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Genetic Differences in Spatially and Temporally Isolated Populations: Winter and Spring Populations of Pelagic Mollusk *Clione* (Mollusk: Gymnosomata), Southern Okhotsk Sea, Japan

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Abstract

The pelagic mollusk *Clione* is a naked pteropod with a sympatric two-species distribution in the southern Okhotsk Sea, Japan, consisting of the morphologically and genetically distinct *C. elegantissima* and *C. okhotensis*. *Clione elegantissima* appears in both winter and spring, and body length differs between the winter coastal population (WCP, January to March, 10–20 mm) and the spring offshore population (SOP, April to July; up to 30 mm). This body size difference and temporal-spatial separation of the populations suggests that the SOP is either a cryptic species or *C. limacine* drifted from the Subarctic Atlantic Ocean or an interspecies of *C. elegantissima* resulting from reproductive isolation. We investigated the taxonomic positions of both populations using morphological and genetic analyses and identified both as *C. elegantissima* with very high genetic similarity. We explain the occurrence of spatio-temporal isolated populations using the water mass dynamics in the Okhotsk Sea. Warm water entering the southern Okhotsk Sea around Japan through the Soya Straits is divided into the Soya Warm Water (SWW: June to November) and the Forerunner of the SWW (FSWW: March to May); cold water entering the Okhotsk Sea around Japan, east of Sakhalin Island, is divided into the East Sakhalin Current Water (ESCW: November to April). The Cold Water Belt (CWB) is frequently formed off the SWW during summer and autumn and comprises upwelling cold water originating from either subsurface water of the Japan Sea off Sakhalin or Okhotsk Sea bottom water. We present the temporal-spatial isolation mechanism of WCP and SOP per the SWW, FSWW, ESCW, and CWB dynamics.

Keywords Taxonomy · Clione · Genetics · Water currents

Introduction

The Okhotsk Sea, located in the northwestern corner of the North Pacific Ocean, is one of the largest semi-enclosed seas in the world (Fig. 1). Whereas sea ice is typically present in the Antarctic and Arctic waters during the winter when it covers an area of up to 7% of the earth's surface (Comiso et al. 1990), in the North Pacific Ocean sea ice occurs only during a part of the winter (Ruth and Patrick 1998). From spring to autumn, *Clione elegantissima* (Dall 1871) lives at a depth of approximately 200 m in the Okhotsk Sea, whereas in winter this species migrates toward the coast of the southern Okhotsk Sea with drift ice (Hamaoka 2002).

Despite being important members of zooplankton communities, the Gymnosomata are poorly understood (Lalli and Gilmer 1989; Sromek et al. 2015). Although their anatomy and ecology have been well studied (Martens 1675; Wanger 1885; Smith 1902; Massy 1932; Morton 1958; Mileikovsky 1970; Van Der Spoel 1976; Lalli and Conover 1976; Lalli and Gilmer 1989; MClintock and Janssen 1990; Gilmer and Lalli 1990; Richter and Seapy 1999; Van Der Spoel and Dadon 1999; Suprenand et al. 2015), the genetics of these pteropods have received little attention (Jennings et al. 2010; Sromek et al. 2015; Yamazaki and Kuwahara 2017). The characteristics of the family Clionidae

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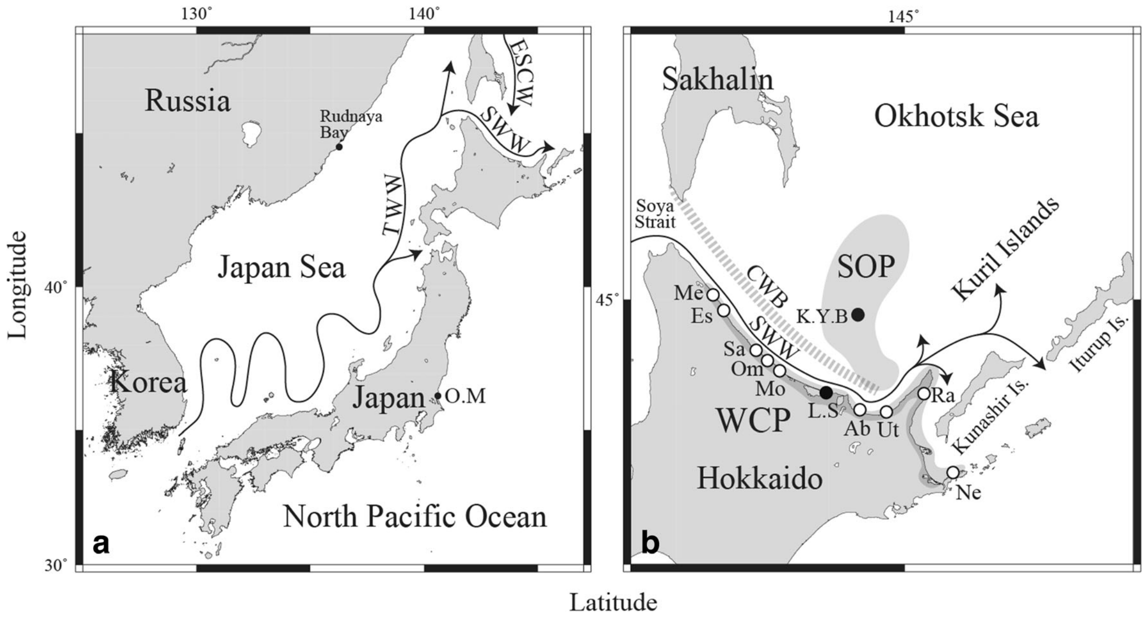

Fig. 1 **a** Distribution of the southern limit of *Clione elegantissima,* **b** Distribution of the winter coastal population (WCP) and spring offshore population (SOP) and sampling location. WCP areas were based on Yamazaki and Kuwahara (2017) and the collected record points are shown as ○. SOP areas based on Suzuki et al. (2005) and Kunihiro (1995). Lake Saroma (N44°13, E143°97) and K.Y.B (N44°46, E144°23) points indicated with ● are sampling locations. Abbreviation: TWW, Tsushima Warm Water; SWW, Soya Warm Water; ESCW, East Sakhalin Current Water; CWB, Cold Water Belt; O.M, Oarai Marina; Me, Menashidomari; Es, Esashi; Sa, Sawaki; Om, Omusaro; Mo, Monbetsu; L.S, Lake Saroma; Ab, Abashiri; Ut, Utoro; Ra, Rausu; Ne, Nemuro; K.Y.B, Kitami-Yamato Bank

include a thin integument and the presence of anterior tentacles, which are mostly transparent and typically very small. The defining characteristic of the genus *Clione* is three pairs of buccal cones (Van Der Spoel 1976; Lalli and Gilmer 1989). Members of the genus have been called “sea angels” owing to their characteristic swimming behavior (Wrobel and Mills 1998). *Clione* species are an important component of pelagic food webs in temperate and polar waters, providing a food source for baleen whales, salmon, and other fish species (Martens 1675; Phipps 1774; Lalli and Gilmer 1989; Azuma 1995; Davis et al. 2009; Haigh et al. 2015).

The genus *Clione* consists of four known species with a bipolar distribution: the Northern and Southern Hemispheres have three and one species, respectively (Yamazaki and Kuwahara 2017). The genus is distributed in Japan and the adjacent waters of the southern limit, Oarai Marina (Fig. 1a, O.M), Ibaraki prefecture in the North Pacific Ocean, (Inaba and Okutani 1999), and Rudnaya Bay Japan Sea (Chichvarkhin 2016) (Fig. 1a).

The Northern Hemisphere *C. limacina* (Phipps 1774) and South Hemisphere *C. antarctica* (Smith 1902) are considered as synonyms or subspecies (Gilmer and Lalli 1990). Sromek et al. (2015) suggested that the two species are genetically distinct. The taxonomical complexes of the northern and southern populations could be distinguished using morphological and genetic approaches.

The Northern Hemisphere *C. limacina* consists of two known populations: the North Pacific and the Arctic to North Atlantic Ocean populations. The two populations differ morphologically in body size and swimming speed (Gilmer and Lalli 1990), and Yamazaki and Kuwahara (2017) suggested they are genetically distinct. The taxonomy of the Northern Hemisphere *Clione* species is organized into the Subarctic Atlantic Ocean population and North Pacific Ocean population, which were identified respectively as *C. limacina* and *C. elegantissima* (Dall 1871) by Yamazaki and Kuwahara (2017). Yamazaki and Kuwahara (2017) described a new species, *C. okhotensis*, from the southern Okhotsk Sea with evidence of its morphological and genetic features.

The southern Okhotsk Sea has two sympatric *Clione* species, *C. elegantissima* and *C. okhotensis* (Yamazaki and Kuwahara 2017). *C. elegantissima* appears in two seasons: winter (January to March) in coastal waters, and spring (April to July) in the offshore area. There are clear differences in the body lengths of the winter coastal population (WCP) and spring offshore population (SOP), which are 10–20 mm and up to 30 mm, respectively (Suzuki et al. 2005). The maximum adult body length of *Clione* is known to be 70–85 mm in *C. limacina* (Conover and Lalli 1972; Lalli and Wells 1978; Gilmer and Lalli 1990), whereas it is <30 mm in *C. elegantissima* (Agersborg 1923; McGowan 1963; Gilmer and Lalli 1990). The spatio-temporal isolation and different body lengths suggest that a cryptic species or *C. limacina* could have drifted from the Subarctic Atlantic Ocean to the southern Okhotsk Sea and without being damaged by the sea ice because of their medium size. In this study, the taxonomical positions of both the WCP and SOP were investigated morphologically and genetically. Additionally, isolation mechanisms were discussed based on the water dynamics of adjacent waters.

Materials and Methods

Sampling

For the sampling, the WCP was collected from January 30 to February 3, 2017 during the spring tide time from shallow waters at a depth range of 0–50 cm at the entrance of Lake Saloma (N44°13, E143°97), in the southern Okhotsk Sea (Fig. 1, L.S, ●WCP). In addition, the SOP was collected from May to June 2017 from night-time shallow waters at a depth range of 0–50 cm at Kitami-Yamato Bank (N44°13, E143°97), from breaktime fishermen fishing Kichiji rockfish (Fig. 1, K.Y.B, ●SOP). The individual specimens were placed and observed in 150 mm × 150 mm × 20 mm viewing aquariums and preserved in 99.9% ethanol as DNA samples.

Morphology

The external morphology of the live animals was observed in situ in the aquarium and under a stereomicroscope (Nikon SMZ18). The radula of three specimens of WCP and SOP was extracted from the buccal mass and cleaned in 1 N sodium hydroxide (NaOH) solution in a 50 °C water bath for 30 min. Sputter coating was performed for 5 min at 20 mA (JEOL JEC-3000FC; EC-30020RTS) and samples were observed using a stereomicroscope (Nikon SMZ18) and a scanning electron microscope (SEM, JEOL JCM-6000Plus NeoScope) at an acceleration voltage of 5 kV.

Molecular Sequence Analysis

Eleven specimens were used for DNA extraction, amplification, and sequencing, consisting of six and five specimens of the WCP and SOP. The DNA was extracted using the QIAGEN DNeasy blood and tissue kit (Qiagen). The cytochrome c oxidase subunit 1 (*COI*) gene was amplified using the universal DNA primers LCO-1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO-2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). Polymerase chain reactions (PCRs) were performed using the GeneAmp PCR System 9600 (Applied Biosystems). Reactions were run using the following schedule: initial denaturation, 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 50 °C for 45 s, 72 °C for 1 min; and final extension, 72 °C for 5 min (Jennings et al. 2010; Yamazaki and Kuwahara 2017). PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were performed using a BigDye Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems) using a GeneAmp PCR System 9600 (Applied Biosystems). Reaction products were purified using ethylenediaminetetraacetic acid (EDTA), sodium acetate, and ethanol precipitation, and then electrophoresed using an ABI PRISM 3100 genetic analyzer (Applied Biosystems). Sequences were aligned and edited using the MEGA7 program (Kumar et al. 2016). There were 569 positions in the final dataset.

New sequences have been deposited in the DNA Data Bank of Japan (accession numbers: LC341683-LC341693). One and six COI sequences of *C. elegantissima* and *C. limacine*, respectively, which were already available in GenBank were included in the calculation of the genetic distance. Two COI sequences for pseudothecosome pteropods were downloaded from GenBank and used as an outgroup to root the tree (see Fig. 4 for accession numbers).

The molecular phylogenetic analysis was conducted using the maximum likelihood (ML) method based on the Kimura 2-parameter (K2P) model (Kimura 1980). Initial trees for the heuristic search were obtained by applying the Neighbor-Ioining and BioNJ algorithms to a matrix of pairwise distances estimated using the PhyML ML, and then selecting the topology with superior log likelihood values. A bootstrap consensus tree was calculated from 10,000 replicates and branches found in <50% of the bootstrap replicates were collapsed. The analysis involved 28 nucleotide sequences and the evolutionary analyses were conducted using MEGA 7 (Kumar et al. 2016).

Results

Morphology

The total materials were collected for up to 1000 individuals and up to 2000 individuals for both the WCP and SOP, respectively. The WCP live materials had a length of 8 to 23 mm, whereas SOP materials had a length of up to 45 mm (Fig. 2); the specimens shrank by approximately two-thirds, but almost no shrinkage of the viscera occurred in 99.9% of the ethanol-preserved specimens. The radula morphology of WCP was approximately 500 μm long, the central teeth lacked approximately 9 lines of the anterior rows, the posterior to anterior consisted of approximately 30 lines (Fig. 3a), and a crescent-like morphology was observed (Fig. 3b). The lateral teeth consisted of 11 lines (Fig. 3c) and an L-like morphology, whereas the old part of the anterior showed desquamative central teeth and was spread out in a fan-like form outside (Fig. 3a). In contrast, SOP was approximately 600 μm long, the central teeth lacked approximately 10 lines of the anterior rows, the posterior to anterior consisted of approximately 40

Fig. 2 The live condition of two body size types *Clione* in the viewing aquarium. Above (small individual): winter coastal population (WCP), approximately 12 mm body length. Below (big individual): spring offshore population (SOP), approximately 45 mm body length. Scale 10 mm

lines (Fig. 3d), and a crescent-like morphology was observed (Fig. 3e). The lateral teeth consisted of 11 lines (Fig. 3f) and an L-like morphology (Fig. 3d).

Molecular Analysis

The K2P distances observed within and between the two populations were 0.005–0.014 within WCP, 0.007–0.016 within SOP, and 0.005–0.019 between WCP and SOP (Table 1).

The PhyML software generated a tree with a topology very similar to that of the bootstrap consensus tree obtained from MEGA (Fig. 4). The monophyly of the ingroup (genus *Clione*) and the sister relationship between the WCP (Fig. 4○) and SOP (Fig. 4◎) each formed an unsupported clade (Fig. 4).

Discussion

Morphology

The SOP specimens showed the longest body length (up to 45 mm), which has been the focus of considerable attention from the news media such as in the coverage of the "Giant *Clione*" at the habitat museum (Fig. 2 below individual).

In the North Pacific Ocean, the *C. elegantissima* population has an average body size of 28 mm according to its original description. Subsequently, Gilmer and Lalli (1990) reported a size of <30 mm in the North Pacific regions.

SOP was collected at night, when it appears at the sea surface at depths of 0–50 cm, using nets. This suggests that they inhabit the areas of the East Sakhalin cold current (at a depth range of 0 to 200 m; see next paragraph), in the Soya warm

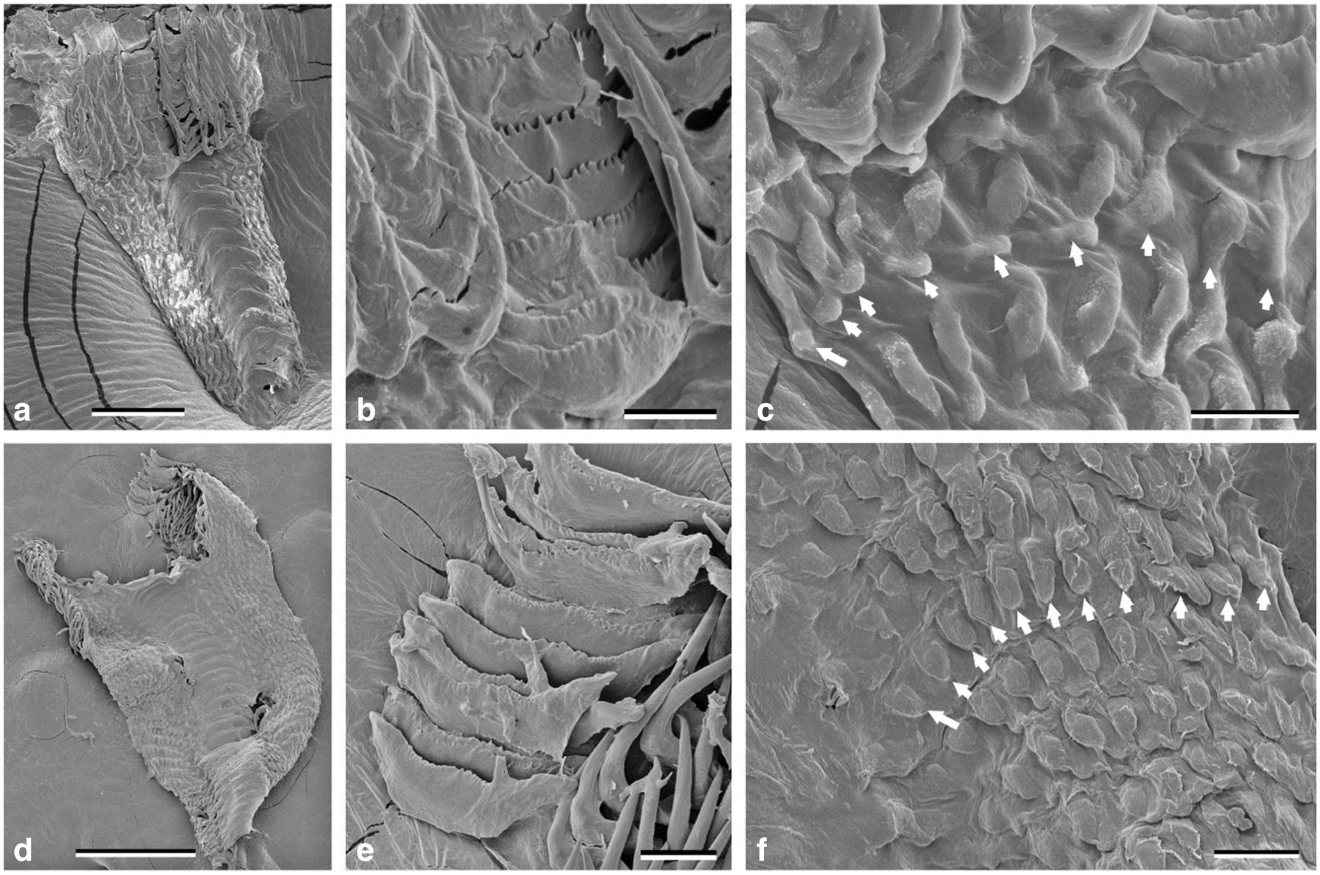

Fig. 3 Back side of the radula of *Clione elegantissima* winter coastal population (WCP; **a**–**c**) and spring offshore population (SOP; D-F): (**a**, **d**) Overall, (**b**, **e**) central teeth, and (**c**, **f**) lateral teeth. Arrows indicate lateral teeth inner to outer on one line. Scale 50 μm (**a**) and 10 μm (**b** and **c**), 200 μm (**d**), 20 μm (**e** and **f**)

Table 1 Estimates of genetic divergence between sequences of *Clione* species. The number of base substitutions per site between sequences is shown. Analyses were conducted using the Kimura 2-parameter (K2P) model. The analysis involved 28 nucleotide sequences

	Clione limacina AB859614	Clione limacina AB859613	Clione limacina AB859615	Clione limacina AB859612	Clione limacina AB859611	Clione limacina AB859616	*Clione elegantissima* LC341688	Clione elegantissima LC341684	Clione elegantissima LC341683	Clione elegantissima LC341687
Clione limacina AB859614										
Clione limacina AB859613	0.010									
Clione limacina AB859615	0.019	0.016								
Clione limacina AB859612	0.019	0.019	0.028							
Clione limacina AB859611	0.023	0.023	0.028	0.010						
Clione limacina AB859616	0.016	0.016	0.021	0.021	0.021					
Clione elegantissima LC341688	0.060	0.060	0.066	0.062	0.058	0.049				
Clione elegantissima LC341684	0.070	0.070	0.077	0.064	0.064	0.060	0.016			
Clione elegantissima LC341683	0.064	0.064	0.070	0.062	0.058	0.054	0.010	0.012		
Clione elegantissima LC341687	0.066	0.066	0.073	0.064	0.060	0.056	0.009	0.014	0.009	
Clione elegantissima LC341686	0.064	0.064	0.070	0.062	0.058	0.054	0.010	0.016	0.010	0.009
Clione elegantissima LC341690	0.064	0.064	0.070	0.062	0.058	0.054	0.010	0.016	0.010	0.009
Clione elegantissima LC341693	0.070	0.070	0.077	0.068	0.060	0.060	0.012	0.014	0.012	0.010
Clione elegantissima LC341692	0.064	0.064	0.070	0.062	0.054	0.054	0.014	0.019	0.014	0.012
Clione elegantissima LC341685	0.060	0.064	0.070	0.062	0.054	0.054	0.010	0.016	0.010	0.009
Clione elegantissima LC341691	0.068	0.068	0.075	0.066	0.058	0.058	0.010	0.016	0.010	0.009

Table 1 (continued)

Clione elegantissima LC341689	0.066	0.062	0.073	0.064	0.056	0.056	0.009	0.014	0.009	0.007
Clione okhotensis LC144855	0.100	0.094	0.100	0.094	0.097	0.097	0.108	0.117	0.117	0.114
Clione okhotensis LC144853	0.117	0.111	0.117	0.117	0.120	0.114	0.120	0.123	0.129	0.120
Clione okhotensis LC144851	0.111	0.105	0.111	0.111	0.114	0.114	0.117	0.120	0.126	0.117
Clione okhotensis LC144852	0.117	0.111	0.111	0.117	0.120	0.120	0.109	0.106	0.118	0.109
Clione okhotensis LC144854	0.111	0.105	0.111	0.111	0.114	0.114	0.109	0.112	0.118	0.109
Clione antarctica AB859618	0.238	0.242	0.238	0.235	0.222	0.235	0.213	0.210	0.210	0.210
Clione antarctica AB859619	0.238	0.241	0.238	0.231	0.219	0.241	0.225	0.216	0.222	0.222
Clione antarctica AB859617	0.232	0.235	0.232	0.235	0.222	0.242	0.225	0.216	0.222	0.222
Clione antarctica GU227107	0.235	0.238	0.235	0.232	0.219	0.238	0.222	0.213	0.219	0.219
Peracle valdiviae FJ876940	0.709	0.681	0.709	0.681	0.681	0.702	0.659	0.640	0.640	0.640
Peracle bispinosa FJ876939	0.747	0.721	0.740	0.727	0.734	0.727	0.734	0.747	0.747	0.753

	Clione elegantissima LC341686	Clione elegantissima LC341690	Clione elegantissima LC341693	Clione elegantissima LC341692	Clione elegantissima LC341685	Clione elegantissima LC341691	Clione elegantissima LC341689	Clione okhotensis LC144855	Clione okhotensis LC144853
Clione limacina AB859614									
Clione limacina AB859613									
Clione limacina AB859615									
Clione limacina AB859612									
Clione limacina AB859611									
Clione limacina AB859616									
Clione elegantissima LC341688									

Table 1 (continued)

Clione elegantissima LC341684									
Clione elegantissima LC341683									
Clione elegantissima LC341687									
Clione elegantissima LC341686									
Clione elegantissima LC341690	0.007								
Clione elegantissima LC341693	0.012	0.012							
Clione elegantissima LC341692	0.014	0.014	0.009						
Clione elegantissima LC341685	0.007	0.007	0.012	0.010					
Clione elegantissima LC341691	0.010	0.010	0.012	0.010	0.007				
Clione elegantissima LC341689	0.009	0.009	0.010	0.009	0.005	0.005			
Clione okhotensis LC144855	0.114	0.105	0.114	0.114	0.114	0.114	0.111		
Clione okhotensis LC144853	0.126	0.117	0.126	0.126	0.126	0.126	0.123	0.030	
Clione okhotensis LC144851	0.123	0.114	0.123	0.123	0.123	0.123	0.120	0.024	0.028
Clione okhotensis LC144852	0.115	0.106	0.109	0.115	0.115	0.115	0.112	0.035	0.042
Clione okhotensis LC144854	0.115	0.106	0.115	0.109	0.115	0.115	0.112	0.035	0.037
Clione antarctica AB859618	0.213	0.204	0.210	0.207	0.207	0.207	0.207	0.204	0.223
Clione antarctica AB859619	0.225	0.216	0.216	0.219	0.219	0.219	0.219	0.204	0.223
Clione antarctica AB859617	0.225	0.216	0.216	0.219	0.219	0.219	0.219	0.204	0.216

Table 1 (continued)

Clione antarctica GU227107	0.222	0.213	0.213	0.216	0.216	0.216	0.216	0.200	0.219
Peracle valdiviae FJ876940	0.659	0.646	0.646	0.622	0.646	0.646	0.628	0.659	0.680
Peracle bispinosa FJ876939	0.747	0.721	0.734	0.721	0.734	0.727	0.734	0.735	0.776

	Clione okhotensis LC144851	Clione okhotensis LC144852	Clione okhotensis LC144854	*Clione antarctica* AB859618	Clione antarctica AB859619	Clione antarctica AB859617	Clione antarctica GU227107	Peracle valdiviae FJ876940	*Peracle bispinosa* FJ876939
Clione limacina AB859614									
Clione limacina AB859613									
Clione limacina AB859615									
Clione limacina AB859612									
Clione limacina AB859611									
Clione limacina AB859616									
Clione elegantissima LC341688									
Clione elegantissima LC341684									
Clione elegantissima LC341683									
Clione elegantissima LC341687									
Clione elegantissima LC341686									
Clione elegantissima LC341690									
Clione elegantissima LC341693									
Clione elegantissima LC341692									

Table 1 (continued)

Clione elegantissima LC341685								
Clione elegantissima LC341691								
Clione elegantissima LC341689								
Clione okhotensis LC144855								
Clione okhotensis LC144853								
Clione okhotensis LC144851								
Clione okhotensis LC144852	0.037							
Clione okhotensis LC144854	0.028	0.013						
Clione antarctica AB859618	0.223	0.219	0.219					
Clione antarctica AB859619	0.223	0.211	0.219	0.012				
Clione antarctica AB859617	0.216	0.204	0.212	0.012	0.003			
Clione antarctica GU227107	0.219	0.208	0.216	0.014	0.005	0.005		
Peracle valdiviae FJ876940	0.682	0.642	0.642	0.596	0.601	0.596	0.602	
Peracle bispinosa FJ876939	0.743	0.792	0.784	0.741	0.727	0.727	0.727	0.475

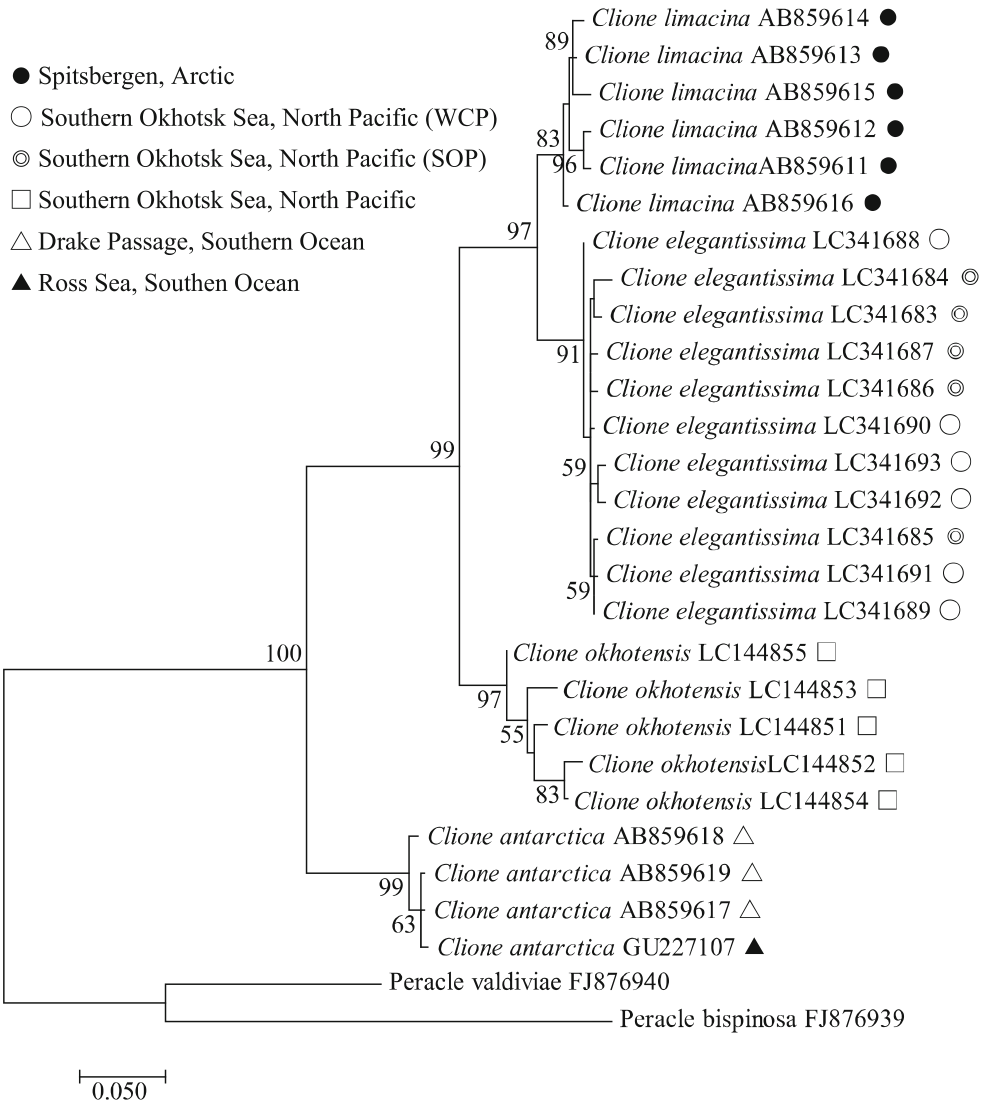

Fig. 4 Maximum likelihood (ML) phylogenetic tree of *Clione* species based on mtDNA individual *Clione elegantissima* (COI) sequences. Numbers at nodes represent bootstrap support

current (Takizawa 1982). *Clione* might exhibit diel vertical migration in the East Sakhalin current and appear at the sea surface at night. Migration is supported by the swimming ability based on the body size. In the Arctic to the North Atlantic Ocean, *C. limacina* species has a body size of 70 mm and swims at a speed of 100 mm/s (Gilmer and Lalli 1990). However, the vertical distribution and swimming ability of both populations are unknown in the North Pacific Ocean and from the Arctic to North Atlantic Ocean.

The radula is a common morphological feature of the genus *Clione* that consists of the central and L-like lateral teeth (Fig. 3). The radula tooth distribution allows the distinction of *C. antarctica* from other species of the genus (Smith 1902). However, the numbers of lateral teeth show usually intraspecific variation (Gilmer and Lalli 1990). In this study, WCP and SOP showed similar variation in radula traits, supporting a conspecific status.

Population Genetics

The K2P distances indicate that the differences observed between populations are of the same magnitude as those observed within populations, and therefore, they show that the genetic differentiation between the two populations is almost nil. Moreover, the COI sequences from the two populations, WCP and SOP, appear in the same clade and exhibit a maximum K2P distance of 1.9% (Table 1). This distance is at the intraspecies level in both populations (Jennings et al. 2010). Therefore, the two populations are conspecific based on the mitochondrial genetic analysis. Therefore, the two populations are intraspecies based on the genetic analysis. However, mtDNA data alone do not provide a full demonstration that the two populations are conspecific, as occasional hybridization can lead to the presence of the same mtDNA in two species that otherwise differ at the nDNA level (e.g., Azuma et al. 2011). An additional study of nuclear DNA sequences or microsatellite marker analysis should be conducted to fully confirm the conspecificity of the WCP and SOP populations.

Emergence of Spatio-Temporal Separated Populations

Because WCP and SOP are genetically similar, a mechanism that allows the separation of the two populations in time and space should be sought. Here, we propose a mechanism based on the pattern of water circulation in the region.

The warm water flowing into the Okhotsk Sea through the Soya Straits is divided into two parts: the Soya Warm Water (SWW: June to November) and the Forerunner of the Soya Warm Water (FSWW: March to May) (Aota 1975; Matsuyama et al. 1999). At the end of May, the SWW flows into the subsurface layer (approximately 200–400 m deep) of the Abashiri Bay. Furthermore, it flows northeastward as a subsurface current just off the coast of the Kuril Islands and reaches the region northwest of Etorofu Island (Takizawa 1982). In addition, the cold water flowing into the Okhotsk Sea around Japan along the east of Sakhalin Island is divided into the East Sakhalin Current Water (ESCW: November to April) (Ohshima et al. 2002). Additionally, the Cold-Water Belt (Fig. 1b, CWB) is frequently formed off the SWW during summer and autumn. The CWB is upwelling cold water that originates from either subsurface water of the Japan Sea off Sakhalin or bottom water of the Okhotsk Sea (Kuma et al. 2014). During the seasonal change, both populations were found based on the pattern of FSWW, SWW, WSCW and CWB. In conclusion, the WCP fades in the SWW, whereas the SOP is protected by the sunken warm current. Therefore, the SOP population is found in the spring. The mechanisms of isolation of the two populations are related to the water currents of both warm and cold temperatures.

Summary of the Results and Conclusions

Two types of *Clione* with different body lengths were found in the southern Okhotsk Sea, which was the emergence of temporal-spatial separated populations. The small type appeared in winter in coastal areas, whereas the large type appeared in spring in offshore areas. The large type of *Clione* was not recorded in North Pacific areas, which suggests cryptic species or drift of middle-sized *C. limacine* from Arctic Atlantic Ocean. mtDNA COI barcodes were applied at the intraspecies level in both populations, and they identified *C. elegantissima*. We found that the isolation mechanism involved specific dynamics of the adjacent waters. However, mtDNA data alone do not provide a full demonstration that the two populations are conspecific, as occasional hybridization can lead to the presence of the same mtDNA in two species, which otherwise differ at the nDNA.

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