

First Record of *Ulva pertusa* Kjellman (Ulvales, Chlorophyta) in the Pacific Coast of Mexico

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Based on samplings during 2006-2007 on the intertidal zone of Playa Tampico, Eréndira and Punta Baja, Baja California, *Ulva pertusa* Kjellman (Ulvales, Chlorophyta) was recorded for the first time for the Pacific coast of Mexico. An alga native to Asia, *U. pertusa* has just recently been recorded in the southern coast of California (USA). The identification of *U. pertusa* was based on the observation of the morphology and reproductive characters of the collected specimens. Furthermore, the species identity was confirmed by molecular comparison between nuclear-encoded ITS2 sequences of the Mexican samples and those of other *Ulva* species in GenBank data. Habitat and distribution of *U. pertusa* along the Pacific coast of North America are also described. Considering our new report of its occurrence in Mexico, we conclude that *U. pertusa* is expanding its geographical distribution by trans-ocean introductions.

Key Words: Baja California, introduced species, morphology, reproduction, *Ulva pertusa* Kjellman

INTRODUCTION

During an ecological study of the flora and fauna associated to the banks of mussels *Mytilus californicus* Conrad along the western coast of Baja California in 2006-2007, a species of *Ulva* with foliar thallus was found growing on diverse substrata, with morphological characteristics not matching with those of *Ulva* species known to date for the Mexican Pacific coast (Aguilar-Rosas *et al.* 2005; Pedroche *et al.* 2005). The morphological and molecular analyses of the collected material revealed that it can be identified as *Ulva pertusa* Kjellman, a species of Asian origin recently recorded in the southern coast of California (USA) (Hayden and Waaland 2004).

Herein, *Ulva pertusa* is recorded for the first time in Mexico, in the west coast of Baja California, being this a new case of a species introduced to Mexican waters. The morphological and reproductive characters of the thallus are described in detail, as well as the habitat and distribution range in the Pacific coast of North America.

MATERIALS AND METHODS

The samples of *Ulva pertusa* were collected in the intertidal zone of Playa Tampico, Ejido Eréndira, and Punta Baja, Baja California, in February, March, June, September and December 2006, and March 2007 (Fig. 1). Specimens were put in polyethylene bags, labeled and preserved in 4% formaldehyde solution in seawater. Cross sections were measured of the preserved material previously stained with 1% blue aniline in water and mounted in a 20% solution of Karo[®] syrup in water. Photographs were taken with a digital camera (Sony DSC-S85, Tokyo, Japan) in a microscope (Zeiss, Axioscop 40, Goettingen, Germany). The identification of thallus was carried out using a composite microscope (Bauch & Lomb), based on the descriptions and illustrations by Kamiya *et al.* (1993), Verlaque *et al.* (2002) and Shimada *et al.* (2003).

Samples for molecular analyses were partially cut from the samples used in the morphological observations, dried and preserved with silica gel crystals. These samples were deposited in the Herbarium CMMEX. DNA was extracted and determined the nuclear-encoded

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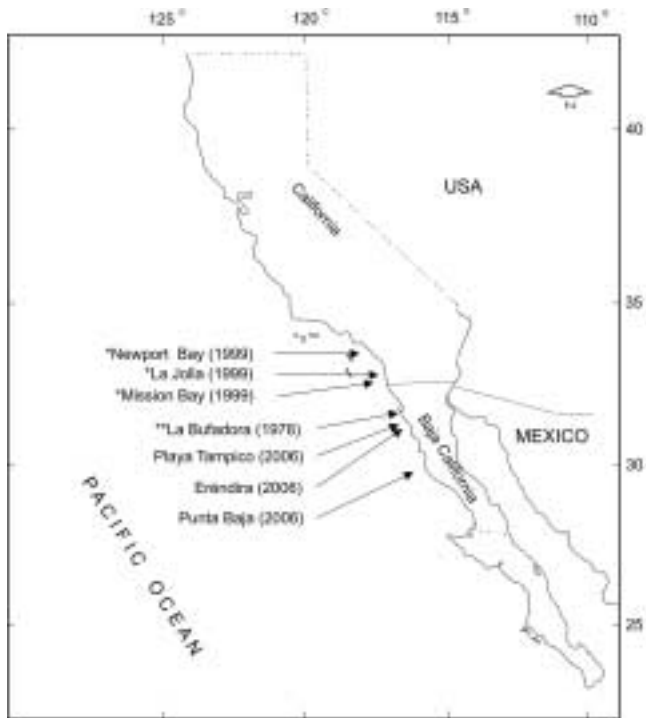


Fig. 1. Study area and collection sites (*reported by Hayden & Waaland 2004; **specimens in Herbarium CMMEX).

internal transcribed spacer 2 region (ITS2) following the protocol established by Shimada *et al.* (2003). Twenty-six ITS2 sequences were downloaded from GenBank and included in the alignment. The ITS2 sequences (290 bp) were aligned by eye with regard to their secondary structure using the mFOLD program (Zuker 1989). Phylogenetic analyses were performed using the maximum likelihood (ML) and maximum parsimony (MP) algorithms available in the computer program PAUP* 4.0 b10 (Swofford 2002). Regions of uncertain homology in the ITS2 alignment (sites 107-119 and 146-169) and identical sequences were excluded from alignments. Takahashi and Nei (2000) suggested that a simple model is better when a large number of short sequences are analyzed. Thus, we used the JC model in the ITS2 analyses. ML tree searches were performed using the estimated model parameters with the following options: starting tree obtained by stepwise addition, and TBR branch swapping algorithm. Bootstrap values (Felsenstein 1985) based on 100 re-samplings in ML and 2000 re-samplings in MP calculated (TBR, full heuristic search option). Specimens were deposited in the CMMEX herbarium of Facultad de Ciencias Marinas of the Universidad Autónoma de Baja California, Baja California, Mexico (Holmgren *et al.* 1990). In addition, almost 100 of laminar specimens *Ulva* deposited in CMMEX, was also

reviewed, with the possibility to understand the history of introduction of *U. pertusa* in Mexican coast.

Examined material

La Bufadora, 10 april 1978, Ma. Elena Sosa Medina, on rocks in tide pools (CMMEX 1021); La Bufadora, 14 june 1979, Ricardo Castro González, on rocks in tide pools (CMMEX 1070); La Bufadora, 25 june 1981, Sergio Elizarrarás, on rocks in tide pools (CMMEX 1080); La Bufadora, 28 june 1981, Mario Castillo Adame, on rocks in tide pools (CMMEX 1081); Playa Tampico, 22 February 2006, R. Aguilar-Rosas, on rocks; Eréndira, 28 March 2006, R. Aguilar-Rosas, on rocks; Punta Baja, 9 March 2006, R. Aguilar-Rosas, on rocks and epizoic on mussels; Punta Baja, 5-6 September 2006, R. Aguilar-Rosas, on rocks and epizoic on mussels; Eréndira, 3 March 2007, R. Aguilar-Rosas, on rocks.

RESULTS

Morphology and reproduction

Thallus membranous blades, distromatic, yellowish-light to shiny-dark green colored, 3-16 cm tall, irregularly lobulated and oval to orbicular in shape. Blades with smooth margins slightly undulated, with frequent perforations of different sizes and shapes (Fig. 2). The base of the blades is cuneiform and thick, with characteristic concentric surface wrinkles around the discoid structure (Fig. 3). Viewed from their surface, cells are irregularly or regularly arranged in small groups, from isodiametrical to elongated and polygonal on occasions with rounded corners, 10-26 μm in length and 8-14 μm wide, with 2 (1-3) pyrenoids (Fig. 4). The cross section of the blade is 200-350 μm thick in its base, and thinner in the margins 40-60 μm , with cylindrical cells, frequently their length doubling their width, and with round apices, 20-35 μm long and 10-14 μm wide. Rhizoidal cells are round and dark, with filamentous hyaline projections oriented towards the center of the blade.

The reproductive tissue is located in the margins of the blade, limited by a thin band yellowish to olive-green colored; when the reproductive agents (spores or gametes) are released to the environment, cells are emptied. Fertile cells measure 14-20 μm in length and 10-14 μm in width viewed from the surface and 16-22 μm in length and 8-14 μm in width viewed in cross section. Twenty to thirty biflagellate gametes were observed in each fertile cell.



Fig. 2. *Ulva pertusa* specimen collected on 5 September 2006 at Punta Baja, Baja California. Scale bar = 2 cm.



Fig. 3. Detail of the concentric wrinkles in the base part of the thalli. Scale = 2 cm.

Molecular Analysis

Three samples of *U. pertusa* collected at Mexico (Mexico 7, 10 and 12) were sequenced for ITS2 analyses. The ITS2 sequences of all three samples at Mexico and Japanese *U. pertusa* (AB097653) were found to be identical. ML analysis of ITS2 sequences with JC model produced a topology shown in Fig. 5 ($-\ln L = 1830.84$). *Ulva pertusa* was clustered with *U. arasaki* Chihara and *U. lactuca* L. (51ML/57MP).

Habitat and Distribution

Plants of *Ulva pertusa* collected in Playa Tampico, Ejido

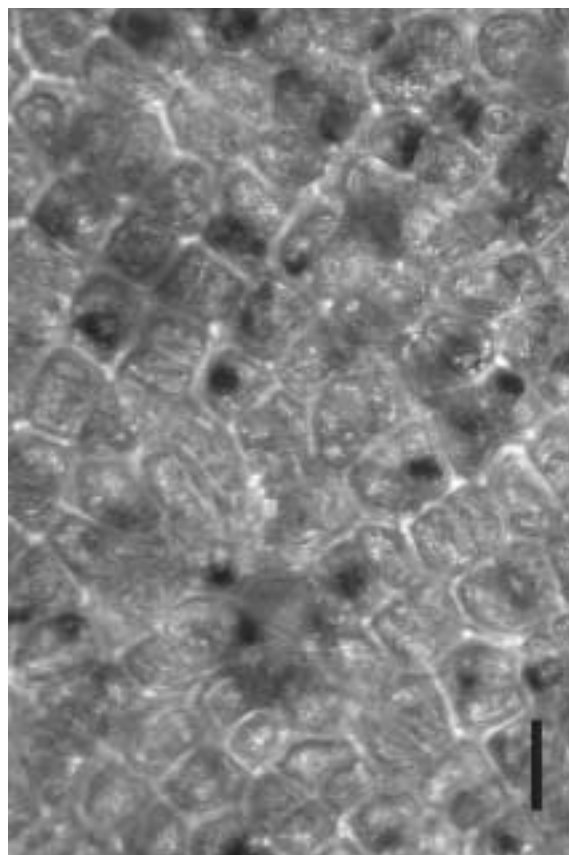


Fig. 4. Surface view of the cells isodiametrical to elongated with rounded corners, without and with local arrangement. Scale = 15 μm .

Erendira and Punta Baja, Baja California, were found growing on rocks in the intertidal zone, epiphytic on a wide variety of algae and epizotic on mussels, with thallus 5-10 cm in length, being one of the dominant species in these areas in association with *Cladophora columbiana* Collins, *Codium fragile* (Suringar) Hariot, *Ulva nematoidea* Bory Saint-Vincent, *Egregia menziesii* (Turner) Areschoug, *Halydris dioica* Gardner, *Hesperophycus californicus* P.C. Silva, *Endarachne binghamiae* J. Agardh, *Silvetia compressa* (J. Agardh) Serrão, Cho, Boo & Brawley, *Corallina vancouveriensis* Yendo, *Chondracanthus canaliculatus* (Harvey) Guiry in Hommersand, Guiry, Fredericq & Leister, *Centroceras clavullatum* (C. Agardh) Montagne, *Gastroclonium subarticulatum* (Turner) Kützing, *Pterocladia capillacea* (S.G. Gmelin) Santelices & Hommersand, *Mazzaella leptorhynchos* (J. Agardh) Leister in Hommersand, Guiry, Fredericq & Leister, *Porphyra perforata* J. Agardh, *Mazzaella affinis* (Harvey) Fredericq in Hommersand, Guiry, Fredericq & Leister, *Prionitis lanceolata* (Harvey) Harvey and meadows of sea grasses *Phyllospadix torreyi* S. Watson and *P. scouleri* Hooker. The populations stud-

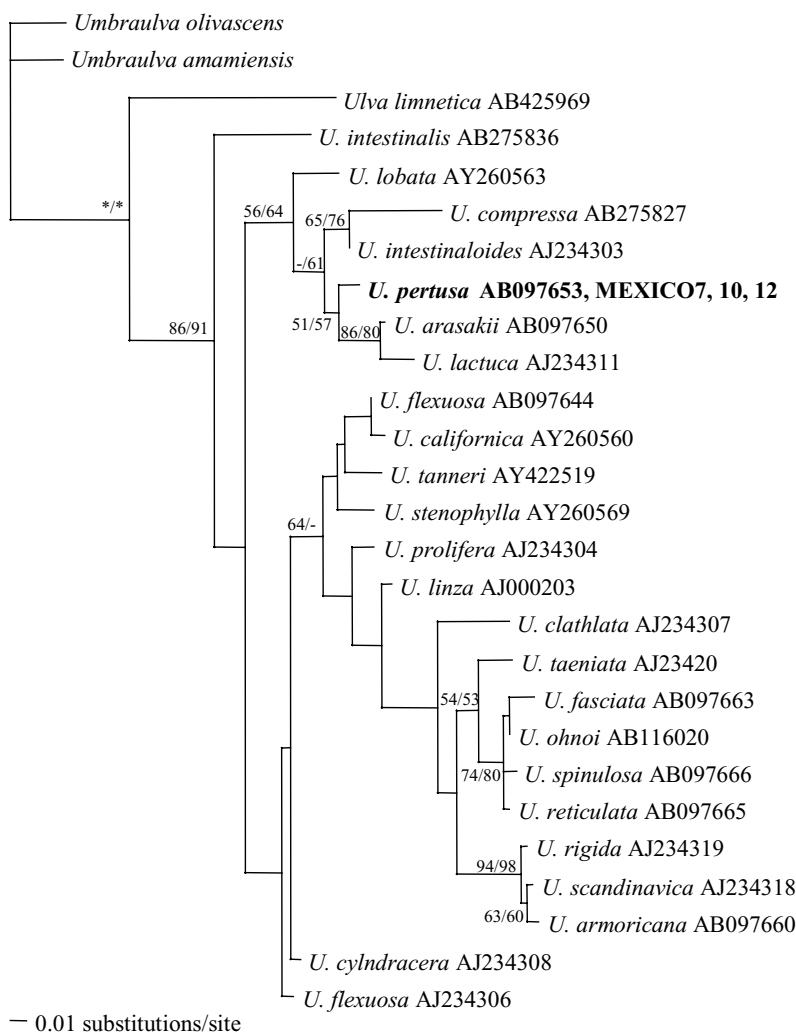


Fig. 5. Phylogenetic tree of the Maximum likelihood (ML) analysis inferred from the nuclear-encoded ITS2 region. Numerals at internal nodes are bootstrap values > 50% for 100 replicates in ML and 2000 replicates in maximum parsimony (MP) analyses (ML/MP). * = 100.

ied in Playa Tampico, Ejido Erendira y Punta Baja, are distributed across the intertidal zone, from semi sheltered to exposed to wave action, with a density of 10 to 65 thallus m^{-2} .

Revision of herbarium *Ulva* specimens of Mexican coast, shows that *U. pertusa* was misidentified under *U. rigida*, *U. lactuca* and *U. dactylifera*. The populations of *U. pertusa* are distributed in the northwest coast of Baja California, being Punta Baja their southern range limit (Fig. 1). Our periodical visits along the western coast of Baja California indicate that *U. pertusa* is present year-round, with fertile specimens occurring in February and June. Apparently this species shows a seasonal high abundance between spring and summer, more likely growing on rocky substrate in the intertidal zone.

DISCUSSION

Ulva pertusa is an Asian species originally described by Kjellman (1897) based on specimens collected in Hakodate, Yonoshima and Yokohama, Japan. It is also widely found in temperate waters around the world including the Pacific Ocean (China, Japan, Korea, Taiwan, the Philippines, Yemen, Singapore, Indonesia), the Indic Ocean (Kenya and Tanzania), the Mediterranean and the Atlantic Ocean (France and the Netherlands) (Teo and Wee 1983; Yoshida *et al.* 1990; Silva *et al.* 1996; Verlaque 2001). The geographic distribution range of *U. pertusa* was recently extended to the Pacific coast of North America with populations reported in southern California (USA), in Newport Bay, La Jolla and Mission Bay in June 1999 (Hayden and Waaland 2004). In this

study *U. pertusa* was recorded in the Pacific coast of Mexico for the first time, extending its known North American distribution range limit 450 km to the south, from Mission Bay to Punta Baja, in the central part of the western coast of Baja California, Mexico (Fig. 1).

The oldest Mexican herbarium specimens studied shows that *U. pertusa* was present in La Bufadora, Baja California until April 1978, under several names like *U. rigida*, *U. lactuca* and *U. dactylifera*. In this material and the specimens collected in this study, the morphological and reproductive characters are consistent with those described for this species in Japan (Arasaki 1984; Kamiya *et al.* 1993; Yoshida 1998; Hiraoka *et al.* 2003), the French Mediterranean (Verlaque *et al.* 2002) and Spain (Baamonde López *et al.* 2007). Among the characters distinguishing *U. pertusa* from other species are the presence of perforations mainly concentrated in its base portion, concentric wrinkles in the base of the blade (Figs 2 and 3), and the great number of reproductive agents produced by each cell (20-30) in the fertile marginal portion of the blade. On the other hand, viewed in cross section the cells are oblongated with round corners, and 2-pyrenoid cells, and blade are absent from the marginal spines. The presence of concentric wrinkles in the base and the absence of marginal spines differentiate this species from *U. rigida*, one of the most similar species to *U. pertusa* in the study area (Abbott and Hollenberg 1976; Aguilar-Rosas and Pacheco Ruíz 1983, 1986).

To date, 19 species of *Ulva* have been recognized in the Pacific coast of Mexico, 13 of which have been recorded for the west coast of Baja California (Pedroche *et al.* 2005; Aguilar-Rosas *et al.* 2005). With the presence of *Ulva pertusa* in the west coast of Baja California the number of *Ulva* species with foliar thallus increases to 9 since *U. fascista* has just been recently recorded for this same area (Aguilar-Rosas *et al.* 2005). However, a comparative study is required to define the cytological, morphological and reproductive criteria to identify the species occurring in the Mexican coasts (Aguilar-Rosas *et al.* 2005). Molecular techniques (ITS-rnDNA and plastid encoded *rbcl* sequencing) have been also useful for discriminating species in this group with species difficult to identify (Coat *et al.* 1998; Malta *et al.* 1999; Hayden and Waaland 2002; Shimada *et al.* 2003), provided the high phenotypic plasticity observed under different environmental conditions and age-related variations of its plants, which in turn are associated with their geographic location, habitat and the season of the year (Mshigeni and Kajumulo 1979; Arasaki 1984; Phillips 1984; Tanner 1986; Stewart

1989; Woolcott and King 1993; Hayden and Waaland 2002; Shimada *et al.* 2003).

The populations of *Ulva pertusa* in the study area are composed by young plants 3 to 10 cm long, and some times reproducing adult plants up to 16 cm long maximum are present. Reproducing plants were observed in repeated occasions in the tidal pools at low tide forming a cloud of reproductive agents (spores and gametes), specially in the warm months of summer and fall when temperatures can reach 24°C (Grijalva-Chon *et al.* 1985). Studies carried out in cultures have proved that the life cycle of *Ulva pertusa* is biphasic with alternating isomorphic generations (Sawada 1972; Okuda 1975). The diploid sporophyte produces tetraflagellate zoospores, whereas haploid gametophytes release biflagellate gametes (Okuda 1975; Hiraoka and Uenosono 1998). Gametes can develop through parthenogenesis (Yabu and Tokida 1960). In general, a biphasic cycle has been observed and described for other species of *Ulva* (Smith 1947; Tanner 1981; Bold and Wynne 1985; van den Hoek *et al.* 1995).

The presence of *U. pertusa* in the coast of Baja California is probably related with the commercial vessel traffic (including large Asian cargo ships), cruise ships, yachts, and sailboats coming to the port of Ensenada, in the Todos Santos Bay, from southern California (Casarrubias Garcia 2001). In Asia (Japan, Korea), *U. pertusa* is considered one of the most common species growing in the intertidal and subtidal zone on diverse substrata and epiphyte. It is considered an opportunistic species (Chihara 1990; Tokuda *et al.* 1994). Potential vectors introducing *U. pertusa* in the Pacific coast of North America (including California, USA and Baja California, Mexico) are related to the arrival of plants being part of fouling and or the presence of gametes and spores in ballast water (Carlton 1985; Sidharthan *et al.* 2004; Hewitt *et al.* 2007).

The geographical distribution of *U. pertusa* determined in this study indicates that populations are located in the northwest coast of Baja California; however, this species is expected to continue its dispersal colonizing new localities provided its great reproductive potential and the broad tolerance of plants to wide changes in temperature, salinity and light intensity (Okuda 1975; Kakinuma *et al.* 2001, 2004; Han *et al.* 2003; Kim *et al.* 2004). A similar pattern was observed in *Sargassum muticum* (Yendo) Fensholt, a species of Japanese origin introduced accidentally in the Pacific coast of North America in the 1940's (Scagel 1956), which colonized the shoreline from

Alaska to Baja California Sur, Mexico (Aguilar-Rosas and Aguilar-Rosas 1993).

The presence of *Ulva pertusa* in the west coast of Baja California brings up the necessity of undertaking population dynamics studies aimed at determining the positive or negative impacts on the communities of marine flora and fauna; and likewise, determining the economic benefits of exploiting this species that can be used for human consumption in soups and salads (Chapman 1961; Trono 1999), as a biofilter (Dongyan *et al.* 2004) and as natural feed for culturing gastropods (Alcantara and Noro 2006). Furthermore, it can be used as medicine to reduce high fever temperatures in the human body (Levring *et al.* 1969).

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