

# FIRST RECORD AND DNA IDENTIFICATION OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS* (THUNBERG, 1793), IN THE SOUTHERN BLACK SEA

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The Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), is an important commercial species and has become the most prominent species for shellfish culture with a worldwide production of over 600,000 tons in 2017 (FAO 2019). As such, it also accounts for 97 % of the world oyster production. Specimens are permanently attached to hard substrates (e.g. on rocks) or live on soft sediments (e.g. muddy sand) in tidal and sub-tidal zones. Due to its rapid growth and broad tolerance to varying environmental conditions, *C. gigas* has become the oyster of choice for cultivation in many regions of the world. Originally, its native area is Japan and South-East Asia, where it has been cultivated for centuries. However, it has been introduced worldwide, most significantly along the western coast of the USA in the 1920's and France in 1966.

These introductions were intended as reinforcement to existing aquaculture programs. But it also allowed the species to expand further along coastlines since the late 1990s, and to progressively invade all European waters. As such, *C. gigas* has also been reported from the northern Black Sea, where it has been both accidentally and deliberately introduced. However, the presence of this species along the Turkish Coast of the Black Sea has hitherto uncertain.

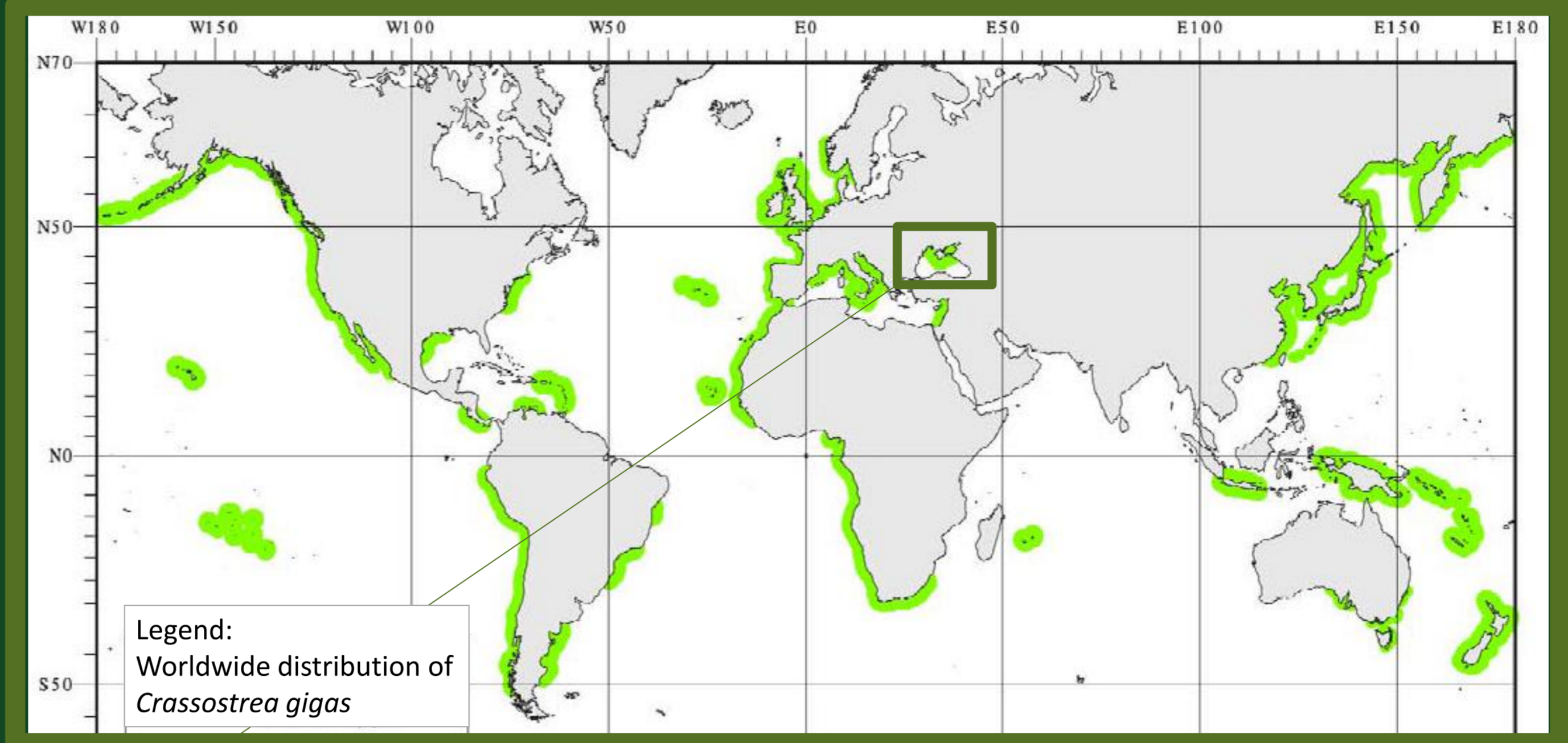
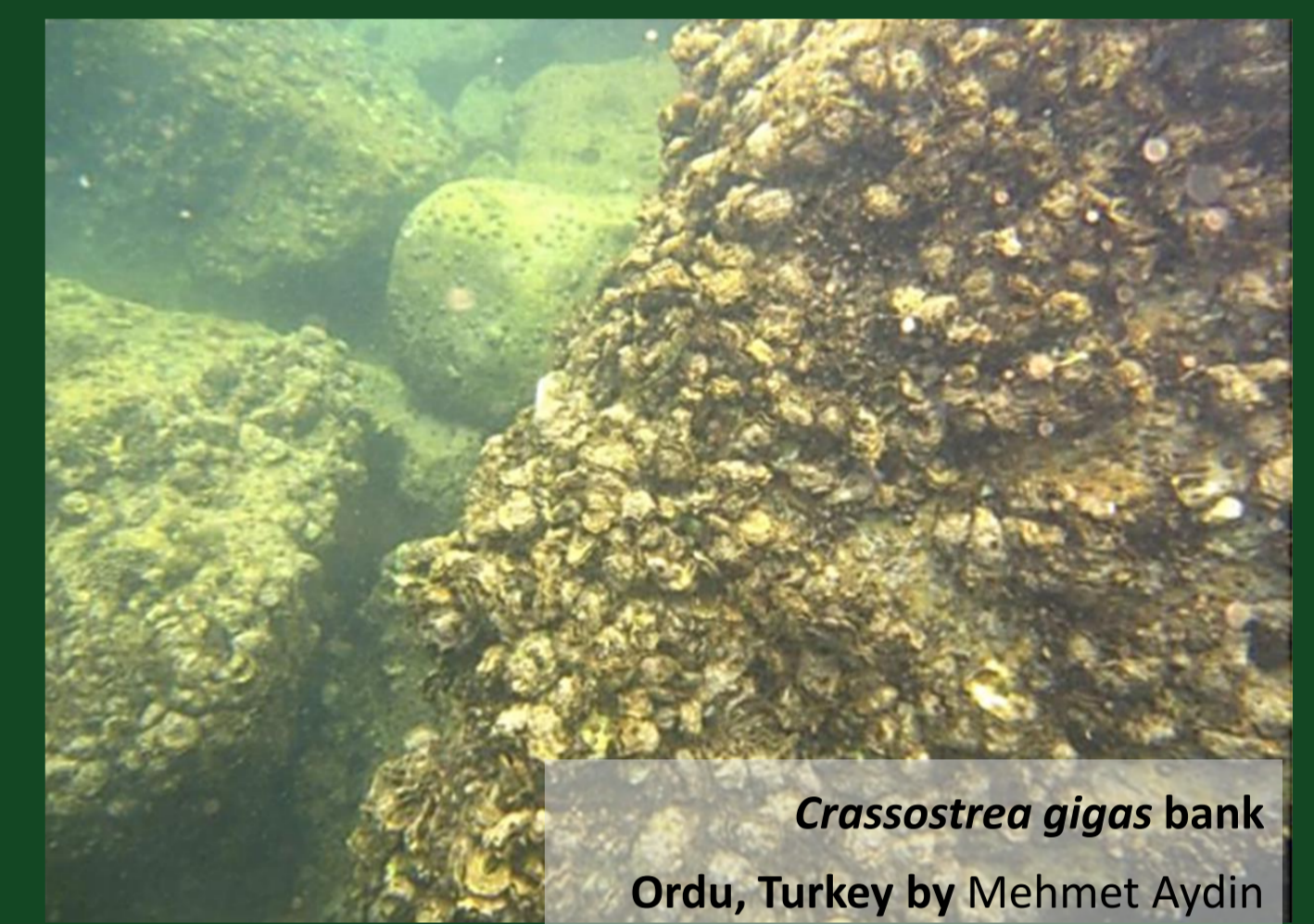


Figure 1. Map showing the worldwide distribution of *Crassostrea gigas* as from Miossec et al. 2009.



Figure 2. Map showing the study location, Ordu (blue star) and current patterns in the Black Sea (modified from Tuzhilkin 2008; Kershaw 2015). The orange stars indicate the distribution of non-native *Crassostrea gigas* (Thunberg, 1793) in the Black Sea (modified from Mitov et al. 2020).

The study site (41°04'44"N; 37°49'00"E) is located 23 km west of Ordu, situated along the Black Sea, northern Turkey (Figure 2). Sampling was performed by handpicking in the southern Black Sea region in 2019 below sea level on rocky shore substrates near the harbour of Ordu. A total of 235 adult specimens was collected in an area of about 200 m<sup>2</sup> of which five were identified with DNA-barcoding.



*Crassostrea gigas* bank Ordu, Turkey by Mehmet Aydin

*Crassostrea gigas* is morphologically very similar to *Crassostrea angulata* (Lamarck, 1819), also known as the Portuguese oyster. Therefore, a genetic analysis is required to verify the identification of *Crassostrea gigas*.

Two mitochondrial fragments were generated by Sanger sequencing. The 649 bp COI fragment was amplified, using LCO1490 and HCO2198 primers (Folmer et al. 1994) and the 488 bp 16S rRNA fragment was amplified using 16Sar and 16Sbr primers (Simon et al. 1994). The generated COI and 16S sequences were compared to the GenBank database using the BLAST search algorithm.

Table 1. Search results from BLAST algorithm with similarity scores.

	<i>C. gigas</i>	<i>C. angulata</i>
COI	99.85 %	97.69 %
16S	100 %	99.39 %

In addition to the BLAST search, Neighbour-Joining trees were constructed using all sequences available in the online databases GenBank and BOLD for members of the genus *Crassostrea*.

For both COI and 16S, the respective species sequences group together with 100 % and 76 % bootstrap support (Fig. 3 and 4). The BLAST results (99.85% and 100% similarity scores, Table 1) resulted in congruent identifications.

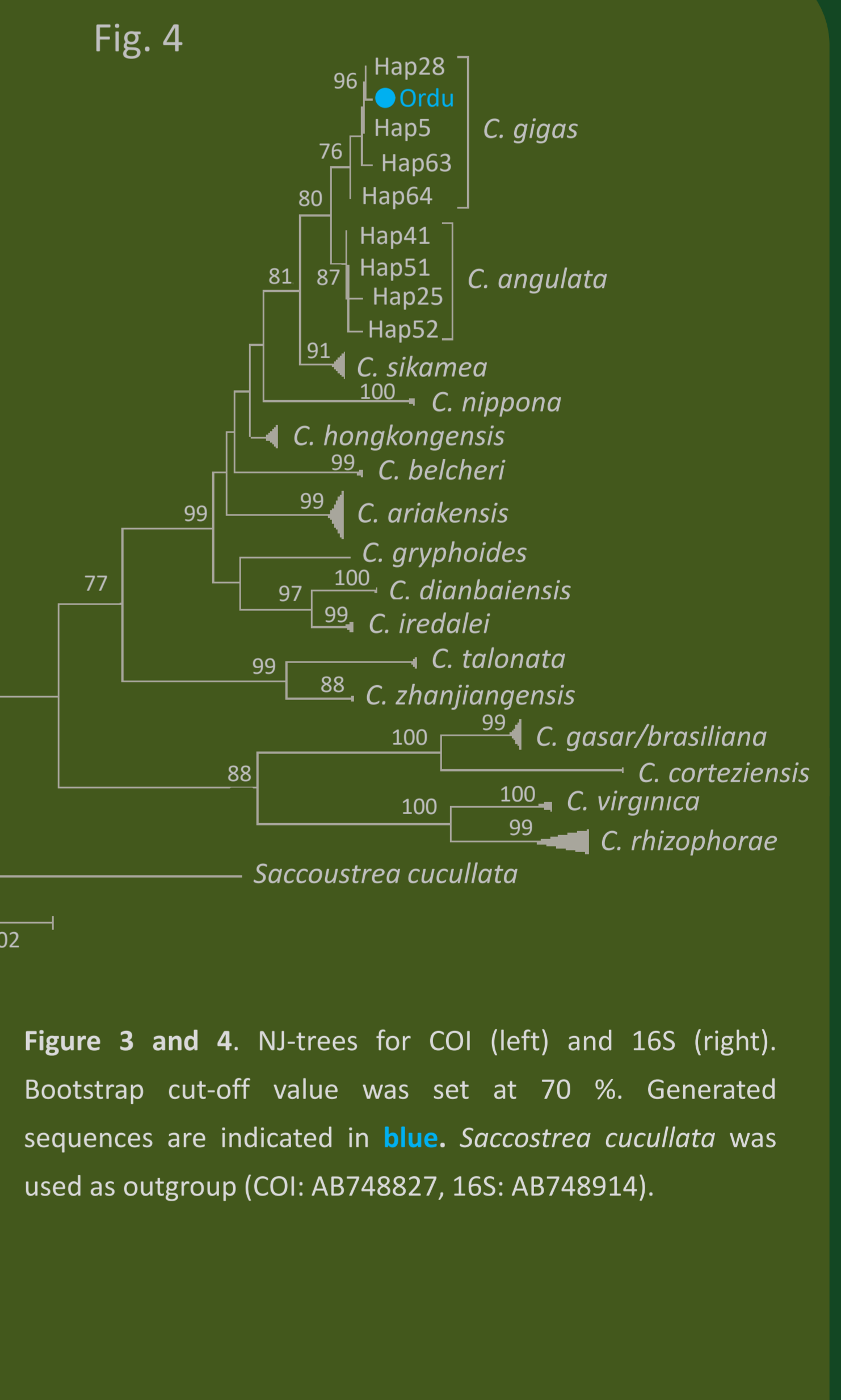
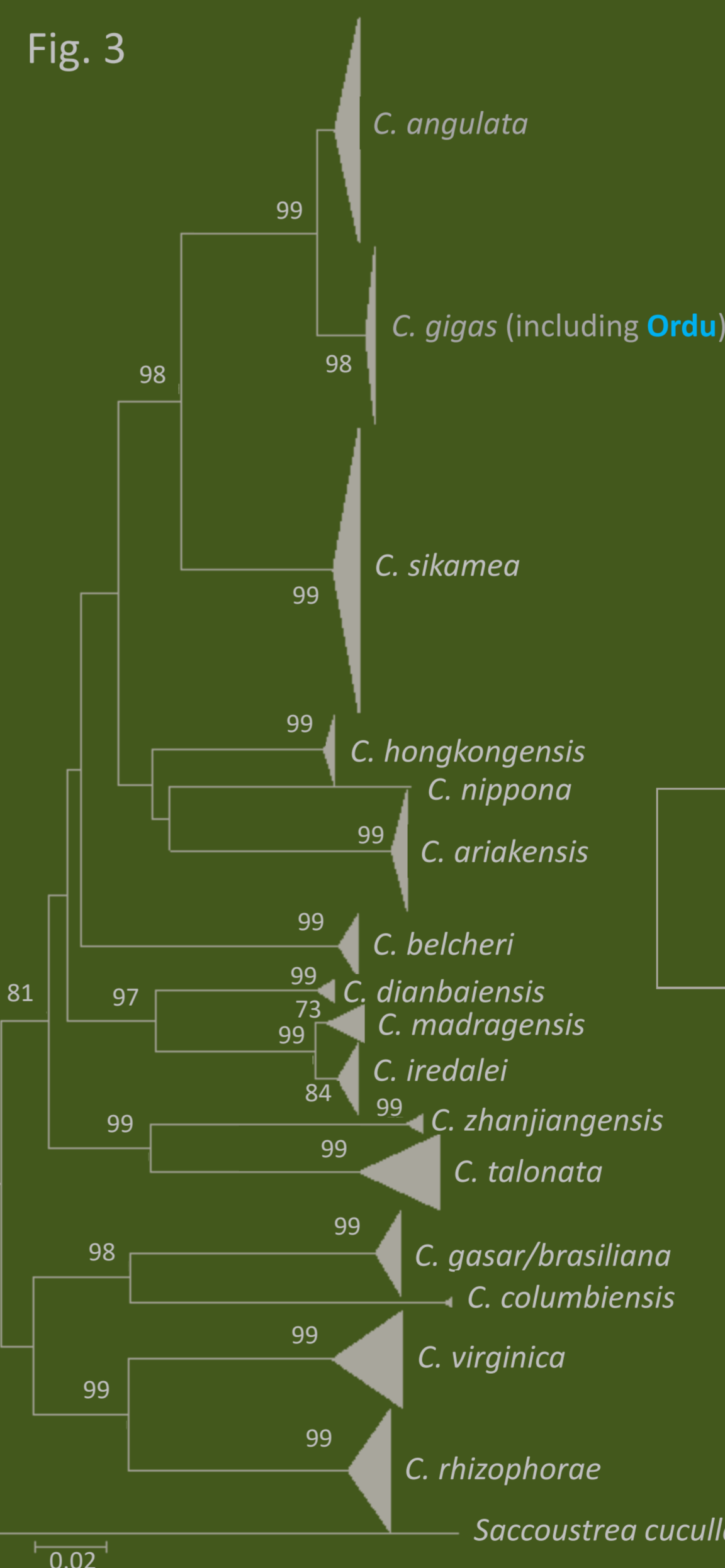


Figure 3 and 4. NJ-trees for COI (left) and 16S (right). Bootstrap cut-off value was set at 70 %. Generated sequences are indicated in blue. *Saccostrea cucullata* was used as outgroup (COI: AB748827, 16S: AB748914).

All new *Crassostrea* sequences from Ordu have been deposited in GenBank under the accession numbers: MT350568–MT350572 for 16S rRNA and MT350630–MT350634 for COI. The five sequenced individuals and additional voucher material have been deposited in the collections in the Fatsa Faculty of Marine Sciences, Ordu University (Turkey).

Results from the DNA-based analyses leave no doubt that the oyster specimens collected at Ordu are *Crassostrea gigas*. The discrimination with respect to *C. angulata* or any other species of the genus was clear (Table 1, Figure 3 and 4). Recent checklists did not mention the occurrence of *C. gigas* along the Turkish Black Sea coast. Therefore, **the present report is the first record of this species in the southern Black Sea**. Furthermore, the species appears to form well-established populations in this area, outside of aquaculture stations, which is a sign of concern as *C. gigas* are notorious for outcompeting native species for food, substrate and other sources.

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INTRODUCTION

RESULTS

CONCLUSION