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RESEARCH ARTICLE **OPEN ACCESS**

Molecular Phylogenetic Analysis of *Vallisneria Spiralis Linnaeus* in Kanyakumari, Tamil Nadu, India

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Abstract:

Background: The plant *Vallisneria spiralis Linnaeus*, also referred to as water celery or tape grass, is classified as a monocotyledonous plant within the Hydrocharitaceae family. The genus *Vallisneria* has a wide geographical range, encompassing temperate, tropical, and subtropical areas, such as Australia, Africa, Europe, Asia, and North America. Except for a limited range of characteristics, there is a lack of consistent physical traits that can function as precise taxonomic indicators for defining species borders within the genus. Hence, *Vallisneria* samples obtained from Kanyakumari in Tamil Nadu, India, were subjected to molecular phylogenetic analysis. *Vallisneria spiralis L.* has been documented in many regions worldwide; however, its existence in Kanyakumari, Tamil Nadu, India, has not been verified by molecular identification.

Aim: This study aimed to identify the *Vallisneria species* found in Kanyakumari, Tamil Nadu, India, through molecular phylogenetic analysis.

Objective: The objective of the molecular identification of *Vallisneria species* was to distinguish between the different species of *Vallisneria* found in Kanyakumari, Tamil Nadu, India. Genetic differences between the *Vallisneria species* were analyzed using molecular markers.

Methods: The specimens were obtained from Kanyakumari, Tamil Nadu, India, and subsequently verified and identified using genetic techniques. The maximum likelihood technique was employed as an optimality criterion to conduct the phylogenetic studies.

Results: The resulting tree had grouping patterns comparable to the preceding phylogenetic tree generated using the Maximum Likelihood (ML) technique. The consistent clustering observed in this study strengthened the reliability and strength of the results of *Vallisneria spiralis L.* from Kanyakumari, Tamil Nadu, India. The placement of *Vallisneria spiralis L.* from Kanyakumari, Tamil Nadu, India, inside the predicted clade, was confirmed through a comparison with published phylogenetic research on the *Vallisneria genus*.

Conclusion: The identification of *Vallisneria* spiralis within the analyzed dataset was supported by grouping the original sequences in the resulting tree. The verification of the existence of *Vallisneria spiralis L.* in Kanyakumari, Tamil Nadu, India, is significant in comprehending the geographical range and biological variety of this species within the Indian context.

Keywords: Aquarium plant, *Vallisneria*, Phylogenetics, Kanyakumari, Tamil Nadu, *Hydrocharitaceae*, Monocotyledonous plants, Taxonomic indicators.

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1. INTRODUCTION

Vallisneria spiralis, as recorded by Linnaeus, serves as the exemplar species within the *Vallisneria genus*, tracing its origins back to its native habitat in southern Europe. Recently, comprehensive global identification of 12 species has been achieved through the utilization of molecular techniques. Furthermore, three additional species have been acknowledged based on morphological differentiations that have exhibited comparable molecular characteristics [1]. *Vallisneria* L., commonly known as water celery or tape grass, belongs to the Hydrocharitaceae family, which is a monocotyledonous plant [2]. The species in question is categorized as a mandatory submerged freshwater aquatic species. Typically, this genus is dioecious and perennial, characterized by grouped male flowers and single pistillate blooms. The genus exhibits a broad distribution over temperate, tropical, and subtropical regions, as observed in Australia, Africa, Europe, Asia, Australia, and North America [3]. *Vallisneria species* demonstrate a unique pollination process, which is categorized as type III-B within the angiosperm classification [4]. This process involves the full separation of male flowers, which are then able to swim and interact with the pistillate flowers through direct physical contact. Pollen is deposited in this procedure by a mechanism that deliberately precludes the utilization of water. Pistillate flowers undergo underwater development. The pistillate bloom exhibits epigynous properties. The elongation of the scape typically persists beyond the point at which the tip of the flower emerges from the water surface, and a sufficient scape is developed to enable the pistil to be positioned horizontally on the water surface at the moment of flower opening. Pollination occurs within the horizontal plane of the pistillate flower [5]. A basal rosette is used by the *Vallisneria species* to generate linear leaves. Fig. (**1**) depicts the taxonomic tree of *Vallisneria spiralis Linnaeus*.

The plant species *Vallisneria* is found in subtropical and tropical regions of the world. Apart from a restricted set of attributes, there is an absence of consistent physical characteristics that can serve as accurate taxonomic markers to demarcate species boundaries within the genus [6]. Numerous vegetative characteristics have been hypothesized to be completely disregarded, owing to their uncertain taxonomic validity. Similarly, numerous floral characteristics, including sepal length, flower color, male scape breadth, and fruit curvature, have been deemed inadequate and unnecessary for taxonomic determinations [7]. The floral characteristics commonly employed to differentiate species in *Vallisneria* are frequently absent or significantly diminished, resulting in the inaccurate identification of taxa within the genus. In this study, we conducted a molecular phylogenetic analysis of specimens collected from Kanyakumari in the Tamil Nadu state of India.

2. MATERIALS AND METHODS

2.1. Sample Collection

Vallisneria samples were collected from Kanyakumari, Tamil Nadu, India. The three specimens that were gathered were subjected to a drying process using herbarium sheets. Each individual was sampled at a minimum distance of a few meters, to ensure that identical genets were not collected. The leaf samples were preserved in a refrigerated environment at 25 °C in airtight sealed plastic bags until DNA extraction was conducted.

Fig. (1). Taxonomic tree of *vallisneria spiralis linnaeus* (*vallisneria spiralis L.*) [2].

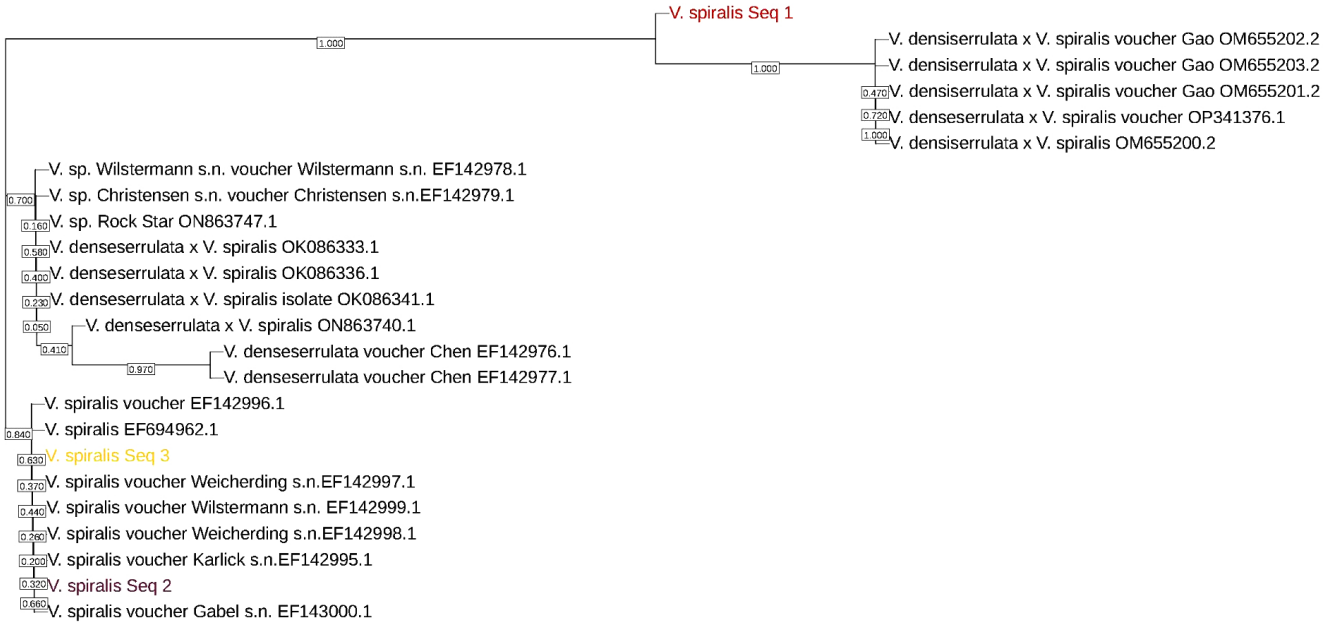

Fig. (2). Evolutionary analysis by maximum likelihood method.

2.2. DNA Sequencing

Genomic DNA was extracted from leaf tissues weighing 50–100 mg of dried leaf samples, according to a previously published procedure [8]. A total reaction volume of 15 µL was used to amplify the polymerase chain reaction (PCR). This volume consisted of 10–20 ng of total DNA, 0.375 units of Taq DNA polymerase, 2 mM $MgCl_2$, 0.1 mM deoxynucleotide triphosphates (dNTPs), 10 mM Tris–HCl (pH 8.3), 50 mM KCl, and 0.15 µM of each primer. Amplification of the double-stranded DNA was performed following an initial incubation at 94°C for 3 minutes. Subsequently, a series of 30 incubation cycles was conducted at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, culminating in a final extension at 72°C for 15 minutes. The amplification of the internal transcribed spacers of nuclear ribosomal DNA (nrITS) regions was conducted and applied to a Genetic Analyzer equipped with a BigDye Terminator v2.0 Cycle Sequencing Kit to sequence the fragments. Identical primers were used for amplification during the sequencing process. A homology search was conducted using the BLAST algorithm *via* blastn on the NCBI for Biotechnology Information website. The target fragments were subjected to DNA sequencing using the M13R and M13F primers.

2.3. Phylogenetic Analysis

Three experimental sequences of *Vallisneria spiralis Linnaeus* samples collected from Kanyakumari, Tamil Nadu, India, were used for Phylogenetic analysis [9-12]. Basic Local Alignment Search Tool (BLAST) was performed [13]. The deduced amino acid sequences of the three gene fragments were individually aligned using ClustalW [14]. The maximum likelihood technique was employed as an optimality criterion to conduct the phylogenetic studies [15]. The Akaike Information Criteria (AIC) were used to evaluate the most suitable models for maximum likelihood analysis, as implemented in the Modeltest [16]. Subsequently, repetitions were conducted using PhyML in the Geneious program, employing the GTR model for nucleotide sequence evolution with maximum likelihood bootstrap analysis [17]. The original sequences were subjected to blasting to generate a multi-FASTA file, which was subsequently utilized to create a Neighbor-Joining (NJ) tree employing the Tamura-Nei model and then selecting the topology with a superior log likelihood value [18]. Evolutionary analyses were conducted in MEGA11 [19]. Branch support was evaluated through bootstrapping [20].

3. RESULTS AND DISCUSSION

The phylogenetic reconstruction (Fig. **2**) successfully identified key clades in the research sequences and similar reference sequences. The research sequences formed a clade that closely grouped with reference sequences containing formerly accessioned *V. denseserrulata* and *V. spiralis* from GenBank. The cluster labeled as *V. spiralis* Seq 1 clade contained reference sequences from GenBank, *i.e.*, voucher OM655202.2, OM655203.2, OM655201.2, OP341376.1, OM655200.2, EF142978.1, EF142979.1, ON863747.1, OK086333.1, OK086336.1, OK086341.1, OK0863740.1, EF142976.1, and EF142977.1. The cluster labeled as *V. spiralis* Seq 2 clade contained reference sequences from GenBank, *i.e.*, voucher EF143000.1. The cluster labeled as *V. spiralis* Seq 3 clade contained reference sequences from GenBank, *i.e.*, voucher

EF142997.1, EF142999.1, EF142998.1, and EF142995.1. This research agrees with previously identified challenges in establishing strong evidence for the classification of this taxon, as well as the evidence supporting the links between the taxa [1]. Our results were in agreement with the findings of Les, Jacobs, and Tippery, 2008, since they demonstrated that the combined molecular dataset had a phylogenetic signal for estimating the phylogeny.

CONCLUSION

Previous studies have reported the presence of *Vallisneria spiralis L.* in various parts of the world, but its presence in Kanyakumari, Tamil Nadu, India, has not been confirmed through molecular identification. Therefore, the aim of this study was to identify and confirm the presence of *Vallisneria spiralis L.* in Kanyakumari, Tamil Nadu, India, through molecular identification. The specimens were collected from Kanyakumari, Tamil Nadu, India, and confirmed through molecular identification as *Vallisneria spiralis L.* Confirmation of the presence of *Vallisneria spiralis L.* in Kanyakumari is important for understanding the distribution and diversity of this species in India. The genetic sequences of the research specimens were grouped together with previously collected *V. spiralis* and V. denseserrulata specimens in a clade. The study specimens occupied an intermediate position in this clade, which is typical of hybrids, and was located between the two species. Previous studies have discovered contemporary hybrid forms of both of these species in Australia, Hungary, and Japan by the confirmation of the nrITS gene *via* subcloning [3]. Hybridization most likely took place in a nursery where both parental species were being treated or in an area where one of the species became established while the other was native, as the two species do not naturally coexist in the wild [6]. Lastly, the sample size of this study was limited to specimens collected from Kanyakumari, Tamil Nadu, India, which may have limited the generalizability of the results.

AUTHORS' CONTRIBUTIONS

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

LIST OF ABBREVIATIONS

ML	=	Maximum Likelihood
dNTPs	=	deoxynucleotide triphosphates
nrITS	=	nuclear ribosomal DNA
BLAST	=	Basic Local Alignment Search Tool
AIC	=	Akaike Information Criteria
NJ	=	Neighbor-Joining

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not Applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analysed during this study are included in this published article.

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CONFLICT OF INTEREST

The authors declared no conflict of interest, financial or otherwise.

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