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Kappaphycus alvarezii (Rhodophyta): New Record of an Exotic Species for the Caribbean Coast of Costa Rica

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Abstract

Kappaphycus alvarezii, a red macroalgal species native to South-East Asia, has been introduced for commercial purposes to more than 20 tropical countries. In 2000, Panama became the first Central American country to develop its cultivation following a pilot-commercial approach. Twelve years after his introduction to Panama, the species was found to the south Caribbean coast. In the present study, we report the first record of *K. alvarezii* collected in Costa Rican waters. Genetic identification of the sample was performed with the mitochondrial marker cox2-cox3 intergenic spacer, and phylogenetic analyses showed that the sample collected grouped into a monophyletic clade with GenBank sequences from Vietnam, Indonesia, the Philippines, Brazil and Venezuela. Here we provide further description of its position with respect to other strains of *K. alvarezii* collected globally. Its possible route of entry to the Caribbean coast of Costa Rica is discussed. To date, it is possible to locate *K. alvarezii* in different areas away from the production fields which show its dispersion, and that the species is expanding; but more studies are needed to know the phase of colonization in which it is located because it is a combination of natural factors and human exploitation that determines the invasive potential of the species.

Keywords

Carrageenophytes, Cultivation, Costa Rica, Dispersion, Exotic, Introduction

1. Introduction

Kappaphycus alvarezii (Doty) Doty ex P.C. Silva is a marine red macroalga with a native range confined to shallow-reef areas of the Sulu archipelago, Philippines [1]. However, because of its fast growth rate and carrageenan content (*i.e.* a viscous polysaccharide used in the formation of gels), the species has been promoted as a maricultural crop across tropical countries worldwide [2] As such, during the last four decades its range of distribution has been substantially expanded, and to date *K. alvarezii* is one of the exotic macroalgae with the broadest distribution in the circumtropical belt [3]. The species has been deliberately introduced into more than 20 countries including areas in Africa, Southeast Asia, Central America, and South America [4]. Currently, there are few to non-risk assessment or environmental impact procedures for the intentional introduction of *Kappaphycus* and scientists have rarely examined the areas adjacent to farms to determine whether populations have been established in the benthos [5].

On a global scale, *Kappaphycus* represents one of the main commercial sources of carrageenan, which rheological properties have various uses in the food, feed, and pharmaceutical industries [6]. While their rapid growth rate and modes of dispersion justify some of the success in large-scale production, this attributes in addition to the cultivation methods used have also facilitated the introduction, establishment, and spreading of *K. alvarezii* into new habitats [7]. Similar to other macroalgal species, a combination of factors including water movement, temperature, nutrient and light levels, and abundance of herbivores may cause differential growth across sites [5]. Under favorable environmental conditions, the species can double its biomass in periods of less than 30 days [8] [9] and can easily spread in the water column by spores or vegetative fragmentation [10]. Studies assessing the adaptive responses of *K. alvarezii* have shown that in regions where the species have been introduced, responses change as a function of the environmental conditions [2] [7] [11] [12] including the type and availability of substrate and current.

For example, on the coast of Brazil, environmental conditions seem to limit the propagation of *K. alvarezii*, as no propagules nor viable spores have been reported [12]. On the contrary, in Bocas del Toro, Panama, propagules have been reported to form benthonic mats of more than 72 m^2 [13]. Although the authors reported several mats, *K. alvarezii* is not yet considered an invasive species for Panama. Although *K. alvarezii* propagules can successfully establish as wild individuals, the species may not necessarily become invasive (see [14]) and, therefore, may not have negative effects on the local biodiversity. In fact, some studies highlight the positive influence that invasive macroalgae can have on colonized sites, as they can ameliorate the environment and provide shelter, substrate, and feed for associated species [15] [16] [17]. However, in the case of *Kappaphycus* spp., the majority of studies emphasize that its propagation and establishment outside its natural distribution negatively impact the community dynamics of colonized marine environments, contributing substantially to their detriment [5].

In Hawaii, for example, *Kappaphycus* spp., became a successful competitor and affecting the indigenous ecosystem by altering the availability of nutrients, light, and substrate [18] [19]. Moreover, campaigns by India to eradicate *K. alvarezii* growing over coral reefs have been unsuccessful. Contrary to the hoped, these campaigns have promoted the territorial expansion of the species that continues to support the decline of native benthonic diversity [20]. Despite the above, some findings cannot be overlooked [2], demonstrated that *Kappaphycus* subject to experimental cultivation in Cuban coastal waters experience significant grazing, limiting the productivity of the farm site and possibly preventing the expansion of the species outside the growing areas. These contrasting results suggest that more research is needed to understand the dynamic response of *Kappaphycus* in the different regions where it has been established in adjacent areas outside farm sites.

In 2000, Panama became the first Central American country to established a pilot-commercial farm site of *K. alvarezii.* The first propagules were introduced in the province of Colón [21] and later in the province of Bocas del Toro. While there is no clear origin of the propagules introduced in Panama, the first propagules introduced in Latin America, with the exception of Cuba, are allegedly of Venezuelan origin. Thirteen years after the first introduction reported for Panama, *K. alvarezii* was reported as established in areas adjacent to abandoned farm sites. Individuals were found in coral reefs and in seagrass and mangroves areas [13]. In 2011, a fragment of K. *alvarezii* was found on the southern Caribbean coast of Costa Rica. The fragment was recognized during a routine macroalgal harvesting expedition in the context of identifying native species for human consumption [22]. Further molecular analyses were conducted to confirm the species identity. It is the first record of an exotic algae for the Caribbean of Costa Rica.

Significant studies on *Kappaphycus* have been made on the basis of molecular analyses [23]-[38]. Several studies indicated that the mitochondrial cox2-cox3 intergenic spacer marker provides sufficient phylogenetic signal to determine the interspecific and intraspecific relationships of *Kappahycus* [12] [24] [25] [26] [27] [30] [33] [34] [35] [38] [39] [40]. This is the first report to the presence of *Kappaphycus* in Costa Rica, which details through molecular phylogenetic analysis the positioning of this fragment with respect to other strains of the species.

2. Material and Methods

2.1. Collection Site

Cahuita National Park (CNP) is located in the southern Caribbean coast of Costa Rica within the tropical rainforest ecosystem, Limón province. It is categorized as a national protected area with an extension of approximately 224 km^2.

The National Park is home to the largest and most studied strip of coral reefs and seagrass beds in the country [41] that extend within a reef lagoon [42]. The National Park is characterized by a rich biodiversity and therefore, there is a constant effort to understand, monitor, and protect its dynamic stability. The specimen was collected on 3.ix.2011 by R. Cabrera and S. Umanzor at Punta Cahuita (9'44'41 53"N, 82'48'32 63"W) on a shore along the beach line (0 m in depth) entangled with a large specimen of *Sargassum polyceratium* var. *ovatum* (Collins) W. R. Taylor (**Figure 1**). After photographing it at the site, the fragment was collected and transported in a cooler for later processing at the Rangers Station within the park. Upon arrival, the fragment was rinsed with seawater to remove any sand, then dried with absorbent tissue, and fragmented for various analyses. One section of the thallus was kept in refrigeration for later use in histological analysis, another section was preserved in silica gel for DNA extraction and phylogenetic analysis, and a third section was prepared for submission to the University of Michigan Herbarium. The specimen of *Kappaphycus* collected weighed 29.3 g (wet weight).

2.2. Morphological Observations

For morphological characterization, transverse and longitudinal cross sections were made manually using a double-edged stainless-steel razor blade. The cross sections were stained with 1% blue aniline, acidified with 1N HCl and mounted in a solution of 80% Karo syrup/distilled water. Macroalgal sketches were created using a camera lucida attached to an Olympus microscope. Sketches were edited using Photoshop CS3 (Adobe Systems). Voucher specimen is deposited in the Herbarium of the University of Michigan, (MICH 701908), Ann Arbor, MI, United States.

2.3. Molecular Analysis

The DNA was extracted from 40 mg of dry tissue that was processed in a Precellys homogenizer using a DNeasy Plant Mini Kit (Qiagen, Valencia, USA). The mitochondrial intergenic marker cox2-3 was amplified with the conditions suggested by [43]. PCR amplifications were performed in a reaction with a volume of 25 μL consisting of 15 μL of ultra-pure water, 5 μL of buffer, 2.5 μL of $MgCl_2$, 2.5 μL of dNTPs, 0.5 μL of each primer, 0.15 μL of Taq polymerase and 0.5 μL of the DNA template. The PCR cycle was programmed according to [43]. All PCR products were analyzed by electrophoresis in 1% agarose to check for product size. PCR products were cleaned using MicroSpin TM S-300 HR columns (GE Healthcare Life Sciences, Piscataway, USA). Sequencing was conducted using a ABI PRISM 3100 (Applied Biosystems) genetic analyzer with a BigDye Terminator Cycle Sequencing Reaction Kit (Applied Biosystems, NJ, USA). Complete sequences were obtained from both DNA strands. Analysis of the sequences was performed using the computer program BioEdit 7.1.3.0 [44]. Finally, the obtained sequence was registered in the NCBI GenBank base, with the MG18880 number.

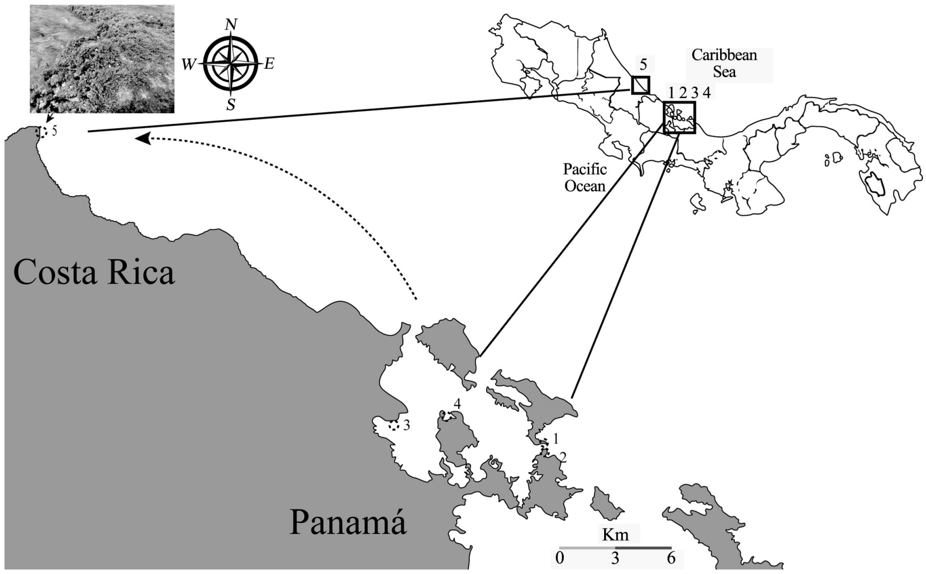

Figure 1. Sites where loose *K. alvarezii* was reported in Bocas del Toro, Panamá (1. Bastimentos; 2. Popas; 3. Almirante; 4. Cristóbal) sites reported by Sellers *et al.* (2015) and for Punta Cahuita in Costa Rica (5) for present study [-----, Hypothetical route from the entrance of *Kappaphycus* to Costa Rica].

2.4. Phylogenetic Analysis

Phylogenetic relationships were inferred with MrBayes v.3.0 beta 4 [45] and MEGA version 5 [46]. The ModelTest version 3.7 program was used to find the model of sequence evolution least rejected for each data set by a hierarchical likelihood ratio test. A Bayesian tree was generated with MrBayes using the GTR + I + G model of nucleotide substitution. For Bayesian analysis, we ran five chains of the Markov chain Monte Carlo (one hot and four cold), sampling one tree every 1000 generations for 10×10^6 generations starting with a random tree. Outgroup species (*Eucheuma denticulatum*) were selected on the basis of close phylogenetic relationship with the ingroup (**Table 1**). The range of divergence values sequences within and among species was calculated using uncorrected "p" distances using MEGA. A total of 22 sequences, including the outgroups, were analyzed (**Table 1**).

3. Results

The specimen collected is reported as:

Phylum Rhodophyta

Family Solieriaceae

Kappaphycus alvarezii (Doty) Doty ex PC Silva., in P. C. Silva, Basson & Moe 1996: 333 ≡ *Eucheuma alvarezii* Doty., in I. A. Abbott & J. N. Norris, 1985: 37. **Figures** correspond to the original description. **Figure 2** is the species in Costa Rica.

3.1. Type Locality

Creagh Reef, south of Semporna, Sabah, Malaysia [47].

Table 1. List of species used for phylogenetic analyses, with their collection data and GenBank accession numbers.

Species name	Country	Genbank Accession Numbers	Reference
Eucheuma denticulatum (N.L. Burman) Collins & Hervey[a,c]	Malaysia	JN980403	Unpublished
E. denticulatum[a,c]	Hawaii	FJ554859	Conklin *et al.* (2009)
E. denticulatum[a,c]	Indonesia	KC905455	Lim *et al.* (2014)
Kappaphycus alvarezii (Doty) Doty ex P.C. Silva[b]	Costa Rica	MG188801	This study
K. alvarezii[c]	Indonesia	JX624072	Tan *et al.* (2012)
K. alvarezii[c]	Indonesia	KC905349	Lim *et al.* (2014)
K. alvarezii[c]	Philippines	JX624073	Tan *et al.* (2012)
K. alvarezii[c]	Philippines	KC905369	Lim *et al.* (2014)
K. alvarezii[c]	Brazil	KC247828	Barros-Barreto *et al.* (2013)
K. alvarezii[c]	Brazil	KC122263	Araujo *et al.* (2013)
K. alvarezii[c]	Venezuela	AY687427	Zuccarello *et al.* (2006)
K. alvarezii[c]	Vietnam	KC905379	Lim *et al.* (2014)
K. alvarezii[c]	Malaysia	KM051559	Unpublished
K. alvarezii[c]	Hawaii	AY687433	Zuccarello *et al.* (2006)
K. alvarezii[c]	Hawaii	AY687432	Zuccarello *et al.* (2006)
K. alvarezii[c]	Hawaii	FJ554861	Conklin *et al.* (2009)
K. alvarezii[c]	Tanzania	AY687436	Zuccarello *et al.* (2006)
K. alvarezii[c]	Tanzania	KT390538	Tano *et al.* (2015)
K. alvarezii[c]	Madagascar	AY687430	Zuccarello *et al.* (2006)
K. alvarezii[c]	Tanzania	JQ713901	Halling *et al.* (2013)
K. cottonii (Weber Bosse) Doty ex P.C. Silva[c]	Philippines	AY687426	Zuccarello *et al.* (2006)
K. striatum Doty ex P.C. Silva 1996[c]	Philliphines	AY687434	Zuccarello *et al.* (2006)

a Outgroups. b Sequences obtained in the present study. c Sequences taked from GenBank.

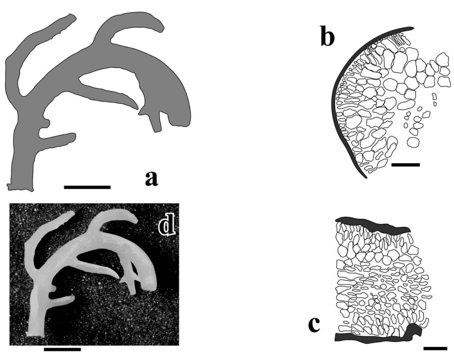

Figure 2. *Kappaphycus alvarezii.* a, Habit (MICH 701908) [scale bar = 3 cm]. b, Transverse section of segment 1 - 1.5 cm from the tip [scale bar = 250 µm]. c, Longitudinal section of the tip [scale bar = 250 µm]. d, Specimen collected [scale bar = 1 cm].

3.2. Description

Multiaxial thallus, bright green in color, cylindrical, thick, succulent, and very firm to contact, about 7 cm in length and without a basal disc. The main axis is moderately straight, devoid of secondary branching near the apical region. The branching is open and irregular. The apices of the secondary branches are slightly flexible, thin, and narrow in the apical portion. In the thickest and thinnest section, the main axis was 153 mm and 12 mm in diameter, respectively. Internally, the entire is pseudo-parenchymatous, composed of cortical and spinose cells. The spinose region consists of large cell layers that are interspersed with smaller cells. No reproductive structures were observed.

3.3. Remarks

Kappaphycus alvarezii is not listed as present for the Atlantic coast of Costa Rica (see [48]). This species is a new record for the country. The fragment collected showed no evidence of grazing or epiphytes.

3.4. Molecular Phylogeny

The phylogenetic analysis (**Figure 3**) showed that the fragment collected corresponds to *Kappaphycus alvarezii,* grouping into a monophyletic clade that includes GenBank samples from Vietnam, Indonesia, the Philippines, Brazil and Venezuela. Strains from Hawaii, Malaysia, and the Philippines were placed in a sister clade. Strains from Tanzania and Madagascar were grouped in a basal clade. The analysis shows that the sample collected in Costa Rica presents low levels of genetic variation from Vietnam, Indonesia, the Philippines, Brazil and

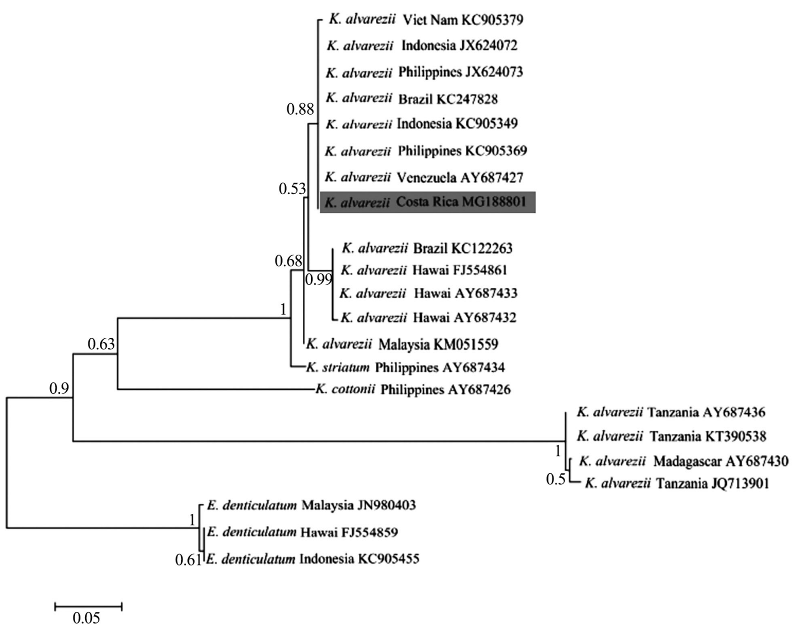

Figure 3. Bayesian phylogeny of *Kappaphycus* representatives: the 50% majority-rule consensus tree resulting from Bayesian analysis of cox2-cox3 intergenic spacer mitochondrial DNA sequences. Bayesian posterior probabilities are shown at the nodes.

Venezuela specimens (0% - 0.3%). It is worth noting that there are no sequences of *Kappaphycus alvarezii* at GenBank from Panama; so it was impossible to include them in the analysis of phylogeny.

4. Discussion

The biodiversity of *Kappaphycus* species is high in the Indo-Pacific regions [31], where most of the carrageenophytes have their center of origin [49]. The cultivation of *K. alvarezii* represents an attractive option to support the economy of coastal communities outside its natural distribution area because the species has a relatively low cost of production, high growth rates, and there is an increase in global demand for carrageenans [50]. While the current global introduction of *Kappaphycus alvarezii* has raised concerns about the effects that the species may have on new environments, its cultivation contributes to the economic income of thousands of families in tropical communities [51]. The productive experience of countries such as the Philippines and Indonesia supports the economic success that can accompany the cultivation of *K. alvarezii* when a market has also been secured.

The specimen found in Costa Rica may correspond to a strain introduced in Panama in 2000. This hypothesis is reinforced by the Bocas del Toro, one of the areas in Panama where the species is known is near Costa Rica (**Figure 1**). However, the lack of DNA records from the strain or strains introduced into Panama precludes further comparisons. The lack of grazing on the specimen analyzed suggests that a short travel time (*i.e.* the donor population should be relatively close as suggested in this investigation). In fact, experimental studies have identified that species within the genus *Kappaphycus* are sensitive to herbivory [19] [50] [52]. It is probable that the specimen collected was dragged by ocean currents. Propagules detached from whole individuals (e.g. during storm events) have the greatest chance of dispersal and successful establishment when compared to spores [11] [53].

Another interesting fact is that the species of *Sargassum* reported as associated at the time of the sighting is a large benthic species with abundant vesicles or floats that facilitate drag by current. Such dragging patterns are similar to other *Sargassum* species with a pelagic life history (e.g. *S. fluitan* (Børgesen) Børgesen and *S. natans* (Linnaeus) Gaillon), which are able to travel thousands of kilometers. The fact is that this red algal species was entangled with *Sargassum*, and this could have facilitated its transportation by ocean currents. Several *Sargassum* species are used by other organisms (e.g. macroalgae, invertebrates) as a dispersal agent (rafting) [54].

The morphology of the only specimen of *Kappaphycus alvarezii* in Costa Rica is consistent with the results of [35], who claimed that vegetative thalli are less robust than cystocarpic specimens. The specimen collected in the vegetative condition is consistent with the results of several authors who stated that reproductive structures in cultivated varieties are rare [9] [47], and even laboratory-produced spores under ideal conditions have very low survival rates [55] [56] [57]. Moreover, the phylogenetic analysis suggests the strain found in Costa Rica is clearly different from the strains currently grown in Tanzania and Madagascar. On the contrary, it is highly related to the strains in Vietnam, Indonesia, the Philippines, Brazil, and Venezuela. It is possible that that considering the ocean current patterns and the vicinity of the countries, the populations of *K. alvarezii* from Panama and Venezuela are related, but the absence of data in GenBank leaves this as a likely relationship within the speculative framework. Panamanian samples will need to be incorporated into future studies to contrast this hypothesis.

The intraspecific divergence values obtained are comparable to those reported by other authors for the *Kappaphycus* genus. [24], recorded null intraspecific divergence values for all examined strains of introduced *Kappaphycus* species in China. [58] recorded intraspecific divergence values for *K. alvarezii* that ranged from 0% to 2.19%, and ranged from 0% to 2.17% for *K. cottonii*, and ranged from 0% to 1.23% for *K. striatus*. [59], recorded intraspecific divergence values for *K. alvarezii* that ranged from 0% to 2.19% and ranged from 0% to 1.2% for *K. striatus*.

Finally, no further specimens have been observed along the beach line in the southern Caribbean coast of Costa Rica. Sampling for the species should also be conducted in subtidal regions where it can establish in case of multiple introductions. The mariculture of *K. alvarezii* could be considered as a productive option that could contribute to the socio-economic development of the southern Caribbean region of the country. However, at least three core aspects must be fulfilled before supporting this endeavor. First, the species must be identified as established in the area. Second, similar to Brazil, the species must be proven to pose no risk to the dynamic equilibrium of the marine ecosystem in Costa Rica, and third, similar to the Philippines, there must be a secured buyer that will handle the biomass after harvest.

Similarly, the mariculture of macroalgae has already been shown to be a viable operation in both the Caribbean and the Costa Rican Pacific [22]. It is clear that more studies are needed to identify potential areas of cultivation where the species does not pose the greatest risk and which on the contrary contributes to the ecosystem. For example, the establishment of exotic species may contribute to the increase of biodiversity by increasing the structural complexity of the habitat [17]. On the other hand, macroalgae mariculture can also contribute to improving water quality in its effect as a bioremediation agent [60].

Some macroalgae float positively and can drift at the mercy of currents hundreds of kilometers [61]. However, most algae float negatively and can float like epiphytes in floating species [62]. [63], or bonded to other floating elements such as timbers and vessels [64] [65]. The transfer of non-floating species by others that are, or without being, have advantages in the flotation process; as set out herein, it is not a minor detail in the dispersion processes that are widely documented [66] [67] [68].

Environmental impact studies in India concluded that the introduction of

Kappaphycus alvarezii as a species for mariculture has translated into more benefits than negative impacts on the environment. Therefore, it is not regarded as an environmental risk in India [50]. On the contrary, other studies are opposed by evidence that the bioinvasion of *K. alvarezii* does pose a risk by having adverse effects on the reefs of the Gulf of Mannar, also in India [20] [53]. In Venezuela, [7] described that the introduced strain was successfully adapted to the physical characteristics of the growing area. To date, it is possible to locate *K. alvarezii* in different areas away from the production fields which show its dispersion, and that the species is expanding; but more studies are needed to know the phase of colonization in which it is located because it is a combination of natural factors and human exploitation that determines the invasive potential of the species [9]. The same results in the dispersal of *Kappaphycus* outside of growing areas were observed in Panama by [13]. In our research, taking phylogeny analyses as a tool, we were able to verify that *K. alvarezii* present in Costa Rica is related to the strain present in Venezuela (**Figure 3**).

5. Conclusion

The morphological data and molecular analyses validate the proper identification of the specimen of *Kappaphycus alvarezii* collected in the southern Caribbean coast of Costa Rica. This species is being commercially exploited at the regional level only in Brazil, and cultivation trials have been done in Venezuela and Panama. We hypothesize that the material collected in Costa Rica was dragged by ocean currents from populations located in Panama. The absence of reproductive structures in the material analyzed suggests that the reproduction mechanism is possibly agamic. The species collected in Costa Rica is grouped in the clade together with strains from Vietnam, Indonesia, Philippines Brazil and Venezuela.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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