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Short communication

# A new record of a second seagrass species from the Hawaiian archipelago: *Halophila decipiens* Ostenfeld

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### Abstract

*Halophila decipiens* Ostenfeld, a new record for the Hawaiian Islands, was collected from three locations spanning the archipelago: Midway Atoll at 3–15 m deep, O'ahu Island at 1–2 m deep, and Hawai'i Island at 40 m deep. These findings expand the biogeographical distribution of this pantropical seagrass into the north central Pacific Ocean. Specimens showed morphological, reproductive and DNA characteristics typical of the species.

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## 1. Introduction

Although, the Hawaiian Islands host a diverse marine macroalgal flora of over 400 species, only one species of seagrass, an endemic, *Halophila hawaiiana* Doty and Stone (Doty and Stone, 1966), has ever been reported from these islands, other than the brackish water indigenous widgeon grass, *Ruppia maritima* L. (Sachet and Fosberg, 1973; Wagner et al., 1990). In 2001, *Halophila decipiens* Ostenfeld was collected from three separate islands, spanning the length of the Hawaiian Islands chain.

*H. decipiens* is reknowned for its pantropic distribution, unique among all seagrass species (Den Hartog, 1970; Phillips and Meñez, 1988). Since the first description of *H. decipiens* from the Gulf of Thailand in 1902, this species has been found along coasts of islands and continents in the Atlantic Ocean, Caribbean Sea, Gulf of Mexico, Red Sea, Indian

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Ocean, South China Sea, East China Sea, and the western Pacific, as well as in Tahiti and Panama (Den Hartog, 1972; Gil-Rodriguez et al., 1982; De Olivieira et al., 1983; Meñez and Calumpong, 1983; Den Hartog, 1989; Den Hartog and Yang, 1990; Mok et al., 1993; Japar Sidik et al., 1995; Kuo and Kirkman, 1995; Kuo et al., 1995; Pavon-Salas et al., 2000; Payri et al., 2000). However, the biogeographical distribution of *H. decipiens*, similar to that of most Indo–Pacific seagrass species, dwindles eastward across the Pacific (Stoddard, 1992), and many islands of the Central Pacific are depauperate in seagrasses (Tsuda et al., 1977; Tsuda and Kamura, 1990; McDermid and Edward, 1999) or apparently lack them altogether, i.e. Palmyra Atoll (McDermid unpublished data, 2000).

The discovery of a second seagrass species in the Hawaiian Islands changes long-standing views about seagrass biogeography, and increases our knowledge of *H. decipiens* distribution and the Hawaiian seagrass flora.

#### 2. Methods

In the course of a research project on *H. hawaiiana* populations, *H. decipiens* was collected by snorkeling or with SCUBA on the island of Hawai'i  $(19^{\circ}45'N, 155^{\circ}30'W))$  at a depth of 40 m off the Kohala coast on 15 January 2001; at Midway Atoll in the Northwestern Hawaiian Islands  $(28^{\circ}01'N, 177^{\circ}25'W)$  at two locations in the lagoon at 3 m (inner harbor) and 10–15 m deep (between Cargo Pier and Turtle Beach) on 17 and 18 May 2001; and on the island of O'ahu  $(21^{\circ}30'N, 158^{\circ}W)$  at 1–2 m deep along the southern Kahala Beach coast on 8 June 2001. Fresh specimens were examined, measured and photographed, and then preserved as dried herbarium vouchers, wet specimens in 4% formalin–seawater solution, or in silica gel for DNA analysis. Dried specimens will be deposited in the Bernice Pauahi Bishop Museum Herbarium in Honolulu.

DNA was extracted from silica gel dried specimens using a DNeasy Plant Mini Kit and protocol (Qiagen, Inc. Valencia, CA, USA). The internal transcribed spacer region 1, 5.8S rRNA gene, and internal transcribed spacer region 2 of the nuclear-encoded rRNA gene (ITS) were amplified with the universal primers described in White et al. (1990) and Baldwin (1992). Amplification reactions were set up in 50  $\mu$ l volumes containing 40–100 ng DNA template, 1.25 mM MgCl<sub>2</sub>, 80  $\mu$ M each dNTP, 0.2  $\mu$ M each flanking primer and HotStarTaq DNA polymerase and reaction buffer as suggested by the manufacturer (Qiagen, Inc. Valencia, CA, USA). The thermocycling protocol followed that of Freshwater et al. (2000) with the addition of an initial enzyme activation step of 15 min at 95 °C. Amplification products were cleaned using a GeneClean II Kit (Q-biogene, Carlsbad, CA, USA), and sequence reactions for both DNA strands were set up using the Big Dye Sequencing Kit and protocol (Applied Biosystems, Foster City, CA, USA). Sequence reactions were run on an ABI Prism 377 DNA Sequencer and the resulting data compiled and edited with Sequencher (Gene Codes Corp., Ann Arbor, MI, USA).

#### 3. Results

The plants from all sites are easily distinguishable from the sympatric species, *H. hawaiiana*, based on leaf shape, leaf margins, hairiness, rhizome diameter, and reproductive



Figs. 1–5. (1) Portion of fertile *H. decipiens* plant with branching rhizome and blades collected at North Kohala, Hawai'i Island (KM5340). Scale bar = 1.5 cm. (2) Serrulated margin of *H. decipiens* from Kahala, O'ahu (KM5352). Scale bar = 0.5 mm. (3) Tip of blade with dense covering of hairs on the surface, (KM5352). Scale bar = 0.5 mm. (4) Male (M) and female (F) flowers at a single node, from Kahala, O'ahu population (KM5353). Scale bar = 1 mm. (5) Fruit of *H. decipiens* from Kahala, O'ahu (KM5354). Scale bar = 1 mm.

characteristics. The plants clearly agree with previous descriptions of *H. decipiens*. The Hawaiian specimens are small with a slender, prostrate, branched rhizome 0.57–0.7 mm in diameter with internodes 2.8–29.8 mm long (Fig. 1). One root and a pair of petiolate leaves subtended by two scales occur at each node. Petioles are short, 4.3–16 mm long, 0.3–0.51 mm in diameter. Scales are transparent, 3.1–4.5 mm long, obovate, keeled and sparsely covered with short hairs. The leaf blades are grass green, elliptic to oblong or Hawaiian canoe paddle-shaped, obtuse at the apex, cuneate to obtuse at the base, 12.9–18.5 mm long, 3.8–6.3 mm wide, with finely serrulated margins visible under low power with a microscope (Fig. 2). Both surfaces of the leaves are densely covered in minute unicellular hairs (Fig. 3) which give the plants a furry texture discernible even with the naked eye on damp leaves exposed to air. Leaves have five to eight pairs of ascending non-forked cross-veins arranged alternately to sub-oppositely which join the intra-marginal veins.

Table 1

Variable sites within a 668-bp alignment of ITS1, 5.8S rRNA, and ITS2 regions of the nuclear-encoded rRNA gene sequences from ten *H. decipiens* specimens

Taxon	Site					
	133	217	218	259	369	560
Florida, USA (AF366407)	Т	С	A	G	G	С
Curacao (AF366413)	Т	С	А	G	С	С
Caribbean Panama (AF366408)	Т	С	А	G	G	С
Caribbean Costa Rica (AF366409)	Т	С	А	G	G	С
Midway (AF395673)	G	С	А	G	G	С
O'ahu (AF395672)	G	С	А	G	G	С
Hawai'i (AF395671)	G	С	А	G	G	С
Dunk Island, Australia (AF366411)	G	С	А	G	G	С
Gladstone Harbour, Australia (AF366410)	G	-	-	А	G	Т
Kuala Setiu, Malaysia (AF366412)	G	-	-	А	G	Т

Geographic location and GenBank accession numbers are given for each specimen. Gaps at sites are coded by a dash (–).

Plants are monoecious. One male (staminate) and one female (pistillate) flower occur at the same node enclosed by a semi-transparent spathe (Fig. 4). Flowers were observed in all three populations. Male flowers have three oblong-elliptic tepals set upon a pedicel. Female flowers bear three long styles atop the hypanthium connected to the ovary. Ovoid to spherical fruits were abundant on plants in all populations (Fig. 5).

Previous DNA sequence analyses have shown that *H. decipiens* may be distinguished from other *Halophila* species by a comparison of ITS sequence data (Waycott et al., in press). Mean sequence divergence values between *H. decipiens* and 11 other *Halophila* species (including *H. hawaiiana*) were a minimum of 6.2-6.3% in ITS comparisons. Direct comparisons of Hawaiian *H. decipiens* and *H. hawaiiana* ITS sequences over a 668 site alignment showed sequence divergences of 6.4-6.6% in this study. In contrast, sequence divergence values for comparisons among *H. decipiens* samples from the Caribbean, Hawaiian Islands and Indo–Pacific were found to be <0.5% (Waycott et al., in press; this study). ITS sequences generated from the Hawaiian Islands specimens were identical to each other and also to the sequence for a specimen from Dunk Island, Australia. Little variation was detected in comparisons of ITS sequences among all the sequenced *H. decipiens* specimens despite their wide geographical distribution (Table 1).

*H. decipiens* appears to form monospecific patches adjacent to, but not intermingling with *H. hawaiiana* plants at Midway and O'ahu. Possible interactions between *H. decipiens* and *H. hawaiiana* are under investigation (C. Unabia personal communication, 2001). At one location (10–15 m deep) at Midway, *H. decipiens* grows in dense meadows, averaging 24,000 blades per square meters. *H. decipiens* is the only seagrass known from the island of Hawai'i where it forms pure stands in deep water.

#### 4. Conclusions

This new record of *H. decipiens* in the Hawaiian archipelago extends the distribution of this species approximately 5300 km eastward into the north Pacific Ocean. The Midway

Atoll population at  $28^{\circ}$ N is the most northern record of this species in the Pacific. With these collections, the seagrass flora of the Hawaiian Islands has been increased from one to two species. The finding of *H. decipiens* in the Hawaiian Islands is remarkable, but could be accounted for by this species' already broad distribution, the greater local awareness of the importance of marine plants in the coastal environment, the increasing level of marine botanical research throughout the archipelago, and more thorough surveying of deeper habitats with SCUBA. *H. decipiens* is probably not a new immigrant to the Hawaiian Islands, since recent re-examination of voucher specimens from marine turtle research found *H. decipiens* in a 1979 reef sample and from Hawaiian green turtle stomach samples (Russell and G. Balazs, personal communication, 2002).

The occurrence of *H. decipiens* in the Hawaiian Islands is comparable to the distribution patterns of several seaweed species, such as *Crouania mageshimensis* Itono, *Kallymenia sessilis* Okamura, *Porphyra vietnamensis* Tanaka et Pham, and *Ptilonia okadae* Yamada known only from the Hawaiian Islands and Japan or South East Asia (Abbott, 1999). Oceanographic currents may partly explain the unexpected biogeography of these marine plants. It will be important to explore other islands in the Hawaiian archipelago to search for *H. decipiens* and understand its role in the reef ecosystem.

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