

First finding of live specimens of non-native copepod *Calanus finmarchicus* (Gunnerus, 1770) in the Sea of Marmara

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ABSTRACT

In April of both 2018 and 2019, one specimen each time of live *Calanus finmarchicus* adult females was found during collections of zooplankton samples near Sivri Ada in the Sea of Marmara. This was the first observation of live *C. finmarchicus* (a native species of northern Atlantic waters) in the eastern Mediterranean Sea. This morphological study illustrates the similarity of *C. finmarchicus* specimens found in the Sea of Marmara with existing illustrations and descriptions of this species. It also shows the clear differences between this species and *Calanus helgolandicus* specimens collected during the same or earlier periods from the Marmara Sea. Transportation of *C. finmarchicus* to the Sea of Marmara is possible either via the lower current from the Aegean Sea or through the ballast waters of vessels. In any case, the occurrence of *C. finmarchicus* as a cold-water species in the Sea of Marmara is striking and attracts further investigations.

Keywords: Calanoid copepod, taxonomy, *Calanus*, Marmara Sea, alien

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INTRODUCTION

Calanus finmarchicus (Gunnerus 1770) is widely distributed in the northern hemisphere and is among the most studied copepod species in the northern Atlantic (for example, Marshall & Orr 1972; Heath *et al.* 2000; Melle *et al.* 2014). This boreal species has dominated the North Atlantic Gyre (Fleminger & Hulsemann 1977), and its regional distribution varies with the North Atlantic Oscillation (Greene & Pershing 2000). The Azores and the Iberian coasts are the lower limit of their range in the North Atlantic. Rare records of this species in the western Mediterranean Sea (Rose 1933) may be due to its introduction into these waters via the southern branch of the North Atlantic Current. However, there has been no reliable evidence of this species being found in the central and eastern Mediterranean (Marshall & Orr 1972). Only two species of copepods of the genus *Calanus* have been found in this part of the Mediterranean

Sea: *C. helgolandicus* and *C. tenuicornis* (Brodskii 1950; Weikert *et al.* 2001), and only one of them, *C. helgolandicus*, permanently lives in the Sea of Marmara and the Black Sea (Svetlichny *et al.* 2006; 2010). In the Black Sea, the population of this species is also referred to as *Calanus euxinus* (Hulsemann 1991), however, according to the results of a morphological (Isinibilir *et al.* 2009) and genetic analysis (Ünal *et al.* 2006; Yebra *et al.* 2011; Figueroa *et al.* 2019), *C. euxinus* does not have differences in species rank from *C. helgolandicus* living in the Mediterranean basin (e.g. the Black Sea, the Sea of Marmara, the Aegean Sea, the Adriatic Sea) and in the North Atlantic.

The Sea of Marmara is a Turkish inland sea connected to the Black Sea via the Bosphorus and to the Aegean/Mediterranean Sea via the Dardanelles straits (Figure 1). It has a surface area of 11,350 km² with the greatest depth of 1,370 m. The Sea of Marmara has two distinct vertical



layers. In the upper layer, due to the continuous inflow of the less saline (18 PSU) Black Sea, the surface salinity is low averaging about 22 PSU (=Practical Salinity Unit). Mediterranean waters having a salinity of 38.5 PSU dominate the bottom layer 20-25 m, which is the depth of the permanent halocline/pycnocline. Except in the two shallow straits (Jarosz *et al.* 2013), these two layers in the Sea of Marmara do not generally mix, as is the case for the Black Sea. During the last few decades, the Sea of Marmara's ecosystem has faced several anthropogenic problems such as land-based pollution, eutrophication, overfishing, loss of habitats, and introduction of invasive species. The large-scale mucilage events occurring during several months in 2021 received great attention from scientists, the media, and the public. Strong stratification combined with pollution in the Sea of Marmara caused oxygen levels to drop to critical levels for metazoan life in the deeper waters.

The high volume of shipping traffic and particularly the discharge of ballast water by ships are the main reasons for the introduction of new species (Öztürk & Albayrak 2016). By 2014, the number of alien taxa found was 95 belonging to 11 different systematic groups, including 7 copepod species.

This paper presents the first finding of a total of two individual females of *C. finmarchicus* in the northeastern part of the Marmara Sea in the spring of 2018 and 2019.

MATERIALS AND METHODS

Zooplankton samples were collected on 3 April 2018 and 5 April 2019 with a closing Nansen net (opening diameter: 50 cm, mesh size: 200 μ m) by vertical hauls from the bottom (150 m) to the surface at a station located (40° 51' 53" N – 28° 57' 44" E) in the northern Marmara Sea south of Sivri Ada, off Istanbul, Türkiye (Figure 1). The total depth of the station was about 300-500 m since it was in a region steeply deepening. Water temperature and salinity were measured using a Sea-Bird CTD probe.

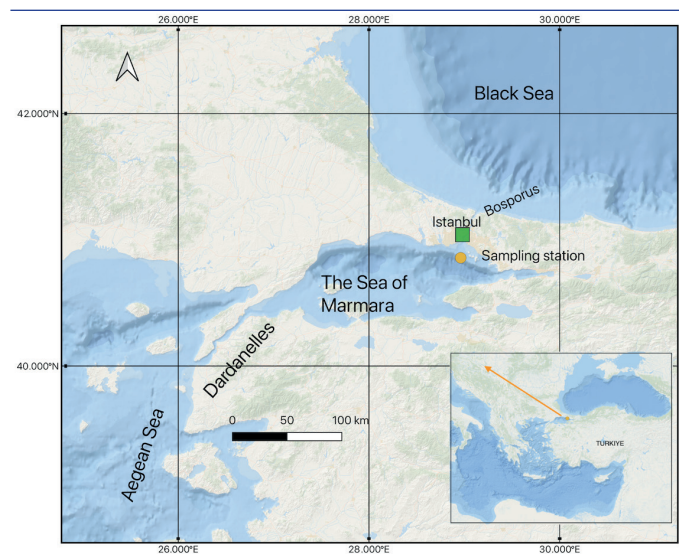


Figure 1. Sampling station (orange circle) in the Sea of Marmara in 2018 and 2019.

Immediately after capture, a sample of the collected species was placed into 5-litre bottles of clean, filtered seawater about 22 PSU and approximately 2 hours after collecting samples, all healthy copepod specimens were sorted individually at the laboratory using a wide pipette and placed in 1-litre volume vessels containing 0.45 μ m filtered seawater (temp. 17°C) for further study.

On 3 April 2018 nine females of the genus *Calanus* were caught, of which one individual looked different and was characterized by slow swimming. This specimen soon died in the laboratory. A preliminary morphological examination allowed us to suggest its taxonomic difference from other individuals belonging to the species *Calanus helgolandicus* living in this area. This specimen was photographed (Figure 2a, b) measured and fixed in 96% ethanol for further taxonomic analysis.

On 5 April 2019 fifteen females of the genus *Calanus* were caught, one of which stood out for its larger size, red color, and slow activity. This individual was anaesthetized with MS-222, photographed (Figure 3), measured and fixed in 96% ethanol. For comparison, females of *Calanus helgolandicus* were selected and fixed in the same catches. A detailed taxonomic analysis of selected individuals, including dissection and photography of their swimming legs and abdomen, was performed at the Department of Invertebrate Fauna and Systematics of I.I. Schmalhausen Institute of Zoology NASU, Kyiv (Ukraine).

The identification of the species was based on original descriptions of *Calanus finmarchicus* and their morphological comparison with *Calanus helgolandicus* and other species of the genus *Calanus* by Rose (1933), Brodsky (1950), Marshall & Orr (1972), Williams (1972) and Frost (1974) and Fleminger & Hulsemann (1977).

RESULTS AND DISCUSSION

As is well known, the Marmara Sea is a transit reservoir between the Black Sea and the Aegean Sea. Its upper layers are formed by the brackish water (about 22 PSU) coming from the Black Sea through the Bosphorus Strait, while deeper than the sharp halocline lies water with high salinity (38.5 PSU) coming with the lower current through the Dardanelles from the Aegean Sea. Plankton fauna is thus distributed in accordance with these currents and water layers. The lower course brings to the Sea of Marmara species typical of the Mediterranean and the North Atlantic, while the upper course replenishes it with Black Sea species.

C. helgolandicus is the only member of the *Calanus* genus both in the Sea of Marmara and the Black Sea. The Black Sea population of *C. helgolandicus* was allocated a separate species, *C. euxinus*, based on the difference in the distribution of supernumerary pores on the second and third urosome segments of adult females (Fleminger & Hulsemann 1987; Hulsemann 1991). Many authors have also used this naming in publications, however, further morphological (Isinibilir *et al.* 2009) and genetic analysis (Ünal *et al.* 2006; Yebra *et al.* 2011; Figueroa *et al.* 2019) has indicated that the supernumerary pores are not sufficient indicators for species differentiation hence the name *C. helgolandicus* should be used.

The female *Calanus finmarchicus* collected on 3 April 2018 (Figure 2a) had a total body length of $L_{tot}=3.60$ mm, prosome length $l_{pr}=2.84$ mm, and prosome width $d_{pr}=0.85$ mm. Respective measurements for the female collected on 5 April 2019 (Figures 3 and 4a) were even higher with $L_{tot}=3.95$ mm, $l_{pr}=3.14$, and $d_{pr}=0.98$. The length of *Calanus helgolandicus* females from the same area (Figure 2d and Figure 4b) were much lower with values of 2.11 ± 0.12 mm for small forms and 2.66 ± 0.14 mm for the large form.

Differences between the morphologies of the two species were also apparent, as follows:

- The ratio of prosome to abdominal length was 3.74 and 3.87 for the two specimens of *C. finmarchicus* whilst an overall lower value of the ratio of 3.43 ± 0.12 was found in *C. helgolandicus*.
- Compared to *C. helgolandicus*, the frontal part of the female head in *C. finmarchicus* was more evenly rounded and its abdomen was relatively thicker (Figures 2a and d as well as Figures 4a and b).
- There were obvious differences in the shape of their genital segment and spermathecae (Figures 2b and 2e as well as Figures 4a and b).
- The inner edge of the coxa of P5 was less curved and the dentate plate on coxopodite had more pointed and shorter teeth, especially in the area of the distal part of the plate (Figures 2c and 2f as well as Figures 5a and b).
- In *C. finmarchicus* collected on 5 April 2019, the spine on the outer margin of the swimming leg basipodite was smaller and located closer to its center, while the marginal spines of the exopodites were thicker compared to those in *C. helgolandicus* (Figure 5c, d).

- There were different proportions of the mandibular gnathobases, as well as different shapes and distances between the opal teeth of the gnathobases (Figure 6).

The first specimen of the female *C. finmarchicus* caught in April 2018 had a prosome length (2.84 mm) as in the largest specimens of *C. helgolandicus* living in the Black Sea, while the prosome length of the female caught in 2019 (3.14 mm) exceeded the known ranges body size of *C. helgolandicus* living in the Black, Mediterranean and North Atlantic seas (Brodskii 1950; Fleminger & Hulsemann 1977, 1987). Besides the total and prosome lengths, ratios of prosome to abdominal length were also much higher in the two specimens found in this study compared to previous studies from the different ratios of prosome to abdominal length (Table 1).



Figure 3. *Calanus finmarchicus*, female collected on 5 April 2019 (front view).

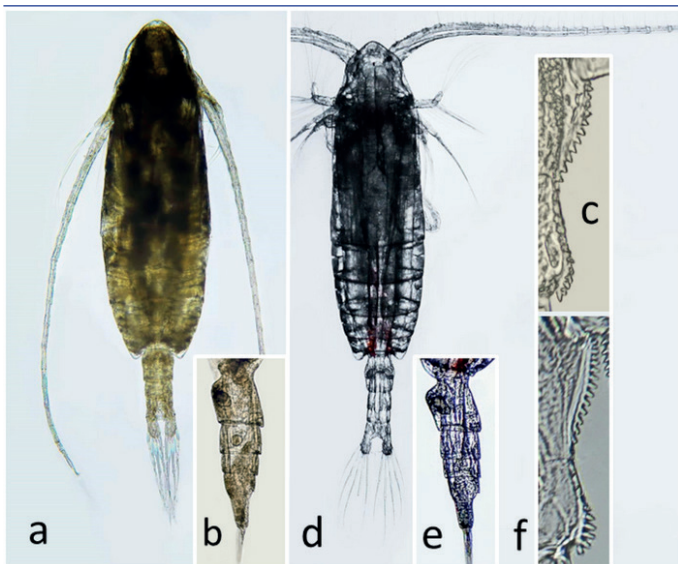


Figure 2. *Calanus finmarchicus*: habitus of female, front view (a); abdomen, side view (b) and coxopodite inner edge of P5 (c). *Calanus helgolandicus*: habitus of female, front view (d); abdomen, side view (e) and coxopodite inner edge of P5 (f). Both specimens were sampled on 3 April 2018.

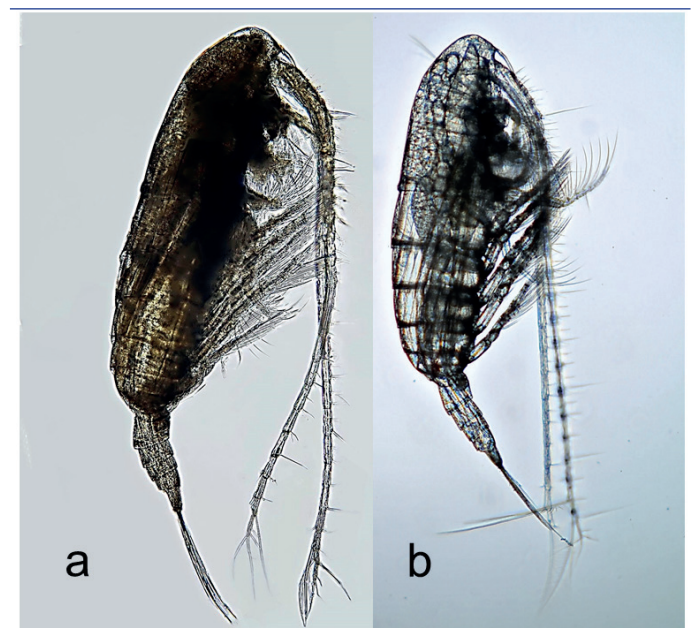


Figure 4. *Calanus finmarchicus* (a) and *Calanus helgolandicus* (b) females, lateral view. Both specimens were sampled on 5 April 2019.

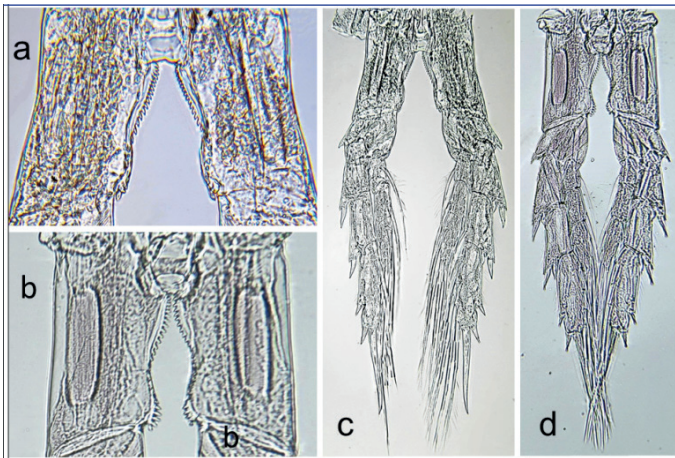


Figure 5. *Calanus finmarchicus*: coxopodite inner edge of P5 (a) and frontal view of P5 (c). *Calanus helgolandicus*: coxopodite inner edge of P5 (b) and frontal view of P5 (d).

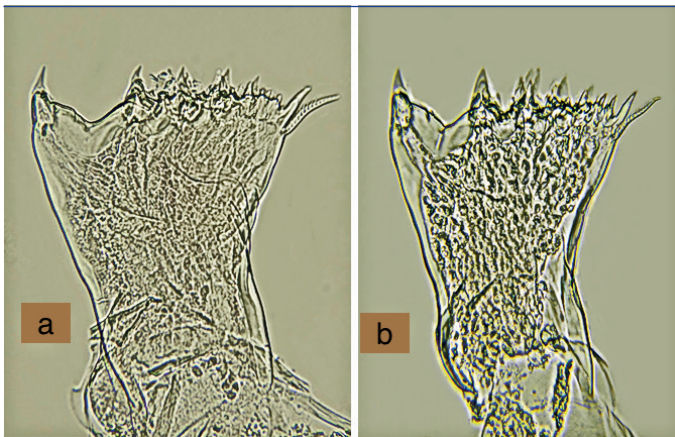


Figure 6. Gnathobase of left mandibula in *Calanus helgolandicus* (a) and *Calanus finmarchicus* (b) from the Sea of Marmara.

The population of *C. helgolandicus* is represented by two size groups of adults in the Sea of Marmara (Isinibilir et al. 2009). In spring, in the upper layers of the Bosphorus region of the Marmara Sea, predominantly large females with a prosome length of 2.67 ± 0.06 mm are found which are believed to be transported from the Black Sea by the strong surface currents, while in the area of the Princes' Islands and Sivri Ada, remote from the Bosphorus, small females with a prosome length of 2.23 ± 0.07 mm predominate. Experimental studies have shown that these two size groups of females lay eggs of the same size around 168–182 μm (Isinibilir et al. 2009), being similar to the typical ranges reported for the Black Sea *C. helgolandicus* population (Sazhina 1987) and *C. helgolandicus* from the North Atlantic (Guisande & Harris 1995; Poulet et al. 1995). Eggs laid by both size groups of females developed identical nauplii and early copepodites. However, starting from the third copepodite stage, individuals developing in the Sea of Marmara experienced growth retardation due to higher water salinity (Svetlichny et al. 2018). The aver-

age length of the prosome of *C. helgolandicus* females inhabiting the Aegean Sea is reported to be even shorter (2.04 ± 0.04 mm) (Fleminger & Hulsemann 1987; see Table 1).

The morphometric and morphological taxonomic characteristics (including those regarding the coxa of P5, the swimming leg basipodite and the mandibular gnathobases) of both individuals caught by us corresponded to the known morphological descriptions of *C. finmarchicus* (Brodsky 1950; Marshall et al., 1953; Frost 1974) and differed from those of *C. helgolandicus*. However, we also suggest that it will be important to utilize molecular tools for confirmation on discriminating between the two species as many authors have stated (Lindeque et al. 2004; Choquet et al. 2017). Unfortunately, we could not undertake molecular analysis since we only had two specimens which were dissected during the morphological analysis.

Both specimens were alive at capture, but inactive after being placed in the surface water of the Sea of Marmara with low salinity, probably due to osmotic shock. The discovery of the living *C. finmarchicus* in the Sea of Marmara indicates the possibility of very deep penetrations or transportation of the North Atlantic species into the eastern part of the Mediterranean basin. However, the vector for this transport is not clear, though it is believed to be via ballast waters of shipping. Because of dense population in the area, particularly in Istanbul which is one of the world's megacities, the Sea of Marmara has a very dense maritime traffic. In 2021, the number of vessels transiting between the Black Sea and Marmara alone was over 38 thousand (<https://www.dailysabah.com/turkey/istanbul/vessels-swarm-turkiyes-marmara-sea-as-bosporus-traffic-heightens>).

It has been reported that in the eastern Atlantic *C. finmarchicus* prefers colder (9°C) and deeper waters, while *C. helgolandicus* dwells in warmer waters (16°C) (Jonasdottir & Koski 2011). Based on long-term Continuous Plankton Recorder (CPR) data, several authors (Planque & Fromentin 1996; Wilson et al. 2016) suggest that *C. finmarchicus* is retreating north from the North Sea and *C. helgolandicus* is gaining ground due to warmer temperatures. However, Beare et al. (2002) observed that despite warming temperatures, *C. finmarchicus* abundance has increased in certain parts of the Atlantic Ocean, and it was therefore suggested that the lower abundance levels observed in shelf waters (North Sea, North Icelandic Shelf Region) are not a pan-Atlantic phenomenon. Similarly, Wilson et al. (2016) stated that temperature alone is not sufficient to explain the differences in the distribution of these two species in the eastern Atlantic and suggested that food quality be a key determinant on their population dynamics.

The Sea of Marmara is one of the most fertile seas within the Mediterranean basin with chl-a values as high as $20.0 \mu\text{g L}^{-1}$ (Yalçın et al. 2017) and production values of $100 \text{gC/m}^2/\text{y}$ (Ergin et al. 1993). Despite the high phytoplankton content of the Sea of Marmara, the establishment of *C. finmarchicus* in it seems difficult though not impossible. This is because *C. finmarchicus* is a cold-water species, but the waters of the Sea of Marmara below 25m are warm at $14\text{--}15^\circ\text{C}$ throughout the year. In order to understand and evaluate the dynamics of *C.*

Table 1. Prosome, urosome lengths (mm) and the ratio of *C. helgolandicus* and *C. finmarchicus*. (*urosome length calculated using the equation $L_{uro} = 0.34 L_{pr}^{0.82}$, by Isinibilir et al. 2009)

Species	Region/Group	Season**	Sample size	Prosome length	Prosome Length Deviation	Urosome Length	Prosome/Urosome	Reference
<i>C. helgolandicus</i>	Black Sea deep	W-SP	465	2.67	0.06	0.76	3.51	Isinibilir et al. 2009*
<i>C. helgolandicus</i>	Black Sea deep	S-A	376	2.57	0.09	0.74	3.49	Isinibilir et al. 2009*
<i>C. helgolandicus</i>	BS Shallow	A	59	2.57	0.16	0.74	3.49	Isinibilir et al. 2009*
<i>C. helgolandicus</i>	NE Marmara, Group1	SP	29	2.01	0.04	0.60	3.34	Isinibilir et al. 2009*
<i>C. helgolandicus</i>	NE Marmara, Group2	SP	46	2.23	0.07	0.66	3.40	Isinibilir et al. 2009*
<i>C. helgolandicus</i>	NE Marmara, Group1	A	23	2.01	0.10	0.60	3.34	Isinibilir et al. 2009*
<i>C. helgolandicus</i>	NE Marmara, Group2	A	64	2.44	0.12	0.71	3.45	Isinibilir et al. 2009*
<i>C. helgolandicus</i>	West N Atlantic		21	2.22	0.02	0.65	3.42	Fleminger & Hulsemann 1987
<i>C. helgolandicus</i>	Mid N Atlantic		20	2.34	0.04	0.67	3.49	Fleminger & Hulsemann 1987
<i>C. helgolandicus</i>	East N Atlantic Europe		20	2.36	0.07	0.68	3.47	Fleminger & Hulsemann 1987
<i>C. helgolandicus</i>	East N Atlantic-Africa		20	2.16	0.05	0.64	3.38	Fleminger & Hulsemann 1987
<i>C. helgolandicus</i>	Western Med.		20	2.31	0.06	0.69	3.35	Fleminger & Hulsemann 1987
<i>C. helgolandicus</i>	Adriatic Sea		20	2.20	0.12	0.69	3.19	Fleminger & Hulsemann 1987
<i>C. helgolandicus</i>	Aegean Sea		23	2.04	0.04	0.63	3.24	Fleminger & Hulsemann 1987
<i>C. helgolandicus</i>	Black Sea		42	2.61	0.03	0.78	3.35	Fleminger & Hulsemann 1987
<i>C. helgolandicus</i>	Off Tunisia		90	1.78	0.05	0.55	3.26	Drira et al. 2018*
<i>C. helgolandicus</i>	Marmara NE, 2018	SP	33	2.11	0.12	0.63	3.36	This study*
<i>C. finmarchicus</i>	Marmara NE, 2018	SP	1	2.84		0.76	3.74	This study
<i>C. helgolandicus</i>	Marmara NE, 2019	SP	46	2.66	0.14	0.76	3.51	This study*
<i>C. finmarchicus</i>	Marmara NE, 2019	SP	1	3.14		0.81	3.88	This study

**Seasons=W = Winter, SP = Spring, S= Summer, and A= Autumn)

finmarchicus in this new environment, this species should be closely followed in the Sea of Marmara through dedicated investigations.

CONCLUSION

A total of two specimens of live *Calanus finmarchicus* adult females (which is native to the north Atlantic) were found for the first time in the Sea of Marmara in 2018 and 2019. Certain morphological characteristics of *C. finmarchicus* differ from the sole *Calanus* species (i.e. *C. helgolandicus*) dwelling in the Sea of Marmara. These differences include an overall larger size, a higher ratio of prosome to abdominal length, the more evenly rounded frontal part of the female head, the shape of their genital segment and spermathecae, the dentate plate on coxopodite, different shapes and distances between the opal teeth of the gnathobases, etc. When more specimens are caught, it will be important to utilize molecular tools for confirmation of this species, also genetically. *C. finmarchicus* specimens may have been transported to the Sea of Marmara either via the lower current from the Aegean Sea or, more plausibly, through the ballast waters of vessels.

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Compliance with Ethical Standard: Authors declare that ethical approval is not required for this type of study.

Conflict of Interests: The authors declare that they have no financial interests or personal relationships that could affect this work, hence no conflict of interest.

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