trnCGAR + trnHpsbA1 + RPB2	99.77	*Atalantia ceylanica*	NC_065396.1
26.	*P. dicoccos*	rbcL1 + Atp + PsbK + RpoB+ trnHpsbA1 + PsbA-trnH2	98.66	*Psydrax obovata*	KY378666.1
27.	*T. agumbensis*	Cn_RbcL + Atp + PsbA-trnH2 + PsbK + RPB2 + RpoB + PsbZ-trnfM	99.85	*Psychotria rubra*	MZ958829.1
Passifloraceae					
28.	*A. hondala*	RbcL1+ trnHpsbA1 + Atp + psbZ_trnfM + RpoB + RpoB_trnCGAR+ trnHpsbA2	98.97	*Adenia mannii*	KF724302.1

Discussion

Our findings indicated that the dissimilarities between species become more disparate when more independent loci are used. In this study, our aim was to generate DNA barcode sequences for endangered, threatened, and vulnerable forest species that are challenging to identify morphologically. The success rate of this study shows that barcoding markers can accurately distinguish between species within a family. In our study, the highest amplification rates were detected for rbcL1 (40 species), psbtrnH2 (36 species), and PsbA-trnH1 (33 species) (Table 3). These findings suggested that these markers could serve as promising universal identifiers for the majority of the selected forest species. Similar results were reported by Kang et al. (2017) and Huang et al. (2015), indicating the potential universality of the *rbcL and* PsbA-trnH1 barcodes. However, the majority lacked available DNA barcodes. Furthermore, in cases where DNA barcodes were available, the number of sequences was limited. In our study, we expanded upon existing DNA barcodes by developing additional markers for species with available sequences. Additionally, we successfully generated DNA barcode sequences for the majority of endangered species in the Western Ghats region for which barcode sequences were previously unavailable. For the 19 species with existing DNA barcodes and sequences, we expanded the available data by generating additional DNA sequences using a broader array of DNA barcoding markers. According to the literature, some DNA barcodes were accessible for the following species: viz; *R. serpentina*; trnL-trnF, ITS2, matK, rbcL and trnL (Eurlings et al. (2013); Cahyaningsih, et al. (2022); *C. nagbettai*; rbcL, matK, psbA-trnH, rpoC, rpoB, psbKpsbI, atpF-atpH and RPB2 (Kurian, et al. (2020); (Table 1); *O. indicum*; ITS2, psbA-trnH (Cui, et al. (2020); *G. indica*; rbcL, ITS, psbA-trnHf, RpoB-trnCGAR, Matk (Seethapathy et al. 2018); C. *circinalis*; ITS2 (Gao, et al. (2010); *S. roxburghii*; ITS1 and ITS2; psbM, rbcL, psbH, rpoC2 (Osathanunkul, et al. (2021); *H. parviflora;* MatK, rbcL (data unpublished); *V. chinensis;* rbc However, in our study, we developed additional markers beyond those that currently exist. We observed that certain existing markers were not amplified in our samples, possibly due to sequence mixing, as evidenced in species such as *R. serpentina, G. indica, C. circinalis, S. roxburghii*, and *P. santalinus* (Table 1). Furthermore, our study revealed lower amplification results with the ITS marker compared to findings reported in the literature (Selvaraj et al. 2014). Our efforts resulted in successful DNA barcoding for 28 species previously lacking representation in the database. The accuracy of the DNA barcodes was determined by comparing the molecular identification results obtained via BLAST searches against available reference databases. The number of matched genes was greatest at the genus level for all amplified and sequenced markers (Table 4; Supplementary file 1). The analysis revealed that the most precise level of identification closely resembled that of their respective neighboring species within the same family across all the amplified and sequenced markers (Supplementary file 1). However, while some species were confidently matched at the genus level, others could only be identified up to the family level. This discrepancy might stem from variations in genetic markers or the availability of reference sequences. Conversely, when individually analyzing the sequences, specific markers such as *A. wightii, V. indica*, and *D. bourdiollon* provided genus-level matches. Additionally, some species, including *G. wightii, D. indicus, D. malabaricum* and *M. bourdillonii*, unexpectedly showed matches with different families (Supplementary file 1).

Conclusion

The findings of this study contribute to the establishment of a database encompassing threatened forest species not only in western Ghats but also in other regions. This study can improve forest biodiversity monitoring by detecting species at the molecular level. Such a database can play a pivotal role in enhancing conservation endeavors by facilitating the precise identification and monitoring of endangered species of western Ghats. In our research, the most significant amplification rates were noted for rbcL1 (40 species), psbtrnH2 (36 species), and PsbA-trnH1 (33 species). DNA amplification ranged from 11.76–94.11%. This highlights the ongoing need to expand DNA barcoding initiatives to encompass a wider array of species, thereby improving species identification accuracy and supporting conservation endeavors.

Declarations

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Data availability statement: The authors declare that all the data supporting the findings of this study are available from GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

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Conflict of interest: The authors declare that they have no conflicts of interest.

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Figures

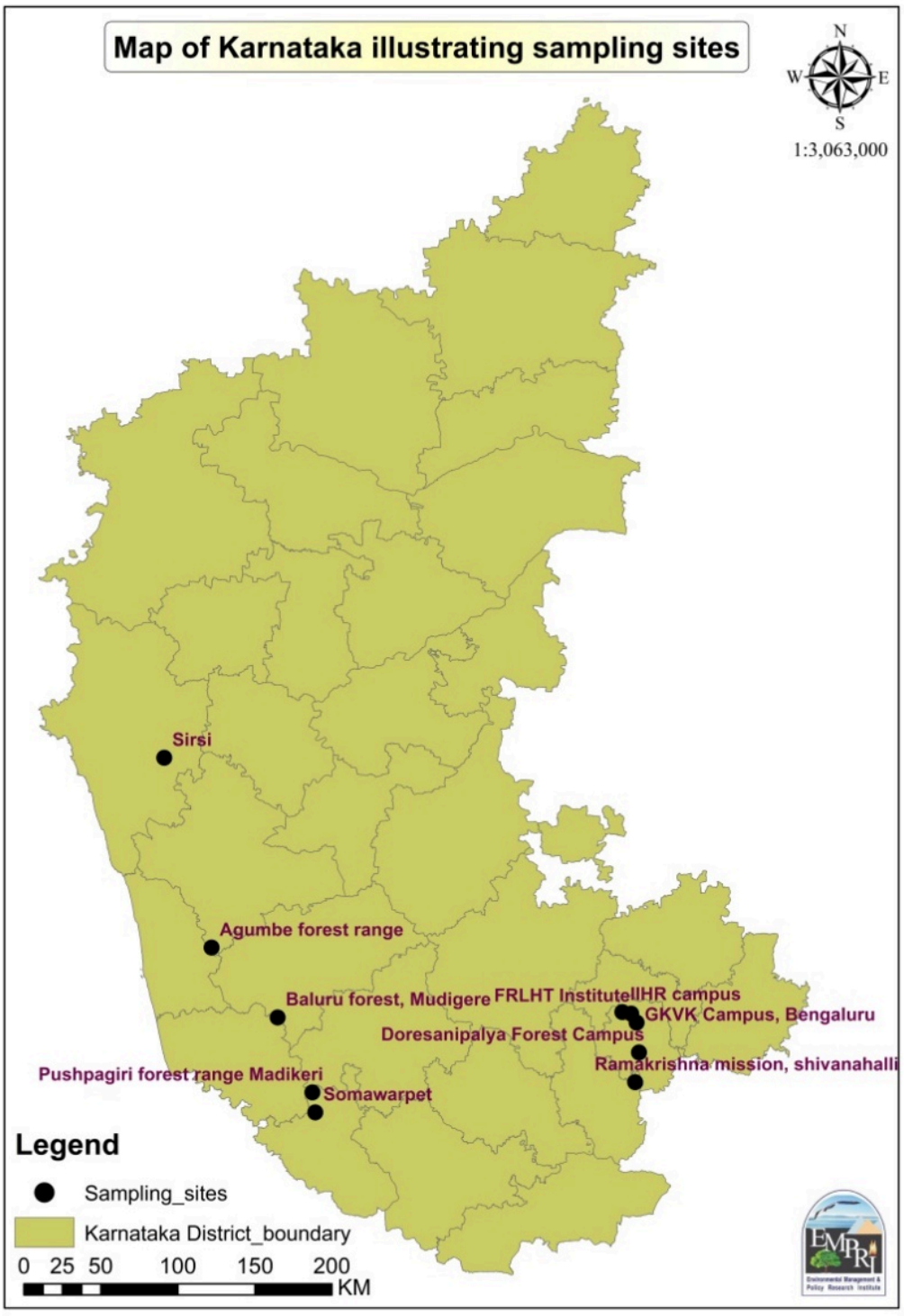

Figure 1

Map of Karnataka showing the sampling sites

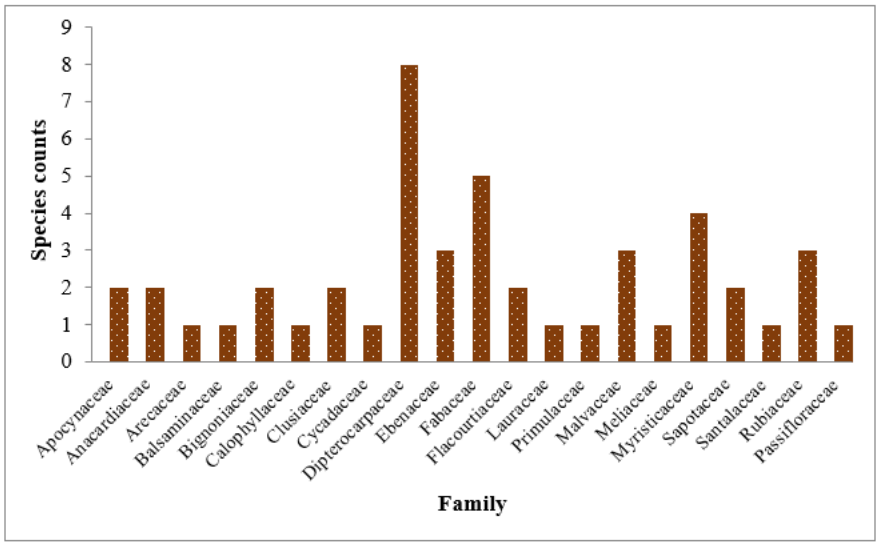

Figure 2

Species count based on the family

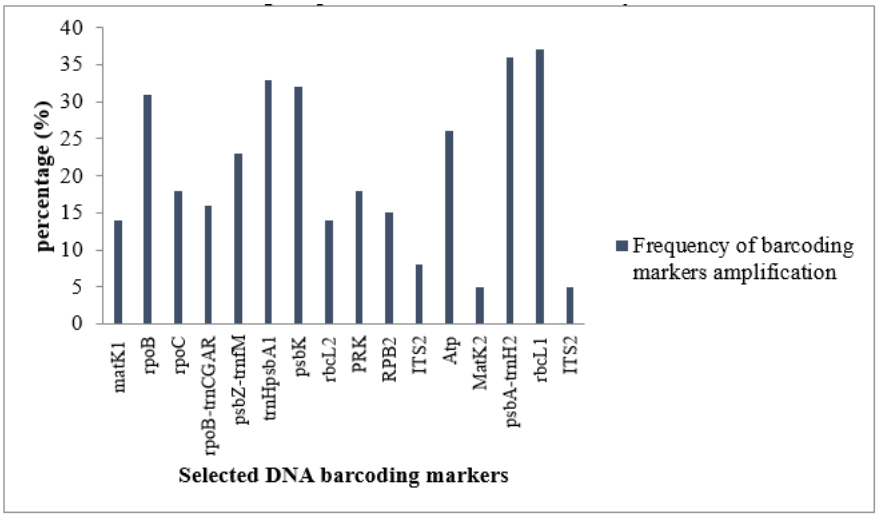

Figure 3

Amplification frequency of the selected DNA barcoding markers

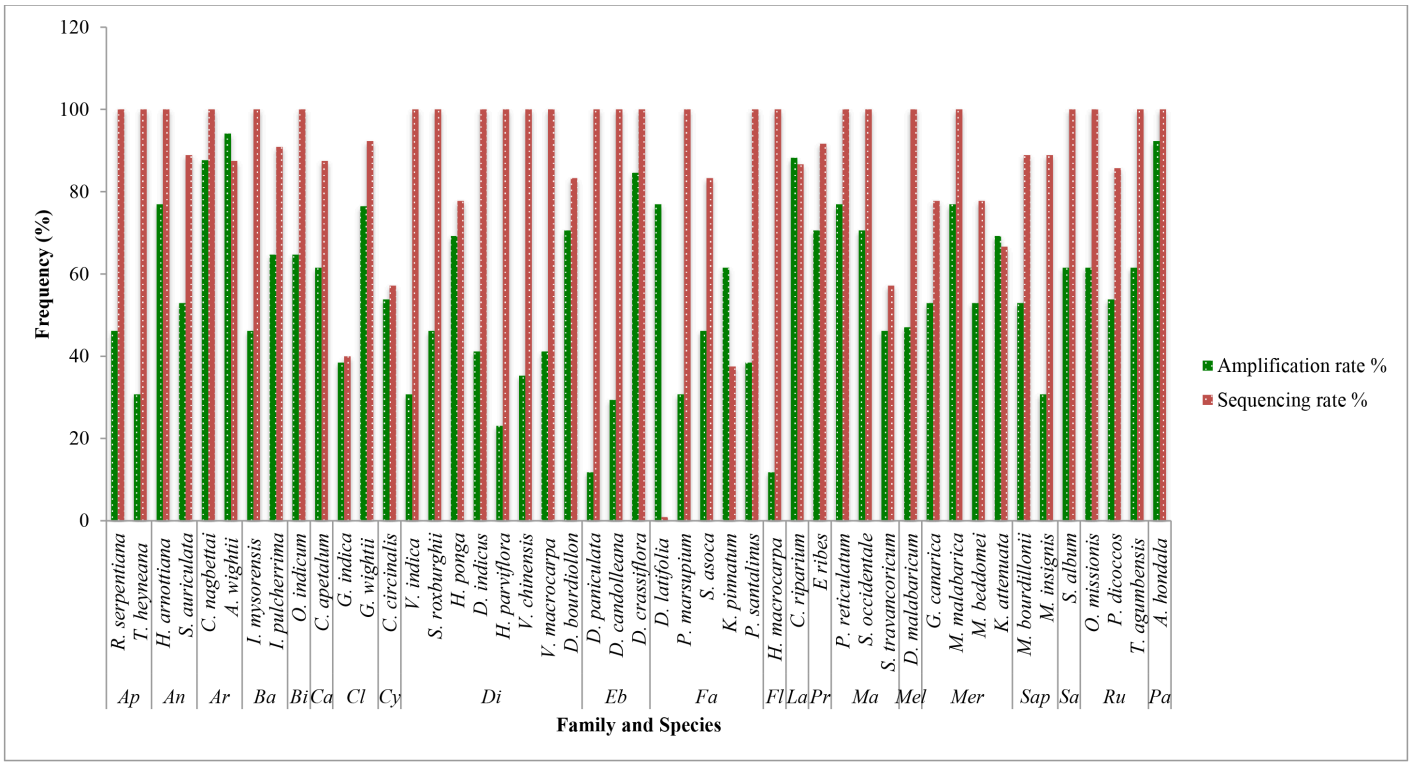

Fig. 4. The successs rate of PCR amplification and sequencing of the sixteen barcode fragments across the 47 species from 21 families

Figure 4

The successs rate of PCR amplification and sequencing of the sixteen barcode fragments across the 47 species from 21 families

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