

Journal of Coastal Life Medicine

New record of *Dictyota bartayresiana*, a marine brown algal species revealed from Rich Seaweed Diversity Area of South India

Received:12 December 2022, **Revised:**11 January 2023, **Accepted:**23 January 2023

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Keywords

Macroalgal species, Phylogenetic analysis, Indian coastal region, *D.bartayresiana* , rbcL gene sequencing.

Abstract

In the current paper, *Dictyota bartayresiana* account for the earliest time, a novel record for the Indian macroalgal flora. A present species of *Dictyota*, previously recognized as *D. bartayresiana*, is illustrated from southeast coast region of India. Morphological observation and phylogenetic analysis, as well to molecular sequence data of specimens collected from Mandabam and species were evaluated and facilitated us to authenticate that *D. bartayresiana*. There were 38 species of *Dictyota* species used for the comparison.

1. Introduction

Seaweed is found as an inhabitant in submerged oceanic waters. Based on their chemical composition and structure, they are classified as Chlorophytes, Rhodophytes, and Phaeophytes. Phaeophytes are mostly submerged in seawater, resulting in unicellular and multicellular

architecture. Chlorophyll a and c, Beta carotene, Lutein, Fucoxanthin, and Dioanthin are some of the respiratory and phytochemical pigments.

Brown algae can tolerate adverse environmental conditions due to the presence of bioactive compounds and oxidizing capacity [1]. Secondary metabolites have contained excellent biological

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activity besides providing resistance against other organisms. As far as, more than 2400 seaweed bioactive compounds are identified that acting as blueprint for developing novel drugs. The bioactive compounds be grouped as aldehydes, alcohols, halogenates and terpenoids that synthesized from algal species and acting as antibacterial, antifungal, antibiofouling agents [2, 3].

Seaweeds are definitely the most promising bioactive molecules and still paved the way of research and exploitation to be fully implemented in human health benefits. However, further studies are needed to evaluate the efficacy of the seaweed on medicinal purpose in pharmaceutical applications [4, 5].

Dictyota bartayresiana is rare seaweed that is multicellular and belongs to the Phylum Phaeophytes. *Dictyota bartayresiana* extracts contain essential phenolic compounds such as alkaloids, steroids, phenols, flavonoids, saponins, tannins, glycosides, and sugars, and it also aids in the scavenging of free radicals, as evidenced by in vitro studies, and it also acts as an antioxidant. The crude extract of *D. bartayresiana* has shown cytotoxicity and antibacterial activity against the HT29 cancer cell line as well as urinary pathogens [6]. Because of external morphological similarities, species limitations, and the need for analytical natures, the Dictyota family has a long and difficult taxonomic history.

Our indulgent of species range has been progressed, and the biogeographical variety of individual species has been purified, thanks to current research combining DNA-support species restricted techniques and characteristic morphological scrutiny. The molecular systematic aids to Dictyota organization may be useful in allocating taxonomists to improve recognizing of species diversity at the genus level [7, 8].

Species identification is important for some industries to invest in technology depends on the final product of algal extraction. The following industries are utilizing algal products such as Pharmaceuticals, food and agro industries. There have been various techniques employed for identification of algal species [9]. The *rbcl* gene sequencing is one among the method which targets

chloroplast gene and facilitates the further phylogenetical studies. Amplification of *rbcl* gene can be easy than other techniques and that uniquely present in photosynthetic organisms [10].

This research uncovers new evidence of the rare Mandabam species *Dictyota bartayresiana*. A molecular phylogeny demonstrates the spot of *D. bartayresiana* presented the genus and in similarity to a closely relay genus, and the morphology, locality, and environments of the species are illustrated. They are the first *rbcl* gene sequences discovered in this careful species. These species were discovered over a century ago, but due to their rarity, they have yet to be investigated.

Dictyota bartayresiana are widely distributed in southeast coast area of India exclusively pudumadam, seeniappa darga, krusadai island, pamban and thonithurai and other areas including goa, okha, Lakshadweep, bhatkal, malvan. This species is distributed in the tropical western central Pacific Ocean and the tropical western Atlantic Ocean, as well as the Gulf of Mexico which occurs at depths of 108 m.

2. Methodology

Brown algae *Dictyota bartayresiana* prefers the submerged portion of the ocean column. It was collected for the experiment from Mandabam in Kanyakumari district (Figure 1) and washed in running tap water for 15 min before soaking to remove unwanted soil residues. Live algae are preserved in order to better understand their morphological structure and compare them to other species for future research.

Finally, distilled water is used to obtain purified seaweeds. The authentication code of our sample is BSI/SRC/5/23/2019/Tech/3070, provided by Botanical Survey of India, Govt of India.

3. Structure Analysis

External structure of *Dictyota bartayresiana* clearly indicates that internodal segments are less than 4 breadth long; upper sinuses broad, but sinuses usually narrower in other Dictyota family. The lamoroux are usually variable, intermingled masses form the bushy and dense structure. Stipes are not present, instead of stipes, holdfast helps the attachment to substratum by their irregularly

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shaped structure. The holdfast usually binds with rhizoids.

4. Polymerase Chain Reaction

DNA extraction was done by using Xpress DNA plant kit as per manufacturing protocol with minor modifications. Amplification of isolated genome carried out by PCR techniques. The 25 µl PCR reaction mixture contains following components such as 1.5 units of Taq polymerase, 0.2µM of each dNTP and primer, 2mM MgCl₂ with reaction buffer (Applied Biosystems), 1µl of temFig DNA, final volume made with double distilled water. The universal primers of rbcl-68F [11] and rbcl-708R [12] were used and the PCR band size approximated to be 520bp.

PCR amplification was carried out through Applied Biosystem to accomplish 40 cycles following an early denaturation cycle at 95°C for 10 min. Every cycle comprised of a denaturation step for 30sec at 95°C subsequent to an annealing step for 20 sec at 50°C further extension step for 30 sec at 72°C. The final extension was continued for 15 mins at 72°C. The resulting product was resolved by electrophoresis technique in 1.5% agarose gel and purified with purification kit (Roche diagnostics). Sequencing was performed using ABI BigDye terminator (Applied Biosystems).

The evolutionary distances were calculated by means of the Kimura 2-parameter method. The sequences were analyzed by MEGA X package and submitted to gen bank [13]. The rbcl PCR primers were doing well to intensify and fabricate PCR product with anticipated band size (≈ 520 bp).

5. Results

Dictyota bartayresiana extended up to the height of 9 to 14 cm and thallus widens around 6 to 10 mm. In young stage of growth, thallus is branched but the further development which become bent and twisted for attaining typical structure of dichotomous in Indian coastal water. The dichotomous structure are equally angled the ranges 45°-90°, mostly that exhibits in 80°. The thallus of *Dictyota bartayresiana* lamouroux have seems very soft, membranous and brittle nature which does not contain any midribs, 2-7mm broad [11].

The collected raw seaweed materials were washed thoroughly with seawater for removing sand particles and unwanted debris. Double distilled water used for washing the seaweeds to make germ free material which avoid bias during sequencing process. The resulting material was chopped into thin pieces and immersed with preservatives for further studies until which kept by vials and stored at -40°C. Approximately 1050 bp of rbcl genes sequence was recovered for *Dictyota bartayresiana* seaweed. First fifteen sequences were selected from blast tool based on maximum score of identity and aligned using multiple alignment software program ClustalW [11].

The rbcl sequences of dictyota species shared similarity score higher than 93% with sequences of *Dictyota bartayresiana* in genbank database which is previously sequenced from Canary Islands (GQ425107.1). Hence, it has been concluded that the seaweeds collected in present study is *Dictyota bartayresiana*. The sequence were uploaded in NCBI and got accession number: MT015586 for further reference (Table 1).

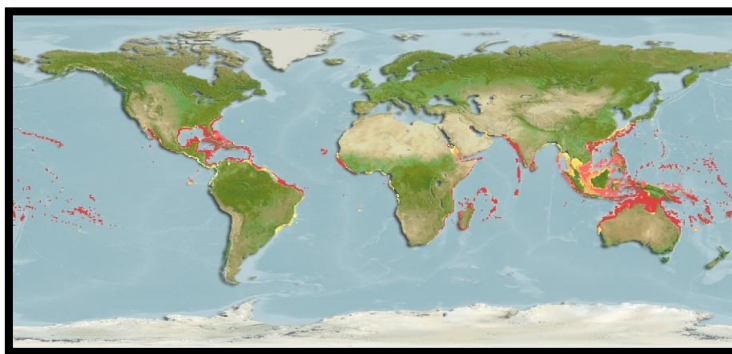


Figure 1: A map published on Aquamaps, showing the Distribution range colours indicate degree of suitability of habitat which can be interpreted as probabilities of occurrence.

(Source: https://www.aquamaps.org/imagethumb/cached_maps/pointmap/pic_SLB-131834.jpg)

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Figure 2: Habit of *Dictyota bartayresiana*

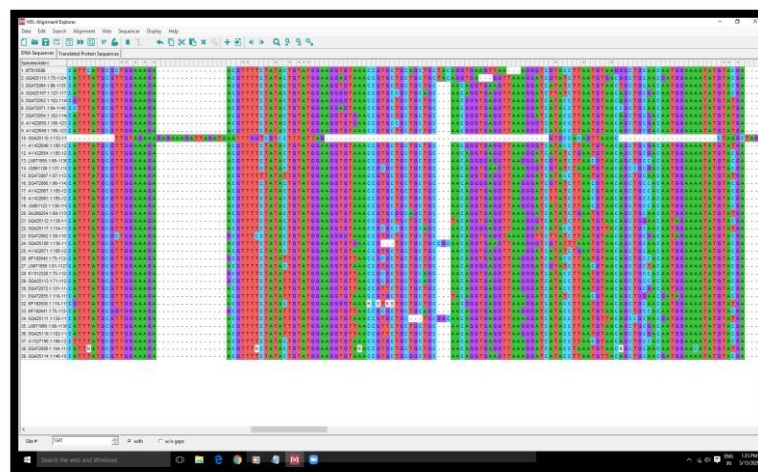


Figure 3: Base pair of *rbcL* gene sequence alignment through software program

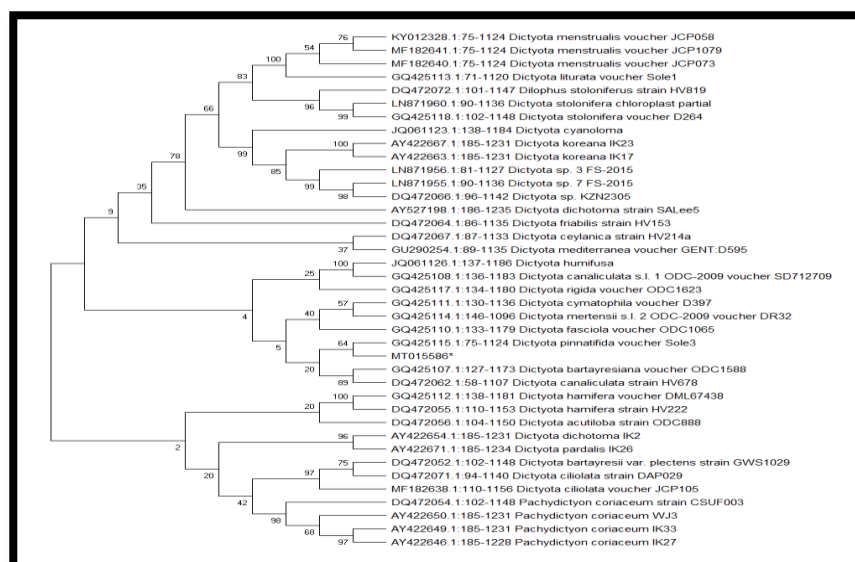


Figure 4: Bayesian consensus tree for the alignment of *rbcL* sequences of brown algal species with bootstrap values for Maximum Parsimony (1000), Neighbour Joining (1000) analyses overlaid on the branches.

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Table 1: The sequences used in the concatenated alignment are listed with their Gen Bank accession numbers

Species	Rbcl gene sequence
<i>Dictyota menstrualis</i>	KY012328
<i>Dictyota menstrualis</i>	MF182641
<i>Dictyota menstrualis</i>	MF182640
<i>Dictyota liturata</i>	GQ425113
<i>Dictyota stolonifera</i>	DQ472072
<i>Dictyota stolonifera</i>	LN871960
<i>Dictyota stolonifera</i>	GQ425118
<i>Dictyota cyanoloma</i>	JQ061123
<i>Dictyota koreana</i>	AY422667
<i>Dictyota koreana</i>	AY422663
<i>Dictyota sp.</i>	LN871956
<i>Dictyota sp.</i>	LN871955
<i>Dictyota sp.</i>	DQ472066
<i>Dictyota dichotoma</i>	AY527198
<i>Dictyota friabilis</i>	DQ472064
<i>Dictyota ceylanica</i>	DQ472067
<i>Dictyota mediterranea</i>	GU290254
<i>Dictyota humifusa</i>	JQ061126
<i>Dictyota canaliculata</i>	GQ425108
<i>Dictyota rigida</i>	GQ425117
<i>Dictyota cymatophila</i>	GQ425111
<i>Dictyota mertensii</i>	GQ425114
<i>Dictyota fasciola</i>	GQ425110
<i>Dictyota pinnatifida</i>	GQ425115
<i>Dictyota bartayresiana</i>	GQ425107
<i>Dictyota canaliculata</i>	DQ472062
<i>Dictyota hamifera</i>	GQ425112
<i>Dictyota hamifera</i>	DQ472055
<i>Dictyota hamifera</i>	GQ425112
<i>Dictyota hamifera</i>	DQ472055
<i>Dictyota acutiloba</i>	DQ472056
<i>Dictyota dichotoma</i>	AY422654
<i>Dictyota pardalis</i>	AY422671
<i>Dictyota bartayresiana</i>	DQ472052
<i>Dictyota ciliolata</i>	DQ472071
<i>Dictyota ciliolata</i>	MF182638
<i>Pachydictyon conaceum</i>	DQ472054
<i>Pachydictyon conaceum</i>	AY422650
<i>Pachydictyon conaceum</i>	AY422649
<i>Pachydictyon conaceum</i>	AY422646

6. Discussion

In order to strengthen the results, phylogenetic analysis was included in the present study. The evolutionary history was inferred using the Neighbor-Joining method [14]. The bootstrap consensus tree inferred from 100 replicates is taken to signify the evolutionary account of the taxa analyzed. Branches corresponding to dividers

reproduced in fewer than 50% bootstrap imitates are collapsed. The percentage of replicate trees in which the associated taxa gathered together in the bootstrap test (100 replicates) are revealed after that to the branches. The evolutionary remoteness was figured by the Kimura 2-parameter method and in the components of the numeral of base replacements per site. This study occupied 39 nucleotide sequences [15]. Codon spots comprised were 1st+2nd+3rd+Noncoding sequence. All indefinite spots were eliminated for every sequence pair (pairwise deletion option). There were a whole of 1508 spots in the concluding dataset. Evolutionary analyses were carried out in MEGA X which shows in Figure 2, 3 and 4 [13].

7. Conclusion

The present study concluded that rbcl gene sequences useful to understand the family and genus level relationship with evolutionary lineage of seaweed. The molecular identification of brown algal species was performed by using rbcl as marker gene. Phylogenetic analysis with aligned rbcl sequences were done by neighbor joining method. Our investigations give you an idea about the rare species of *Dictyota bartayresiana* (Indian Ocean), its evolutionary significance, and the locality was not yet completely recognized by previous researcher. There is need to explore the species of *Dictyota bartayresiana* occurring in Indian sea coastal region using combined morphological and molecular approaches.

Acknowledgement

Thank you to Mr. Rajendra Kumar and the Algae Research Center for the assistance in securing the permit to collect seaweed and for being supportive in the conduct of this study. The authors would also like to thank PAR LAB Private LMD, Trichy, Tamilnadu, India

Conflict Of Interest

Authors declare no conflict of interests.

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