
uni Research
Climate
Health
Energy
Society
Environment

Uni Research AS

- Owned by the University of Bergen
- 50-55 M€ turnover
- 460 employees; 75% of scientific staff with PhD
- Aquaculture
- Oil & Gas
  - Reservoir
  - Impact research and monitoring
  - CO2 Storage

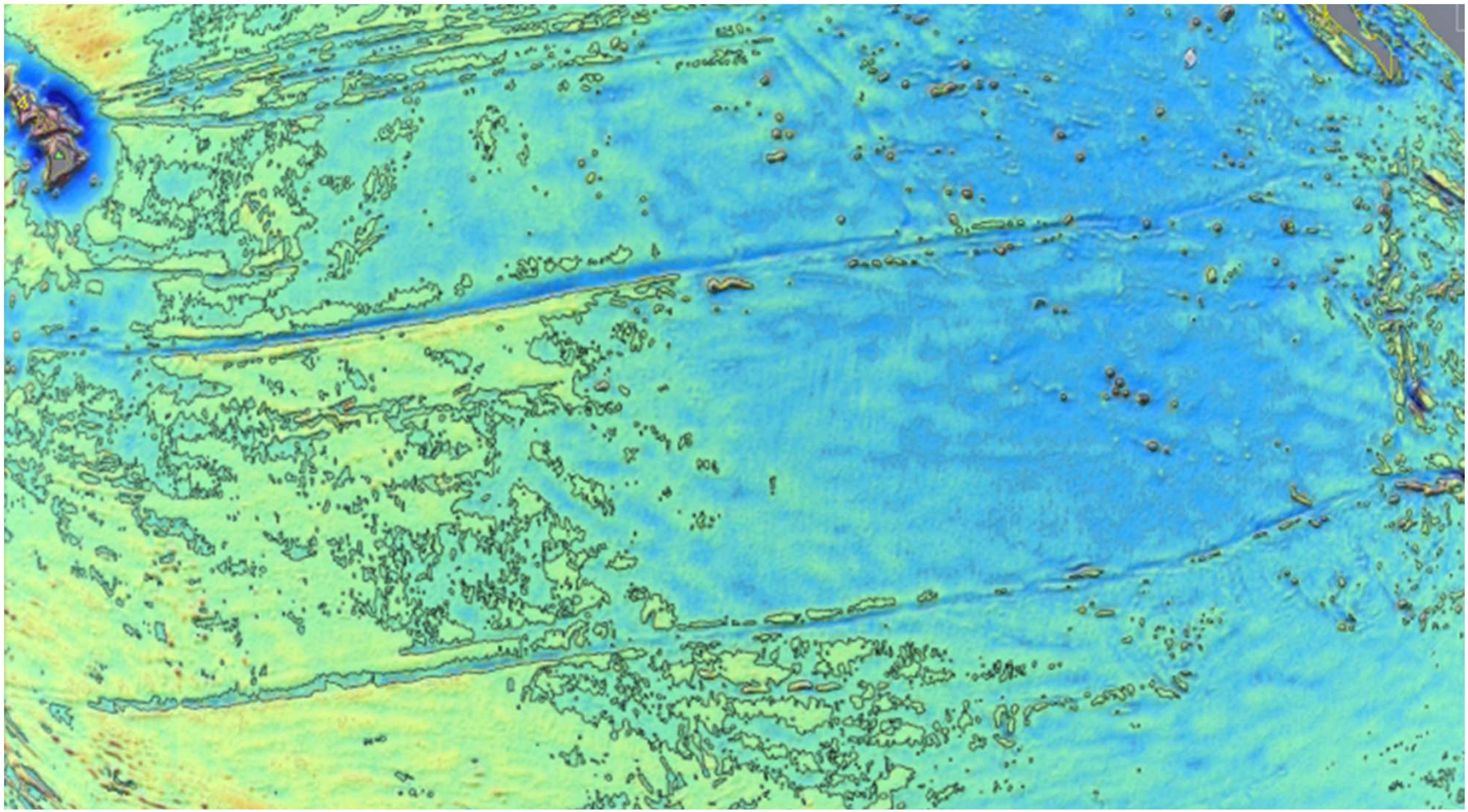
Advances in molecular methodologies and the application and interpretation of molecular methodologies for macrofauna classification

Their relevance to environment assessment and monitoring

Outline

- The deep sea taxonomic knowledge gap
- Deep sea taxonomy and challenges
- Examples of molecular methodologies and connectivity
- Biodiversity monitoring and applications to molecular taxonomy

Clarion Clipperton Fracture Zone (CCZ)

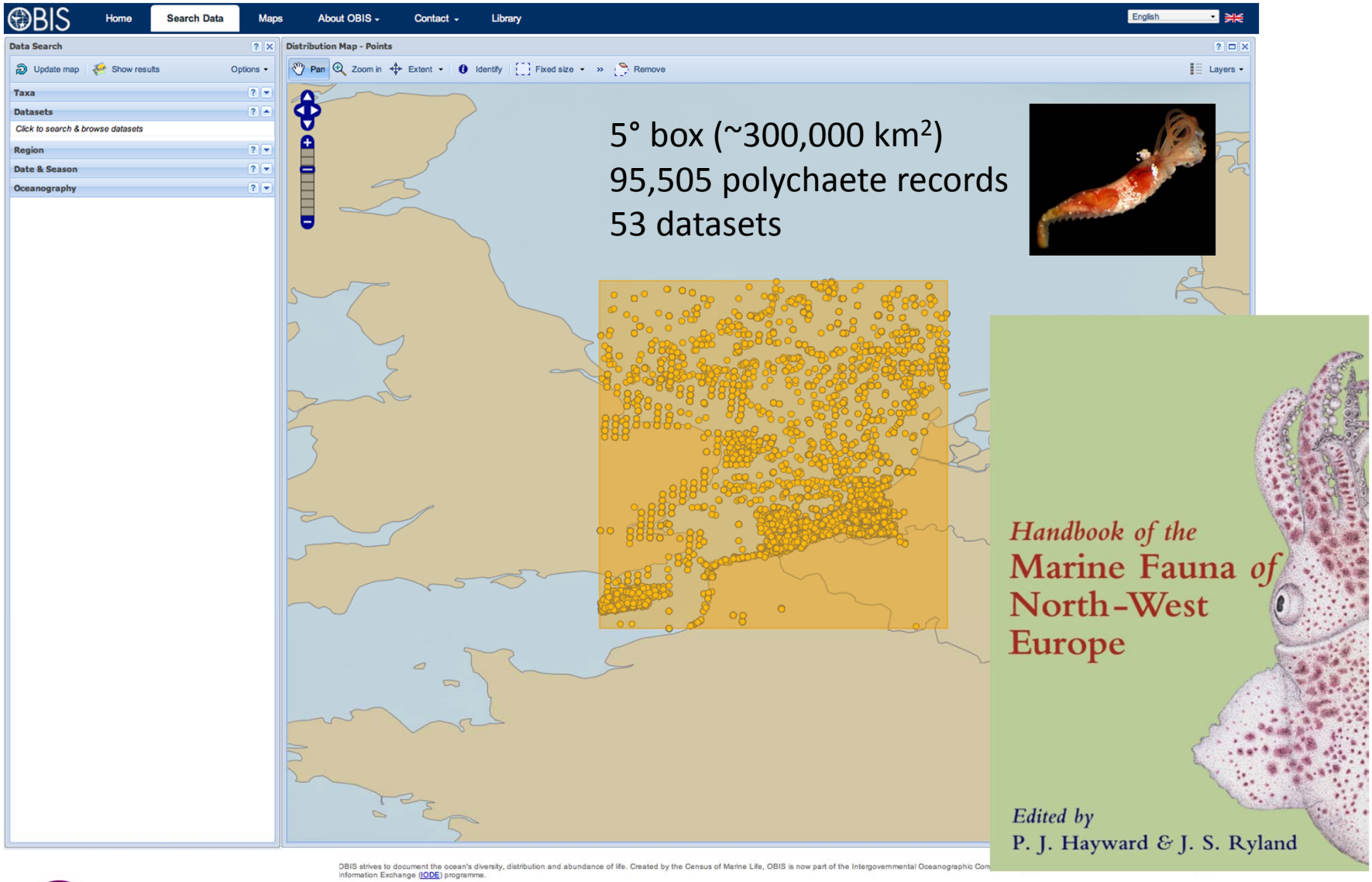


Baseline study objectives

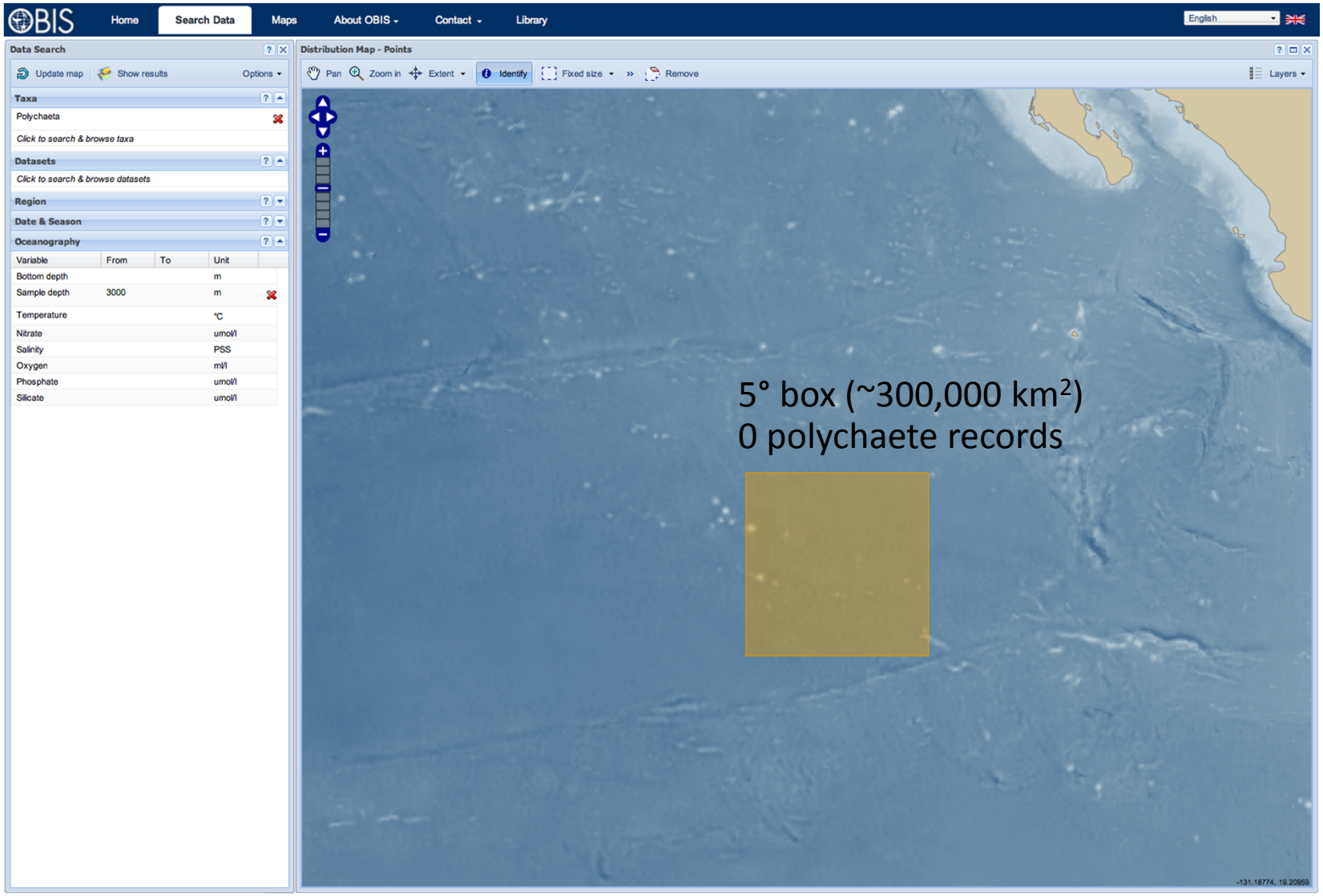
- What is the degree of **population connectivity** of animals across the Clarion-Clipperton Zone (CCZ) at scales of 100-1000 km?
- What is the degree of variation in **biodiversity** levels across the CCZ?
- What is the degree of variation in **community structure** across the CCZ?

Our main parameter is taxonomy

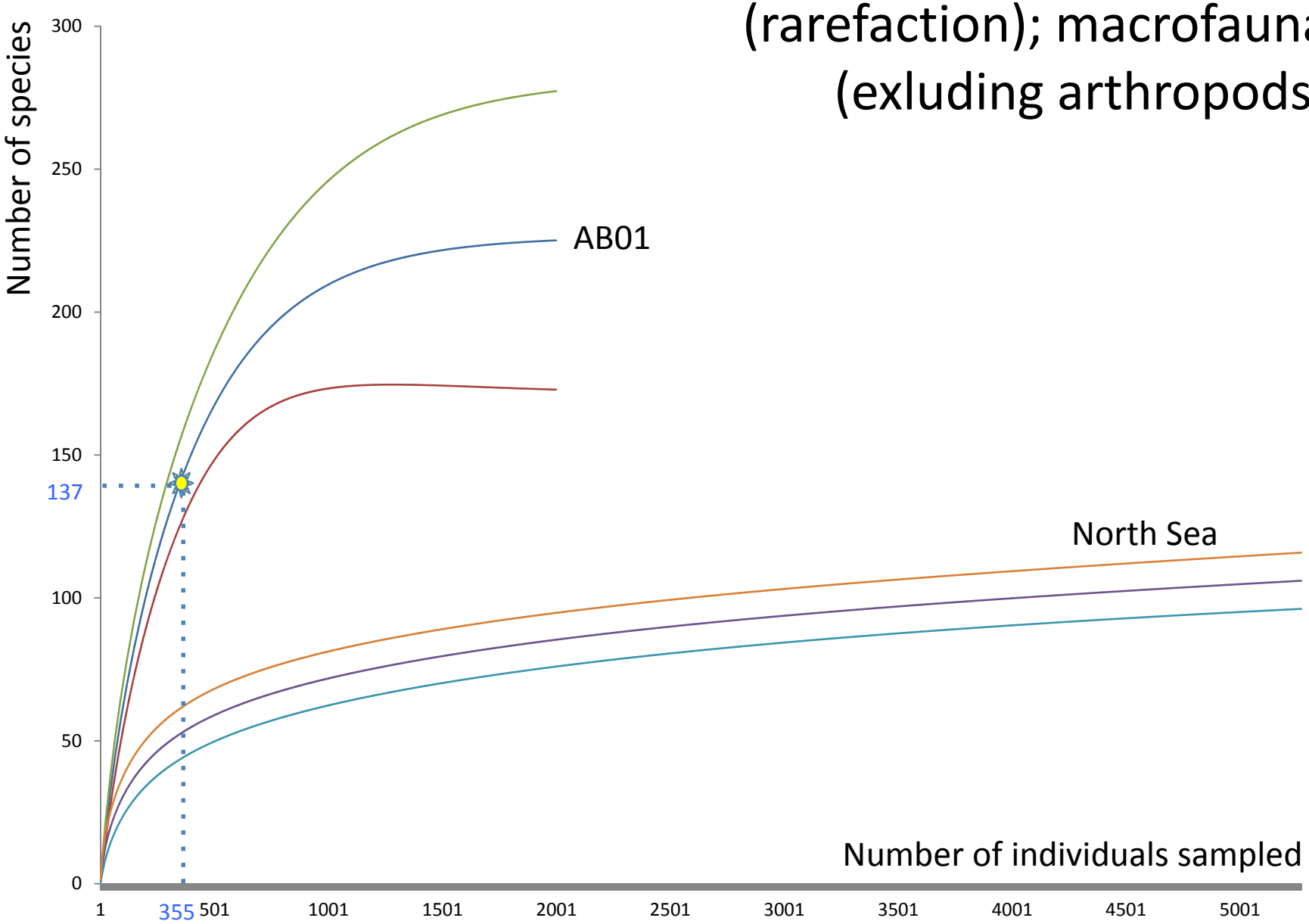
The taxonomy problem



The taxonomy problem



Biodiversity estimate (rarefaction); macrofauna (exluding arthropods)



Scientific cruises to the CCZ

Country	Cruises
USSR & Russia	many
Japan	many
UK	?
Germany	≈30 (6 since 2006)
Korea	several
USA	≈50
France	≈50
Others (eg China, Tonga, Belgium)	many

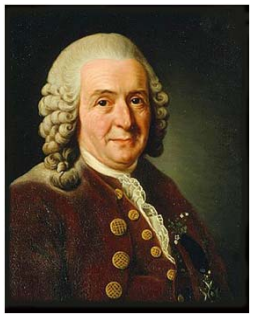
Nimmo 2013

- No lack of sampling effort
- Traditional taxonomical praxis too slow
  - Need to be more pragmatic?

Macrofauna classification

- Why taxonomy?
  - Taxonomy is the scaffolding of biological science

If the names are unknown, knowledge of the things also perishes
– Carl Linnaeus



  - UN Convention on Biological Diversity

"Conscious of the intrinsic value of biological diversity and of the ecological, genetic, social, economic, scientific, educational, cultural, recreational and aesthetic values of biological diversity and its components"

UNITED NATIONS
1992

International Commission on Zoological Nomenclature

Search... SEARCH

All Taxonomy

ABOUT | THE CODE | BULLETIN | PUBLICATIONS | OUTREACH | FAQS | ITZN | ZOOBANK | CURRENT CASES AND LIST OF AVAILABLE NAMES | A NEW LEASE OF LIFE FOR THE ICZN | SUBMITTING A CASE | SPONSORS

Welcome to the ICZN

The International Commission on Zoological Nomenclature (ICZN) acts as adviser and arbiter for the zoological community by generating and disseminating information on the correct use of the scientific names of animals. The ICZN is responsible for producing the International Code of Zoological Nomenclature - a set of rules for the naming of animals and the resolution of nomenclatural problems.

I WANT TO...

- Submit a Case
- Comment on a Case
- View/comment on the Rotifer part of the List of Available Names

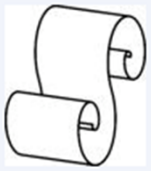
Passing of Commissioner Susan Lim

We are deeply saddened by news of the passing of Commissioner Susan Lim (Parasitology; Malaysia) on 2 August 2014.

ZooBank Progress Report

The ZooBank progress report for the first quarter of 2013 is now available.

- 148,625 total registrations
- 92,543 registered Nomenclatural Acts
- 18,555 registered Authors
- 37,527 registered Published Works



List of open cases updated

The list of open cases has been updated.

ICZN

- The description should include *'a **description or definition** that states in words characters that are purported to differentiate the taxon'*

- The **type specimen** should be deposited *'in an institution that maintains a research collection, with proper facilities for preserving them and making them accessible for study*'

- The taxonomic work *'must have been produced in an edition containing simultaneously obtainable copies by a method that assures numerous identical and durable copies or **widely accessible** electronic copies with fixed content and layout.'*

Deep-sea taxonomy

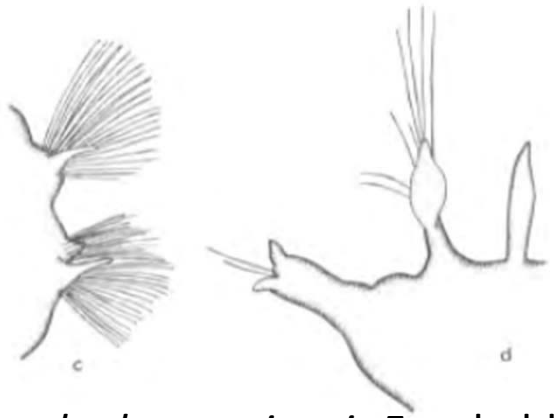
- Records are rare. No (OBIS) records for annelids in the CCZ
- Type material of described deep-sea species often in a poor state
- Type localities not that accurate
- Lack of DNA data
- New sampling is a major effort

Taxonomic challenges

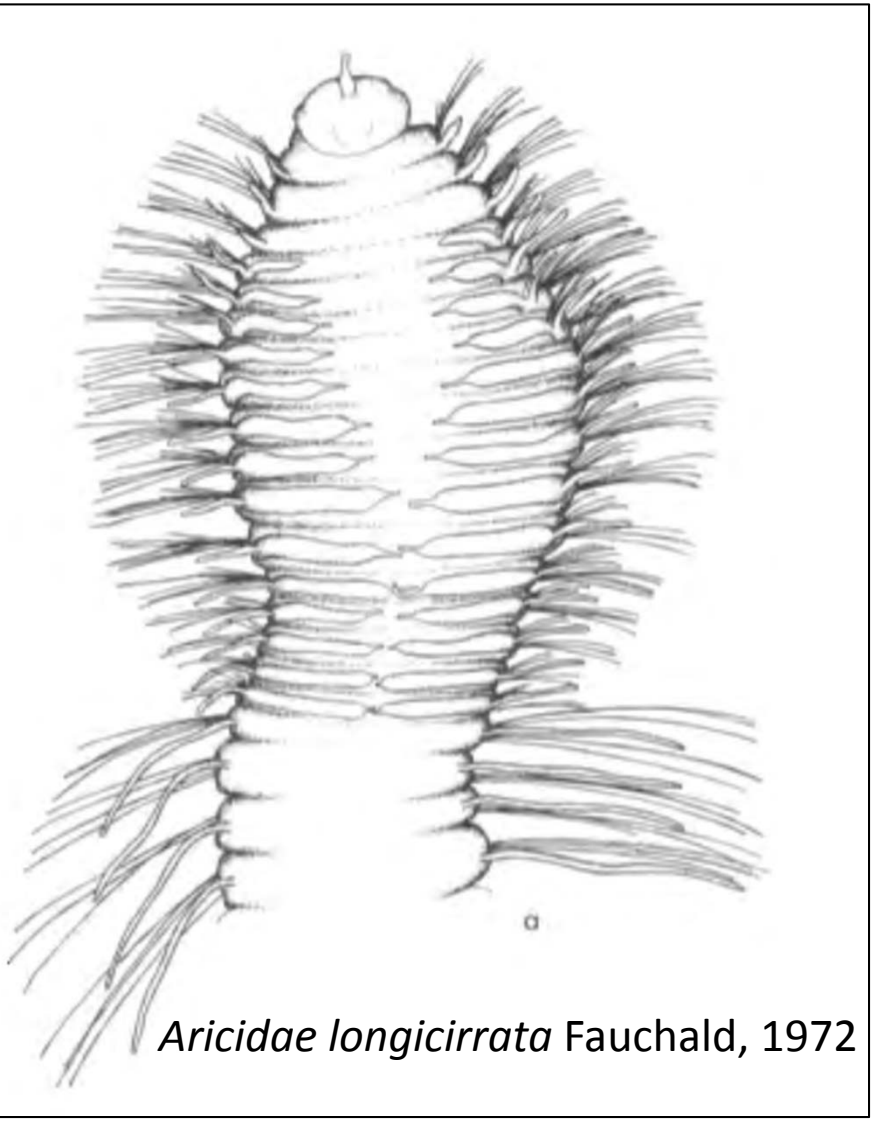
- Species hypothesis formulation
  - Intra-species diversity range
  - The “barcode gap”
  - Representative morphology (larvae, adult, egg)
  - The legacy of old names (is the “new species hypothesis” valid?)
    - Incomplete morphological description
    - Holotype in poor state
    - Poorly defined type locality
    - No DNA sequence data

“State of the art” morpholocical work – Fauchald 1972

- Polychaetes from off Western Mexico
- Stations at shelf and slope
- Material from e few “new” cruises
- Plus the Albatross cruises 1889-91, 1911
- Fauchald recognised 227 species
- 76 new species described



Haploscoloplos mexicanis Fauchald, 1972



Aricidae longicirrata Fauchald, 1972

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Advances in molecular methodologies

- Barcoding initiatives
- Phylogenetic analyses
- Databases (crosslinked to Genbank, BOL)
- Sequencing speed and cost
- Bioinformatics (sequence data and microcomputers)

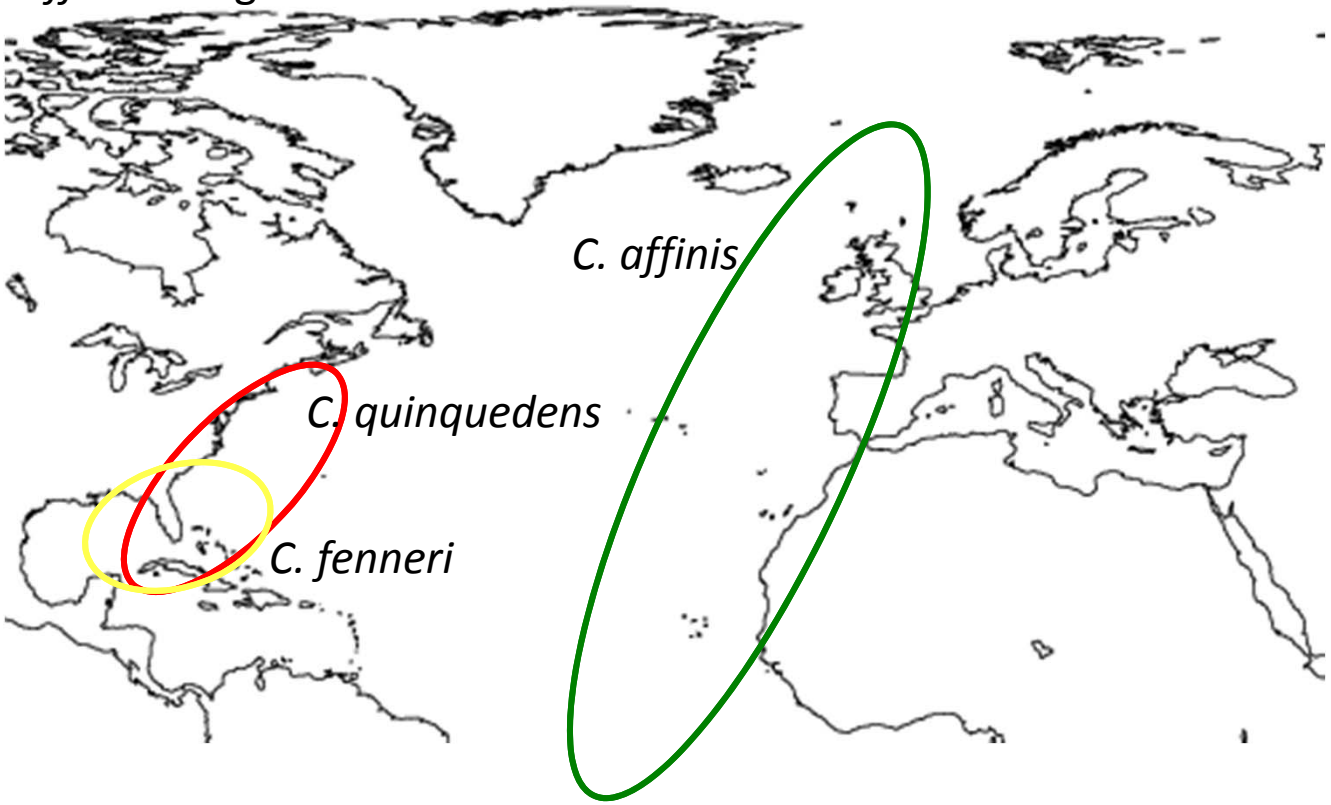
-> A ‘new species hypothesis’ inclusive of DNA barcode data is increasingly easy to falsify!

Connectivity

- Species diversity and community structure
- Scale of species ranges (resilience to disturbance)
- Population structure
  - Identification of suitable model species

Deep Sea Crabs

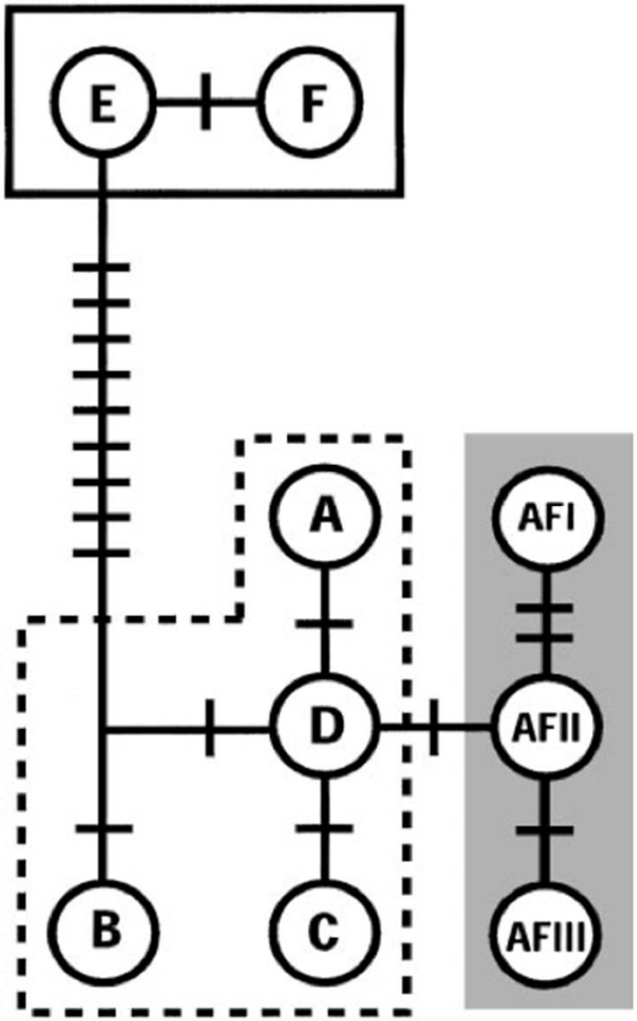
Chaceon quinquedens “Atlantic Deep-Sea Red Crab”, *Chaceon fenneri* “Golden Crab”, *Chaceon affinis* “King Crab”



Weinberg, Dahlgren, Trowbridge, Halanych 2003

Deep Sea Crabs

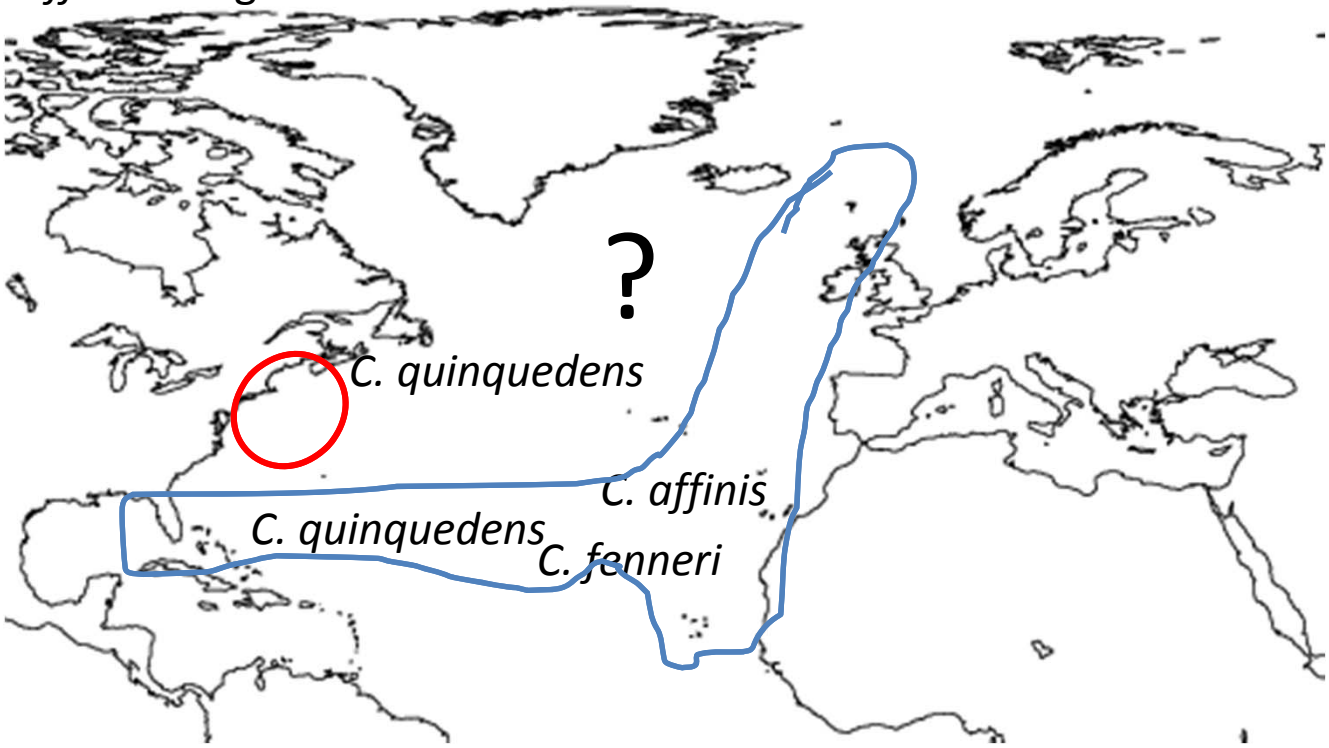
New England *Chaceon quinquedens*



GoM *Chaceon quinquedens*
Atlantic Florida *C. fenneri*
East Atlantic *C. affinis*

Deep Sea Crabs

Chaceon quinquedens “Atlantic Deep-Sea Red Crab”, *Chaceon fenneri* “Golden Crab”, *Chaceon affinis* “King Crab”



Weinberg, Dahlgren, Trowbridge, Halanych 2003

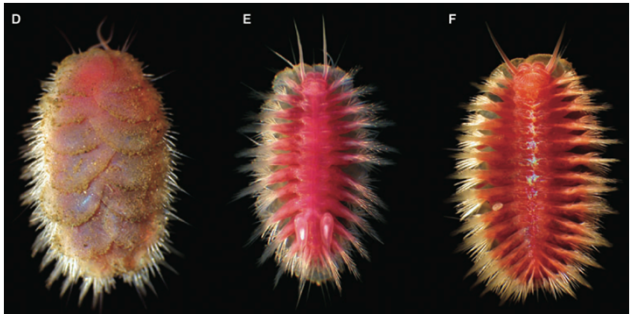
Deep Sea Crabs

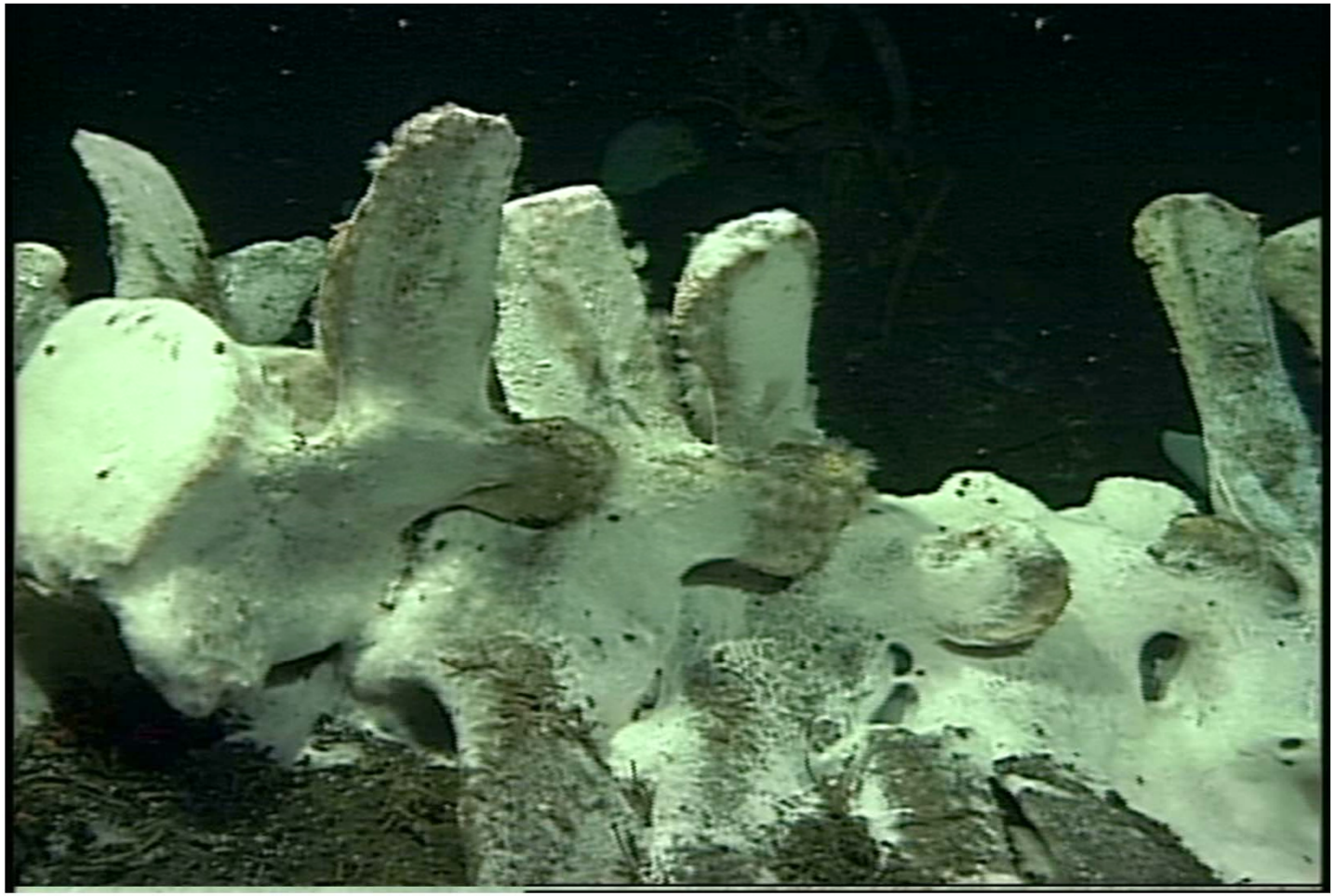
Weinberg et al. 2003

- Three species may be two species
- Deceptive (convergent) morphology
- Long distance gene flow

Deep Sea Annelids

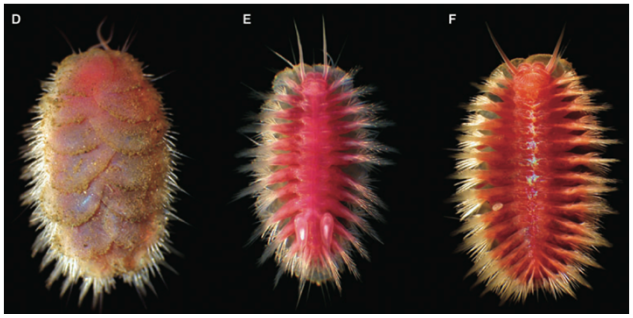
Glover et al. 2005

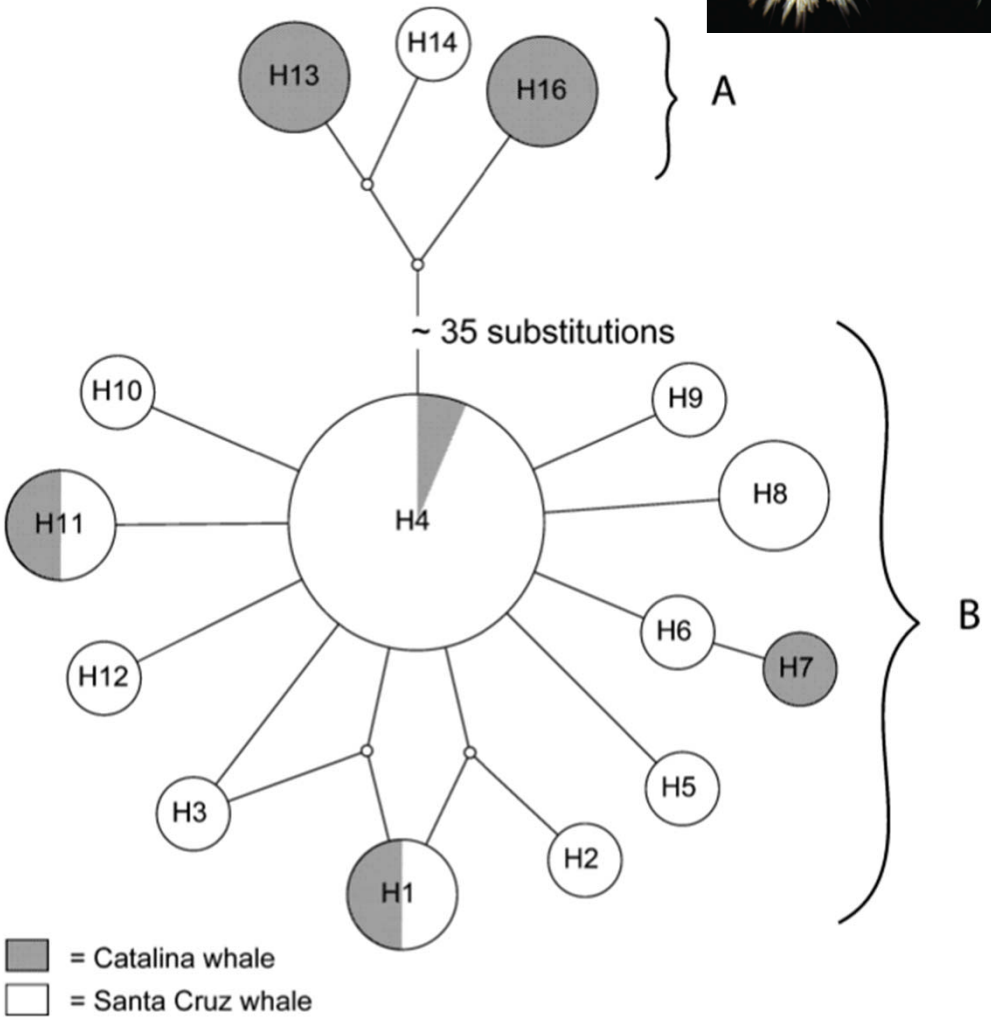




Deep Sea Annelids

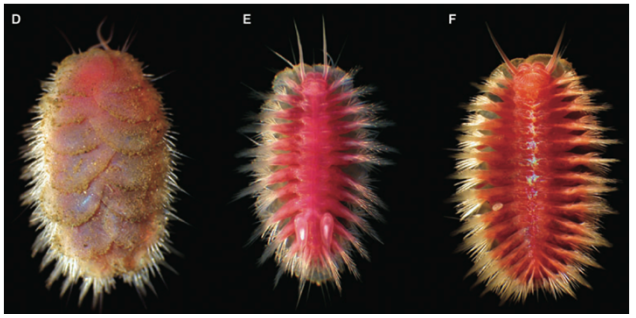
Glover et al. 2005





Deep Sea Annelids

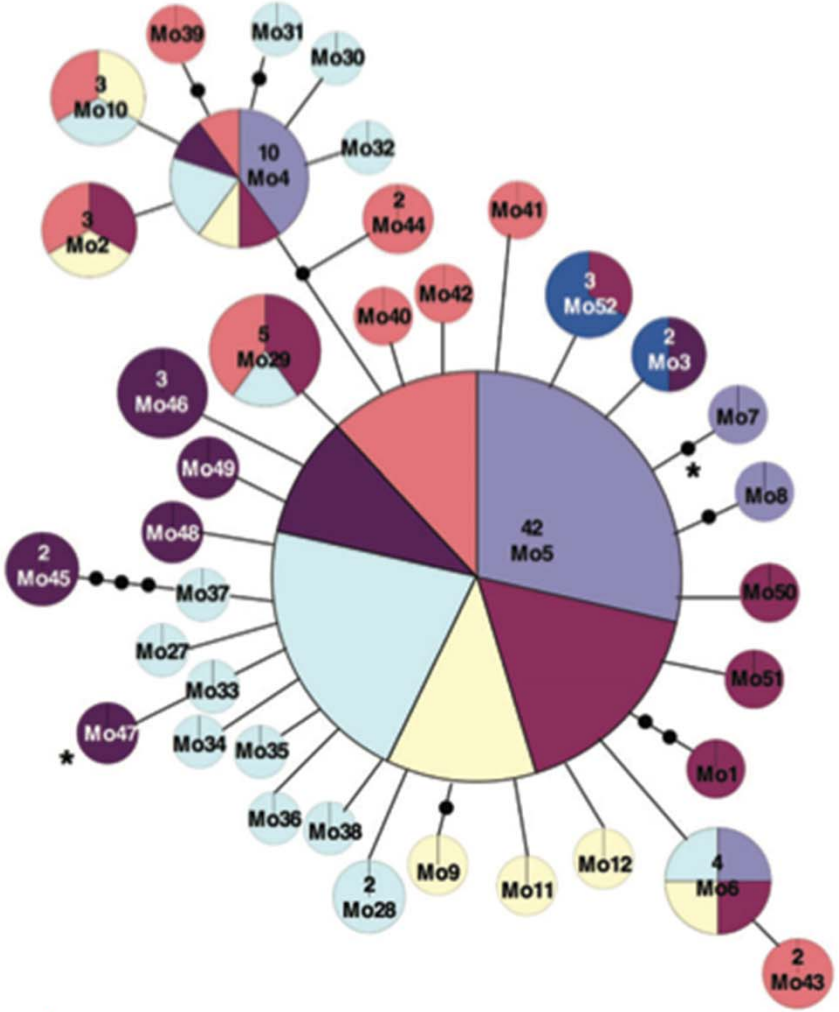
Glover et al. 2005

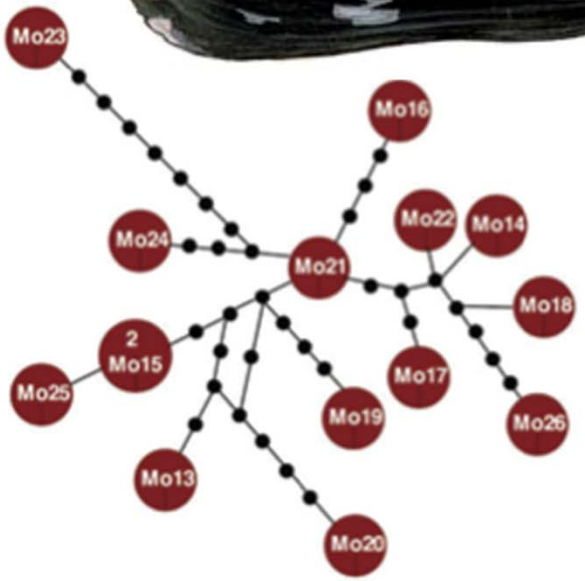


- One species may be two species
- Cryptic diversity
- E.g. through sympatric speciation

Horse Mussel

Halanych et al. 2013





uni Research

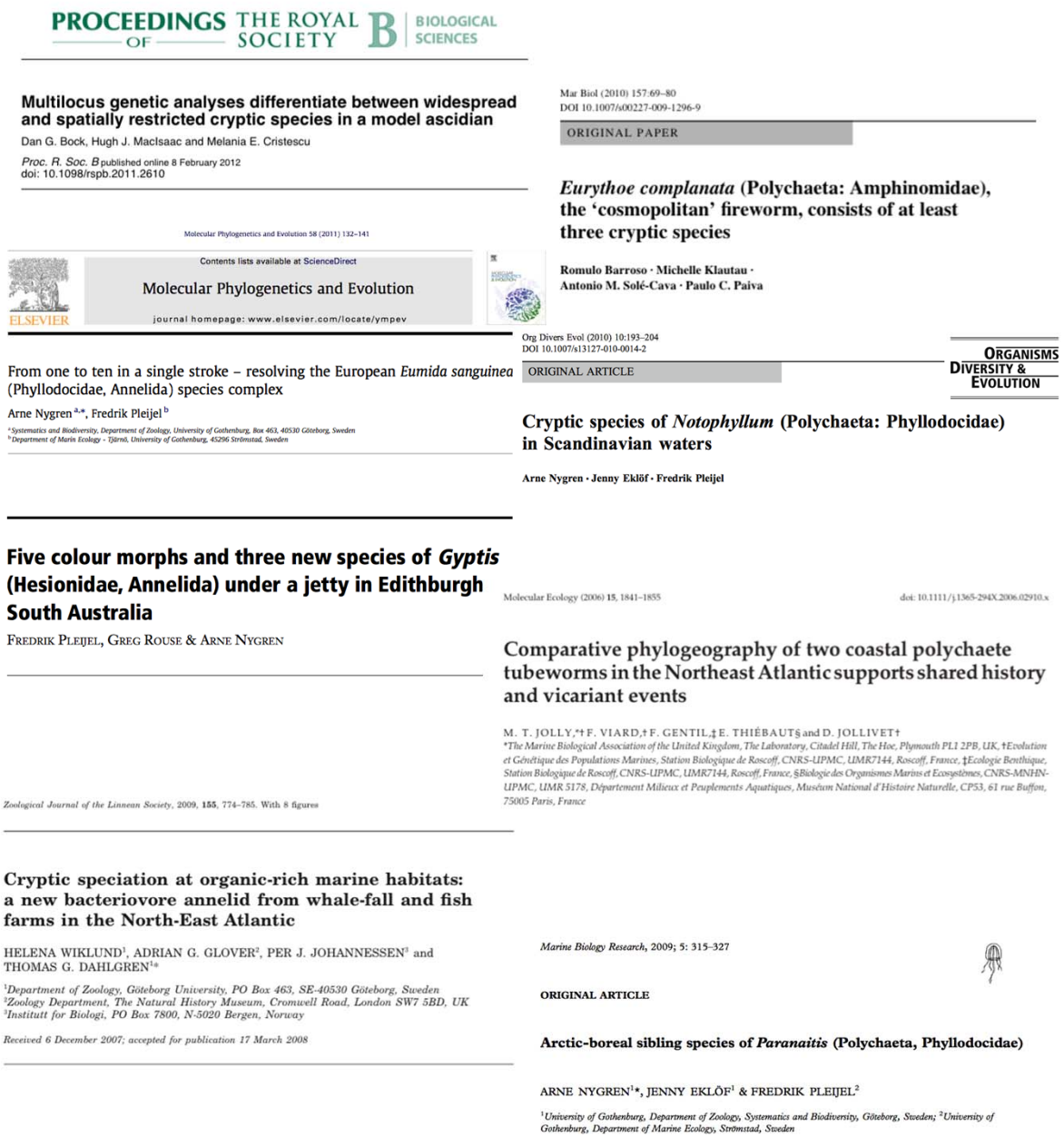
Horse Mussel

Halanych et al. 2013

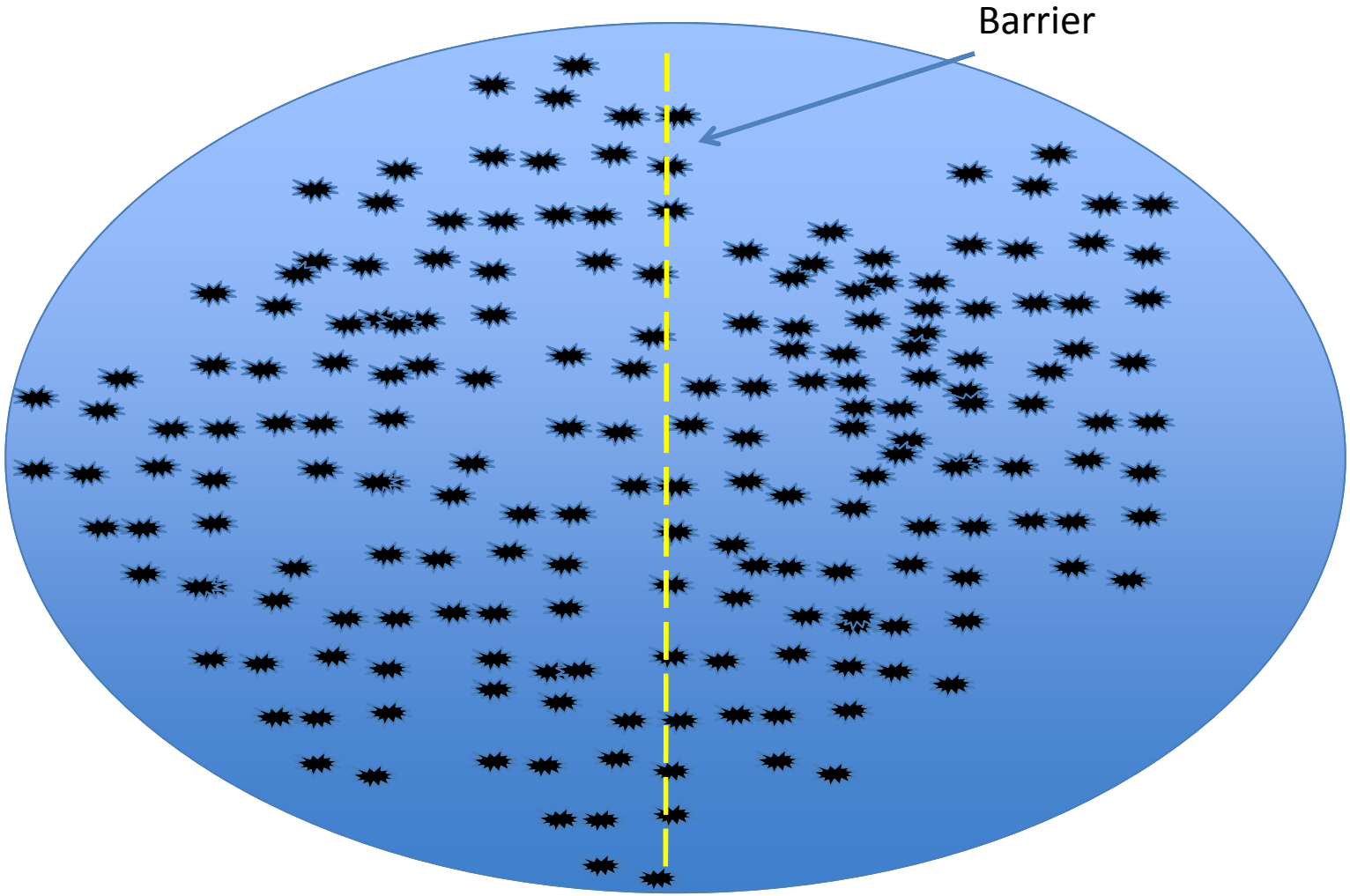
- One species may be two allopatric species
- Cryptic diversity
- Separation by distance

Cryptic diversity common also in shallow water and for well known “species”

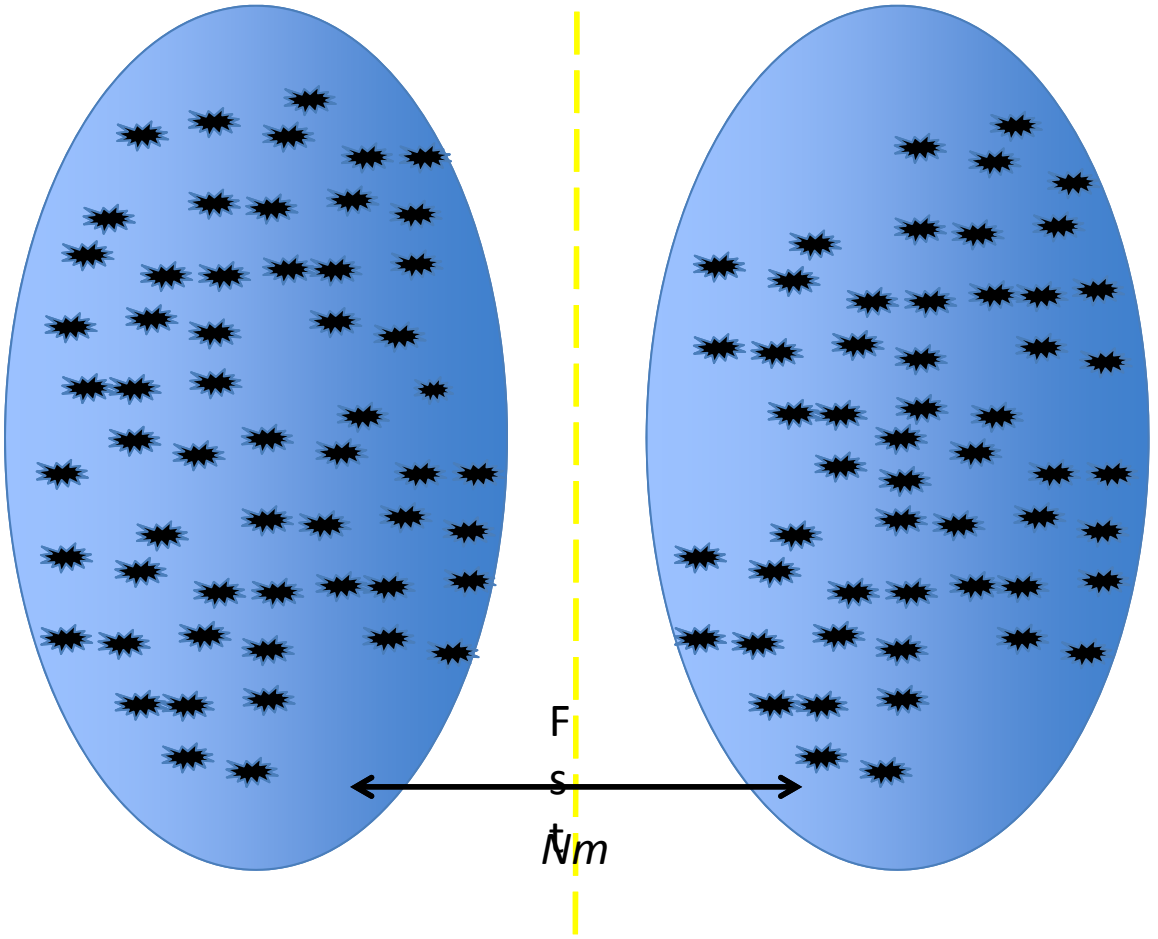
- Barroso, R., Klautau, M., Solé-Cava, A. M., & Paiva, P. C. (2010).
- Bock, D. G., MacIsaac, H. J., & Cristescu, M. E. (2012).
- Jolly, M. T., Viard, F., Gentil, F., Thiebaut, E., & Jollivet, D. (2006).
- Nygren, A., & Pleijel, F. (2011).
- Nygren, A., Eklöf, J., & Pleijel, F. (2009).
- Nygren, A., Eklöf, J., & Pleijel, F. (2010).
- Pleijel, F., Rouse, G., & Nygren, A. (2009).
- Wiklund, H., Glover, A., Johannessen, P., & Dahlgren, T. (2009).



Model species population



Population Structure



POPULATION GENETICS, DEMOGRAPHIC CONNECTIVITY, AND THE DESIGN OF MARINE RESERVES

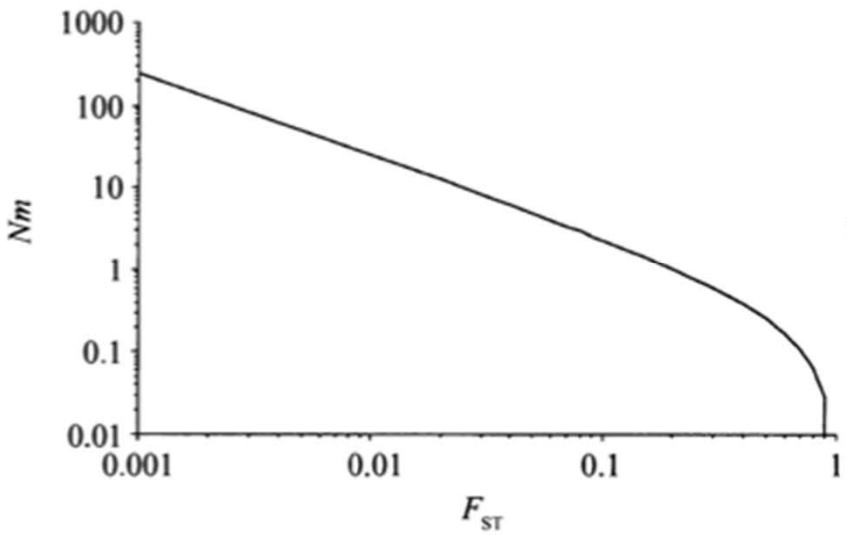
STEPHEN R. PALUMBI[1]

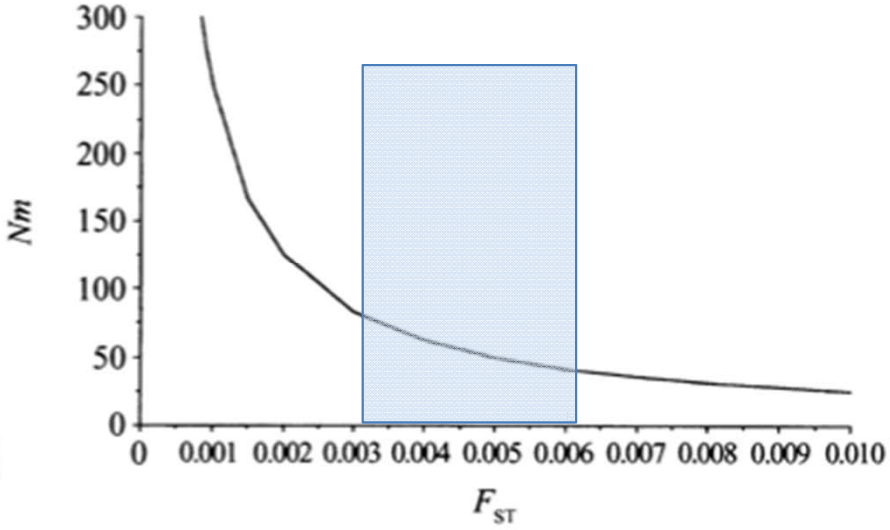
Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138 USA

Abstract. Genetic analyses of marine population structure often find only slight geographic differentiation in species with high dispersal potential. Interpreting the significance of this slight genetic signal has been difficult because even mild genetic structure implies very limited demographic exchange between populations, but slight differentiation could also be due to sampling error. Examination of genetic isolation by distance, in which close populations are more similar than distant ones, has the potential to increase confidence in the significance of slight genetic differentiation. Simulations of one-dimensional stepping stone populations with particular larval dispersal regimes shows that isolation by distance is most obvious when comparing populations separated by 2–5 times the mean larval dispersal distance. Available data on fish and invertebrates can be calibrated with this simulation approach and suggest mean dispersal distances of 25–150 km.

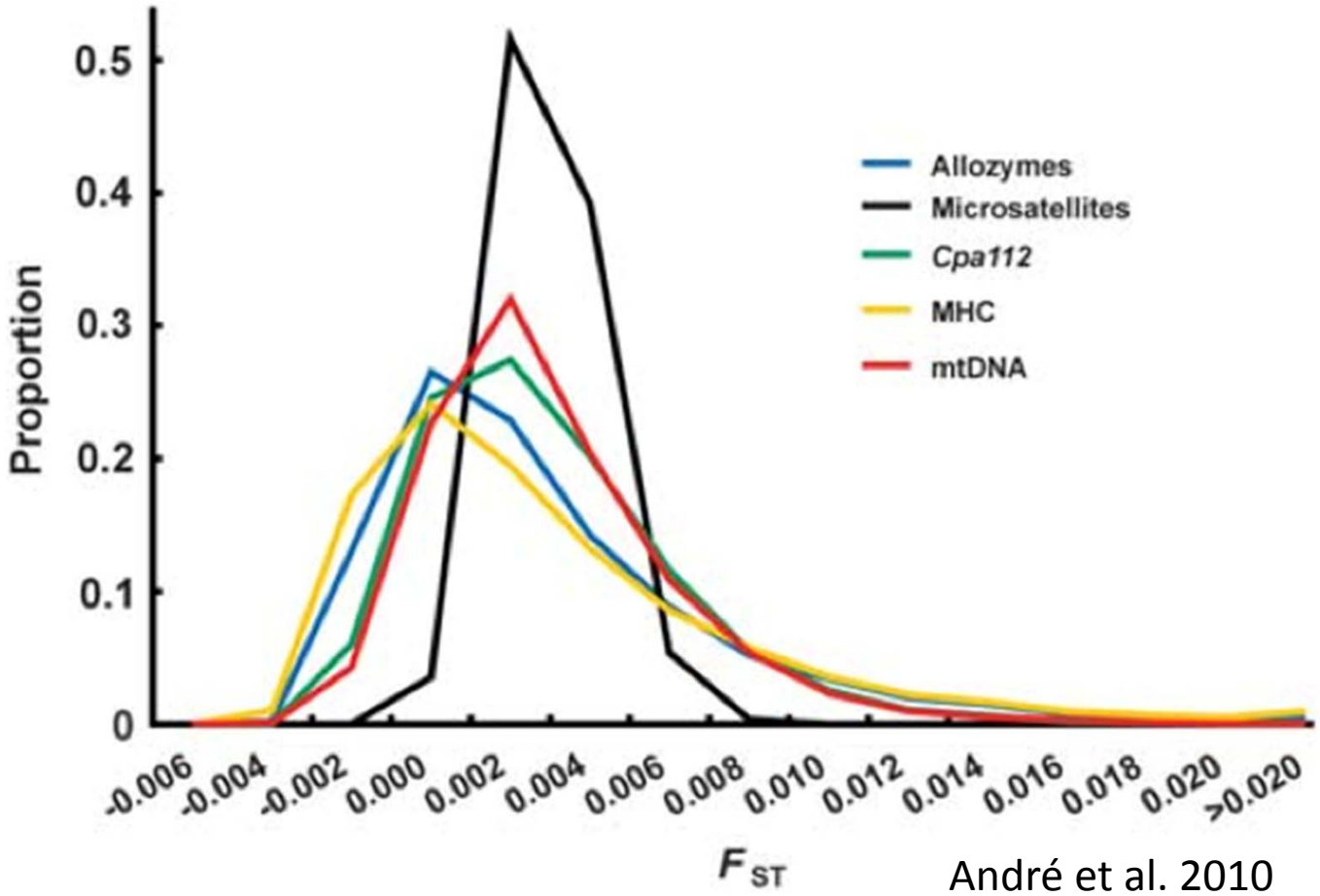
Design of marine reserve systems requires an understanding of larval transport in and out of reserves, whether reserves will be self-seeding, whether they will accumulate recruits from surrounding exploited areas, and whether reserve networks can exchange recruits. Direct measurements of mean larval dispersal are needed to understand connectivity in a reserve system, but such measurements are extremely difficult. Genetic patterns of isolation by distance have the potential to add to direct measurement of larval dispersal distance and can help set the appropriate geographic scales on which marine reserve systems will function well.

Gene flow and genetic separation





mtDNA is “OK”



André et al. 2010

mtDNA is “OK”

F_{ST}	*Micro-satellites 8 loci*	*Allozymes 11 loci*	*mtDNA*	*MHC*	*Cpa112*	*Median of individual microsatellite loci*
0.0000	0.063	0.057	0.063	0.064	0.049	0.054
0.0001	0.125	0.056	0.072	0.068	0.070	0.065
0.0010	0.958	0.342	0.455	0.240	0.386	0.366
0.0020	1.000	0.774	0.866	0.572	0.799	0.770
0.0050	1.000	0.998	1.000	0.982	0.998	1.000
0.0100	1.000	1.000	1.000	1.000	1.000	1.000

Abbreviations: MHC, major histocompatibility complex; mtDNA, mitochondrial DNA.

André et al. 2010

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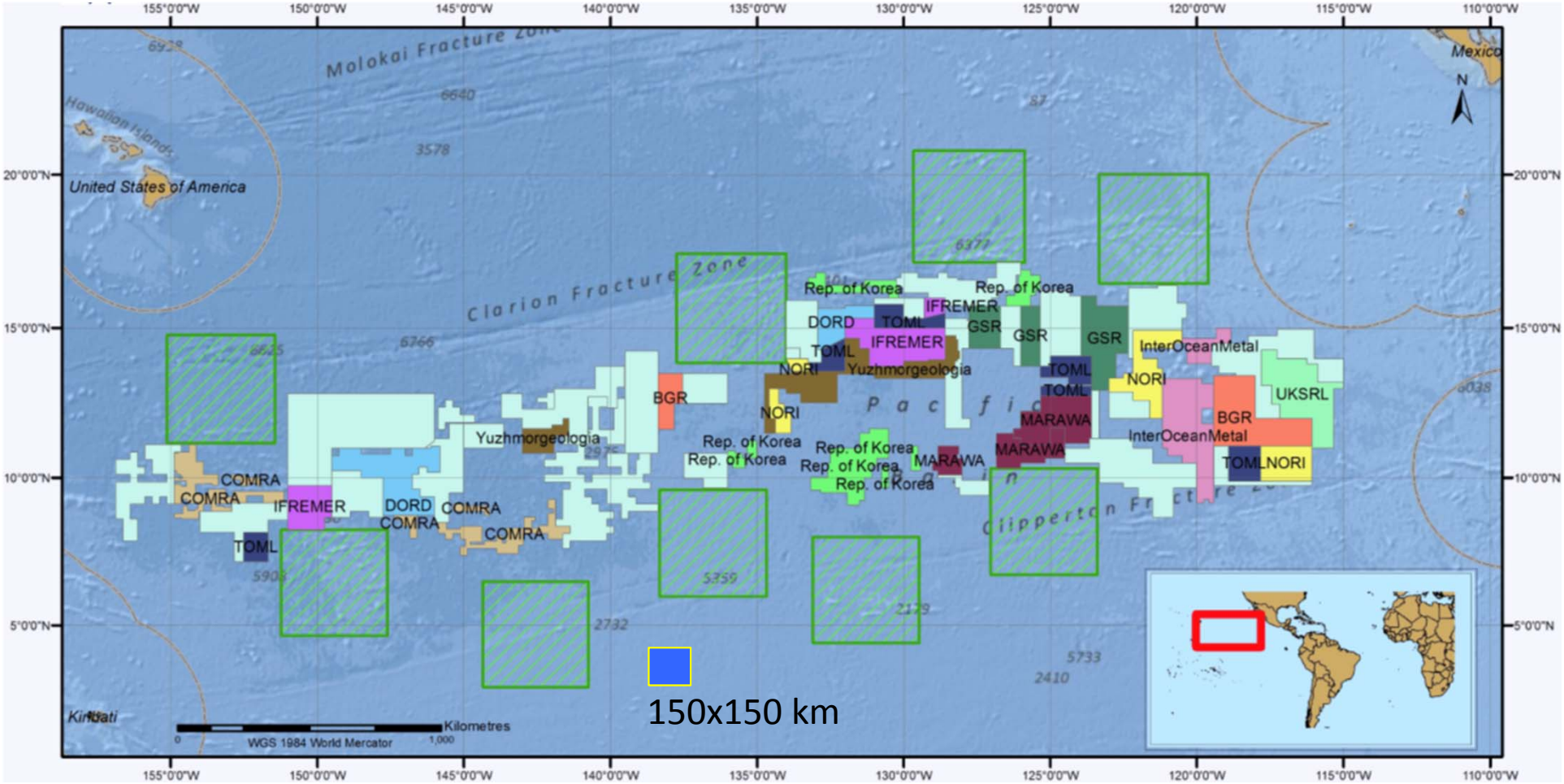
SCALE?


Molokai Fracture Zone
Clarion Fracture Zone
Clipperton Fracture Zone
United States of America
Mexico
Hawaiian Islands
Kiribati
Pacific
Rep. of Korea
IFREMER
DORD
TOML
GSR
NORI
Yuzhmorgeologia
BGR
MARAWA
InterOceanMetal
UKSRL
TOMLNORI
COMRA
150x150 km
Kilometres
WGS 1984 World Mercator

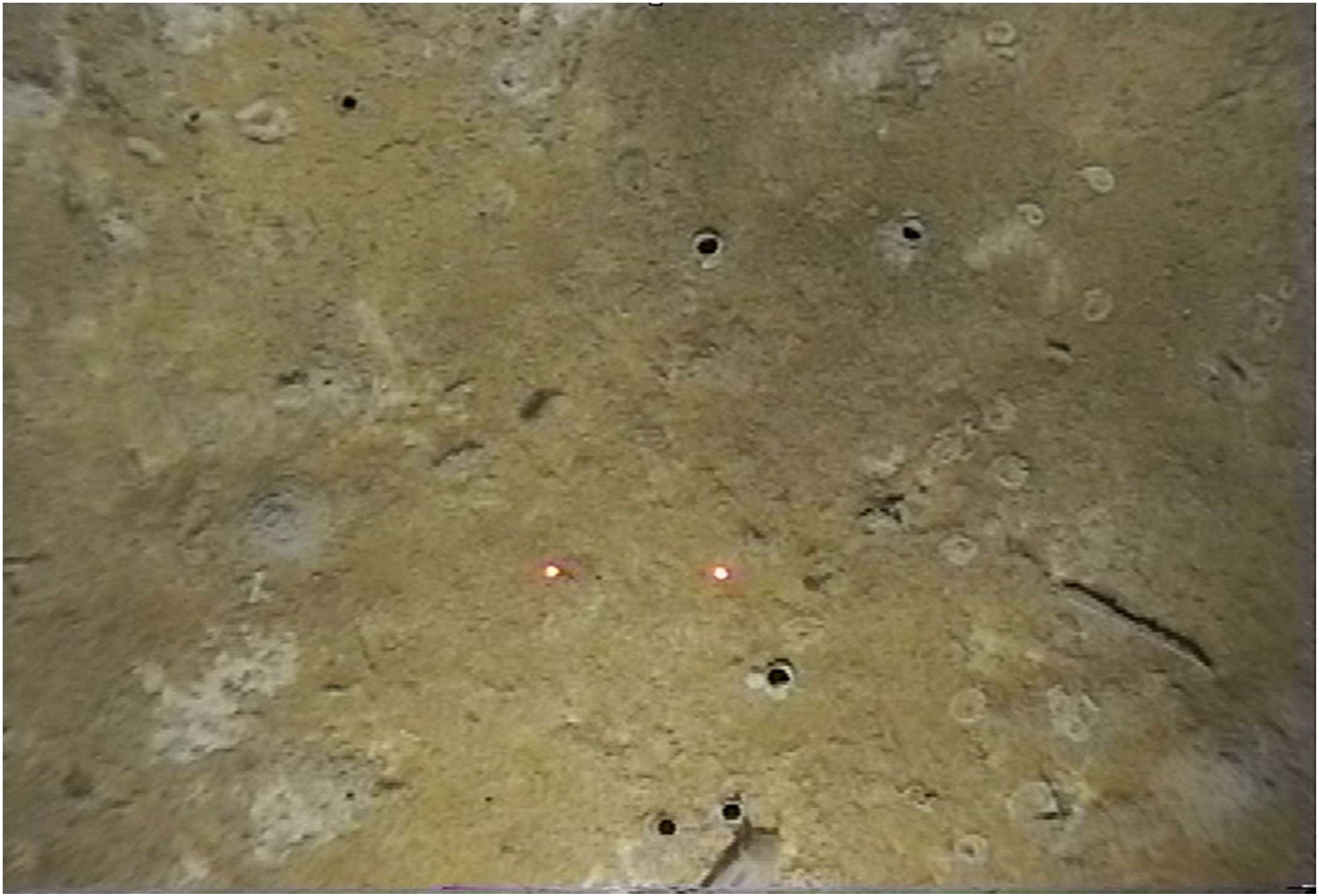
Summary

- Cryptic diversity is common
- mtDNA can be used to discover cryptic diversity
- The scale of connectivity is important in conservation genetics
- mtDNA can be used to analyze population genetic samples with an “OK” resolution
- Scales of genetic structure at around 150 km call for an ambitious sampling effort needed in the CCZ !

Example of how DNA barcode taxonomy data can be used

- DNA barcode taxonomy
  - Analyses of samples
    - Samples from single specimens
    - Bulk sample identification test using a DNA barcode library of tissue from all individuals separated from the sample (Hajibabaei et al. 2012)

      - 87% of taxa present in a sample was detected from analyzing the ethanol used to bulk preserve them
      - 89% in conventional tissue extracted DNA (mixed tissue)
      - 100% success for all taxa represented with more than 1% individuals for both DNA sources
- eDNA
  - Analyses of environmental DNA and taxonomical databases



LEBENSPUR in biodiversity assessments

- Assessment of biodiversity
- Mainly megafauna
- Low taxon resolution
- Large sample size
- Possibility for automatic analyses through image recognition
- No taxon database available

- Papers:
  - Jones et al. 2007
  - …..

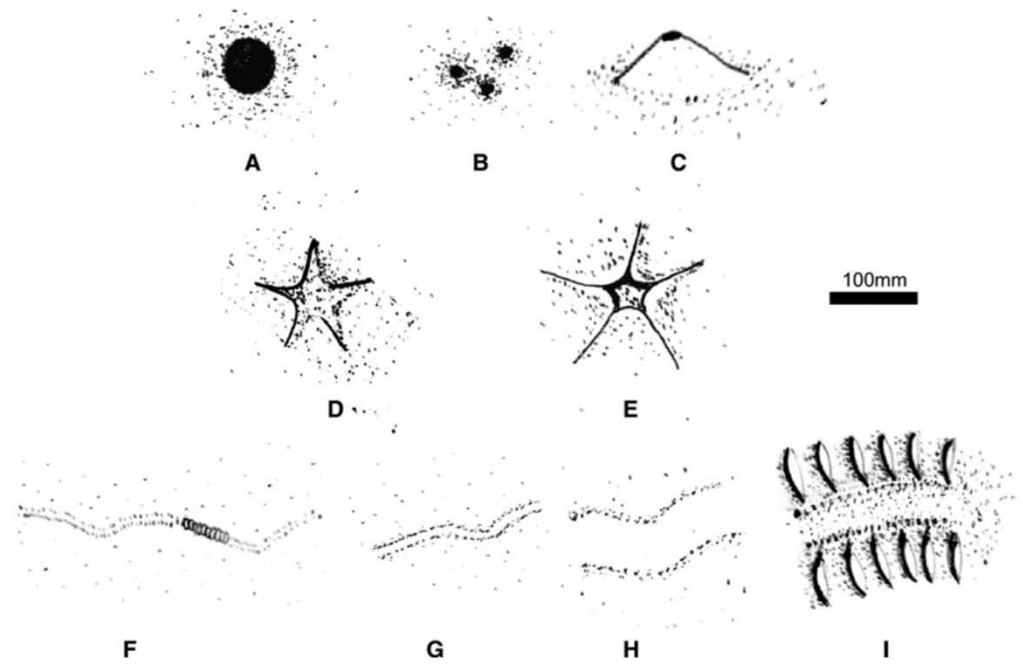


Figure 5. Megafaunal traces recorded in seabed photographs of the bathyal Kangerdlugssuaq area, Greenland. (A) Large circular hole. (B) Small circular hole. (C) Molpadid mound. (D) Asteroid trace. (E) Ophiuroid trace. (F) Holothurian faecal plough. (G) Narrow plough. (H) Wide plough. (I) Crenulated plough.

Jones et al. 2007

eDNA in biodiversity asessments

- Potential to asses diversity levels
- Community structure data if matched with DNA barcode database (but abundance data?)
- No database available for deep sea taxa
- 1 paper tested macro- meio- and micro diversity levels
- 6 meiofauna diversity levels only (+ microbial eukaryotes)

Chariton et al. 2010

Creer et al. 2010

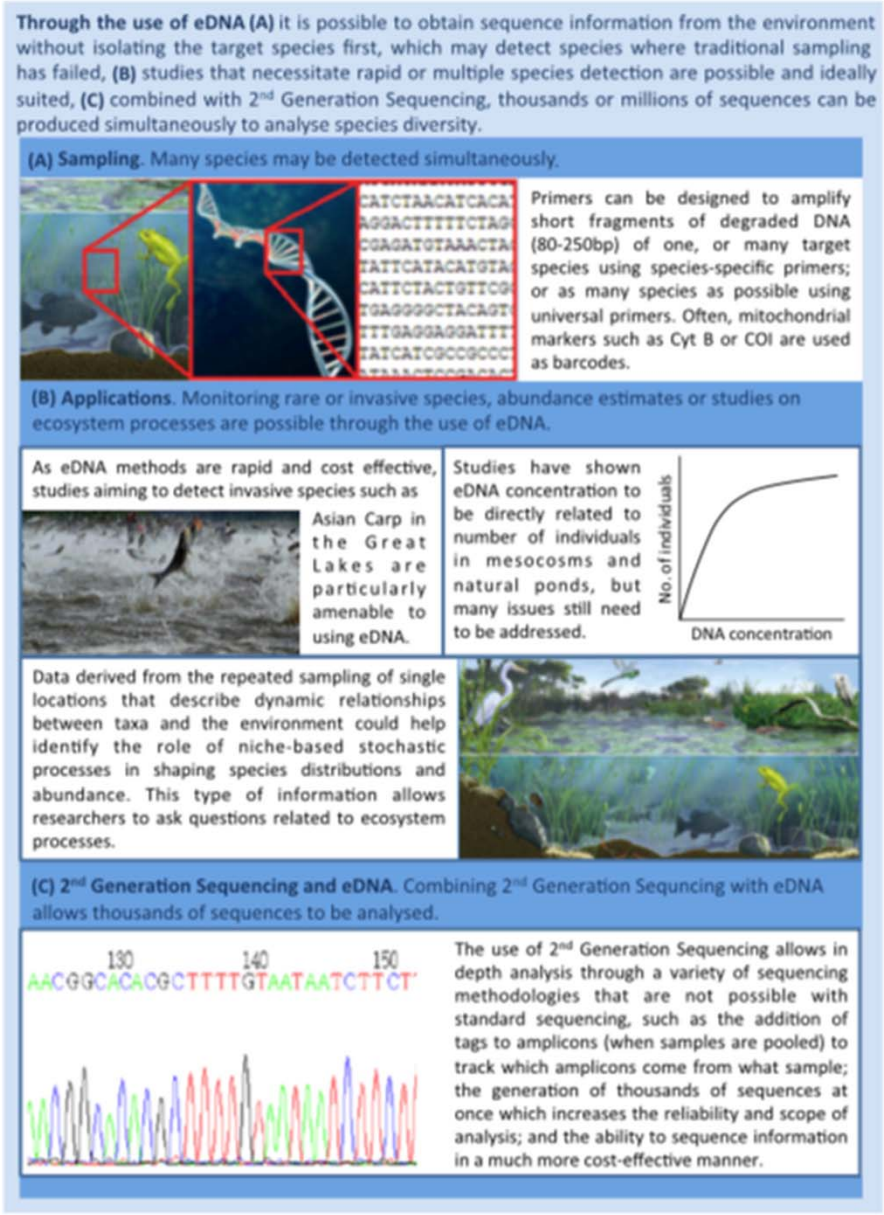
Fonseca et al. 2010

Bik et al. 2011

Bik et al. 2012

Pawlowski et al. 2011

Pawlowski et al. 2014



Bohmann et al. 2014

RESEARCH COMMUNICATIONS RESEARCH COMMUNICATIONS

Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA

Anthony A Chariton[1*], Leon N Court[2], Diana M Hartley[2], Matthew J Colloff[2], and Christopher M Hardy[2]

Biodiversity assessment underpins our understanding of ecosystems and determines environmental management decisions on resource use and conservation priorities. Recently, a new discipline – environmental or ecological genomics (ecogenomics) – has emerged from major advances in sequencing technologies, such as pyrosequencing (a technique based on the detection of pyrophosphate during nucleotide incorporation), and enabled extraordinary progress in the way biodiversity can be assessed. Since 2008, numerous high-impact microbial metagenomic sequencing studies, which have relied on both classical and next-generation sequencing, have been published. As a result, many previously unrecognized taxa and biota have been identified, but none of these studies explored eukaryote diversity. Here, we illustrate the power of applying next-generation pyrosequencing to identify and enumerate eukaryote species assemblages in the context of assessing the impacts of human activity on ecosystems.

Front Ecol Environ 2010; 8(5): 233–238, doi:10.1890/090115 (published online 7 Apr 2010)

Chariton *et al.* 2010

- Shallow water Sydney Harbor
- Control and Impact (but no BACI)
- Phylum (order) level resolution
- Impact on macrofauna but meiobiota less clear

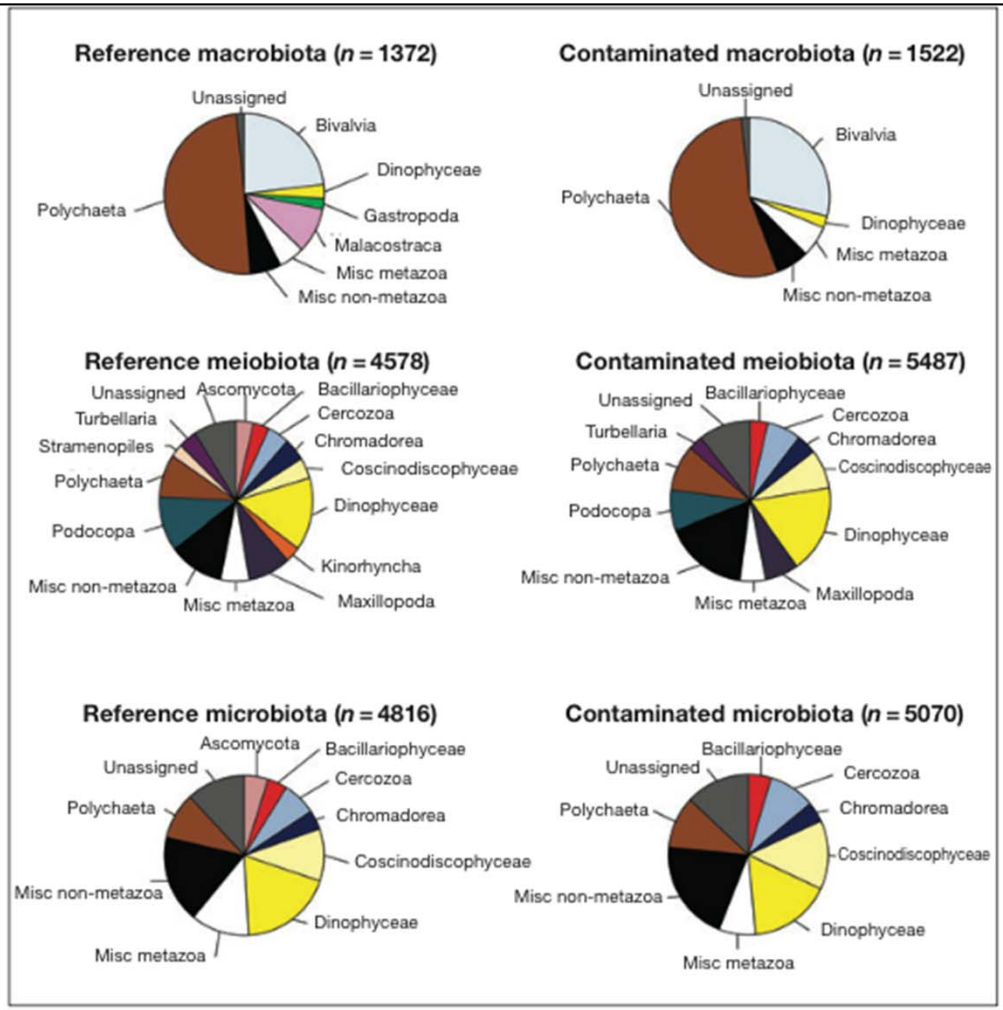


Figure 2. *The relative proportions of eukaryote OTUs, grouped by Class or higher taxonomic level, in macro, meio, and micro fractions from reference and contaminated locations.* n = *total number of OTUs in the combined samples. Miscellaneous (Misc) metazoa/non-metazoa contains the taxa represented by < 2.5% of the total OTUs. Stramenopiles = all OTUs assignable to this unranked taxonomic group, apart from the Phylum Bacillariophyta.*

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Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises

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Creer et al. 2010

- Meiofauna only
- Shallow water British beach
- High richness of nematodes

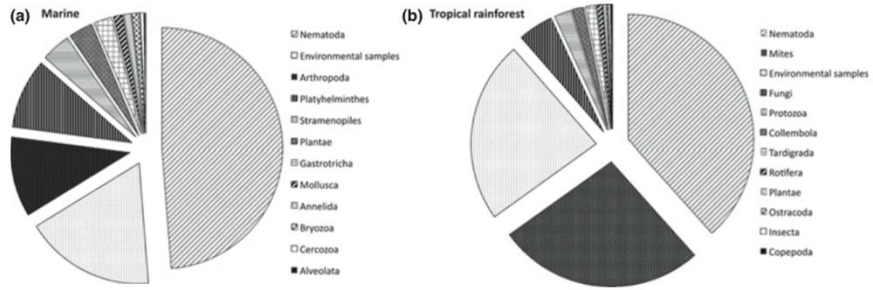

Fig. 5 Pie chart illustrating the relative proportion of OCTUs (clustered at 97% similarity) belonging to each taxonomic grouping found in (a) the marine littoral benthos and (b) the tropical rainforest case studies. BLAST hits to 'environmental samples' represent unclassified taxa.

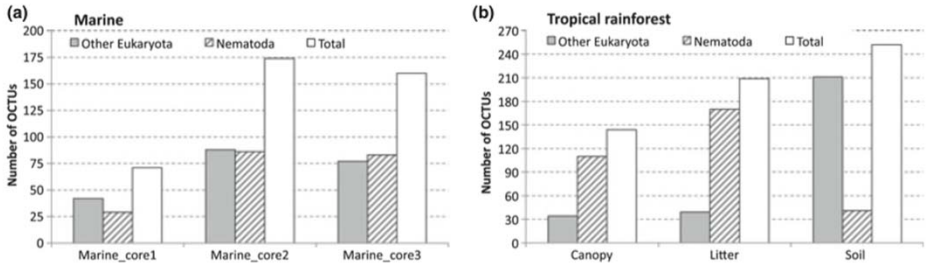

Fig. 4 Number of putative non-chimeric OCTUs (clustered at 97% similarity) found (a) in the marine littoral benthos and (b) tropical rainforest case studies for sample site. Data are provided for totals, Nematoda and other Eukaryota (including OCTUs with BLAST hits to 'environmental samples' representing unclassified taxa).

nature COMMUNICATIONS

ARTICLE

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Second-generation environmental sequencing unmasks marine metazoan biodiversity

Vera G. Fonseca[1], Gary R. Carvalho[1], Way Sung[2], Harriet F. Johnson[1], Deborah M. Power[3], Simon P. Neill[4], Margaret Packer[5], Mark L. Blaxter[6], P. John D. Lambshead[7], W. Kelley Thomas[2] & Simon Creer[1]

Fonseca et al. 2010

- Shallow water Scottish beach
- Meiofauna only
- Phylum level comparisons
- Nematods most divers

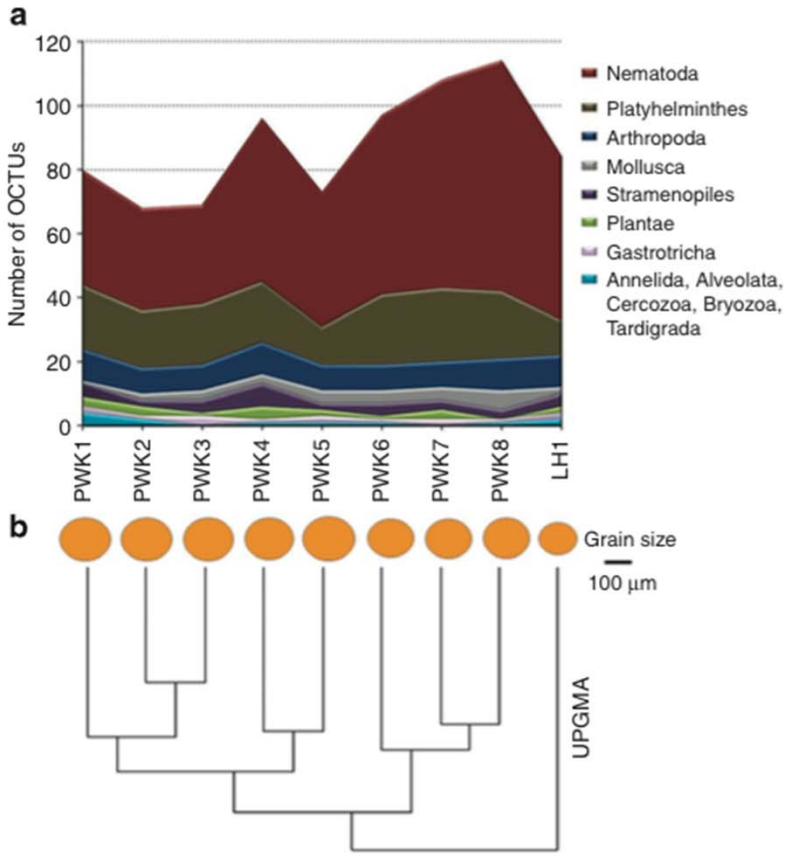

Figure 2 | Taxon richness and community similarity in relation to ecology and space. (**a**) Number of different OCTUs per sample for each phylum after data standardization derived from the Prestwick (eight sampling sites) and Littlehampton (one sampling site) marine littoral benthos; (**b**) grain size represents the relative 50% cumulative median grain size (µm) per site, and cluster analyses (UPGMA) using Sorensen's Coefficient represent the number of shared OCTUs between the nine independent samples. The positive relationship between grain size and sample richness is highly significant (Spearman's correlation coefficient, $n=9$, $\rho=-0.83$, $P=0.0108$).

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Eukaryotic Richness in the Abyss: Insights from Pyrotag Sequencing

Jan Pawlowski[1]*, Richard Christen[2], Béatrice Lecroq[3], Dipankar Bachar[2], Hamid Reza Shahbazkia[4], Linda Amaral-Zettler[5], Laure Guillou[6]

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Pawlowski et al. 2011

- Deep Sea
- Sediment samples
- DNA from pelagic and benthic organisms
- Much of the DNA from organisms not present

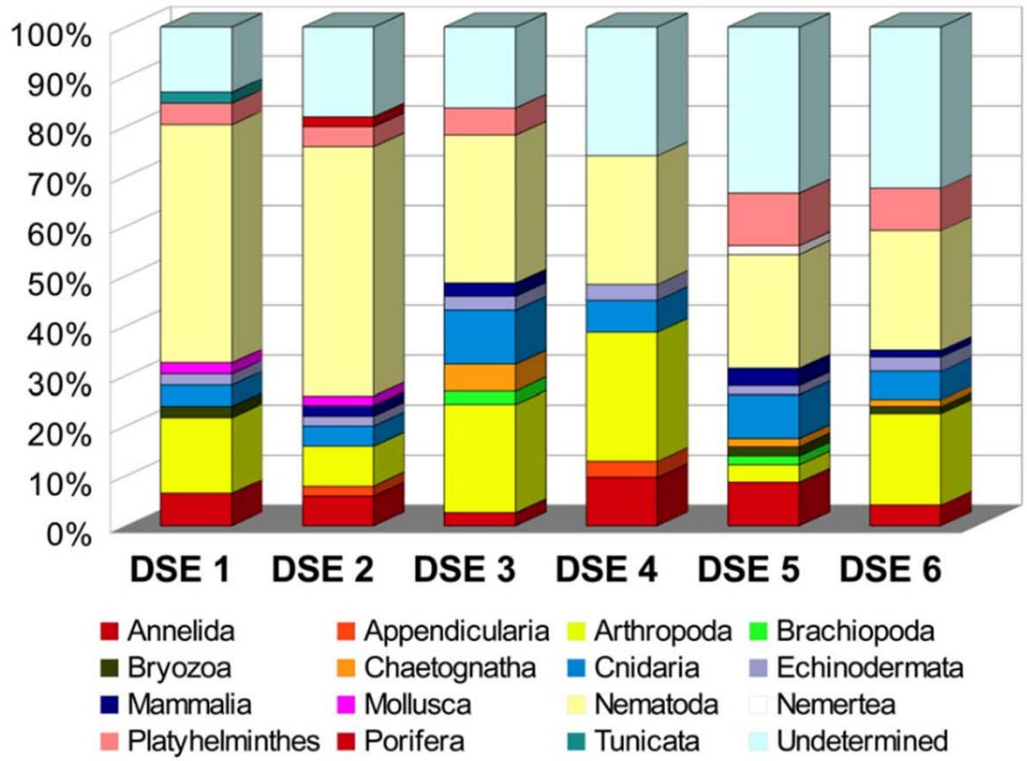

Figure 2. Taxonomic distribution of OTUs assigned to Metazoa.
doi:10.1371/journal.pone.0018169.g002

Review

CellPress

Environmental DNA for wildlife biology and biodiversity monitoring

Kristine Bohmann[1,2*], Alice Evans[3*], M. Thomas P. Gilbert[1,4], Gary R. Carvalho[3], Simon Creer[3], Michael Knapp[3], Douglas W. Yu[5,6], and Mark de Bruyn[3]

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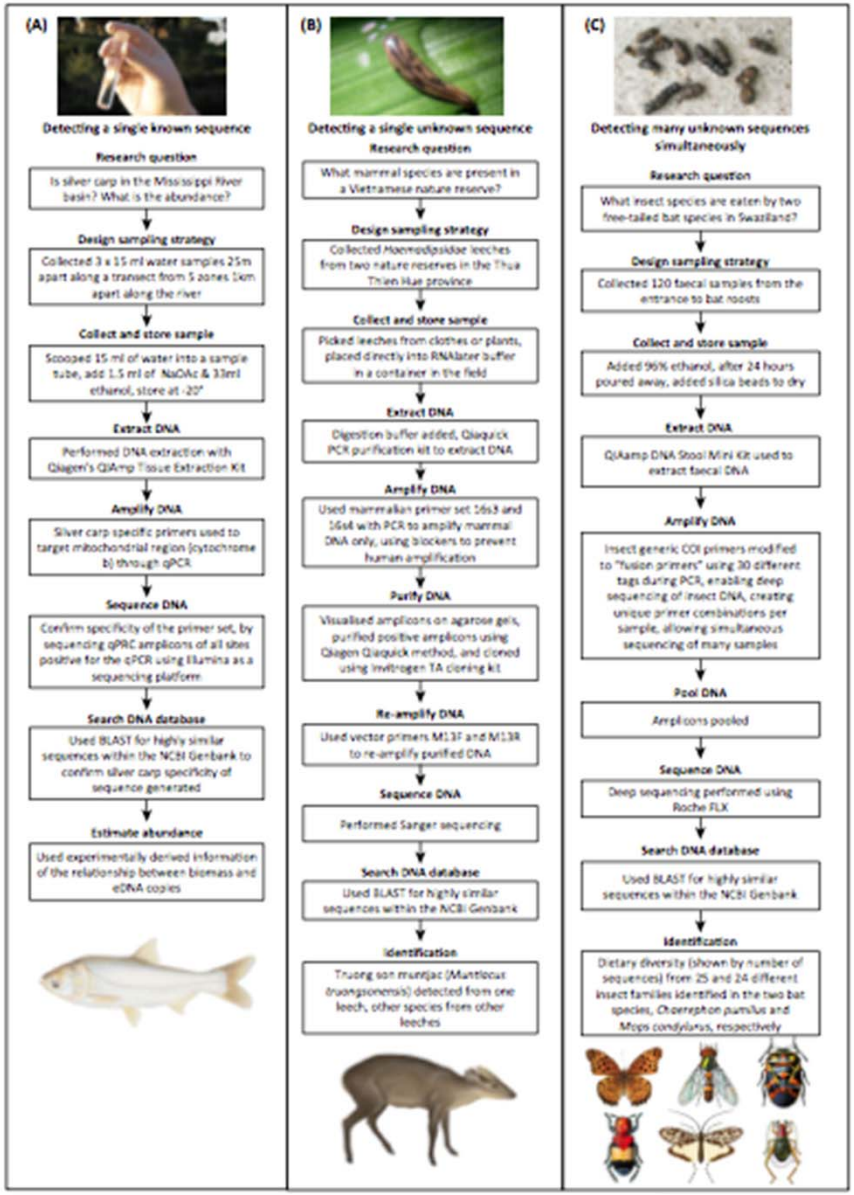
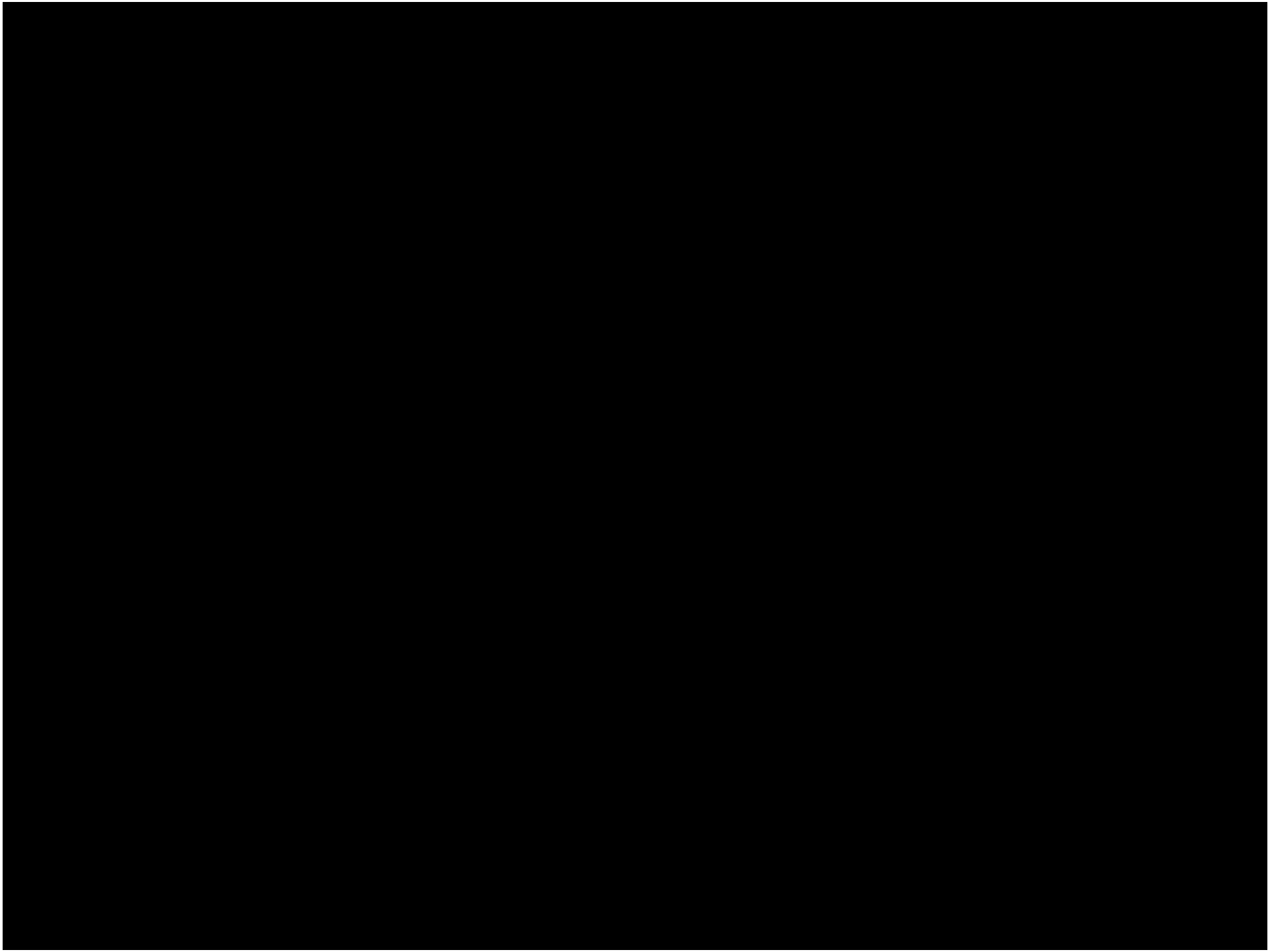
- Can we catalogue the variables that will affect eDNA half-life and can we set standards to determine whether the samples are degraded past the point of use?
- How do we best preserve samples for later analyses of eDNA?
- What are the dispersive properties of eDNA in various environments?
- How readily is eDNA transported between horizons and environments?
- How can we more rapidly and cost-effectively analyse field samples?
- How can we more powerfully and reliably define and assign taxonomies to eDNA sequences?
- How quantitative is eDNA data – can conversion factors be meaningfully implemented to account for sampling, biomass, and amplification biases?



summary

- Enormous taxonomical data gap
- Major challenge is the legacy of old names with poor morphology and no DNA data
- DNA barcode data allow for more pragmatic ‘turbo’ taxonomy
- ‘standard’ and widely used markers such as mtDNA fragments and 18S, is ok
- Population genetic studies show that cryptic species are common
- When barcodes are available, DNA barcode taxonomy data can be analyzed using e.g. eDNA sampling



“BARCODE GAP”

- “Coalescent depth vary among species
- Overlap between intra- and interspecific may be the roule
- Will not compromise identification success (the local gap) for a specific species”

– Dirk Steinke

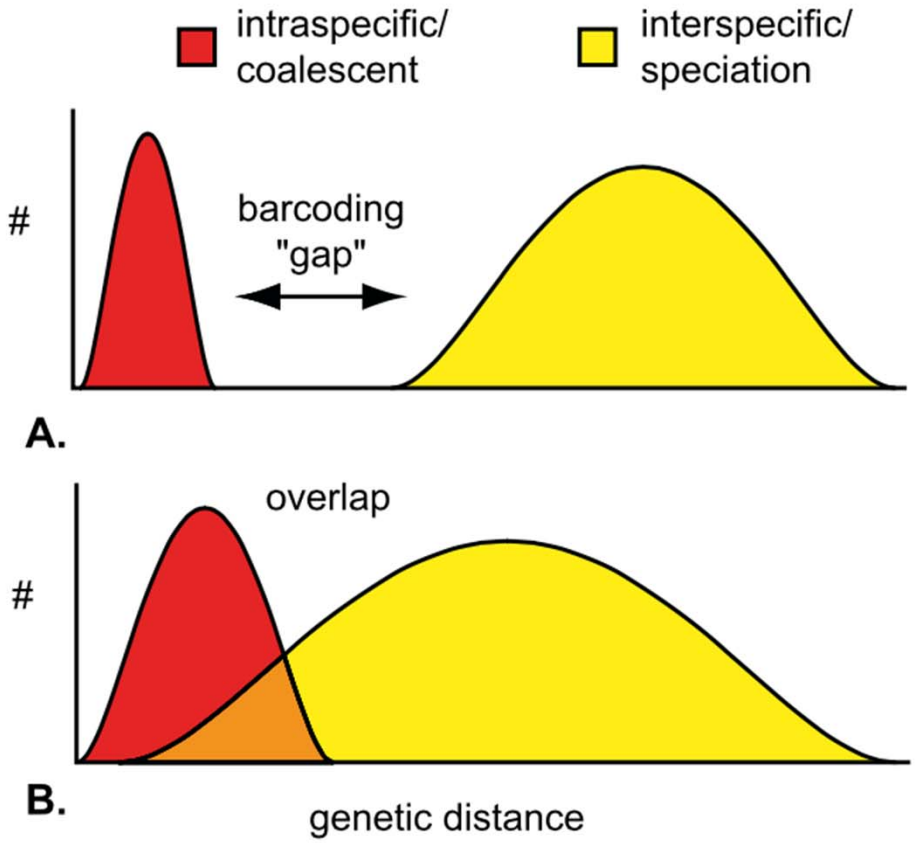


Figure 2. Schematic of the Inferred Barcoding Gap