KLIMENT'S DAYS
2020
FACULTY OF BIOLOGY

PACIFIC OYSTER INVASION ALONG BULGARIAN BLACK SEA COAST

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Introduction

Pacific oyster *Magallana gigas* (Thunberg, 1793) (= *Crassostrea gigas*) originates from the coastlines of the Japanese Sea (Laugen et al. 2015). The Pacific oyster is an invasive species which has been widely dispersed, both intentionally (for aquaculture) and unintentionally and now has an almost worldwide distribution (Mortensen et al. 2018). It has been introduced in many countries following collapse of native oyster culture (e. g. *Ostrea edulis* Linnaeus, 1758).

After the massive introduction of the Pacific oyster *Magallana gigas* in the Black Sea from the Japanese Sea in the period 1980-1991 and the active attempts for its acclimatization in the period 1989-2016 as a potential object for mariculture (along Romanian coast: near Constanta; along Ukrainian, Crimean and Caucasian coast) (Orlenko 2012, Popov & Shurov 2019, Pereladov 2020), individual specimens of this oyster since 1989 began to be found in natural Black Sea habitats, outside farms and places of their introduction (Micu 2004, Scolka & Gomoiu 2004, Kovtun & Zolotarev 2008, Orlenko 2012, Popov & Shurov 2019, Pereladov 2005, 2020) (Fig. 1).

Fig. 1. Distribution map of the wild non-native Pacific oyster *Magallana gigas* (Thunberg, 1793) in the Black Sea based on literature records and present data.

In the last ten years, however, reports of not only single but also wild micropopulations from the Pacific oyster in the Black Sea have become more frequent. They were found along the Ukrainian coast (2009-2011) (Orlenko 2012), along the Romanian coast (2017) (Krapal et al. 2019); in Crimean coast (2015), and in Caucasian coast (2018) (Pereladov 2020) (Fig. 1).
The species began to occur more and more often in the wild on the Bulgarian Black Sea coast, with the first finding in 2010 (Sts. Constantine and Helena Resort), followed by those in 2015 (Burgas) (Fig. 2).

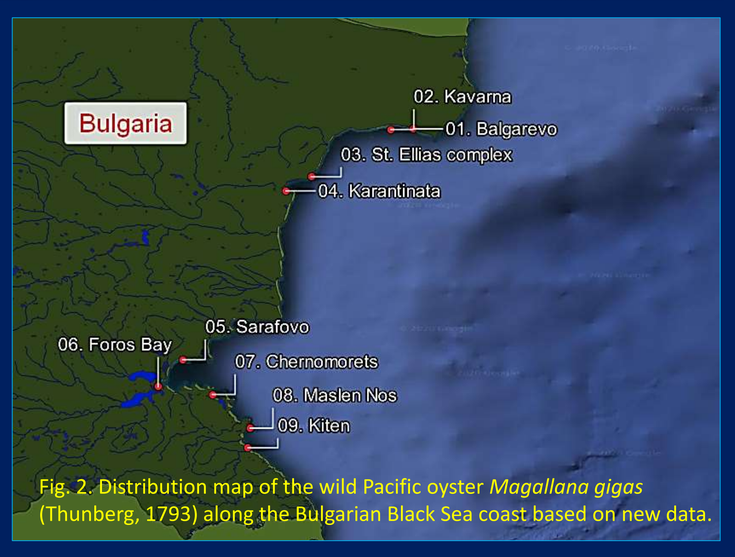

Fig. 2. Distribution map of the wild Pacific oyster *Magallana gigas* (Thunberg, 1793) along the Bulgarian Black Sea coast based on new data.

Goals

In this regard, the focus of our research is 1) to document and present in detail all data on live oysters found so far, identified by morphological features such as Pacific oyster *Magallana gigas* and 2) to confirm the species identity of this invasive oyster by molecular methods.

Material and methods

The collected material (80 specimens) was morphologically identified as *Magallana gigas* in accordance with Otero et al. (2013) and Amaral & Simone (2014).
The oyster shell parameters [height (H), length (L), and width (W)] were measured with a Vernier caliper (± 0.02).
The underwater photographs were made with Olympus TG-5, Panasonic DMC-FT4 and GoPro HERO5 Black cameras.
Part of the collected alive oyster specimens were conserved in 95% ethanol for further DNA analyses.
Total DNA was isolated from the gill tissue of one *M. gigas* specimen (collected from seawaters of Kiten Town and conserved in 95% ethanol) with a commercial kit accordingly to the manufacturer's instructions, and it was quantified on Quantus™ fluorometer (Promega) while the quality of the DNA was monitored electrophoretically on 1.0 % agarose gel in TBE buffer system. Part of the cytochrome oxidase I (COI) gene was amplified with primers LCO1490 and HCO02198 (Folmer et al. 1994) using Phusion™ polymerase (Thermo Scientific). The purified PCR product was Sanger-sequenced with primers LCO1490 and HCO02198 at Macrogen, and the forward and the reverse reads were assembled with SeqMan™ II software (DNA Star).

Fig. 3. The diver Hristo Petrov collecting *Magallana gigas,* Port of Kiten (Bulgarian Black Sea coast), 04.09.2020. Photograph by L. Kenderov.

Results and Disscusion

The collecting efforts have significantly extended the knowledge about the range of the wild *Magallana gigas* along the Black Sea coast. This species is now known from 9 localities, located along both northern and southern parts of the coasts of Bulgaria (see Fig. 2). These sites include, from North to South, the seawaters of: Balgarevo Village (locality 1), Kavarna Town (locality 2), Sts. Constantine and Helena Resort (locality 3), Varna Town (Karantinata locality) (locality 4), Burgas Town (Sarafovo district (locality 5), Port of Bourgas Shipyard and ship-repair factory (locality 6), Chernomorets Village (locality 7), Cape of Maslen Nos (locality 8), and Port of Kiten Town (locality 9). Oysters were found attached to a hard substrate like boulders and bedrock, breakwater tetrapods, mussel collectors, a hull of a boat, and props of a quay.
Morphological data were collected for 80 specimens of *M. gigas* from all localities. Shell height (H) varied between 1.84 and 13.7 cm. In addition to single isolated specimens, along Bulgarian coast were found for the first time two relatively small wild colonies of *M. gigas* – in the seawaters of Burgas Town and Kiten Town, included 19 and 30 alive specimens, respectively (Fig. 2, 3).
These all facts shows that the acclimatization of the Pacific oyster in the Black Sea is at a very advanced stage and some authors (Orlenko 2012) already define *Magallana gigas* as a permanent allochthonous species for the Black Sea fauna.

Below is the list of sites (in chronological order) where the newly recorded Pacific oyster was sampled; for each site, the number of collected and examined oyster shells is provided (see Plates 1-2).

The obtained sequence was subjected to BLAST search at the NCBI which resulted in sequence identities from 99.50 % to 100.00 % with E values of 0.0 with the *Magalana/Crassostrea gigas* sequences available in the database (see Table 1)

With this newly recorded bivalve the number of species of the Bulgarian marine bivalve fauna increases to 46 species (compare with Hubenov, 2015; excluding the uncertain species).

Table 1

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Manage columns — Show 100

select all *100 sequences selected* — GenBank | Graphics | Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Crassostrea gigas isolate WF34 mitochondrion, complete genome	1210	1210	97%	0.0	100.00%	KJ855245.1
Crassostrea gigas isolate YK05 mitochondrion, complete genome	1210	1210	97%	0.0	100.00%	KJ855244.1
Crassostrea gigas isolate CgJap23 mitochondrion, complete genome	1210	1210	97%	0.0	100.00%	KJ855241.1
Crassostrea gigas voucher LBDM 000385 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1210	1210	97%	0.0	100.00%	FJ717608.1
Crassostrea gigas mitochondrial DNA, complete genome	1210	1210	97%	0.0	100.00%	AF177226.1
Crassostrea gigas isolate YK01 mitochondrion, complete genome	1205	1205	97%	0.0	99.85%	KJ855243.1
Crassostrea gigas isolate JN14 mitochondrion, complete genome	1205	1205	97%	0.0	99.85%	KJ855242.1
Crassostrea gigas isolate 618 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	1205	1205	97%	0.0	100.00%	HM626169.1
Crassostrea gigas cytochrome oxidase subunit I gene, partial cds; mitochondrial gene for mitochondrial product	1205	1205	97%	0.0	99.85%	AF280608.1
Crassostrea gigas isolate ORCg-4 mitochondrion, complete genome	1194	1194	97%	0.0	99.54%	EU672831.1
Crassostrea gigas mitochondrial COI gene for cytochrome c oxidase subunit 1, complete cds, isolate: 1997-6	1190	1190	96%	0.0	99.85%	AB904884.1
Crassostrea gigas isolate EU1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	1186	1186	95%	0.0	100.00%	MN862563.1

Conclusions

Magallana gigas is an ecologically plastic species (Krapal et al. 2019 and literature therein). This might have allowed it to reach relatively easily the Bulgarian coast, most probably through ballast waters of vessels, or might happen naturally through the specific currents in the shelf zone of the Black Sea and through the Danube current system in the sea facilitating the planktonic larvae of the species to reach more suitable habitats. This process, together with other vectors for secondary introduction and dispersal (legal farming, illegal introduction and farming of diploids), could lead to a further rapid expansion of *Magallana gigas* throughout the Black Sea basin.
The information given in this article can be used as a basis and reference point for future targeted studies on this bivalve species on Bulgarian seacoast. Also, this can be used in monitoring's projects and projects related to biosecurity, because the long-term effects of current oyster invasion in our Black Sea-habitats at this moment are unknown.

Acknowledgments:

The authors thank all those who provided information on the topic, photographs and oyster material (shells, live and fixed specimens) for analysis: Mr. Nayden Stanev (Black Sea Shells Ltd., Kavarna), Mr. Nikolay Hristakiev (Varna), Mr. Milen Tanev (Burgas), Mr. Hristo Mavrodiev (Primorsko), Mr. Veselin Prokopiev (Balgarevo, Dalboka mushel farm), Mr. Krassimir Ivanov (Varna), Mr. Rumen Zhelezarov (Diving Center "Marina", Kiten), Mr. Hristo Petrov (Diving Center "Marina", Kiten), Mr. Simeon Donchev (Marine administration, Burgas), Mr. Angel Shishkov (Varna), Dr. Svetla Dalakchieva (Natural History Museum, Burgas), Dr. Dimitar Berov (IBER-BAS, Sofia).

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Chronology of the finding, location and habitats /microhabitats of the wild *Magallana gigas* specimens along the Bulgarian Black Sea coast

PLATE 1

2010

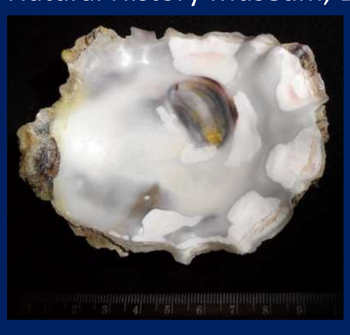
Northern Bulgarian Black Sea coast:

Locality № 3. – Yacht Haven of Complex St. Ellias (Bunata) (Sts. Constantine and Helena Resort, Varna District), in a concrete block's niche, approx. 1 m depth, 43°13'29.3"N 28°00'47.7"E [43.224800, 28.013240], the summer of 2010, P. Yanachkov (pers. comm.); collected in 2020, leg. P. Mitov. – 1 alive specimen (shell height (H): 12.54 cm, shell length (L): 9.4 cm, shell width (W): 5.5 cm) (in malacological collection of P. Mitov).

August 2015

Southern Bulgarian Black Sea coast:

Locality № 5. – Sarafovo district (Burgas Municipality), the exact collecting point in the coastal zone is unknown, 42°33'26.1"N 27°32'34.9"E [42.557245, 27.543020] (putatively place), depth 5-6 m, August 2015, leg. S. Zagorchinov. – 1 alive specimen (H: 8.2 cm, L: 7.1 cm, W: 4.05 cm), attached to rocks. (in malacological collection of Natural History Museum, Burgas).



August 2016

Northern Bulgarian Black Sea coast:

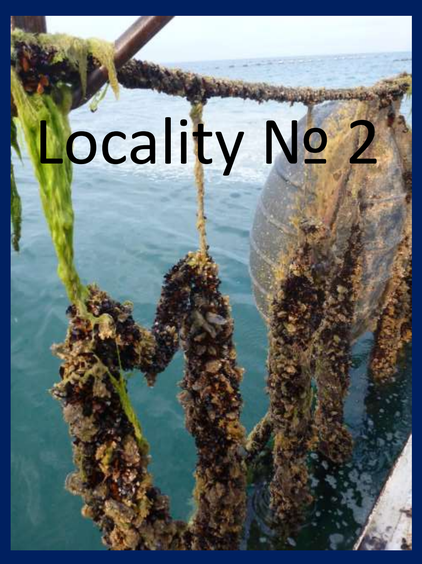
Locality № 4. – locality Karantinata, Varna Bay (Asparuhovo District, Varna Municipality), close to the quay of the military, 43°10'27.0"N 27°55'14.3"E [43.174163, 27.920638] (putatively place), hard bottom: shell sand with fine mud and stones; 1.8 m depth, August 2016, leg. K. Ivanov. – 4 alive specimens (H: 10.24-12.7 cm, L: 6.16-6.86 cm, W: 3.55-3.82 cm), attached to stones. (In personal collection of K. Ivanov (Varna)).

Southern Bulgarian Black Sea coast:

Locality № 8. – Burgas District, Cape of Maslen Nos, South (Kopar) Bay, 42°18'25"N27°47'23"E [42.306944, 27.789722], in early August 2016, depth 1.5 m, leg. Milen Tanev. – 1 alive specimen (H: 5.63 cm, L: 4.28-5.0 cm, W: 2.57 cm) attached to rocks. (in personal collection of Hristo Mavrodiev (Primorsko)).

July 2017

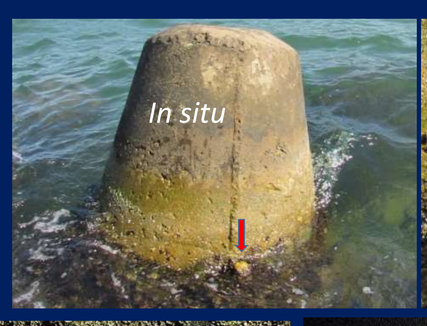
Northern Bulgarian Black Sea coast:

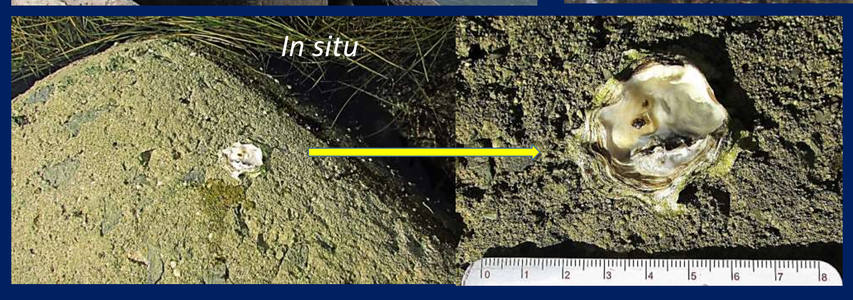


Locality № 2. – Kavarna, Black Sea Shells - mussel farm (*Mytilus galloprovincialis* Lamarck, 1819), 43°23'54.4"N 28°18'15.6"E [43.398436, 28.304344], 03.07.2017, leg. N. Stanev:. – 1 specimen (H: 4.7 cm, L: 3.3 cm) attached to the mussel collectors (the specimen is not preserved, we only have a photos taken by Nayden Stanev (Black Sea Shells Ltd., Kavarna)).

September 2017

Southern Bulgarian Black Sea coast:





Locality № 6. – Foros Bay, Port of Bourgas Shipyard and ship-repair factory, 42°27'28.7"N 27°27'10.4"E [42.457973, 27.452887], 15.09.2017, leg. P. Mitov. - 2 alive specimens (H: 6.37-8.35 cm, L: 5.66-6.12 cm, W:2.69-3.61 cm), and one empty left shell (H: 3.95 cm, W: 4.11 cm) attached to breakwater tetrapods and stones at the water level. (in malacological collection of P. Mitov).

April/May 2018

PLATE 2

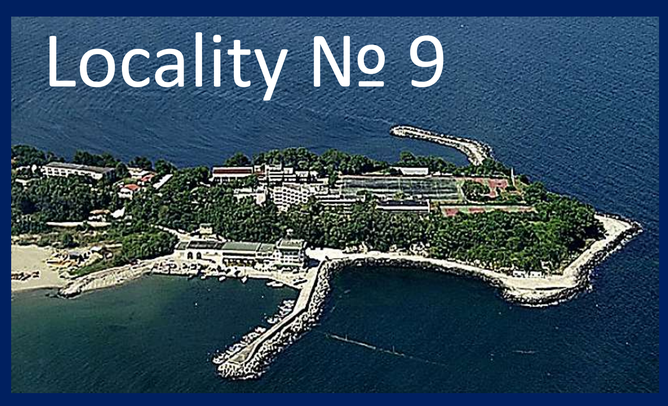
Northern Bulgarian Black Sea coast:

Locality № 1. – Balgarevo Village, locality Dalboka, mussel farm (*Mytilus galloprovincialis* Lamarck, 1819) rack "Dalboka", 43°24'03.7"N 28°23'12.3"E [43.401014, 28.386742], 264 m off shore, April/May of 2018, V. Prokopiev, pers. comm. – 3 alive specimens (with shell height 7-8 cm, V. Prokopiev, pers. comm.) attached to the mussel collectors (non vidi, the specimens are not preserved).

July 2019

Southern Bulgarian Black Sea coast:

Locality № 7. – Southeast of Chernomorets Village, Sozopol Bay, Cape Chervenka, 42°25'47.5"N 27°39'07.1"E [42.429871, 27.651977], hard bottom: mud-sand and stones, depth 0.8-2 m, 01.07.2019, leg. S. Donchev. –2 specimens: one alive (H: 8.73 cm, L: 6.72 cm, W: 5.42 cm) attached to props of a quay (in malacological collection of Natural History Museum, Burgas), and the other is dead – only shells are preserved (H: 9.82 cm, L: 7.51 cm, W: 5.48 cm)), attached to a stone (in personal collection of S. Donchev).

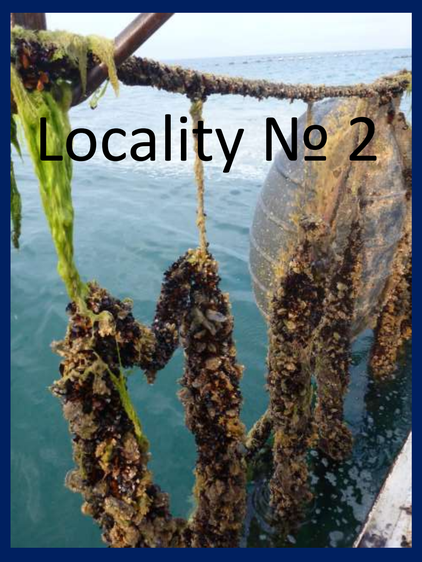


Southern Bulgarian Black Sea coast:

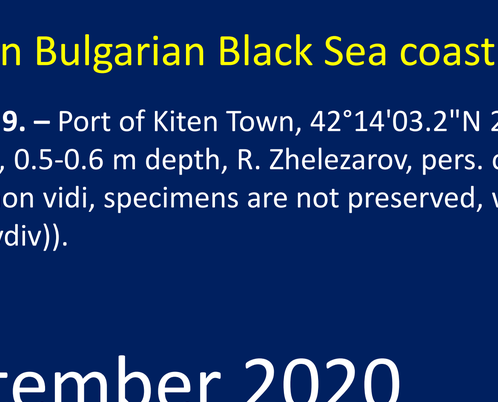
Locality № 9. – Port of Kiten Town, 42°14'03.2"N 27°46'55.5"E [42.234217, 27.782075], 24.07.2019, 0.5-0.6 m depth, R. Zhelezarov, pers. comm. – 20 alive specimens attached to a hull of a boat (non vidi, specimens are not preserved, we only have a photo taken by Konstantin Dakov (Plovdiv)).

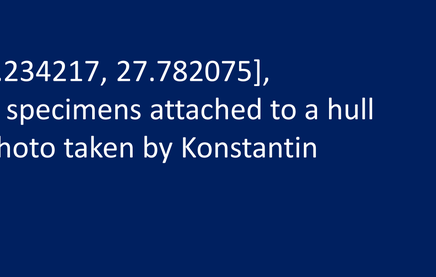
May-September 2020

Northern Bulgarian Black Sea coast:

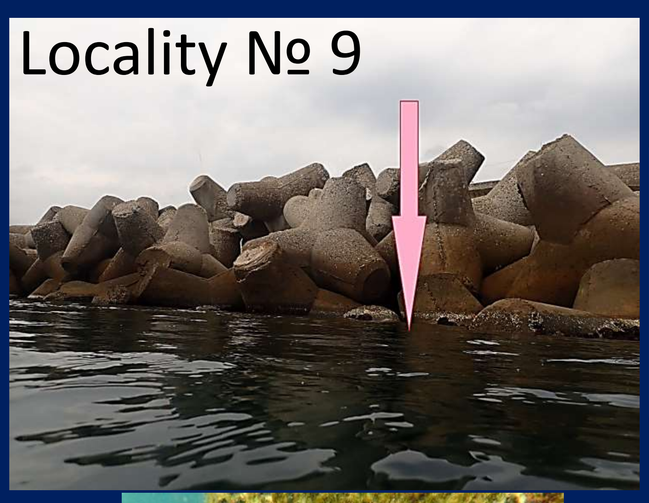


Locality № 2. – Kavarna, Black Sea Shells - mussel farm (*Mytilus galloprovincialis* Lamarck, 1819), 43°23'54.4"N 28°18'15.6"E [43.398436, 28.304344], 49 alive specimens, the all attached to the mussel collectors, leg. N. Stanev: 23.05.2020 – 2 specimens [the specimens are not preserved, we only have a photos taken by Nayden Stanev (Black Sea Shells Ltd., Kavarna)]; 27.06.-28.08.2020 – 23 specimens (H: 3.25-8.35 cm, L: 3.47-5.57 cm, W: 1.36-2.75 cm), 28.08.-11.09.2020 – 21 specimens(H: 3.65-8.25 cm, L: 2.62-5.0 cm, W: 1.73-3.0 cm), 25.09.2020 – 3 specimens (H: 5.24-5.9 cm, L: 3.8-4.3 cm, W: 2.07-2.7 cm). (in malacological collection of P. Mitov).





July-September 2020



Southern Bulgarian Black Sea coast:

Locality № 9. – Port of Kiten Town, 0.5-1.5 m depth: 42°14'03.2"N 27°46'55.5"E [42.234217, 27.782075], 01.07.2020, leg. R. Zhelezarov – 1 alive specimen (H: 13.7 cm, L: 11.32 cm, W: 3.9 cm), attached to a hull of a boat; 42°14'03.71"N 27°46'56.86"E [42.234364, 27.782461], 04.09.2020, leg. H. Petrov & L. Kenderov. – on the spot 9 alive specimens, attached to breakwater tetrapods [3 of them (H: 7.27-9.6 cm, L: 6.26-7.48 cm, W: 2.93-3.29 cm) collected for analysis]. (in malacological collection of P. Mitov).

Southern Bulgarian Black Sea coast:

Locality № 6. – Foros Bay, Port of Bourgas Shipyard and ship-repair factory, 42°27'28.7"N 27°27'10.4"E [42.457973, 27.452887], 26.09.2020, leg. P. Mitov. - 17 alive specimens (H: 1.84-5.4 cm, L: 1.35-3.48 cm, W: 0.73-1.62 cm) attached to breakwater tetrapods, above and at the water level. (in malacological collection of P. Mitov).