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# Molecular confirmation and bromoform content of the economically important species *Asparagopsis taxiformis* (Bonnemaisoniales, Rhodophyta) from northern Philippines

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**ABSTRACT**

The Philippine archipelago is among the most biodiverse countries in the tropical Pacific Ocean in terms of its seaweed resources. However, the Philippine seaweed industry has been heavily relying on very few species, while some seaweeds remain underdeveloped. Among these is the bromoform-producing red seaweed *Asparagopsis taxiformis*, which has been receiving growing attention due to its capacity to reduce the amount of methane that is belched by cows when added to their feeds. We contribute herein to the development of *A. taxiformis* as a resource base for the Philippine seaweed industry by generating foundational information on their taxonomy, phylogeny, genetic diversity, and bromoform content. Our molecular-assisted taxonomic studies provide the first molecular-based confirmation of the presence of *A. taxiformis* in the Philippines and our genetic analyses suggest the presence of both a widely distributed and putative new genetic lineages in northern Philippines. Meanwhile, the bromoform contents of *A. taxiformis* from northern Philippines were relatively lower but comparable to other reports elsewhere and the stark differences in bromoform contents of samples collected from different seasons suggest potential seasonality in bromoform production. Taking together, our work points to the potential of expanding the resource base of the Philippine seaweed industry through *A. taxiformis* we underscore here the need to conduct further studies on the biology, ecology, and biochemistry of *A. taxiformis* for us to maximize the potential of this resource in support of the sustainability of the Philippine blue economy.

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## INTRODUCTION

The warm coastal waters of the Philippines are host to a diverse assemblage of seaweeds with over 900 species documented so far (Lastimoso & Santiañez 2021). Of these, about 20 species are widely utilized; and, in particular, the Philippine seaweed

**KEYWORDS**

Pacific Ocean, red algae, seaweed farming

industry is heavily reliant on three carrageenan-producing red seaweed species (i.e., *Eucheuma denticulatum* (N.L. Burman) Collins & Hervey, *Kappaphycus alvarezii* (Doty) Doty, and *Kappaphycus striatus* (F. Schmitz) Doty, leaving hundreds of economically important species remaining underutilized (Trono & Largo 2019). Considering the growing and anticipated challenges associated with the carrageenan-based seaweed industry, the Philippines need to lessen its dependence on carrageen-producing species and diversify its seaweed farming industry by developing other economically important but underutilized seaweed resources (Delmendo et al. 1992).

Among the underdeveloped and underutilized seaweed resources of the Philippines (and in tropical areas in general) is the red seaweed *Asparagopsis taxiformis* (Delile) Trevisan (Bonnemaisoniales, Rhodophyta). In many areas, *A. taxiformis* is consumed as human food or is fed to livestock as fodder. In Hawaii, the species is collected from the wild by hand and commands a high price, making it among the most expensive vegetables worldwide (Trono 2001). *A. taxiformis* produce a wide variety of bioactive chemicals that act as a deterrent to herbivorous fishes or sea urchins. However, these bioactives are also potent against microbial pathogens (Trono 2001) including those that affect culture organisms like shrimps and fishes (Genovese et al. 2012). The toxic chemicals that they produce can also be tapped for their potential for production of medicinal, pharmaceutical, and nutraceutical products, among others. The growing interest in *A. taxiformis* was primarily due to the presence of brominated compounds that were shown to reduce the amount of methane that is belched by cows (Machado et al. 2014, 2015; Kinley et al. 2020). Studies also suggest that a mere 0.20% *Asparagopsis* addition to feeds can contribute to as much as 98% decrease in methane release from cows while promoting weight gain among those fed with it (Kinley et al. 2020). Consequently, this alleviates the contribution of livestock to greenhouse gas emissions. However, the culture technology for the commercial scale biomass production of *A. taxiformis* is yet to be developed. Building a solid infrastructure for the mariculture of seaweeds is reliant on generating foundational knowledge on aspects of their biology and biochemistry. In this work, we contribute to the *A. taxiformis* mariculture infrastructure by providing information on its taxonomy, molecular phylogeny, and preliminary data on their bromoform content based on specimens from the tropical waters of northern Philippines.

## MATERIALS AND METHOD

The macroscopic gametophyic thallus of *A. taxiformis* were collected from the shallow subtidal areas of Bolinao-Anda Reef Complex in Pangasinan, northwestern Luzon, Philippines (Figure 1) from 2021 to 2024. Samples were cleaned off of epiphytes and subsamples were obtained for taxonomic observations, molecular analyses, and analysis of bromoform content. Subsamples for morphological and anatomical studies were preserved in hypersaline solution. For molecular analyses, algal subsamples were preserved in either silica, alcohol, or hypersaline solution. For bromoform analysis, Specimen A was collected from Cangaluyan, Anda, Pangasinan on February 27, 2024, which corresponds to the coldest season in the region, while Specimen B was collected from Balingasay, Bolinao on June 3, 2024, during the warmer season. Dried samples were homogenized using a blender prior to further drying until constant weight was achieved. The samples were stored in scintillation vials in dry conditions prior to analysis. Voucher specimens were deposited at the Gregorio T. Velasquez Phycological Herbarium (MSI) of the Marine Biodiversity Resources and Information System, Marine Science Institute, College of Science, University of the Philippines, Diliman, Quezon City, Philippines.

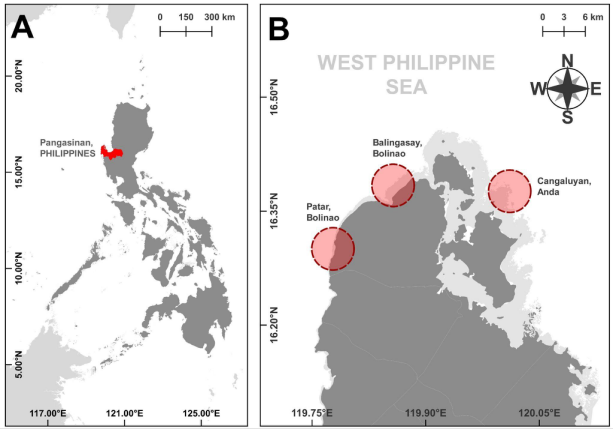


**Figure 1:** Map of the collection sites of *Asparagopsis taxiformis* used in this study. (A) Location of the province of Pangasinan in the Philippines. (B) Collection areas (red circles) of *A. taxiformis* within the Bolinao-Anda Reef Complex in Pangasinan, northwestern Luzon, Philippines

Morpho-anatomical observations of macroscopic and microscopic features of *A. taxiformis* were done under a Ken-A-Vision VisionScope T2600-230 stereomicroscope (Ken-A-Vision, Inc., Kansas City, United States) and Ken-a-Vision Comprehensive Scope 2 Binocular compound microscope (Kansas City, Missouri, USA). Cross-sections were made by hand. Whole mounts and tissue sections were stained with 1% aniline blue with acetic acid, and mounted on glass slides using 50% glycerol. Photomicrographs were obtained using a Nikon D500 (Nikon Inc., Tokyo, Japan) mounted on the compound microscope.

Genomic DNA (gDNA) was extracted using QuickExtract FFPE (Lucigen, USA) using the methods described by Santiañez et al. (2018) or using the E.Z.N.A. Plant DNA kit (Omega Biotek, USA) or DNeasy plant mini kit (Qiagen, Germany) following the manufacturer's protocol with some modifications for the former. In brief, a small fragment of *A. taxiformis* was placed in a sterile 1.5 mL tube and incubated with a lysis buffer for 24 h with rigorous homogenization by either vortex and/or mechanical mortar and pestle. Approximately 10, 30, and 200 µL of eluted DNA were obtained for the FFPE, E.Z.N.A. kit, and DNeasy kit methods, respectively. Eluted DNA samples were analyzed in a 1% TAE-agarose gel at 100 V for 30 minutes to visualize the quality of gDNA extracted. The plastid RuBisCo spacer (rbcS) gene marker was amplified using the primers and PCR conditions described by Dijoux et al. (2014) with few modifications. Mitochondrial *cox*1 gene marker was amplified following the method described by Sherwood et al. (2008), and mitochondrial *cox*2-*cox*3 intergenic spacer marker was amplified according to Zuccarello et al. (1993) and Kurihara et al. (2016). PCR products were analyzed in a 1.0% TAE-agarose gel stained with 0.8 µL GelRed (Intron, South Korea) at 100 V for 45 minutes and UV-visualized for photo-documentation and determination of DNA bands. PCR products were then cleaned using Monarch® PCR and DNA Cleanup Kit (5 µg) following the manufacturer's protocol with few modifications. The final volume of eluted PCR product was set to 30 µL with 3 minutes of 16,000 × *g* centrifugation at room temperature. This was done to ensure that a high concentration of PCR products was obtained. Samples with positive amplification of *rbc*S, *cox*1, and *cox*2-c*ox*3 spacer markers were sent to Macrogen, Inc. (South Korea) for bidirectional capillary sequencing. Raw sequence reads were manually checked, aligned, and trimmed using BioEdit v7.2.5 or MEGA11 to create good-quality contigs. Newly generated sequences along with publicly deposited records of Order Bonnemaisoniales in GenBank were aligned using MUSCLE (Edgar, 2004) in MEGA11 (Tamura et al.

2021), with members of Order Peyssonneliales or *A. armata* serving as outgroups. For each gene marker, phylogenetic relationships between conspecifics from different marine ecoregions were examined by creating a maximum likelihood (ML) tree in IQTREE2 v.2.2.0 (Minh et al. 2020). The appropriate evolutionary model for each marker was determined using the integrated ModelFinder (Kalyaanamoorthy et al. 2017). Uncorrected sequence divergence (p-distance) was also calculated to evaluate the genetic distance between lineages. Bayesian inference (BI) analysis was also conducted in MrBayes v.3.2.7 (Ronquist et al. 2012) to calculate the Bayesian posterior probabilities (BPP). Three (3) independent runs of Markov chain Monte Carlo (MCMC) were run for 2.5 million generations, with a burn-in value of 10%, and sampled every 1000 generations until a split frequency of 0.01 is reached. In addition, a haplotype network was also constructed in PopART v. 1.7 (http://popart.otago.ac.nz) (Leigh & Bryant 2015) using the default settings of the TCS network parameter. Lastly, consensus trees for each of the three makers will be visualized in FigTree v.1.4.4 and annotated in Adobe Illustrator 2024 (Adobe Inc., USA).

Bromoform extraction was performed on duplicate aliquots (100 mg, weighed to the nearest 0.1 mg) of dried and ground Asparagopsis taxiformis samples using 1.5 mL of analytical-grade n-hexane (95%, RCI Labscan). Chromatographic analysis was conducted using a Shimadzu GC-2030 gas chromatograph (Japan) equipped with an electron capture detector (ECD) and a SH-I-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas. A 5 µL injection volume was employed in 1:10 split mode, with injector and detector temperatures set at 180 °C and 200 °C, respectively. The oven temperature was programmed to begin at 80 °C (held for 2 minutes), ramped to 180 °C at 80 °C/min, and held at 180 °C for 2 minutes. Bromoform was identified based on retention time matching against a standard solution of bromoform (RCI Labscan) in hexane, and quantified using a calibration curve generated from standard solutions ranging from 0.1 to 20 µg/L. Peak areas were corrected against a solvent blank. The method detection limit (MDL) was estimated using seven replicate standard solutions at 4 µg/L. The analytical method was adapted from Romanazzi et al. (2021), with adjustments to extraction volume, solvent, and chromatographic conditions.

## RESULTS AND DISCUSSION

### Morphological observations

The gametophytic *A. taxiformis* is characterized by having rosy pink to dark red thallus, composed of upright, soft, and plumose fronds, 2–5 cm in height, arising from creeping stolons that are attached to the rocky substrate via rhizoidal holdfasts (Figure 2A). Branches are feather-like, alternating and distichously arranged along the percurrent main axis. Main axis is composed of pigmented cortical cells broadly obovate, 18–30 × 11–22 µm in size, bounding clear and larger medullary cells; the latter progressively becoming larger towards the hollow axial center, wherein an axial filament traverses throughout the branch (Figure 2B–D). Elongated cell rows forming the axial filament enlarged at each end, 601–774 µm in size (Figure 2B). Cystocarps are globose, stalked, 447–494 µm in diameter and found near the base of lateral branches (Figure 2E). Spermatangia are clavate, 387–470 µm long, 134–173 µm broad near the tips, tapering to 57–98 µm near the bottom; these are commonly found in clusters near the branch apices (Figure 2F). Pangasinan specimens are monoecious. Tetrasporic plants are red in color, small, filamentous, growing as amorphous tufts near gametophytic plants. Both gametophytic and tetrasporophytic plants grow on rocks in clumps, exposed to relatively strong water movement.

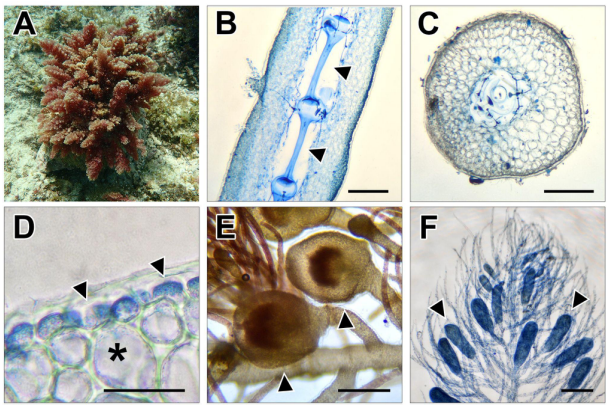


**Figure 2:** Habit and morpho-anatomy of *Asparagopsis taxiformis* (Delile) Trevisan from Bolinao, Pangasinan. (A) In situ photo of gametangial thallus. (B) Longitudinal section of the main axis showing the axial filament (arrowheads) that runs through the whole axis. (C) Transverse section of the main axis highlighting the presence of the axial filament in the center. (D) Transverse section highlighting the small, broadly obovate and pigmented epidermal (arrowheads) and the larger ovate to obovate clear medullary cells (asterisk). (E) Stalked and globose cystocarps (arrowhead) found in female gametophytic plants. (F) Spermatangial branches (arrowheads) that are densely aggregated near the apex of a male plant. Scale bar: B,C,E,F = 350 µm; D = 70 µm.

The *A. taxiformis* specimens we examined in this study shared the typical characteristics of the species except that these are relatively shorter from those reported in the Philippines (7–13 cm; Trono (1997), Calumpong & Meñez (1997)), California, USA (45 cm; Norris (2014)), South Africa (8 cm; Anderson et al. 2016) but have similar sizes with those found in Thailand and Sri Lanka (2–4 cm; Coppejans et al. 2010), although those from southern Thailand may reach up to 11 cm (Coppejans et al. 2009; Coppejans et al. 2017). Similarly, the shape and nature of the cystocarps and spermatangia of *A. taxiformis* from Pangasinan were comparable with those from other localities. The cystocarps of *A. taxiformis* from Pangasinan are about the same size as those from California, but their spermatangia are considerably shorter (to 470 µm long in the former, 550 µm long in the latter) (Norris, 2014).

### Molecular phylogeny

A total of 27 new sequences (*cox*1: 18; *cox*2-*cox*3: 2; *rbc*S: 7) were generated in this study, constituting the first molecular dataset for *A. taxiformis* populations from the Philippines. Our molecular-assisted taxonomic work on *A. taxiformis* also confirms the previous bibliographic reports of this species in the Philippine archipelago (Dijoux et al. 2014; Lastimoso & Santiañez 2021).

Our molecular phylogenetic studies based on three gene regions suggested that our *A. taxiformis* specimens formed a highly supported clade with its conspecifics from other marine ecoregions. Our haplotype network analyses based on *cox*1 and cox2-*cox*3 sequences also showed the presence of cryptic lineages within *A. taxiformis* as earlier reported by Andreakis et al. (2007) (Figure 3). Populations from the Philippines grouped together with specimens from the Eastern Indo-Pacific (EIP), Central Indo-Pacific (CIP), Temperate Northern Atlantic (TNA), Temperate Northern Pacific (TNP), and Temperate Southern Africa (TSA) under *Lineage 2*. This lineage is currently the most widespread lineage of *A. taxiformis* and is considered to be invasive in most temperate regions (Sherwood et al. 2008; Dijoux et al. 2014; Andreakis et al. 2004, 2016; Kurihara et al. 2016). *Lineage 2* is believed to have originated from the Indo-Pacific Ocean region and was later on introduced

to the TNA, TNP, and TSA through maritime trade (Andreakis et al. 2007; 2009; Zanolla et al. 2014). Intraspecific divergences within this lineage were 0–0.16 for *cox*1 and 0–0.069 for *cox*2-*cox*3. Interestingly, specimens from Balingasay, Bolinao belonged to a distinct and putative new haplotype as observed in the *cox*2-*cox*3 network (Figure 3B). The Balingasay haplotype is only one mutational step away from the major haplotype within *Lineage 2*. This presents a potentially untapped genetic resource for *A. taxiformis* which may possess desirable traits for mariculture or bioprospecting. Unfortunately, the five cryptic lineages were not observed in the tree inferred from *rbc*S dataset (Figure S1). Based on our analyses of the *rbc*S gene marker, *A. taxiformis* specimens from the Philippines formed two separate clades, one of which is a large group composed of conspecifics from Japan, Taiwan, Australia, South Africa, New Zealand, the Azores, and other smaller islands in the Western Indo-Pacific region; meanwhile, the second group formed a haplotype with other specimens from Hawaii and New Caledonia. This failure in recognizing the cryptic lineages among *A. taxiformis* populations may be due to the conserved nature of most plastidial genes (Freshwater et al. 1994; Yoon et al. 2004). The slow evolutionary rate in *rbc*S would often result in low variability (Andreakis et al. 2007), making it difficult to infer recently diverged lineages; thus, this marker is more suitable for exploring deep phylogenies among red algae.

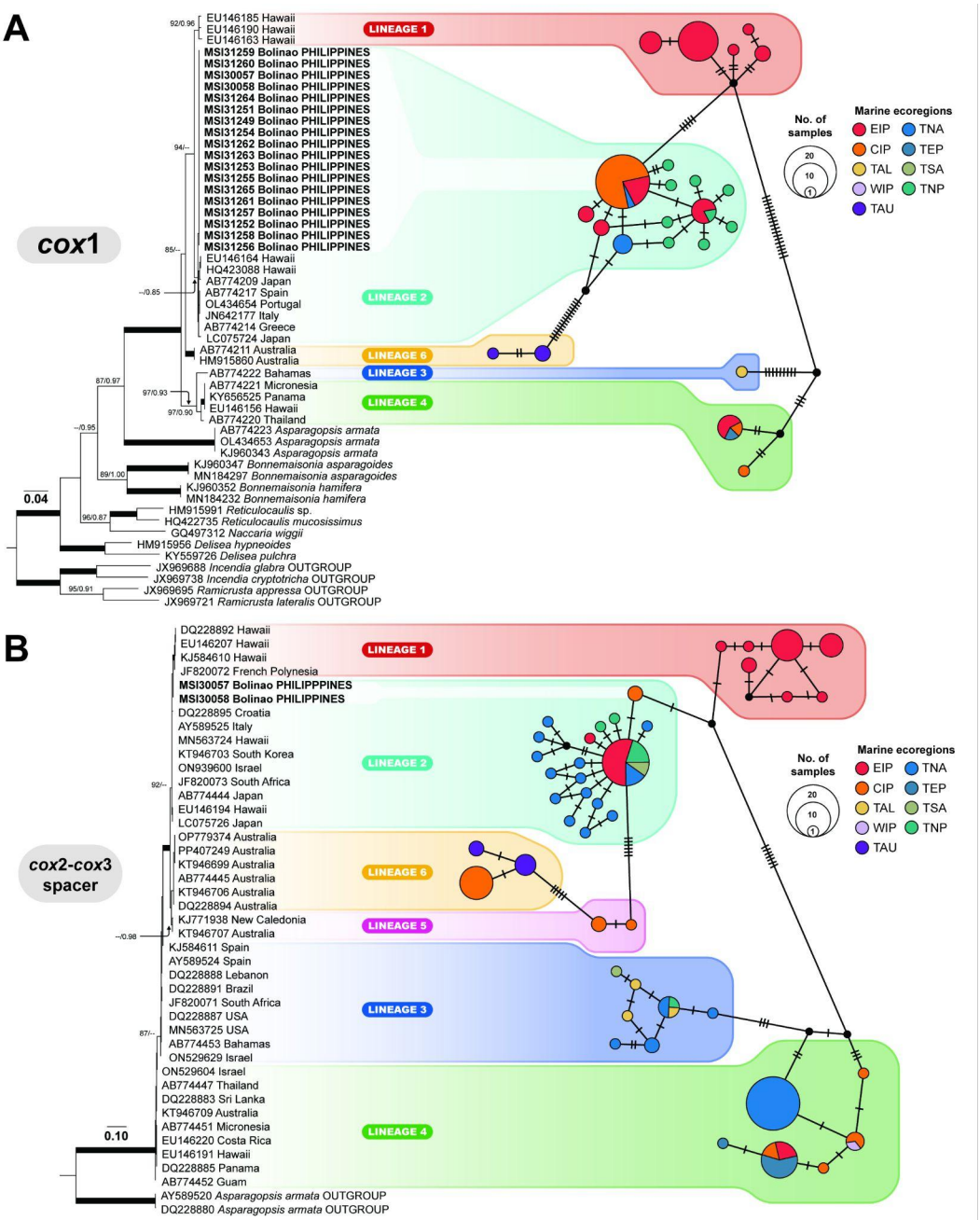


**Figure 3:** Maximum likelihood (ML) tree of order Bonnemaisoniales based on the mitochondrial *cox*1 gene (**A**) and the non-coding *cox*2-*cox*3 intergenic spacer (**B**) alongside their corresponding haplotype network. Newly generated sequences for the *Asparagopsis taxiformis* specimens from Pangasinan, Philippines are in **boldface**. Support values in the form of ultrafast bootstrap percentages (UFBS) and Bayesian posterior probabilities (BPP) are shown at each node. Thickened lines indicate highly supported nodes (UFBS: ≥95 / BPP: ≥0.95). Values <85 UFBS and <0.85 BPP were removed. Members of the order Peyssonneliales serve as outgroups for the *cox*1 tree while sequences of *A. armata* were used for the tree inferred from the *cox*2-*cox*3 intergenic spacer. Cryptic lineages of *A. taxiformis* are labelled and highlighted. Specimens in each haplotype are grouped per marine ecoregion (Spalding *et al.* 2007) wherein: **EIP** (Eastern Indo-Pacific); **CIP** (Central Indo-Pacific); **TAL** (Tropical Atlantic); **WIP** (Western Indo-Pacific); **TAU** (Temperate Australia); **TNA** (Temperate Northern Atlantic); **TEP** (Temperate Eastern Pacific); **TSA** (Temperate Southern Africa); and **TNP** (Temperate Northern Pacific).

**Preliminary assessment of bromoform from *A. taxiformis***

Chromatographic analysis confirmed the presence of bromoform in our *A. taxiformis* samples, with a characteristic peak at approximately 2.85 minutes matching the bromoform standard (Figure 4). Quantitative analysis revealed a marked difference between Specimen A (94.80 µg/g DW) and Specimen B (215.10 µg/g DW), suggesting variability in compound accumulation even within the same species. Nonetheless, *A. taxiformis* from Pangasinan exhibited relatively low bromoform concentrations compared to other populations of *A. taxiformis* from other countries, which possess bromoform contents of up to 297.0 µg/g (DW) (Cheong et al. 2024; Machado et al. 2016). These lower concentrations may reflect biological and environmental influences such as light, temperature, or nutrient availability—factors known to affect halogenated compound biosynthesis in red algae (Juneja et al. 2013; Vega et al. 2024).

It is also important to consider methodological influences on the measured bromoform levels. Although extraction protocols were standardized to ensure sample comparability, our use of a relatively small sample size (0.1 g dry weight) and limited solvent volume (1.5 mL hexane) may have contributed to reduced extraction efficiency. A low solvent-to-sample ratio can hinder full compound recovery, particularly in matrices where bromoform may be bound or unevenly distributed (Jacobsen et al. 2019; Machado et al. 2016). Additionally, the high volatility of bromoform increases the risk of analyte loss during sample handling, especially if precautions against evaporation were not strictly followed (Romanazzi et al. 2021). While care was taken to minimize heat exposure and avoid prolonged delays between collection and drying—by air-drying samples in shaded, ventilated conditions—we recognize that passive drying may still have resulted in partial bromoform loss. Storage conditions further complicate bromoform quantification, as bromoform is known to degrade or volatilize over time. Variations in storage duration and handling may thus have contributed to the relatively low concentrations observed in this study. The choice of extraction solvent is also a critical factor. While hexane was employed in this study for its selectivity and compatibility with the analytical method, most previous studies have used methanol, which may facilitate greater solubilization of bromoform and improved recovery (Cheong et al. 2024). However, these studies were predominantly conducted using gas chromatography with mass spectrometric detection (GC-MS), which differs in sensitivity and matrix tolerance compared to the method applied here. Furthermore, as shown in Figure 5, issues related to co-elution and matrix interferences may have further compromised quantification accuracy, particularly at lower concentration thresholds (Quitério et al. 2022).

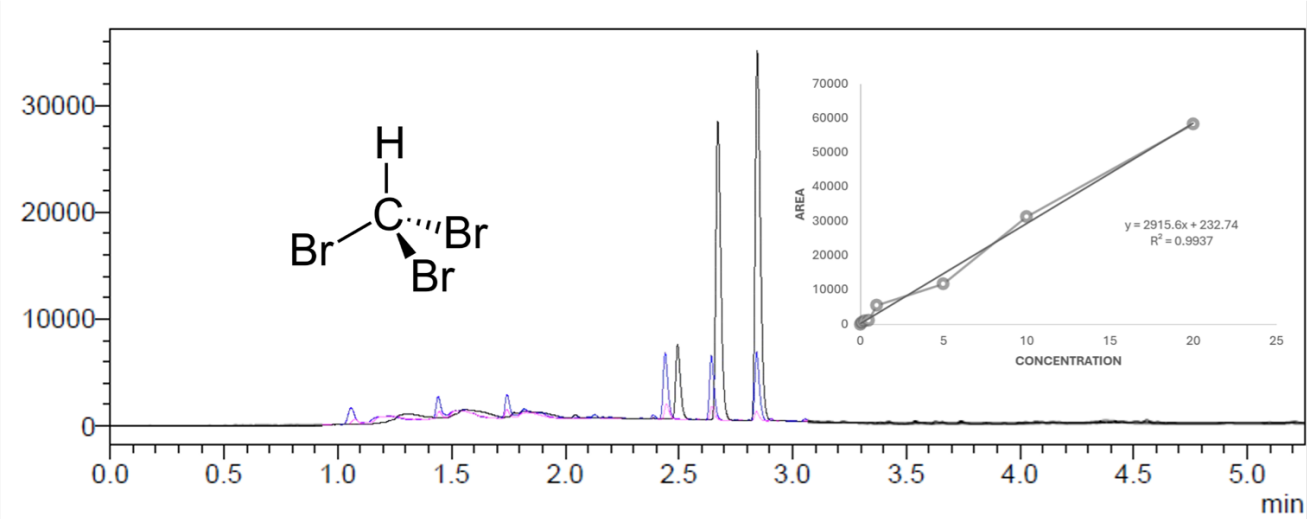


**Figure 4:** Overlay of chromatograms for standard bromoform (black), Sample A (blue), and Sample B (pink).

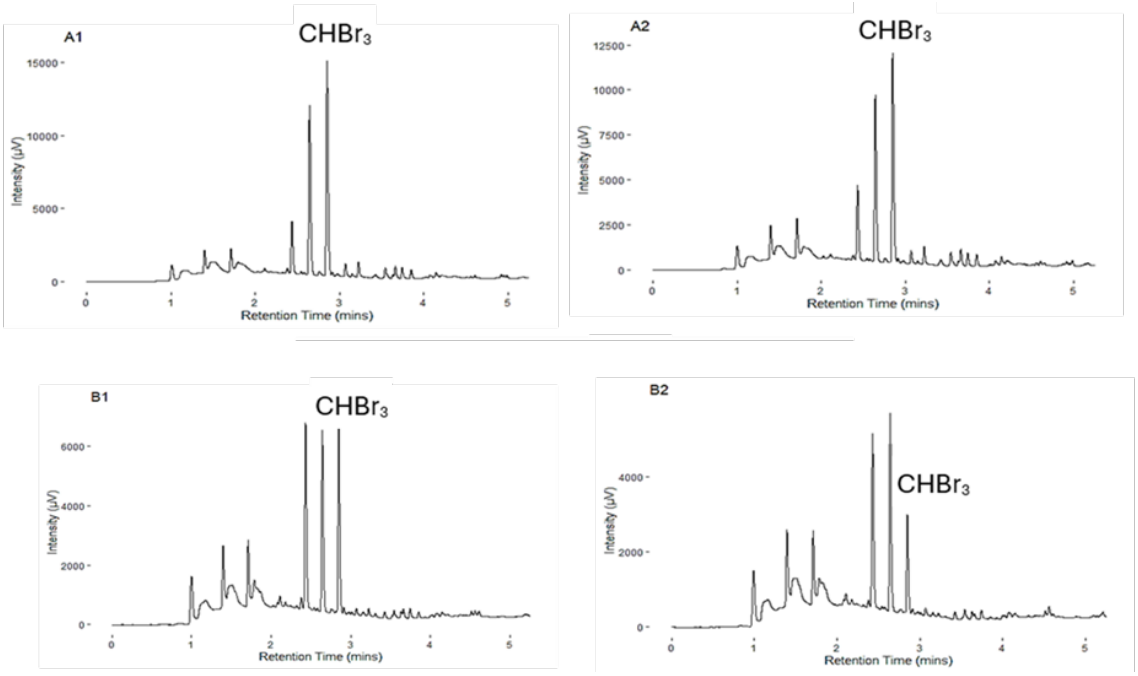


**Figure 5:** Chromatograms of Specimen A and B, illustrating the elution of bromoform with a retention time of 2.85 minutes.

Additionally, the observed difference between Specimens A and B may reflect the biology of the species, which is related to seasonal differences. Specimen A was collected in February, when Bolinao waters are relatively colder, while Specimen B was collected in June, when waters are relatively warmer. Shifts in environmental stressors, such as changes in irradiance or water chemistry between these periods, may have affected metabolic activity and bromoform production (Hargrave et al. 2024). Typically, A. *taxiformis* in Bolinao are mature and reproductive during the colder months; meanwhile, they are young and immature during warmer months (personal observation). Seaweed halocarbons including bromoforms serve as antibacterial and anti-herbivory compounds (De Bhowmick & Hayes 2023) and it is likely highly expressed in mature and reproductive individuals to ensure and increase reproductive success. This highlights a potential temporal or phenological influence on secondary metabolite expression in *A. taxiformis*. Given the limited number of samples analyzed in this study (n = 2), it was not possible to fully disentangle the effects of these methodological, environmental, and biological variables. However, these findings underscore two key directions for future research: (1) optimizing extraction and detection methods to ensure maximum recovery and analytical sensitivity, and (2) integrating molecular and chemical data to explore whether genetic variation—however slight—may be linked to bromoform production potential. As global interest in *A. taxiformis* continues due to its application in reducing livestock methane emissions, understanding both its phylogeographic structure and chemical profiles across different populations is essential. Our results contribute to this growing body of knowledge and support the need for comprehensive studies that combine genetic identity, environmental factors, and metabolite expression in this ecologically and economically valuable red alga.

## CONCLUSION

Our work lays the foundation on the sustainable development of the ecologically and economically important red seaweed *A. taxiformis* in the Philippines by providing the first studies on its molecular-assisted taxonomy, population genetics, and biochemistry (i.e., bromoform content). We confirmed here the occurrence of *A. taxiformis* in the Philippines based on multiple gene markers and report for the first time a distinct haplotype from Balingasay in Pangasinan. Our work also confirmed the presence of bromoform in *A. taxiformis* from Pangasinan, Philippines, though concentrations were notably lower than those reported elsewhere. The marked variability between specimens, alongside possible methodological limitations including solvent choice, extraction efficiency, and sample handling, highlights the complexity of accurately quantifying this volatile compound. Furthermore, biological and environmental factors, particularly seasonal variation and developmental stage, appear to influence bromoform accumulation. While the small sample size limits broader generalization, our findings emphasize the need for improved analytical methods and integrated approaches that consider genetic, environmental, and phenological influences. Nonetheless, we anticipate that our current work will facilitate and support the development of *A. taxiformis* as a new commodity for the Philippine seaweed industry and, as an economic resource, *A. taxiformis* will be integral in advancing sustainable Philippine blue economy.

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characterization of macroalgae for food and high-value products (PhycoPRO)" project.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## CONTRIBUTIONS OF INDIVIDUAL AUTHORS

WJES: Conceptualization, data analysis, manuscript writing and editing, funding acquisition
JMLL: Data collection, data analysis, manuscript writing and editing
LBL: Data collection, data analysis, manuscript writing and editing
IPBT: Data collection and analysis
KAC: Data collection and analysis
MAMF: Data collection and analysis
IBR: Conceptualization, data analysis, manuscript writing and editing, funding acquisition

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**SUPPLEMENTARY INFORMATION**

**Table S1:** Collection details, herbarium accession numbers, and GenBank codes of specimens used in this study.

| Species and collection details | Herbarium Code | GenBank Code | | |
|---|---|---|---|---|
| | | *rbc*S | *cox*1 | *cox*2-*cox3* |
| *Asparagopsis taxiformis* (Delile) Trevisan | | | | |
| Balingasay, Bolinao Pangasinan; 23.iii.2023 | MSI30057 | | * | * |
| Balingasay, Bolinao Pangasinan; 23.iii.2023 | MSI30058 | | * | * |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31249 | | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31250 | * | | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31251 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31252 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31253 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31254 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31255 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31256 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31257 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31258 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31259 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31260 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31261 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31262 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31263 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31264 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31265 | * | * | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31266 | * | | |
| Patar, Bolinao, Pangasinan; 21.i.2021 | MSI31267 | * | | |
| Cangaluyan, Anda, Pangasinan; 25.ii.2021 | MSI31268 | * | | |
| Cangaluyan, Anda, Pangasinan; 25.ii.2021 | MSI31269 | * | | |
| Cangaluyan, Anda, Pangasinan; 25.ii.2021 | MSI31270 | * | | |
| Cangaluyan, Anda, Pangasinan; 25.ii.2021 | MSI31271 | * | | |
| Cangaluyan, Anda, Pangasinan; 25.ii.2021 | MSI31272 | * | | |

**Table S2:** Bromoform concentrations in specimens A and B, expressed in micrograms per gram (µg/g) dry weight.

| Specimen | | Bromoform Concentration (µg/g dry seaweed) |
|---|---|---|
| A | 1 | 88.4230 |
| | 2 | 101.1751 |
| B | 1 | 313.5766 |
| | 2 | 116.6187 |

**Table S3:** Comparative bromoform concentrations in Asparagopsis taxiformis from different locations.

| Location | Bromoform Concentration (µg/g DW) | Notes / Conditions | Reference |
|---|---|---|---|
| **Pangasinan, Philippines** | 94.80 – 215.10 | Wild samples; reef area | This study |
| **California, USA** | 20,000-45,000 | Wild samples; with light stress | Hargrave et al. 2024 |
| **Queensland, Australia** | 297.0 | Cultivated and wild | Machado et al. 2016 |
| **New Zealand** | 2,040 | Wild samples; rocky reefs | Romanazzi et al., 2021 |
| **Queensland, Australia** | 4,400-18,000 | Cultivated and wild | Zhao et al. 2025 |

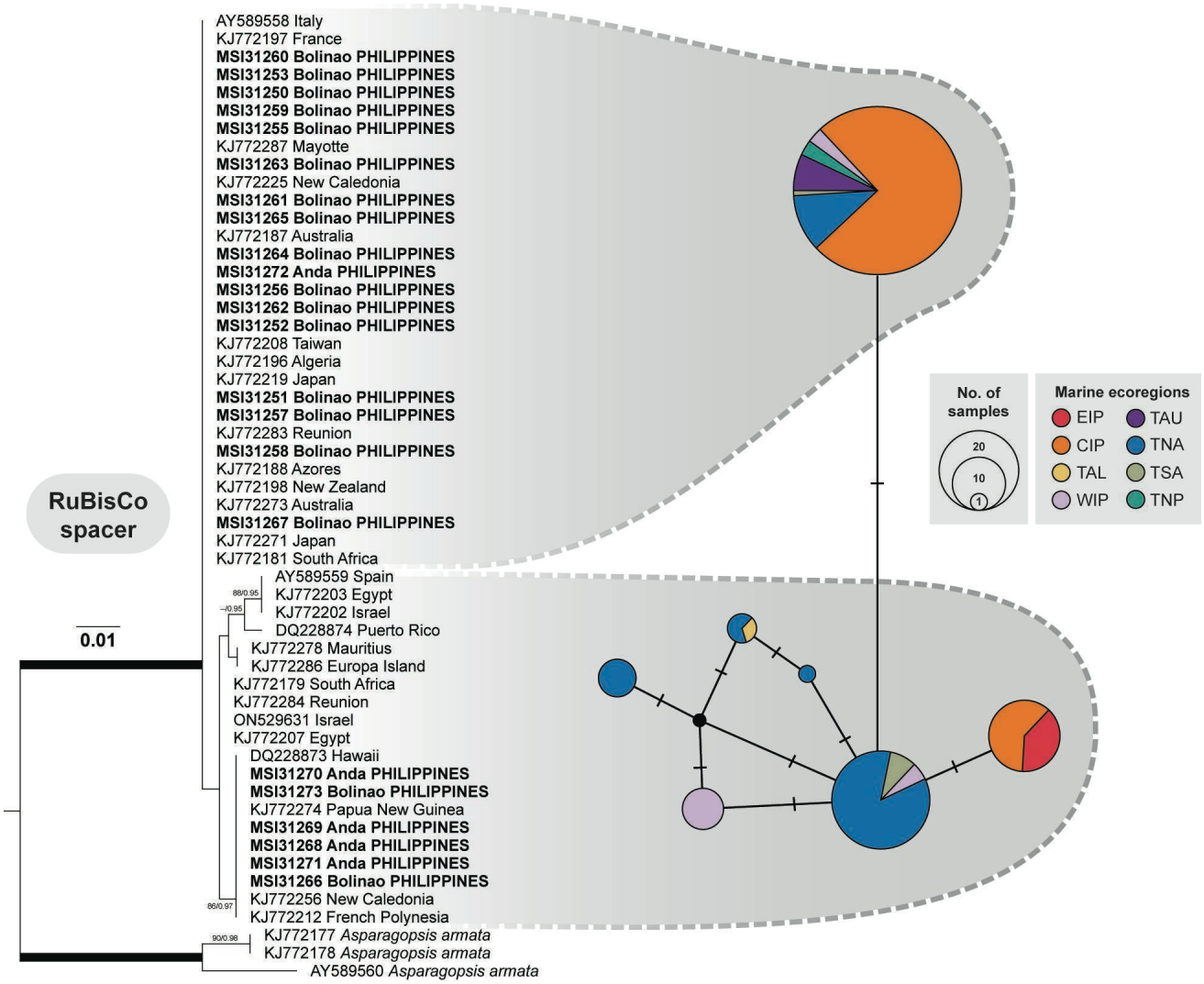


Figure S1: Maximum likelihood (ML) tree of *Asparagopsis* species based on the plastidial RuBisCo spacer (*rbc*S) alongside its corresponding haplotype network. Newly generated sequences for the *Asparagopsis taxiformis* specimens from Pangasinan, Philippines are in **boldface**. Support values in the form of ultrafast bootstrap percentages (UFBS) and Bayesian posterior probabilities (BPP) are shown at each node. Thickened lines indicate highly supported nodes (UFBS: ≥95 / BPP: ≥0.95). Values <85 UFBS and <0.85 BPP were removed. Sequences of *A. armata* were used as the outgroup. Only two superficial groupings are highlighted. Specimens in each haplotype are grouped per marine ecoregion (Spalding *et al*. 2007) wherein: **EIP** (Eastern Indo-Pacific); **CIP** (Central Indo-Pacific); **TAL** (Tropical Atlantic); **WIP** (Western Indo-Pacific); **TAU** (Temperate Australia); **TNA** (Temperate Northern Atlantic); **TEP** (Temperate Eastern Pacific); **TSA** (Temperate Southern Africa); and **TNP** (Temperate Northern Pacific).