Science & Technology Development Journal 2024, 26(SI):38-44

Open Access Full Text Article

Research article

Morphological and preliminary molecular data on freshwater red algae in Tam Dao National Park, Vietnam

Nguyen Hoai An, Cao Thi Hong Anh, Nguyen Anh Duc, Nguyen Thuy Lien*

ABSTRACT

Rhodophyta is one of the oldest and largest groups of algae and is dominated by marine species, while freshwater species are relatively scarce. Although the diversity and distribution of rhodophytes in freshwater plants have been studied worldwide, there are few published records of freshwater red algae in Vietnam. Therefore, this study aimed to preliminarily investigate the morphology and analyze the phylogeny of freshwater red algae in Vietnam. This report provides information about red algal specimens collected in July 2018 and 2022 in the Tam Dao National Park watershed forests. Morphological analysis of the specimens revealed similar characteristics to those of *Batrachospermum*; however, phylogenetic analysis of the Tam Dao specimens and *Batrachospermum* placed them into different clades. Data relating to both morphological and molecular characteristics suggest that Tam Dao specimens may represent a new clade within Batrachospermaceae.

Key words: Batrachospermum, Batrachospermales, Rhodophyta, Thoreales, Freshwater red algae

Faculty of Biology, VNU University of Science, 334 Nguyen Trai, Thanh Xuan, Ha Noi, Viet Nam

Correspondence

Nguyen Thuy Lien, Faculty of Biology, VNU University of Science, 334 Nguyen Trai, Thanh Xuan, Ha Noi, Viet Nam

Email: nguyenthuylien@hus.edu.vn

History

- Received: 2023-10-16
- Accepted: 2024-01-24
- Published Online: 2024-6-30

DOI :

https://doi.org/10.32508/stdj.v26iSI.4201

INTRODUCTION

Red algae (Rhodophyta) are among the earliest and largest groups of multicellular algae and can be found in both oceans and freshwater bodies. While marine species make up the majority of the Rhodophyta division, freshwater red algae account for less than 5%, with two-thirds of all known species belonging to Batrachospermales and Florideophyceae[1]. Freshwater rhodophytes are distinguished from others by unique characteristics, as the predominant photosynthetic pigments in their chloroplasts are phycocyanin or phycoerythrin, resulting in cells that appear blue or red in color. Research on the distribution of red algae has shown that freshwater species occur mainly in oligotrophic running waters. The presence of these bacteria primarily indicates that water reservoirs contain low nutrient concentrations and are minimally contaminated[2].

In recent years, the number of research studies on freshwater red algae has increased globally due to the necessity of obtaining molecular knowledge to study the phylogeny and taxonomy of taxa that were previously based solely on morphological features. The combination of morphological and molecular data has significantly contributed to a better understanding of the relationships among genera and species within those genera. A notable outcome of this combined approach was the proposal of six orders: Balbianiales, Thoreales, Acrochaetiales, Batrachospermales, Nemaliales, and Palmariales. Another significant collaborative effort among freshwater red algal experts led to a comprehensive phylogenetic analysis of the order Batrachospermales, incorporating members from genera and sections of Batrachospermum with the aim of establishing better-defined taxonomic categories, especially to address the issue of paraphyly within the genus *Batrachospermum*. These collaborations led to the suggestion of numerous new genera, including *Kumanoa, Sheathia, Torularia, Virescentia, Acarposporophycos, Visia, Montagnia, and Paludicola*. Under this revised classification approach, *Kumanoa* and *Sheathia* were the two genera with the highest numbers of species within the freshwater red algae[1].

Despite significant advancements in rhodophyte taxonomy worldwide, there is limited published information regarding the distribution of red algae in freshwater bodies in Vietnam. Notifications about the occurrence of freshwater red algae in Vietnam are primarily conveyed through oral communication among researchers, often supplemented by occasional observations in certain national parks. The Checklist of Plant Species of Vietnam (2001) records only one species, *Batrachospermum moniliforme*, within several national parks, such as Ba Be National Park (Bac Kan), Phia Oac – Phia Den National Park (Cao Bang), Tam Dao National Park (Vinh Phuc), and Chu Mom Ray National Park (Kon Tum)[3]. In 2010, Nguyen Thuy Lien reported the presence of two species of freshwater rhodophytes in the Ma Da area within

Cite this article : An N H, Anh C T H, Duc N A, Lien N T. **Morphological and preliminary molecular data on freshwater red algae in Tam Dao National Park, Vietnam**. *Sci. Tech. Dev. J.* 2024; 26(SI):38-44.

the Dong Nai Biosphere Reserve in southern Vietnam[4]. However, these occurrence records are relatively vague and lack parameters for molecular analysis.

From that perspective, in this report, we present information about red-algal samples collected from the Tam Dao National Park watershed forests during annual summer schools in 2018 and 2022. This study aimed to provide morphological data and molecular evidence for phylogenetic analysis, with the goal of clarifying the taxonomy of freshwater rhodophytes in Vietnam.

MATERIALS AND METHODS

Sample collection

Samples were collected from the headwater streams of Tam Dao National Park at coordinates 21°49'61.71" North latitude and 105°62'69.93" East longitude. Sample collection took place in two phases: on July 21st, 2018 (TD18), and on July 17th, 2022 (TD22). The collected algae were divided for various purposes, including DNA extraction, morphological examination, and herbarium voucher preparation. Specimens intended for molecular analyses were rapidly dried using silica gel, while those for morphological and anatomical observations were preserved in 5% formalin. Voucher herbarium specimens have been deposited at the VNU University of Science Herbarium.

Morphological analyses

The morphological characteristics were observed and documented using a Primo Star microscope with an attached camera system (Carl Zeiss, Germany) following the methods described by Chapuis et al. (2017), Entwisle et al. (2009), Evans et al. (2017), Fischer et al. (2020), Rossignolo et al. (2020), Suzuki & Kitayama (2021), and Vis et al. (2012, 2021)[1,5–11]. Morphological characteristics, including the whorl diameter, fascicle cell number, carposporophyte and carposporangium dimensions, spermatium diameter, and carpogonium dimensions, were measured in 10 to 15 replicates. Algal identification was performed based on observation features following the methods of the Freshwater Algae of North America[2] and Freshwater Red Algae[1].

Molecular analyses

Silica-dried plant material was ground in 200 μl of CTAB using a mortar and pestle. The solution was then vortexed using two glass beads (ø 5 mm) for 30 seconds. DNA was subsequently extracted from the obtained solution following the CTAB extraction protocol[7].

Molecular assays for sample analysis focused on multilocus sequence analysis (MLSA) following the methods of Lam et al., (2016)[12]. In addition to *rbcL, COI-5P (cox1), psaA, psbA, psaB, EF2, cob, 18S rDNA*, and *28S rDNA*, four other *genes—16S rDNA*, *23S rDNA*, *tilS* (tRNA(Ile)-lysidine synthase), and *trpA* (tryptophan synthase alpha subunit)—were included in the study. The typical PCR mixture (25 μl) consisted of 2X OneTaq® DNA Polymerase (New England Biolabs, USA), 0.5 μM primers, and 2 μl of DNA. Thermocycler incubation was carried out using a Mastercycler® Nexus-PCR Thermal Cycler (Eppendorf, Germany) following the following general conditions: 94 °C for 2 min; 35 cycles of 94 °C for 40 s, 60 °C for 45 s, and 68 °C for 90 s; and 1 cycle at 68 °C for 5 min, followed by holding at 4 °C. The integrity of the PCR products was assessed by electrophoresis for 45 min at 90 V in 1% (w/v) agarose gels in TAE buffer. DNA sequencing was performed by 1st BASE (Axil Scientific Pte. Ltd., Singapore).

Phylogenetic analyses

The sequences were submitted to GenBank and analyzed using the Nucleotide Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The individual genes and a concatenated alignment were subjected to maximum likelihood (ML) analysis using PHYML in the Geneious Prime® 2023 plugins with 1000 bootstrap support replicates.

RESULTS

Morphological characteristics

The specimen was undisturbed by human activity and consisted of freshwater streams with shallow rocky soil that were lightly shaded (Figure 1a). The collected thalli ranged from olive-green in full sunlight to dark blue–green in shaded areas. The morphological characteristics of the specimens from both phases of collection were similar.

Macroscopic gametophytic thalli are olive-green in color, heterotrichous, and uniaxial, with whorls of branches that give rise to a bead-like appearance on the thallus. The thallus lacks an outer cortex and has a mucilaginous consistency. The thalli are monoecious, very mucilaginous, 5 to 17 cm in height, and 600 to 1000 μm wide. They are abundantly and irregularly branched and appear olive green (Figure 1b). Carposporophytes are small and pedunculate. The whorls are ellipsoidal and separated, contiguous and more or less compressed, measuring 50 to 100 μm in diameter, with 1 to 2 peripheral carposporophytes (indicated by the arrow in Figure 1c). The carpogonial branch is composed of cells that are similar in shape and size to primary fascicle cells and pedunculate carposporophytes. Primary branchlets are abundantly branched, with 10 to 13 cell stories. The proximal cells are cylindrical, measuring 8 to 10 μm wide and 50 to 90 μm long. Carpogonium-bearing branches are 60 to 90 μm long and consist of 8 to 11 cells arising from pericentral cells. Carposporangia are ovoidal, measuring 10 to 12 μm wide and 19 to 22 μm long. Carposporophytes are spherical, with a diameter of 100 to 150 μm (indicated by the arrow in Figure 1e). The spermatangia are spherical, with 3 to 10 cell stories (indicated by the arrow in Figure 1f). Carpogonium-bearing branches are 60 to 100 μm long and consist of 8 to 11 cells (Figure 1d). The trichogyne is 15 μm long with a spermatium (Figure 1g).

Molecular analyses

This study designed and utilized 13 pairs of primers following previous studies and based on red algal data from the NCBI. However, only two fragments of *18S rDNA* and *trpA* showed bands in agarose gels, measuring 1500 and 1000 base pairs, respectively. Amplifications for *18S rDNA* and *trpA* were performed through PCRs using the following primers: *18S rDNA*.RA. F (5'- CACCTGGTTGATCCTGCCAG -3'), *18S rDNA*.RA. R (5'- CTTGTAAGCGTGGGTCATCAG -3'), *trpA*.RA. F (5'- TCCCTTTCACGGAGGTAACG -3'), and *trpA*.RA. R (5'- ACTCTACCATTGAGTTAGCAACC -3'). Alignment searches on NCBI indicated that both TD18 and TD22 belong to Batrachospermaceae. For *18S rDNA*, the sequences covered more than 99% of the gene fragment, showing homology of more than 98%. Similarly, for *trpA*, the figures were approximately 81.5% for gene coverage and 77.5% for pairwise similarity.

Separate ML phylogenetic trees for *trpA* and *18S rDNA* were constructed based on the sequences of the samples and Batrachospermaceae taxa to identify the taxonomy of TD18 and TD22 (Figures 2 and 3). For the concatenated phylogenetic tree, as not all taxa had corresponding sequences for *18S rDNA* and *trpA*, the concatenated alignment was assembled for genera based on sequences of *18S rDNA* and *trpA* extracted from species in the same genus. Following trimming and concatenation, the final alignment used for examination comprised 2363 bases and was analyzed to determine the relationship between TD18 and TD22 and other genera in Batrachospermaceae (Figure 4).

DISCUSSION

The morphological characteristics of the two Tam Dao samples revealed that these samples were closely related to species of *Batrachospermum* sensu stricto. These features include large, multibranched filaments in a mucilaginous or gelatinous mass; undifferentiated straight carpogonial branches arising from both fascicle and pericentral cells; carpogonia with club- to urn-shaped trichogynes; and small, globose, pedicellate carposporophytes at various distances from the whorl axis.

In recent years, the taxonomy of the genus *Batrachospermum* has undergone significant changes. Several sections have been elevated to the genus level, such as *Kumanoa* (formerly sections Contorta and Hybrida) with the type species is *K. virgatodecaisneana* (Basionym: *Batrachospermum virgatodecaisneanum*), *Sheathia* (formerly section Helminthoidea) with the type species is *S. boryana* (Basionym: *Batrachospermum boryanum*), *Torularia* (synonym *Atrophycus*, formerly section Setacea) with the type species is *T. dillenii* (Basionym: *Batrachospermum puiggarianum*), *Virescentia* (formerly a section with the same name) with the type species is *Virescentia helminthosa* (Basionym: *Batrachospermum helminthosum*), *Acarposporophycos* (formerly section Acarposporophytum) with the type species is *Acarposporophycos brasiliensis* (Basionym: *Batrachospermum brasiliense*), *Visia* (formerly section Aristata) with the type species is *Visia cayennensis* (Basionym: *Batrachospermum cayennense*), *Montagnia* (formerly section Macrospora) with the type species is *Montagnia macrospora* (Basionym: *Batrachospermum macrosporum*), and *Paludicola* (formerly section Turfosa) with the type species is *Paludicola turfosa* (Basionym: *Batrachospermum turfosum*)[1].

There was only one species of freshwater red algae recorded in Vietnam, and this species was published in the Checklist of Plant Species of Vietnam (2001): *Batrachospermum moniliforme*, which is now considered a synonym of *B. gelatinosum*. The characteristics of the carpogonial branches, carposporophytes, and other features of the Tam Dao specimens indicate that their morphological phenotypes are relatively similar to those of the genus *Batrachospermum*. However, the trichomes of these specimens had an obovoidal shape, which means that they differ from the description of the trichogyne of *B. gelatinosum*, which is clavate or lanceolate in shape[1].

In terms of the taxonomy of *B. moniliforme*, only *B. moniliforme* var. *pilosissimum* has been identified as the true *B. moniliforme*. Other varieties of this species

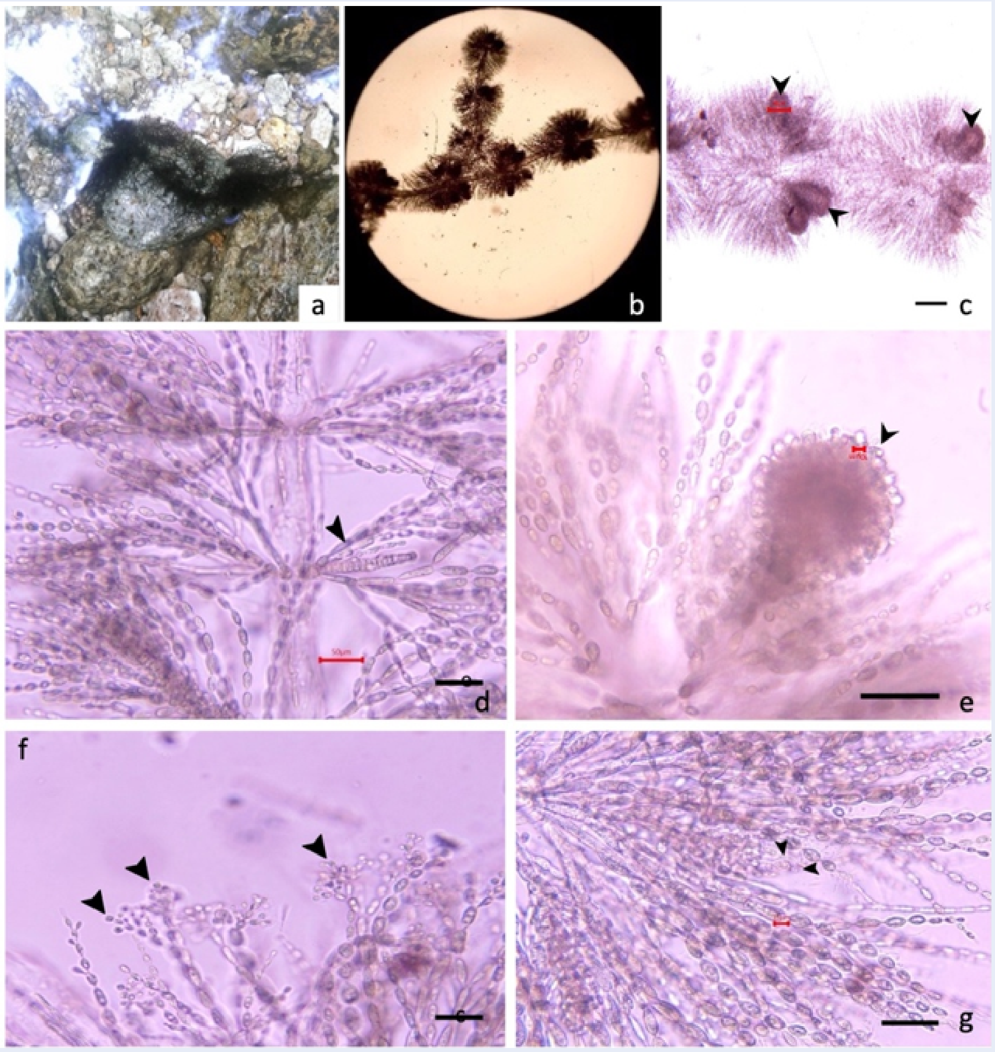

Figure 1: Morphological characteristics of fresh water algal samples from Tam Dao. a. Habitat of the specimen; b. Whorls of thallus; c. The whorls of a female gametophyte showing carposporophytes within the whorls and exerted (arrowheads); d. Carpogonium-bearing; e. Carposporophytes; f. Spermatangia (arrowheads) produced terminally on the fascicle; g. The trichogyne with a spermatium. Scale bars: 50 μm.

are now regarded as synonyms of different species. For example, the species *B. moniliforme* and most of its varieties are currently classified as *B. gelatinosum*, the type species of *Batrachospermum*. *B. moniliforme* var. *stagnale* has been reclassified as *Sheathia boryana*, whereas *B. moniliforme* var. *confusum* and *B. moniliforme* var. *condensatum* are now known as *Sheathia confusa*. Similarly, *B. moniliforme* var. *nodiflorum* has been redirected to *Kumanoa nodiflora*, and both *B. moniliforme* var. *dillenii* and *B. moniliforme* var. *detersum* have been changed to *Torularia atra*[13]. The taxonomy of the genus *Batrachospermum* has changed significantly, and Vietnamese freshwater red algae have also been subjected to these changes.

Identifying closely related species in *Batrachospermum* sensu stricto based on morphology has proven difficult and remains challenging due to morphological similarities. For instance, the separation of *Batrachospermum* and *Sheathia* has relied primarily on the occurrence of heterocortication, which is unique to the majority of species in *Sheathia*; however, this feature is absent in *S. arcuata*[1]. Therefore, in this situation, only DNA sequence data provide a feasible approach.

Both ML phylogenies depicting the relationships of TD18 and TD22 with other genera of Batrachospermaceae, based on 18S rDNA and trpA, revealed that the Tam Dao specimens formed a separate clade with

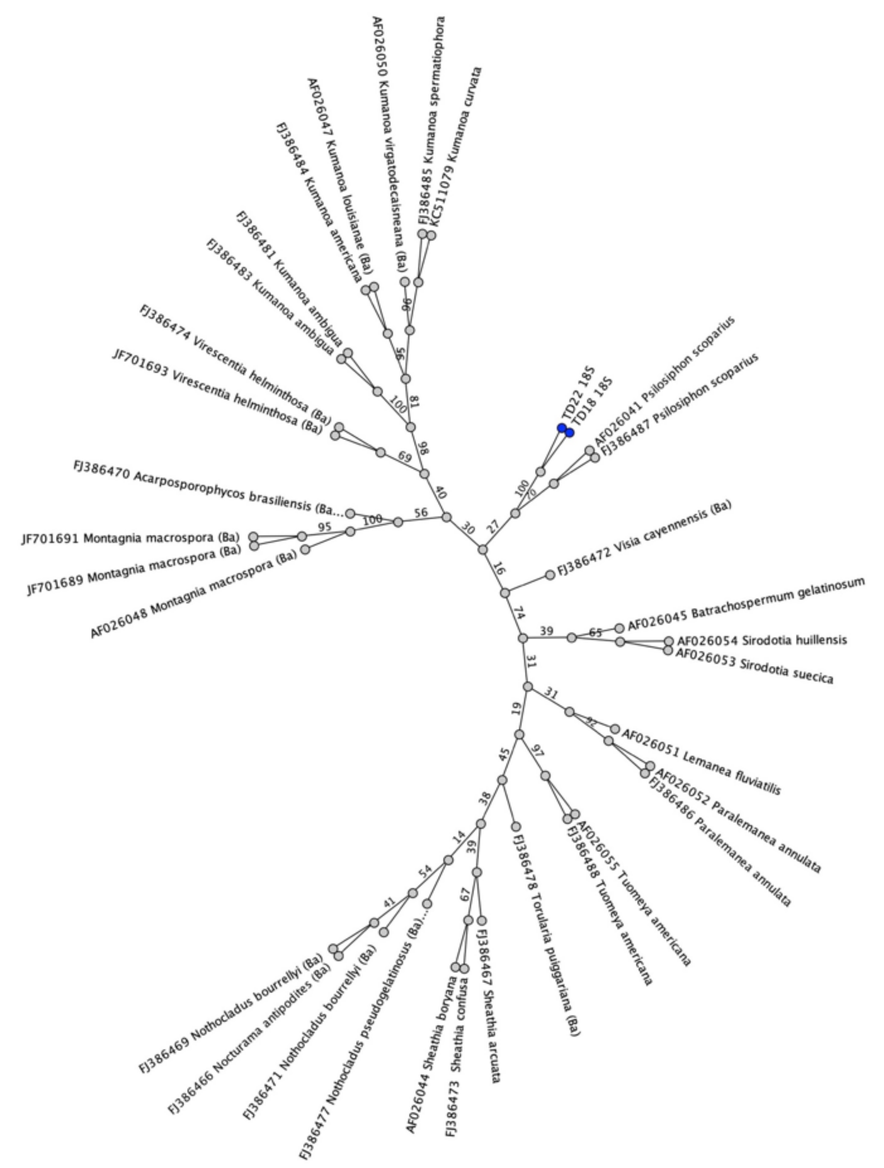

Figure 2: ML phylogeny depicting the relationship of TD18 and TD22 with other genera of Batrachospermaceae based on *18S rDNA* using PHYML in the Geneious Prime® 2023 plugins with 1000 bootstrap support replicates.

their nearest neighbors, Psilosiphon (Figure 2) and Kumanoa (Figure 3). In Figure 2, the TD samples plot on the same branch as the PsiloSiphon samples; however, the morphological features of the PsiloSiphon samples are completely different from those of the TD samples. TD specimens possessed *Batrachospermum* sensu stricto characteristics, similar to *Kumanoa* or *Sheathia*, whereas *Psilosiphon* species have cartilaginous thalli, no division into node and internode regions; they are normally unbranched with putative spermatangia, no carpogonia observed, cortex composed of ellipsoidal to obovoidal photosynthetic cells of uniform size in distinct outward radiating rows or filaments; dense medullary filaments composed of cylindrical cells, colorless, in between the outer cortex and the uniaxial central filament. Reproduction is based on putative monosporangia that develop in chains from cortical filaments on the outside of the thallus or adventitious plantlets[1].

In recent years, many new species have been recorded in neighboring countries of Vietnam, including China, Thailand, Japan, and India[1]. These species are mainly found in Kumanoa and Sheathia. Therefore, freshwater algae are likely related to these species.

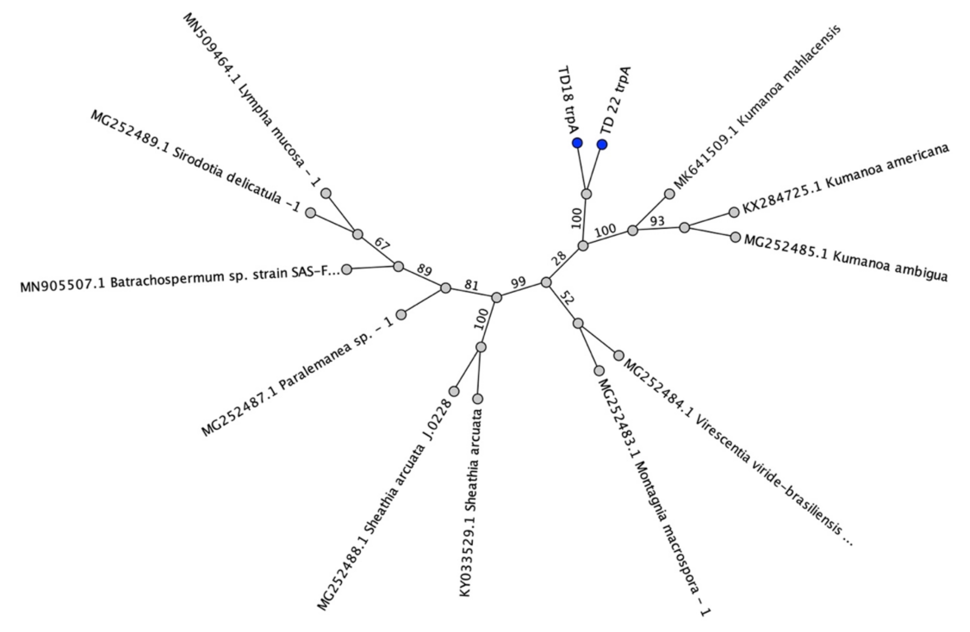

Figure 3: ML phylogeny depicting the relationship of TD18 and TD22 with other genera of Batrachospermaceae based on *trpA* sequences using PHYML in the Geneious Prime® 2023 plugins with 1000 bootstrap support replicates.

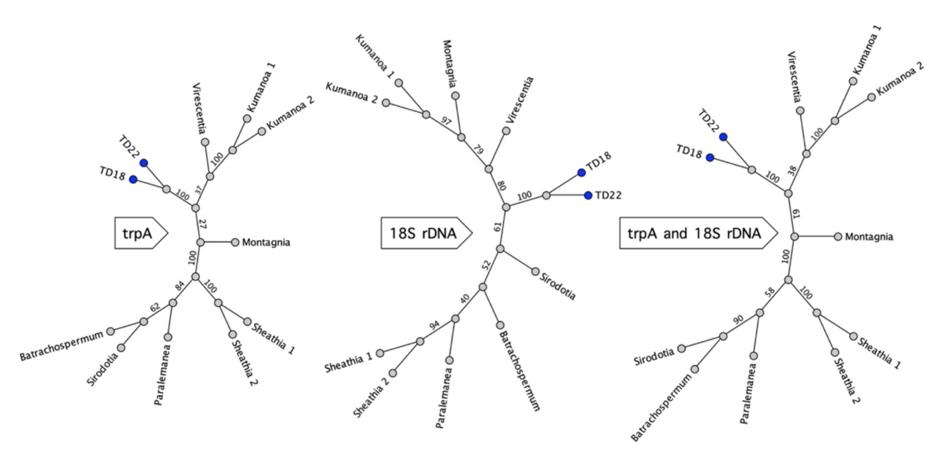

Figure 4: *trpA*, *18S rDNA* and concatenated *trpA-18S rDNA* ML phylogeny depicting the relationships of TD18 and TD22 with seven genera of Batrachospermaceae

However, the phylogenetic trees in Figure 2 and Figure 3 revealed that the Tam Dao samples, *Batrachospermum*, *Kumanoa* and *Sheathia* were separated into different clades. Similarly, the concatenated ML phylogeny representing the association of TD18 and TD22 with several genera of Batrachospermaceae revealed that Tam Dao specimens were more closely related to *Kumanoa* and *Montagnia* than to *Batrachospermum* and *Sheathia*. Although there are limited data on the *18S rDNA* (nuclear DNA) and *trpA* (chloroplast DNA) genes in freshwater algae, the results of morphological and molecular analysis suggest that Tam Dao specimens form a new clade in *Batrachospermum* sensu stricto. Additional evidence from DNA sequences, such as rbcl, may be instrumental in fully characterizing the freshwater red algae of Vietnam.

CONCLUSION

Recently, taxonomic research based on the combination of DNA sequence data and morphological characteristics has revealed the species diversity of freshwater red algae, especially within the family Batrachospermaceae. These studies shed light on the phylogenetic analysis of the family Batrachospermaceae and simplified the taxonomic categories. By providing both morphological and molecular data in the first report on freshwater rhodophytes in Vietnam, this research contributes information about rhodophyte diversity in South Asia and may provide new genus data within the family Batrachospermaceae.

ABBREVIATIONS

None.

ACKNOWLEDGMENTS

This study was financially supported by the Biofund, Faculty of Biology, VNU University of Science.

AUTHOR CONTRIBUTIONS

All authors significantly contributed to this work, read and approve the final manuscript for publication.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

The data and materials used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Vis ML, Necchi O. Freshwater Red Algae: Phylogeny, taxonomy and biogeography. Springer. Springer International Publishing; 2021. 350 p;.
2. Wehr JD. Freshwater Algae of North America: Ecology and Classification (Aquatic Ecology). Academic Press; 2nd edition. 2015. 1066 p;.
3. VNU-CRES. Checklist of plant species of Vietnam [Internet]. Agriculture Publishing House; 2001. (Checklist of plant species of Vietnam);.
4. Nguyen TL. New data of freshwater Red Algae (Rhodophyta) at Ma Da area, Dong Nai province. VNU J Sci Nat Sci Technol Sci. 2010;26(1):43-6;.
5. Chapuis IS, Necchi O, Zuccarello GC, Xie SL, Aboal M, Castillo PMS, et al. A new genus, Volatus and four new species of Batrachospermum sensu stricto (Batrachospermales, Rhodophyta). Phycologia. 2017;56(4):454-68;Available from: https://doi.org/10.2216/16-73.1.
6. Entwisle TJ, Vis ML, Chiasson WB, Necchi O, Sherwood AR. Systematics of the Batrachospermales (Rhodophyta)A synthesis. J Phycol. 2009;45(3):704-15;PMID: 27034046. Available from: https://doi.org/10.1111/j.1529-8817.2009.00686.x.
7. Evans JR, Chapuis IS, Vis ML, Evans JR, Chapuis IS, Vis ML. Adding to the freshwater red algal diversity in North America: Lympha mucosa gen. et sp. nov.(Batrachospermales, Rhodophyta). Algae. 2017 Sep 15;32(3):171-9;Available from: https://doi.org/10.4490/algae.2017.32.9.8.
8. Fischer E, Gerlach J, Killmann D, Quandt D. The freshwater red algae (Batrachospermales, Rhodophyta) of Africa and Madagascar I. New species of Kumanoa, Sirodotia and the new genus Ahidranoa (Batrachospermaceae). Plant Fungal Syst. 2020;65(1):147-66;Available from: https://doi.org/10.35535/pfsyst-2020-0010.
9. Rossignolo NL, Yasmin F, West JA, Ganesan EK, Necchi Junior O. Molecular and morphological evidence for a new species in the genus Sirodotia (Batrachospermales, Rhodophyta) from the State of Assam, India. Phytotaxa. 2020;437(3):121-34;Available from: https://doi.org/10.11646/phytotaxa.437.3.1.
10. Suzuki M, Kitayama T. A new species of the genus Sheathia (Batrachospermaceae, Rhodophyta) from Japan. Phycologia [Internet]. 2021;60(4):368-74;Available from: https://doi.org/10.1080/00318884.2021.1946643.
11. Vis ML, Necchi O, Chiasson WB, Entwisle TJ. Molecular phylogeny of the genus Kumanoa (Batrachospermales, Rhodophyta). J Phycol. 2012;48(3):750-8;PMID: 27011092. Available from: https://doi.org/10.1111/j.1529-8817.2012.01141.x.
12. Lam DW, Verbruggen H, Saunders GW, Vis ML. Multigene phylogeny of the red algal subclass Nemaliophycidae. Mol Phylogenet Evol. 2016;94:730-6;PMID: 26518739. Available from: https://doi.org/10.1016/j.ympev.2015.10.015.
13. Guiry MD, Guiry GM. AlgaeBase. Worldwide electronic publication. Galway: National University of Ireland; 2022;.