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Short communication

First report of the intentionally introduced kelp, *Saccharina japonica*, in the Pacific coast of southern Chile

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ABSTRACT

We report for the first time the occurrence of the Japanese kelp *Saccharina japonica* on the Pacific coast of southern Chile following an illegal introduction for aquaculture purposes. In November 2020, a citizen complaint indicated that the non-native kelp was being illegally farmed in Canal Caicaén. Specimens of the non-native kelp were collected during successive surveys for molecular and morphological analyses, and reproductive viability tests. The species was determined using two mitochondrial molecular markers, *COI* and *trnW-L*. Phylogenetic analysis confirmed the taxonomic identity of the specimen as *S. japonica* and revealed a genetic similarity with *S. japonica* \times *S. latissima* hybrid cultivars Sanhai and Rongfu. In April 2021, several adult specimens became fertile at the farm site and the laboratory and released meiospores were able to develop into embryos after 15–20 days of incubation. These findings underline the risk for this kelp to disperse and colonize in the natural surrounding habitat, with potential impacts on local coastal ecosystems.

1927 via sporelings and young plants attached to logs imported from northern Japan, during the Japanese occupation in China [6]. Soon

after, the summer sporeling method was devised and since then, the

cultivation of the species has spread rapidly supported by the develop-

ment of new breeding techniques (e.g., vegetative gametophyte propa-

gation, parthenogenesis induction, hybridization) and high-yield

cultivars [7]. To date, more than 10 cultivars of S. japonica produced in

China are commercially used for their improved growth rates, yields,

iodine contents and thermal resistance [7,8]. Since the intensive culti-

vation of S. japonica, conspicuous spontaneous populations of the spe-

cies have been established in subtidal zones, which are thought to be

derived from local farmed populations [9,10]. Interestingly, literature

on the potential risk of genetic contamination between wild and farmed

population is still scarce (see 9, 10), and their ecological impact or socio-

ecological consequences remains unclear. For instance, the invasive kelp

Undaria pinnatifida, seems to cause a wide variety of ecological impact in

invaded locations, but the evidence suggests that it does not appear to

1. Introduction

The global transfer of marine species by human-mediated means is of significant concern for biodiversity conservation and the sustainable development of coastal and oceanic resources [1]. Some introduced species can become invasive, and their impacts on local ecosystems might be devastating [2]. Introduced species from all major animal, plants and alga phyla have been detected around the globe [1]. At least 346 macroalgae species were introduced out of their native distribution range [3]. The primary vectors for macroalgal introduction are hull fouling (i.e., species attached to oceanic vessels), shellfish farming (i.e., macroalgae growing on or associated with cultured shellfish) and aquaculture (i.e., species intentionally introduced for cultivation) [4]. A well-documented case of macroalgal introduction is the Japanese kelp *Saccharina japonica* (Areschoug) Lane, Mayes, Druehl & Saunders into China. *S. japonica* is native to the coast of Japan, northwestern Korea and Far Eastern Russia [5]. It was unintentionally introduced into China in

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drive ecosystem change in invaded regions such as Australia, New Zealand, and Argentina. Instead, significant impacts seem linked to site-specific contexts, and anthropogenic disturbances (see Refs. [11,12] and references therein). Potential impacts of invasive species such as *Undaria* and *Saccharina* include changes to the functioning and quality of the ecosystem through the introduction of parasites or pathogens into the new environment, producing genetic changes in populations due to hybridization with native species, modifying physico-chemical conditions and energy flows and organic matter cycles in the native habitat [13–15].

As the global demand for macroalgal biomass continues to increase, together with the availability of high yield strains, interests in expanding the cultivated areas will likely promote the intentional introduction of commercial macroalgae species to new geographical areas. A recent case of a macroalgae introduction for aquaculture purposes occurred in southern Chile. In November 2020, the National Fisheries and Aquaculture Service (SERNAPESCA, by its acronym in Spanish), the government entity in charge of protection and inspection of the hydrobiological resources, received a citizen complaint about an illegal farming of a non-native kelp species in Canal Caicaén, Calbuco, southern Chile [16]. The kelp farm was set in an area of intense aquaculture of salmon, native macroalgae and bivalves. This introduction was illegal because it did not comply with the current regulations for the importation of hydrobiological species in Chile (see Refs. [17,18]). Consequently, national authorities declared the area of Canal Caicaén as a "plague zone" because of the presence of this alien kelp, and closed the farm, forcing the owner to remove the standing biomass [16].

Given the environmental risks and socio-ecological consequences that the introduction of this species might have on Chilean ecosystems if it spreads beyond the marine farm [19], we aimed to report this intentional introduction, determine the taxonomic identity of the alien kelp, and provide preliminary assessment of its capacity to propagate in the surrounding environment.

2. Materials and methods

The citizen complaint (November 2020) indicated the introduction of a non-native kelp in a macroalgal aquaculture farm of about 5 ha in Canal Caicaén, Calbuco ($41^{\circ} 47' 38.7'' S; 73^{\circ} 09' 51.1''$ W, Fig. S1). To confirm this, three surveys were carried out in December 2020, January and April 2021 at the farm site. During the first survey, the presence of the alien kelp sporophytes was confirmed after visually analyzing three individuals sampled from the farm (Fig. 1a). The alien kelp was interspaced within a long-line farming system of the giant kelp, *Macrocystis pyrifera* (Linnaeus) C. Agardh. Before the second survey in January 2021, most of the farmed biomass was harvested by the aquaculture



Fig. 1. Specimens of the non-native *Saccharina japonica* illegally farmed in Canal Caicaén, Calbuco (a) collected in December 2020, and (b) found on long-lines in January 2021. (c) Some specimens kept under laboratory conditions produced sorus (*S*) in March 2021 and released viable meiospores that developed into male and female gametophytes, development into (d) embryos after 25 d.

company, but several long-lines with the alien species remained sunken at 5–10 m depth within the farm site (Fig. 1b). Scientific divers determined that >40 individuals remained attached to the ropes after the biomass harvest. At this point, our divers were not allowed to remove all these remains, due to it was the mandated duty of the farmer. We collected vegetative tissue samples (two samples from each of three individuals) for genetic analyses, and whole adult sporophytes (n = 14) to measure morphological characters (lamina length, width and basal angle, stipe length and width, fascia width, and holdfast width). At the laboratory, collected individuals were held in 1-µm filtered seawater inside a culture tank (1 m³) under controlled conditions (15 \pm 1 °C, PAR of 150 \pm 5 μmol photon m^{-2} $s^{-1}\!,$ photoperiod of 12 L/12D) for 4 months. Seawater was not nutrient-enriched and was renewed every 4-5 days. To avoid propagation of meiospores through the wastewater pipes, culture seawater was discharged on land after the addition of chlorine (5% v/v). During March, the three collected specimens became fertile at the laboratory (Fig. 1c), forming sori containing viable meiospores. Three samples of the sorus from each adult individual were excised to quantify the release of meiospores and percentage of germination according to Leal et al. [20]. Viable meiospores were released and disposed into six-well plates to determine germination and development into microscopic male or female gametophytes for 30 days of incubation (12 °C, PAR of 50 \pm 3 µmol photon m⁻² s⁻¹, photoperiod of 12 L/12D). Statistical analyses were not performed for these data. In the last survey conducted on April 2021, we analyzed the remaining biological material in situ, focusing on identifying living parts of sporophytes and their reproductive status.

For genetic analyses, tissue samples were placed in 2-mL cryogenic tubes, ice-packed and transported to the laboratory within 2 h of collection. In the laboratory, tissue samples were stored at -80 °C until analyses. In total 6 samples were analyzed. Total genomic DNA was extracted using the Purelink plant DNA purification kit (Invitrogen, ThermoFisher Scientific, Texas, USA) following manufacturer's protocol. Two mitochondrial regions were amplified, COI and trnW-L genes, using primers and protocols described by [21,22], respectively. Forward and reverse sequences were edited and assembled in a consensus sequence for each sample in Bioedit v7.1. software [23]. The COI and trnW-L sequences have been deposited in GenBank under accession numbers OM621879 - OM621881 and OM621882 - OM621884 for COI and trnW-L, respectively. COI and trnW-L sequences were treated independent and concatenated (COI + trnW-L). They were aligned with GenBank reference sequences, considering species of Saccharina and Laminaria for which the taxonomic status is recognized according to Algaebase [24] and recent phylogenetic studies [25,26]. Phylogenetic reconstructions were performed using Maximum Likelihood method in MEGA v.7 [27] after selecting the best substitution model based on Akaike information criterion. Nodal support was calculated using a bootstrap method with 1000 repetitions, and trees were rooted with M. pyrifera and Nereocystis luetkeana. Pairwise distance between sequences was calculated using pdistance in MEGA v.7.

3. Results

The global alignment of *COI* sequences was 1738 bp long and 413 bp for *trnW-L* including 19 and 16 sequences, respectively, retrieved from GenBank (Table S1), plus 6 newly generated sequences from Chilean samples. The six Chilean samples corresponded to 3 different individual sporophytes (i.e., the sequences were obtained in duplicate for each individual). The Chilean *COI* sequences shared the same haplotype with all wild *S. japonica* and all cultivated hybrids involving *S. japonica*. *trnW-L* sequences revealed two haplotypes, and all Chilean samples shared the same haplotype together with cultivars Sanhai and Rongfu and an unspecified hybrid *S. japonica* × *S. latissima*. The concatenated data analysis (Fig. S2) confirmed the same results. The best-fit nucleotide substitution model for phylogenetic analyses was Hasegawa-Kishino-Yano (HKY + G) and Tamura 3-parameter (T92) for *COI* and *trnW-L*.

respectively. The ML *COI* trees suggested a strongly supported group containing Chilean samples together with *S. japonica* and *S. japonica* cultivars (Rongfu, Ailunwan, Dongfang No.3 and No.6, Xinbenniu, Sanhai, Shichang, Pengza, Pingbancai and Haiyi) (Fig. 2A). The other *Saccharina* species and *Laminaria yezoensis* and *L. setchelli* appeared as basal branches with p-distances between 0.045 for *S. angustata* to 0.114 for *L. setchellii* (Table S2). All sequences within the *S. japonica* group showed p-distance equal to zero (Table S2). The ML *trnW-L* tree revealed a similar pattern, a well-supported *S. japonica* group including Chilean samples together with the three abovementioned hybrid cultivars (Fig. 2B). The rest of the *S. japonica* cultivars (i.e., Ailun, Dongfang No3. and No6. Xinbenniu, Shichang, Pengza No2, Pingbancai and Haiyi No1.) and the Japanese *S. japonica* appeared external to this group, with p-distances = 0.003 (Table S3).

During the first and second surveys, individuals of the alien kelp showed holdfasts with thick stipes bearing unique, linear-lanceolate laminae with a fascia covering about the half of the lamina (Fig. 1a, b; Table 1). The average size of these individuals was about 1.0 m long and 18 cm wide (Table 1). In April 2021, most of the adult sporophytes of the non-native kelp were fertile at the farm site (Fig. 1c), and those collected in January 2021 also turned fertile under laboratory conditions (Fig. 1c), releasing viable meiospores. Meiospore release and germination success (expressed as percentage) from the three fertile adults of *S. japonica* ranged from 1.44×10^4 to 1.75×10^4 cells·mL⁻¹·cm⁻² of sorus and 64.33% to 72.33% (Table 2), respectively. Germination occurred after 2 days, gametophytes were formed at day 15–17, and the first embryos were recorded on day 22–25 (Table 2).

4. Discussion

The phylogenetic analysis confirmed that the introduced species is Saccharina japonica, for which it is the first report in the southeastern Pacific Ocean. Similarly, the different morphological characteristics of the sampled sporophytes is within the range described for the genus Saccharina from the non-digitate group of the family Laminariaceae: a unique linear-lanceolate lamina joint to a holdfast by a thick, and a median flat band or fascia that occupies 1/3 to 1/2 the width of lamina [28-30]. The farmed alien sporophytes were smaller than those reported in the literature [28,29], but had likely reached a bigger size because the individuals were damaged in the apical part of the lamina prior to sampling (Fig. 1b), which occurred after the cultivator harvested the standing biomass. The analysis based on the trnW-L mitochondrial marker revealed a single nucleotide difference between the native species S. japonica and the hybrid cultivars Sanhai and Rongfu, which was also detected in the Chilean samples. This result suggests therefore an introduction of one of these cultivars. Yet, the haplotype has not been reported in natural populations, and an alternative origin, eventuating from a natural population in China, Japan or Korea, cannot be ruled out. More polymorphic genetic markers, such as microsatellite markers [5], are required to provide a stronger statistical support to this conclusion, and further determine which cultivar or natural strain was the source of the introduction. However, the observation of hybrid cultivars in Chile is coherent with the fact that the species was introduced for cultivation purposes.

The establishment and maintenance of kelp populations depend on settlement in a suitable habitat for the development of both the gametophytic and sporophytic stages [31–33]. Sporogenesis in Saccharina spp. is favored by summer conditions such as elevated temperatures, high light, and nutrient availability [34,35]. This may explain the fertile stage that the introduced kelp reached during late summer at the illegal farm site. This kelp also showed a high release of meiospores (>1.44 × 10⁴ cells·mL⁻¹·cm⁻² of sorus) in laboratory conditions, suggesting that those sporophytes remaining at the farm site (and detected in April 2021) were continuously releasing similar amount of meiospores to the local environment, with an associated risk of establishing an alien population. Kelps such as *S. japonica* require hard substratum such as rocky reef or



Fig. 2. Maximum likelihood inference tree showing the phylogenetic relationships of *Saccharina japonica* individuals found in Chile, based on (A) *COI* sequences and (B) *trnW-L* sequence data. Numbers above branches indicate bootstrap values.

aquaculture structures for meiospore settlement and development of the gametophyte and sporophyte stages [31–33]. Although the substratum of Canal Caicaén is mainly muddy and sandy, it is necessary to identify hard-bottom areas surrounding the farming site to determine whether the alien kelp was able to settle there. However, other possible hard substratum (e.g., ropes, buoys and anchoring blocks) can be found in

abundance around the area due to the intensive aquaculture activities [36]. Additionally, the successful release of meiospores by the alien species highlights its ability for reproduction under the local (Chilean) environmental conditions and alerts its potential ability to hybridize with other native brown macroalgae. For example, interfamilial hybridization between *M. pyrifera* and *Lessonia spicata* has been confirmed

Table 1

Morphological characters measured from the non-native kelp species (n = 14) illegally farmed in Canal Caicaén, Calbuco.

Morphology character	Length (cm)	Width (cm)	Basal angle (°)
Lamina	100.57 ± 64.69	17.86 ± 5.78	93.45 ± 37.78
Fascia	n.a.	$\textbf{8.25} \pm \textbf{1.70}$	n.a.
Stipe	5.04 ± 1.26	$\textbf{0.86} \pm \textbf{0.18}$	n.a.
Holdfast	n.m.	$\textbf{3.82} \pm \textbf{2.07}$	n.a.

n.a. = not applicable; n.m. = not measured.

Table 2

Release and 3-d germination of meiospores obtained from three sporophytes of *Saccharina japonica* that turned fertile under laboratory conditions. Mean \pm SD, n = 3 for meiospore release and n = 6 for meiospore germination. It is also indicated the number of days (d) of culture when gametogenesis and embryogenesis occurred.

Fertile individual	Developmental stage				
	$\begin{array}{l} \text{Release} \\ (\text{cells } \text{mL}^{-1} \\ \text{cm}^{-2}) \times 10^4 \end{array}$	Germination (%)	Gametogenesis (d)	Embryogenesis (d)	
1	1.75 ± 0.89	$\begin{array}{c} 64.33 \pm \\ 6.42 \end{array}$	15	23	
2	1.45 ± 0.28	$\begin{array}{c} 69.38 \pm \\ 4.33 \end{array}$	17	25	
3	1.45 ± 0.56	$\begin{array}{c} \textbf{72.33} \pm \\ \textbf{6.79} \end{array}$	16	22	

to occur in wild populations [37,38].

As ecosystem engineers, kelps are especially problematic when introduced in a new area because they have strong potential to alter both the ecosystem structure and its function [39,40]. For instance, one of the most successful invasive kelps, U. pinnatifida, is now established on the coastlines of 13 countries across four continents [12,41-43]. For U. pinnatifida, besides its introduction into France for aquaculture purposes, it was accidentally introduced in the Mediterranean Sea (Thau Lagoon, France) through the mollusk aquaculture industry [40,44]. This area is dominated by sandy and muddy bottom and the introduced species was initially found on aquaculture infrastructures. Nevertheless, U. pinnatifida was actively removed, and no population settlement was further observed in the Mediterranean Sea [44]. However, the region of southern Chile where S. japonica was introduced is much similar to its native environment in terms of temperature and light (i.e., same latitude), and according to the results obtained in this study, it is expected that these conditions might be favorable for sporophyte growth and reproduction. Moreover, it is a region of intense aquaculture activity, with dense arrays of artificial structures on which S. japonica may establish. Altogether, these conditions seem potentially favorable for the proliferation of this alien kelp.

Any introduction poses a potential threat to the ecosystem, and two important unknowns are the impact of the alien species on the recipient community, and its performance in the new ecosystem [40]. To date, 51 species are catalogued as introduced species in Chile, of which 14 macroalgae are successfully established along the Chilean coasts [44-46]. However, evidence regarding their ecological effects or invasive potential is scarce. One particular aspect generally excluded from surveys relates to the potential role of introduced algae as a source of pathogenic organisms or other microbial species. Infectious diseases in farmed Saccharina spp. have been systematically studied in China, Korea and Japan, evidencing losses up to 30% of the harvested biomass [47]. Macroalgal farms often act as reservoirs of pests and pathogens, as the result of their dense and genetically depauperate constitution [48]. If the origin of the introduced material is an Asian farm/hatchery, the risk of introducing pathogenic organisms and other associated microbial species into the newly colonized system is a particularly strong threat. Finally, prediction of future invasiveness is not possible, but estimating

potential dispersal distances of meiospores and detached sporophytes might be useful for this purpose. However, it is necessary to recognize that Chilean seaweed aquaculture regulations have shown to have several flaws in relation to environmental impact assessment, biosecurity prevention and management policies established for macroalgal farming in Chile [49]. Indeed, after initial confirmation of the illegal introduction by SERNAPESCA in November 2020, the farmer did collect the standing biomass, but living material was incidentally left in sunken lines at the bottom of the farm site. The detected living and fertile material during the 3rd survey was removed and disposed following biosecurity protocols, but 5 months after the harvest. This raises concerns about who should take the responsibility of removing introduced organisms and the appropriate methods to do so, in order to ensure a rapid and efficient eradication. In this case, for example, the farmer refused to remove the sporophytes and floating structures citing lack of funds, consequently, the authority had to assume the removal at the expense of the state. So far, introduction prevention remains the most effective method of limiting biological invasions, as well as the correct management of human activities both directly implicated (Aquaculture), or those who facilitate the spread of introduced species (pollution, eutrophication or species loss). The monitoring and eradication of this introduced alien kelp in southern Chile is in process and more surveys and rapid control measures will be needed to ensure no further establishment occurs.

CRediT authorship contribution statement

PPL, LH-A, SC, DU and PFC were responsible for surveys, sample collection and laboratory analyses; CC, JV, SF and PAF were responsible for molecular analysis; PFC and AHB were responsible for funding acquisition for field trip and laboratory analyses. All authors contributed to the interpretation of data, writing-review & editing of the paper.

Declaration of competing interest

On behalf of all the authors involved in this study, I declare that no conflict of economic interest or others exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2022.102750.

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