

First Report of *Cyamus Boopis* from A Humpback Whale (*Megaptera novaeangliae*) in the Coastal East China Sea

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Short report

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Abstract

Background: *Cyamus boopis* is an amphipod crustacean that obligately parasitizes the body surface of the humpback whale, *Megaptera novaeangliae*. The life cycle of *C. boopis* does not include a swimming stage, and the crustacean spends its entire life on the body of *M. novaeangliae*.

Methods: On November 15, 2017, a male humpback whale was found stranded on the coast of the cape of Yuan Tuo, Qidong, Nantong, JiangSu province, China. Parasites were collected from the carcass of this whale and identified by morphological techniques and molecular analysis.

Results: A total of 15 *C. boopis* specimens were collected, and eight females and seven males were morphologically identified. A phylogenetic tree of cytochrome c oxidase subunit 1 revealed that the collected specimens clustered together with previously reported *C. boopis* sequences from the Northern Hemisphere.

Conclusion: This is the first report of *C. boopis* in a humpback whale from the East China Sea and supplements data from humpback whales found off the coast of China. In addition, our data provide supplementary data on the migration paths of humpback whales.

Background

Amphipod crustaceans, or whale lice, are common ectoparasites found in cetaceans. However, whale lice remain among the least understood crustaceans, despite their specialized biological features. To date, 28 species of Cyamidae from eight genera, including at least 15 species of *Cyamus*, *Isocyamus*, *Neocyamus*, *Platycyamus* and *Syncyamus*, have been reported all over the world [1]. Some cyamid species parasitize several species of cetaceans. However, *Cyamus boopis* is found only on humpback whales (*Megaptera novaeangliae*) [2–6].

Acute ventral processes formulas and maxilliped palp characters are usually used to identify cyamids [2, 7–10]. However, a lack of available descriptions and variations in morphological characteristics have led to the incorrect identification of some *Cyamus* juveniles [2, 7]. In particular, identification of *C. boopis* over the decades has been controversial. *C. boopis* was first described by Lütken, who collected specimens from *M. novaeangliae* in 1870 [11]. In the following few years, *C. boopis* was transferred to the new genus *Paracyamus* based on the uni-articulate maxilliped palp present in adults [12]. Later *C. boopis* was returned to the *Cyamus* genus [2]. At the genus level, *C. boopis* and *Cyamus catodontis* are similar, as both lack ventral processes on pereonites 3 and 4 and have a smooth grinding surface on the right lacinia mobilis [7, 10]. This has led previous authors to confuse these two species. Therefore, in addition to morphological identification, molecular methods are required for proper identification.

The life cycle of whale lice does not include a swimming stage, and the parasite spends its entire life on the body of its whale host [4, 13]. Therefore, whale lice are a potential tool for studying humpback whale migratory routes around the world [6, 14]. Although there have been persistent reports of whale lice in the

Northern Hemisphere, molecular data on these parasites are still relatively scant [5, 14, 15]. *M. novaeangliae* are one of the most cosmopolitan whales in the world [16]. The migration patterns of humpback whales have been extensively studied over the last few decades. However, there is still a gap in data on *M. novaeangliae* in the Northern Hemisphere, especially near China.

In this study, we describe the first known case of whale lice collected from a humpback whale stranded on the coast of the East China Sea. Based on morphological identification and cytochrome c oxidase subunit 1 (COI) gene sequence analysis, we confirmed that the lice belonged to *C. boopis*. This report supplements data on *C. boopis* and its humpback whale hosts off the coast of China and provides information on the possible migration paths of humpback whales.

Methods

Whale lice (JS isolate) were collected from the carcass of a male humpback whale (body length, 6.62 m) on the coast of the East China Sea on the cape of Yuan Tuo, Qidong, Nantong, Jiangsu province, China (N 31°44'28", E 121°56'10") on November 15, 2017 (Graphical Abstract).

Parasites were fixed and preserved in 70% ethanol. Microscopic examinations were performed using an Olympus BX53 (Olympus, Tokyo, Japan) with an adapted Canon EOS R camera (Canon, Tokyo, Japan). Morphological characteristics and descriptions were used to identify parasites according to previous reports [2, 7, 10]. Drawings were digitally prepared using Adobe Illustrator CS6 (Adobe Inc., San Jose, CA, USA).

Genomic DNA was extracted from the pereopod of each specimen using a QIAamp Fast DNA Tissue Kit (Cat No.51404, QIAGEN, Hilden, Germany) following the manufacturer's instructions. DNA samples were stored at -20°C until polymerase chain reaction (PCR) amplifications were performed. The COI gene was amplified using the following primers: CO1F, 5'-TGGCATCGTTTCTCACGTAG-3'; and CO1R, 5'-GGGTGATACCATTCTACAGCG-3'. Primer synthesis and PCR product sequencing were performed by Sangon Biotech (Shanghai, China).

To examine phylogenetic relationships, homologous sequences were selected and multiple alignments were performed using ClustalX2. A maximum likelihood tree was constructed using the online program PhyML 3.0 (<http://www.atgc-montpellier.fr/phyml/>) with Smart Model Selection [17]. The Akaike Information Criterion model was selected with Subtree Pruning and Regrafting as the type of tree improvement. The number of bootstrap replicates was 100. All sequences included in the phylogenetic tree were retrieved from the GenBank database (Table 1).

Table 1
Sequence information included in the phylogenetic tree

No.	Accession No.	Ocean	Hemisphere	Reference
Cyamus boopis				
1	MT458501	WNP	NH	present study
2	DQ095150.1	NP	NH	Kaliszewska,Z.A. <i>et al</i> , 2005
3	FJ751159.1	NP	NH	Callahan,C.M. <i>et al</i> ,2008
4	FJ751158.1	NP	NH	
5	MG720511.1	WSP	SH	Iwasa-Arai,T. <i>et al</i> ,2018
6	MG720513.1	WSP	SH	
7	MG720500.1	ESA	SH	
8	MG720497.1	WSA	SH	
9	MG720490.1	WSA	SH	
10	MG720494.1	WSA	SH	
11	MG720519.1	WSA	SH	
12	MG720533.1	WSA	SH	
13	MG720523.1	WSA	SH	
14	MG720528.1	WSA	SH	
15	MG720479.1	WSA	SH	
Cyamus erraticus				
16	DQ095128.1	NP	NH	Kaliszewska,Z.A. <i>et al</i> , 2005
17	DQ095129.1	SO	SH	
18	DQ095139.1	SO	SH	
19	DQ095134.1	SO	SH	
20	DQ095135.1	SO	SH	
21	DQ095107.1	NA	NH	
22	DQ095127.1	NA	NH	—
23	DQ095106.1	NA	NH	—
<p>WNP: Western north pacific; NP: North Pacific; WSP: Western South Pacific; ESA: Eastern South Atlantic; WSA: Western South Atlantic; NP: North Pacific; SO: Southern ocean; NA: North atlantic; SH: South Hemisphere; NH: North Hemisphere.</p>				

No.	Accession No.	Ocean	Hemisphere	Reference
	Cyamus gracilis			
24	DQ095104.1	—	—	—
25	DQ095096.1	—	—	—
26	DQ095087.1	SO	SH	—
27	DQ095100.1	—	—	—
28	DQ095092.1	—	—	—
29	DQ095048.1	NA	NH	—
30	DQ095089.1	SO	SH	—
31	DQ095102.1	SO	SH	—
32	DQ095050.1	NA	NH	—
33	DQ095049.1	NA	NH	—
WNP: Western north pacific; NP: North Pacific; WSP: Western South Pacific; ESA: Eastern South Atlantic; WSA: Western South Atlantic; NP: North Pacific; SO: Southern ocean; NA: North atlantic; SH: South Hemisphere; NH: North Hemisphere.				

Results

All whale lice exhibited specific features that allowed for sex and species identification. In total, eight female and seven male *C. boopis* specimens were identified. *C. boopis* adults differ from other *Cyamus* species by the number of ventral processes on pereonites 5–7. Sex was assigned based on several characteristics: pereonites 3 and 4 are broader than those of males; subequal in width to pereonites 5 and 6; and the pleons of females lack pleopods (Fig. 1).

The phylogenetic tree showed that the sequence of the collected specimens (JS isolate) clustered with all other reported *C. boopis* COI sequences but was separate from other *Cyamus* species (Fig. 2). The sequence was deposited in the GenBank database (accession number, MT551876).

Discussion

To the best of our knowledge, this is the first report of *C. boopis* in a humpback whale in Chinese waters. In general, research on humpback whales and whale lice in China is rare. There has been only one report of a North Atlantic right whale, *Eubalaena glacialis*, stranded on the coast of the Yellow Sea in China (N 38°55', E 123°40') in 1977 on which three species of whale lice (*Cyamus erraticus*, *Cyamus scammoni* and *Cyamus ovalis*) were identified [18]. Our report extends findings of humpback whales and whale lice in China.

Using morphological identification, the specimens collected from the humpback whale stranded on the coast of the East China were found to belong to *C. boopis*. Combined with mitochondrial DNA analysis of the COI sequence, we found that the *C. boopis* specimens clustered together with all other specimens from the Northern Hemisphere. A previous study compared *C. boopis* from three *M. novaeangliae* breeding stocks from the Southern Hemisphere with *C. boopis* from the Northern Hemisphere using the COI gene sequences and found that *C. boopis* from the two locations differed greatly in genetic structure [6]. However, data from the Northern Hemisphere was based on only three samples of *C. boopis*. Therefore, data on *C. boopis* and humpback whales in the Northern Hemisphere are lacking compared with those from the Southern Hemisphere.

Some studies have suggested that, in addition to *C. boopis*, *C. elongatus* may also parasitize humpback whales [13]. However, molecular studies of Brazilian *C. boopis* confirmed that all specimens of whale lice collected from *M. novaeangliae* were from the same species [2, 7]. Our study confirmed that the specimen collected from the humpback whale in China was also *C. boopis*. Therefore, more data are needed to support the presence of *C. elongatus* on humpback whales.

Whale lice can provide valuable information about whale population histories. Because whale lice have no alternative hosts or free-living stages and have a long history of living on their whale hosts [4, 19]. In addition, because of their short generation time [19] and large population size [20], synonymous sequence divergence of homologous markers of Cyamidae can be ten times faster than that of their whale hosts [14]. The genetic structure of cyamids could also reveal encounters between whales of different stocks [6, 13]. Although records have been collected of whale lice from Cetacea in coastal waters all over the world, molecular information on whale lice is still limited. Therefore, the collection of whale louse samples and their genetic data could provide a scientific basis for the classification and identification of whale lice and provide information for the further study of whale populations and migration.

Conclusions

To the best of our knowledge, this is the first report of *C. boopis* in humpback whale from China. This study extends the track of humpback whale over the world. The record of *C. boopis* provides important supplementary details and data for of studying the migration paths of humpback whales and population communication.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YQ wrote and designed the manuscript; XWM and XYC performed the morphology description and draw the figures; BYC collected and contributed the sample of the parasite; SPZ performed the molecular identification of the parasite; All authors read and approved the final manuscript.

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Figures

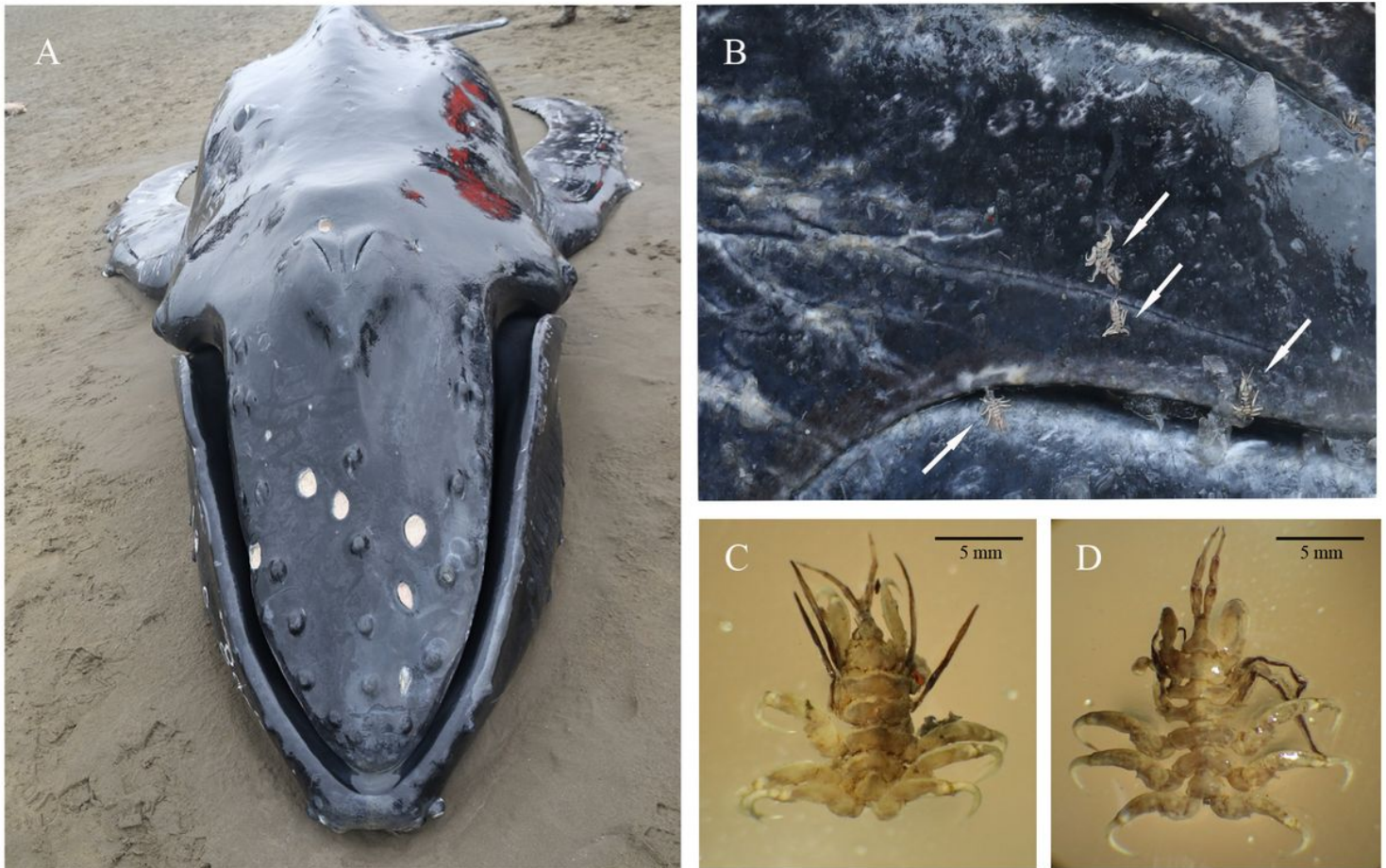


Figure 1

Whale lice collection from the carcass of a male humpback whale on the coast of the East China Sea. A) humpback whale; B) Whale lice on humpback whale, white arrow indicates the whale lice; C) *Cyamus boopis* Lütken, 1870. Female. Scale bar: 5 mm; D) *Cyamus boopis* Lütken, 1870. Male. Scale bar: 5 mm

Colored ranges

- *Cyamus boopis*
- *Cyamus erraticus*
- *Cyamus gracilis*

bootstrap

- 12
- 33.75
- 55.5
- 77.25
- 99

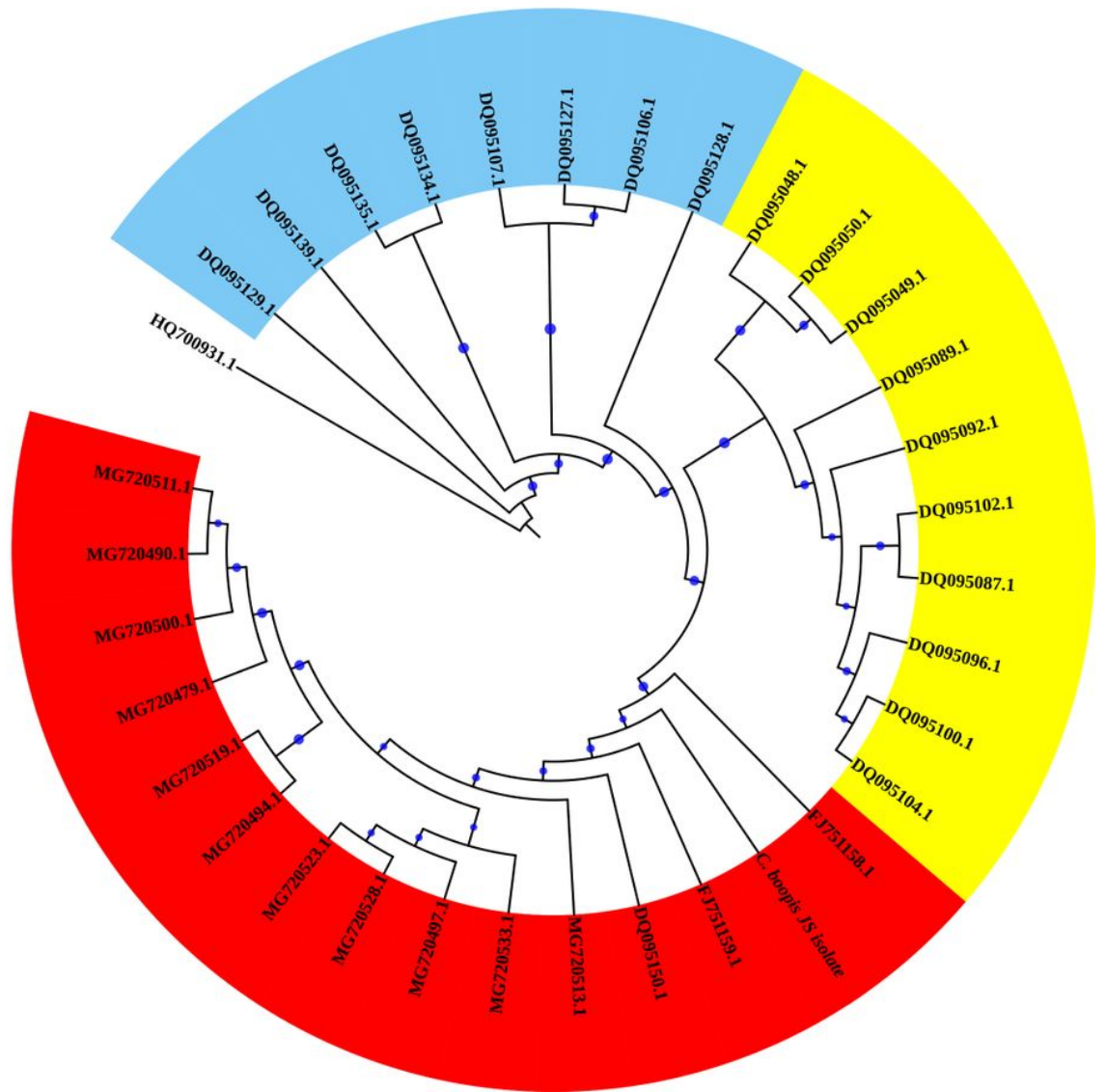


Figure 2

Phylogenetic tree of *Cyamus boopis* COI sequences constructed with maximum likelihood method using the online program PhyML 3.0. Node values represent the bootstrap confidence from 100 replicates. Red indicates *Cyamus boopis*. Yellow indicates *Cyamus gracilis*. Blue background indicates *Cyamus erraticus*