

**Rapid Communication****First record of the non-native jellyfish *Chrysaora pacifica* (Goette, 1886) (Cnidaria, Scyphozoa) in Liaodong Bay, Bohai Sea, China**Yantao Wang<sup>1,2</sup>, Nan Wang<sup>1,2</sup>, Song Sun<sup>1,2,3,\*</sup>, Junjian Wang<sup>4</sup> and Xin Jin<sup>1,2</sup><sup>1</sup>Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology; Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao 266071, P. R. China<sup>2</sup>Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, P. R. China<sup>3</sup>University of Chinese Academy of Sciences, Beijing 100049, P. R. China<sup>4</sup>North China Sea Marine Forecasting Center of State Oceanic Administration, Qingdao 266061, P. R. China

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**Citation:** Wang Y, Wang N, Sun S, Wang J, Jin X (2022) First record of the non-native jellyfish *Chrysaora pacifica* (Goette, 1886) (Cnidaria, Scyphozoa) in Liaodong Bay, Bohai Sea, China. *BioInvasions Records* 11(1): 206–214, <https://doi.org/10.3391/bir.2022.11.1.21>

**Received:** 1 June 2021**Accepted:** 17 December 2021**Published:** 7 February 2022**Handling editor:** David Hudson**Thematic editor:** Andrew David**Copyright:** © Wang et al.

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**OPEN ACCESS****Abstract**

The occurrence of *Chrysaora pacifica* (Goette, 1886) (Cnidaria, Scyphozoa) is reported from most Japanese and Korean coastal waters. This is the first record of the alien jellyfish *Chrysaora pacifica* from the Liaodong Bay, Bohai Sea, China. Eight specimens of *Chrysaora* sp. were collected. Based on the evidence from morphology (bell, size, umbrella pattern, number of tentacles, and lappets) and further molecular analysis (mitochondrial DNA sequences comparison), we identified the *Chrysaora* sp. jellyfish as *Chrysaora pacifica* which belonged to Cnidaria, Scyphozoa. The distribution of *C. pacifica* in the Bohai Sea is currently unknown, however, there is a high potential risk for the spread of this species to the whole Bohai Sea by the ocean currents circulation system. As *C. pacifica* is a bloom-forming species, we recommend further work to assess the distribution of this species and its impact on the local fauna and flora.

**Key words:** scyphomedusa, new records, morphology, molecular Tools, China Sea**Introduction**

Under the pressure of global changes and human activities, mass occurrences of jellyfish are frequently reported in the coastal waters in recent decades, causing great concern due to the effect of their blooms on fisheries, aquaculture, tourism, and coastal plants (Purcell 2012). The class Scyphozoa includes approximately 201 accepted species worldwide (Daly et al. 2007; Daglio and Dawson 2017). Only 35 species of scyphomedusae, belonging to 20 genera in 16 families, have been recorded in Chinese seas. Of these, 7 species belong to the order Coronatae, 8 to Semaestomeae, and 20 to Rhizostomeae (Gao et al. 2002; Hong and Lin 2010). In recent years, the genus *Versuriga* and the species *V. anadyomene* (Maas, 1903) and *Phyllorhiza* sp. were recorded in Chinese seas (Dong et al. 2019; Sun et al. 2019). There are four species of scyphomedusae in the Bohai Sea and Yellow

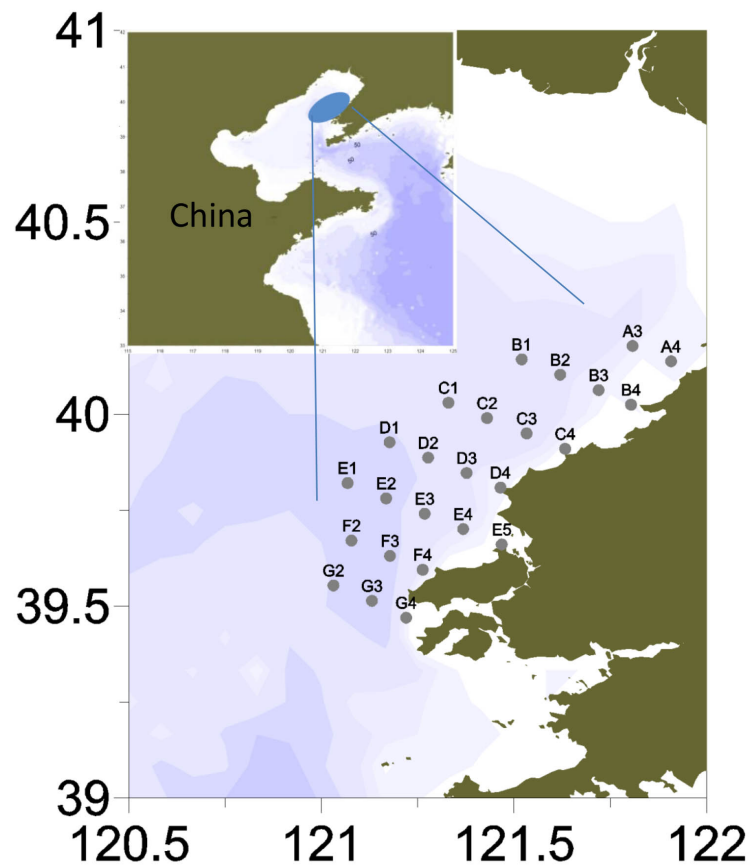
Sea of China. *Rhopilema esculentum* (Kishinouye, 1891) are economically exploited as a food resource, blooms formed by *Aurelia coerulea* (von Lendenfeld, 1884) (formerly mistaken for *A. aurita* (Linnaeus, 1758)), *Cyanea nozakii* (Kishinouye, 1891), and *Nemopilema nomurai* (Kishinouye, 1922) have proven to be detrimental to fisheries, tourism, and aquaculture (Dong et al. 2010; Sun et al. 2015).

The scyphozoan medusa genus *Chrysaora* (Péron & Lesueur, 1810) (Semaestomeae: Pelagiidae) is composed of many species, and frequently occurs in coastal waters around the world. Currently, species of *Chrysaora* are discriminated by morphological characteristics such as the tentacle number, shape of radial septa, order of tentacle development, coloration, and the form of nematocyst capsules. Recently, Morandini and Marques (2010) analyzed morphological features of various *Chrysaora* specimens and found 16 valid and two doubtful species in genus *Chrysaora* (Bayha et al. 2017; Collins et al. 2021). *Chrysaora pacifica* (Goette, 1886) is distributed in the North Pacific Ocean (most Japanese coastal waters) and most coastal waters in Korea (mainly southern and eastern) and usually appeared from May to September (Lee et al. 2016). In Tokyo Bay, which is adjacent and on the east side of Sagami Bay, *C. pacifica* is a common member of the jellyfish community during the springtime (Kinoshita et al. 2006). It is known that the South China Sea waters is the type locality of *Chrysaora chinensis* (Vanhöffen, 1888), none of the *C. pacifica* have previously been reported in China.

During a field survey in the Bohai Sea China Sea, samples of jellyfish which had not been previously recorded in Chinese seas were collected. These specimens were identified as *Chrysaora pacifica* (Cnidaria, Scyphozoa) based on a combination of morphological characteristics, COI and 16S sequences data. This is the first record of *C. pacifica* in Liaodong Bay, the Bohai Sea, which is also the first record for China. The Bohai Sea is the epicontinental sea of China, its maximum depth is less than 100 m. Many rivers converge here, the Liaodong Bay is at the northern part of the Bohai Sea, which is one of the most important fishing grounds of China.

## Materials and methods

Jellyfish monitoring cruises were carried out at 25 stations in the northeast part of LDB, 4 times yr<sup>-1</sup> from May to September 2018 to 2019, the distance of two station is about 10 km (Figure 1). These surveys focused on *A. coerulea* and *N. nomurai*, as these cause fisheries damage and affect the cooling system of power station in LDB. As the jellyfish grew, drift nets of different mesh sizes (1, 3, and 10 cm for small, large juvenile, and adult, respectively) were used. They were placed across the current flow with a system of floats and sinkers for 30 minutes for each station. The length of each net was 30 to 60 m with a height of 8 to 12 m. The depth of the station is from 5 m to 38 m.



**Figure 1.** Sampling location in the present study in the Bohai Sea, China.

Sea surface and bottom temperature and salinity were measured at each site with JFE-Advantech CTD (ASTD102). For field measurements of diameter, we adopted the standard bell diameter (BD): distance from lappet to lappet of opposite sides for advanced metaephyra, and distance from one edge to the other edge of the bell for juvenile and medusa.

Morphological characters were examined by using the preserved specimens collected. We observed morphologies, including exumbrella, subumbrella, oral arms, marginal lappets, rhopalia, tentacles. The number of tentacles and lappets were counted and expressed as the number per octant (Chae et al. 2018).

For molecular analysis, medusa tissue, clipped from the bell margin, was preserved in 95% ethanol and stored at  $-20^{\circ}\text{C}$  until DNA extraction. Prior to genomic DNA extraction, alcohol-preserved jellyfish samples were washed in distilled water to remove all ethanol, and these procedures were repeated several times. The total genomic DNA was extracted using the TIANamp Marine Animals DNA Kit (TIANGEN). The sequencing was completed using the Illumina NovaSeq sequencing platform (Illumina, San Diego, CA). The genome sequence was assembled and analyzed using A5-miseq v20150522 (Coil et al. 2015) and SPAdes v3.9.0 (Bankevich et al. 2012). The pilon v1.18 (Walker et al. 2014) software was used to correct the results to obtain the final mitochondrial sequence. Annotation of the complete



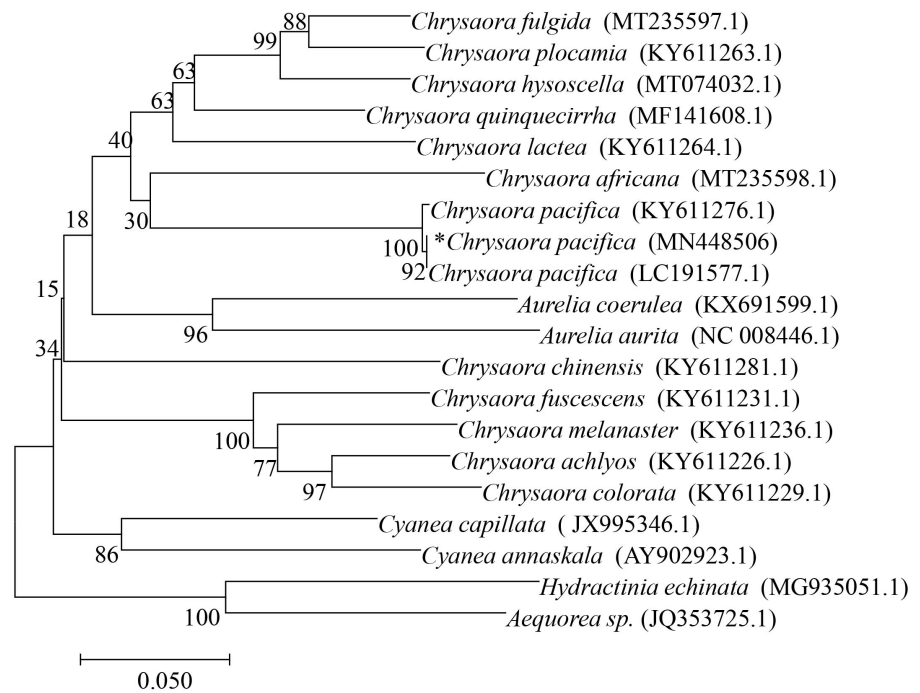
**Figure 2.** *Chrysaora* sp. specimen freshly collected in Liaodong Bay, Bohai Sea, China A July 2018, B June 2019, C July 2019. Photographs by Yantao Wang.

mitochondrial genome sequence was performed with the MITOS web server (<http://mitos.bioinf.uni-leipzig.de/>) (Bernt et al. 2013). The sequences were blasted in NCBI to confirm their identities. Additionally, closely related sequences were obtained from GenBank for phylogenetic analyses. Neighbor-joining analysis of COI and 16S data was performed using the K80 model with 1,000 bootstrap replicates. Phylogenetic analyses were conducted with MEGA5.0 (Ballard and Melvin 2010).

## Results

Our survey demonstrated the occurrence of the scyphozoan *Chrysaora* sp. in Liaodong Bay, Bohai Sea, China (Figure 2). A total of 8 *Chrysaora* sp. medusae (none juveniles medusae) were collected in all the investigations during the two years. On July 18, 2018, two individuals of large jellyfish (umbrella diameter 10 and 15 cm) were observed in one station (D4), and on June 17, 2019, two individuals of large jellyfish (umbrella diameter 5.5 and 7 cm) were observed in one station (A4 and C1), on July 13, 2019, four individuals of large jellyfish (umbrella diameter 8~18 cm) were observed in two stations (A3 and C3) at a depth of approximately 20 m.

The umbrella is almost hemispherical (Figure 2A, C) and divided into eight sectors. The surface of exumbrella is finely granulated with 32 radial stripes, mouth is at the center of subumbrella, with 4 oral arms (Figure 2B, C). Oral arms are approximately two-three times longer than the diameter of umbrella. The marginal lappets are rounded (Figure 2C) and there are six lappets per octant. The total number of lappets is 48 and there are numerous nematocyst warts on its external surface. The total number of tentacles is 40. The tentacle is extremely elastic and the length of tentacles is two-three times longer than the diameter of umbrella in live samples (Figure 2C). The combination of the morphological characteristics allowed to assign this specimen to *C. pacifica* (Cnidaria, Scyphozoa) (Morandini and Marques 2010).



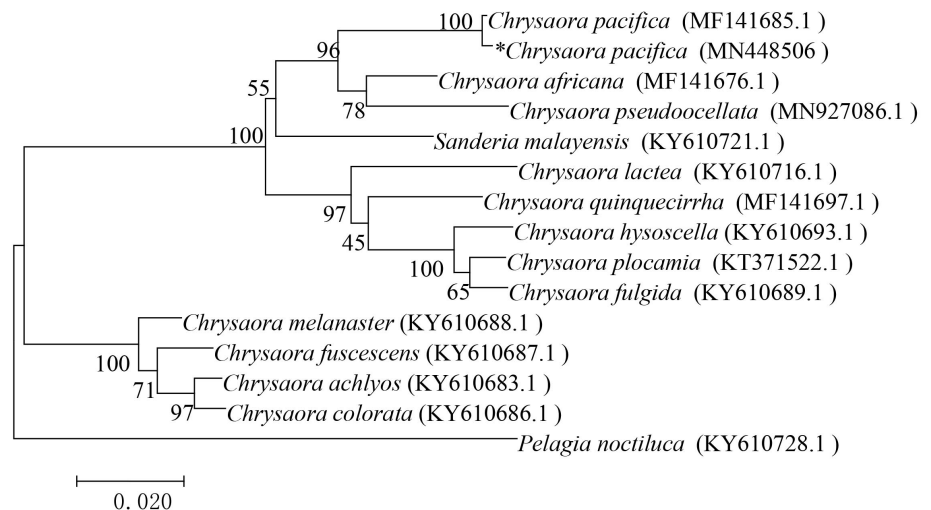
**Figure 3.** Neighbor-joining tree for mitochondrial COI fragments. Bootstrap values are shown above the branches.

The complete mitochondrial genome of *C. pacifica* was linear with 16,964 bp in length and the GenBank accession No. is MN448506). A BLAST search of the GenBank database revealed that the mtDNA COI sequences determined in our study nested among the genus *Chrysaora*, and were most similar to those of *C. pacifica* from the coastal waters of Japan (LC191577.1). The group including our sequences from China and the Japan specimen is the sister group of *C. pacifica* from Monterey Bay Aquarium USA; this grouping is supported by a 100% bootstrap value in neighbor-joining trees for mitochondrial COI fragments (Figure 3).

The group including our sequences from China is the sister group of *C. pacifica* from Monterey Bay Aquarium USA; this grouping is supported by a 100% bootstrap value in neighbor-joining trees for mitochondrial 16S fragments (Figure 4).

## Discussion

Four species of scyphomedusae frequently occur in the Bohai Sea: *A. coerulea*, *R. esculentum*, *C. nozakii*, and *N. nomurai* (Dong et al. 2010). Our survey was carried out since 2017, these four species were found in this area. After the first detection of two *C. pacifica* in 2018, an additional six samples in summer 2019 confirmed the presence of *C. pacifica* population in the Liaodong Bay, Bohai Sea, China. This study represents the first record of the scyphozoan *C. pacifica* population in the Liaodong Bay, Bohai Sea, China. It is still unknown whether the *C. pacifica* has established populations in the Liaodong Bay, due to the absence of information on the polyp and ephyra



**Figure 4.** Neighbor-joining tree for mitochondrial 16S fragments. Bootstrap values are shown above the branches.

stages of this jellyfish species. *Chrysaora pacifica* is a common species in the North Pacific Ocean, usually appeared from May to September in Japanese coastal waters and most coastal waters in Korea, and sometimes a nuisance to fisheries (Kinoshita et al. 2006; Lee et al. 2016). The mtDNA COI sequences determined in our study was most similar to those of *C. pacifica* from Maizuru Bay, Kyoto (LC191577.1) (Minamoto et al. 2017), suggesting that the jellyfish in the Bohai sea may have come from these area. The dispersal of the scyphozoans can be achieved in two ways: local dispersal by physical transport in the pelagic medusa stage and global transport via ships, barges, and offshore drilling platforms in the benthic polyp stage (Vodopivec et al. 2017; Bayha and Graham 2008). There are no certainties regarding possible pathway(s) of the arrival of *C. pacifica* in the Bohai Sea. However, along the coast of the Bohai Sea, there are several huge international ports (Yingkou Port, Tianjin Port, and Tangshan Port), shipping from nearby populations in the North Pacific Ocean constitutes the most probable ones. And as there are several aquariums (Dalian Aquarium, Qinhuangdao Aquarium, and Tianjin Aquarium) around the Bohai Sea, jellyfish may also be an expansion from aquariums. Many jellyfish species, including *C. pacifica*, have complex life cycles, with the occurrence of distinct benthic and pelagic life stages (i.e. the tiny sessile polyp and some cyst-like hidden propagules, the more conspicuous, free-living medusa, and the almost invisible planula larva stage) and all species need to be considered as a whole comprising both benthic and planktonic stages when considering possible routes of introduction (Boero et al. 2008).

Introduced jellyfish species are considered an important threat to biodiversity and ecosystem function in marine environments (Bax et al. 2001). The species *Phyllorhiza punctata* (von Lendenfeld, 1884) in the Gulf of Mexico is an example of a successful jellyfish invasion. Blooms of this species caused economic losses of up to US \$10 million to the shrimp

fishery in the Gulf of Mexico in 2000 (Graham et al. 2003). *Phyllorhiza* sp. appeared in an aquaculture pond on the coast of the southern Yellow Sea, fishermen needed to catch the medusae using hand nets to prevent damage to the shrimp *Penaeus japonicus* (Spence Bate, 1888) (Dong et al. 2019). Although the density of *C. pacifica* is not high in our survey in the Bohai Sea, the bloom possibility and the possible impacts of *C. pacifica* on other species and the ecosystem of the introduced area deserve further studies. So far, it is not known whether the specimens belong to a self-sustaining population in the area. No ephyrae of the species were ever identified in the net samples, while specimens observed were all juveniles (bell diameter 5.5~18 cm). During the *C. pacifica* occurrence period, the water surface temperature ranged from 17.91 °C (June 2019) to 26.59 °C (July 2019), and the water surface salinity varied from 28.52 (June 2019) to 32.66 (June 2019). Polyps of *C. pacifica* were found on sediments sampled from the sea bottom in Sagami Bay, Japan, they are recorded to strobilate from October to November in the laboratory of the Asamushi Marine Biological Station in northern Japan, and they strobilated when the temperature was lowered from room temperature (22–23 °C) to 5–10 °C in the previous study (Toyokawa 2011). Studies will be needed in the future to reveal more about the location and habitat conditions that polyps can survival in the field. And more attention should be paid to the jellyfish, through scientific surveys as well as citizen-based records (Douek et al. 2020). Further investigation is also needed to assess the factors that support *C. pacifica* proliferations as well as its impact on the Bohai Sea trophic resources and other gelatinous species populations.

### Acknowledgements

We are grateful to the reviewers for the helpful comments on an earlier version of this manuscript.

### Funding declaration

This research was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA23050301), the National Key R & D Program of China (No. 2017YFC1404401), the National Natural Science Foundation of China (No. 41506144).

### Authors' contribution

Yantao Wang: sample design and methodology, data analysis and interpretation, writing – original draft, writing – review and editing; Nan Wang: writing – review and editing; Song Sun: research conceptualization, funding provision; Junjian Wang: investigation and data collection; Xin Jin: species identification.

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