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A molecular systematic study of *Ulva* (Ulvaceae, Ulvales) from the northeast Pacific

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Species delineation in the genus *Ulva* is difficult due to a lack of distinguishing morphological characters and the high degree of phenotypic plasticity observed in these algae; thus, species descriptions are necessarily based on a limited set of characters. The present study uses molecular data to test species hypotheses for and explore species diversity in *Ulva* in the northeast Pacific. Samples of 21 taxa were collected from Valdez, Alaska to San Diego, California, additional Pacific locales outside this region, and Europe. Sequences from ITS nrDNA and the *rbc*L gene were analysed separately and simultaneously using maximum parsimony to reconstruct phylogeny. Molecular data resolve many of the species recognized by early and more recent treatments, reveal unanticipated potentially conspecific taxa and suggest that certain *Ulva* species are more widely distributed than may have been recognized previously. At least 12 species of *Ulva* were found in the northeast Pacific based on the present data: *Ulva californica, U. intestinalis, U. lactuca, U. linza, U. lobata, U. pertusa, U. prolifera, U. pseudocurvata, U. rigida, U. stenophylla, U. taeniata* and *U. tanneri.* Other *Ulva* species previously reported in this region were not encountered during the present study. In addition to providing insights into the systematics of *Ulva,* this paper indicates areas for additional research for this ubiquitous genus.

INTRODUCTION

Species of the morphologically simple green algal genus *Ulva* Linnaeus are ubiquitous members of coastal marine floras worldwide. They are found in a diversity of habitats from exposed rocky shores to protected bays and estuaries, where they can thrive attached to solid substrata or while floating freely. Many species tolerate wide ranges of salinity, temperature and water quality and grow rapidly in nutrient-rich habitats causing 'green tides' and marine fouling (Fletcher 1996). *Ulva* species are also common in experimental systems where they have been used as model organisms for studies of algal physiology (e.g. Johnston 1991; Vershinin & Kamnev 1996; Larsson & Axelsson 1999), spore adhesion (e.g. Dillon *et al.* 1989; Fletcher & Callow 1992) and as bioindicators of marine pollution (e.g. Ho 1990; Favero *et al.* 1996).

The genus *Ulva* was one of the first named by Linnaeus (1753) and initially included many unrelated algae. In the 1800s, the *Ulva* of Linnaeus was split into several genera. The name *Ulva* was maintained for green seaweeds with distromatic blades, and *Enteromorpha* Link was established for tubular green seaweeds (Link 1820). Recently, *Enteromorpha* was reduced to synonymy with *Ulva* based on molecular data (Hayden *et al.* 2003); thus, the name *Ulva* is used here to refer to this current concept of the genus (i.e. green seaweeds with distromatic blades or tubular morphologies), whereas *Ulva sensu stricto* is used to refer to the earlier concept (i.e. green seaweeds with distromatic blades only). The actual number of *Ulva* species is uncertain; however, approximately 85 are currently recognized (Guiry & NicDonncha 2002; Hayden *et al.* 2003). Refer to Tables 1 and 2 for authorities of taxa mentioned herein.

The first major treatments of these seaweeds in the northeast Pacific (Setchell & Gardner 1920a, b) recognized 13 *Ulva s.s.* and 16 *Enteromorpha* species from Alaska to southern California. Subsequent studies in this region recognized some additional taxa while reducing others to synonyms (Doty 1947; Scagel 1966; Chihara 1968; Hollenberg 1971; Norris 1971; Tanner 1979). Tanner (1979) conducted a comprehensive study of *Ulva s.s.* from northern British Columbia to southern California. He recognized six species of *Ulva s.s.* and reduced six others to synonyms. His species concepts are followed today (e.g. Gabrielson *et al.* 2000). Tanner also described a monotypic genus, *Chloropelta,* based on developmental features (Tanner 1980). Like *Enteromorpha,* the monotypic *Chloropelta* has been reduced to synonymy with *Ulva* based on molecular data (Hayden *et al.* 2003).

Assignment of tubular green seaweeds to *Enteromorpha* species in the northeast Pacific (Scagel 1966; Abbott & Hollenberg 1976; Gabrielson *et al.* 2000) was based primarily on studies of European taxa (Bliding 1963, 1968; Kornmann & Sahling 1977; Blomster *et al.* 1998, 1999). In recent accounts, six *Ulva s.s.,* six *Enteromorpha* and one *Chloropelta* species are recognized from southeast Alaska to southern California (Table 1) (Abbott & Hollenberg 1976; Gabrielson *et al.* 2000).

Initially, *Ulva* species were described entirely by morphological and anatomical characters. Although these seaweeds are morphologically simple, they exhibit considerable plasticity in response to environmental factors (Vinogradova 1974; Titlyanov *et al.* 1975; Steffensen 1976; Tanner 1979), a situation that has probably contributed to the description of a large number of described species and infraspecific taxa. Later, researchers incorporated characters considered to be more stable, including details of reproduction, development and hy-

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Table 1. Species of *Ulva* currently recognized in the northeast Pacific.

Taxon	Earlier synonym (if different)
Ulva californica Wille *in* Collins, Holden & Setchell[1,2,3]	
Ulva compressa Linnaeus[2,3]	*Enteromorpha compressa* (Linnaeus) Nees
Ulva fasciata Delile[1]	
Ulva fenestrata Postels & Ruprecht[1,3]	
Ulva flexuosa Wulfen[2,3]	*Enteromorpha flexuosa* (Wulfen) J. Agardh
Ulva intestinalis Linnaeus[2,3]	*Enteromorpha intestinalis* (Linnaeus) Nees
Ulva linza Linnaeus[2,3]	*Enteromorpha linza* (Linnaeus) J. Agardh
Ulva clathrata (Roth) C. Agardh[3]	*Enteromorpha clathrata* (Roth) Greville
Ulva prolifera O.F. Müller[2,3]	*Enteromorpha prolifera* (O.F. Müller) J. Agardh
Ulva rigida C. Agardh[1,2]	
Ulva stenophylla Setchell & Gardner[1,2,3]	
Ulva taeniata (Setchell *in* Collins, Holden & Setchell) Setchell & Gardner[1,2,3]	
Ulva tanneri Hayden & Waaland[1]	*Chloropelta caespitosa* Tanner

[1] Tanner (1979).
[2] Abbott and Hollenberg (1976).
[3] Gabrielson *et al.* (2000).

bridization potential, in combination with morphological and anatomical features, to delimit species (Føyn 1955; Cauro 1958; Dangeard 1958, 1959; Bliding 1963, 1968; Chihara 1968, 1969; Kapraun 1970; Vinogradova 1974; Tanner 1979; Koeman & van den Hoek 1981; Hoeksema & van den Hoek 1983; Womersley 1984; Phillips 1988). These studies resulted in extensive revisions, yet this group of algae continued to be systematically and taxonomically difficult (e.g. van den Hoek *et al.* 1995; Gabrielson *et al.* 2000).

Molecular data are increasingly being used to test systematic hypotheses for challenging groups such as *Ulva*. Although these data provide insights into genetic variation, delineating species boundaries remains difficult and arbitrary. Many competing species concepts have been proposed (e.g. Mayr 1969; van Valen 1976; Cracraft 1989). Although a discussion regarding the relative merits of these is beyond the scope of the present paper, it is important to recognize the concepts and criteria employed to delimit species (Luckow 1995). The present analysis follows the phylogenetic species concept described by Donoghue (1985) in which species, like other taxonomic units, are recognized as monophyletic groups based on shared derived characters. Sequence divergence between taxa within monophyletic groups is used as a secondary criterion for determining boundaries for species rank. Such an approach has been taken in the green algae (though perhaps not explicitly stated) using sequences of the fast-evolving internal transcribed spacers of nuclear ribosomal DNA (ITS nrDNA) in cases where morphological traits are highly variable. For example, Marks & Cummings (1996) delimited freshwater species of *Cladophora* Kützing (Ulvophyceae), in which they found no correlation between ITS genotypes and morphology, habitat and geographical distribution. Blomster *et al.* (1998) delimited two morphologically similar species *U. intestinalis* and *U. compressa* (as *E. intestinalis* and *E. compressa*), though they were unable to correlate ITS nrDNA data with either sample morphology or geographical distribution.

The present study uses molecular data to test species hypotheses for northeast Pacific *Ulva*. Taxa were sampled from Valdez, Alaska to San Diego, California, additional Pacific locales outside this region and Europe. Sequences from two genomic regions were sampled on the basis of their utility in previous studies: ITS nrDNA (e.g. Blomster *et al.* 1998, 1999) and the large subunit of the chloroplast-encoded RUBISCO gene (*rbc*L) (e.g. Nozaki *et al.* 1995; Hayden *et al.* 2003). ITS nrDNA and *rbc*L sequences were analysed separately and simultaneously.

The results of the present study provide a clearer understanding of the species diversity and distribution of *Ulva* in the northeast Pacific and indicate areas for further systematic study of these ubiquitous green seaweeds.

MATERIAL AND METHODS

Collection and identification of samples

Northeast Pacific *Ulva* was sampled at sites in Alaska, British Columbia, Washington, Oregon and California from 1997 to 2000 (Table 2). Efforts were made to collect samples from type localities or locations near them. Geographical coordinates were recorded using a hand-held Garmin 45 GPS unit (http://www.garmin.com). Habitat information was also recorded, including a description of available substrata, wave exposure and dominant algae (Dethier 1990).

Each sample consisted of several morphologically similar thalli collected from an area of less than 25 cm^2. Samples were wrapped in damp paper towels and stored in plastic bags in the dark at 4–15°C until detailed laboratory observations could be made. Algae were identified to species using the following references: Setchell & Gardner (1920a, b), Smith (1944), Doty (1947), Bliding (1963, 1968), Scagel (1966), Chihara (1968, 1969), Vinogradova (1974), Abbott & Hollenberg (1976), Kornmann & Sahling (1977), Tanner (1979, 1980, 1986), Koeman & van den Hoek (1981, 1982a, b), Hoeksema & van den Hoek (1983), Phillips (1988), Blomster *et al.* (1998, 1999), Dion *et al.* (1998) and Gabrielson *et al.* (2000). Characters used for identification were: (1) thallus habit (colour, shape, length, width, branching pattern and form of attachment); (2) features of cells in surface and transectional views (shape, height, width and arrangement); (3) thallus thickness; (4) number of pyrenoids per chloroplast; (5) chloroplast position in cells; (6) ecology (habitat type and location of sample in habitat); and (7) miscellaneous features (e.g. presence of fenestrae or microscopic teeth along blade

Table 2. Species and sample names of *Ulva* used in this study with source, collection details, GenBank accession numbers for the ITS nrDNA and *rbc*L sequences, and herbarium accession numbers. Culture details for University of Washington (UWCC) and University of Texas (UTEX) culture collections are included in source when available. The geographical area from which each sample was collected is indicated in the sample name: AK, Alaska; AU, Australia; BC, British Columbia; NCA, northern California, CCA, central California; SCA, southern California; CE, Chile; EU, Europe; JN, Japan; KA, Korea; OR, Oregon; TX, Texas; WA, Washington. CCA includes Marin to San Luis Obispo Counties. The ITS nrDNA sequences of European samples are from source references, and *rbc*L sequences for these samples are from the present study.

Sample name	Species	Source	Collection locality	Collection date	Latitude, longitude	ITS nrDNA	*rbc*L	Herbarium accession
Ulva armoricana EU	*Ulva armoricana* Dion, de Reviers & Coat	Coat *et al.* (1998)	Roscoff, France	NA	48°43.0′N, 4°1.0′W	NA	NA	NA
Ulva californica BC	*Ulva californica* Wille *in* Collins, Holden & Setchell	field collection	Botany Bay, Vancouver Island, BC	29 Jun. 1999	48°31.7′N, 124°27.2′W	AY422515	AY422558	344786[1]
Ulva californica WA	*Ulva californica*	field collection UWCC MA712	Cattle Point, San Juan Island, San Juan County, WA	15 Jun. 1998	48°27.0′N, 122°57.7′W	AY422518	AF499667	344789[1]
Ulva californica I OR	*Ulva californica*	field collection UWCC MA713	Fogarty Creek, Lincoln County, OR	14 May 1999	44°50.4′N, 124°3.2′W	AY422512	AY422555	344790[1]
Ulva californica I NCA	*Ulva californica*	field collection	Coast Guard Cove, Humboldt Bay, Humboldt County, CA	19 Jun. 2000	40°45.8′N, 124°13.2′W	AY422516	AY422559	344791[1]
Ulva californica II NCA	*Ulva californica*	field collection	Coast Guard Cove, Humboldt Bay, Humboldt County, CA	19 Jun. 2000	40°45.8′N, 124°13.2′W	AY422517	AY422560	344793[1]
Ulva californica I CCA	*Ulva californica*	field collection	South Point Cabrillo, Monterey, Monterey County, CA	17 Jun. 1999	36°36.6′N, 121°53.7′W	AY422513	AY422556	344795[1]
Ulva californica II CCA	*Ulva californica*	field collection UWCC MA714	Moss Landing Harbor, Monterey County, CA	18 Jun. 1999	36°48.5′N, 121°47.2′W	AY422514	AY422557	344796[1]
Ulva californica SCA	*Ulva californica*	field collection	Casa Cove, La Jolla, San Diego County, CA	14 Jun. 1999	32°50.9′N, 117°16.7′W	AY260560	AY255866	344798[1]
Ulva californica EU	*Ulva californica*	Tan *et al.* (1999), as *Ulva* sp.	Stromness Harbour, Orkney, Scotland	NA	59°0′N, 3°0′W	AJ234323	NA	E00068522[2]
Ulva clathrata EU	*Ulva clathrata* (Roth) C. Agardh	Blomster *et al.* (1999), as *E. muscoides*	Los Toruños, Puerto Réal, Cádiz, Spain	2 May 1998	36°30.0′N, 6°20.0′W	AF127170	AY422563	NA
Ulva compressa EU	*Ulva compressa* Linnaeus	Blomster *et al.* (1998), as *E. compressa*	Portaferry, Strangford Lough, N. Ireland	12 Apr. 1996	54°22.7′N, 5°34.6′W	AF035350	AY255859	NA

Table 2. Continued.

Sample name	Species	Source	Collection locality	Collection date	Latitude, longitude	ITS nrDNA	*rbc*L	Herbarium accession
Ulva fasciata TX	*Ulva fasciata* Delile	UTEX LB1859	Port Aransas, Nueces County, TX	NA	27°50.0′N, 97°3.7′W	AY422523	NA	NA
Ulva fasciata I HI	*Ulva fasciata*	field collection by L. Hodgson	Kihei, Maui County, HI	6 Feb. 2000	20°47.0′N, 156°28.0′W	AY260561	AY255867	344799[1]
Ulva fasciata II HI	*Ulva fasciata*	field collection by L. Hodgson	Maalaea, Maui County, HI	6 Feb. 2000	20°47.8′N, 156°30.9′W	AY422524	AY422565	344800[1]
Ulva fasciata AU	*Ulva fasciata*	field collection by G. Zuccarello	Tamarama, Sydney, Australia	9 Aug. 1999	33°53′S, 151°12′E	AY260567	AY255872	344801[1]
Ulva fasciata KA	*Ulva fasciata*	Tan *et al.* (1999), as *U. pertusa*	Guryongpo, east coast of Korea	NA	NA	AJ234321	NA	E00068521[2]
Ulva flexuosa EU	*Ulva flexuosa* Wulfen	Tan *et al.* (1999), as *E. flexuosa*	Sweden	NA	NA	AJ234306	NA	A00277[3]
Ulva intestinalis AK	*Ulva intestinalis* Linnaeus	field collection by T. Klinger	Sheep Bay, Valdez-Cordova County, AK	8 Jul. 1997	60°39.7′N, 146°0.5′W	AY422508	AY422552	344772[1]
Ulva intestinalis BC	*Ulva intestinalis*	field collection	Botany Bay, Vancouver Island, BC	29 Jun. 1999	48°31.7′N, 124°27.2′W	AY422506	AF499671	344773[1]
Ulva intestinalis NCA	*Ulva intestinalis*	field collection	Bodega Head, Sonoma County, CA	17 Jun. 2000	38°18.2′N, 123°3.9′W	AY422507	AY422551	344774[1]
Ulva intestinalis EU	*Ulva intestinalis*	Blomster *et al.* (1998), as *E. intestinalis*	Woolwich, Thames, London, UK	9 Nov. 1996	51°30.0′N, 0°5.0′W	AF035342	AY255860	NA
Ulva intestinaloides EU	*Ulva intestinaloides* (Koeman & van den Hoek) Hayden, Blomster, Maggs, Silva, Stanhope & Waaland	Tan *et al.* (1999), as *E. intestinaloides*	Dunbar, East Lothian, Scotland	NA	56°0′N, 2°31′W	AJ234303	NA	E00068505[2]
Ulva lactuca AK	*Ulva lactuca* Linnaeus	field collection	Petersburg, Wrangell-Petersburg County, AK	6 Sep. 1998	56°48.8′N, 132°57.6′W	NA	AY422546	344802[1]
Ulva lactuca BC	*Ulva lactuca*	field collection	Botanical Beach, Vancouver Island, BC	30 Jun. 1999	48°32.0′N, 124°26.0′W	AY422499	AY422543	344805[1]
Ulva lactuca WA	*Ulva lactuca*	field collection UWCC MA715	Cattle Point, San Juan Island, San Juan County, WA	15 Jun. 1998	48°27.0′N, 122°57.7′W	AY260562	AF499668	344806[1]
Ulva lactuca I OR	*Ulva lactuca*	Tan *et al.* (1999), as *U. californica*	Otter Crest, Lincoln County, OR	NA	44°45.7′N, 124°3.8′W	AJ234315	NA	E00068515[2]

Table 2. Continued.

Sample name	Species	Source	Collection locality	Collection date	Latitude, longitude	ITS nrDNA	*rbc*L	Herbarium accession
Ulva lactuca II OR	*Ulva lactuca*	field collection UWCC MA717	Fogarty Creek, Lincoln County, OR	14 May 1999	44°50.4′N, 124°3.2′W	AY422500	AY422544	344811[1]
Ulva lactuca III OR	*Ulva lactuca*	field collection UWCC MA718	Seal Rock, Lincoln County, OR	15 May 1999	44°30.0′N, 124°5.0′W	AY422498	AY422542	344814[1]
Ulva lactuca NCA	*Ulva lactuca*	field collection	Coast Guard Cove, Humboldt Bay, Humboldt County, CA	19 Jun. 2000	40°45.8′N, 124°13.2′W	AY422501	AY422545	344815[1]
Ulva lactuca EU	*Ulva lactuca*	Tan *et al.* (1999)	Ballyhenry Island, Strangford Lough, N. Ireland	NA	54°25.9′N, 5°31.9′W	AJ234310	AF499669	F11621[4]
Ulva linza NCA	*Ulva linza* Linnaeus	field collection	Coast Guard Cove, Humboldt Bay, Humboldt County, CA	19 Jun. 2000	40°45.8′N, 124°13.2′W	AY260557	AY255861	344776[1]
Ulva linza EU	*Ulva linza*	Tan *et al.* (1999), as *E. linza*	Ythan estuary, Aberdeenshire, Scotland	NA	57°7′N, 2°6′W	AJ000203	NA	E00068506[2]
Ulva lobata OR	*Ulva lobata* (Kützing) Harvey	field collection UWCC MA716	North Jetty, Yaquina Bay State Park, Lincoln County, OR	16 May 1999	44°37.4′N, 124°3.7′W	AY260563	AY255868	344808[1]
Ulva lobata CCA	*Ulva lobata*	field collection	San Simeon, San Luis Obispo County, CA	16 Jun. 1999	35°36.9′N, 121°9.0′W	AY422505	AY422550	344817[1]
Ulva pertusa I SCA	*Ulva pertusa* Kjellman	field collection	Lower Newport Bay, Newport, Orange County, CA	15 Jun. 1999	33°37.1′N, 117°56.0′W	AY260568	AY255873	344819[1]
Ulva pertusa II SCA	*Ulva pertusa*	field collection UWCC MA719	Perez Cove, Mission Bay, San Diego County, CA	14 Jun. 1999	32°46.0′N, 117°13.8′W	AY422502	AY422547	344822[1]
Ulva pertusa III SCA	*Ulva pertusa*	field collection UWCC MA720	Bird Rock, La Jolla, San Diego County, CA	14 Jun. 1999	32°48.9′N, 117°16.4′W	AY422503	AY422548	344824[1]
Ulva pertusa JN	*Ulva pertusa*	field collection by H. Kawai	Yura, Hyogo Prefecture, Japan	21 Mar. 2000	34°17′N, 134°57′E	AY422504	AY422549	NA
Ulva procera EU	*Ulva procera* (Ahlner) Hayden, Blomster, Maggs, Silva, Stanhope & Waaland	field collection by J. Blomster	Suomenlinna, Helsinki, Finland	NA	60°36′N, 21°25′E	NA	AY255863	NA

Table 2. Continued.

Sample name	Species	Source	Collection locality	Collection date	Latitude, longitude	ITS nrDNA	*rbc*L	Herbarium accession
Ulva procera JN	*Ulva procera*	field collection by H. Kawai	Osaka Bay, Japan	26 Apr. 2000	35°57′N, 137°16′E	AY422521	AY422562	NA
Ulva prolifera WA	*Ulva prolifera* O.F. Müller	field collection	Blakely Island, San Juan County, WA	13 Aug. 1997	48°33.7′N, 122°48.0′W	AY422511	AF499670	344778[1]
Ulva prolifera NCA	*Ulva prolifera*	field collection	Westside Road, Bodega Bay, Sonoma County, CA	17 Jun. 2000	38°18.6′N, 123°3.6′W	AY260559	AY255865	344779[1]
Ulva prolifera SCA	*Ulva prolifera*	field collection by D.M. Dexter	Red Hill Marina, Salton Sea, Imperial County, CA	5 Feb. 2000	33°22.5′N, 116°0.3′W	AY422510	AY422554	344780[1]
Ulva prolifera EU	*Ulva prolifera*	Tan *et al.* (1999), as *E. prolifera*	Ythan estuary, Aberdeenshire, Scotland	NA	57°7′N, 2°6′W	AJ234304	AY255864	E00068508[2]
Ulva pseudocurvata SCA	*Ulva pseudocurvata* Koeman & van den Hoek	field collection	North Star Beach, Newport, Orange County, CA	15 Jun. 1999	33°37.5′N, 117°53.6′W	AY422509	AY422553	344818[1]
Ulva pseudocurvata EU	*Ulva pseudocurvata*	Tan *et al.* (1999)	Ythan estuary, Aberdeenshire, Scotland	NA	57°7′N, 2°6′W	AJ234312	AY255869	E00068512[2]
Ulva reticulata HI	*Ulva reticulata* Forsskål	field collection by L. Hodgson	Lipoa Street, Kihei, Maui County, HI	6 Feb. 2000	20°47.0′N, 156°28.0′W	NA	AY422568	344825[1]
Ulva rigida OR	*Ulva rigida* C. Agardh	Tan *et al.* (1999), as *U. fenestrata*	North Boardman State Park, OR	NA	NA	AJ234316	NA	E00068516[2]
Ulva rigida EU	*Ulva rigida*	field collection by C.A. Maggs	Cádiz, Spain	NA	36°30′N, 6°20′W	AY260565	NA	344826[1]
Ulva rigida CE	*Ulva rigida*	field collection by J.R. Waaland	Pelluco Beach, SE of Puerto Montt, Chile	17 Oct. 2000	41°28′S, 72°56′W	AY422522	AY422564	344827[1]
Ulva rotundata EU	*Ulva rotundata* Bliding	Coat *et al.* (1998)	Roscoff, France	NA	48°43.0′N, 4°1.0′W	NA	NA	NA
Ulva scandinavica EU	*Ulva scandinavica* Bliding	Tan *et al.* (1999)	Langstone Harbor, Portsmouth, England	NA	50°46′N, 1°4′W	AJ234317	AY255870	E00068517
Ulva stenophylla WA	*Ulva stenophylla* Setchell & Gardner	field collection UWCC MA721	Shilshole Bay, Seattle, King County, WA	2 Jun. 2000	47°41.4′N, 122°24.0′W	AY260569	AY255874	344829[1]
Ulva taeniata CCA	*Ulva taeniata* (Setchell *in* Collins, Holden & Setchell) Setchell & Gardner	field collection UWCC MA722	Perkins Park, Monterey, Monterey County, CA	17 Jun. 1999	36°37.6′N, 121°55.1′W	AY262335	AY255875	344830[1]

Table 2. Continued.

Sample name	Species	Source	Collection locality	Collection date	Latitude, longitude	ITS nrDNA	*rbc*L	Herbarium accession
Ulva taeniata I SCA	*Ulva taeniata*	field collection UWCC MA723	Point Loma, San Diego, San Diego County, CA	14 Jun. 1999	32°41.7′N, 117°15.3′W	AY422525	AY422566	344833[1]
Ulva taeniata II SCA	*Ulva taeniata*	field collection	Point Loma, San Diego, San Diego County, CA	14 Jun. 1999	32°41.7′N, 117°15.3′W	NA	AY422567	344834[1]
Ulva tanneri CCA	*Ulva tanneri* Hayden & Waaland	field collection UWCC MA711	South Point Cabrillo, Monterey, Monterey County, CA	17 Jun. 1999	36°36.6′N, 121°53.7′W	AY422519	AF499672	344770[1]
Ulva tanneri JN	*Ulva tanneri*	field collection by H. Kawai	Kobe, Hyogo Prefecture, Japan	22 Mar. 2000	34°43′N, 135°19′W	AY260556	AY255858	NA
Ulva sp. OR	*Ulva* sp.	Tan *et al.* (1999), as *U. taeniata*	Seal Rock, Lincoln County, OR	NA	44°30.0′N, 124°5.0′W	AJ234320	NA	E00068520[2]
Ulva sp. CE	*Ulva* sp.	field collection by J.R. Waaland	Coihuin, SE of Puerto Montt, Chile	17 Oct. 2000	41°28′S, 72°56′W	AY260566	AY255871	344835[1]
Ulva sp. EU	*Ulva* sp.	Tan *et al.* (1999), as *Enteromorpha* sp.	Ythan estuary, Aberdeenshire, Scotland	NA	57°7′N, 2°6′W	AJ234308	NA	E00068510[2]
Ulva sp. NCA	*Ulva* sp.	field collection	Coast Guard Cove, Humboldt Bay, Humboldt County, CA	19 Jun. 2000	40°45.8′N, 124°13.2′W	AY422520	AY422561	344782[1]
Percursaria percursa	*P. percursa* (C. Agardh) Rosenvinge	UWCC MA230	NA	NA	NA	AY260570	AF499658	NA
Umbraulva olivascens	*Ulva olivascens* (Dangeard) Bae & I.K. Lee	field collection by C.A. Maggs	Portaferry, Strangford Lough, N. Ireland	5 May 2000	54°22.7′N, 5°34.6′W	AY260564	AY255876	344837[1]
Ulvaria obscura var. *blyttii*	*Ulva obscura* var. *blyttii* (Areschoug) Bliding	field collection	Padilla Bay National Estuarine Research Reserve, Skagit County, WA	25 Apr. 1997	48°28.7′N, 122°30.2′W	AY260571	AF499657	344838[1]

[1] University of Washington Herbarium.
[2] Royal Botanic Garden Edinburgh Herbarium.
[3] Uppsala University Herbarium.
[4] Ulster Museum Herbarium.

margin). Cell dimensions and thallus thickness were measured with an ocular micrometer at ×400–1000. Several pieces of thalli from identified samples were dried in silica gel for molecular research, and other pieces were placed in culture as described below. The remaining material was pressed for herbarium specimens.

All collections made by the present authors were placed in culture to observe reproductive and developmental characters, including spore and gamete dimensions, presence of a germination tube, number of cells in the germling at first longitudinal division, and basal development. Fertile material was field-collected, or reproduction was induced in pieces of vegetative thalli using a low-to-high-light protocol from Tanner (1979). Unialgal cultures were grown in Guillard's f/2 en-

riched seawater at 15°C in 500 ml glass culture vessels (Pyrex 3250) under 30–50 μmol m^{-2} s^{-1} cool white fluorescent light on a 16 : 8 hour light–dark photoperiod cycle.

Additional collections from Australia, Chile, Hawaii, Spain and Japan were received as silica gel–preserved specimens. Vouchers for these and field collections were deposited in the University of Washington Herbarium (WTU). These collections were compared to herbarium specimens, including many of the type specimens, from the following herbaria: OSC, UC, UBC and WTU (abbreviations follow *Index Herbariorum*: http://nybg.org/bsci/ih/ih.html). These herbarium specimens, particularly those from Tanner (1979), were valuable for interpreting species descriptions, published distributions and geographical and seasonal variation.

Outgroup taxa were chosen on the basis of results from previous studies of the Ulvaceae (Hayden & Waaland 2002).

DNA extraction, PCR amplification and sequencing

Extraction of DNA, polymerase chain reaction (PCR) amplification and sequencing methods followed Hayden *et al.* (2003). Genomic DNA for eight European taxa was supplied by Dr Christine Maggs, The Queen's University of Belfast, Northern Ireland, in order to sequence the *rbc*L gene. ITS nrDNA sequences for these taxa were published elsewhere (Table 2).

Phylogenetic analysis

Sequences for *rbc*L were aligned using Clustal X (Thompson *et al.* 1997) and edited by eye. Sequences of ITS nrDNA were aligned manually using Se–Al version 1.0a1Fat because they contained numerous substitutions and indels. The *rbc*L and ITS nrDNA alignments can be viewed at TreeBASE (http://www.treebase.org/treebase/) under the accession numbers SN1610 and SN1609, respectively. Three regions of ITS 1 and two of ITS 2 were unalignable across all taxa; however, sequences within four of these five regions were alignable within subsets of taxa. In the final alignment, these subsets were offset with corresponding gaps in unalignable sequences following the method of Steane *et al.* (1999). Using this method, all reliable data can be used to resolve relationships within subgroups as well as between these groups for higher-level resolution. One region in ITS 1 that could not be aligned with confidence was removed prior to all analyses. Pairwise percentage sequence divergence values were calculated as uncorrected p-distances for ITS nrDNA and *rbc*L sequences independently. For the ITS nrDNA data set, only those regions alignable across all taxa were included in calculations; thus, divergence values are approximate.

Maximum parsimony (MP) analyses were performed for the ITS nrDNA, *rbc*L and combined data sets using PAUP* version 4.0b8 (Swofford 1999). Prior to analyses of combined data, the incongruence length difference test (ILD) of Farris *et al.* (1994), implemented in PAUP* as the partitions homogeneity test, was performed. This test assesses heterogeneity among user-designated data partitions, in this case the ITS nrDNA vs *rbc*L genes. A nonsignificant result indicates that user-designated data partitions are not significantly different from random partitions of the combined data set. Congruent data partitions may then be combined in a single phylogenetic analysis (de Quieroz *et al.* 1995; Huelsenbeck *et al.* 1996).

Nucleotide positions and character state changes were weighted equally, and gaps were coded as missing data in all analyses. In analyses of the independent data sets, heuristic searches were performed with tree bisection–reconnection (TBR), MulTrees and steepest descent options in effect. Ten replicate searches with randomized taxon input were conducted using the random stepwise-addition option to avoid local optima of most-parsimonious trees (MPT). In analyses of combined data, heuristic searches were performed with TBR and steepest descent (MulTrees off) with 100 replicate searches of randomized taxon input. The ITS nrDNA, *rbc*L and combined data sets were bootstrapped 1000 times (Felsenstein 1985), using closest addition sequence, TBR and steepest descent (MulTrees off), to assess relative support for branches.

The MulTrees option was turned off in parsimony analysis of the combined data and in bootstrap analyses because saving multiple minimal trees for branch swapping proved to be computationally prohibitive. DeBry & Olmstead (2000) and Mort *et al.* (2000) showed that bootstrap analysis with reduced search effort did not inflate bootstrap values. Rather, it resulted in generally lower bootstrap values for nodes with less than 90% bootstrap support (DeBry & Olmstead 2000), or bootstrap values (Mort *et al.* 2000) similar to more rigorous searches.

RESULTS

Boundaries of ITS 1, ITS 2 and 5.8S nrDNA follow Hayden *et al.* (2003). The length of these combined regions ranged from 492 to 540 nucleotides for surveyed taxa. The aligned sequence length without offsetting the highly variable regions in ITS 1 and ITS 2 (see Material and Methods) was 478 nucleotide positions. The alignment including offset regions was 1055 positions. Size ranges of ITS 1, ITS 2 and the 5.8S gene are comparable with other taxa in the Ulvophyceae (Bakker *et al.* 1995a, b; van Oppen 1995; Friedl 1996). Alignment of *rbc*L sequences resulted in 1354 nucleotides, which includes 95% of the gene and required no gaps. This length is similar to most Ulvales sequenced to date and differs from other Ulvophyceae by one amino acid (see Hayden & Waaland 2002 for further discussion).

Estimated sequence divergence of ITS nrDNA ranged from 0% between conspecifics to 11.9–16.8% between ingroup and outgroup taxa. The G + C content of sequences ranged from 59.9 to 66.4%. Nucleotide bias in ribosomal DNA has been shown to confound phylogenetic analysis based on those sequences by grouping taxa with similarly biased compositions when they do not share a recent common ancestor (Hasegawa & Hashimoto 1993); however, a chi-squared test of homogeneity of base frequencies across surveyed taxa was not significant (P = 1.0). Sequence divergence values in the *rbc*L data set ranged from 0% among conspecifics to 3.7–4.9% between ingroup taxa and designated outgroups.

Parsimony analysis of 478 nucleotide positions of ITS nrDNA, which excluded the highly variable regions, yielded 30 equally MPT of 367 steps [Fig. 1a; consistency index (CI) excluding uninformative sites = 0.578, retention index (RI) =

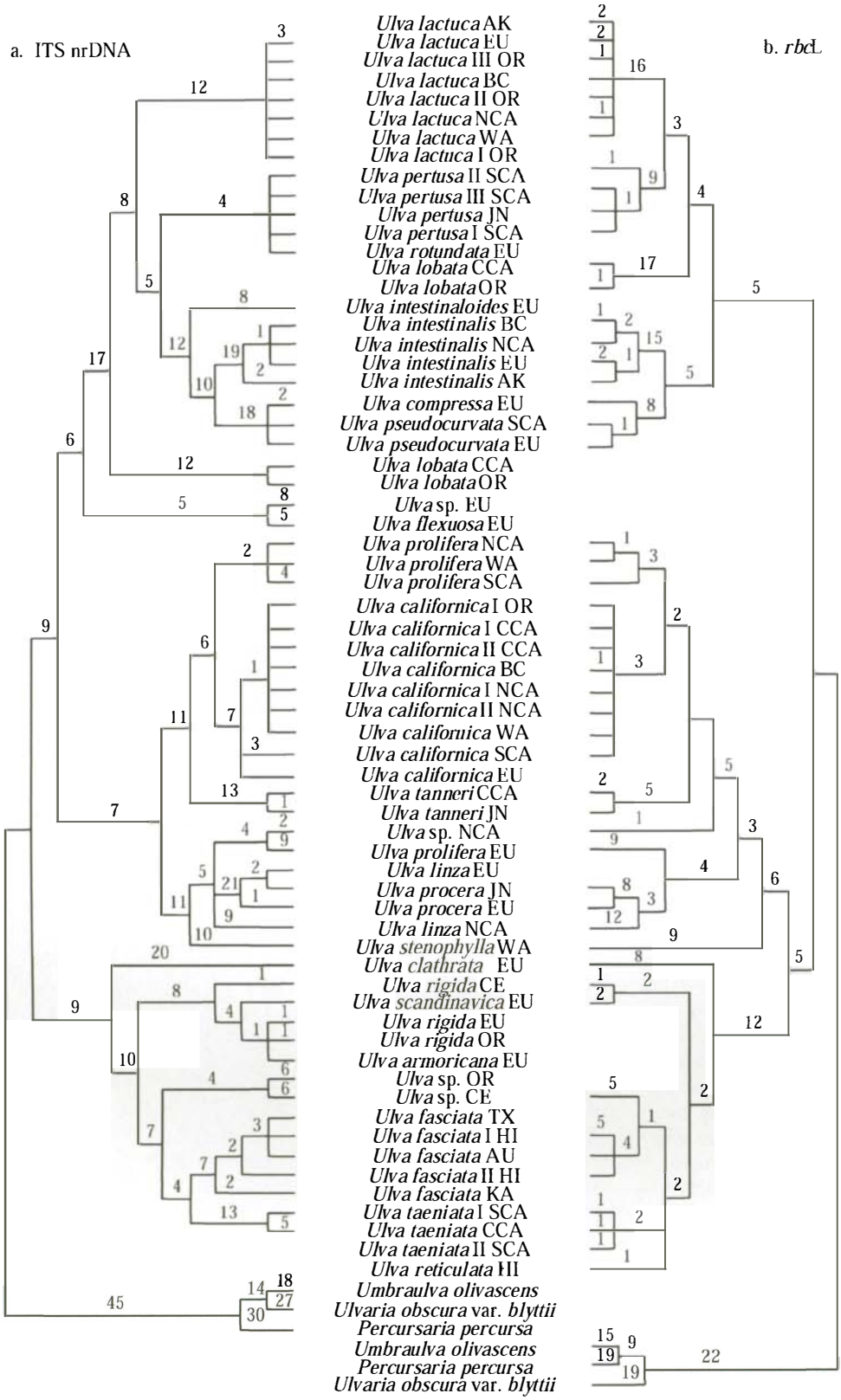

Fig. 1. a. One of 30 most-parsimonious trees of 367 steps derived from cladistic analysis of ITS nrDNA sequence data (478 nucleotide positions). b. One of 16 most-parsimonious trees of 320 steps resulting from cladistic analysis of *rbc*L sequences. Numbers above branches indicate branch lengths. Geographical abbreviations are as in Table 2. The clade comprising *U. lobata* OR and *U. lobata* CCA appears twice because it occupies different locations in the two trees.

0.886]. Parsimony analysis of *rbc*L sequences resulted in 16 MPT of 320 steps (Fig. 1b; CI excluding uninformative sites = 0.565, RI = 0.889). The ITS nrDNA and *rbc*L trees are similar though not identical. Strict consensus trees show that many similar well-supported nodes (> 80% bootstrap values) are resolved in both analyses (Fig. 2); however, some nodes are strongly supported in one tree and are weakly supported or unresolved in the other. These 'soft incongruencies' (Seelanan *et al.* 1997) explain much of the topological differences between trees. For example, the clade consisting of *U. lobata* OR and *U. lobata* CCA appears in two different locations in the strict consensus trees, but its position in the *rbc*L tree is not well supported. Similarly, relationships among outgroup taxa differ between trees, but nodes are not well supported in either tree. The remaining topological differences can be explained by differences in surveyed taxa.

The results of the ILD test indicated that the length of the original partition (ITS nrDNA vs *rbc*L) was not significantly different from random partitions of characters from the combined data set ($P = 0.44$). Given this result, the two data sets were combined for further phylogenetic analysis. Parsimony analysis of combined data, including 478 nucleotide positions of ITS nrDNA plus 1354 *rbc*L positions, resulted in 95 trees of 695 steps (not shown; CI excluding uniformative sites = 0.540, RI = 0.884). Of the 1832 characters, 235 were parsimony informative. The tree based on combined data is more resolved than the ITS nrDNA- or *rbc*L-based trees, and bootstrap values in the combined data tree are generally higher.

Sequences in the highly variable regions of ITS 1 and ITS 2 were alignable within five clades in the combined data tree (see Fig. 3, Clades I–V). Clade I was subdivided into smaller groups of taxa (Ia–If) for alignment in one region of ITS 1 in which sequences were not clearly alignable across all Clade I taxa. Outgroups were aligned together as a separate group (VI). This method of aligning variable sequence regions within subgroups incorporates assumptions about relationships between taxa. Thus, primarily moderate and well-supported nodes were used to delimit alignment groupings. Clades I–V and Clade I subgroups received > 50% bootstrap support in the separate and combined data trees, with the exception of Clade I which received < 50% bootstrap support in the *rbc*L tree, and Clade II which does not exist in the *rbc*L tree because the gene was not sequenced in these taxa. The combined data set was reanalysed including these aligned regions. Of the 2401 characters included, 299 were parsimony informative. MP analysis resulted in 98 trees of 860 steps (Fig. 3; CI excluding uniformative sites = 0.570, RI = 0.885). The topology of this tree is the same as that resulting from analysis of the smaller combined data set (not shown). Bootstrap values in the two trees also were similar though support for certain nodes was slightly higher (e.g. Clade III) or lower (e.g. Clade II) in the tree based on the reanalysed data.

DISCUSSION

Phylogenetic analysis of ITS nrDNA, *rbc*L and combined sequences yielded similar trees. Numerous clades are well supported, particularly in the combined data trees. Some of these clades comprise geographic isolates of recognizable taxa, such as *U. californica* and *U. intestinalis*. Sequence divergence values within these clades are relatively low, up to 0.7% in ITS nrDNA and up to 0.4% in *rbc*L. This range for ITS nrDNA sequences is comparable to that in a previous study of *U. clathrata* [as *E. muscoides* (Blomster *et al.* 1999)] and is smaller than that in a previous study of *U. intestinalis* and *U. compressa* in which intraspecific divergence was as large as 2.3% [as *E. intestinalis* and *E. compressa* (Blomster *et al.* 1998)]. Other well-supported terminal clades comprise multiple taxa, such as *U. pertusa* with *U. rotundata*.

Each of the currently recognized northeast Pacific *Ulva* species is discussed below in the order that they appear in the trees. Percent bootstrap support values for clades in the tree based on the larger combined data set (Fig. 3) are noted in parentheses. This discussion is summarized Table 3. This table also includes references to taxonomic treatments detailing the morphological and developmental traits that best characterize each species based on observations by the present authors. For a discussion of morphological synapomorphies, see Hayden *et al.* (2003).

Clade I

Postels & Ruprecht (1840) established the species *U. fenestrata* based on algae with perforated, expanded blades collected in Kamchatka. Although subsequent research suggested that the perforations are a result of herbivory (Vinogradova 1974; Tanner 1979), Tanner (1979) recognized this species and considered all expanded, distromatic blades in the northeast Pacific as *U. fenestrata,* a view that has persisted to the present day (e.g. Gabrielson *et al.* 2000). Such expanded blade algae from the present study appear in several clades: Clade Ia (100%) includes algae from Alaska to northern California and *U. lactuca* from Europe; Clade Ib (100%) comprises algae from southern California, *U. pertusa* from Japan and *U. rotundata* from Europe; Clade Ic (100%) comprises algae from central California and Oregon; and Clade If (100%) includes an alga from southern California and *U. pseudocurvata* and *U. compressa* from Europe. Sequence divergence between these clades is outside the range of conspecifics, whereas divergence between taxa within Clades Ia, Ib, Ic and If is < 0.5% for ITS nrDNA and < 0.2% in *rbc*L sequences. Strong bootstrap values for these clades, coupled with relatively high sequence divergence between clades and low sequence divergence between taxa within them, suggest that there are several genetically distinct algae in the northeast Pacific that Tanner (1979) and current treatments of expanded blade algae (Scagel *et al.* 1993; Gabrielson *et al.* 2000) recognize as *U. fenestrata.* This finding of potentially cryptic species is not unique to this group of algae (e.g. Stiller & Waaland 1993).

ULVA LACTUCA (CLADE IA): Tan *et al.* (1999) sequenced several geographically distinct isolates of *U. lactuca,* the type species of *Ulva*. One of these was included here for comparison purposes. In Clade Ia, algae first identified as *U. fenestrata* group with *U. lactuca.* According to Setchell & Gardner (1920b), *U. lactuca* is distributed from Alaska to the Gulf of California, and *U. fenestrata* is distributed from southeast Alaska to Puget Sound, Washington. Abbott & Hollenberg (1976) included *U. lactuca* in their treatment of the California flora. Doty (1947), Scagel (1966) and Tanner (1979) recognized *U. fenestrata* but not *U. lactuca* in their studies of *Ulva* from Alaska to Oregon. Tanner (1979) conducted a very lim-

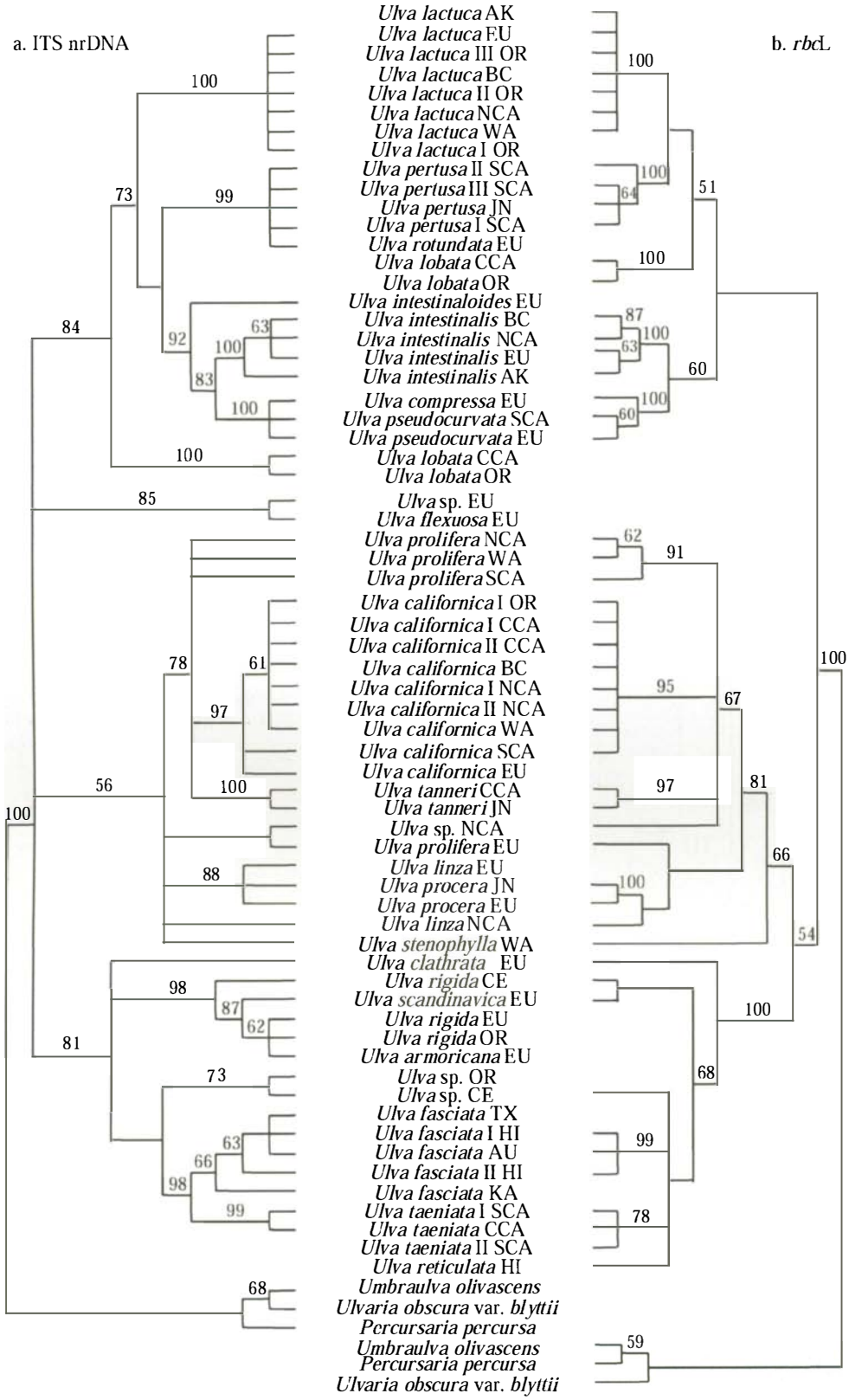

Fig. 2. Comparison of strict consensus trees derived from: a. ITS nrDNA (478 nucleotide positions); and b. *rbc*L sequences. Bootstrap percentages (> 50%) are shown above branches. Species identification and geographical abbreviations are as in Table 2. The clade comprising *U. lobata* OR and *U. lobata* CCA appears twice because it occupies different locations in the two trees.

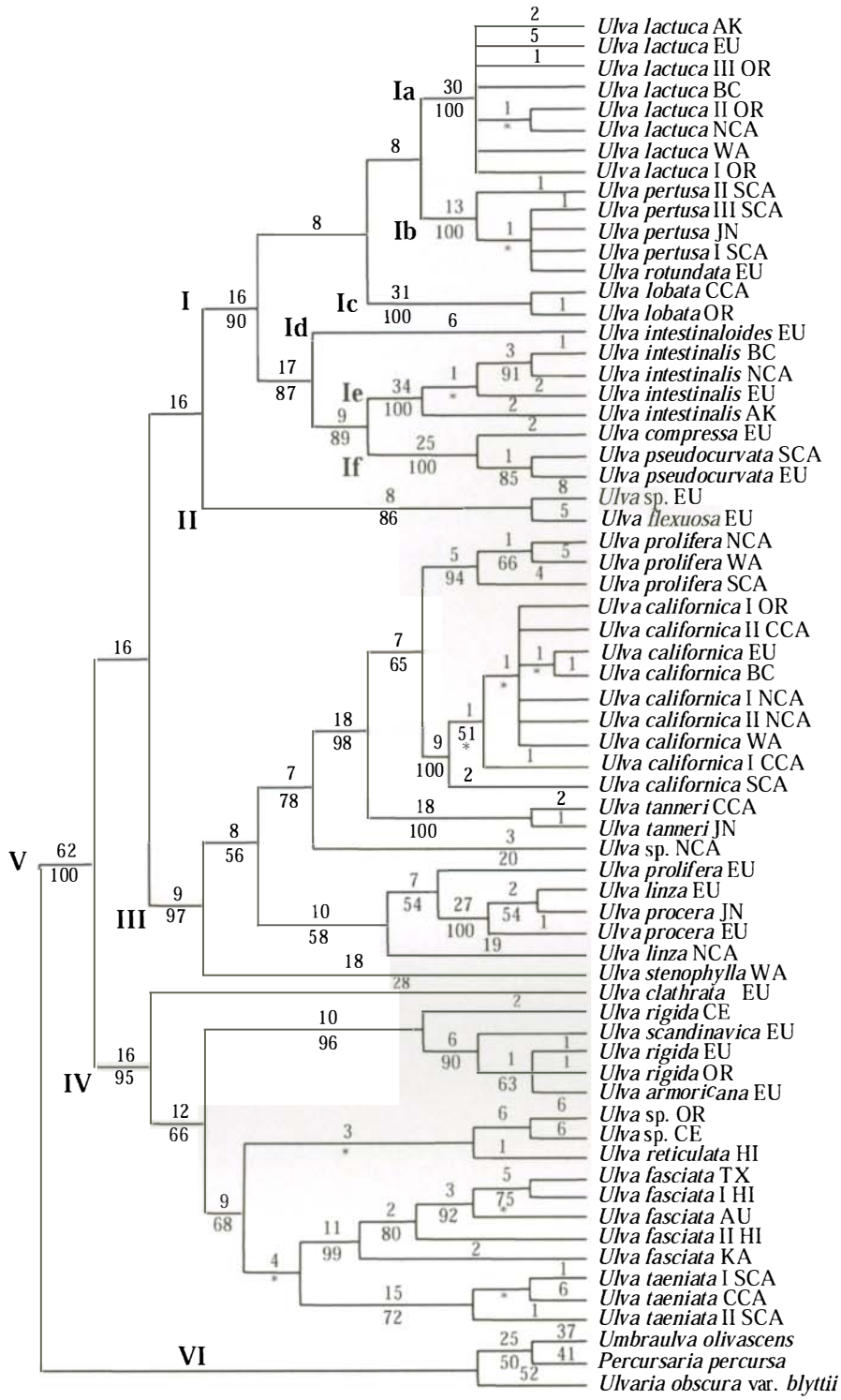

Fig. 3. One of 98 most-parsimonious trees of 860 steps derived from the reanalysed combined data set including ITS nrDNA (1047 nucleotide positions) and *rbc*L sequences. Branch lengths are indicated above branches and bootstrap percentages (> 50%) are shown below. Asterisks denote branches that collapse in the strict consensus tree. Roman numerals I–VI correspond to clades in which subsets of taxa were aligned in highly variable regions of ITS 1 and ITS 2. Groups Ia–If are subgroups within Clade I that correspond to alignment groupings in one region of ITS 1 in which sequences were not alignable across all Clade I taxa. Species identification and geographical abbreviations are as in Table 2.

Table 3. Summary of northeast Pacific *Ulva* species recognized in the present study.

Taxon	Observed northeast Pacific distribution	Reference for identification	Comments
Ulva californica	British Columbia to southern California	Tanner (1986)	synonymy with *U. angusta* Setchell & Gardner and *U. scagelii* Chihara is supported. Range extended to Scotland. Exhibits a variety of morphological forms from tufted, cuneate blades in upper littoral to lanceolate/oblanceolate blades in midlittoral zone. Development is distinct from other *Ulva* species.
Ulva fasciata	not collected in this region	Womersley (1984)	phylogenetically distinct from *U. taeniata*. According to Tanner (1979), *U. fasciata* can be distinguished by the uniform thickness, from the central axis to margin in transection, of multiple planular lacinae.
Ulva intestinalis	Alaska to southern California	Blomster *et al.* (1998), as *E. intestinalis*	common in the northeast Pacific, particularly in freshwater seeps in the upper littoral zone. Plants are tubular or flattened, usually unbranched and have apically oriented chloroplasts in surface view.
Ulva lactuca	Alaska to central California	Bliding (1968)	includes plants resembling typical *U. fenestrata*, i.e. expanded blades with perforations growing in subtidal and intertidal zones. Small, upper littoral plants may be difficult to distinguish from *U. californica* without observing development.
Ulva linza	Alaska to southern California	Abbott & Hollenberg (1976), as *E. linza*	monophyly of northeast Pacific and European *U. linza* not strongly supported. Common in the northeast Pacific midtidal to low intertidal zones. Plants have a tubular or compressed base with distromatic distal blade portion that often maintains hollow margins.
Ulva lobata	Oregon to central California	Setchell & Gardner (1920b)	plants are lobed or divided, attenuate to a cuneate, crispate base. Mature blades are noticeably thicker in the centre than at margins.
Ulva pertusa	southern California	Shimada *et al.* (2003)	range extended to northeast Pacific. Plants have distinctive large, rounded cells. European *U. rotundata* may be conspecific with *U. pertusa*.
Ulva prolifera	Washington to southern California	Abbott & Hollenberg (1976), as *E. prolifera*	northeast Pacific and European *U. prolifera* not monophyletic. Northeast Pacific accessions similar to European *U. radiata* (J. Agardh) Hayden, Blomster, Maggs, Silva, Stanhope & Waaland [as *E. radiata* J. Agardh, in Koeman & van den Hoek (1982), and as *E. prolifera* subsp. *radiata* (J. Agardh) Bliding, in Bliding (1963)]. Common in sheltered habitats. Plants have distinct, tubular or compressed main axis with numerous lateral branches. Chloroplast fills cell in surface view.
Ulva pseudocurvata	southern California	Koeman & van den Hoek (1981)	observed only drift, fragmented specimen with missing base. Large (>40 cm), expanded blade is membranous and lubricous.
Ulva rigida	Oregon	Bliding (1968)	*Ulva armoricana* and *U. scandinavica* are probable synonyms. Expanded blades have distinctive microscopic, marginal teeth.
Ulva stenophylla	Washington	Gabrielson *et al.* (2000)	relatively rare in the northeast Pacific. Plants are linear with undulate margins and planular central axis.
Ulva taeniata	Oregon to southern California	Abbott & Hollenberg (1976)	synonymy with *U. dactylifera* Setchell & Gardner and *U. nematoidea* Bory [as *U. costata* (Howe) Hollenberg] is supported. Blades are divided into narrow, often spirally twisted lacinae that are thicker along the central axis than at margins. Blades often marginally dentate.
Ulva tanneri	central California	Tanner (1980), as *Chloropelta caespitosa*	potentially rare in the northeast Pacific. Superficially resembles *U. californica*; however, has distinctive peltate blade and unique development.

ited hybridization experiment with European *U. lactuca* and algae he identified as northeast Pacific *U. fenestrata,* and interpreted a delay in fusion and a small number of fused gametes as a negative mating response (following Bliding 1963). Based on results from this study, Tanner's conclusion that *U. lactuca* does not occur in the northeast Pacific is rejected. Phylogenetic analyses suggest that some algae in this region considered to be *U. fenestrata* are conspecific with *U. lactuca.*

Ulva lactuca collections in Clade Ia represent what is considered typical northeast Pacific *U. fenestrata,* except *U. lactuca* III OR and *U. lactuca* BC, which are small plants from the upper littoral zone. These accessions fit Tanner's (1979) concept of northeast Pacific *U. conglobata,* an alga with similar morphology to *U. californica,* which he suggested may be a high intertidal form of *U. fenestrata.* Given their position in Clade Ia, these collections represent high intertidal forms of northeast Pacific *U. lactuca.*

Ulva lactuca II OR (as *U. californica* in Tan *et al.* 1999) in Clade Ia is believed to originally have been misidentified by Tan *et al.* (1999). Given the morphological plasticity ex-

hibited by *U. lactuca* and *U. californica,* overlapping distribution ranges and ecology [as *U. fenestrata* and *U. californica* in Tanner (1979, 1986) and Gabrielson *et al.* (2000)], it is not unreasonable that an individual of *U. lactuca* would be misidentified as *U. californica.* Further, the definitive characters that separate these species are developmental, yet *U. lactuca* II OR was not placed in culture prior to identification (Tan *et al.* 1999).

ULVA PERTUSA (CLADE IB): Three *Ulva* samples from southern California have nearly identical ITS and *rbc*L sequences to *U. pertusa* collected in Japan. Furthermore, the large size and rounded shape of cells in these Californian collections are consistent with those of *U. pertusa* (Yoshida 1998; Shimada *et al.* 2003), although there is considerable overlap in these characters across *Ulva* taxa (Vinogradova 1974; Titlyanov *et al.* 1975; Steffensen 1976; Tanner 1979; Phillips 1988). Based on these molecular and morphological data, the southern California samples are considered to be *U. pertusa.* This species has been previously reported from the Mediterranean, northwest and southwest Pacific and Indian Ocean. The California collections extend the range of *U. pertusa* to the northeast Pacific.

Ulva rotundata from Europe is also found in this *U. pertusa* clade, a grouping similar to that found in a recent study of northwest Pacific *Ulva* (Shimada *et al.* 2003). Both species are described as having large, rounded cells with chloroplasts located at the outer or side walls (Bliding 1968; Shimada *et al.* 2003); however, *U. rotundata* is considered to be restricted to the northeast Atlantic and Mediterranean. Bliding (1968) established *U. rotundata* on the basis of its large, rounded cells and its development in culture, in which the upper thallus grows into a discoid blade with a well-developed stipe. Subsequent authors (Hoeksema & van den Hoek 1983; Coat *et al.* 1998) further noted that *U. rotundata* thalli have a 'metallic gloss' not present in other *Ulva* species. Collections from southern California did not follow the developmental pattern for *U. rotundata* noted in Bliding (1968). A metallic gloss was also not recorded; however, this trait may have been overlooked because this character was not noted in any accounts of northeast Pacific *Ulva.* Given these discrepancies in development and limited sampling, additional comparative studies of European *U. rotundata* and *U. pertusa* are necessary to test the hypothesis that these species are conspecific.

ULVA LOBATA (CLADE IC): Clade Ic comprises two collections: obovate, lobed plants collected from the lower littoral zone in Oregon (*U. lobata* OR) and tufted plants with deeply divided blades collected from the upper littoral zone in central California (*U. lobata* CCA). Prior to Tanner's (1979) study, northeast Pacific *Ulva* specimens with expanded blades were divided into several species, including *U. expansa, U. fenestrata, U. lactuca* and *U. lobata.* Thallus habit and ecology of *U. lobata* OR is similar to Setchell & Gardner's (1920b) description and collections of *U. lobata.* Furthermore, the anatomy of this collection is similar to *U. lobata* CCA and overlaps that of *U. lobata,* though the same caveat applies here as with *U. pertusa* discussed above. Phylogenetic analyses suggest that these algae are distinct from other expanded-type samples. Based on this evidence, these algae are considered to be *U. lobata,* contrary to Tanner's (1979) suggestion that this taxon be synonymized with *U. fenestrata.* Morphological differences between these collections are probably a result of environmental conditions because there is a general trend in *Ulva* towards smaller thalli where desiccation is greater (Tanner 1986; H.S. Hayden, personal observation).

ULVA INTESTINALIS (CLADE IE): *Ulva intestinalis* (as *E. intestinalis*) was among the 16 *Enteromorpha* taxa recognized by Setchell & Gardner (1920a, b), and it is recognized in the northeast Pacific flora to the present day (Gabrielson *et al.* 2000). A recent study of European *U. intestinalis* showed this taxon to be genetically distinct from the morphologically similar *U. compressa* [as *E. intestinalis* and *E. compressa* (Blomster *et al.* 1998)]. The present study, including *U. intestinalis* collections from Alaska, British Columbia, northern California and Europe, further supports this conclusion. These collections group with strong bootstrap support (100%). Sequence divergence among them is near the upper limit for conspecifics in this study, up to 0.7% in ITS nrDNA and up to 0.4% in *rbc*L sequences, but this ITS nrDNA value is within the bounds observed by Blomster *et al.* (1998). This taxon is common in the northeast Pacific, particularly in freshwater seeps in the upper littoral zone.

ULVA PSEUDOCURVATA (CLADE IF): In Clade If, ITS nrDNA and *rbc*L sequences for an alga collected in southern California are identical to those of *U. pseudocurvata* from Europe. As with *U. lactuca,* Tan *et al.* (1999) sequenced several samples of this taxon, and one was included in the present study for comparative purposes. *Ulva pseudocurvata* SCA was collected as a drift specimen from mudflats in Newport Bay, California. It is typical of *Ulva* frequently encountered in protected bays in the northeast Pacific (H.S. Hayden, personal observation), but these blades are atypical of *U. fenestrata sensu* Setchell & Gardner (1920b). The thallus morphology and anatomy of *U. pseudocurvata* SCA overlaps that described for European *U. pseudocurvata* (Koeman & van den Hoek 1981); however, the characteristically curved nature of *U. pseudocurvata* thalli was not readily apparent in this collection because the base of the plant was absent. Reproductive and developmental characters could not be compared for these taxa because pieces of the *U. pseudocurvata* SCA thallus persisted in a vegetative state in culture despite light and temperature alterations. Given that sequences for these taxa are identical and that the morphology of this sample is atypical for *U. fenestrata sensu* Setchell & Gardner (1920b), it is reasonable to refer this alga to *U. pseudocurvata.* Until now, this taxon has been considered a northeast Atlantic species.

The presence of *U. compressa* (formerly *E. compressa*) in this clade and the observed low ITS and *rbc*L sequence divergence values between this species and *U. pseudocurvata* are discussed in Tan *et al.* (1999) and Hayden *et al.* (2003).

Clade III

ULVA PROLIFERA: Setchell & Gardner (1920b) reported the presence of *U. prolifera* (as *E. prolifera*) in the northeast Pacific, and this alga has been reported by more recent authors (e.g. Doty 1947; Abbott & Hollenberg 1976; Gabrielson *et al.* 2000). It is relatively common in this region in protected bays and estuaries and can become quite prevalent in these environments in late summer in Puget Sound, Washington (Hayden & Waaland 1998, 1999). Collections of *U. prolifera* from

Washington and northern and southern California form a well-supported clade (94%); however, this clade does not include the European sample of *U. prolifera*, which is only distantly related. The northeast Pacific collections of *U. prolifera*, particularly *U. prolifera* WA and *U. prolifera* NCA, are typical *U. prolifera sensu* Abbott & Hollenberg (1976) and Gabrielson *et al.* (2000). When compared to descriptions in the European literature, these accessions fit the description of *E. prolifera* subsp. *radiata* (Bliding 1963), which is recognized in a more recent study by Koeman & van den Hoek (1982b) as *E. radiata*. Given this similarity, it is hypothesized that northeast Pacific *U. prolifera* is conspecific with European *U. radiata*; however, molecular data for European *U. radiata* are needed to test this hypothesis.

ULVA CALIFORNICA: Tanner (1979, 1986) investigated morphological variation in *U. californica* through morphological studies of field and herbarium material and culture studies. He concluded that *U. californica* is a distinct species with a wide range of thallus size and habit, from a tufted, cuneate form that grows in the upper littoral zone to a lanceolate to oblanceolate blade that grows in the midlittoral zone. As a result, he synonymized two taxa with *U. californica*: *U. angusta* and *U. scagelii* (Tanner 1986). Descriptions of these species differ primarily in thallus shape and size: *U. angusta* has linear to oblanceolate blades to 15 cm long and 1.5 cm wide, *U. californica* has a triangular or reniform thallus to 2 cm long and 1.5 cm wide, and *U. scagelii* has a linear to oblanceolate or cuneiform thallus to 16 cm long and 7 cm wide. Plants fitting the descriptions for typical *U. californica* (*U. californica* WA, *U. californica* I OR, *U. californica* I NCA, *U. californica* I CCA, *U. californica* SCA), *U. angusta* (*U. californica* BC, *U. californica* II NCA) and *U. scagelii* (*U. californica* II CCA) were collected for the present study. Phylogenetic analysis yielded a strongly supported (100%) clade comprising all these collections. Within this clade there is little sequence divergence in either ITS nrDNA (< 0.4%) or *rbc*L (< 0.1%). Molecular data appear to support Tanner's conclusions; however, no collections were made at Moss Beach, California, or Kitsilano Beach, Vancouver, Canada, the type localities for *U. angusta* (Tanner 1986) and *U. scagelii* (Chihara 1968), respectively. Thus, algae fitting the descriptions of these species should be collected from their type localities and sequenced before Tanner's hypotheses can be verified with molecular data.

Tanner (1986) considered *U. californica* to be a distinct species on the basis of its development in culture. This species produces a germination tube and extensive basal rhizoidal discs, though Tanner found that these traits may be influenced by growth conditions. These traits were observed in all cultured samples in this clade, including *U. californica* SCA, which was collected in La Jolla, California, the type locality.

Prior to this study, the distribution of *U. californica* was considered to be restricted to the northeast Pacific. *Ulva californica* EU (as *Ulva* sp. in Tan *et al.* 1999) groups with other *U. californica* samples in all trees with strong bootstrap support (97–100%) and has sequence divergence from other members of this clade within the range of conspecifics as noted above; thus, this collection extends the distribution of *U. californica* to Stromness Harbour, Scotland.

ULVA TANNERI: Tanner (1979, 1980) established the monotypic genus *Chloropelta* on the basis of its unique developmental pattern, which is very distinctive in culture. Using ITS nrDNA and *rbc*L sequence data, Hayden *et al.* (2003) showed that these developmental traits were not synapomorphies and transferred *C. caespitosa* Tanner to *Ulva* as *U. tanneri*. *Ulva tanneri* has been reported from as far away as South Africa and Japan, but its distribution in the northeast Pacific was considered to be restricted to southern California until the present study, in which *U. tanneri* CCA was collected at Monterey. Although it was not distinguishable from neighbouring *U. californica* in the field, its peltate blade was identified upon closer inspection, and its development in culture confirmed this identification. Early in development, cells in the tubular germling of *U. tanneri* undergo one division producing a distromatic tubular germling not seen in other Ulvaceae. Rupture of the apical end of the germling and continued growth eventually result in a peltate, distromatic blade (Tanner 1980). Development was not observed in *U. tanneri* from Japan.

Ulva tanneri is recognized here as a distinct species on the basis of strong bootstrap support (100%) for the node uniting the California and Japan collections and the relatively large divergence values between sequences for these collections and other surveyed taxa: > 3% for ITS nrDNA and > 0.7% for *rbc*L.

ULVA LINZA: Setchell & Gardner (1920b) reported the occurrence of *U. linza* in the northeast Pacific. This alga is very common in this region; however, difficulties in PCR amplification prevented the inclusion of more than one sample in the present study. *Ulva linza* NCA groups with *U. linza*, *U. procera* and *U. prolifera* from Europe and Japan, but support for the node uniting these taxa is weak (58%). Sequence divergence between *U. linza* NCA and the remaining taxa in this clade is approximately 2.0% for ITS nrDNA and > 1.5% for *rbc*L. Although the ITS nrDNA value is within the range for *U. intestinalis* and *U. compressa* (Blomster *et al.* 1998), the *rbc*L value is well above the divergence among conspecifics observed in the present study. This suggests that northeast Pacific *U. linza* may not be conspecific with European *U. linza*; however, additional European and northeast Pacific samples should be sequenced to confirm this finding.

European *U. linza* groups with *U. procera* from Europe and Japan with strong bootstrap support (100%), and there is low sequence divergence between these collections. This result is surprising given the different morphologies of these two taxa, yet is consistent with a previous study including only the European samples (Tan *et al.* 1999).

ULVA STENOPHYLLA: Setchell & Gardner (1920a) described *U. stenophylla* on the basis of morphologically distinct plants growing in the lower littoral and upper sublittoral zones in central California. Subsequent authors recognized this species (e.g. Doty 1947; Scagel 1966; Tanner 1979; Gabrielson *et al.* 2000), though there was some confusion initially over its identity due to inaccuracies in the original description and an atypical holotype (Smith 1944; Abbott & Hollenberg 1976; Tanner 1979). Although its linear thallus with undulate margins and planular central axis makes *U. stenophylla* one of the easier species to identify in the northeast Pacific, it was difficult to find in the field and only one collection was made during the present study.

Ulva stenophylla WA is basal in Clade III in phylogenetic

trees on the basis of combined data. Sequence divergence values between *U. stenophylla* and nearby clades suggest that it is distinct from other surveyed taxa, and it is recognized here as a separate species. *Ulva stenophylla* has been reported in the northeast Pacific from Vancouver Island, British Columbia to Santa Barbara County, California (Gabrielson *et al.* 2000).

Clade IV

ULVA RIGIDA: Setchell & Gardner (1920b) and more recent authors (Scagel 1966; Abbott & Hollenberg 1976; Tanner 1979) considered *U. rigida* to be a member of the northeast Pacific marine flora. This species was described by Agardh (1822) on the basis of material from southern Spain and is very common in some areas along northeast Atlantic shores (Hoeksema & van den Hoek 1983). A sample of *U. rigida* from the type locality (*U. rigida* EU) was obtained for this study. It appears in a strongly supported clade (92%) with a sample of *U. rigida* CE, *U. rigida* OR (as *U. fenestrata* in Tan *et al.* 1999) and two other European taxa. Divergence values among sequences in this clade are within the range for conspecifics. Divergence in ITS nrDNA sequences is 1% between *U. rigida* CE and other taxa in this clade but is 0.2% among *U. rigida* EU, *U. armoricana* EU and *U. scandinavica* EU. Divergence in *rbc*L sequences is 0.2% between *U. rigida* CE and *U. scandinavica* EU. This result is consistent with a previous molecular study including these *U. armoricana* and *U. scandinavica* sequences and different *U. rigida* samples (Tan *et al.* 1999). The separation of these taxa from each other is based on very few molecular and morphological characters (Bliding 1968; Coat *et al.* 1998; Dion *et al.* 1998). For example, Coat *et al.* (1998) suggest that cell shape in surface view of the middle and apical regions may help distinguish *U. armoricana* with polygonal cells from *U. rigida* with rounded cells. Dion *et al.* (1998) further suggest that *U. armoricana* may be differentiated from *U. scandinavica* by the presence in the former of longitudinal ribs formed by packed bundles of rhizoids, except in the youngest thalli. Molecular data suggest that these observed morphological and anatomical differences are not diagnostic. Further study may prove *U. armoricana* and *U. scandinavica* to be candidates for synonymy with the older *U. rigida*; however, additional collections are required for such taxonomic revision.

ULVA FASCIATA: *Ulva fasciata* is a warm water species occurring in tropical and subtropical waters around the world. Although Tanner (1979) included this taxon in his treatment of northeast Pacific *Ulva,* he did not collect this species from the field and based conclusions on two herbarium specimens from southern California. Algae fitting the description of *U. fasciata* were not observed in warmer California waters during the present study; however, samples were obtained from Hawaii and the Texas Gulf Coast. This species is morphologically similar to *U. taeniata* (discussed below) though environmental conditions have been shown to influence thallus morphology (Mshigeni & Kajumulo 1979). Setchell & Gardner (1920a, b) separated *U. taeniata* from *U. fasciata* on the basis of blade morphology: lacinae of *U. taeniata* are usually spirally twisted, whereas those of *U. fasciata* are planular. Tanner (1979) followed Setchell & Gardner and further distinguished these taxa using anatomical characters. He found that *U. taeniata* is sparsely and irregularly branched and that the margins of lacinae are thinner than the central axis in transection. Conversely, he found that *U. fasciata* tends to be highly branched from the base into linear lacinae of similar lengths and that these planular lacinae have uniform thickness from the central axis to margin in transection. Molecular data support the separation of these species. The clade comprising *U. fasciata* collections has 99% bootstrap support. Divergence between these collections is no more than 0.6% for ITS nrDNA and $< 0.4\%$ for *rbc*L sequences, whereas divergence between *U. fasciata* and *U. taeniata* sequences is $> 4.0\%$ for ITS nrDNA and $> 0.5\%$ for *rbc*L. Based on the present data, the separation of *U. fasciata* from *U. taeniata* by Setchell & Gardner (1920a, b) is supported.

ULVA TAENIATA: Setchell & Gardner (1920a) established *U. taeniata* on the basis of algae with long, simple or segmented, dentate thalli found in central California. They described a second species, *U. dactylifera,* for smaller algae in southern California with similar morphology, but lacking dentition and having larger cells in a thicker midrib along the lacinae. A third species with similar morphology but a more distinct midrib, *U. nematoidea,* has also been described. Tanner (1979) suggested that *U. dactylifera* and *U. nematoidea* (as *U. costata*) were conspecific with *U. taeniata* and that observed differences were due to environmental conditions (thallus size) or the physiological state of cells (thickened midrib). He has been followed by recent authors (Scagel *et al.* 1993; Gabrielson *et al.* 2000). Collections of taxa in this assemblage were made in central and southern California, including at the type locality in Monterey, California. Algae fitting the description for this taxon were not encountered north of Monterey; however, *U. taeniata* has been reported as far north as southern British Columbia (Gabrielson *et al.* 2000).

The California collections form a clade. Although this clade does not have strong support in the combined analysis trees, *U. taeniata* I SCA and *U. taeniata* CCA are grouped with strong support in the ITS nrDNA tree (Fig. 2a). ITS nrDNA data are lacking for *U. taeniata* II SCA, but it groups with the other California samples in *rbc*L trees (Figs 1b, 2b). Sequence divergence between these three collections is within the range of conspecifics. *Ulva taeniata* CCA has morphology typical of *U. taeniata* and was collected from the type locality. *Ulva taeniata* II SCA is more typical of *U. dactylifera,* though some specimens have a more distinct midrib similar to *U. nematoidea. Ulva taeniata* I SCA was initially identified as *U. rigida* because it lacks lacinae; however, these algae were growing higher in the intertidal than most *U. taeniata* and may have been dwarfed by desiccation stress.

Sequence for an alga originally identified as *U. taeniata* (Tan *et al.* 1999), renamed here as *Ulva* sp. OR, is located in a clade with an alga collected in Chile. Sequence divergence between these samples and the clade of Californica *U. taeniata* is 3.5% for ITS nrDNA and 0.7% for *rbc*L, which are outside the ranges of conspecifics. The habit of the Chilean *Ulva* sp. CE is different from the typical California *U. taeniata.* The former has a blade that is simple and broad, whereas the latter has blades divided into several long divisions that are deeply ruffled and often twisted. The herbarium specimen for the Oregon accession *Ulva* sp. OR was not observed; however, the collector confirmed that this accession has a morphology typical of *U. taeniata* (G. Hansen, personal commu-

nication). This suggests that *U. taeniata* may be polyphyletic and should be investigated further.

The remaining *Ulva* species reported in the northeast Pacific and represented here by European samples, *U. compressa* (Clade If), *U. flexuosa* (Clade II) and *U. clathrata* (Clade IV), were not encountered during the present study. One unidentified collection, *Ulva* sp. NCA (Clade III) did not group strongly with any other surveyed taxa. This alga has tubular thalli up to 35 cm long and 0.5 cm wide with proliferations at the base. Chloroplasts appear hood-like in surface view, similar to *U. compressa, U. pseudocurvata, U. intestinalis* and *U. intestinaloides* (Koeman & van den Hoek 1981, 1982a; Blomster *et al.* 1998). In ITS nrDNA trees, *Ulva* sp. NCA groups with *U. prolifera* EU but only with weak support. Sequence divergence between *Ulva* sp. NCA and *U. prolifera* EU is 1.3% for ITS nrDNA and 1.4% for *rbc*L. Thus, its identity remains uncertain.

CONCLUSIONS

The focus of the present study was to test species hypotheses and to gain a better understanding of species diversity of *Ulva* in the northeast Pacific. To date, it is the largest study of *Ulva* from this region using molecular data. The results provide insights into the systematics of these taxa and point to areas for additional research.

Many of the species recognized by early and more recent treatments for this region are resolved by molecular data. This demonstrates the value of these data in species delimitations in these morphologically simple algae. In particular, molecular data were useful for discerning species among *Ulva* with expanded-type thalli. Tanner's (1979) hypothesis that all northeast Pacific expanded-type *Ulva* are *U. fenestrata* is rejected. Rather, several species, recognized by earlier authors, can be distinguished. However, his hypotheses for *U. californica, U. stenophylla* and *U. taeniata* are supported.

The present phylogenetic analyses revealed unanticipated potentially conspecific taxa, such as European *U. rotundata* and *U. pertusa*. Strong bootstrap values for such clades and low sequence divergence within them suggests that more detailed studies of these taxa are warranted. Phylogenetic analyses also revealed that closely related taxa can be morphologically similar (e.g. *U. fasciata* and *U. taeniata*) or very different (e.g. *U. californica* and northeast Pacific *U. prolifera*). This is in accord with previous studies that showed that it is difficult to identify morphological and developmental traits reflecting interspecific relationships (Tan *et al.* 1999; Hayden *et al.* 2003).

Another result of this study is the finding that certain *Ulva* species are more widely distributed than may have been recognized previously. Many species described in Europe are inferred to occur in the northeast Pacific (e.g. *U. lactuca* and *U. intestinalis*) and vice versa (e.g. *U. californica*). Given their tolerance for wide ranges of salinity, temperature and water quality, it is not surprising that these species are widely distributed. Furthermore, given their role as fouling organisms, these algae may move around the globe via human activities such as shipping and commercial shellfish transport.

Based on results from the present study, 12 species of *Ulva* occur in the northeast Pacific (see Table 3 for additional comments): *U. californica, U. intestinalis, U. lactuca, U. linza, U. lobata, U. pertusa, U. prolifera, U. pseudocurvata, U. rigida, U. stenophylla, U. taeniata* and *U. tanneri*. Other *Ulva* species reported in this region were not encountered during the present study.

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