Diversity of marine planktonic ostracods in South China Sea: a DNA taxonomy approach

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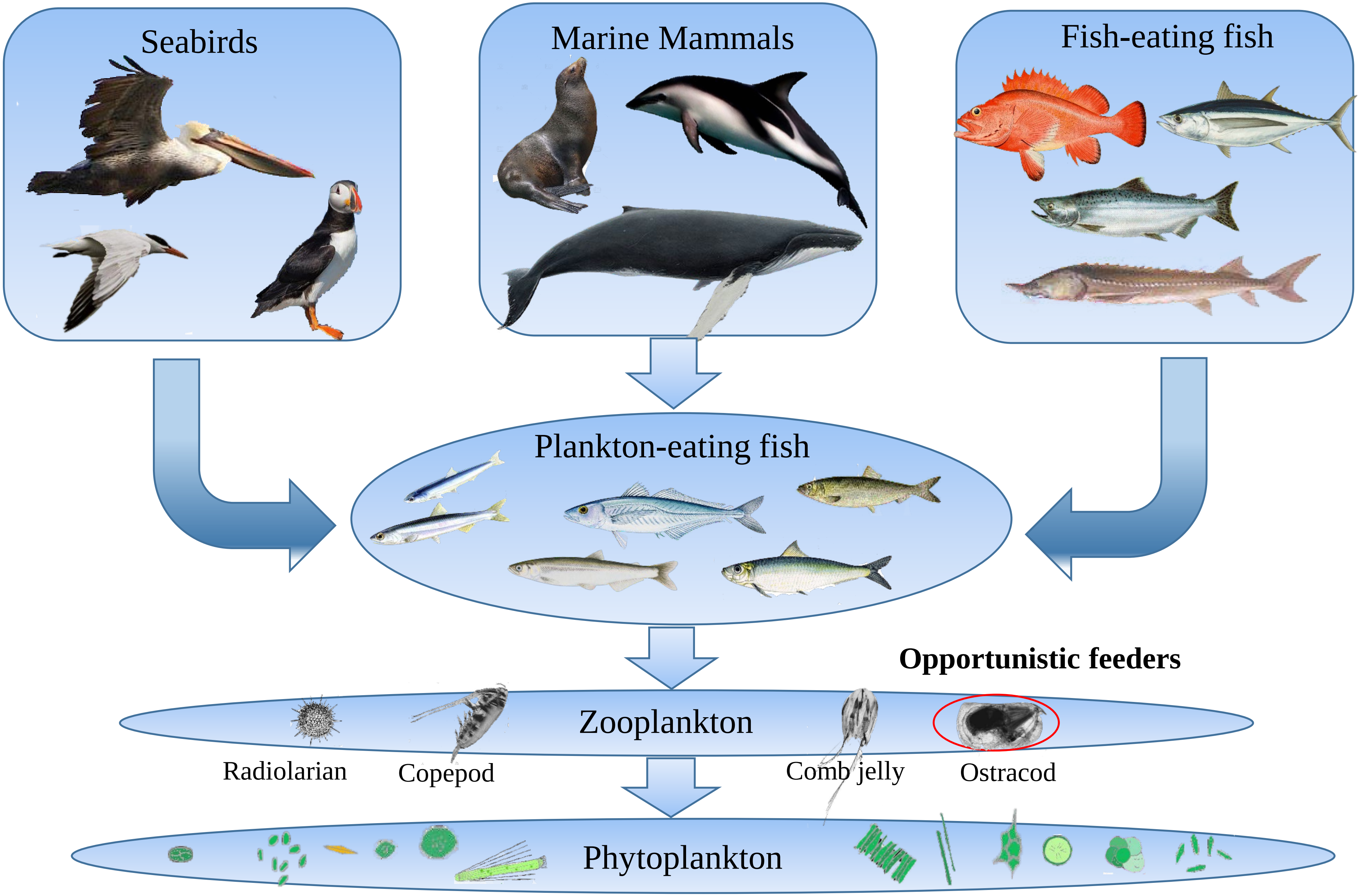
Outline

01 Introduction

02 Materials and methods

03 Results and conclusions

Introduction



Seabirds

Marine Mammals

Fish-eating fish

Plankton-eating fish

Opportunistic feeders

Zooplankton

Radiolarian

Copepod

Comb jelly

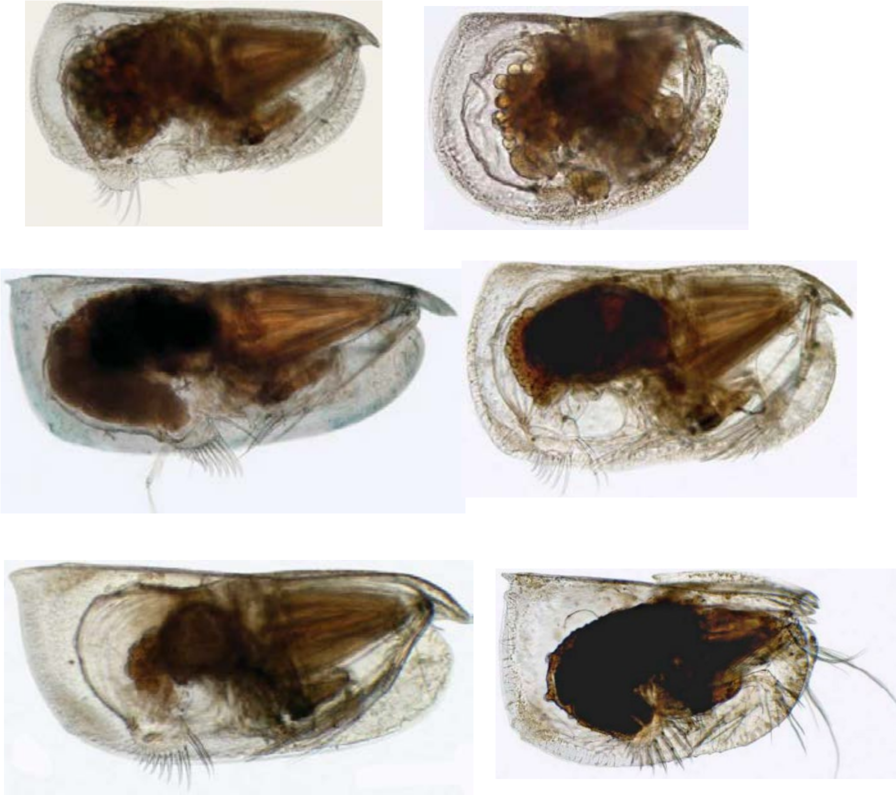
Ostracod

Phytoplankton

Introduction

Ostracods (Crustacea, Ostracoda) are small crustaceans, contributing over 200 described species to the marine zooplankton community.

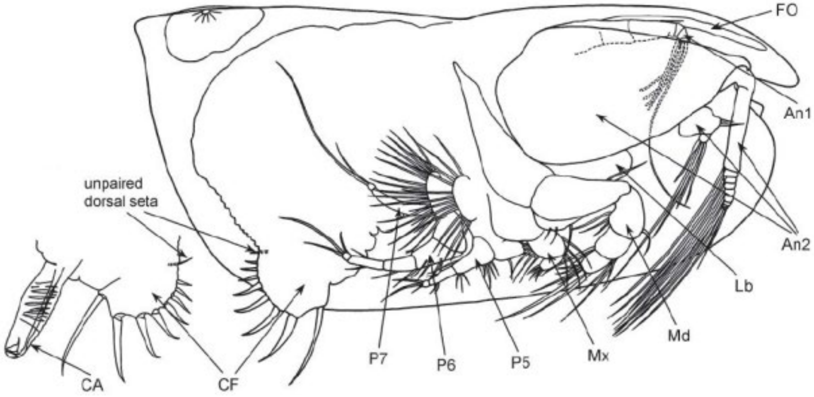
They are widely distributed and are relatively abundant components of the zooplankton.



Drapun and Smith (2012)

Introduction

Previous fragmentary taxonomic studies of ostracods in the South China Sea, were based solely on morphology.

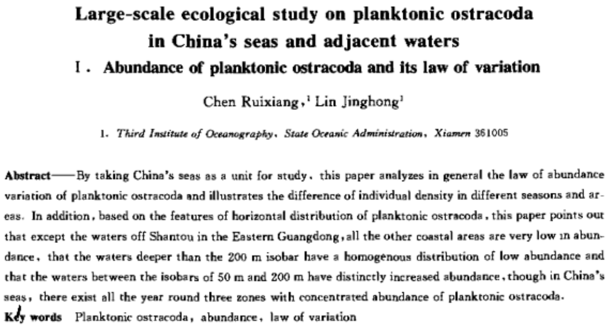



中国海洋浮游介形类

PELAGIC OSTRACODA IN CHINA SEAS

陈瑞祥 林景宏 著

海洋出版社


Large-scale ecological study on planktonic ostracoda in China's seas and adjacent waters

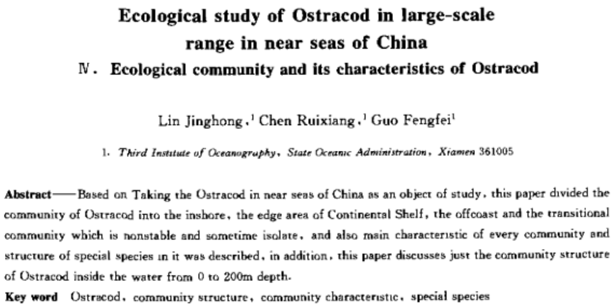
Ⅰ. Abundance of planktonic ostracoda and its law of variation

Chen Ruixiang,[1] Lin Jinghong[1]

1. *Third Institute of Oceanography, State Oceanic Administration, Xiamen* 361005

Abstract——By taking China's seas as a unit for study, this paper analyzes in general the law of abundance variation of planktonic ostracoda and illustrates the difference of individual density in different seasons and areas. In addition, based on the features of horizontal distribution of planktonic ostracoda, this paper points out that except the waters off Shantou in the Eastern Guangdong, all the other coastal areas are very low in abundance, that the waters deeper than the 200 m isobar have a homogenous distribution of low abundance and that the waters between the isobars of 50 m and 200 m have distinctly increased abundance, though in China's seas, there exist all the year round three zones with concentrated abundance of planktonic ostracoda.

Key words Planktonic ostracoda, abundance, law of variation


Ecological study of Ostracod in large-scale range in near seas of China

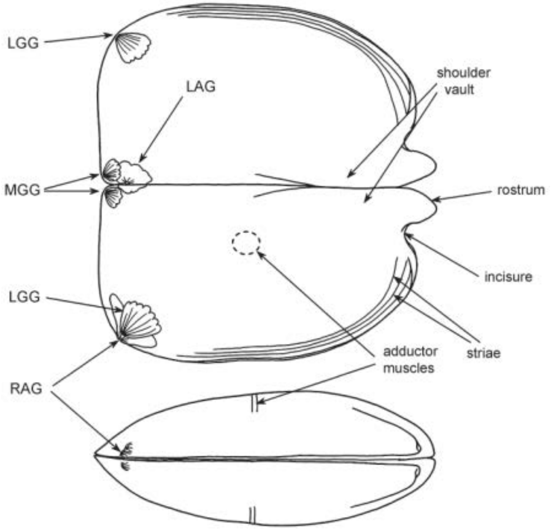
Ⅳ. Ecological community and its characteristics of Ostracod

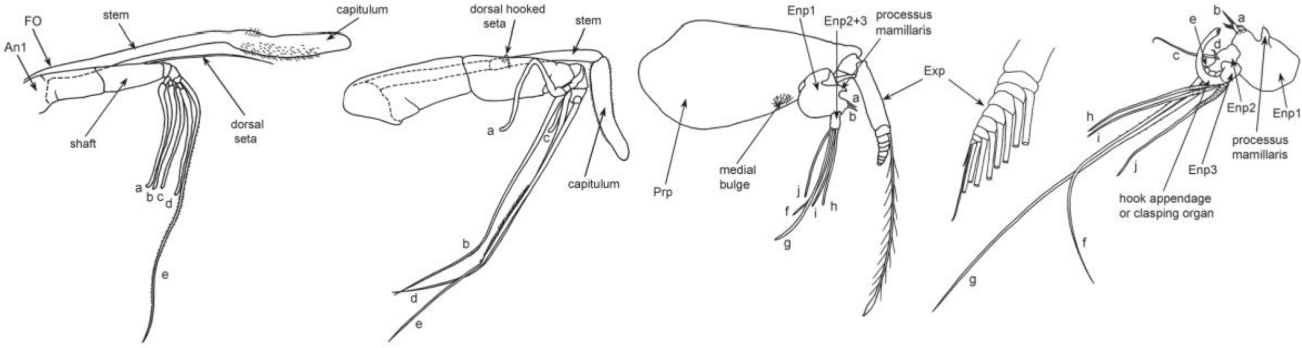
Lin Jinghong,[1] Chen Ruixiang,[1] Guo Fengfei[1]

1. *Third Institute of Oceanography, State Oceanic Administration, Xiamen* 361005

Abstract——Based on Taking the Ostracod in near seas of China as an object of study, this paper divided the community of Ostracod into the inshore, the edge area of Continental Shelf, the offcoast and the transitional community which is nonstable and sometime isolate, and also main characteristic of every community and structure of special species in it was described, in addition, this paper discusses just the community structure of Ostracod inside the water from 0 to 200m depth.

Key word Ostracod, community structure, community characteristic, special species





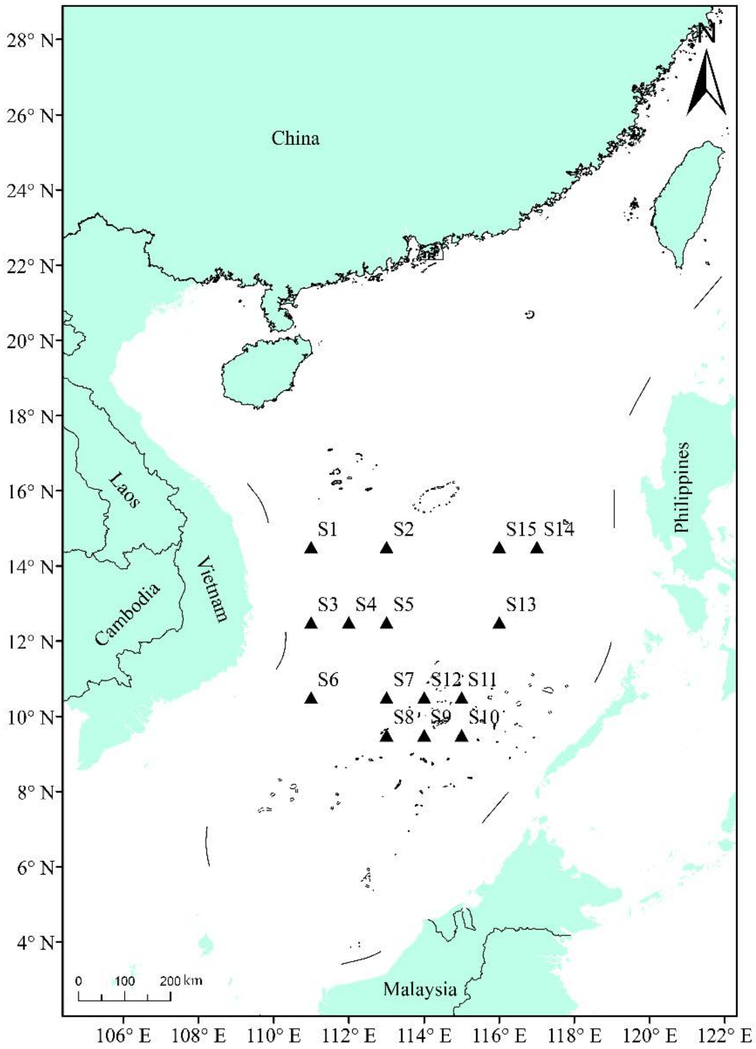
Question and targets

1. Use DNA taxonomy approach to investigate the species diversity of ostracods from South China Sea.

2. We estimated the existence and number of cryptic species in ostracods from South China Sea.

3. We assessed wether long distance dispersal impacts seascape genetic structure in common species of ostracod in South China Sea ?

Materials and methods



Fifteen sampling sites from South China Sea were investigated in 2017 from an investigation area covering a large range (>360,000 km^2).

Species identification follow the previously documented diagnostic morphological characters from South China Sea.

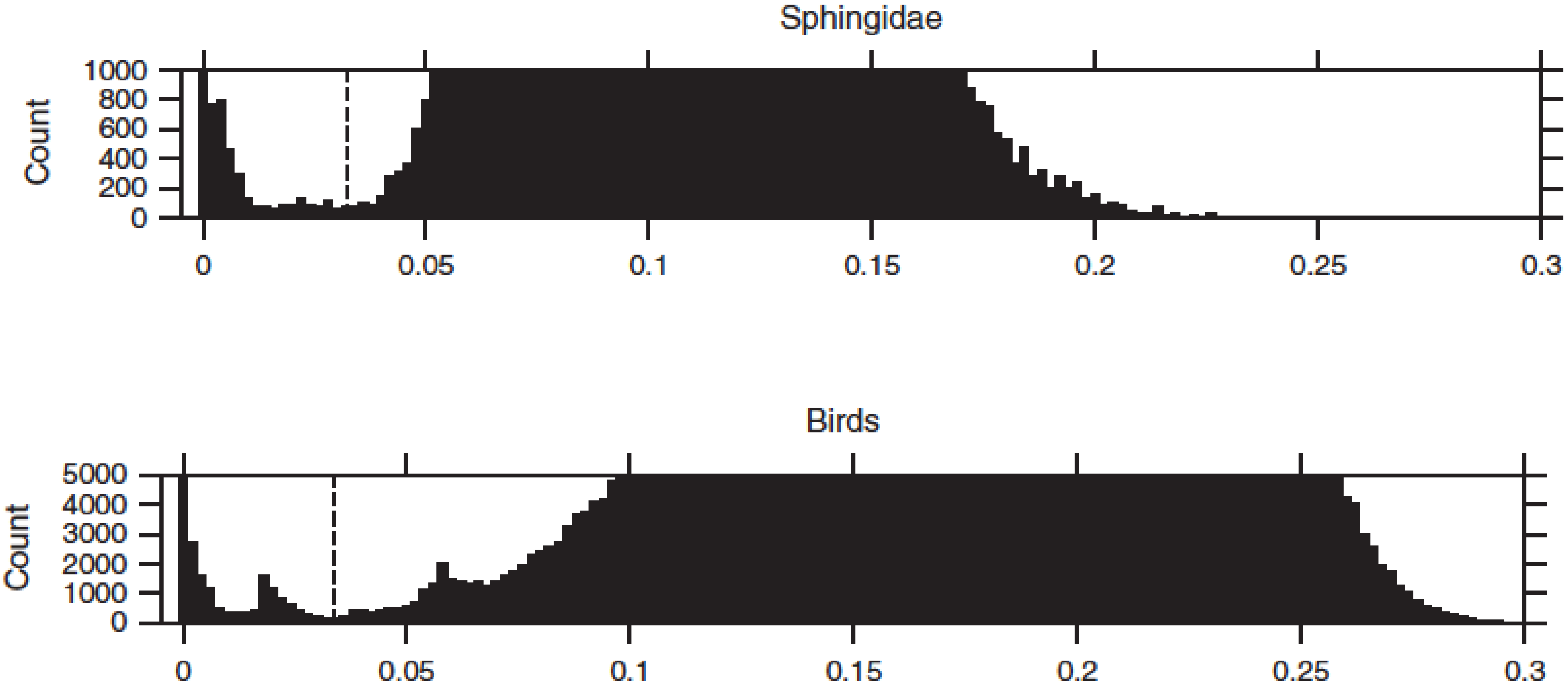
Total genomic DNA was extracted.

Mitochondrial COI gene was amplified and sequenced.

Materials and methods

DNA taxonomy approach

1) Barcoding gap, Automatic Barcode Gap Discovery (ABGD).

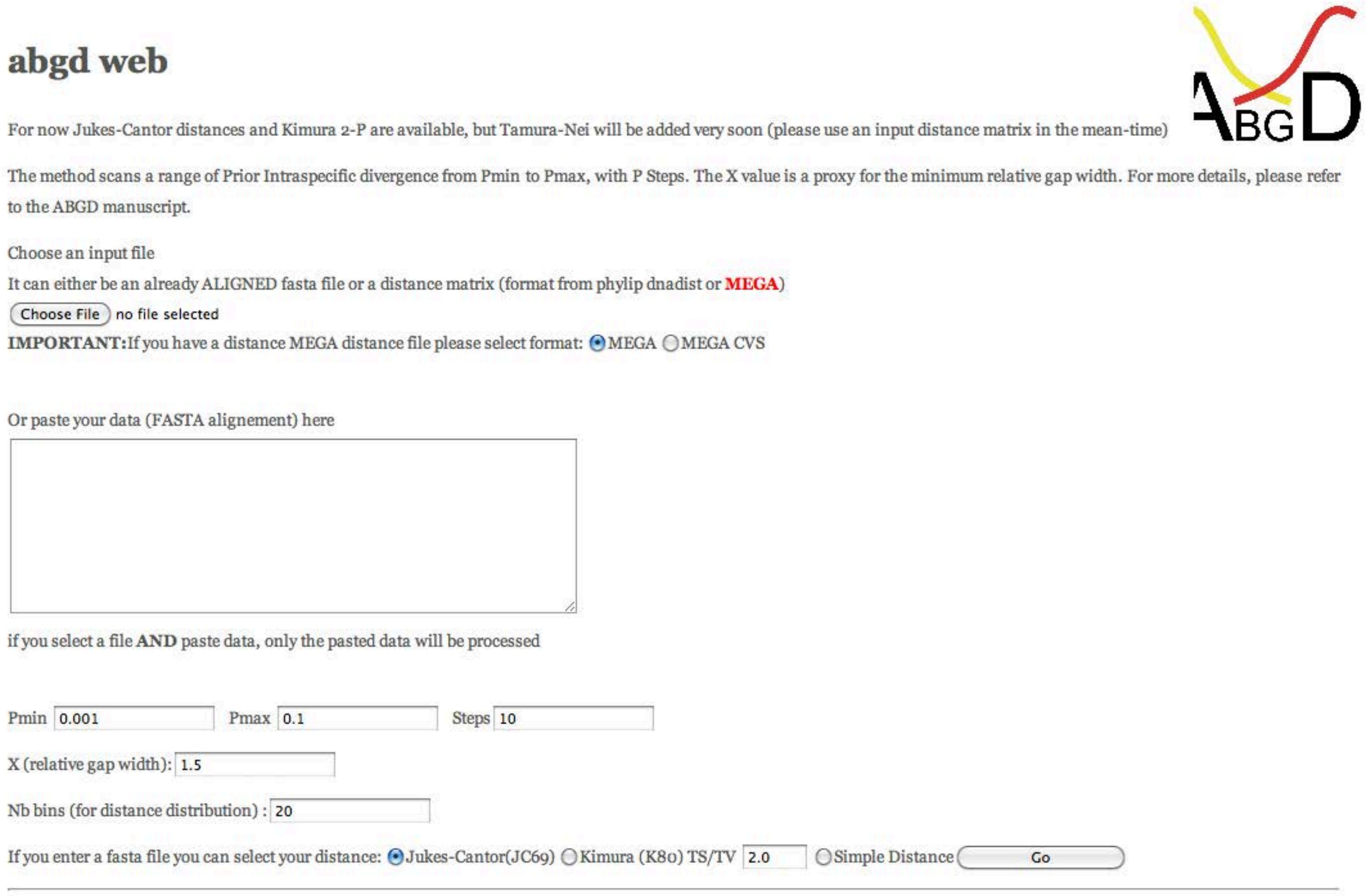


(Puillandre et al., 2012: Molecular Ecology)

Materials and methods

DNA taxonomy approach

1) Barcoding gap, Automatic Barcode Gap Discovery (ABGD).



abgd web

For now Jukes-Cantor distances and Kimura 2-P are available, but Tamura-Nei will be added very soon (please use an input distance matrix in the mean-time)

The method scans a range of Prior Intraspecific divergence from Pmin to Pmax, with P Steps. The X value is a proxy for the minimum relative gap width. For more details, please refer to the ABGD manuscript.

Choose an input file
It can either be an already ALIGNED fasta file or a distance matrix (format from phylip dnadist or **MEGA**)
Choose File no file selected
IMPORTANT: If you have a distance MEGA distance file please select format: ● MEGA ○ MEGA CVS

Or paste your data (FASTA alignement) here

if you select a file **AND** paste data, only the pasted data will be processed

Pmin 0.001 Pmax 0.1 Steps 10

X (relative gap width): 1.5

Nb bins (for distance distribution) : 20

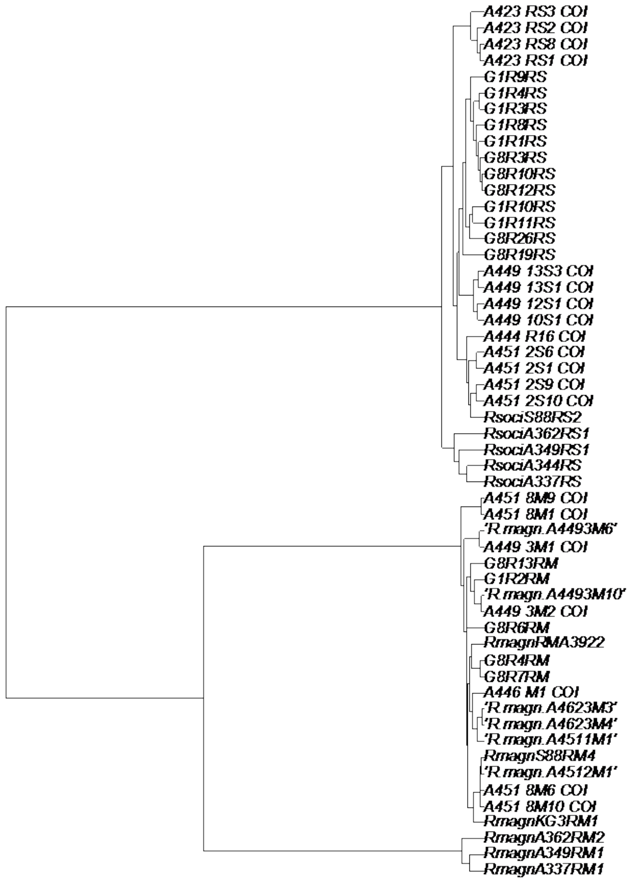
If you enter a fasta file you can select your distance: ● Jukes-Cantor(JC69) ○ Kimura (K80) TS/TV 2.0 ○ Simple Distance

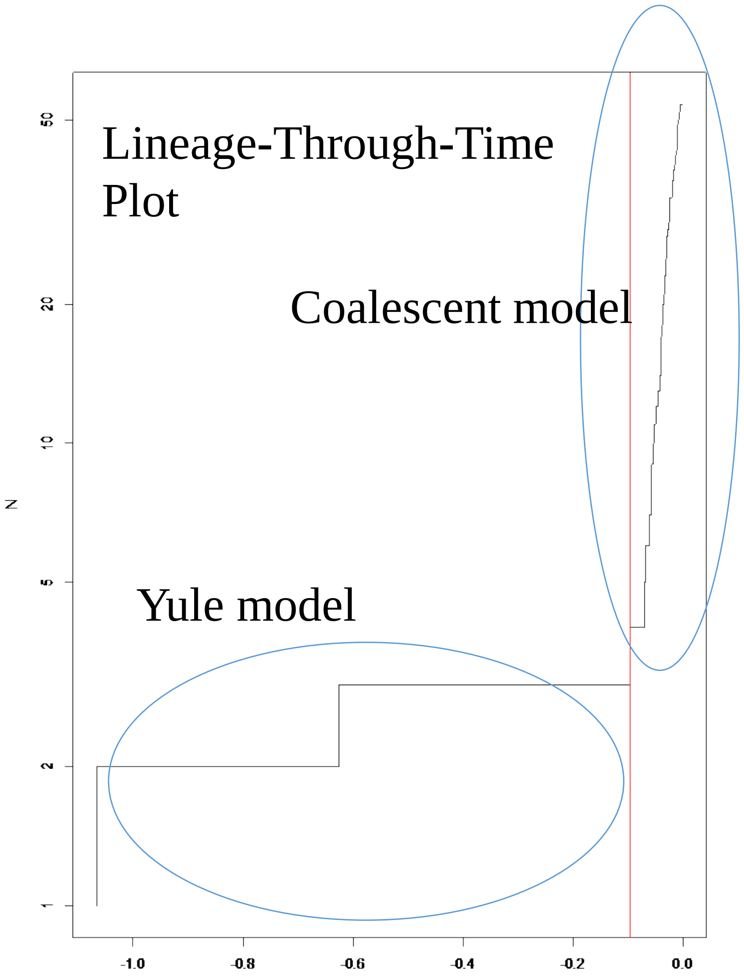
(Puillandre et al., 2012: Molecular Ecology)

Materials and methods

DNA taxonomy approach

2) Tree topology, Generalized Mixed Yule Coalescent model (GMYC).





Lineage-Through-Time Plot

Coalescent model

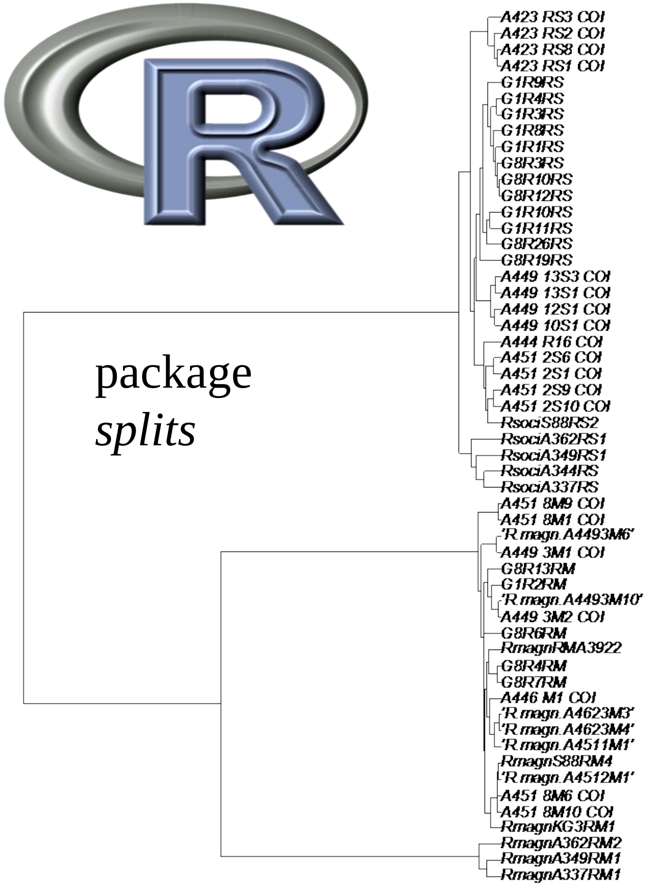
Yule model

(Fujisawa & Barraclough, 2013: Systematic Biology)

Materials and methods

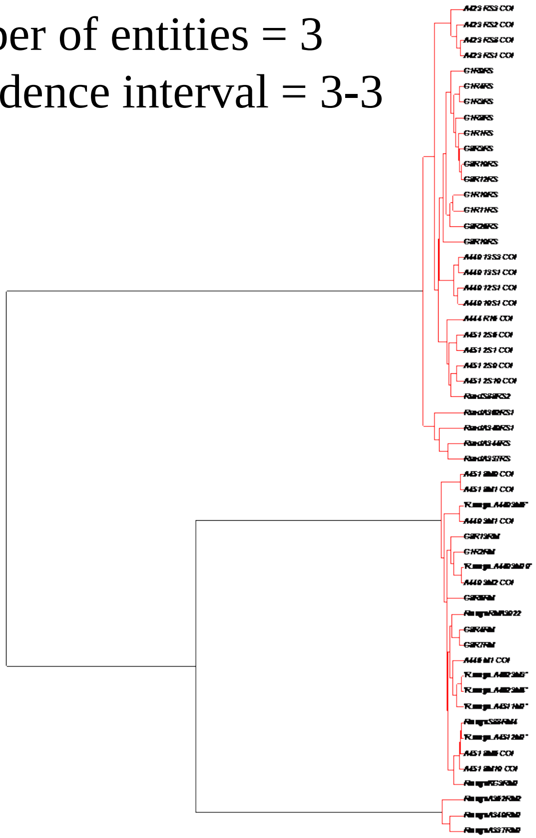
DNA taxonomy approach

2) Tree topology, Generalized Mixed Yule Coalescent model (GMYC).



package
splits

Number of entities = 3
Confidence interval = 3-3



(Fujisawa & Barraclough, 2013: Systematic Biology)

Materials and methods

Data analysis:
—haplotype, Φ_{ST}, Mantel test

- Network analysis is performed to estimate gene genealogies using **HAPLOVIEWER** , which turns trees built from traditional phylogenetic methods into haplotype genealogies (Salzburger et al. 2011).

- We calculated genetic distance (Φ_{ST}) determined at COI gene, using an infinite allele model in **Arlequin version 3.5** (Excoffier & Lischer, 2010) between fifteen populations.

- We made scatterplots of pairwise Φ_{ST} values and geographic distances. Mantel test correlations were calculated in the **vegan package** version 2.2-1.

Materials and methods

Data analysis:
—PcoA, MEM and partial RDA in R

- We examine genetic patterns caused by spatial structure by redundancy analysis (**RDA**) with distance-based Moran eigenvector maps (**MEM**) as independent variables and the nuclear genetic data as dependent variables.

- Genetic distance (Φ_{ST}) determined at COI gene is calculated in **Arlequin version 3.5** between all populations, and this distance matrix was used to calculate principal coordinates (**PCo**) of the genetic data (Oksanen et al. 2010).

- We partition the genetic variance into purely environmental (E) and spatial (S) components **with RDA** after Peres-Neto and Legendre (2010).

Results

Table 1 List of populations examined with, coordinates and Morphological type identified.

Sampling site	Latitude (N)	Longitude (E)	Morphological type
S1	14.5	111	*Halocypris brevirostris*; *Paraconchoecia echinata*; *Proceroecia* sp.; *Porroecia spinirostris*
S2	14.5	113	*Paraconchoecia oblonga*; *Conchoecia magna*; *Metaconchoecia subinflata*; *Paraconchoecia echinata*; *Orthoconchoecia secernenda*; *Conchoecetta acuminata*; *Proceroecia* sp.; *Porroecia spinirostris*
S3	12.5	111	*Paraconchoecia oblonga; Porroecia spinirostris*
S4	12.5	112	*Porroecia spinirostris*
S5	12.5	113	*Paraconchoecia oblonga*; *Paraconchoecia echinate; Porroecia spinirostris*
S6	10.5	111	*Halocypris brevirostris*; *Discoconchoecia elegans*; *Orthoconchoecia secernenda*
S7	10.5	113	*Proceroecia* sp.; *Porroecia spinirostris*
S8	9.5	113	*Conchoecia magna*; *Discoconchoecia* sp.; *Conchoecetta giesbrechti; Porroecia spinirostris*
S9	9.5	114	*Metaconchoecia subinflata*; *Orthoconchoecia secernenda*; *Proceroecia procera*; *Porroecia spinirostris*
S10	9.5	115	*Paraconchoecia oblonga*; *Metaconchoecia subinflata*; *Proceroecia procera; Porroecia spinirostris*
S11	10.5	115	*Metaconchoecia subinflata*; *Metaconchoecia inflata*; *Paraconchoecia echinata*; *Orthoconchoecia secernenda*; *Conchoecetta acuminate; Porroecia spinirostris*
S12	10.5	114	*Paraconchoecia oblonga*; *Conchoecia magna*; *Porroecia* sp.; *Porroecia spinirostris*
S13	12.5	116	*Orthoconchoecia secernenda*; *Porroecia spinirostris*
S14	14.5	117	*Euconchoecia* cf. *chierchiae; Porroecia spinirostris*
S15	14.5	116	*Paraconchoecia echinata*; *Discoconchoecia* sp.; *Porroecia spinirostris*; *Conchoecetta giesbrechti*

Our survey of sampling sites across South Chine Sea identified sixteen morphological species of the Ostracoda.

The most common morphospecies was *Porroecia spinirostris*, found in all sampling sites.

Results

Genetic diversity, assessed by Kimuras two-parameter distance (median, in %) within/between the ten genus of Ostracoda with uniform rates

	1	2	3	4	5	6	7	8	9	10
1	8.78±0.86									
2	22.37±1.81	1.99±0.41								
3	21.66±1.68	21.80±1.79	8.82±1.01							
4	35.75±2.63	32.04±2.64	29.14±2.27	-						
5	23.22±1.87	25.99±2.14	21.54±1.70	29.41±2.38	4.91±0.87					
6	23.21±1.83	22.61±1.89	22.21±1.80	31.15±2.38	22.29±1.83	4.58±0.47				
7	21.27±1.79	22.70±1.94	20.95±1.83	30.27±2.50	22.52±1.92	23.53±2.07	0.52±0.020			
8	22.79±1.61	21.05±1.56	20.73±1.48	30.11±2.18	23.66±1.71	21.64±1.64	20.89±1.57	14.09±1.15		
9	27.99±1.81	27.49±1.83	29.13±2.00	32.60±2.31	30.38±2.06	30.31±2.00	25.83±1.76	28.33±1.72	18.44±1.45	
10	27.99±2.08	24.69±1.94	25.05±1.95	34.32±2.61	26.23±1.96	26.00±1.99	24.94±2.02	25.75±1.87	29.88±1.94	8.78±0.86

1 *Conchoecetta* 2 *Conchoecia* 3 *Discoconchoecia* 4 *Euconchoecia* 5 *Halocypris* 6 *Metaconchoecia* 7 *Orthoconchoecia* 8 *Paraconchoecia* 9 *Porroecia* 10 *Proceroecia*

The uncorrected K2P pairwise distances among genus varied between 20.73% and 35.75% , average pairwise distances is 25.92%.

Results

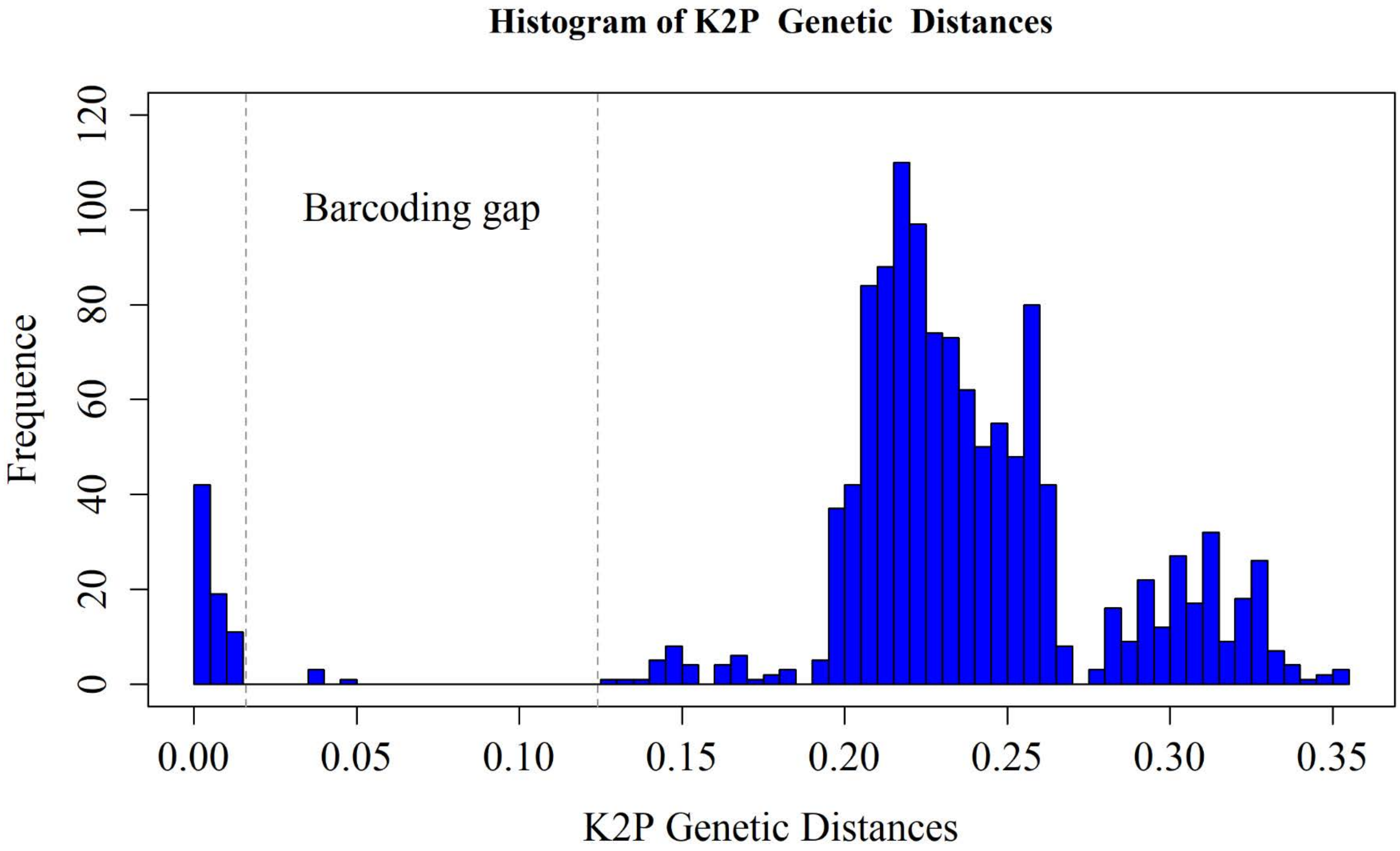
Genetic diversity, assessed by Kimuras two-parameter distance (median, in %) within/between the sixteen morphological species of the Ostracoda with uniform rates

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0.62±0.24															
2	14.15±1.4	1.08±0.38														
3	22.90±2.0	21.57±1.9	1.99±0.39													
4	22.23±1.9	20.38±1.7	21.41±1.9	0.62±0.30												
5	22.01±1.9	22.00±1.9	22.58±1.9	12.93±1.4	-											
6	35.82±2.8	35.65±2.7	32.04±2.5	29.05±2.4	29.33±2.4	-										
7	23.14±2.0	23.35±2.0	25.99±2.0	21.21±1.8	22.20±1.8	29.41±2.3	4.91±0.84									
8	22.10±1.9	22.72±2.0	21.51±2.0	21.90±2.1	23.53±2.1	31.58±2.5	21.99±1.9	-								
9	22.48±1.9	24.62±2.1	22.76±1.9	22.22±2.0	22.08±1.9	31.09±2.5	22.34±1.8	17.60±1.6	0.23±0.12							
10	21.43±1.8	21.02±1.8	22.70±1.9	21.35±1.9	20.15±1.7	30.27±2.4	22.52±1.9	23.15±2.0	23.59±2.0	0.52±0.19						
11	25.08±2.1	21.69±1.8	21.51±1.8	23.01±1.9	17.74±1.6	32.05±2.6	23.15±2.0	22.83±2.0	20.92±2.0	21.44±1.8	0.52±0.18					
12	21.71±1.8	22.49±1.6	20.66±1.6	19.60±1.6	21.70±1.7	28.50±2.2	24.08±1.8	22.64±1.8	21.96±1.8	20.44±1.6	21.50±1.7	8.29±0.89				
13	30.56±2.4	31.73±2.3	29.75±2.2	33.42±2.6	33.11±2.6	31.31±2.3	33.17±2.4	27.24±2.2	33.61±2.7	29.52±2.1	31.63±2.3	31.04±2.2	0			
14	22.57±1.9	24.72±2.0	24.10±2.0	22.73±2.0	23.08±2.0	34.54±2.7	26.21±2.1	26.15±2.2	26.62±2.1	20.29±1.8	24.63±2.1	23.23±1.8	30.55±2.3	1.08±0.42		
15	29.30±2.1	25.68±2.2	24.06±2.0	24.48±2.1	24.01±2.1	33.81±2.6	26.17±2.2	24.94±2.0	25.14±2.0	24.03±2.1	25.81±2.1	24.83±2.0	31.93±2.3	24.83±2.0	0.93±0.35	
16	0.293±2.3	29.30±2.3	25.01±2.1	25.95±2.2	24.34±2.1	34.57±2.7	26.25±2.1	26.07±2.1	26.50±2.2	25.39±2.1	26.48±2.2	25.59±2.0	33.05±2.4	26.13±2.1	16.01±1.5	0.46±0.18

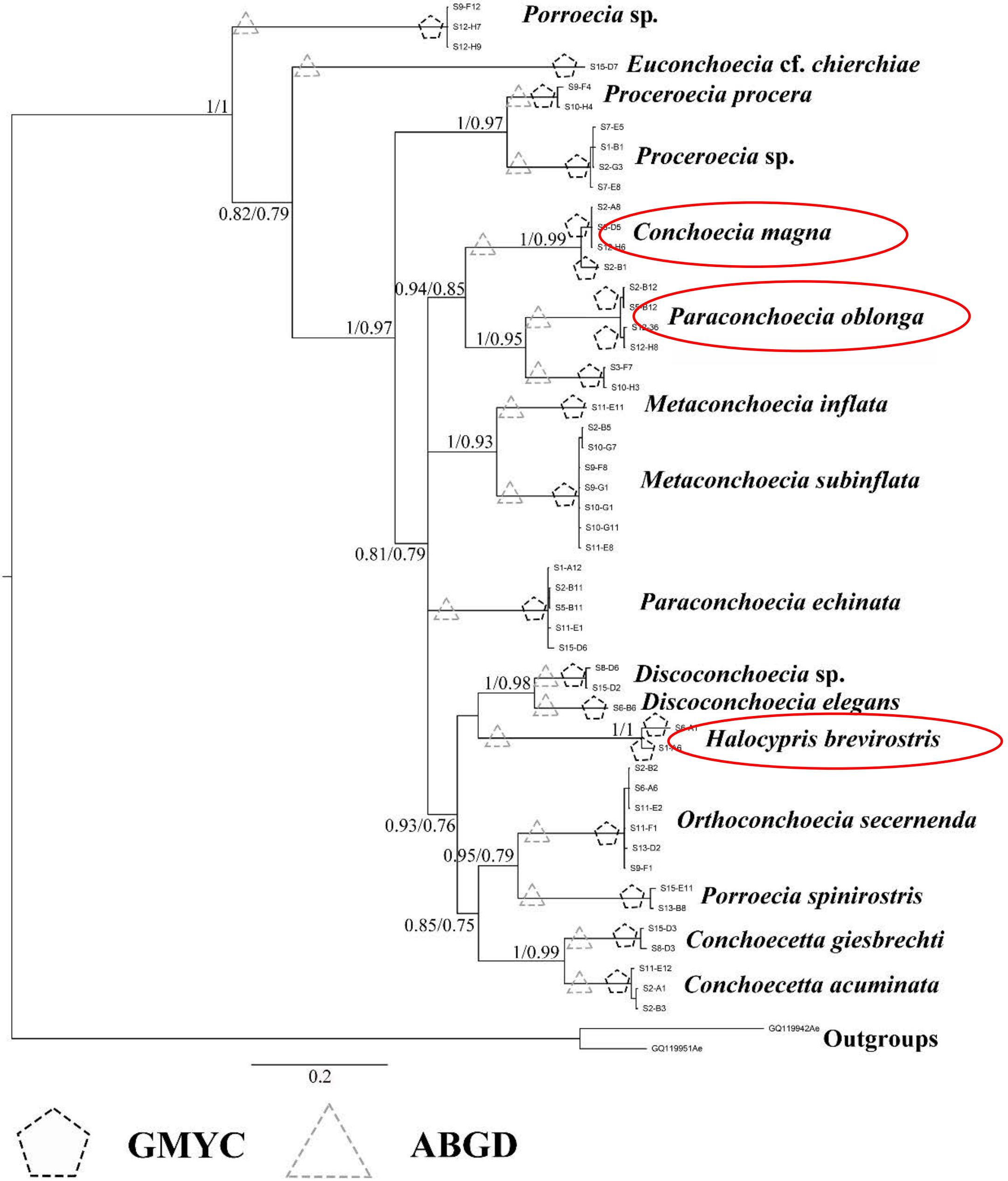
1 *Conchoecetta acuminata* 2 *Conchoecetta giesbrechti* 3 *Conchoecia magna* 4 *Discoconchoecia* sp. 5 *Discoconchoecia elegans* 6 *Euconchoecia* cf. *chierchiae* 7 *Halocypris brevirostris* 8 *Metaconchoecia inflata* 9 *Metaconchoecia subinflata* 10 *Orthoconchoecia secernenda* 11 *Paraconchoecia echinata* 12 *Paraconchoecia oblonga* 13 *Porroecia* sp. 14 *Porroecia spinirostris* 15 *Proceroecia procera* 16 *Proceroecia* sp.

The uncorrected K2P pairwise distances among species varied between 12.93% and 35.82% and average pairwise distances is 25.14%. The uncorrected K2P pairwise distances within species varied between 0 and 8.29%.

Results


Histogram of K2P Genetic Distances
Barcoding gap
Frequence
0
20
40
60
80
100
120
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
K2P Genetic Distances

Results

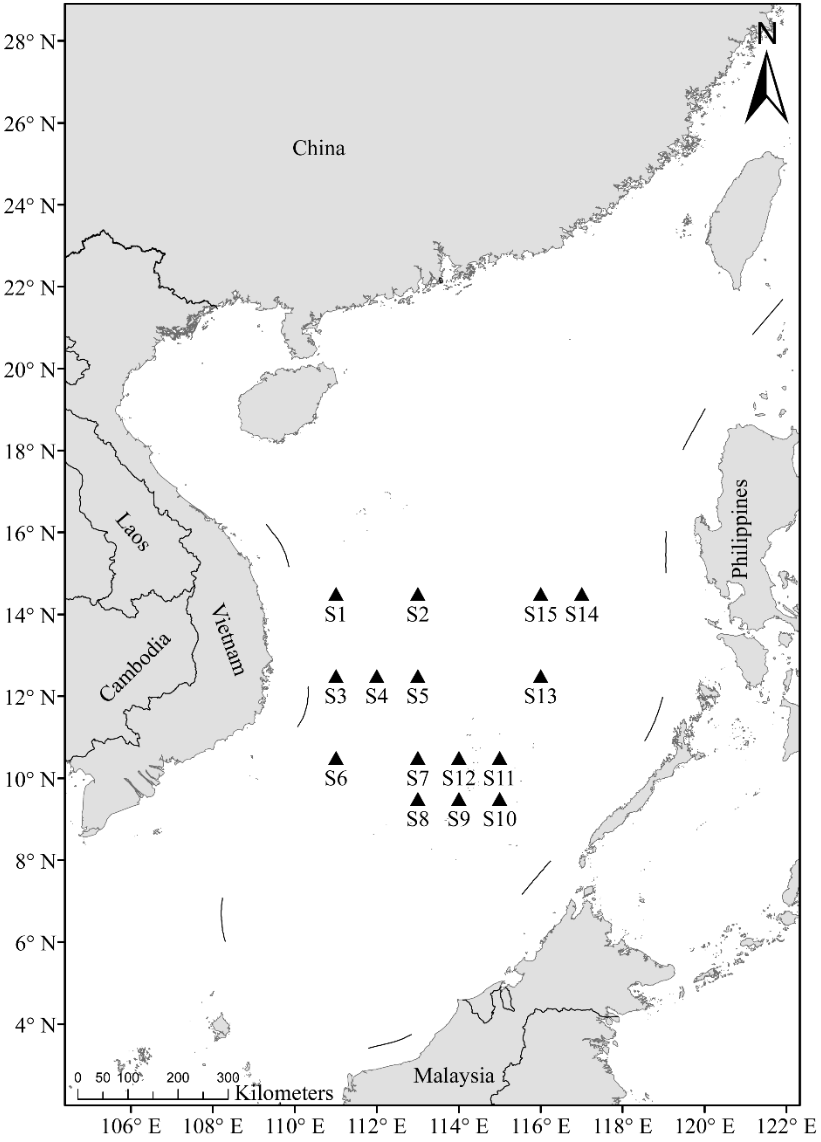


The Bayesian inference COI phylogenetic tree for Ostracoda in South China Sea.

DNA taxonomy from ABGD and GMYC are showed on the branches.

Clade *Conchoecia magna*, *Paraconchoecia oblonga* and *Halocypris brevirostris* contains two distinct genetic clusters.

Results



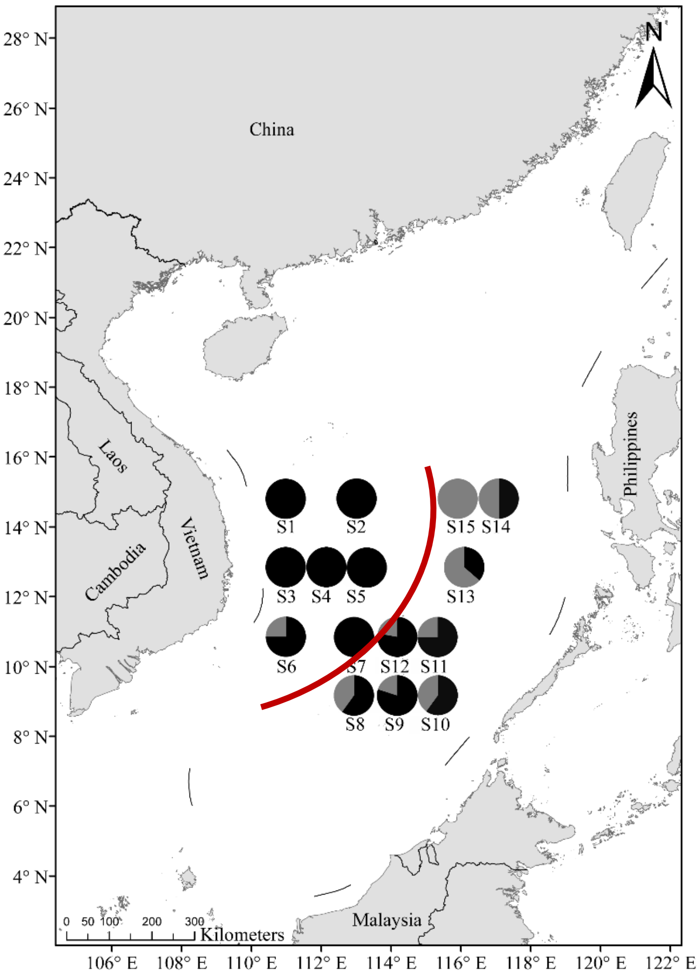
Porroecia spinirostris is common species of ostracods in South China Sea.

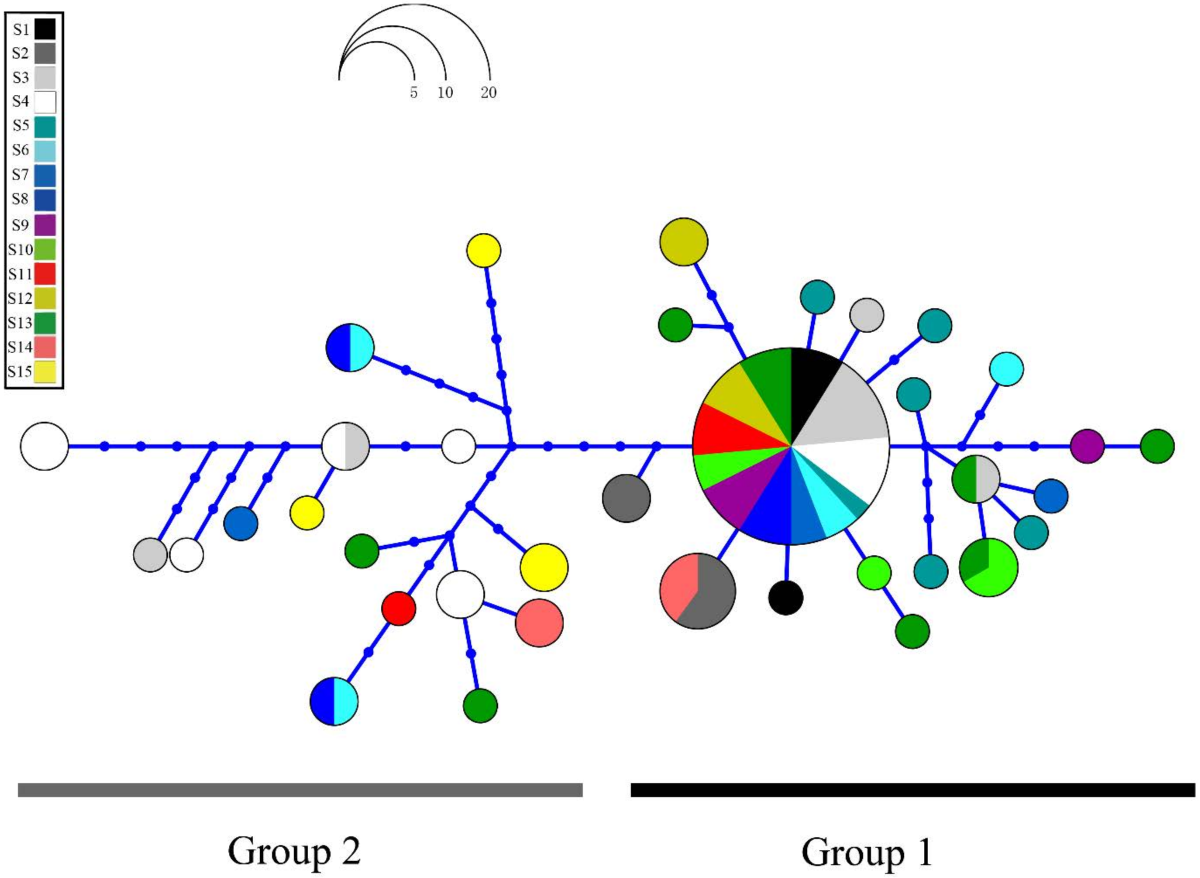
Results

Overview of the sampled sites, location, mean temperature, Chl a, salinity, wind speed and sample sizes for COI haplotype analysis of 15 *Porroecia spinirostris* populations across South China Sea.

Code	Longitude (E)	Latitude (N)	Chl a (mg/m^3)	Mean temperature (°C)	Sanility	Wind speed (m/s)	Nm	nH	Haplotype
S1	111	14.5	0.142	28.29	33.32	4.39	4	2.	H1, H2.
S2	113	14.5	0.11	28.66	33.41	0.93	5	2.	H3, H4.
S3	111	12.5	0.141	28.98	34.11	2.39	9	5	H1, H5, H6, H7, H8.
S4	112	12.5	0.132	28.85	33.25	0.93	11	6	H1, H7, H9, H10, H11, H12
S5	113	12.5	0.0976	28.98	33.45	0.72	6	6	H1, H13, H14, H15, H16, H17
S6	111	10.5	0.127	29.11	33.84	1	5	4.	H1, H18, H19, H20.
S7	113	10.5	0.0989	29.27	33.25	2.65	4	3.	H1, H21, H22
S8	113	9.5	0.118	29.3	33.53	2.25	5	3.	H1, H19, H20
S9	114	9.5	0.103	29.39	33.06	3.12	4	2.	H1, H24
S10	115	9.5	0.138	29.42	33.32	3.11	5	3	H1, H24, H25
S11	115	10.5	0.0977	29.38	33.36	2.33	4	2	H1, H26
S12	114	10.5	0.101	29.35	33.25	2.72	5	2.	H1, H27
S13	116	12.5	0.107	29.26	33.44	3.07	10	8	H1, H6, H25, H28, H29, H30, H31,H32
S14	117	14.5	0.128	28.95	33.64	5.51	4	2	H3, H33
S15	116	14.5	0.119	28.91	33.56	5.52	4	3	H34, H35, H36

Results

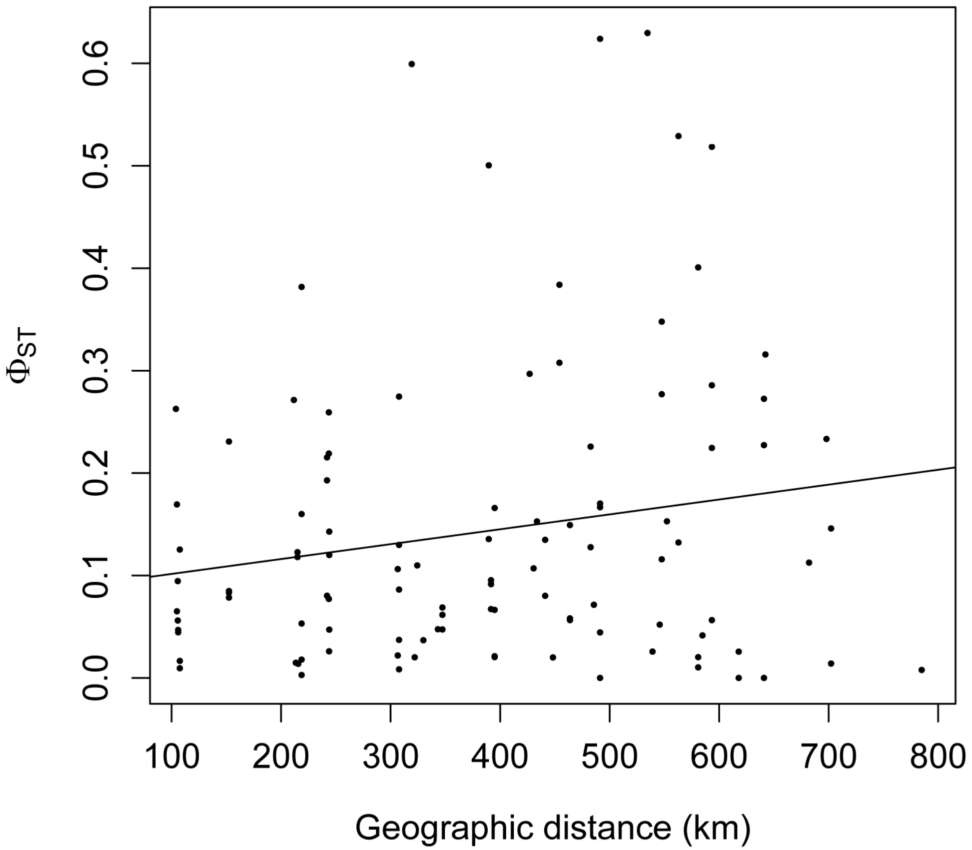




The dominant haplotype was found in twelve sampling sites.

Porroecia spinirostris shows mild but consistent differentiation in COI sequences grouping in eastern and western groups of South China Sea.

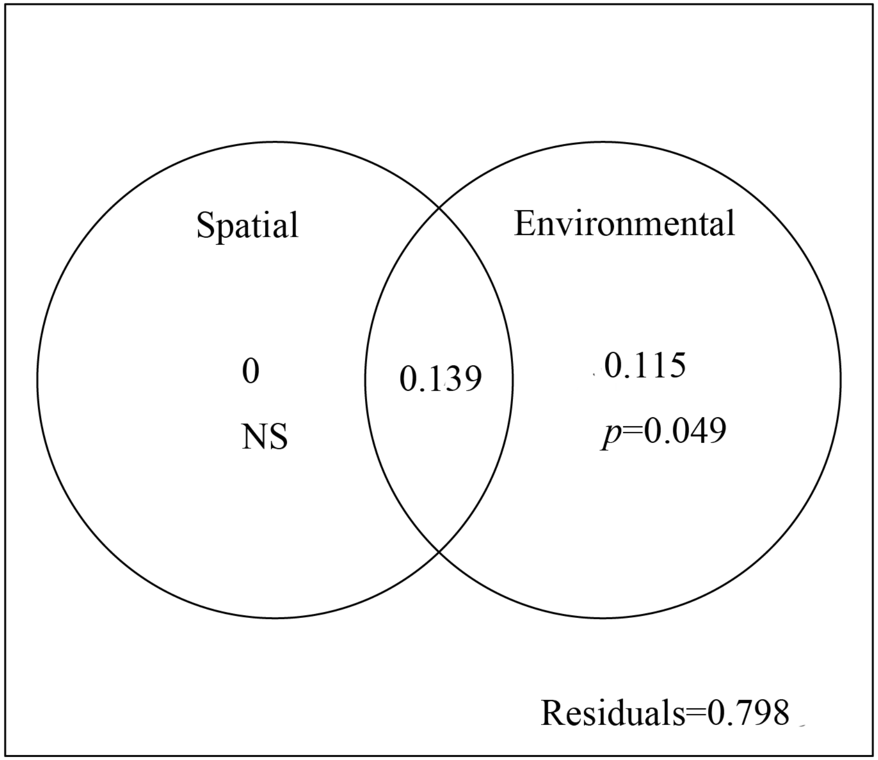
Results



Plotting pairwise genetic distance against geographical distances among sites resulted in no significant positive correlations by Mantel tests (Mantel statistic $r = 0.1165$; $p = 0.167$).

Results

RDA model	R^2	R^2_{adj}	P
S			
Global model	0.496	0.216	NS
E			
Global model	0.492	0.288	0.05
FS	0.472	0.328	0.03
Wind speed	0.170		0.05
Salinity	0.244		0.014
S+E	0.544	0.201	0.03
S|E	0.076	0	NS
E|S	0.327	0.115	0.049
Shard		0.139	
Unexplained		0.798	



Spatial and environmental variables explained 20.1% of the genetic structure.

Pure environmental variation (E|S) still explain 11.5%.

The spatially structured environmental variance (shared) of 13.9% and 79.8%, was unexplained.

Conclusions

1. COI barcode region was shown to be a valuable character for identification and discovery of cryptic species of marine planktonic ostracods. Three potential cryptic species were found.

2. The COI sequence variation between species were consistent with other groups of crustaceans. A large range, from 12.9% to 35.8%.

3. The genetic structure of *Porroecia spinirostris* reflects both the importance of long distance dispersal as well as of reduced levels of gene flow, likely caused by colonization events.

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