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First Report of the Asian Seaweed *Sargassum filicinum* Harvey (Fucales) in California, USA

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ABSTRACT

We report the occurrence of the brown seaweed *Sargassum filicinum* Harvey in southern California. *Sargassum filicinum* is native to Japan and Korea. It is monoecious, a trait that increases its chance of establishment. In October 2003, *Sargassum filicinum* was collected in Long Beach Harbor. In April 2006, we discovered three populations of this species on the leeward west end of Santa Catalina Island. Many of the individuals were large, reproductive and senescent; a few were small, young but precociously reproductive. We compared the sequences of the mitochondrial *cox3* gene for 6 individuals from the 3 sites at Catalina with 3 samples from 3 sites in the Seto Inland Sea, Japan region. The 9 sequences (469 bp in length) were identical. *Sargassum filicinum* may have been introduced through shipping to Long Beach; it may have spread to Catalina via pleasure boats from the mainland.

Key words: California, *cox3,* invasive seaweed, Japan, macroalgae, *Sargassum filicinum, Sargassum horneri*

INTRODUCTION

The brown seaweed *Sargassum muticum* (Yendo) Fensholt, originally from northeast Asia, was first reported on the west coast of North America in the early 20th c. (Scagel 1956), reached southern California in 1970 (Setzer & Link 1971) and has become a common component of California intertidal and subtidal communities (Ambrose and Nelson 1982, Deysher and Norton 1982, Wilson 2001, Britton-Simmons 2004). Since 2000, the Asian kelp *Undaria pinnatifida* (Harvey) Suringar and the cosmopolitan red alga *Caulacanthus ustulatus* (Mertens ex Turner) Kützing have been introduced to southern California and continue to flourish (Silva et al. 2002, Zuccarello et al. 2002). A weedy strain of the tropical green alga *Caulerpa taxifolia* (M. Vahl) C. Agardh was introduced in 2000 and declared eradicated in 2005 (Merkel and Associates 2005).

Sargassum filicinum was first collected in October 2003 by biologists conducting surveys in inner Long Beach Harbor (33º42'N, 118º14'W) (Marine Biological Consultants, pers. comm.; Fig. 1). By October

2005, the population had spread within Long Beach Harbor, occurring on both the Terminal Island and the Long Beach side of the channel (Marine Biological Consultants, pers. comm.). The Long Beach plants were attached to the substrate or epiphytic on *Sargassum muticum* (Fig. 2). We examined small specimens, lacking vesicles, and identified them as *Sargassum horneri* (Turner) C. Agardh, an Asian species very closely related to *S. filicinum* (Yoshida 1983; Stiger et al. 2003).

Both *Sargassum horneri* and *S. filicinum* have deeply incised, often notched vegetative leaves with spiny branches, but mature *S. horneri* bears cylindrical vesicles while those of *S. filicinum* are spherical to elliptical (Yoshida 1983; Tseng et al. 1985; Fig. 3a and 3b). *Sargassum horneri* is broadly distributed in Japan, Korea, Hong Kong, Taiwan and China (Tseng et al. 1985), often forming extensive meadows in sheltered areas, while *S. filicinum* has a narrower distribution in western Japan (Yoshida 1983; Tseng et al. 1985) and Korea (Lee & Yoo 1992).

Here we report the introduction of *Sargassum filicinum* to Santa Catalina Island, a pristine habitat relative to the mainland harbors. To confirm our identification based on morphology and to determine the origin of the Santa Catalina plants, the cytochrome oxidase subunit III gene (*cox3*) was isolated and sequenced from our California specimens and compared with sequences from specimens from the Seto Inland Sea, Japan (Table 1).

MATERIALS AND METHODS

After the initial discovery of *Sargassum filicinum* at Santa Catalina Island on 8 April 2006, we conducted further searches using scuba on 20-24 April 2006. Apical portions of fresh specimens were dried in silica gel. In the lab, vesicles with a coronal leaf were hydrated and cleaned of contaminants. Less than 1 cm of a vesicle or a coronal leaf was used for DNA extraction (DNeasy plant mini kit [Qiagen, Hilden, Germany] according to the manufacturer's protocols). DNA was purified (Geneclean [Bio101, Vista, CA, USA]) and used as a template for PCR amplification of the *cox3* gene using primers caf4A and car4A (Kogame et al. 2005). The thermal profile for PCR amplification included: initial denaturation at 95 °C, for 2 min; 45 cycles of denaturation at 95 °C for 30 sec; annealing at 45 °C, for 30 sec; and extension at 75 °C for 45 sec;

final extension at 75 °C for 5 min; followed by storage at 4 °C or 15 °C. PCR products were purified by polyethylene glycol precipitation and used as templates for cycle sequencing reactions (CEQ cycle sequencing kit [Beckman-Coulter, Fullerton, CA, USA]) using the same primers as those for PCR amplification. Sequencing was accomplished with a CEQ8000 gene analyzer (Beckman-Coulter) according to the manufacturer's instructions. The resulting sequences lacked insertions/deletions and were easily aligned by eye.

RESULTS

On 8 April 2006, we discovered a population of *Sargassum filicinum* at the Intake Pipes (33°26'N, 118°29'W), a rocky point north of Big Fisherman Cove near the University of Southern California's Wrigley Marine Science Center and the town of Two Harbors, on Santa Catalina Island, CA (Fig.1). We observed more than 30 plants (0.3-1.5 m tall) at depths between 4-12 m, some of which were young, with symmetrical, spiny leaves (Fig. 4a) and many of which were mature, bearing elongate receptacles and the characteristic spherical-elliptical vesicles of *Sargassum filicinum* (Fig. 4b). Individuals were also observed in sheltered habitats at Cherry Cove (2 plants at 4 m and 13 m depths) and Emerald Bay (4 plants at 7 m depth), both nearby sites on the leeward west end of the island (1.8 km and 3.0 km NW of Intake Pipes) (Fig 1).

Plants at all locations were attached to rock and found associated with other seaweeds, including *Macrocystis pyrifera*, *Cystoseira neglecta*, *Dictyopteris undulata*, and two other *Sargassum* species, the native *S. palmeri* and the introduced *S. muticum*. Most plants at these locations were reproductive, including young plants without vesicles (Fig. 4a). Many older plants had shed vegetative leaves, leaving axes bearing only vesicles and ripe receptacles. Some post-reproductive individuals were deteriorating.

The *cox3* sequence in *S. filicinum* was 469 base pairs in length. Samples from 3 sites at Santa Catalina Island shared the same sequence (GenBank accession number AB264797) as samples from 3 sites in the Seto Inland Sea, Japan.

DISCUSSION

Sargassum filicinum, like *S. muticum*, is well adapted for widespread dispersal to and rapid colonization of new areas (Nyberg & Wallentinius 2005). Fertile fragments are buoyant due to air-filled vesicles and can readily disperse locally. It is possible that the three populations at Santa Catalina Island are the result of a single introduction and subsequent local dispersal. Like *S. muticum*, this species is monoecious, bearing both male and female conceptacles; individuals are thus capable of self-fertilization. Its establishment, like that of *S. muticum* and *Undaria pinnatifida*, may be promoted by its precocious fertility, which is presumably related to its essentially annual growth pattern.

Some of the mature plants at Santa Catalina Island were 1-2 m in length and encrusted with bryozoa, indicating that they had been present for several months. Because we did not observe this species during annual monitoring dives in April 2005 or earlier, and because others diving at these popular locations have not reported it, we suspect that this introduction is recent.

The mode of introduction of *S. filicinum* to California is unknown, but possibilities include hitch-hiking on ships' hulls or by transport of embryos in ballast water (Critchley et al. 1990). Long Beach and the nearby Port of Los Angeles together constitute the 5th busiest international shipping port in the world, with more than 5,000 cargo and tanker ships arriving per year. China, Japan, Taiwan, Thailand and South Korea are the chief sources of ships to these ports (Ports of Los Angeles and Long Beach, 2006).

Because the Catalina populations share *cox3* sequences with Japanese populations, we assume that the California populations originated there, although genetic diversities of Korean populations are unknown. We do not know if the Catalina populations were introduced from Asia or from Long Beach Harbor. There are potential vectors from both places: the island lies near major shipping lanes in the San Pedro Channel, and there is considerable pleasure and small commercial boat traffic across the 20 miles from the mainland ports to the Two Harbors region of Santa Catalina Island.

Although *Sargassum filicinum* has not been reported from other California insular or mainland locations, it may have spread undetected in the several years since its initial discovery in Long Beach Harbor. If an eradication program is possible, there is a very short time window in which to implement it. In any case, it is important to document the spread of this potentially invasive species, evaluate its ecological impacts, and determine its mode of introduction to develop programs to minimize future introductions.

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FIGURE LEGENDS

Figure 1. Map showing California collecting sites (black dots) at Long Beach Harbor and Santa Catalina Island.

Figure 2. Photograph of plant collected in Long Beach Harbor on 23 October 2003.

Figure 3a. *Sargassum filicinum* from Japan (University Herbarium, University of California at Berkeley). Note spherical-elliptical vesicles and cigar-shaped receptacles.

Figure 3b. *Sargassum horneri* from Japan (University Herbarium, University of California at Berkeley). Note elongate vesicles and cigar-shaped receptacles.

Figure 4a. Mature *Sargassum filicinum* with receptacles and elliptical vesicles from Santa Catalina Island.

Figure 4b. Juvenile *Sargassum filicinum* from Santa Catalina. Island. Note spiny axes.

Table 1. Collection sites and number of samples for *cox3* sequences.

Country	Site	n
USA	Intake Pipes, Santa Catalina Island (8 April 2006)	1
	Intake Pipes, Santa Catalina Island (20 April 2006)	3
	Cherry Cove, Santa Catalina Island (21 April 2006)	1
	Emerald Bay, Santa Catalina Island (24 April 2006)	1
JAPAN	Mukaishima Island, Onomichi, Hiroshima Prefecture	1
	Mukuchi-jima Island, Kurashiki, Okayama Prefecture	1
	Iwaya, Awaji, Hyogo Prefecture	1

Figure 1. Map showing California collecting sites (black dots) at Long Beach Harbor and Santa Catalina Island.

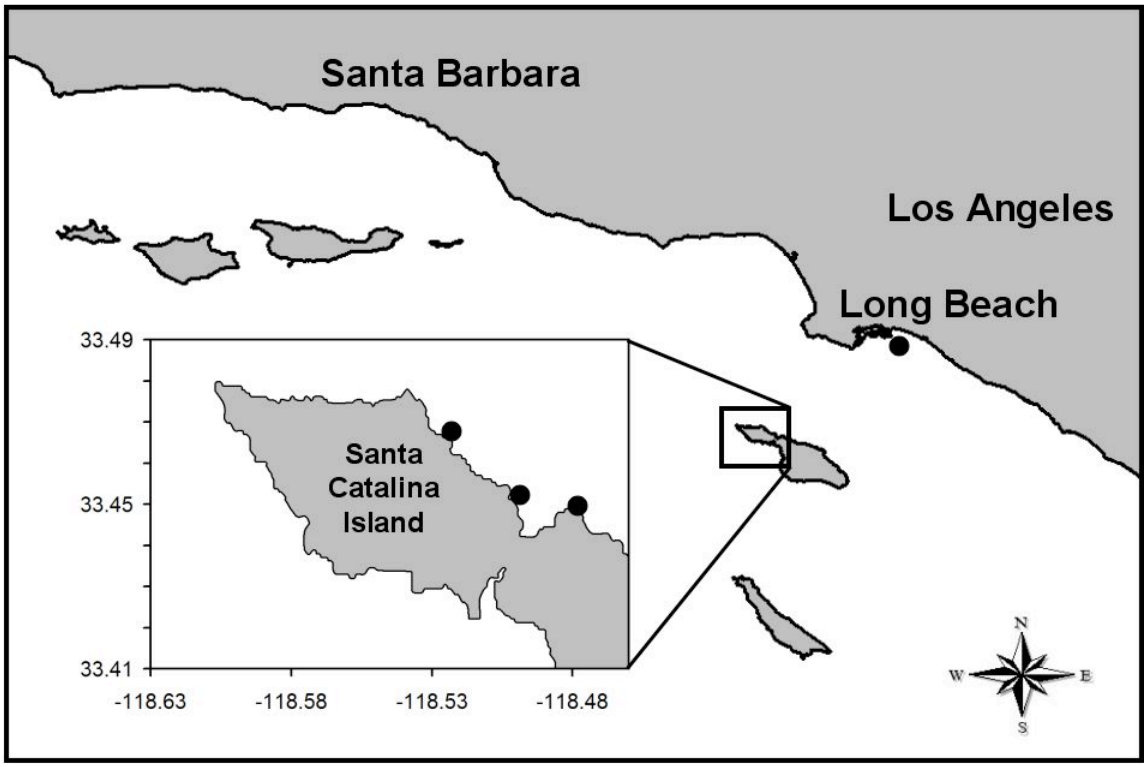
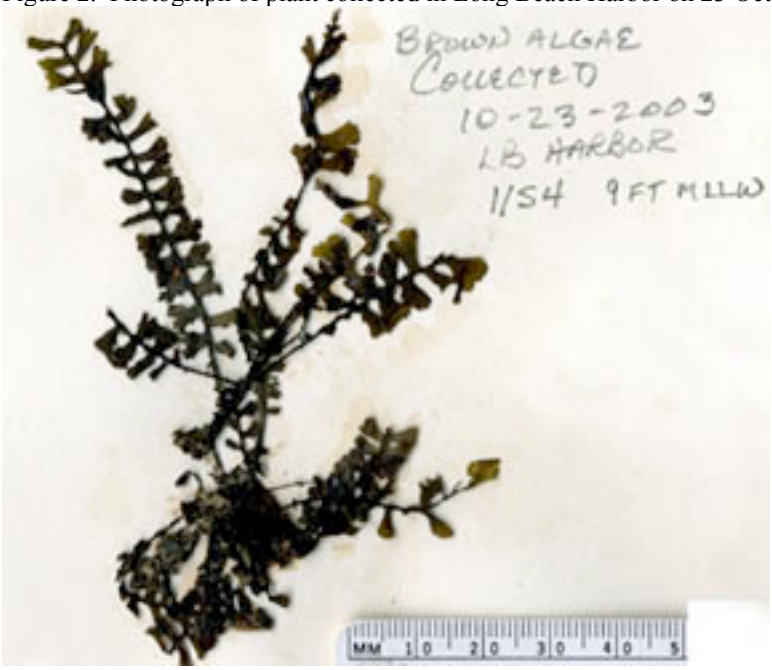


Figure 2. Photograph of plant collected in Long Beach Harbor on 23 October 2003.



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