Chapter 9
Evolution and Biogeography of Laminarialean Kelps

Hiroshi Kawai, Takeaki Hanyuda and Shinya Uwai

Abstract This review covers the evolution of Laminariales and recent phylogeographic studies focusing on *Chorda* and *Undaria*. In Laminariales, the phylogenetic relationships between basal families (i.e., Akkesiphycaceae, Pseudochordaceae, and Chordaceae) with simple thallus structures, and derived families (i.e., Alariaceae, Laminariaceae, and Lessoniaceae) with large elaborate sporophytes, have remained unclear. Derived Laminariales have been suggested to consist of three major clades roughly corresponding to Alariaceae, Agaraceae (=Costariaceae), and Laminariaceae/Lessoniaceae. Recently, a novel species *Aureophycus aleuticus,* basal to all derived Laminariales, was found in the Bering Sea, and was shown to be basal to all derived Laminariales. Geographically, the majority of derived families in the Laminariales only occur in the Northern Hemisphere, and the Laminariales are therefore considered to have originated in the Northwestern Pacific and spread to the other regions including the Atlantic and the Southern Hemisphere. The limited distributional range of the basal families Akkesiphycaceae and Pseudochordaceae in northeastern Asia, and that of Aureophycaceae in the Bering Sea, supports the notion that the Laminariales originated in the Northwestern Pacific and evolved to giant taxa such as *Macrocystis* in the course of dispersal to the Northeastern Pacific, perhaps through the Aleutian Archipelago. Chordaceae has wide distributional ranges both in the Atlantic and Pacific oceans, but of the four clearly recognized species of *Chorda*, *C. asiatica*, *C. rigida,* and *C. kikonaiensis* are found only in the Northwestern Pacific, whereas *C. filum* is also found in the North Atlantic. In addition, the genetic diversity within *C. asiatica* is greater than that of *C. filum*, and it is suggested that *Chorda* also originated and diverged in the Pacific, then spread into the Atlantic. In the genus *Undaria* (Alariaceae), three species, *U. pinnatifida*, *U. undarioides,* and *U. peterseniana* have been traditionally recognized based on morphological characters, and *U. crenata* was recently described. Based on the genetic studies, *U. crenata* was

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considered to be conspecific with *U. pinnatifida* or *U. peterseniana*, although the three species were shown to be genetically rather close, at present we suggest retaining the species-level taxonomy. Based on genetic analyses, we discuss the likely geographic origin and dispersal pathways for nonindigenous populations of *U. pinnatifida.*

Keywords *Aureophycus* · Biogeography · *Chorda* · Laminariales · Phylogeography · *Undaria*

9.1 Background

Members of the order Laminariales (often referred to as the kelps) are the largest marine macroalgae in terms of physical size, constituting a significant ecological element of coastal ecosystems in temperate and colder seas, for example, by providing habitat for diverse organisms (Bold and Wynne 1985; Dayton 1985; Graham and Wilcox 2000). They are the most structurally complex macroalgae with two distinctive generations, large sporophytes and microscopic gametophytes. Sporophytes of the largest laminarialean species such as *Macrocystis* and *Nereocystis* show distinctive differentiation between stipe and blade, as well as buoyancy structures to maintain the distal portion at the water's surface for photosynthesis. Their remarkably heteromorphic life history is explained as an adaptation to seasonality, enabling tolerance of warm and low water transparency summer conditions as small gametophytes and rapid winter growth as the large sporophytes. Although Laminariales has been considered a distinctive order characterized by the above-mentioned elaborate anatomy and life history pattern, there are considerable gaps in morphological complexities and thallus sizes among the major basal lineages such as *Akkesiphycus*, *Pseudochorda*, and *Chorda*, and other derived families with complex thallus morphology such as Alariaceae, Laminariaceae, and Lessoniaceae (Setchell and Gardner 1925; Tilden 1935; Kawai and Kurogi 1985; Henry and South 1987).

Most members of the order are distributed only in the Northern Hemisphere, with only four genera (*Ecklonia*, *Eisenia*, *Lessonia,* and *Macrocystis*) reported from the Southern Hemisphere as indigenous populations. Furthermore, because of the higher biodiversity in the Pacific Ocean, the Laminariales are considered to have originated in the North Pacific Ocean and spread to other regions including the Atlantic Ocean and the Southern Hemisphere (Lüning and tom Dieck 1990; Bolton 2010). Occurrence of basal taxa such as *Akkesiphycus* and *Pseudochorda* only in the Northwestern Pacific Ocean supports this notion. The distributional ranges of the basal taxa Akkesiphycaceae and Pseudochordaceae are remarkably narrow, and to date, the monotypic species *Akkesiphycus lubricus* has been reported only from the eastern coast of Hokkaido. *Pseudochorda nagaii* has a somewhat broader distribution, but is restricted to the Sea of Okhotsk coasts in Japan and Russia, and *P. gracilis* has been recorded only from the Sea of Japan coast of Hokkaido. In contrast, the other basal taxon, Chordaceae, is distributed both in the Northern Pacific and Northern Atlantic Ocean. Historically, the traditional inclusion of Phyllariaceae Tilden and *Halosiphon tomentosum* (=*Chorda tomentosa*; since moved to Stschapoviales) hindered elucidation of the biogeographical origin of the Laminariales (for details see below), because their occurrence only in the Atlantic Ocean appeared to contradict a Pacific origin of the order (Lüning and tom Dieck 1990) (Fig. 9.1).

Because of the lack of any significant fossil record and incomplete molecular phylogeny, the evolution of laminarialean specialization and history of dispersal have remained unclear. The divergence time of Laminariales from its sister taxon Ectocarpales has been suggested to be relatively recent (<100 Ma), but the phylogeny is still poorly understood (Silberfeld et al. 2010; Kawai et al. 2015a). However, detailed analyses of the genetic diversities in local populations of selected laminarialean taxa have revealed the geographical origin of these taxa, and their dispersal history.

In order to discuss the biogeography and dispersal history of each taxon, it is essential that the phylogeny and the species boundaries are clearly understood. This review aims to: (1) summarize the current status of higher rank taxonomy of Laminariales, focusing on molecular phylogenies, and (2) to review phylogeographical studies of two representative genera, with a focus on the work carried out by the present authors.

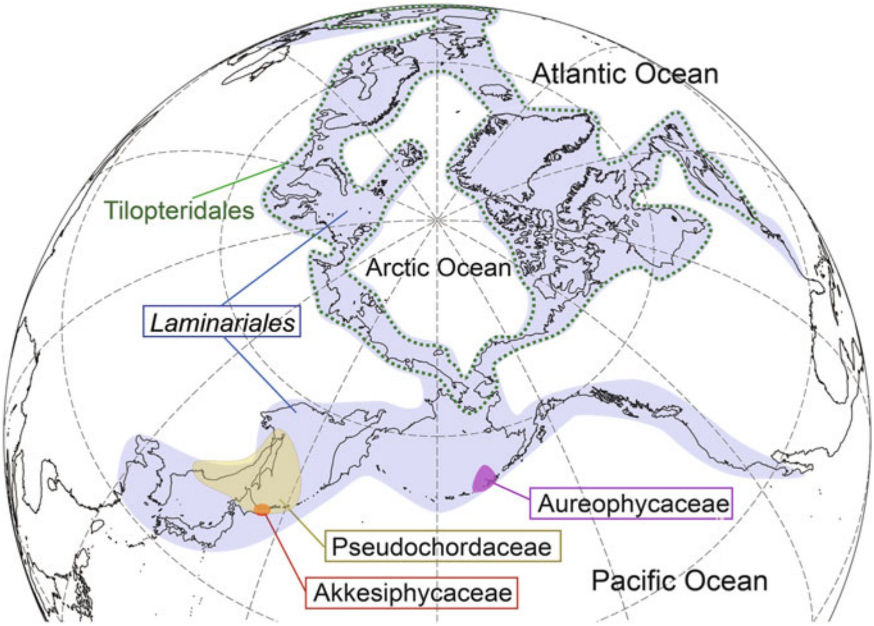


Fig. 9.1 Geographical distributions of Laminariales and Tilopteridales in the Northern Hemisphere. Distributional ranges of the basal laminarialean taxa Akkesiphycaceae, Pseudochordaceae, and Aureophycaceae are separately indicated. The other basal family Chordaceae is shown in Fig. 9.4

9.2 Historical Review of the Taxonomy of Laminariales

Fossil evidence of the evolution of the Phaeophyceae is scant, because of their generally soft tissues composed of polysaccharides such as alginates, fucoidans and cellulose, very limited occurrence of calcified taxa, preference for exposed habitats where sedimentation is not common, and relatively recent evolution compared with red and green algae. Therefore, it is very difficult to assess the divergence times of phaeophycean taxa. However, Lim et al. (1986) estimated the divergence of Phaeophyceae from Bacillariophyceae to be around 200 Ma based on molecular phylogenetic analyses using rRNA sequences. Later Silberfeld et al. (2010) published a time-calibrated molecular phylogenetic tree based on combined DNA sequence data, and suggested the branching time of Laminariales from Ectocarpales to be around 100 Ma, and that of Chordaceae and derived families to be around 85 Ma. Kawai et al. (2015a) reexamined the time-calibrated molecular phylogenetic tree adding the sister and basal taxa of Phaeophyceae (i.e., Schizocladiophyceae and Discosporangiales, respectively) and suggested that the branching times were more recent: 90 Ma (Laminariales/Ectocarpales) and 75 Ma (Chordaceae/derived Laminariales).

Among the Phaeophycean orders, the order Laminariales Migula (1909) has long been regarded as a well-defined order characterized by a heteromorphic life history, alternating between a large parenchymatous sporophyte and filamentous, oogamous gametophytes (Kylin 1916; Sauvageau 1916; Oltmanns 1922; Setchell and Gardner 1925; Fritsch 1945; Bold and Wynne 1985). Laminariales traditionally included four families, Alariaceae Setchell et Gardner, Chordaceae Dumortier, Laminariaceae Bory de Saint-Vincent, and Lessoniaceae Setchell et Gardner. Tilden (1935) proposed Phyllariaceae Tilden, including *Saccorhiza* and *Phyllariopsis* (=*Phyllaria*), and this family was considered a basal member of Laminariales based on morphology and life history studies (Henry and South 1987; Henry 1987). The family was later transferred to Tilopteridales Bessey based on molecular phylogeny (Sasaki et al. 2001). Similarly, *Halosiphon tomentosus* (=*Chorda tomentosa*), which used to be placed in Chordaceae, Laminariales, was transferred to Tilopteridales in its own new family Halosiphonaceae (Sasaki et al. 2001) based on molecular phylogeny, and further moved to a newly proposed order Stschapoviales Kawai (Kawai et al. 2015a).

Kawai and Kurogi (1985) proposed a new family Pseudochordaceae Kawai et Kurogi in Laminariales for *Pseudochorda nagaii*, and later added a second species *P. gracilis* (Kawai and Nabata 1990). Furthermore, Kawai and Sasaki (2000) extended the definition of the order to include a species (*Akkesiphycus lubricus*) with plano-anisogamy (Kawai 1986) by the establishment of Akkesiphycaceae Kawai et Sasaki and its inclusion in the order.

Lane et al. (2006) examined the molecular phylogeny of derived Laminariales, and suggested that they consist of three major clades roughly corresponding to Alariaceae, Agaraceae Postels et Ruprecht (=Costariaceae C.E. Lane, C. Mayes, Druehl et G.W. Saunders), and Laminariaceae/Lessoniaceae. Later, Kawai et al.

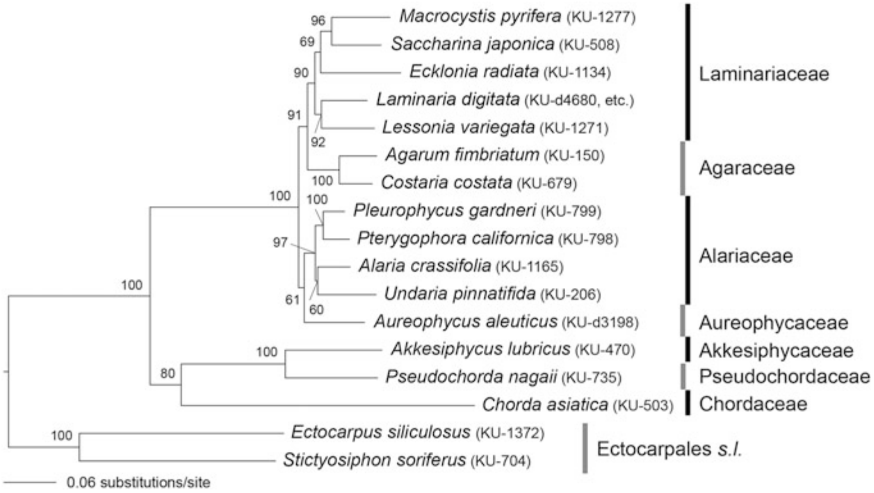


Fig. 9.2 Maximum likelihood phylogenetic tree of Laminariales based on the concatenated DNA sequences of chloroplast *atp*B, *psa*A, *psa*B, *psb*A, *psb*C, *rbc*L, and mitochondrial *cox*1 and *cox*3 genes. Node numbers indicate bootstrap values (only values >50 % are shown). KU-### means culture strains housed in Kobe University Macroalgal Culture Collections (KU-MACC), and KU-d### means silica gel dried field-collected specimens housed in Kobe University Research Center for Inland Seas

(2008) described a novel genus/species *Aureophycus aleuticus*, which is distinct from any known laminarialean species, from islands in the Bering Sea, and later proposed a new family Aureophycaceae H. Kawai, T. Hanyuda, Lindeberg et S.C.Lindstrom to accommodate this species (Kawai et al. 2013). Molecular phylogeny suggested that *Aureophycus* is basal to all derived Laminariales, and this notion was supported by its remarkably simple sporophyte morphology, lacking a rhizoidal meristematic holdfast hapteron and any mucilaginous organs. The limited distributional range of *Aureophycus* in the Bering Sea, together with the limited distribution of the basalmost taxa of the order in the Northwestern Pacific, supports the hypothesis that Laminariales originated in the Northwestern Pacific Ocean, and evolved to giant taxa such as *Macrocystis* and *Nereocystis* in the course of their dispersal to the Northeastern Pacific Ocean, perhaps along the Aleutian Archipelago (Fig. 9.2).

9.3 Systematics and Phylogeography of Selected Genera

9.3.1 Phylogeny and Congeneric Phylogeography of Chorda

In spite of its remarkably different thallus morphology, *Chorda* has been regarded as a member of Laminariales because of the anatomical similarity of its sori, composed of unicellular paraphyses and unilocular zoidangia (Reinke 1892).

However, in addition to the simpler sporophyte anatomy lacking differentiations between blade and stipe, *Chorda* has been regarded as a basal ('primitive') member of the order because of the differences in the following features: lack of a meristematic rhizoidal holdfast haptera; annual nature of sporophytes; lack of mucilage organs such as mucilage gland cells and ducts; lack of mucilage caps on paraphyses; presence of eyespots in zoospores; and occurrence of monoecious gametophytes in *C. tomentosa* = *H. tomentosus*.

The placement of *H. tomentosus* in the family Chordaceae has been controversial because the species differs from the generitype *C. filum* in various basic features: occurrence of long assimilatory filaments instead of unicellular paraphyses; absence of an intercalary meristem; absence of trumpet-shaped hyphae or an obvious differentiation between cortical layer and peripheral (meristodermal) layer; occurrence of monoecious gametophytes; and presence of different sexual pheromones (Maier 1984, 1995; Kogame and Kawai 1996). More recently Peters (1998) established that there is a relatively substantial genetic distance between *C. filum* and *H. tomentosus* based on molecular phylogenetic data. Consequently, Peters (1998) reinstated the generic name *Halosiphon*, suggesting the possibility that *Halosiphon* was incorrectly placed in the Chordaceae, but did not present a formal taxonomic treatment. Only one *Chorda* species, *C. filum* (except for some doubtful species such as *C. munuta*), was then recognized after the transfer of *C. tomentosa* to *Halosiphon* (as *H. tomentosus*; Peters 1998). Later, *Halosiphon* was placed in an independent family Halosiphonaceae Jaasund ex Kawai et Sasaki (Sasaki et al. 2001) in Tilopteridales.

Later, within *Chorda*, an additional species *C. rigida* was described based on morphological and molecular phylogenetic data (Kawai et al. 2001). This species is similar to *C. filum* in habit, but it differs by its more robust thallus with an intercalary meristem even at maturity. Later, largely based on molecular phylogeny, further taxonomic divergence within the genus became evident: A third species, *C. kikonaiensis* was described, and Asian *C. filum* was shown to be independent from Atlantic *C. filum* and was therefore designated as *C. asiatica* (Sasaki and Kawai 2007). *C. kikonaiensis* resembles *C. filum* and *C. asiatica*, but is distinguished by the shorter (0.4–1.3 m) and softer sporophyte, and the thinner cortex composed of fewer (2–4) cells. The independence of this species is further supported by molecular phylogenetic analyses using *rbc*L gene and ITS rDNA sequences (Fig. 9.3). *C. asiatica* is more variable in morphology (length of erect thallus and number of cell layers composing the cortex) than *C. kikoniensis* and *C. rigida*, and is difficult to distinguish from *C. filum* based on morphology, but is clearly separated from the other species (*C. filum, C. kikoniensis* and *C. rigida*) based on ITS rDNA data. *C. filum* is distributed in the Atlantic, whereas *C. asiatica*, *C. kikonaiensis,* and *C. rigida* are distributed in the Pacific Ocean. It is interesting that Atlantic *C. filum*, which is considered based on molecular phylogenetic data to have diverged more recently compared to Asian taxa (Kawai et al. 2015b), demonstrates considerably greater morphological diversity in thallus anatomy (South and Burrows 1967) than is observed within each Asian species, and this hindered recognition of the species divergences in the Pacific Ocean (Sasaki and Kawai 2007).

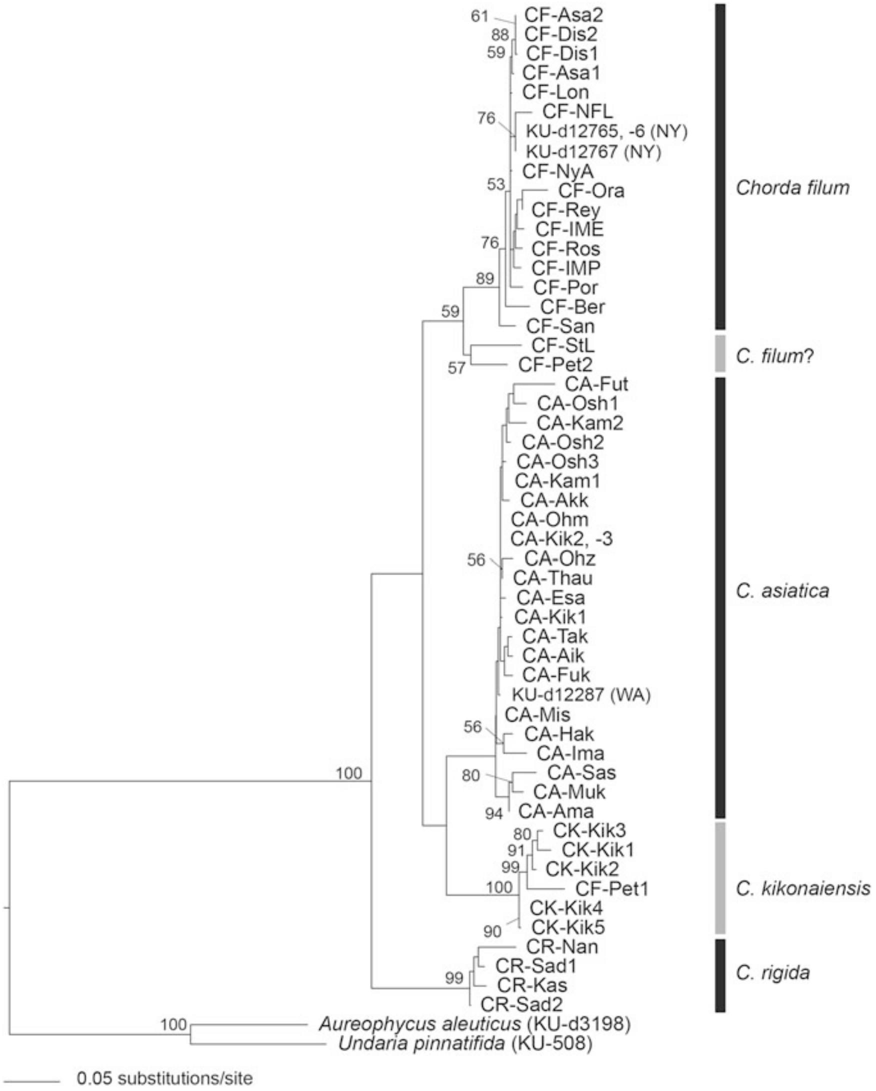


Fig. 9.3 Maximum Likelihood phylogenetic tree of Chordaceae based on ITS1-5.8S-ITS2 region of rDNA sequences. Bootstrap values (>50 %) based on 10,000 replicates are shown. For specimen codes, see Kawai et al. (2015b). KU-###, KU-MACC culture strains; KU-d####, silica gel dried field-collected specimens housed in Kobe University Research Center for Inland Seas

Sasaki and Kawai (2007) demonstrated the occurrence of genetically distinct populations of *Chorda* species on the northwestern American coast based on the sequence data of specimens collected in Puget Sound, WA, USA. In contrast, examination of multiple newly collected specimens from the same locality (Hood Canal, Puget Sound) gave the same ITS rDNA sequence, which was placed in the clade of *C. asiatica*. Considering the historical introductions of oysters from Japan, Kawai et al. (2015b) concluded that they are most likely anthropogenically introduced populations, associated with the widespread practice in the shellfish industry of Puget Sound from 1903 until the 1970s of bringing in oyster spat (*Crassostrea gigas*) on adult shells from Japan (Bonnott 1935; Steele 1964). Similar (and possibly simultaneous) introductions of *C. asiatica* (reported as *C. filum*) associated with young oysters from Japan have been reported in the Mediterranean Sea (Riouall 1985; Boudresque and Verlaque 2002; Kawai et al. 2015b).

Furthermore, the presence of one or two additional cryptic species is suggested in the northern Pacific based on the molecular data. Therefore, it is shown that the genus *Chorda* has considerably greater taxonomic and genetic diversity in the Pacific than in the Atlantic. Although no molecular data are available for other eastern Asian (southeastern Russian coast, Korea and China) *Chorda* species, they are likely referable to *C. asiatica,* based on morphology and geographical distributions. *C. kikonaiensis* grows on upper intertidal rocks of relatively sheltered, somewhat muddy, wide flat rock beds, whereas *C. asiatica* typically grows on upper subtidal rocks. Young sporophytes of *C. asiatica*, retaining the intact distal portion of the intercalary meristem, are found in early March, and they retain the intercalary meristem throughout the spring. The sporophytes reach their maximum size (normally up to 0.8 m, but sometimes attaining 1.3 m in length, and 2.5 mm in diameter) during April and May, become fertile in April, and disappear in July–August.

Water temperature is the primary environmental factor delimiting the distributional ranges of most marine macroalgae. The sporophytes of *C. rigida* grew well in culture under 10 to 25 °C conditions, but did not grow well below 5 °C. They became fertile at 15 °C forming unilocular sporangia among unicellular paraphyses. In contrast, the strains of *C. filum* (Bergen, Norway) showed relatively good growth at 5 to 10 °C, and they did not grow in higher temperatures of 15 and 20 °C respectively. *C. kikonaiensis* grew well at 2 to 15 °C but did not grow at 20 and 25 °C. In contrast, the sporophytes of *C. asiatica* grew at 5, 10 and 15 °C, but did not grow at 2 °C (Sasaki and Kawai 2007).

In the cooler parts of the distributional range of *C. filum*, (e.g., northern Europe where monthly average surface water temperature in August is 11–13 °C; South and Burrows 1967), the growth of *C. filum* may be slow and the sporophytes often persist over summer (Lund 1959; South and Burrows 1967). However, even in such localities, the sporophytes do not remain vegetative after attaining their maximum length in summer (South and Burrows 1967; Russell 1985).

Of the four clearly recognized species of *Chorda*, *C. asiatica*, *C. rigida*, and *C. kikonaiensis* are distributed only in the Northwest Pacific (Kawai et al. 2001, 2015b). In addition, genetic diversity within *C. asiatica* is greater than that of Atlantic *C. filum*, although the data for *C. asiatica* are based only on Japanese specimens. This suggests that *Chorda* originated and diverged in the Pacific Ocean, and spread into the Atlantic (Fig. 9.4). These findings are consistent with the notion that the Laminariales originated in the Pacific Ocean, based on the rich diversity of laminarialean taxa in the Pacific, the occurrence of basal taxa and the limited

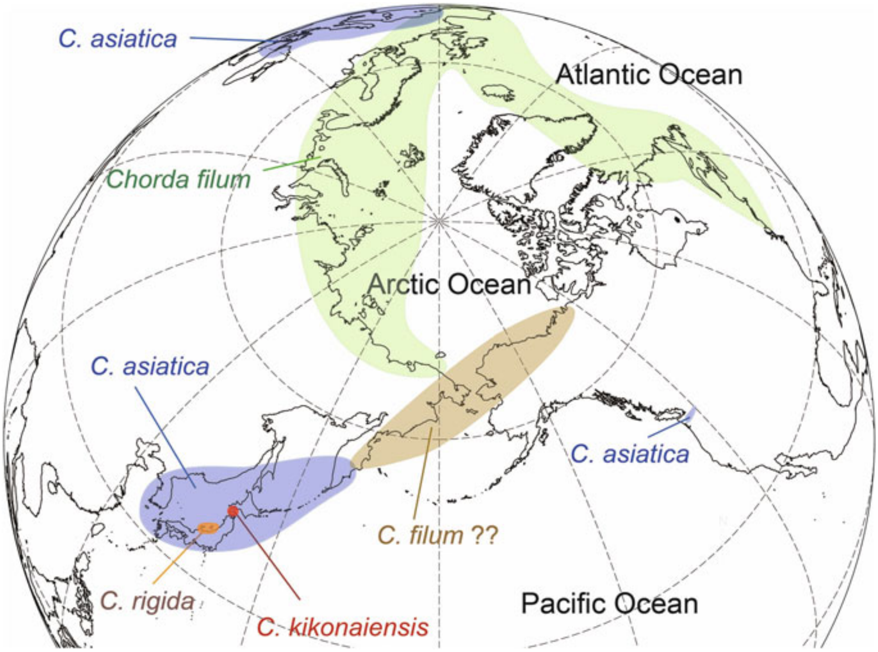


Fig. 9.4 Geographical distribution of *Chorda* species including introduced populations of *C. asiatica* in the Mediterranean Sea and Northwestern America

distribution of the Pseudochordaceae (Kawai and Kurogi 1985; Kawai and Nabata 1990; Lüning and tom Dieck 1990) and Akkesiphycaceae (Kawai 1986; Kawai and Sasaki 2000) in this area.

9.4 Phylogeny of Laminarialean Crown Taxa

Family-level taxonomy of derived Laminariales based on morphology has been reexamined by many authors using different genetic markers, raising questions about the monophyly of each family (Fain et al. 1988; Saunders and Druehl 1992; Boo et al. 1999).

Yoon et al. (2001), using *rbc* spacer and rDNA ITS sequences, obtained eight clades (corresponding to the genera *Hedophyllum*, *Macrocystis*, *Alaria*, *Agarum, Ecklonia, Lessonia*, *Laminaria*, and *Egregia*) and showed that some of the clades corresponded to the Tribes suggested by Kützing, Bory de Saint Vincent, Setchell, and Setchell and Gardner (Setchell and Gardner 1925). The authors discussed subdivision of the order into eight families, although they did not provide a taxonomic treatment. Lane et al. (2006) suggested that Laminariales consist of three major clades roughly corresponding to Alariaceae, newly proposed Costariaceae, and Laminariaceae/Lessoniaceae.

As mentioned above, there has been a large gap between basal and derived Laminariales but the discovery of *Aureophycus aleuticus* (Kawai et al. 2008) and its familial assignment (Kawai et al. 2013) provided a clue for elucidating the relationship between them. In addition to the remarkably simple morphology of the sporophytes, molecular phylogeny based on the concatenated sequences of eight genes (*rbc*L, *atp*B, *psa*A, *psa*B, *psb*A, *psb*C, *cox*1, and *cox*3) suggested that *Aureophycus* is either basal to all derived Laminariales, or to the clade of Alariaceae including *Alaria, Undaria, Pterygophora*, and *Pleurophycus*. However, based on the results of character mapping of representative taxonomic features onto the two topology options, the authors concluded that *Aureophycus* was basal to all derived Laminariales (Kawai et al. 2013). The limited distribution of *Aureophycus* in the Bering Sea and the Aleutian Archipelago suggest the importance of this area as the center for the differentiation of the family-level lineages of derived Laminariales: evolution of the genera with very large and elaborate sporophytes such as *Macrocystis*, *Egregia* and *Nereocystis* could have occurred in the process of eastward dispersal, whereas evolution of taxa adapting to warmer habitats such as *Undaria* and *Ecklonia* could have occurred in the course of westward dispersal from the region.

9.5 Phylogeny and Phylogeography of *Undaria*

9.5.1 Taxonomy of Undaria Species

The genus *Undaria* Suringar (1873) is distinctive from other laminarialean genera, and is characterized by the soft annual sporophytes forming reproductive structures at the basal portion of the membranous thalli (blades) and/or sporophylls at the basal part of the stipe. Three to four species are currently recognized in the genus and they have relatively limited geographical distributions in the temperate regions in northeastern Asia. Because of the high morphological plasticity and close genetic relationships among the reported taxa, their species-level and intraspecific taxonomy has been somewhat confused (Yendo 1911; Okamura 1915; Castric-Fey et al. 1999; Cecere et al. 2000).

Three species, *U. pinnatifida*, *U. undarioides* and *U. peterseniana*, have been traditionally recognized (Okamura 1915, 1926) based on morphological characters. These species have been distinguished by combinations of characters such as presence/absence of pinnae on the blade, presence/absence of sporophyll, and presence/absence of a midrib. *U. pinnatifida* typically has a pinnately lobed blade (membranous thallus) with a midrib throughout the entire length, and a wrinkled sporophyll on the flattened stipe. *U. peterseniana* has a long foliose blade without a midrib, forming sori on the basal and middle portion of the blade. *U. undarioides* has an intermediate morphology between *U. pinnatifida* and *U. peterseniana,* having an elliptical to ovate blade without pinnae, and the presence of midrib is variable. *U. undarioides* sometimes has sporophylls on the basal stipe in addition to the sorus on the blade (Yendo 1903, 1911; Okamura 1915). Furthermore, the occurrence of sori on the basal part of the blade and midrib in *U. pinnatifida* has also been found in the field (H. Kawai, unpublished data).

U. pinnatifida has the broadest distribution, along the Sea of Japan and the Pacific coast of Japan, whereas *U. peterseniana* and *U. undarioides* are distributed in narrower ranges within that of *U. pinnatifida*. Later a new species *U. crenata* Y. P. Lee was described from Korea, having a blade with laciniate margin, considered to be an intermediate between *U. pinnatifida* and *U. peterseniana* (Lee and Yoon 1998). Additionally, Lee (1998) established *Undariella* to accommodate *Undariella peterseniana*, although *Undariella* was later reduced to synonymy with *Undariopsis* Miyabe et Okamura (Okamura 1902) by the author (Lee 1999).

Muraoka and Saito (2005) reported genetic distinctness between *U. pinnatifida* and *U. undarioides* based on the mitochondrial 23S rDNA sequence. Later, Uwai et al. (2007) examined morphology and genetic diversity using mitochondrial *cox*3 sequences among *Undaria* species collected from throughout their distribution around Japan (Fig. 9.5). In the statistical parsimony network, *U. peterseniana* haplotypes were divided into two lineages (type-c17, -c18 and -c19), and they were closely related to haplotypes found in *U. pinnatifida* in the Sea of Japan and type-c15, -c16 found in *U. undarioides*, respectively. Similarly, type-c12, found in *U. undarioides*, was closely related to type-c13 and -c14 found in *U. pinnatifida*. In the network, direct connection among *U. peterseniana* haplotypes (between type-c17, -c18 and type-c19) and among *U. undarioides* haplotypes (between type-c15, -c16, and -c12) were not observed. In the ML molecular phylogenetic tree based on the *cox*3 sequences, none of the three species were monophyletic, and the statistical support for the clades was moderate (<80 %, data not shown).

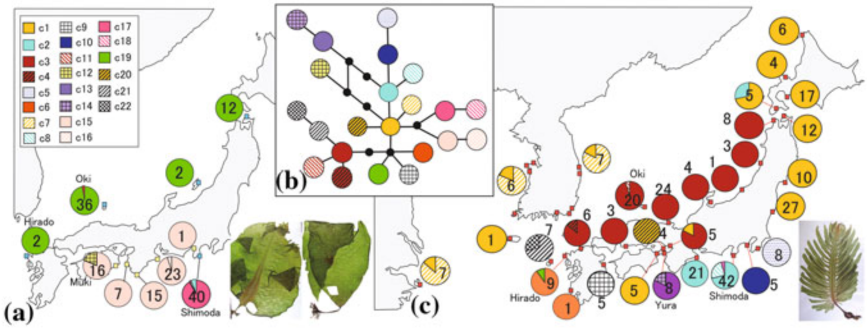


Fig. 9.5 Network and geographic distribution of the mitochondrial *cox*3 haplotypes of *Undaria* spp. **b** haplotypes are illustrated by different combinations of color and pattern and identified with a haplotype code (*inset* in **a**). In the maps, pie charts represent local haplotype frequencies, with numerals indicating the number of samples in each site for (**a**) *U. undarioides* (*yellow*) and *U. peterseniana* (*blue*), and (**c**) *U. pinnatifida*. Plants with the typical morphology of *U. undarioides* (**a**, *left*), *U. peterseniana* (**a**, *right*), and *U. pinnatifida* (**c**), are also shown

Geographical distributions of each haplotype elucidated in Uwai et al. (2007) are shown in Fig. 9.5. Each of three species had unique *cox*3 haplotypes, with a few exceptions, and showed apparent geographical structure within species. Each population of *U. undarioides* and *U. peterseniana*, as well as *U. pinnatifida* populations, had only one or two haplotypes. A *U. peterseniana* population on the Pacific coast (Shimoda, Shizuoka, Japan) had type-c17 and -c18, both of which were unique in this population, in addition to type-c2 which was found from *U. pinnatifida* populations in the same region. *U. peterseniana* populations along the Sea of Japan had unique type-c19. Almost all *U. undarioides* had species-specific type-c15 in addition to type-c16 and type-c12 as minor haplotypes.

Specimens of each species with atypical morphology did not show unique *cox3* haplotypes. For example, the specimens with well-developed sporophyll had type-c17 (*U. peterseniana* collected in the Pacific coast) and type-c15 (*U. undarioides*), identical to the sporophytes having blade sori in *U. peterseniana* and *U. undarioides*, respectively (Uwai et al. 2007). Similarly, the *U. pinnatifida* specimens morphologically similar to *U. crenata*, collected at Oki, central region of the Sea of Japan, had type-c3, which was commonly distributed along the *U. pinnatifida* populations in the Sea of Japan. On the other hand, the sporophytes of *U. pinnatifida* collected in Hirado, Northern Kyusyu, had type-c19, which was identical to that of *U. peterseniana* in the Sea of Japan. Exceptionally, a Pacific sample with typical *U. peterseniana* morphology showed type-c2, found in a *U. pinnatifida* population in that region (Shimoda). Three sporophytes of six *U. undarioides* with dentate margin collected in Muki, the westernmost sampling site for the species, had a unique haplotype, type-c12, which was closely related to the haplotypes found in nearby *U. pinnatifida* populations (Yura, Awaji Island). However, three other sporophytes with dentate margin had type-c15, which was identical to *U. undarioides* with typical morphology.

These results based on mitochondrial *cox*3 haplotypes strongly suggest that there is, in principle, genetic distinctness among the Japanese *Undaria* species. Almost all samples examined were easily identified based on morphological features, especially based on presence or absence of pinnae on the blade, and the *cox*3 haplotypes were different depending on the species. This result supports the present species-level taxonomy of *Undaria*, especially the significance of the blade shape (Okamura 1915); *U. peterseniana* and *U. undarioides* plants with well-developed sporophylls did not show any differences in *cox*3 haplotype when compared with sporophytes with typical sori.

On the other hand, an exceptional *cox*3 haplotype was observed in some of the sporophytes with intermediate morphologies. An *U. pinnatifida* specimen from Oki that had only short pinnae and a rudimentary midrib had a haplotype that was identical to those of *U. pinnatifida* in the Sea of Japan, however, an *U. pinnatifida* specimen from Hirado, morphologically similar to the specimen from Oki, had the same haplotype as *U. peterseniana*. Interestingly, those *U. pinnatifida* were similar to *U. crenata* (Lee and Yoon 1998) as well as the experimental hybrids that resulted from crosses between *U. pinnatifida* and *U. peterseniana* (Migita 1967; Saito 1972). Therefore, Uwai et al. (2007) considered that *U. crenata* is conspecific with

U. pinnatifida or *U. peterseniana*; those plants could be considered as one extreme example of the morphological variations of *U. pinnatifida* or *U. peterseniana*. Alternatively, it is also possible that *U. crenata* and the *U. pinnatifida* samples from Oki and Hirado were hybrids originating from crosses between *U. pinnatifida* and *U. peterseniana* (Kikuchi et al. 1996). Similarly, three *U. undarioides* plants with dentate margin and type-c12 also could be considered a result of interspecies hybridization, rather than resulting from a polyphyletic origin of species or lineage sorting (discussed below), although type-c12 has not been found from *U. pinnatifida*. More significantly, occurrence of interspecies hybridization was suggested based on a sporophyte collected in Shimoda, Japan, with typical *U. peterseniana* morphology but having the *cox*3 haplotype that was common in *U. pinnatifida* in that region. These results suggest frequent occurrence of interspecies hybridizations among *Undaria* spp.

In addition to the exceptional haplotypes, the phylogeny of the *cox*3 haplotypes suggests that taxonomic problems still remain in the genus. Conspecific populations had different haplotypes depending on their geographical origins, and such haplotypes, in some cases, had no direct connection in the network, both of which suggest that each species includes two to several sibling species. An alternative explanation for the observed polyphyly is incomplete lineage sorting (Avise 2000); through speciation, each daughter species could take a subset of haplotypes, which are not necessarily monophyletic, from the ancestral species, and therefore the daughter species is polyphyletic in the gene tree for a certain period after speciation. The reported small genetic distance (Muraoka and Saito 2005; Uwai et al. 2007) suggests this could be the case for *Undaria* species. Besides the polyphyly, the haplotypic differentiation between conspecific populations also suggests the presence of sibling species, or an ongoing speciation event. Only little has been known about the isolation mechanisms between such local groups, as well as species; based on laboratory culture experiments with strict temperature control, Morita et al. (2003a, b) reported differences in temperature requirements between parapatric *U. pinnatifida* and *U. undarioides* populations, which possibly functions as the isolation mechanism between them. Gene flow between conspecific populations, as well as between species, and studies on the isolation mechanism are warranted to evaluate the taxonomic status of local groups.

Within *U. pinnatifida*, several intraspecific taxa have been recognized as varieties (Suringar 1873) and forms (Miyabe 1902; Yendo 1911; Okamura 1915). The two forms *U. pinnatifida* f. *typica* and *U. pinnatifida* f. *distans* (Yendo 1911; Okamura 1915) are distinguished by the distance between the sporophyll and blade, and the depth of the incisions of the blade. Besides these forms, Yendo (1911) recognized *U. pinnatifida* f. *narutensis*, characterized by a short stipe with less-folded sporophylls, although Okamura (1915) regarded this as a synonym of f. *typica*. Later, Lee and Yoon (1998) suggested the reinstatement of *U. pinntifida* var. *elongata* and var. *vulgaris* for those forms because of nomenclatural priority. Relationships between these morphological varieties/forms and the local groups characterized by the haplotypes remain unknown.

All molecular analyses for interspecies-level phylogeny (Muraoka and Saito 2005; Uwai et al. 2007) have shown close genetic relationships among members of *Undaria*. The number of substitutions among *Undaria* spp. is up to 1.9 % (nine of 470 bp) in the mitochondrial *cox*3 gene, which is remarkably smaller than those often observed within single species; up to 10.3 % in *Scytosiphon lomentaria* (Kogame et al. 2005), 5.9 % in *Ishige okamurae* (Lee et al. 2012), and 3.7 % in *Colpomenia peregrina* (Lee et al. 2014). Several genera have been established to accommodate *Undaria* species; Lee (1998) established *Undariella* (=*Undariopsis*) based on *U. peterseniana*; *Hirome* for *U. undarioides* by Yendo (1903); *Undariopsis* for *U. peterseniana* by Miyabe et Okamura (Okamura 1902). As Okamura (1915) concluded, however, morphological variations of *Undaria* spp. could be considered continuous when three species (*U. pinnatifida, U. undarioides*, and *U. peterseniana*) were compared. Therefore, we suspend any changes in the present taxonomic system and classify three species in the single genus *Undaria*.

In addition to taxonomic research, because of the commercial importance of *Undaria*, there have been a number of studies examining the influences of genetic and environmental traits on the morphological plasticity of *Undaria* species (Saito 1972; Stuart et al. 1999; Choi et al. 2009; Nanba et al. 2011; Park et al. 2012). By the application of genetic analyses, intraspecific taxonomy has been updated, although the species-level taxonomy still appears to need further studies.

9.5.2 Phylogeography of Undaria Pinnatifida

Several studies on the molecular diversity of *U. pinnatifida* have been done for the purpose of deducing the geographic origin of introduced populations (Voisin et al. 2005; Uwai et al. 2006a), to establish a method for authenticating the origin of commercial products (Endo et al. 2009), and to analyze the phylogeographic history of the species (Uwai et al. 2006b), based on mitochondrial DNA sequences and the nuclear ribosomal ITS regions. All authors commonly reported high genetic diversity within indigenous populations as well as introduced ones.

9.5.3 Indigenous Populations

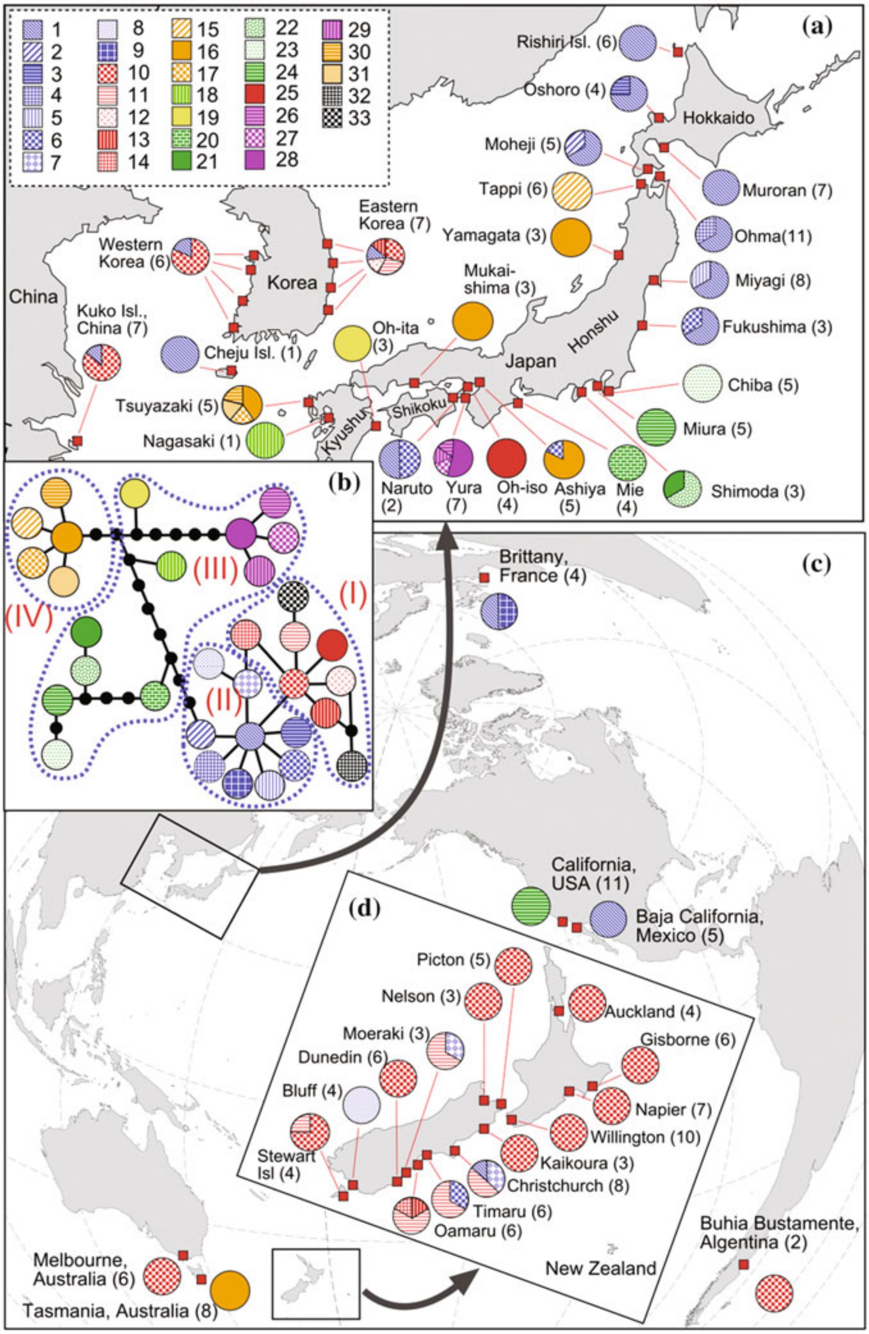
The native distributional range of *U. pinnatifida* has been regarded as temperate waters of northeastern Asia (Japan, Korea and China) (Akiyama and Kurogi 1982; Tseng 1983), although Chinese populations are regarded as introduced by Lutaenko et al. (2013). Techniques for *U. pinnatifida* mariculture were established in the 1950s, and intentional introductions within the indigenous region for mariculture have been a concern in relation to genetic disturbance through escape and hybridization between introduced and native populations. High and geographically structured genetic diversity has been reported for the “wild” populations, especially along Japanese coasts (Voisin et al. 2005; Uwai et al. 2006a, b; Endo et al. 2009), suggesting limited genetic disturbance by anthropogenic movement, although the Bay of Osaka is an exceptional area (discussed below).

Uwai et al. (2006a) reported a strong geographic structure throughout the native range of *U. pinnatifida* based on concatenated sequences (ca. 900 bp) of the mitochondrial *cox*3 and *tat*C-*trn*L region. Haplotypes found on Japanese coasts were divided into three local types based on their geographic distributions and phylogenetic relationships in the statistical parsimony network (Fig. 9.6): (1) Pacific central Japan type from northern Kyushu to central Honshu, (2) northern Japan type from Hokkaido and the Pacific coast of northern Honshu, and (3) the Sea of Japan type found from the Sea of Japan coast of Honshu. Besides these local types, the populations in Korea and China had unique major haplotypes (continental type), in addition to the northern Japan haplotype as a minority. The northern Japan haplotype and the continental type had sequences similar to each other, but differed by a long (20 bp) indel in the intergenic spacer region between *trn*W and *trn*I. Haplotypes comprising each local type differed by only a few base pairs, except for the Pacific central Japan type. Their geographical distributions in northeastern Asia are shown Fig. 9.6a.

Based on the small number of steps and the star-like topology among haplotypes of the Sea of Japan type, Uwai et al. (2006a) concluded that regional populations there have expanded recently and linked this notion with geological events in this region. Ohba et al. (1991) concluded, based on the microfossil assemblages in piston core samples, that a large input of freshwater into the surface layer and stratification of the water column caused severe anoxic conditions in the Sea of Japan during the middle period of the last glacial age (27,000–20,000 years ago). These perturbations led to the extinction of benthic fauna in the Sea of Japan, and are likely to have impacted the seaweed flora of this region as well. Both seaweed flora and benthic fauna of the Sea of Japan might have been established after the beginning of the inflow of seawater after the Last Glacial Maximum, especially after the inflow of the Tsushima warm current through the Tsushima strait from 8000 years ago. A similar colonization pattern into the Sea of Japan was reported for the Japanese turban shell (Kojima et al. 1997) and the ulvophycean genus *Blidingia* (Woolcott et al. 2000). The diverged haplotypes of the central Pacific type, especially those of central Pacific Honshu, show clear contrast with the Sea of Japan type and might indicate differences in population demographics between both sides of Japan.

9.5.4 Nonindigenous Populations

Undaria pinnatifida has been introduced into Europe in the 1970s associated with young oysters introduced for mariculture, initially to the French Mediterranean coast. Within a decade the species had spread to a broader area in Europe (Perez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Oshoro (4)
Hokkaido
Moheji (5)
Muroran (7)
Tappi (6)
Ohma(11)
Yamagata (3)
Miyagi (8)
Fukushima (3)
Eastern Korea (7)
Western Korea (6)
Korea
China
Kuko Isl., China (7)
Cheju Isl. (1)
Mukai-shima (3)
Oh-ita (3)
Honshu
Japan
Chiba (5)
Miura (5)
Tsuyazaki (5)
Nagasaki (1)
Kyushu
Shikoku
Naruto (2)
Yura (7)
Oh-iso (4)
Ashiya (5)
Mie (4)
Shimoda (3)
(b)
(I)
(II)
(III)
(IV)
(c)
Brittany, France (4)
California, USA (11)
Baja California, Mexico (5)
(d)
Picton (5)
Nelson (3)
Auckland (4)
Moeraki (3)
Gisborne (6)
Dunedin (6)
Bluff (4)
Napier (7)
Willington (10)
Stewart Isl (4)
Kaikoura (3)
Christchurch (8)
Timaru (6)
Oamaru (6)
New Zealand
Buhia Bustamente, Algentina (2)
Melbourne, Australia (6)
Tasmania, Australia (8)

◀ **Fig. 9.6** Parsimony network and geographical distributions of mitochondrial DNA haplotypes (*cox*3 and *tat*C-*tLeu* regions) of *Undaria pinnatifida* in the native and introduced populations. **b** Haplotypes are illustrated by different combinations of color and pattern and identified with an haplotype code (*inset* in **a**). Pie charts depict haplotype frequencies in each populations, with numerals in parentheses representing the number of samples in each population in **a** Native range, **c** Worldwide introduced populations except New Zealand, and **d** Introduced populations in New Zealand. Reprint from Uwai et al. (2006a) with permission

et al. 1981; Boudouresque et al. 1985; Castric-Fay et al. 1999; Fletcher and Farrell 1998). In the late 1980s the species was reported worldwide, in New Zealand (Hay and Luckens 1987; Hay 1990), Tasmania, Australia (Sanderson 1990), Argentina (Casas and Piriz 1996), Victoria, Australia (Campbell and Burridge 1998), California, USA (Silva et al. 2002) and in Baja California, Mexico (Aguilar-Rosas et al. 2004).

Apart from the introductions to Europe (non-intentional introductions associated with oyster farming), processes allowing introductions into other regions are not clearly understood. Although ballast water and hull fouling have been considered probable dispersal vectors to other areas, there has been limited scientific information supporting these assumptions. Furthermore, possible secondary introductions within the same ranges have not been investigated.

Voisin et al. (2005) as well as Uwai et al. (2006a) studied the genetic diversity of *U. pinnatifida* populations based on newly collected specimens worldwide using mitochondrial gene sequences. Uwai et al. (2006a) also examined historical voucher specimens collected from various localities in New Zealand since the time of early introduction and discussed the succession of the dominant haplotypes in populations in New Zealand. Based on the analyses, they suggested multiple introductions in *U. pinnatifida*, because all local types were found in the introduced populations; the population in Brittany, France and that in Baja California, Mexico, as well as some of the New Zealand populations, had the northern Japan types; almost all populations in the Southern Hemisphere (most New Zealand populations, the Argentina population and the Melbourne population), had the continental types; one population in California, USA and in Tasmania, Australia, had the central Japan type and the Sea of Japan type, respectively (Fig. 9.6c). Furthermore, an analysis of populations throughout almost all introduced areas in New Zealand, indicated that the populations have origins in continental Asia as well as northern Japan (Uwai et al. 2006a). Historical samples from the times of the early introductions (1987–1990) showed that an introduced population at Wellington had the continental types (type-10 and -11), whereas those in Timaru and Oamaru, both in central region of the South Island, had the northern Japan type (type-1). Nine haplotypes, 7 from 28 historical samples (1987–2003) and seven from contemporary samples (five the same as those in historical samples), were found in the New Zealand samples. This suggests that possibly more than two introductions have occurred in this country, especially on the South Island (Uwai et al. 2006a). In addition to New Zealand, the Sea of Japan type was found in the Tasmania population, suggesting that another equator-crossing introduction occurred. Since

Undaria gametophytes are relatively tolerant to high temperatures up to 30 °C (tom Dieck 1993), they could be transported alive across the tropics on ships' hulls and/or in ballast tanks (Sanderson 1990). *Undaria* sporophytes were collected from the hull of a Korean trawler in New Zealand in 1987, suggesting the introduction across the equator was achieved in this way.

In contrast to high haplotype diversity on the South Island, only a single haplotype (type-10) was found on the North Island, suggests successive introductions, possibly through domestic vessel transport (Stuart 2004), although scientific evidence for such hull fouling as an introduction vector, as well as successive secondary introductions, has been limited. This is possibly true for the populations in Argentina and Melbourne, i.e., they could be carried from New Zealand. The optimal temperature for growth of both gametophytes (Akiyama 1965; Morita et al. 2003a) and sporophytes (Morita et al. 2003b; Gao et al. 2013) are below 23 °C; at 25 °C or above, growth is stunted. The survival of *Undaria* gametophytes and sporophytes is regarded as much more likely in the course of ship operations within the temperate region than those crossing the high temperature tropical regions.

Although the haplotypes found in the Bay of Osaka area showed high haplotype divergences, and included all of the local types reported in Uwai et al. (2006a), this is explained as the result of intentional introductions for breeding of strains used for mariculture in the area.

9.6 Future Work

The familial taxonomy of the Laminariales has been considerably revised in the last two to three decades by the discoveries of new key taxa and the application of molecular phylogenetic approaches. Based on this new information, combined with existing biogeographic information, it has been possible to elucidate the phylogeography of the basal taxa, especially their global dispersal history. Despite these advances, the familial or generic level phylogeographies of the derived Laminariales remain poorly understood. For example, although Laminariales is considered to have originated in cool temperate or cold water regions, adaptation to warm temperate habitat has occurred in some lineages but it is not clear where these evolutionary events occurred (Uwai et al. 2007; Rothman et al. 2015). We consider that detailed phylogenetic analyses of such derived families and genera will provide insights for understanding the processes driving genetic and morphological diversification in Laminariales. For example, development of large and elaborate sporophytes could have resulted from adaptations to drastic changes of sea temperatures and sea levels with plate tectonic movements and global climate change cycles.

As summarized above, phylogeographic patterns at the genus and species levels have suggested greater species diversities within of *Chorda* and *Undaria* species than formerly estimated by morphological taxonomy alone, and the occurrence of cryptic species has been demonstrated in both genera. These findings suggest there may also be unrecognized, cryptic species in other laminarialean genera and species. Species-level taxonomy needs further revisions especially in the genera such as *Alaria* (Kraan and Guiry 2000), *Saccharina* (Yotsukura et al. 2008), and *Ecklonia* (Rothman et al. 2015). However, clarification of species boundaries is difficult if natural hybridization is possible, and both experimental (Migita 1967; Lewis and Neushul 1995; Kraan and Guiry 2000) and observational (Coyer et al. 1992; Kikuchi et al. 1996) studies indicate that hybridization among laminarialean species can occur.

On the other hand, a considerable range of morphological and physiological adaptations could be expected within a single species having a wide distributional range, as suggested in the population structure of *Undaria pinnatifida*. Therefore, in addition to detailed molecular phylogenetic analyses for each genus and species, population genetic analyses and ecophysiological comparisons between local populations are necessary for understanding species-level phylogeographic patterns. Such studies would also provide useful information for discussing the possible impacts of rising seawater temperatures on existing populations of the laminarialean species, many of which are fundamental elements of coastal ecosystems.

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