



CoCoNet Project  
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# CoCoNet

*Towards COast to Coast NETworks of marine protected areas (from the shore to the high and deep sea), coupled with sea-based wind energy potential.*

## **“Collection of tissues samples for genetic analysis” WP10 D10.2**

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## ***EXECUTIVE SUMMARY***

The aim of this document is to present a synthesis of the work dedicated to collection of tissues samples for genetic analysis conducted in WP10 “Black Sea Pilot Project”, Task 1. “Multi scale mapping of geological, biological, oceanographic features characteristic for different habitats”, within Subtask 1.2 “Biological sampling of offshore and coastal areas”.

This document, released at the end of sampling effort , provides the reference information for all the tissues samples collected in WP10 and updates the first two CoCoNET Interim Scientific reports of WP10 and the Milestone MS15.

The present document aims to:

1. Recall the work planned for collection of samples for genetics in WP10 Subtask 1.2.
2. Describe the activity of collection of samples for genetics performed in the subtask.
3. Summarize the final results of the sampling.
4. Provide details on samples collected for each species in the Black Sea pilot areas.

## 1. Background for genetic sampling in the Black Sea

Genetic analysis of certain species will be performed to test for population structure and gene flow but also to identify origin of migrants, when possible. Testing for genetic heterogeneity is done on the assumption that genetic differentiation indicates breaks in connectivity within the metapopulation of a species. A number of species was selected for genetic analysis, based on the coverage of a broad number of taxa, their role in the ecosystems functioning (priority to habitat formers or characteristic species), their presence in the two pilot areas (Black Sea and Adriatic Sea) and life-history traits that maximize the amount of useful information which can be inferred through their analysis.

To assess genetic connectivity throughout the Mediterranean and Black seas a few key species from different taxonomic groups, with different features in terms of ecological roles and of dispersal mechanisms, will be sampled at two geographic levels: at a large scale within the Mediterranean and at a refine scale within the two pilot areas. Genetic analyses are ideal to test for population structure and gene flow but also might identify origin of migrants as well as major discontinuity in connectivity. Genetic heterogeneity assessed in species with different dispersal capabilities on the base line that genetic differentiation indicates break in connectivity can provide relevant information on the role of networks of MPAs. Marine populations are open systems and thus inferring genetic connectivity through multiple codominant molecular markers (microsatellites or SNPs) for the same key species collected in the same sampling sites, not only in the pilot areas but also at a more global scale, can rule out confounding factors of gene flow (i.e. low power to detect differences, insufficient time to accumulate genetic differences, or selection acting on the genetic markers used). If different areas are connected, then management decisions for one area may affect other areas as well and these impacts need to be considered in decision making.

In the context of MPA network design, studying the connection of all marine populations both among the MPAs and the MPAs and the surrounding areas, which are part of the network, is impossible and far too expensive. Therefore, there is necessity of selecting a proper sample of species to study their genetic structure and diversity in a given area.

During the WP3 workshop on genetic (Subtask1.1, Barcelona, May 2012) a primary list of 20 target species has been established between partners involved in genetic analyses together with coordinators of WP10 (Black Sea Pilot Project) and WP11 (Adriatic Sea Pilot Project). Following the WP3-WP10-WP11 coordination meeting in Paris (Dec 2012) and the first general assembly in Rome (Jan 2013) this list has been updated to 18 target species (cf. Table 1). Participants agreed to focus on a reduced number of species in order to be able to study each of them in detail using different, but carefully selected, genetic markers and techniques. The same molecular markers will be used for a given species across all the sampling areas.

The criteria suggested for the selection of target species were:

- species must cover a broad number of taxa
- species of interest for each major habitat be selected (e.g. habitat formers)
- species role in the ecosystems functioning
- species must be present in the two pilot areas (Black Sea and Adriatic Sea)

Each species should be easily sampled, resources quantitatively available for representative sampling and biological information readily available

There are 9 species that occur in both pilot areas (# 1-9 in the table below), one species (#10) is only present in the Black Sea and four species (#11-14) only in the Adriatic Sea. Four additional species in red (#15-18) only present in the Adriatic Sea will be collected opportunistically. The 9 species that occur in both pilot areas (# 1-9) have been identified as “priority” species. To facilitate the use of the same molecular markers per species, each species has been assigned to a reference team in charge of the overall genetic analysis for this species, as listed in table 1.

Table 1 Species list, sampling area and team agreed to be in charge of the genetic analysis

	Taxon group	Scientific name	Sampling (BS=Black Sea AS=Adriatic Sea)	Analysis team assignment
1	Algae	<i>Cystoseira barbata</i>	BS and AS	CNRS (Nice)
2	Seagrass	<i>Zostera noltii</i>	BS and AS	CoNISM a (SZU)
3	Mollusca	<i>Mytilus galloprovincialis</i>	BS and AS	CoNISM a
4	Mollusca	<i>Donacilla cornea</i>	BS and AS	CNRS
5	Mollusca	<i>Gibbula divaricata</i>	BS and AS	CSIC
6	Mollusca	<i>Cyclope neritea</i>	BS and AS	CNRS
7	Crustacea	<i>Pachygrapsus marmoratus</i>	BS and AS	CoNISM a
8	Fish	<i>Scorpaena porcus</i>	BS and AS	CNRS
9	Fish	<i>Symphodus tinca</i>	BS and AS	CSIC
10	Algae	<i>Phyllophora crispa</i>	BS	IO-BAS
11	Seagrass	<i>Posidonia oceanica</i>	AS	CoNISM a (SZU)
12	Sponge	<i>Hemimycale columella</i>	AS	CSIC
13	Cnidaria	<i>Desmophyllum dianthus</i>	AS	CSIC
14	Echinodermata	<i>Paracentrotus lividus</i>	AS	CoNISM a
15	Cnidaria	<i>Cladocora caespitosa</i>	AS	CSIC
16	Cnidaria	<i>Paramuricea clavata</i>	AS	CNRS
17	Echinodermata	<i>Arbacia lixula</i>	AS	CSIC
18	Fish	<i>Tripterygion delaisi</i>	AS	CSIC

Note: the species originally indicated for the *Symphodus* genus was *Symphodus roissali* that, in preliminary field surveys, resulted difficult to obtain at the Adriatic sites. The species has been replaced with *S. tinca*, after joint discussion of WP3, WP10 and WP11 relevant partners.

For the Black Sea sampling only the first 10 species are of interest; 50 specimens will be collected for each species from each sampling site. The number of sampling sites has been increased in order to better be able to discern genetic connectivity at different scales, as well as breaks in connectivity around the Black Sea basin. There are now two kinds of sampling sites:

- pilot sites where, besides genetic sampling, benthic sampling for habitat mapping, as well as other WPs will also be done – these are the same as in the DOW
- secondary, non-pilot sites, where only genetic sampling will take place – additional non-DOW sites were designated for Bulgaria, Ukraine, Russia, Georgia and Turkey, preferentially in MPAs, where possible.

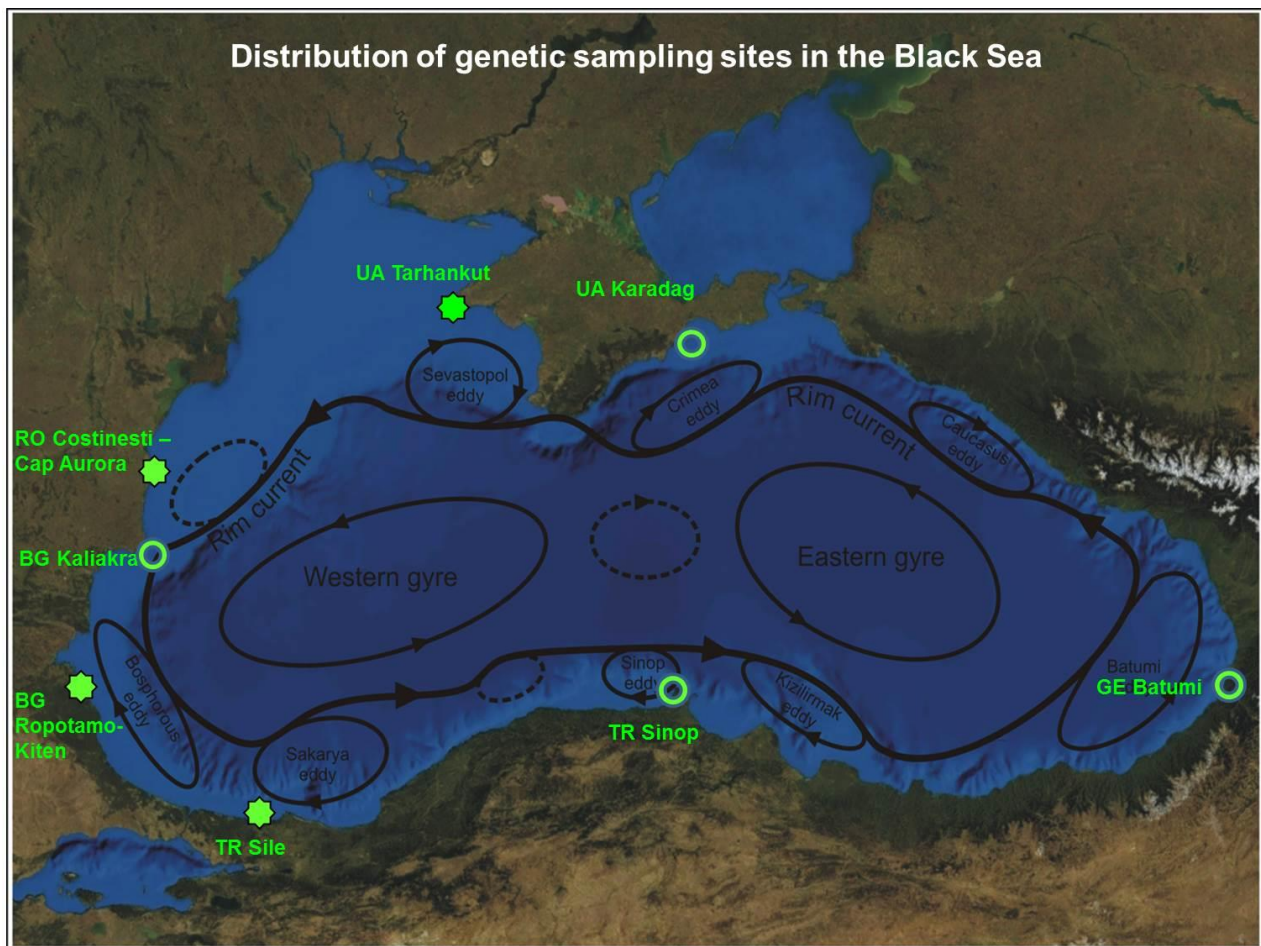


Fig. 1 Distribution of genetic sampling sites in the Black Sea. Pilot sites are marked with stars, while secondary, non-pilot sites are marked with circles.

The workplan defines sites at which connectivity analyses will be performed in the Black Sea in relation to established or potential MPAs and according to the DoW, it identifies the species of interest for genetic analysis based on WP3 inputs and joint discussion with WP10-WP11 leaders, and it prioritizes them based on their occurrence also in the Mediterranean and planned research. In addition, detailed protocols have been provided for collection of tissues for genetic analysis based on participant partners inputs. These protocols served as guidelines for work in the field and included indications on the optimal sampling scheme needed for downstream genetic analyses.

During the Barcelona WP3 workshop dedicated to the genetic work (Subtask 1.1) and according to the DOW, participants agreed to cover a minimum of 6-7 coastal locations per pilot area. During the WP3-WP10-WP11 coordination meeting in Paris, 9 locations in the Black Sea (cf. Figure 1) and 8 locations in the Adriatic Sea were considered to be sampled for genetic connectivity analysis.



## 2. Collection of samples for genetics in the Black Sea pilot areas

Trough several workshops (Barcelona subtask 1.1, Castiglioncello subtask 1.2) and meetings (Paris -Dec-2012, Rome-Jan 2013) the Coconet partners involved in the WP3, 10 and 11 have designed a joint genetic sampling workplan for the two pilot project areas. A short summary of the workplan is presented below. The detailed workplans are available on the deliverables D10.1 and D11.1 for the refine scale studies in the 2 pilot project areas. For large scale study, a detailed workplan can be found in the deliverable D3.4. Basically, the sampling requires 50 individuals per species and per location for each pilot area. This will lead to a total of:

- 8 locations in the Adriatic: 400 individuals per species and about 5200 individuals (13 species) if the sampling ends up without gap; with additional sampling 200 individuals (2 species) in only two locations.
- 9 locations in the Black Sea: 450 individuals per species and about 4500 individuals (10 species) if the sampling ends up without gap.
- 8 locations in the large scale connectivity analyses in the Mediterranean Sea: 400 individuals per species and about 6000 individuals (15 species) if the sampling ends up without gap. The species are the same being sampled in the Adriatic Sea.

17 locations for sampling species for genetic analysis are considered for a correct assessment of the genetic connectivity at a regional scale in the two pilot areas. The 9 species common to both pilot areas are priorities for sampling at a wider scale in the Mediterranean Sea.

Regarding storage, the protocol is essentially the same for the different species with a general storage in ethanol for animal taxa and dehydrated in silicagel for plant and algae taxa. A detailed sampling protocol for each target species is available in the deliverable D3.4 (cf. D3.4. part 5. "Protocols for collection").

Taking into consideration the uneven distribution of genetic sampling capacity among the Black Sea partners, it was agreed during the WP3-WP10 coordination meeting in Paris (Dec 2012) that the WP3 and WP10 leaders will conduct a series of sampling expeditions around the Black Sea. The main aim of the expeditions is to make sure that the sampling collection is completed, but there is also the aspect of direct cooperation with local partners, knowledge transfer and capacity building during these expeditions.

### 3. Genetic samples obtained in the Black Sea pilot project

Two species, *Phyllophora crispa* and *Symphodus tinca*, were not present at most of the 8 Black Sea sampling sites, or they were present but so rare that it did not allow for effective sample collection. Other species, although present in the area, could not be found by the sampling teams.

Geographical location of the sampling points for each target species are presented below. Details of collected samples are reported in ANNEX 1.

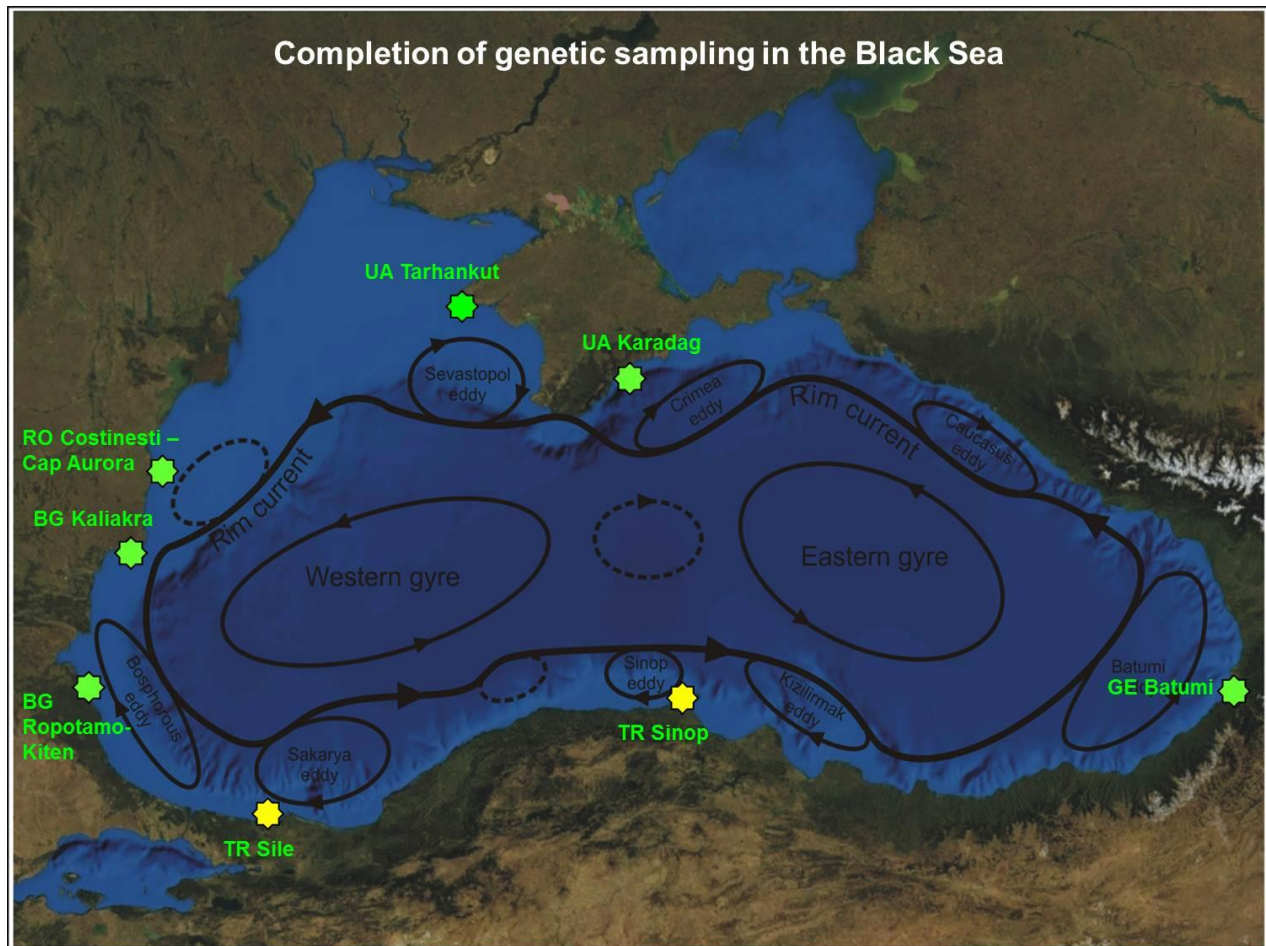


Fig. 2 Sites with complete sampling are marked with green stars, incomplete ones with yellow.

Due to its complexity, the sampling activity involved a large number of persons, whose list is provided in ANNEX 2. Partners from RO, BG, TR sampled on their own, while partners from UA and GE were assisted by WP3-WP10 joint expeditions.

In the Black Sea the 10 target species present (#1-10 in Table 1) plus 2 extra species (*Symphodus roissali* and *Nassaria nitidus*) have been collected; for a total of 3930 individuals.

The distribution of the number of samples per target species per sampling site is presented in Table 2 below.

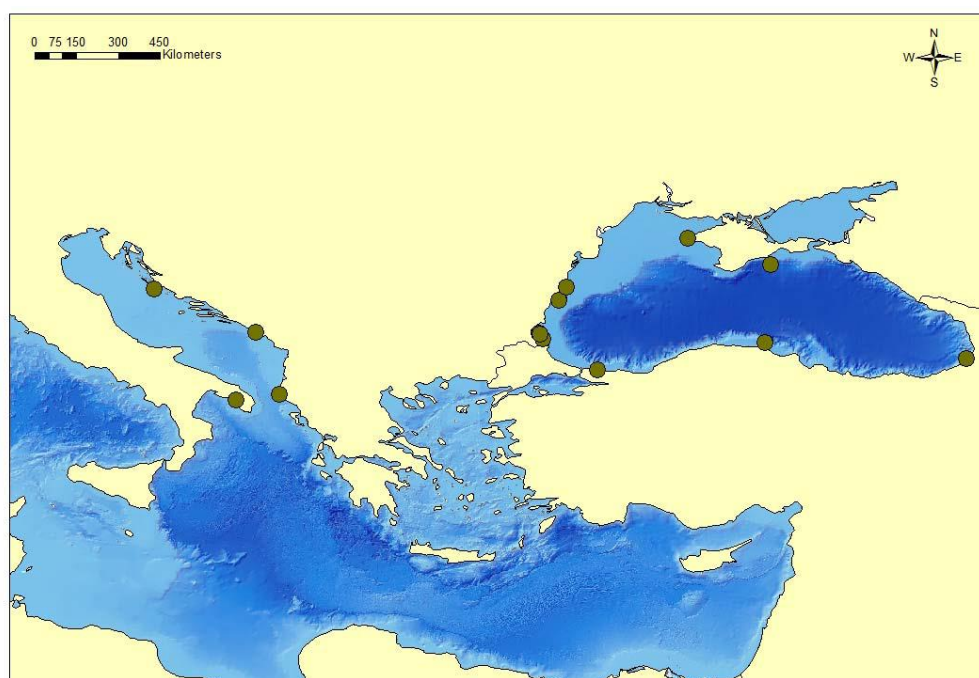


Table 2. Genetic sampling synopsis for the Black Sea

Scientific name	Karadag (UA)	Tarhankut (UA)	Costinesti-Cap Aurora (RO)	Kaliakra (BG)	Ropotamo-Kiten (BG)	Sile (TR)	Sinop (TR)	Batumi (GE)
<i>Phyllophora crispa</i>	50	50	Not present	Not present	51	50	Not present	Not present
<i>Cystoseira barbata</i>	50	50	50	50	51	50	50	50
<i>Zostera noltii</i>	50	50	150	55	47	50	50	Not present
<i>Gibbula divaricata</i>	50	50	50	50	50	Not sampled	Not sampled	50
<i>Cyclope neritea</i>	50	50	150	100	50	50	Not sampled	Not present
<i>Mytilus galloprovincialis</i>	50	50	100	50	50	50	50	50
<i>Donacilla cornea</i>	100	100	50	60	50	Not sampled	50	100
<i>Pachygrapsus marmoratus</i>	40	53	50	52	50	50	57	50
<i>Scorpaena porcus</i>	50	57	50	60	50	50	61	50
<i>Symphodus tinca</i> *	55	51	Not present	Not present	1	11	21	4

### 3.1 *Cystoseira barbata*

401 individuals of *Cystoseira barbata* have been collected in the Black Sea (Fig.3). The species has been collected at all 8 sites.


Fig. 3 Location of the sampling points of *Cystoseira barbata*

### 3.2 *Phyllophora crispa*

A total of 201 individuals of *Phyllophora crispa* have been sampled in the Black Sea (Fig. 4). The species has been collected at 4 sites and confidently assessed as “not present” in Romania and Georgia, also at the sites Cape Kaliakra (BG) and Sinop(TR).

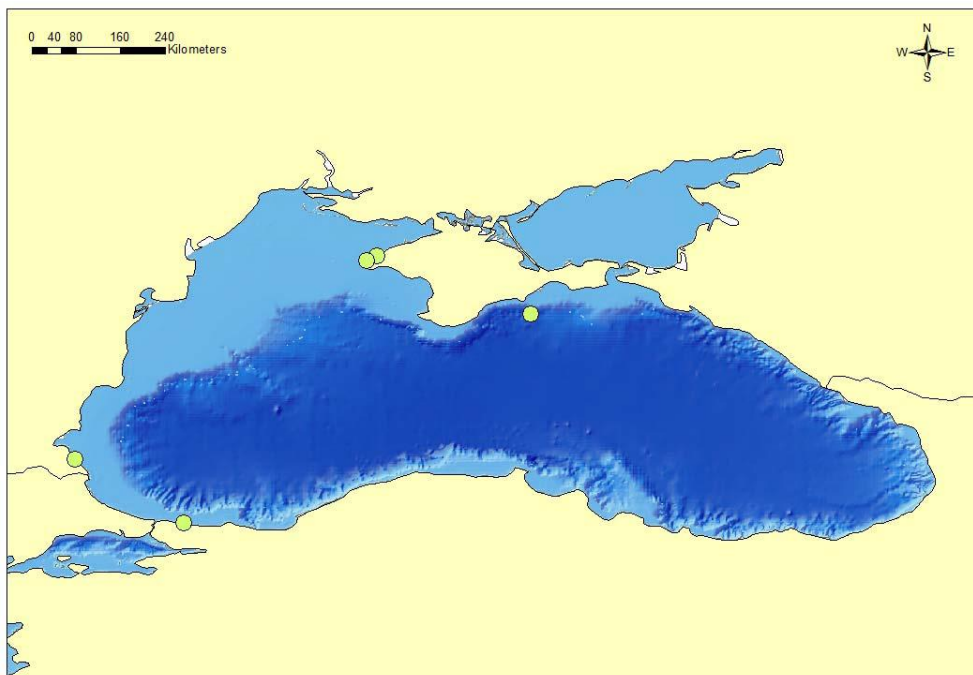


Fig. 5 Location of the sampling points of *Phyllophora crispa*

### 3.3 *Zostera noltii*

A total of 452 individuals of *Zostera noltii* have been sampled in the Black Sea (Fig. 5). The species has been collected at 7 sites and confidently assessed as “not present” in Georgia.

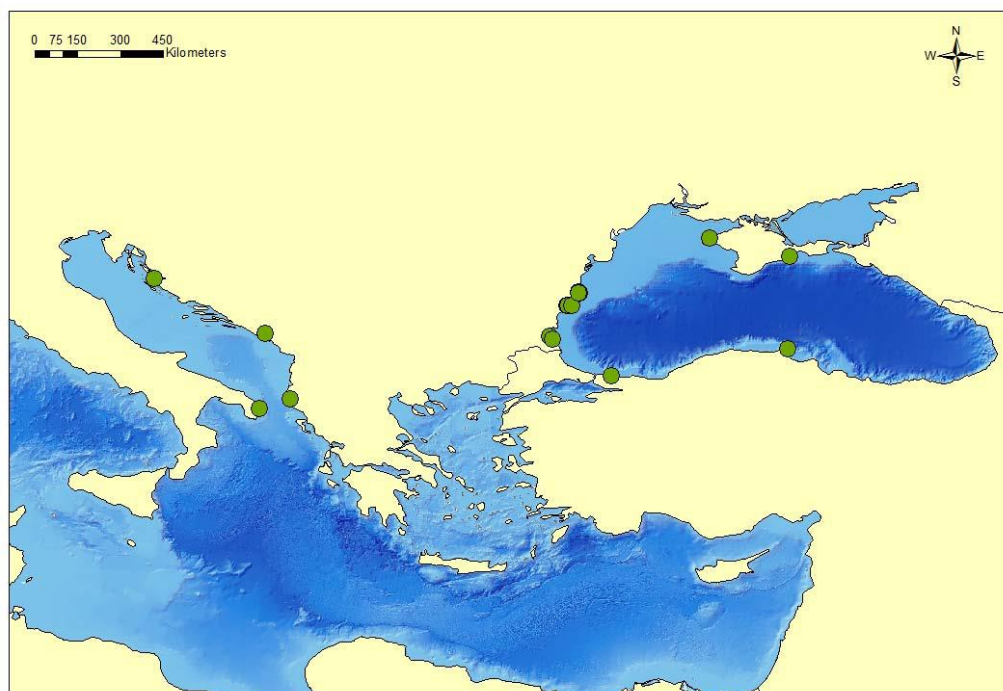


Fig. 5 Location of the sampling points of *Zostera noltii*

### 3.4 *Gibbula divaricata*

For *Gibbula divaricata*, 300 individuals have been collected in the Black Sea (Figure 6) at all sites except those two from Turkey. The species, although present in Turkey, could not be found by the local sampling teams.

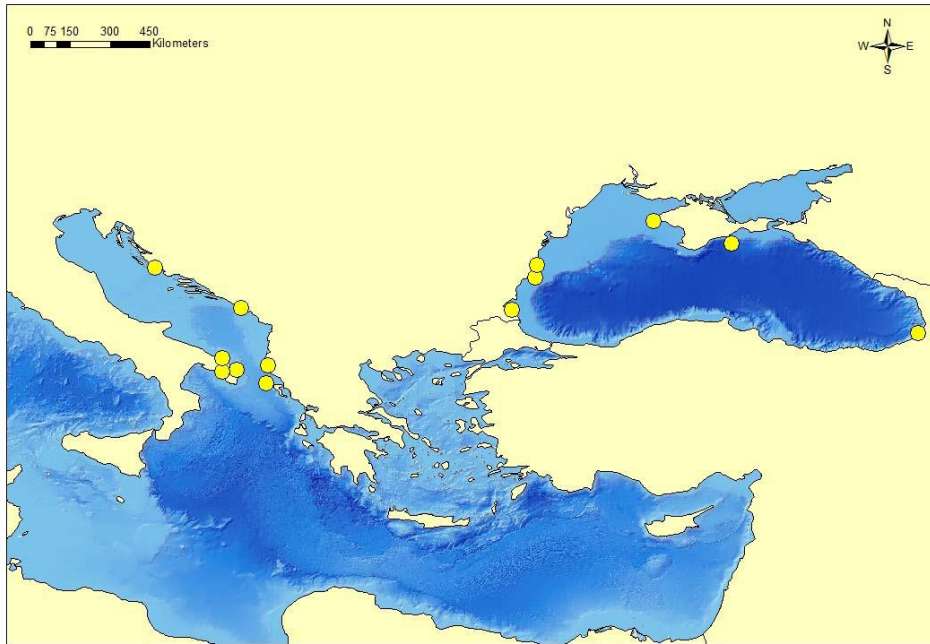


Figure 6. Location of the sampling points of *Gibbula divaricata*

### 3.5 *Cyclope neritea*

A total of 450 individuals of *Cyclope neritea* have been sampled in the Black Sea at 6 sites (Figure 7). The species has been assessed as “not present” in Georgia and, although present, it could not be found by the local sampling team at Sinop.

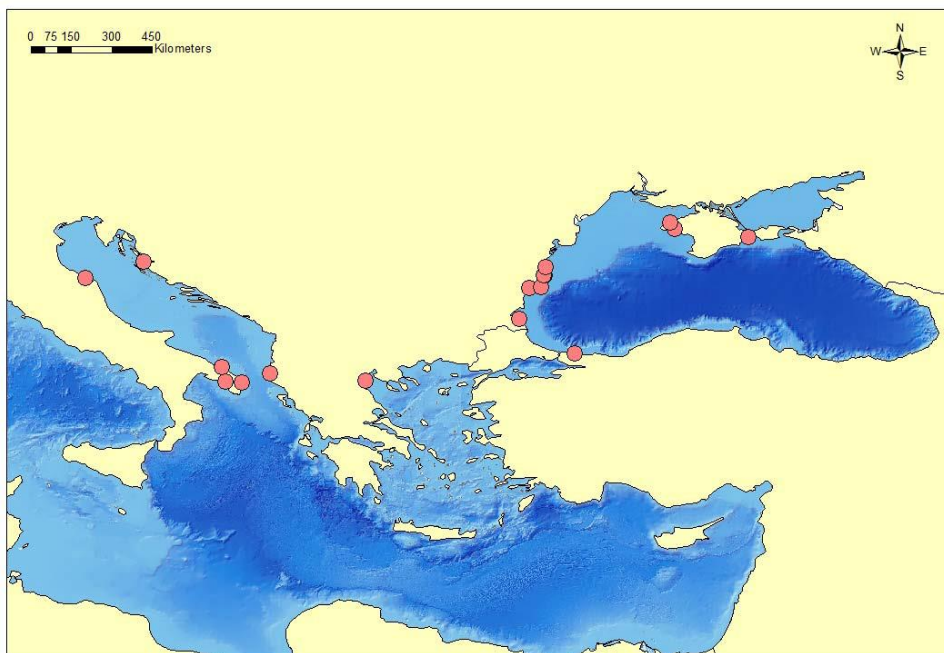


Fig. 7. Location of the sampling points of *Cyclope neritea*



### 3.6 *Mytilus galloprovincialis*

*Mytilus galloprovincialis* has been sampled at all sites from the Black Sea, with a total of 450 individuals collected.

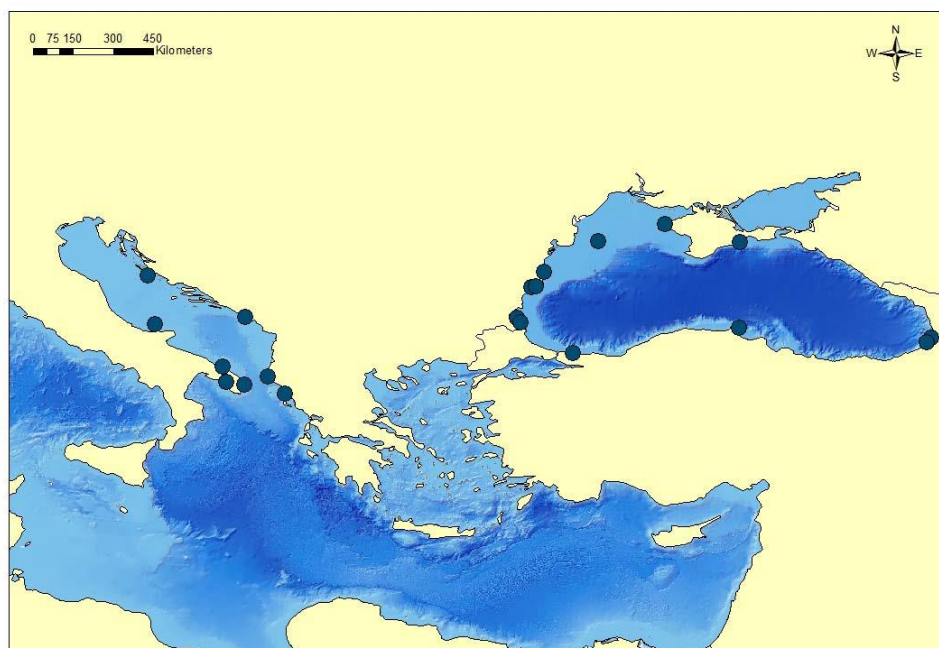


Fig. 7. Location of the sampling points of *Mytilus galloprovincialis*

### 3.7 *Donacilla cornea*

For *Donacilla cornea*, 510 individuals have been collected in the Black Sea (Figure 6) at all sites except Şile (TR). The species, although present in Turkey, could not be found by the local sampling team.

Additionally, 150 individuals have been collected from Greece (150 in Aegean Sea and 50 in Ionian Sea) by Dragos Micu. For the moment no *Donacilla cornea* have been found in Adriatic Sea (Figure 8).

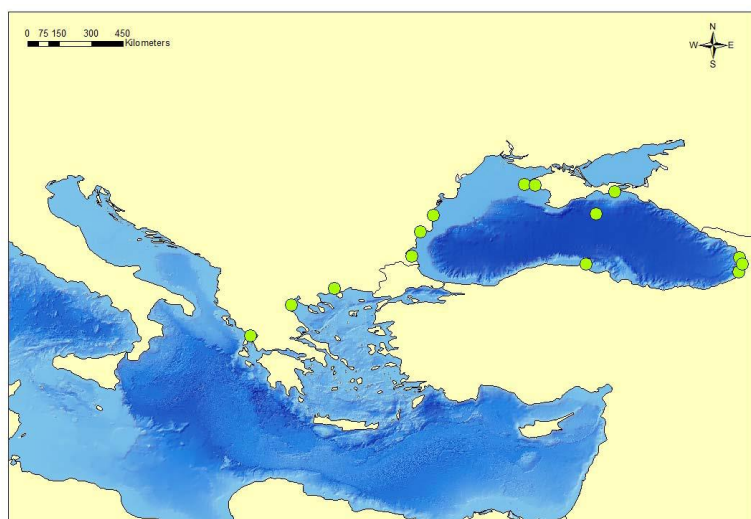


Fig. 8. Location of the sampling points of *Donacilla cornea*

### 3.8 *Pachygrapsus marmoratus*

For the crab *Pachygrapsus marmoratus*, 402 individuals have been collected at 8 all sites from the Black Sea (Figure 9).

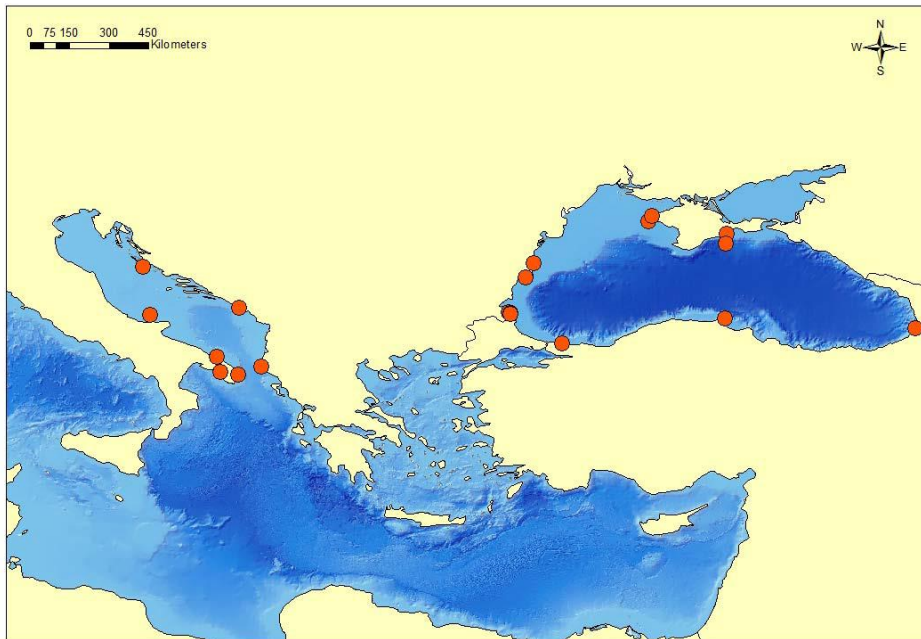


Fig. 9. Location of the sampling points of *Pachygrapsus marmoratus*

### 3.9 *Scorpaena porcus*

Concerning *Scorpaena porcus*, 428 individuals have been collected at all sites in the Black Sea (Figure 10).

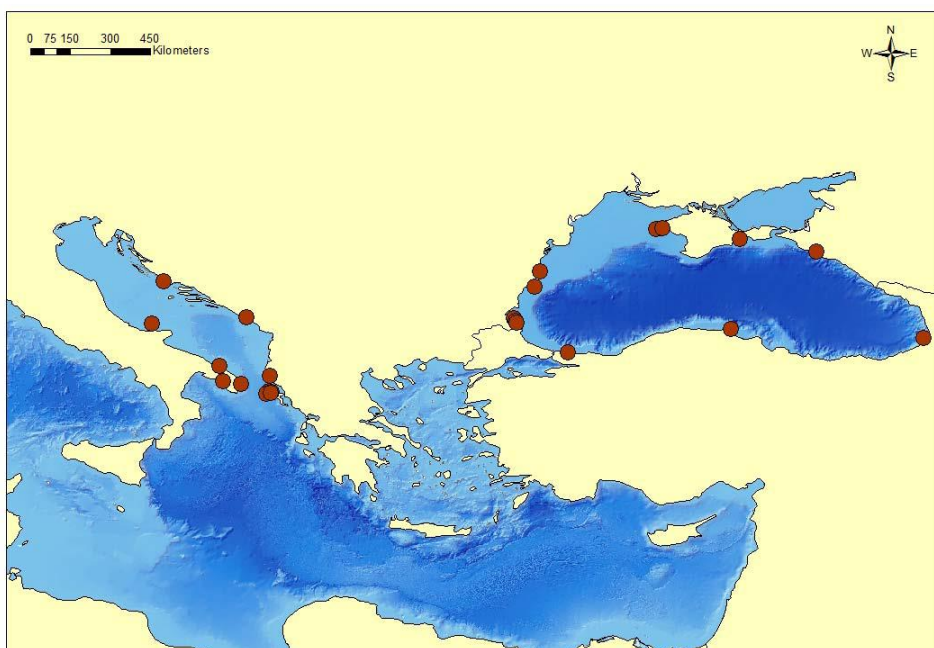


Fig. 10. Location of the sampling points of *Pachygrapsus marmoratus*



### 3.10 *Symphodus tinca*

The species *Symphodus tinca* could not be sampled at most locations around the Black sea. It is absent from Romania and Northern Bulgaria (Cape Kaliakra) and it was extremely rare in Turkey, Georgia and Southern Bulgaria, so only a few specimens could be sampled. Sufficient samples have been obtained only from Crimea (Figure 11)

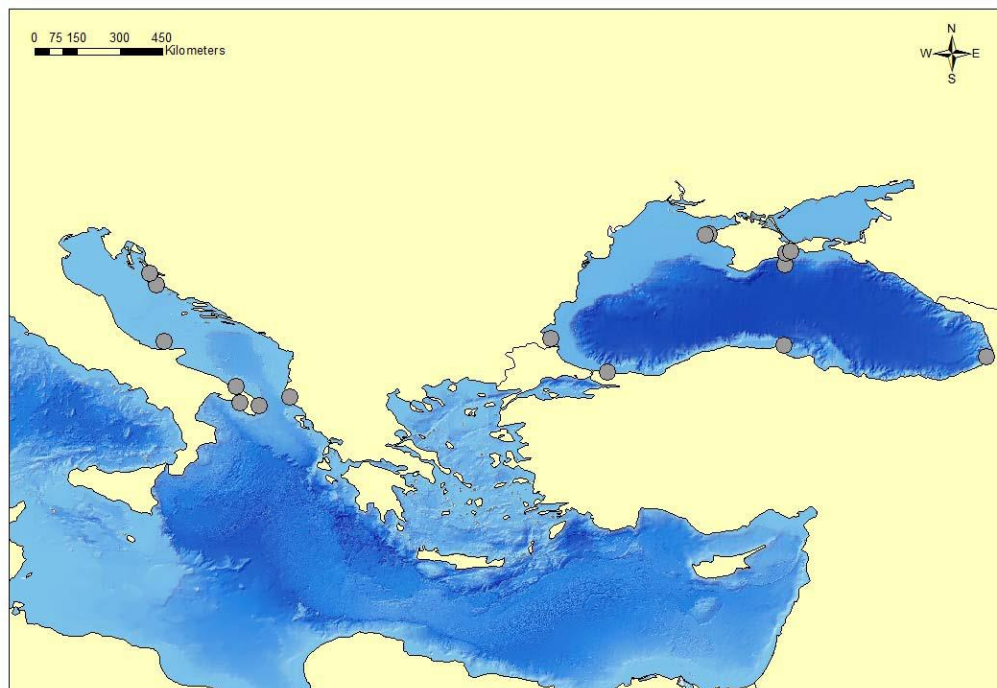


Fig. 11. Location of the sampling points of *Symphodus tinca*

## ANNEX I DETAILS OF GENETIC SAMPLES OBTAINED IN WP10 „BLACK SEA PILOT PROJECT“

Note: Grayed-out species are absent from the respective country and so the sampling is considered complete even without them.

	Sub site	GPS	Zostera noltii	Cystoseira barbata	Phyllophora crispa	Mytilus galloprovincialis	Donacilla cornea	Cydope neritea	Gibbula divaricata	Pachygrapsus marmoratus	Symphodus tinca	Scorpaena porcus
<b>UKRAINE</b>	Crimea - Site 1											
	Olenivka beach	45°22'19 N / 32°30'50 E					50					
	TARHANKUT											
	Cape Atlesh	45°20'03 N / 32°33'09 E							50	5		40
	Donuzlav lagoon	45°20'16 N / 32°58'17 E					50					
	Donuzlav beach	45°20'08 N / 32°58'10 E						50				
	Mejduvodnoe harbour	45°33'24 N / 32°47'57 E						14			2	
	Chernomorske bay	45°31'14 N / 32°42'34 E	50		25	50						
	Chernomorske W cape	45°31'22 N / 32°41'41 E								48	49	
	Ternovaya balka	45°26'43 N / 32°31'58 E		50								
	Bolshoi castel	45°27'23 N / 32°32'33 E			25							
	Okunivka (Fishermen)	45°21'58 N / 32°46'34 E										17
<b>Crimea - Site 2</b>	Karadag MPA - Golden Gate	44°54'37 N / 35°15'33 E				50				35	16	
	KARADAG			50	50				50	5	20	
	Karadag MPA - jetty	44°34'39 N / 35°12'12 E										
	Prymorsky - Golden beach	44°07'38 N / 35°32'32 E					50					
	Feodosia - City beach	45°04'33 N / 35°24'47 E						50				
	Koktebel Bay	44°57'06 N / 35°14'58 E	50								17	
	Feodosia-Quarantine Bay	45°01'26 N / 35°24'15 E									2	50
	Kerch - Yakovenka	45°03'33 N / 36°19'35 E					50					
<b>TOTAL</b>			<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>150</b>	<b>114</b>	<b>100</b>	<b>93</b>	<b>106</b>	<b>107</b>

	Sub site	GPS	Zostera noltii	Cystoseira barbata	Phyllophora crispa	Mytilus galloprovincialis	Donacilla cornea	Cydope neritea	Gibbula divaricata	Pachygrapsus marmoratus	Symphodus tinca	Scorpaena porcus
<b>ROMANIA</b>	COSTINESTI-CAP AURORA											
	Mangalia Broken Pier	43°48'15.59 N / 28°35'31.51 E	50					50				
	Mangalia Inner Bay	43°49'00.65 N / 28°35'24.96 E	50									
	2 Mai	43°46'45.81 N / 28°34'56.81 E	50					50				
	Venus	43°50'40.52 N / 28°36'01.29 E		50					50			
	ZPF (shelf offshore Sulina)	44°56'49.10 N / 30°27'13.30 E				50						
	Costinesti Monastery	43°55'32.02 N / 28°38'26.54 E				50				50		50
	Eforie beach	44°03'29.27 N / 28°38'31.66 E					50	50				
<b>TOTAL</b>			<b>150</b>	<b>50</b>		<b>100</b>	<b>50</b>	<b>150</b>	<b>50</b>	<b>50</b>		<b>50</b>

	Sub site	GPS	Zostera noltii	Cystoseira barbata	Phyllophora crispa	Mytilus galloprovincialis	Donacilla cornea	Cydope neritea	Gibbula divaricata	Pachygrapsus marmoratus	Symphodus tinca	Scorpaena porcus
<b>BULGARIA</b>	Site 1 - CAPE KALIAKRA											
	Bolata Bay	43°22'54.50 N / 28°28'14.61 E						50				
	Albena	43.361308/28.083861					60	50				
	Balchik	43.399555/28.216750	4			26						
	Byala laguna	43.404367/28.235353	32									
	Kavarna	43.411717/28.356953	19	50		24				52		
	Yayla	43.433917/28.542525							50			
	Zelenka	43.381997/28.440639										60
	Site 2 - ROPOTAMO - KITEN											
	Agalina	42.375633/27.717717				15						
	Arkutino	42.3348/27.731133					50					13
	Camping Gradina	42.4281/27.649933	10									
	Kiten reef	42.228467/27.80635			51					25	1	23
	Lozenets	42.19255/27.847033				35				11		14
	North of Tsarevo	42.186983/27.845117		2						14		
	Ropotamo	42.375633/27.717717	37	49				50	50			
<b>TOTAL</b>			<b>102</b>	<b>101</b>	<b>51</b>	<b>100</b>	<b>110</b>	<b>150</b>	<b>100</b>	<b>102</b>	<b>1</b>	<b>110</b>

	Sub site	GPS	Zostera noltii	Cystoseira barbata	Phyllophora crispa	Mytilus galloprovincialis	Donacilla cornea	Cydope neritea	Gibbula divaricata	Pachygrapsus marmoratus	Symphodus tinca	Scorpaena porcus
<b>TURKEY</b>												
Site 1 - ŞİLE	F1	41°11'184 N / 29°34'656 E			50						11	50
	Plaka	41°10'986 N / 29°36'736 E				50				50		
	Sta.3	41°10'815 N / 29°36'668 E		50								
	Beach	41°10'541 N / 29°35'974 E						50				
	Harbour	41°10'702 N / 29°36'092 E	50									
Site 2 - SINOP	Karakum beach	42° 0'56.37"N- 35°11'48.73"E	50							57	21	
	Hamsilos	42° 3'39.01"N- 35° 2'38.97"E		50								
	Gazi Rocks	42° 0'43.73"N- 35°12'22.54"E				50						
	Mobil Beach	42° 0'23.04"N- 35° 7'1.77"E					50					
	Papazlar Beach	41°58'28.56"N- 35° 6'18.44"E										61
<b>TOTAL</b>			<b>100</b>	<b>100</b>	<b>50</b>	<b>100</b>	<b>50</b>	<b>50</b>	<b>0</b>	<b>107</b>	<b>32</b>	<b>111</b>

	Sub site	GPS	Zostera noltii	Cystoseira barbata	Phyllophora crispa	Mytilus galloprovincialis	Donacilla cornea	Cydope neritea	Gibbula divaricata	Pachygrapsus marmoratus	Symphodus tinca	Scorpaena porcus
<b>GEORGIA</b>												
BATUMI	Sarpi	41°31'35.93 N / 41°32'35.93 E		50		50			50			
	Kulevi	42°15'37.03 N / 41°38'14.47 E					50					
	Ureki	41°59'17.86 N / 41°45'31.49 E					50					
	Batumi										4	50
	Mcvane Kondkhi									50		
<b>TOTAL</b>				<b>50</b>		<b>50</b>	<b>100</b>		<b>50</b>	<b>50</b>	<b>4</b>	<b>50</b>

## ANNEX II CONTRIBUTORS TO SAMPLING

UKRAINE: IBSS (team of Nataliya Milchakova), CNRS (Serge Planes), INCDM (Dragos Micu)

ROMANIA: INCDM (team of Dragos Micu)

BULGARIA: IOBAS (team of Valentina Todorova), CNRS (Serge Planes), INCDM (Dragos Micu)

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