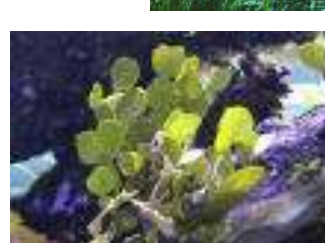
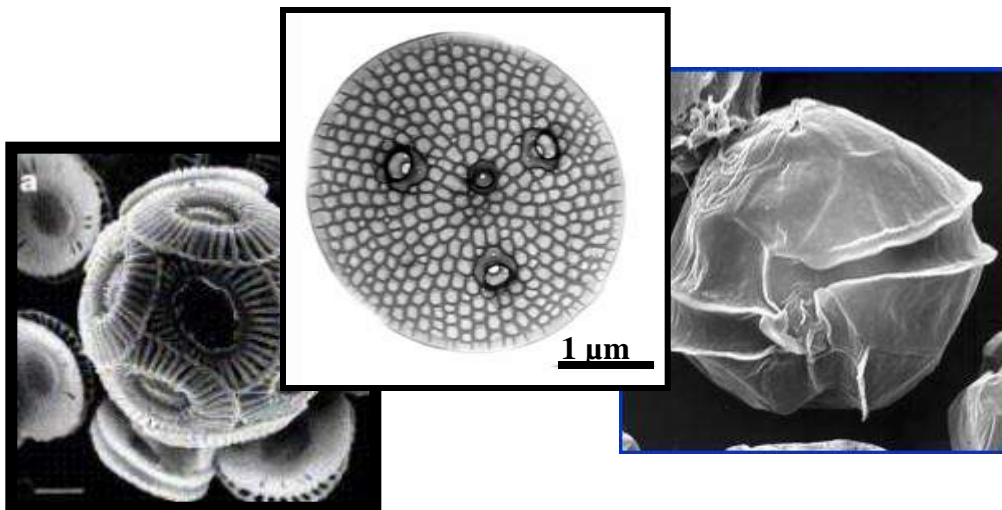


# Le risorse genetiche marine della Stazione Zoologica Anton Dohrn

Gabriele Procaccini

Laboratorio di Ecologia Funzionale ed Evolutiva



Gli organismi marini svolgono **ruoli chiave** in processi biogeochimici di fondamentale importanza per la biosfera.

Essi forniscono una quantità di **prodotti**

- *cibo,*
- *sostanze naturali per le biotecnologie e l'industria farmaceutica,*
- *attrazione per il turismo*

e svolgono una serie di **funzioni**

- *produzione e mineralizzazione della materia organica,*
- *accumulo di carbonio, buffering per i cambiamenti climatici,*
- *protezione della linea di costa, ecc...*

La varietà dei prodotti e l'efficienza nello svolgimento delle funzioni dipendono dalla interazione fra gli organismi e fra organismi ed ambiente, e quindi dalla **biodiversità**.

L'ambiente marino, nonostante possa apparire omogeneo rispetto all'ambiente terrestre, presenta una **enorme varietà di habitat**.

La diversità di **scale spaziali e temporali** lungo le quali gli organismi marini sono distribuiti fa sì che la biodiversità marina sia elevatissima e, per un insieme di cause diverse, in parte inesplorata.

Inaccessibilità di molti ambienti

Convergenza evolutiva di molti caratteri morfologici

**Una stima recente suggerisce che solo nelle comunità abissali ci potrebbero essere circa 10 milioni di specie ancora non descritte, rispetto ai circa 12,5 milioni di specie note sulla terra.**

**In ambiente marino è presente una elevata diversità ad alto livello filogenetico**



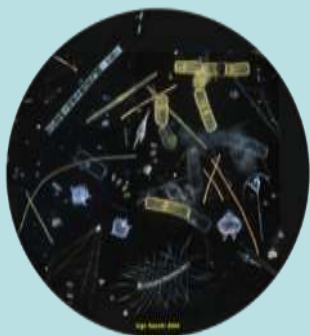
***“Oceanic and coastal marine ecosystems provide a wide range of goods and services that are fundamental to continued human well-being. All ecosystem services ultimately derive from ecosystem functions, that is the processes, products or outcomes arising from the interactions of organisms with their environment activities in the ecosystem ... Marine biodiversity underpins the functioning of marine ecosystems and their provision of services - **without biodiversity there would be no ecosystem services.**”***

*From the* THE VALENCIA DECLARATION. A PLEA FOR THE PROTECTION OF MARINE BIODIVERSITY  
(Conferenza mondiale sulla biodiversità marina – Valencia, 2008-11-15)

## Alla Stazione Zoologica Anton Dohrn:

### Specie

Microalgae



Macroalgae



Tunicati

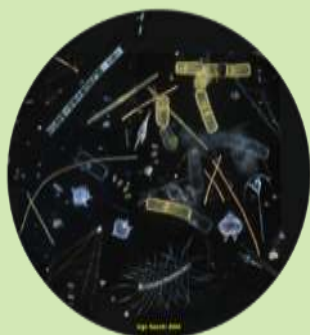


Invertebrati



### Popolazioni

Microalgae



Macroalgae



Fanerogame marine



Tunicati



Rettili marini

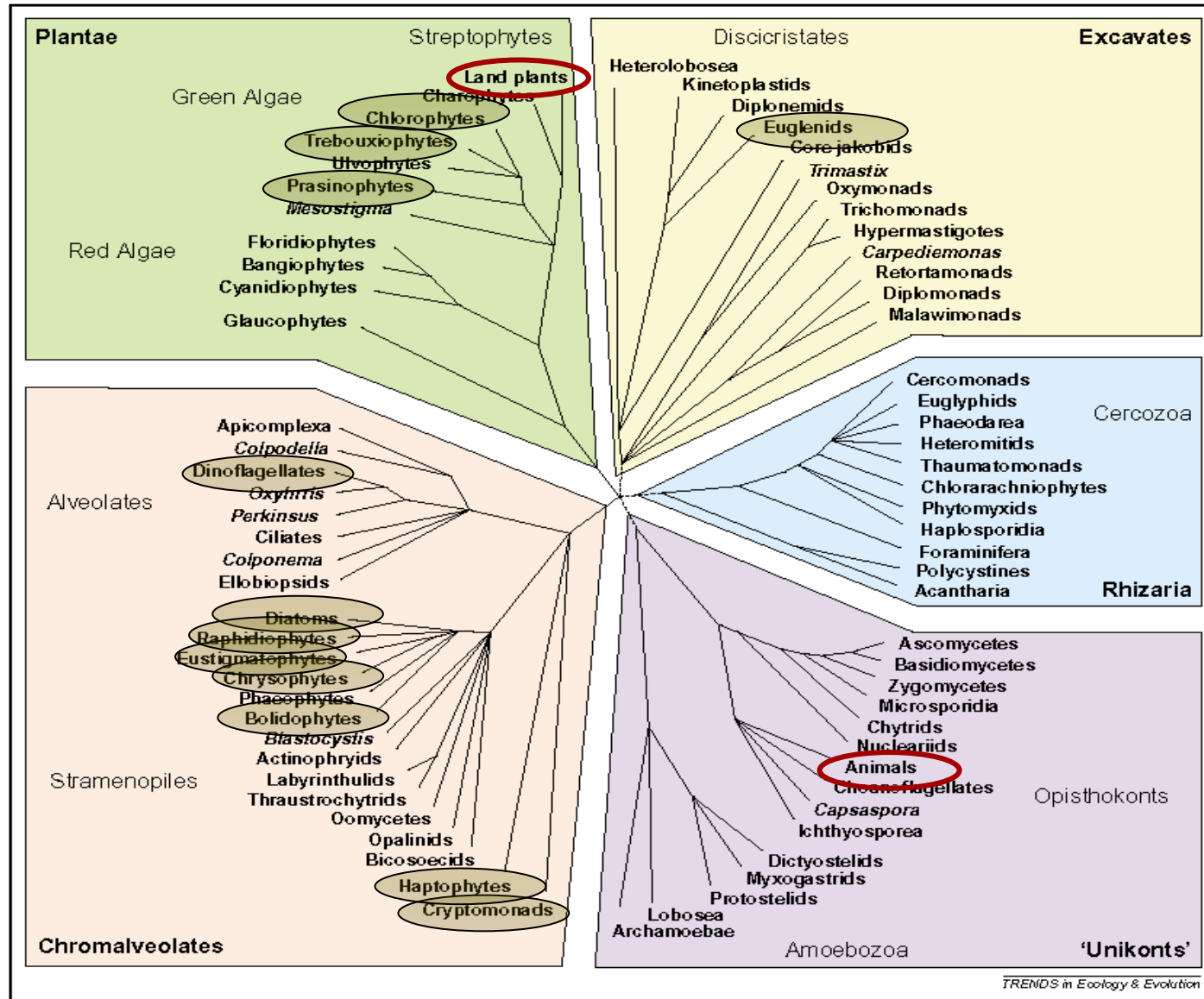


Invertebrati





## Diversità tassonomica delle microalghe marine





## New species 1987-to date

### DIATOMS

- ***Chaetoceros thronsdensei*** Marino, Montresor et Zingone 1987
- ***Lennoxia faveolata*** Thomsen et al 1993
- ***Bacteriastrum parallelum*** Sarno, Zingone et Marino 1997
- ***Amphicocconeis*** De Stefano & Marino 2003\*
- ***Cocconeis multiperforata*** De Stefano et al. 2000
- ***Cocconeis pseudonotata*** De Stefano et Marino 2001
- ***Campyloneis juliae*** De Stefano 2003
- ***Talaroneis posidoniae*** Kooistra et De Stefano 2004\*
- ***Skeletonema marinoi*** Sarno et Zingone 2005
- ***S. grethae*** Zingone et Sarno 2005
- ***S. dohrnii*** Kooistra et Sarno 2500
- ***S. japonicum*** Zingone et Sarno 2005
- ***S. grevilleii*** Sarno et Zingone 2007
- ***S. ardens*** Sarno et Zingone 2007
- ***Pseudo-nitzschia mannii*** Amato & Montresor
- ***Pn autumnalis*** Sarno & Zingone in prep
- ***Leptocylindrus aporus*** Nanjappa & Zingone
- ***Tenuicylindrus belgicus*** Nanjappa & Zingone
- ***Leptocylindrus convexus*** Nanjappa & Zingone
- ***Leptocylindrus hargravesii*** Nanjappa & Zingone

### DINOFLAGELLATES

- ***Protoperidinium parthenopes*** Zingone et Montresor 1988
- ***Scrippsiella precaria*** Montresor et Zingone 1988
- ***Scrippsiella ramonii*** Montresor 1995
- ***Prorocentrum nux*** Puigserver et Zingone 2002
- ***Polarella glacialis*** Montresor et Stoecker 2003 \*
- ***Alexandrium tamutum*** Montresor, Beran et John 2004
- ***Protoperidinium vorax*** Siano et Montresor 2008
- ***Woloszinskia cincta*** Siano & Zingone 2009
- ***Azadinium dexteroporum*** Percopo & Zingone 2013
- ***Pelagodinium béyii*** Siano et al 2010

### PRASINOPHYTES

- ***Dolichomastix tenuilepis*** Thronsdensei et Zingone 1997
- ***Crustomastix stigmatica*** Zingone 2002
- ***Throndsenia aranea***. Eikrem et Zingone in prep.\*

### PRYMNESIOPHYTES

- ***Phaeocystis cordata*** Zingone 1999
- ***Phaeocystis jahnii*** Zingone 1999
- ***Phaeocystis sp.*** Colonial, in prep.
- ***Phaeocystis sp.*** single in prep.

### PINGUIOPHYCEANS

- ***Phaeomonas sp*** in prep.

### CRYPTOPHYTES

- ***Rhodomonas sp.*** in prep. Cerino et Zingone
- ***Cryptochloris sp.*** in prep Cerino et Zingone

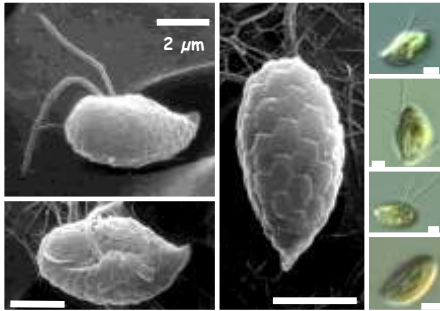
\*new genus



## Approcci metagenomici

Così come le specie criptiche, anche le specie dominanti negli oceani, la componente autotrofa eucariotica dell' ultraplankton (<5 µm), non sono distinguibili in MO ed a volte neanche in ME

Probes e primers per regioni specifiche del 16S rRNA plastidiale



0 *Fuller et al.*

Table 2. 16 S rDNA oligonucleotide probes used in this study. T<sub>d</sub> indicates dissociation temperature.

Probe	T <sub>d</sub> (°C)	Sequence (5'-3')	Target organisms	Reference
CHLA768	49	CCA TTC TCT CCC CTC GCT	Chlorarachniophyceae	This study
CHRY1037	52	GCA CCA CCT GTG TAA GAG	Chrysophyceae	This study
CRYP862	42	GGA TAC TTA ACG CCT TAG	Cryptophyceae	This study
EUB908	35	CCG TCA ATT CCT TTG AGT TT	Eubacteria	(Edwards et al. 1989)
EUST985	49	CAC TTC TAG CAA ACC CTG	Eustigmatophyceae	This study
PAVL665	37	TAG AAA TTC CTC CTA CCC	Pavlovophyceae	This study
PELA1035	52	ACC ACC TGT GTG TGT CTA	Pelagophyceae	This study
PING1024	47	ACG TAT TCC TTA CGG CAC	Pinguicophyceae	This study
PRAS826	58	GAT TCG CGT ATC CCC TAG	Prasinophyceae clade VI	This study
PRYM666	43	CTA GAA ATT CCC TCT ACC	Prymnesiophyceae	This study
TREB708	44	CCT TTG GTG TTC CTC CCG	Trebouxiophyceae (CCMP243, RCC9, and Prototheca clusters)	This study

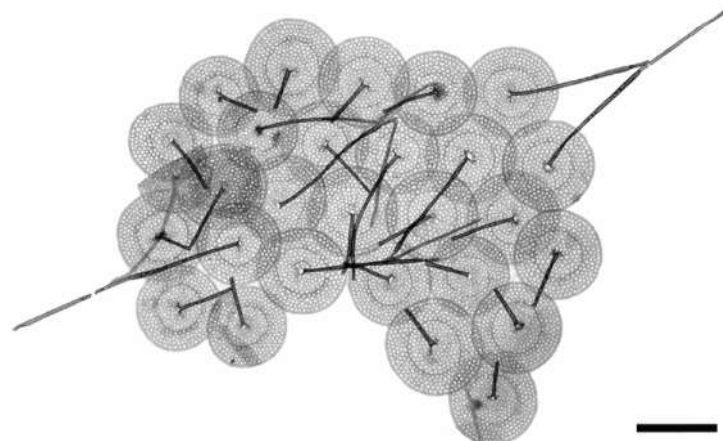
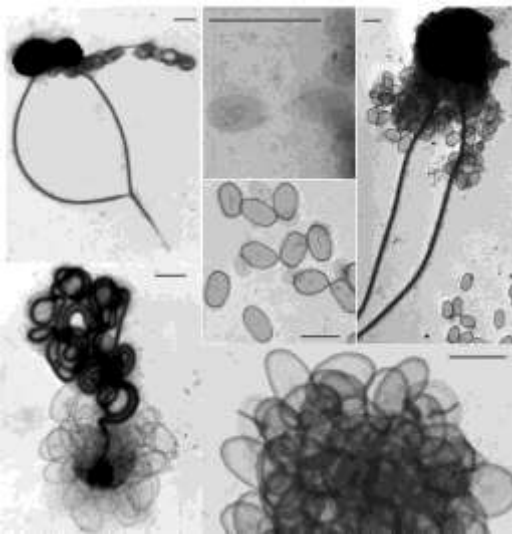
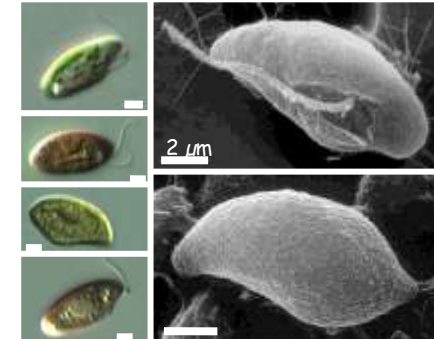
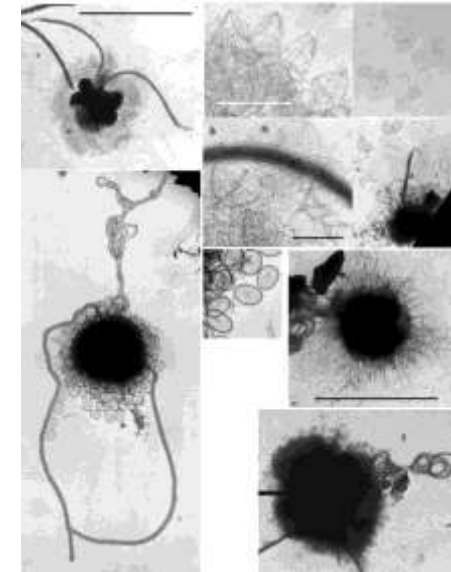


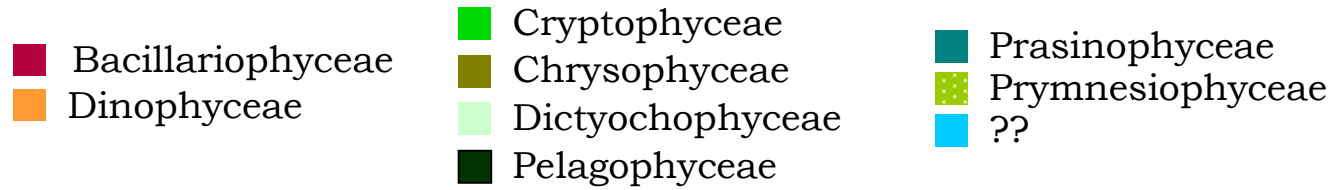
Foto: A. Zingone





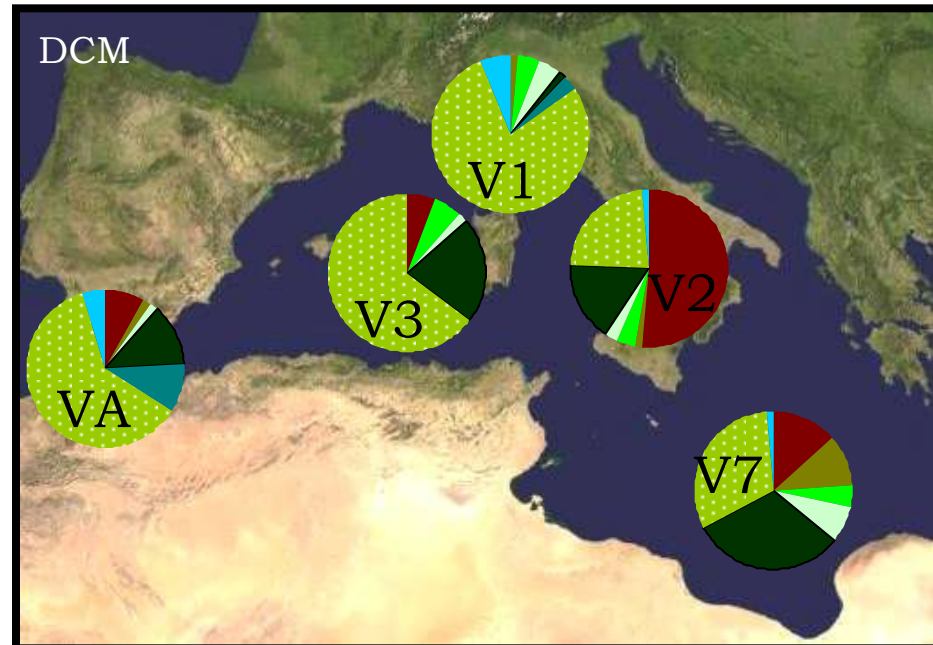
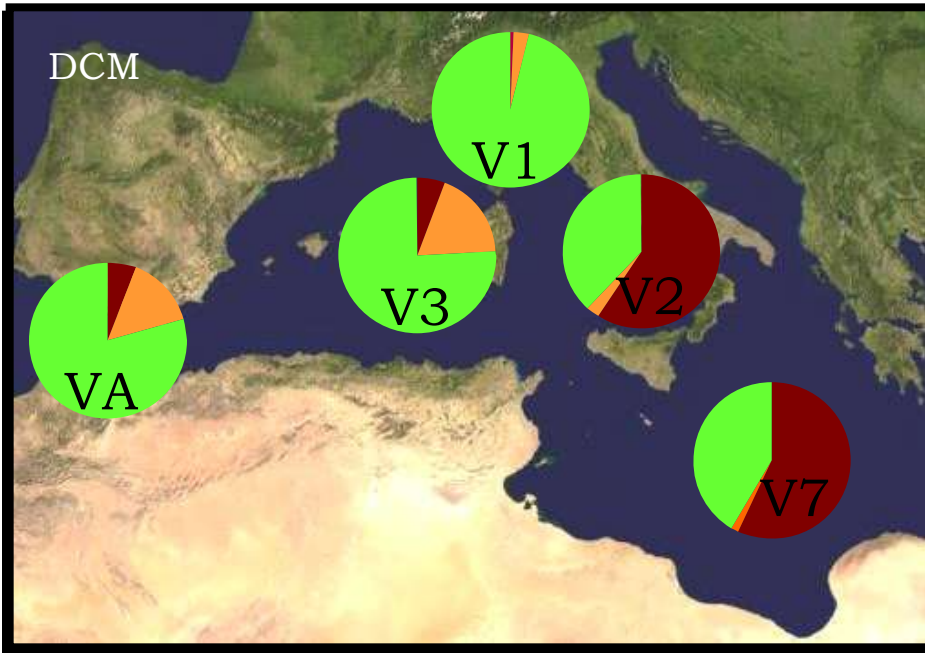


## Clonal libraries- Transmed VECTOR summer 2007



% densità (microscopia)

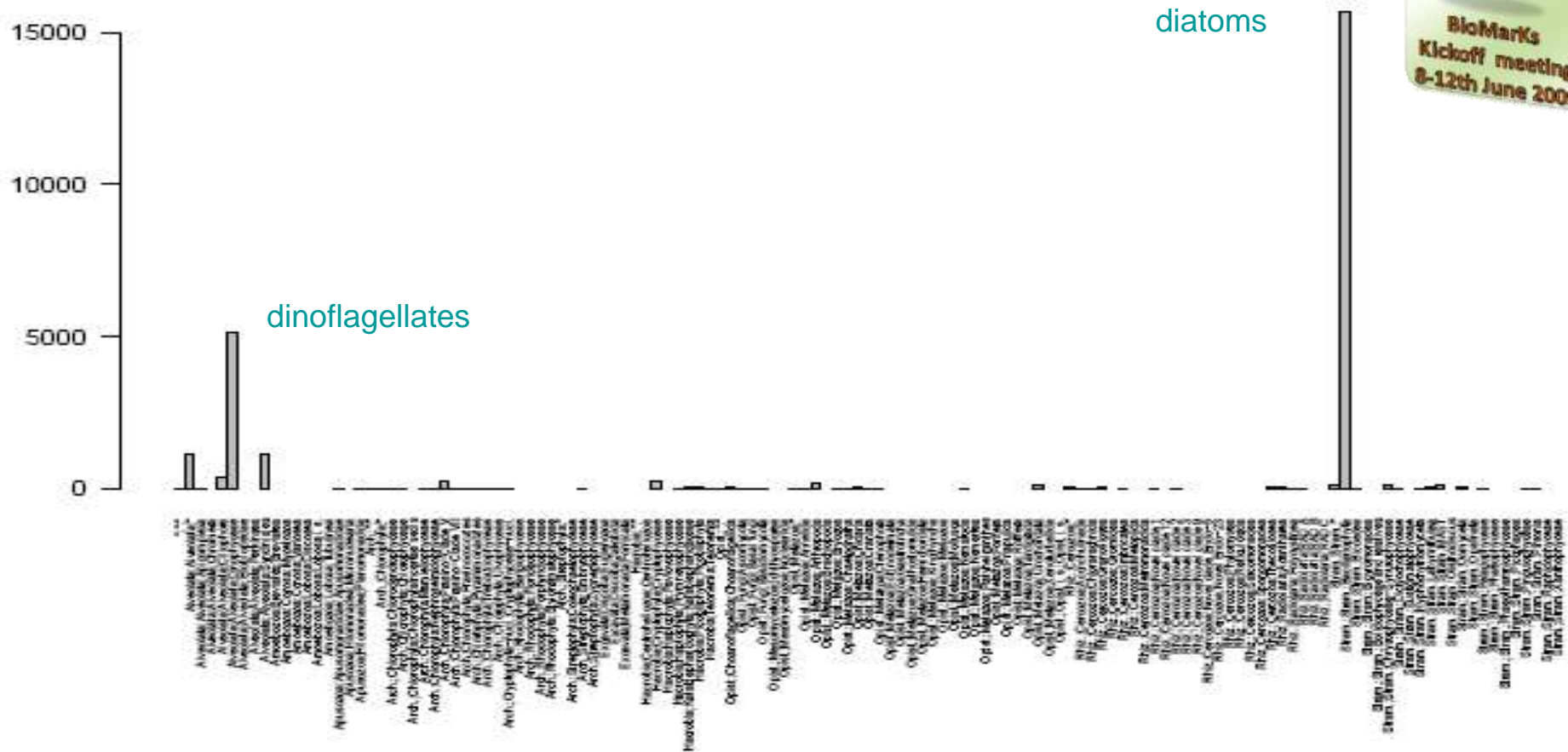
# sequences





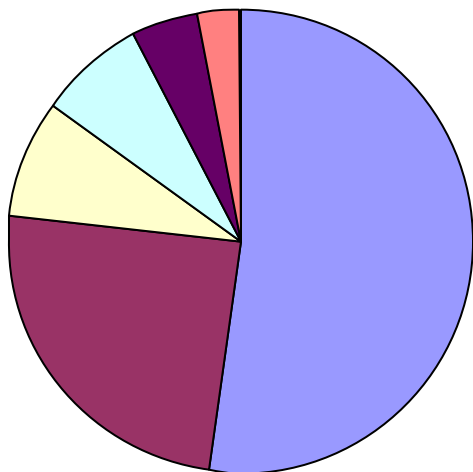
# LTER-MC, Gulf of Naples,

October 2009, m 2, 3-20 um size fraction - Roche 454



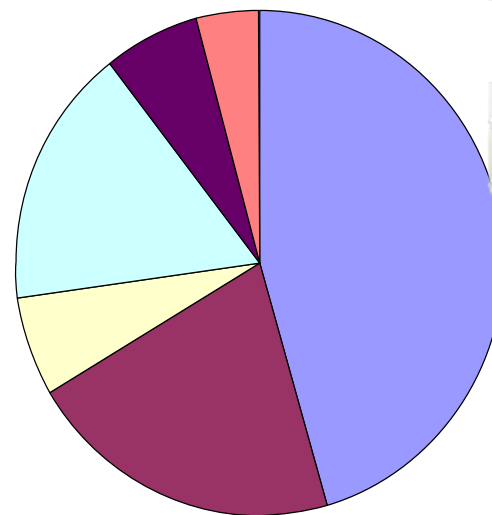


m 2, 454 data

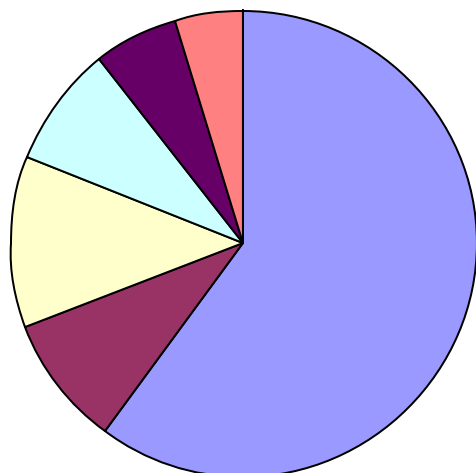


- Centric-diatoms
- Thalassiosira-group
- Skeletonema-group
- Pseudo-nitzschia
- Chaetoceros-group
- Other diatoms

m 2, microscope counts

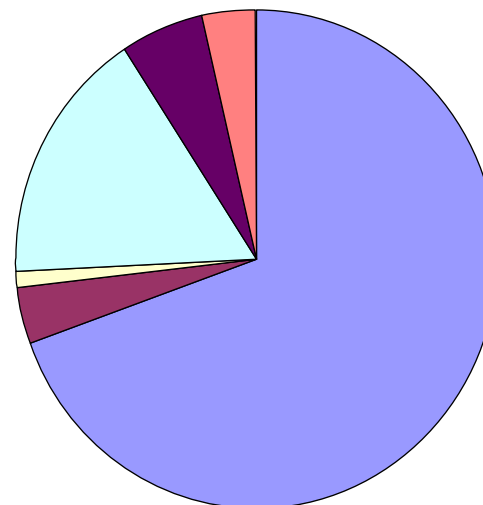


m 23, 454 data



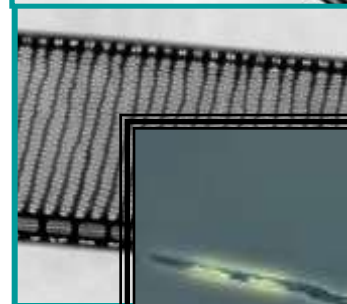
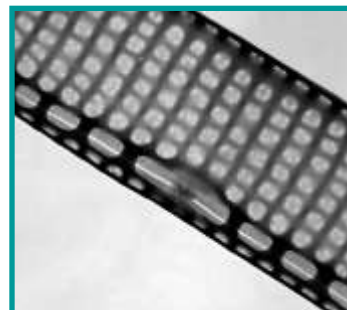
- Centric-diatoms
- Thalassiosira-group
- Skeletonema-group
- Pseudo-nitzschia
- Chaetoceros-group
- Other diatoms

m 23, microscope counts

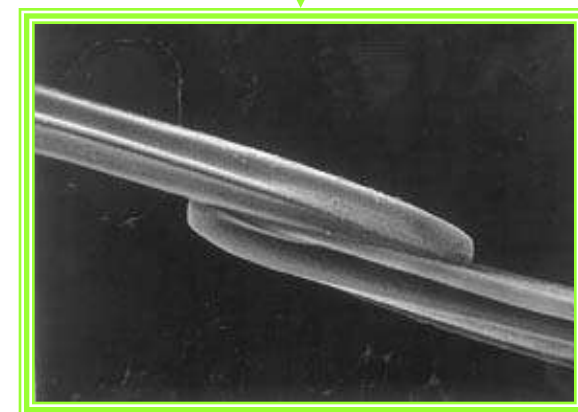
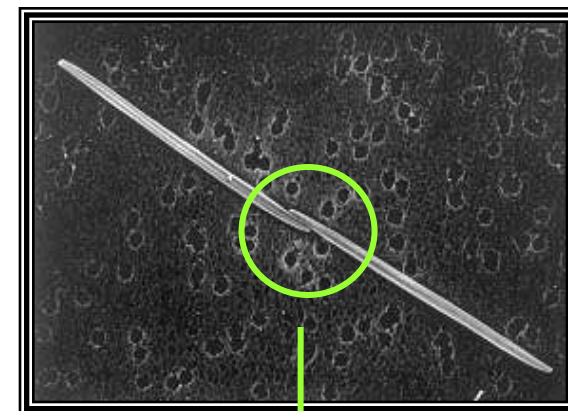




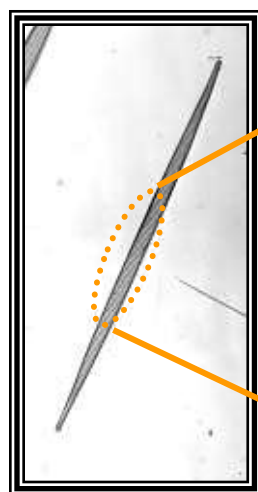
*The Pseudo-nitzschia genus and  
Pseudo-nitzschia delicatissima*



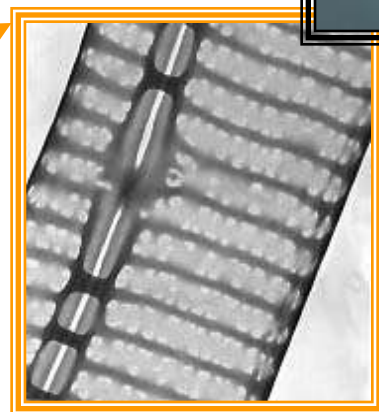
LM

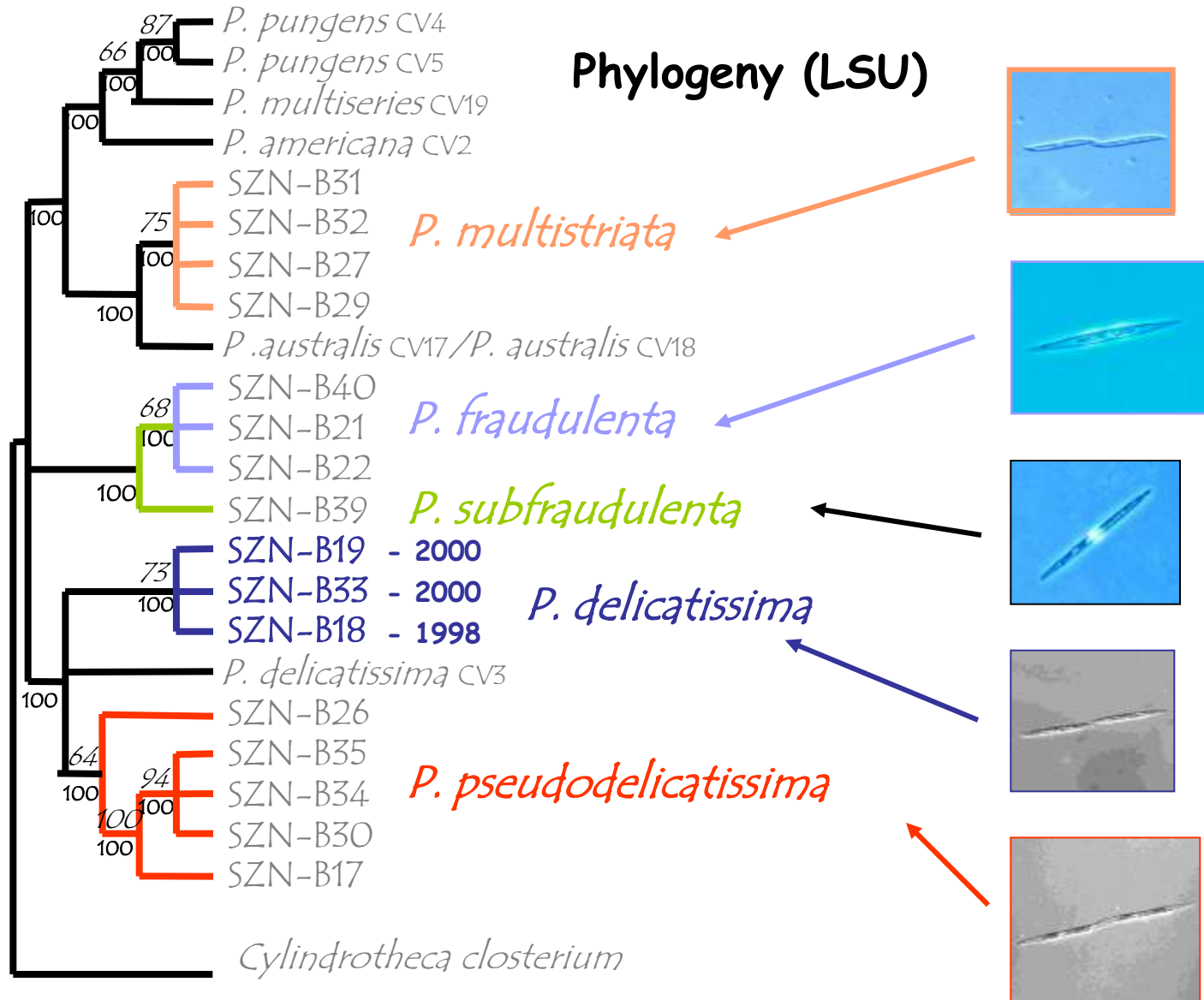


SEM

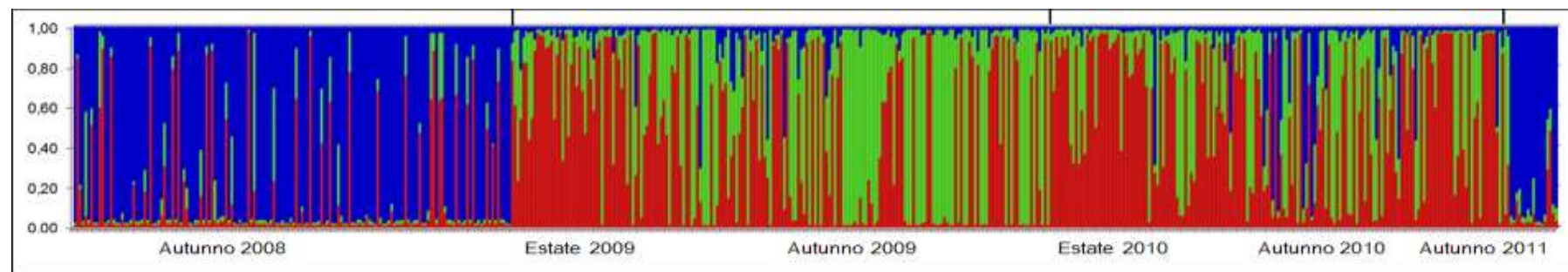
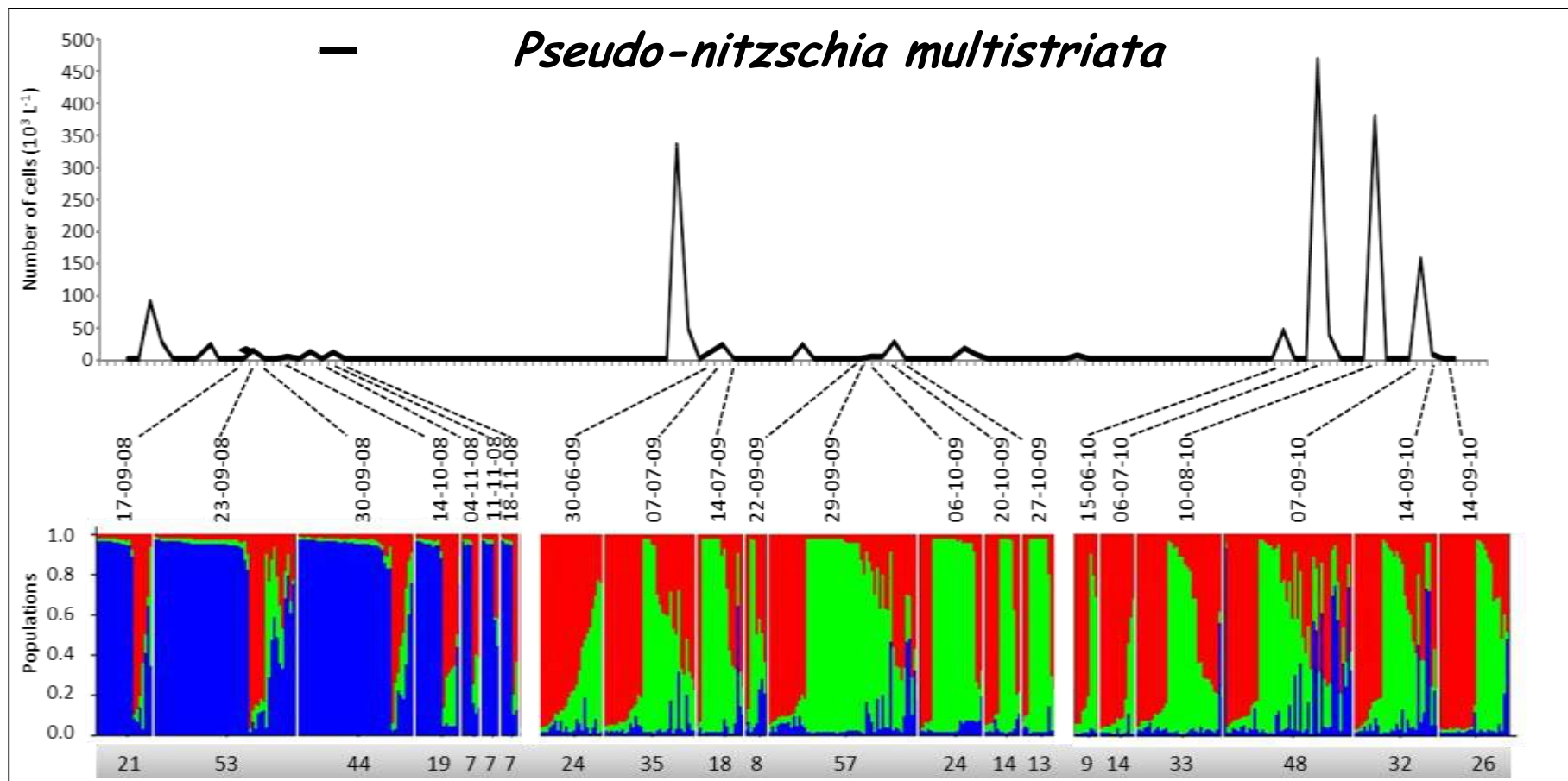


TEM





Parsimony tree-1000 bootstrap







***Asparagopsis armata***  
**e**  
***Asparagopsis taxiformis***

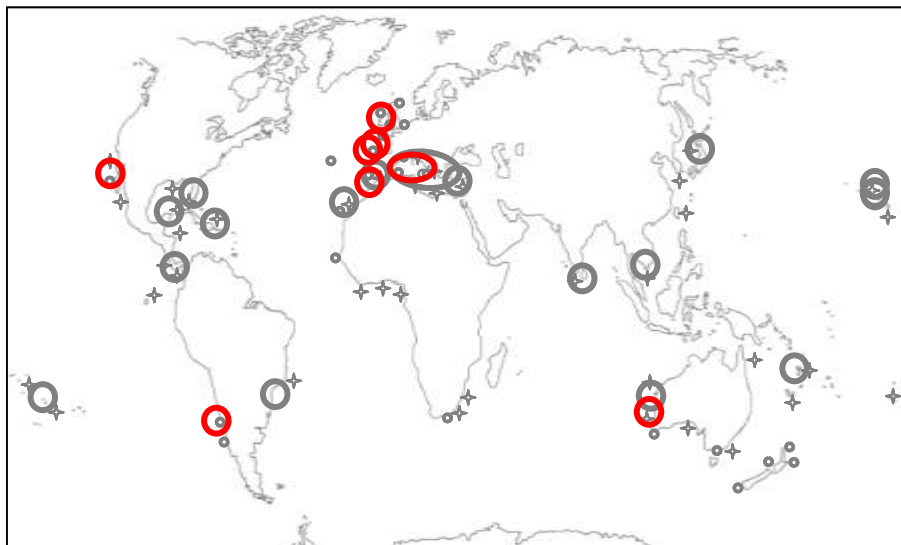


**Introduced species  
in the Mediterranean**

***Caulerpa racemosa***  
**e**  
***Caulerpa distichophylla***

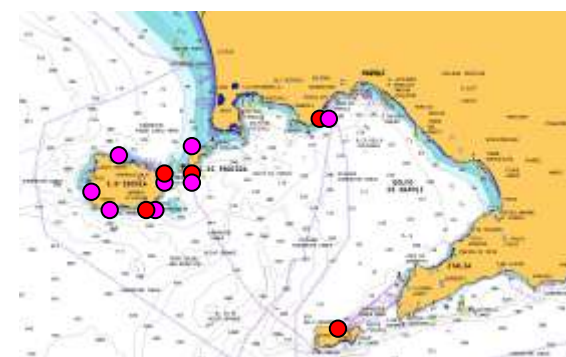


***A. armata* and *A. taxiformis***  
Sampling stations

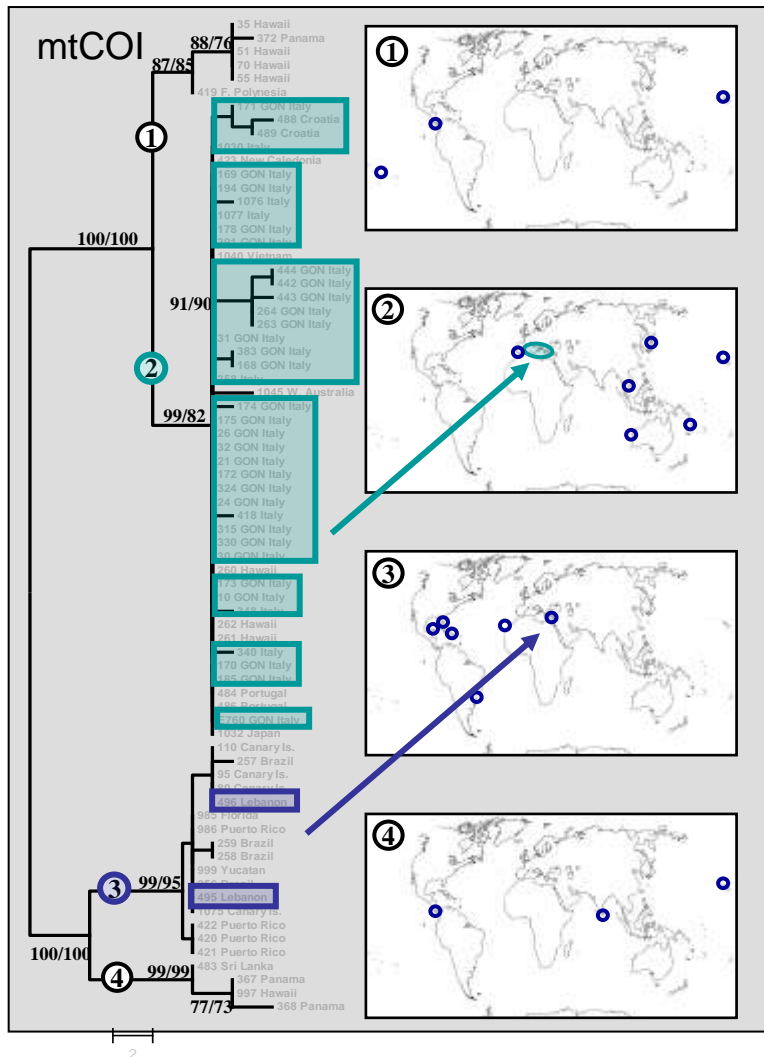


<i>A. taxiformis</i> ✦	<i>A. armata</i> ○	Distribution
<i>A. taxiformis</i> ○	<i>A. armata</i> ○	Sampling stations

***C. racemosa***  
Sampling stations



## *Asparagopsis taxiformis* – struttura genetica intraspecifica



Presenza di 4 cladi mitocondriali,  
di cui due presenti in  
Mediterraneo.

Specie criptiche?

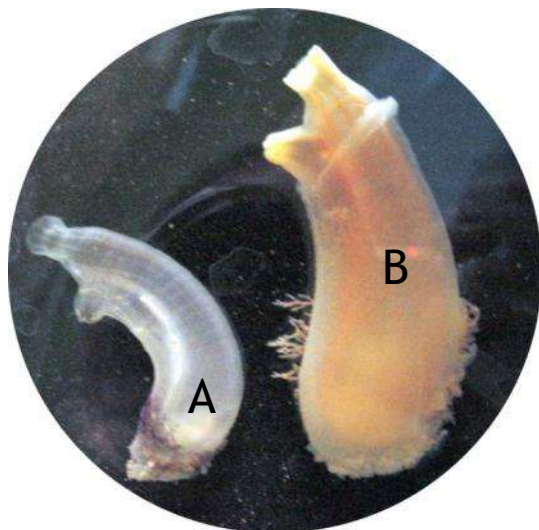
**Clade indopacifico:  
ballast waters?**

**Clade atlantico:  
migrazione attraverso lo  
stretto di Gibilterra?**

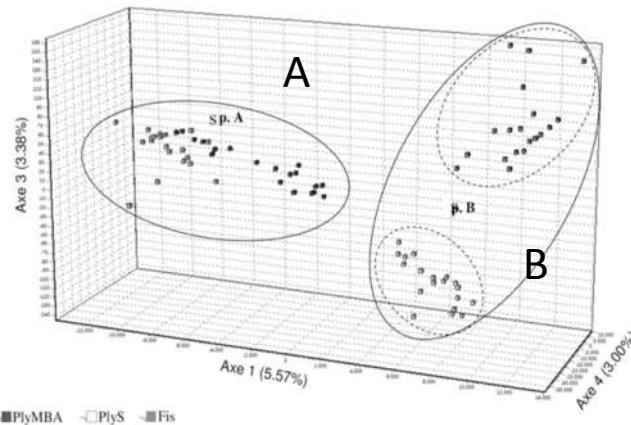


# Speciazione criptica in *Ciona intestinalis*

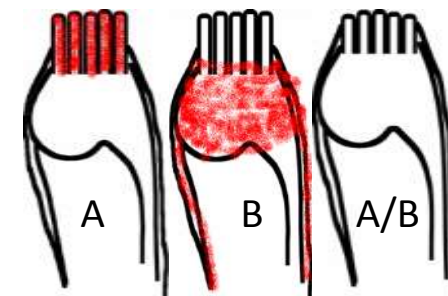
Risorse disponibili



Deep phylogenetic divergence

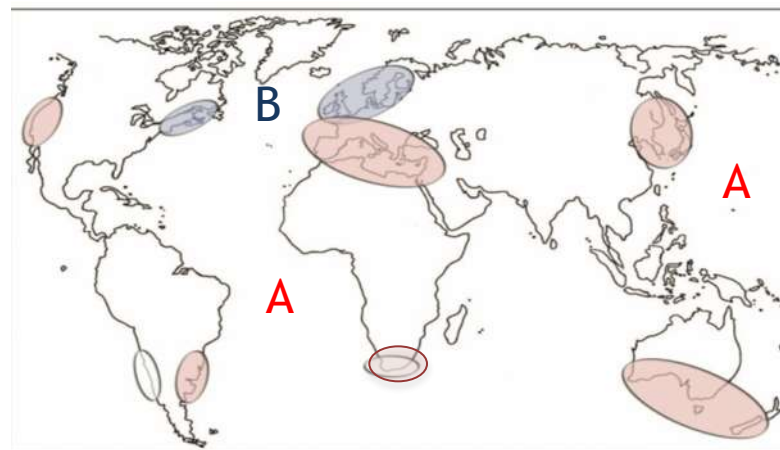


Low morphological difference:  
spermiduct pigmentation



Hox (*Hox5* and *Hox13*)  
ParaHox (*Gsx*)  
mtCOI

Geographical distribution





***Posidonia oceanica***



***Cymodocea nodosa***



***Zostera noltii***

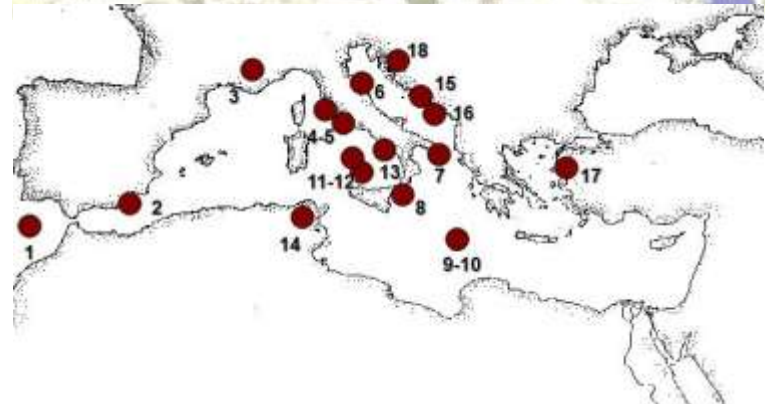




*Posidonia oceanica*



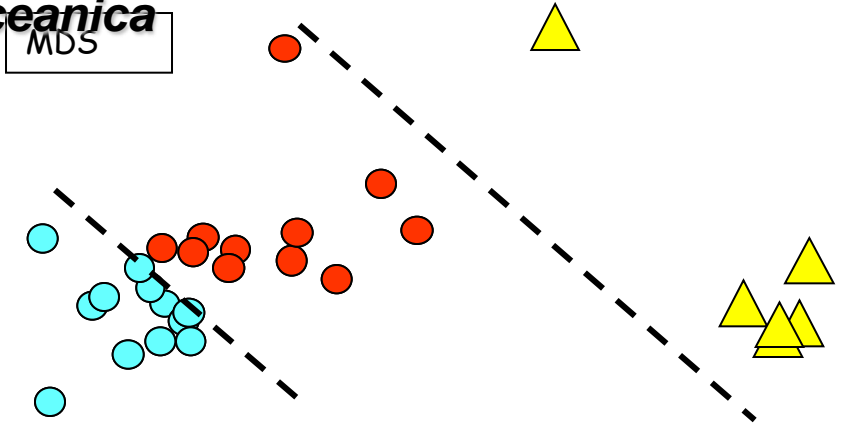
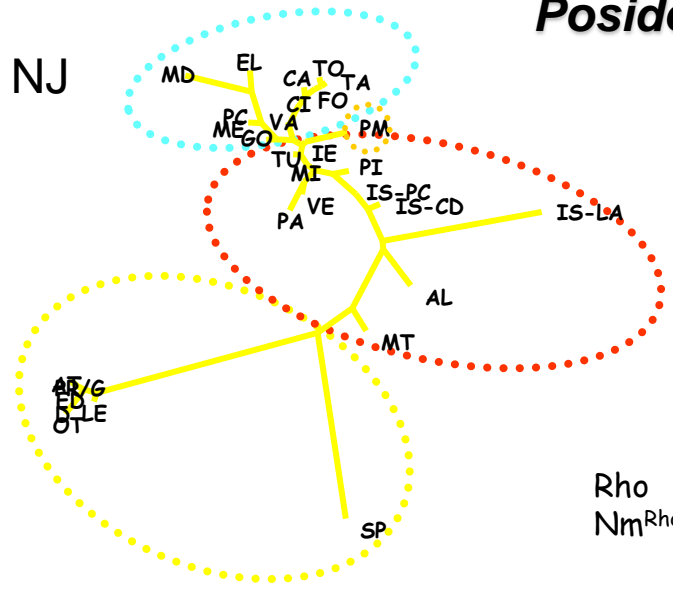
*Cymodocea nodosa*



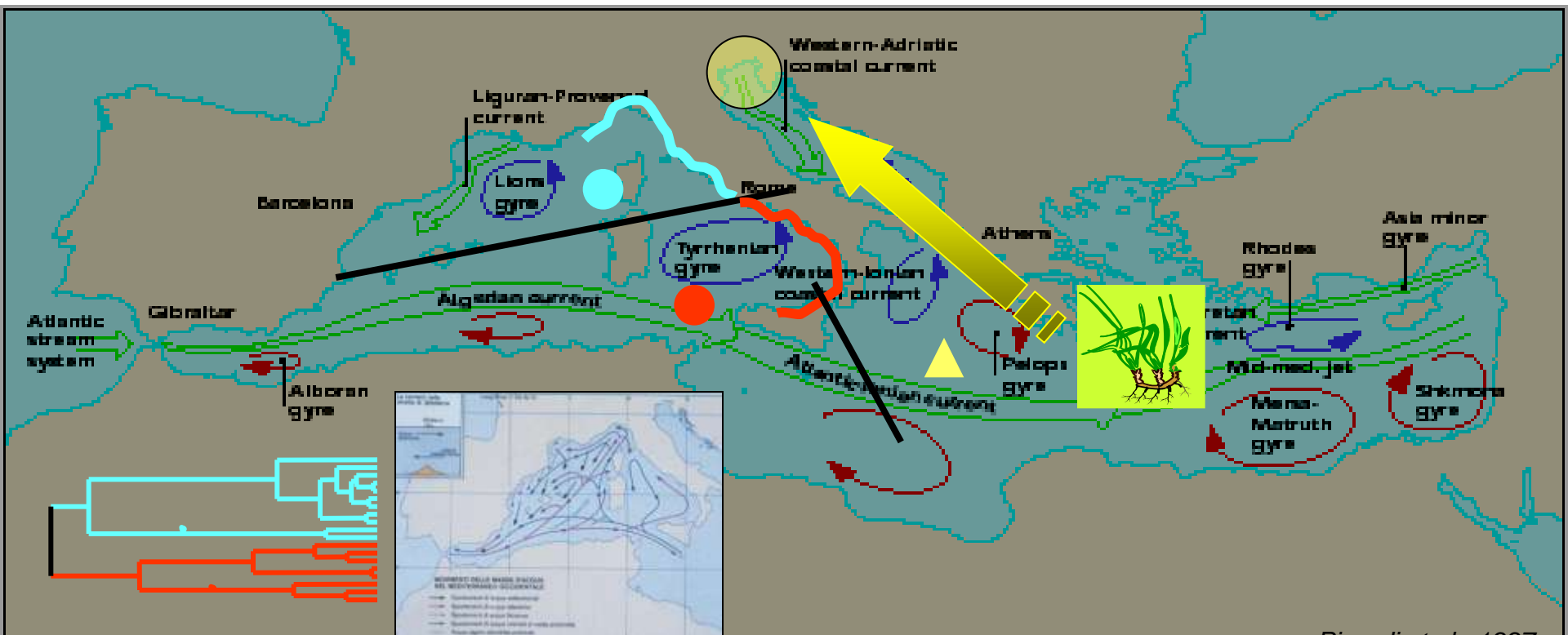
*Zostera noltii*



# Posidonia oceanica



Rho = 0.451, p=0.00  
Nm<sup>Rho</sup> = 0.304, p=0.00



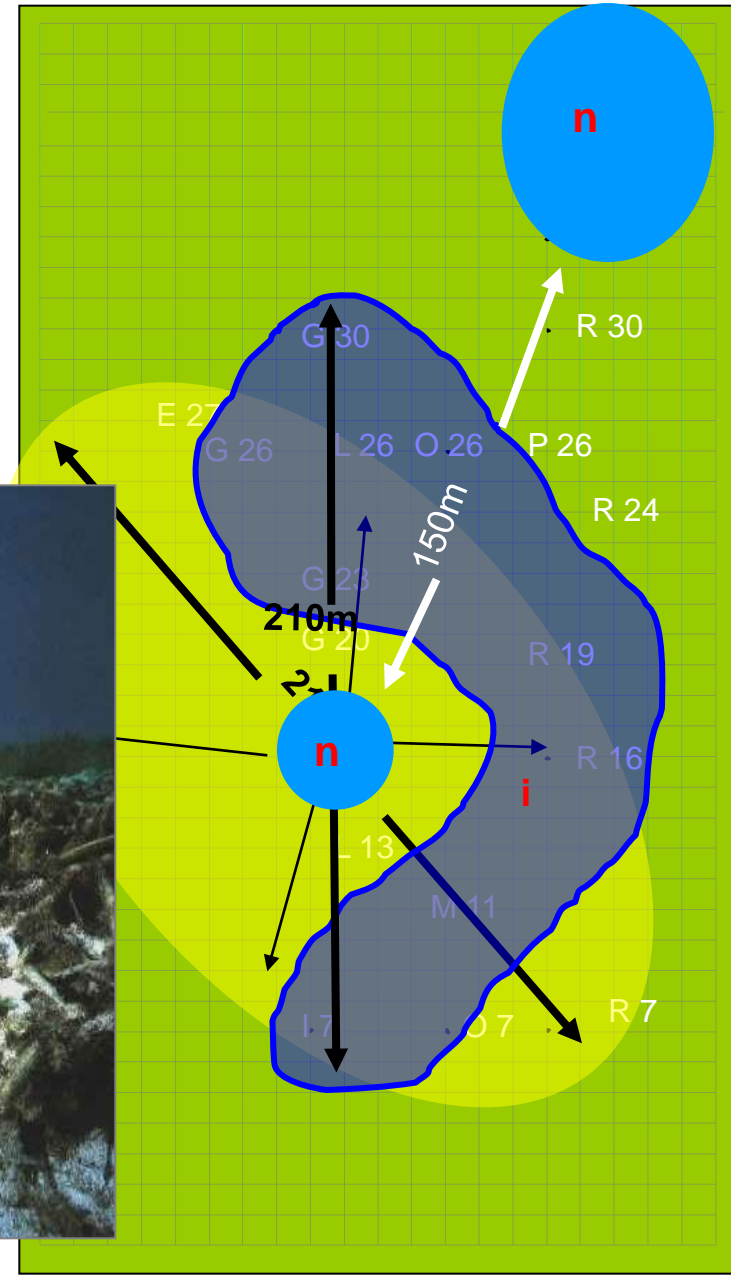
# Clonal structure

# *Posidonia oceanica*

# Clones age and size



Rhizome elongation ranges from 1.1 to 7.4 cm/y at shallow depth. Hypothesizing the expansion of each clone from its central point, clones age range from 9000 to 1350 years.



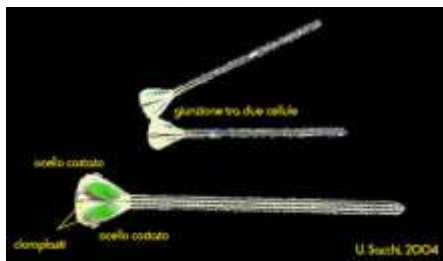
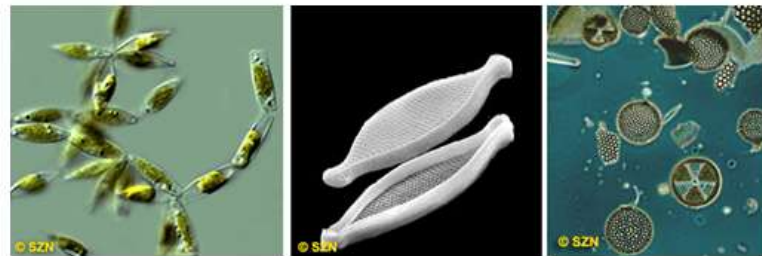
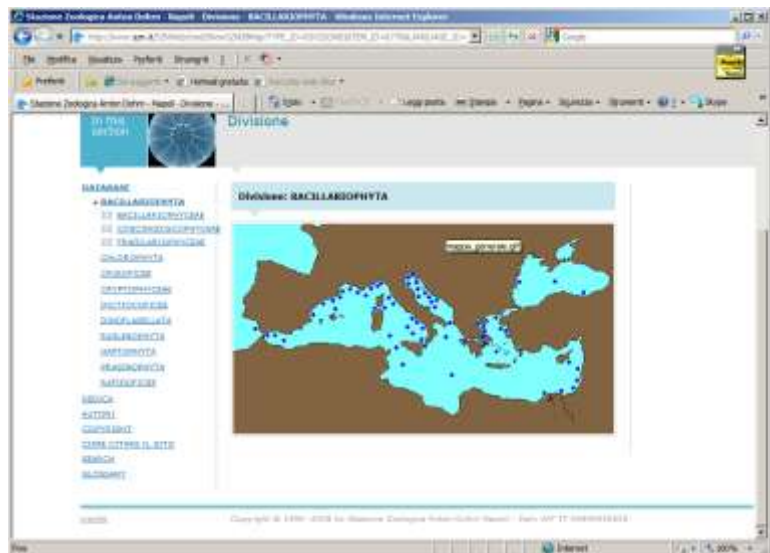




		SPECIE/GENERE	STAZIONI	NUM. SPECIE	MARCATORE	Num Individui	
Microalghe	DIATOMEE	<i>Pseudo-nitzschia multistriata</i>	1		7 microsatelliti	600	
		<i>Pseudo-nitzschia multistriata</i>	F1		7 microsatelliti	250	
		<i>Pseudo-nitzschia spp.</i>	1	20	ITS1 ITS2	60	
		<i>Pseudo-nitzschia spp.</i>	1	30	LSU	200	
		<i>Pseudo-nitzschia spp.</i>	1	0	18S	4	
		<i>Pseudo-nitzschia spp.</i>	1	20	rbcl	50	
		<i>Fragilariopsis</i>	varia	3	ITS1 ITS2	10	
		<i>Fragilariopsis</i>	varia	3	LSU	20	
		<i>Fragilariopsis</i>	varia	0	18S	10	
		<i>Fragilariopsis</i>	varia	3	rbcl	10	
		<i>Skeletonema</i>	20	12	ITS1 ITS2	5	
		<i>Skeletonema</i>	20	12	LSU	100	
		<i>Skeletonema</i>	20	12	18S	20	
		<i>Skeletonema</i>	20	12	rbcl	30	
		<i>Chaetoceros</i>	6	0	ITS1 ITS2	0	
		<i>Chaetoceros</i>	6	14	LSU	100	
		<i>Chaetoceros</i>	6	4	18S	10	
		<i>Chaetoceros</i>	6	0	rbcl	0	
			Diatoms general	varia	200	18S	300
			Pseudo-nitzschia clone libraries			LSU	>2000
	DINOFLAGELLATES						
	Gymnodiniaceae	1	6	LSU	20		
	Suessiaceae	1	2	LSU	6		
	other groups			16S rDNA	ca 50		
	Autotrophic protists	clonal libraries	37		16S rDNA	ca 3000	

		SPECIE/GENERE	STAZIONI	NUM. SPECIE	MARCATORE	Num Individui
Macroalghe	Rhodophyta	<i>Asparagopsis taxiformis</i>	15		8 microsatelliti	270
		<i>Asparagopsis taxiformis</i>	15		cox2-3 spacer	45
	Chlorophyta	<i>Caulerpa racemosa</i>	22		11 microsatelliti	350
Fanerogame		<i>Posidonia oceanica</i>	>100		6-29 microsatelliti	>4000
		<i>Cymodocea nodosa</i>	40		7 microsatelliti	>1500
		<i>Cymodocea nodosa</i>	18		ITS2	67
		<i>Cymodocea nodosa</i>	18		trnT-L	55
		<i>Cymodocea nodosa</i>	18		trnL-F	59
		<i>Cymodocea nodosa</i>	18		atpB-rbcL	41
		<i>Zostera noltii</i>	7		9 microsatelliti	>300
Invertebrati	Molluschi	<i>Octopus vulgaris</i>	15		16S rDNA	446
		<i>Octopus vulgaris</i>	15		COIII mtDNA	481
	Policheti	<i>Lysidice ninetta</i>	11		COI mtDNA	60
		<i>Lysidice collaris</i>	10		COI mtDNA	38
		<i>Lysidice ninetta</i>	11		ITS1	136
		<i>Lysidice collaris</i>	10		ITS2	72
Tunicati		<i>Ciona intestinalis</i>	19		8-12 microsatelliti	1200
		<i>Ciona intestinalis</i>	23		COX-1	255
		<i>Ciona intestinalis</i>	23		Hox-13	255
		<i>Ciona intestinalis</i>	23		Hox-5	275
		<i>Ciona intestinalis</i>	23		ITS-2	255
		<i>Ciona intestinalis</i>	15		GSX	221
Rettili marini		<i>Caretta caretta</i>	4		380 bp mtDNA	395
		<i>Caretta caretta</i>	3		815 bp mtDNA	205

## Risorse disponibili sulla biodiversità Website on Mediterranean Phytoplankton





*Hans von Marées, 1873*

GRAZIE