



RESEARCH ARTICLE

First records of Ruan, *Carapa guianensis* Aublet., from Barda Hill, Porbandar - Gujarat, India, using DNA barcoding

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Abstract

There are several hot spots of plant biodiversity, many of them are well explored; identification of taxa becomes important when the species of plant are in demand for their different properties. We came across one such taxon, which is being used by the local healers, Rabari and Maldhari communities of Barda Hill, Gujarat, in the district of Porbandar. The DNA barcoding approach for the identification of genera and species was performed. The rbcL gene of the taxon 'X', locally known as Ruan, was amplified and the amplicon was used for the sequence analysis using the bioinformatics identification tools. Sequence analysis revealing 100 % identity with the taxa not yet reported from India – the *Carapa guianensis* Aublet. The morphology of the plant is matching with the *Carapa guianensis* of Meliaceae (Mahogany family). The plant is commonly known as Andiroba, is native to the Amazon. Ruan is used for the fastest relief from swelling & inflammation, wound healing, diabetes and high blood pressure.

Keywords: Barda hill; Carapa; DNA barcode; Meliaceae; mahogany; Ruan

Introduction

We learnt about a plant tree (Fig. 1) from Barda Hill, Porbandar-Gujarat, India, called locally as Ruan, which is not included in the published literature on the flora of the Barda Hills (1-4). It might have been introduced by some natural means since there are no records of its cultivation by the forest department or any person. Barda Hill is about 25 km away from Porbandar district, where we meet people who know the plants of Barda Hill. Muru ata, goes to Barda Hill to collect medicinal plants and he also supplies plants according to their diseases. The local public near Barda Hill prefers to take medicinal plants as medicine given by them (3). The people of Barda Hill are not aware of technology, Muru ata knows plants in Barda Hill. He says Ruan is used for the fastest relief from swelling & inflammation; wound healing, diabetes and high blood pressure (a tribal knowledge having no documentation). Currently, the study of traditionally used ethno-medicinal plants has increased. Studies on the use of plants by traditional healers of the nomadic tribes are being added in the search for new therapeutic molecules. Barda's steep woodland is preventing the area from being further salinized. In February 1979, this region was designated as a sanctuary. Its floral diversity includes many therapeutic species and is characterized by its abundance. The sanctuary's high percentage of rare and endangered plants is sizable. Throughout the year, there is no excess water available and the vegetation is dry.

There are several ethnobotanical uses of the plants of Barda Sanctuary. The Rabari tribe, who reside in the remote Ness of Barda Hills, uses 38 medicinal plants (1). 368 species, belonging to 268 genera and 80 families of angiosperms (2). 25 plant species from 16

families were reported, who focused on identifying the vegetation, habit, habitat, flowering season and medicinal use of the plants (3). In Barda Sanctuary, Gujarat, India, recognized the medicine men who have been using plants as medicines. Maldhari medicine men frequently use 51 kinds of plants with therapeutic qualities, spread throughout 47 genera and 31 plant families (4). Other common vegetation includes Euphorbia scrub, dry deciduous scrub and dry bamboo brakes. Barda Hills has several forest subtypes, including northern tropical thorn forest, southern tropical forest and southern dry mixed deciduous forest. The sanctuary's green space contributes to ecological balance by reclaiming groundwater and enhancing the local water system.

Ecology

Carapa guianensis (Andiroba) occurs in upland and floodplain forests and the wood and oil of its seeds have multiple uses. *Carapa guianensis* is a typical component of the sub canopy or canopy layer of evergreen to semi-evergreen rainforests in South America. Mostly found along rivers and in areas that have floods or swamps, but also on higher terrain and low hills. Two varieties of wood are identified by South American foresters: "red" or "hill crabwood" and "white crabwood" (5). Whereas white crabwood comes from trees in swampy areas, the former is considered superior and comes from trees that grow on higher ground distributed from Central America (Belize) and the Caribbean south to Amazonian Brazil.

Documented species distribution

Fig. 2 shows the Nations on the world map where the distribution of *Carapa guianensis* are found as a native or exotic plant (6).



Fig. 1. Ruan tree, at Barda Hill, Porbandar, Gujarat. The large tree, compound leaves, opposite leaflets, entire.



Fig. 2. The documented biogeography for distribution of *Carapa guianensis*.

Source: <http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp>; [Agroforestry database 4.0] (6)

Native: Brazil, Colombia, Costa Rica, Dominican Republic, Ecuador, French Guiana, Guadeloupe, Guyana, Haiti, Honduras, Panama, Peru, Puerto Rico, Surinam, Trinidad and Tobago, Venezuela

Exotic: Indonesia, Malaysia, Singapore

The purpose of this study is to establish the scientific identity of the unknown taxa of the region and their biogeography. Medicinal plants are currently the subject of contemporary research because of their great biological and chemical diversity as well as the fact that they contain a wide range of chemicals with intriguing biological

properties (7). The primary benefits of adopting herbal remedies are their affordability, low cost and typically lower incidence of adverse effects. Confirming the safety and effectiveness of therapeutic herbs requires extensive research (8). Medicinal plants have gained a lot of attention due to the pressing demand for new therapeutic medicines with higher efficacy and fewer adverse effects (9). The research was carried out with the aforementioned context and the findings are anticipated to serve as a guide for identifying the Ruan tree from Barda Hill in Porbandar, Gujarat, India.

Materials and Methods

A plant tree called locally as Ruan, which has very high ethno-medical applications, was recognized during a field visit to Barda Hill between 2023 and 2024. It is a sanctuary that spans around 192 sq km and is situated in Porbandar, Gujarat, between 21°40' - 21°55' N latitude and 69°40' - 69°50' E longitude. The plant samples were collected and digital photographs of the plants were taken. Voucher specimens were deposited in the personal herbarium at the Department of Biotechnology, L J School of Applied Sciences, Ahmedabad (The voucher specimen's collection number is LJBP 001-23-24 VO). This herbarium has not yet been included in the Index herbarium.

The collected plant specimens were studied to identify them according to morphological characters and their uses. Efforts were also made to find the scientific name of this plant, by doing a literature survey for plants used in inflammation, wound healing, diabetes and high blood pressure and their morphological characters to match with Ruan (10 -13). Further inquired with older local Ayurvedic literature (Ayurved ka amrut kumbh, Aaryabhishak athva Hindustan no Vedra) regarding the morphological description of Ruan but did not find any similarity with Ruan. During the field visit, information was collected on the habitat and plant communities in which the studied taxon grew. The taxonomic identity of the collected plant was revealed by following the molecular taxonomy approach using the *rbcl* gene amplification.

DNA extraction and *rbcl* gene amplification

The genomic DNA was extracted from fresh leaves of the plant "X" (Ruan) that were collected from Barda Hill in Porbandar, Gujarat, by following the protocol of Allen et al. (14). The DNA was amplified with *rbcl* Specific Primer (*rbcl*_LaF 5'-ATG TCA CCA CAA ACA GAG ACT AAA GC-3' and *rbcl*_LaR 5'-GTA AAA TCA AGT CCA CCR CG-3') using Veriti® 96 well Thermal Cycler at Unigenome, A life sciences division of Unipath Specialty Laboratory Limited, Ahmedabad. Thermo Fisher Scientific's BDT v3.1 Cycle sequencing kit was used to perform a bi-directional DNA sequencing reaction of the PCR amplicon using the *rbcl*_LaF and *rbcl*_LaR primers on an ABI 3500Dx genetic analyzer.

Phylogenetic analysis

Bioinformatics programs were used to analyse the data. Bio Edit was employed for DNA alignment and the construction of a phylogenetic tree with Neighbour-joining (NJ) method; BLASTn was utilized to compare the obtained sequence with DNA sequences from GenBank. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site (15). Codon positions included were 1st+2nd+3rd+noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGAX (16).

Results

Botanical description

Carapa guianensis trees are medium to big, monoecious, deciduous or semi-evergreen, up to 35 (maximum 55) m tall, straight and cylindrical, branchless up to 20 (maximum 30) m, at most 100 (maximum 200) cm in diameter, occasionally fluted and have short buttresses up to 2 m high. Light grey to greyish brown or dark brown, occasionally reddish, bark surface flaking into squarish scales or horizontal strips; interior bark fibrous, red or pinkish brown. The plants have tap roots, often develop surface roots.

The leaves are alternating, paripinnate, with an exstipulate, dormant glandular leaflet at the apex; the leaflets are opposite and whorled. During the monocaulous juvenile stage, it has enormous leaves that get smaller as branching begins. White, tiny flowers carried in a wide axillary or subterminal thyrse; tetramerous to pentamerous (maximum sextamerous); calyx lobed nearly to the base; petals somewhat twisted; unisexual but with well-developed traces of the opposing sex. The fruit is a dehiscent, woody, subglobose, pendulous, four-lobed capsule with 2 - 4 seeds in each lobe. Smooth, angular, pale brown seeds with woody sarcotesta.

DNA barcoding

DNA barcoding is one of the ways to determine the taxonomic status of an unknown plant. The barcode of life database aims to collect the reference sequences. This uses variations of short, standardized gene regions to identify new species (17). The total DNA of the Ruan plant was successfully extracted and the purity and concentration of the obtained DNA are presented in Table 1. The table shows that DNA isolation of Ruan plants had 22 ng/μL concentration with a purity of 2.300 ± 1.034. The chloroplast DNA containing the *rbcl* gene was amplified by polymerase chain reaction (PCR) by using forward and backwards primer mentioned as above. The amplification of the *rbcl* gene was tested by electrophoresis (Fig. 3).

DNA band obtained from the amplification of the *rbcl* gene was ± 650 bp. The forward primer **rbcl_LaF** 5'-ATG TCA CCA CAA ACA GAG ACT AAA GC-3' generated a sequence of 549 bp.

40400103114V1_RBCLLAF_FP_A11_01_StdSeq50_POP7_1.seq (549 bp)

>40400103114_V1_RBCLLAF_FP

```
AAGATTATAAATTGACTTATTATACTCTGACTATGTAACCAAGATAC
TGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAGTCCGCC
CGAGGAAGCAGGGGCTGCGGTAGCTGCGGAATCTTCTACTGGTACAT
GGACAAGTGTGGACCGATGGGCTTACTAGCCTTGATCGTTACAAG
GACGATGCTACAACATTGAGCCAGTTGCTGGAGAAGAAAATCAATATA
TATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGTTCTGTTAC
TAACATGTTTACGTCCATTGTGGTAATGTATTTGGTTCAAAGCCCT
GCGCGCTCTACGTCTAGAGGATCTACGAATCCCTCCCGGTATTCTAA
AACTTTCCAAGGCCCGYTCATGGCATCCAAGTTGAGAGAGATAAATTG
AACAAGTATGGTCGTCCTTATTGGGATGTACAATTAACCTAAATTGG
GGTTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGTCTACGGC
GTGGACTTGGATTTTACA
```

Table 1. The quality & quantity of total DNA extracted are shown in the table

Method	OD at 260	OD at 280	OD (260 / 280)	Concentration (ng/μL) Sambrook et al., 1989 (37)
Allen G. C. et al 2006 (14)	0.044 ± 0.020	0.025 ± 0.017	2.300 ± 1.034	22

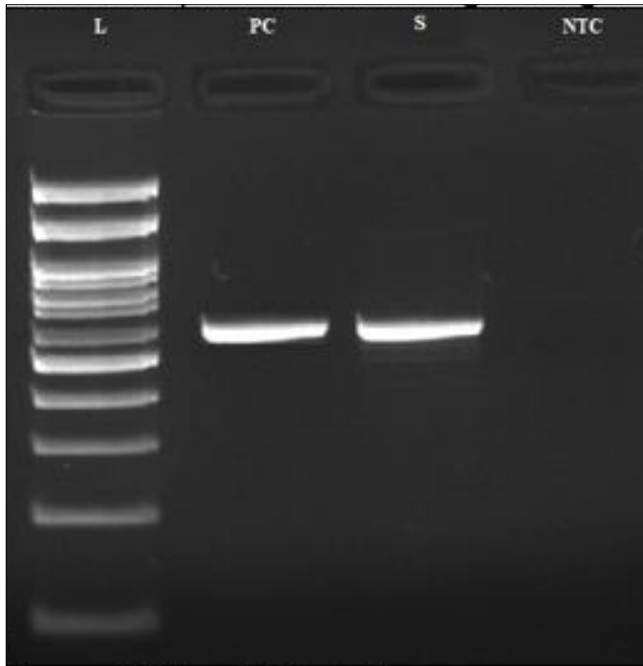


Fig. 3. Electrophoresis results of the *rbcl* gene amplification in Ruan plant 'X' from Barda. Quality of Amplicons on 1.8 % Agarose gel. L= 1000 bp DNA Ladder, PC = Positive control, S = Sample, NTC = Non template control.

The reverse primer **rbcl_LaR 5'**-GTA AAA TCA AGT CCA CCR CG-3' generated a sequence of 536 bp.

40400103114V1_RBCLLAR_RP_B11_02_StdSeq50_POP7_1.seq (536 bp)

>40400103114_V1_RBCLLAR_RP

AACCCCAATTTAGGTTTAAATGTACATCCCAATAGGGGACGACCATACT
TGTTCAATTTATCTCTCAACTGGATGCCATGAGCGGGCCTTGGAA
AAGTTTTAGAATACGCGGGAGGATTCGTAGATCCTCTAGACGTAGAG
CGCGCAGGGCTTTGAACCCAAATACATTACCCACAATGGACGTAAACA
TGTTAGTAACAGAACCTTCTTCAAAAAGGTCTAAAGGTAAGCTACATA
ACATATATATTGATTTTCTTCTCCAGCACTGGCTCAATGTTGTAGCAT
CGTCTTTGTAACGATCAAGGCTAGTAAGCCCATCGGTCCACACAGTT
GTCCATGTACCAGTAGAAGATTCCGCAGCTACCGCAGCCCCGCTCTCC
TCGGGCGGAACCTCCGGGTTGAGGAGTTACTCGGAATGCTGCCAAGAT
ATCAGTATCTTTGGTTACATAGTCAGGAGTATAATAAGTCAATTTATAA
TCTTTAASACCGCTTTGAATCCAACACTGCTTTAGTCTCTGTTTGTG
GGTG

A Consensus sequence of 650 bp of *rbcl* region was generated from forward and reverse sequence data using the aligner software as mentioned below:

Consensus Sequence of 40400103114 (604 bp)

>contig

TTWTGTCACCCACAACAGAGACTAAAGCAAGTGTGGATTCAAAGCC
GGTGTTAAAGATTATAAATTGACTTATTATACTCCTGACTATGTAACCA
AAGATACTGATATCTTGGCAGCATTCCGAGTAACTCCTCAACCCGGAG
TTCCGCCCCGAGGAAGCAGGGGCTGCGGTAGCTGCGGAATCTTCTACT
GGTACATGGACAACCTGTGTGGACCGATGGGCTTACTAGCCTTGATCGT
TACAAAGGACGATGCTACAACATTGAGCCAGTTGCTGGAGAAGAAAAT
CAATATATATGTTATGTAGCTTACCCTTTAGACCTTTTTGAAGAAGGTT
CTGTTACTAACATGTTTACGTCCATTGTGGTAATGTATTTGGGTTCAA
AGCCCTGCGCCTCTACGTCTAGAGGATCTACGAATCCCTCCCGCGTA
TTCTAAAACCTTTCAAGGCCCGCTCATGGCATCCAAGTTGAGAGAGA
TAAATTTGAACAAGTATGGTCGTCCTTATTGGGATGTACAATTAACCT
AAATTGGGGTTATCCGCTAAGAATTACGGTAGAGCAGTTTATGAATGT

CTACGCGGTGGACTTGGATTTTACA

The data from the similarity analysis (BLASTn) from the Ruan, plant 'X', are shown in Table 2. Phylogenetic analysis was carried out to find the location of the taxa based on the similarity and the identity from GenBank using BLASTn method in NCBI. This analysis involved 15 nucleotide sequences. The result of the relationship analysis (phylogenetic) of Ruan, the plant 'X' is shown in Fig. 4.

The evolutionary relation was inferred using the neighbor-joining method (18). The bootstrap consensus tree inferred from 500 replicates (19) is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. Neighbor-joining (NJ) method used in the analysis of phylogeny can describe the clarity of species identification; the difference is limited by cluster and node. The Sample can be in the same cluster even though they are from different areas (20).

Discussion

Species relationship based on genetic similarities are shown in the phylogenetic tree (Fig. 4). According to the BLASTn analysis (Table 2), the Ruan, or plant "X," is the same as *Carapa guianensis* voucher cg70 (Accession ID MK830591.1). Accession IDs MK830590.1, MK830589.1 and MK830588.1 are all associated with cg69, cg68 and cg67. Chloroplast having a 100 % same value for the ribulose 1, 5 - biphosphate carboxylase (*rbcl*) gene, partial cds, cg66 (Accession ID MK830587.1), cg65 (Accession ID MK830586.1), cg64 (Accession ID MK830585.1), cg63 (Accession ID MK830584.1) and Cg62 (Accession ID MK830583.1). Additionally, it has also shown 100 % identity with the *Swietenia mahagoni* voucher Trotta950332. The plant's morphology matches that of *Carapa guianensis*; however, the chloroplast has Accession ID MH550014.1 and the ribulose-1,5-biphosphate carboxylase/oxygenase large subunit (*rbcl*) gene is partial cds.

The *rbcl* gene is used as a DNA barcode (21-24) because its coding region has shown universality and ease in amplifying and analysing (25). Low level of mutation is the superiority of the *rbcl* gene. When the plastid genomes of *Atropa* and *Nicotiana* were compared, the *rbcl* gene had the least amount of divergence (0.83 %) (26). In 2009, the Consortium for the Barcode of Life (27) plant working group proposed the chloroplast gene *rbcl* and *matK* as the core barcodes of plant species, as well as the intergenic sequence *trnH-psbA* and nuclear gene ITS as supplementary barcodes. Presently, *rbcl* genes have been widely used for phylogenetic analysis within the family and subclass of angiosperms and even among the different groups of the seed plants (28). However, variation in *rbcl* sequence mainly exists at the above-species level and variation is seldom found at the species level (25, 29-32) Since *rbcl* is characterized by its universality, easy amplification and comparability, this gene has been proposed as the barcode fragment (33, 34), which becomes a useful tool for biodiversity investigation, monitoring, molecular phylogeny and evolution (35). In a study that compared the species discrimination between 7 leading candidate plastid DNA regions, the result was *rbcl* gene had a 58 - 66 % range of single-locus barcodes (27). In addition, another study also compared the use of the *rbcl* gene to identify genus and

Table 2. rbcl sequence analysis of Ruan, plant 'X' from Barda's Hill in BLAST

S. No.	Accession ID	Description	Scientific name	Max score	Total score	Query coverage	E value	Percent identity
1	OM037434.1	<i>Xylocarpus granatum</i> isolate TR9 ribulose 1,5 biphosphate carboxylose/oxygenase large subunit (rbcl) gene, partial cds; chloroplast	<i>Xylocarpus granatum</i>	1068	1068	97 %	0	99.49 %
2	OM037402.1	<i>Xylocarpus granatum</i> isolate MAT2 ribulose 1,5 biphosphate carboxylose/oxygenase large subunit (rbcl) gene, partial cds; chloroplast	<i>Xylocarpus granatum</i>	1068	1068	97 %	0	99.49 %
3	MT877012.1	<i>Cedrela sp.</i> VS-2021 voucher Berrones Benitez 1 (IBUG) ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; chloroplast	<i>Cedrela sp.</i> VS-2021	1059	1059	96 %	0	99.49 %
4	MG833611.1	<i>Cedrela odorata</i> isolate GDC664 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; chloroplast	<i>Cedrela odorata</i>	1057	1057	95 %	0	99.65 %
5	MN192873.1	<i>Toona sinensis</i> voucher LuJL445 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; chloroplast	<i>Toona sinensis</i>	1053	1053	97 %	0	98.98 %
6	MH550014.1	<i>Swietenia mahagoni</i> voucher Trotta950332 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; chloroplast	<i>Swietenia mahagoni</i>	1051	1051	94 %	0	100.00 %
7	MK830591.1	<i>Carapa guianensis</i> voucher Cg70 ribulose 1,5-bisphosphate carboxylase (rbcl) gene, partial cds; chloroplast	<i>Carapa guianensis</i>	1051	1051	94 %	0	100.00 %
8	MK830590.1	<i>Carapa guianensis</i> voucher Cg69 ribulose 1,5-bisphosphate carboxylase (rbcl) gene, partial cds; chloroplast	<i>Carapa guianensis</i>	1051	1051	94 %	0	100.00 %
9	MK830589.1	<i>Carapa guianensis</i> voucher Cg68 ribulose 1,5-bisphosphate carboxylase (rbcl) gene, partial cds; chloroplast	<i>Carapa guianensis</i>	1051	1051	94 %	0	100.00 %
10	MK830588.1	<i>Carapa guianensis</i> voucher Cg67 ribulose 1,5-bisphosphate carboxylase (rbcl) gene, partial cds; chloroplast	<i>Carapa guianensis</i>	1051	1051	94 %	0	100.00 %
11	MK830587.1	<i>Carapa guianensis</i> voucher Cg66 ribulose 1,5-bisphosphate carboxylase (rbcl) gene, partial cds; chloroplast	<i>Carapa guianensis</i>	1051	1051	94 %	0	100.00 %
12	MK830586.1	<i>Carapa guianensis</i> voucher Cg65 ribulose 1,5-bisphosphate carboxylase (rbcl) gene, partial cds; chloroplast	<i>Carapa guianensis</i>	1051	1051	94 %	0	100.00 %
13	MK830585.1	<i>Carapa guianensis</i> voucher Cg64 ribulose 1,5-bisphosphate carboxylase (rbcl) gene, partial cds; chloroplast	<i>Carapa guianensis</i>	1051	1051	94 %	0	100.00 %
14	MK830584.1	<i>Carapa guianensis</i> voucher Cg63 ribulose 1,5-bisphosphate carboxylase (rbcl) gene, partial cds; chloroplast	<i>Carapa guianensis</i>	1051	1051	94 %	0	100.00 %
15	MK830583.1	<i>Carapa guianensis</i> voucher Cg62 ribulose 1,5-bisphosphate carboxylase (rbcl) gene, partial cds; chloroplast	<i>Carapa guianensis</i>	1051	1051	94 %	0	100.00 %

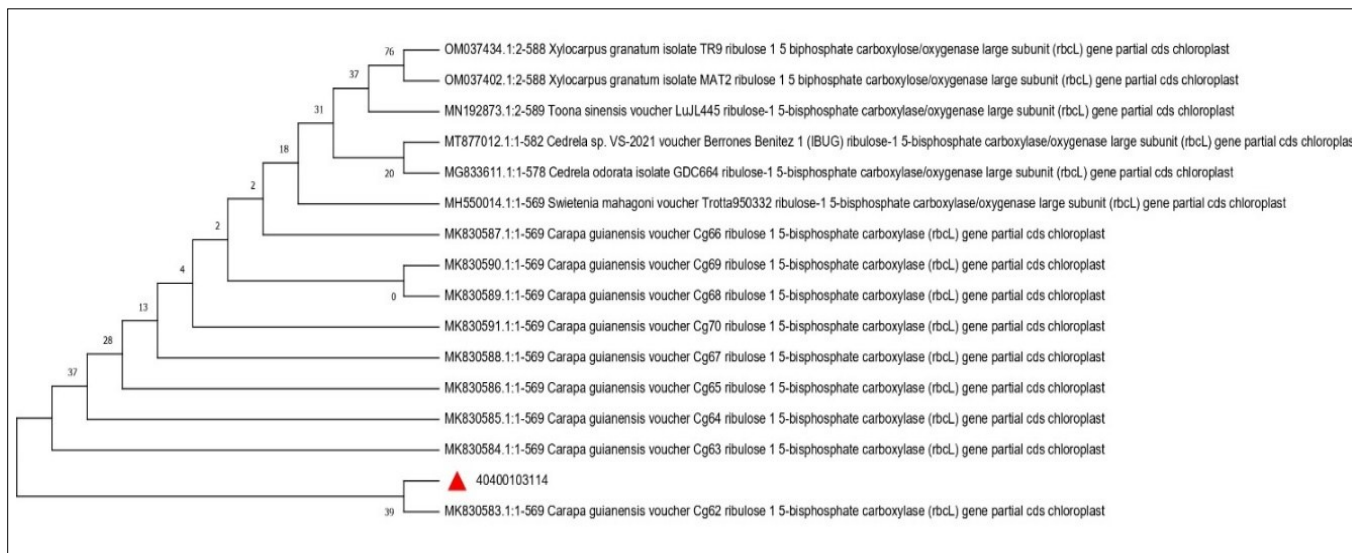


Fig. 4. Ruan, plant 'X' from Barda, was found to be comparable to and close to accession id MK830583.1 with the *Carapa guianensis* voucher Cg62 ribulose 1,5-bisphosphate carboxylase (*rbcl*) gene, incomplete cds; chloroplast, according to phylogenetic analysis.

species level, the results showed that the percentage of correct identification at the genus level was 67.71 % and at the species level was 16.95 % (36).

In the current study, the sequence analysis of the *rbcl* gene of Ruan, the plant 'X', shows 100 % similarity with *Swietenia mahagoni* and *Carapa guianensis*. The sequence "hits", alignment to the query sequence in BLAST, are showing 48 hits for the genus *Carapa* and 33 hits for the *Carapa guianensis* species, whereas the hits for *Swietenia mahagoni* are only 2.

Conclusion

In this investigation, DNA fragment of the *rbcl* gene was effectively amplified from the leaf sample of Ruan, plant 'X' from Barda. The sequence had shown 100 % similarity with *Carapa guianensis* (Accession ID MK830583.1 to MK830591.1) and *Swietenia mahagoni* (Accession ID MH550014.1), but the morphology of the plant matches to that of *Carapa guianensis*. The identified plant has its native to South America and commonly known as Andiroba. This is the first report of the occurrence of *Carapa guianensis* in India at Barda Hill, Porbandar, Gujarat.

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Authors' contributions

The original and final versions of this manuscript were written by NSS, who also conceptualized the study and participated in data processing and visualization. VRO executed the experimental work and collected the sample. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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