

***The pros and cons of barcoding:  
some preliminary results from recent cruises to the CCFZ***

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## DNA barcoding

- initiated by Hebert et al. 2002
- intends to use one or a few reference genes in order to assign unknown individuals to known species and facilitate the discovery of unknown species

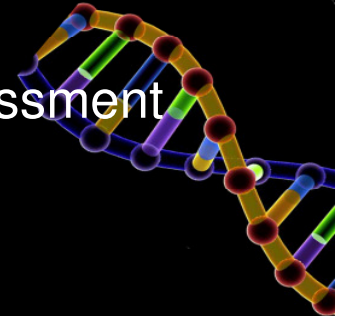
## DNA taxonomy

- proposed by Tautz et al. 2003
- based on the barcoding approach as its practical component
- DNA sequences (not morphological data) should be used as the main criterion for taxonomic decisions
- wants sequences to function as a universal reference systems in biological systematics



reverse taxonomy (Markmann & Tautz 2005)

- integrative molecular-morphological approach:
  - uses initially DNA sequences to construct genetic clusters or molecular operational taxonomic units (MOTUs) based on a similarity threshold
  - subsequently the identity of MOTUs is analyzed using traditional morphological methods
    - in order to test the value of reverse taxonomy for assessment of diversity and species ranges



## why do we need an integrative molecular-morphological approach?

- benthic communities are extremely diverse
- many species occur as singletons
- most species are new to science (~90%)



taxonomic effort required to describe all these species would be tremendous and morpho-species remain provisionally sorted



- hinders morphological comparisons between highly diverse datasets
- the assessment of regional diversity and biogeographic patterns



# SENCKENBERG



Image courtesy IHC Merwede

- is expected to begin within near future



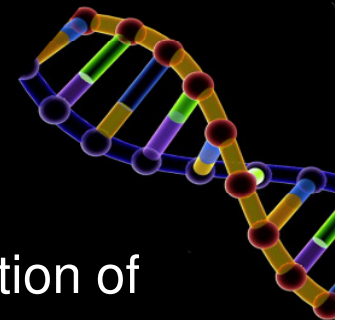
- rapid characterization of the nodule fauna is required

- complementary tools and approaches are needed to speed-up the identification process



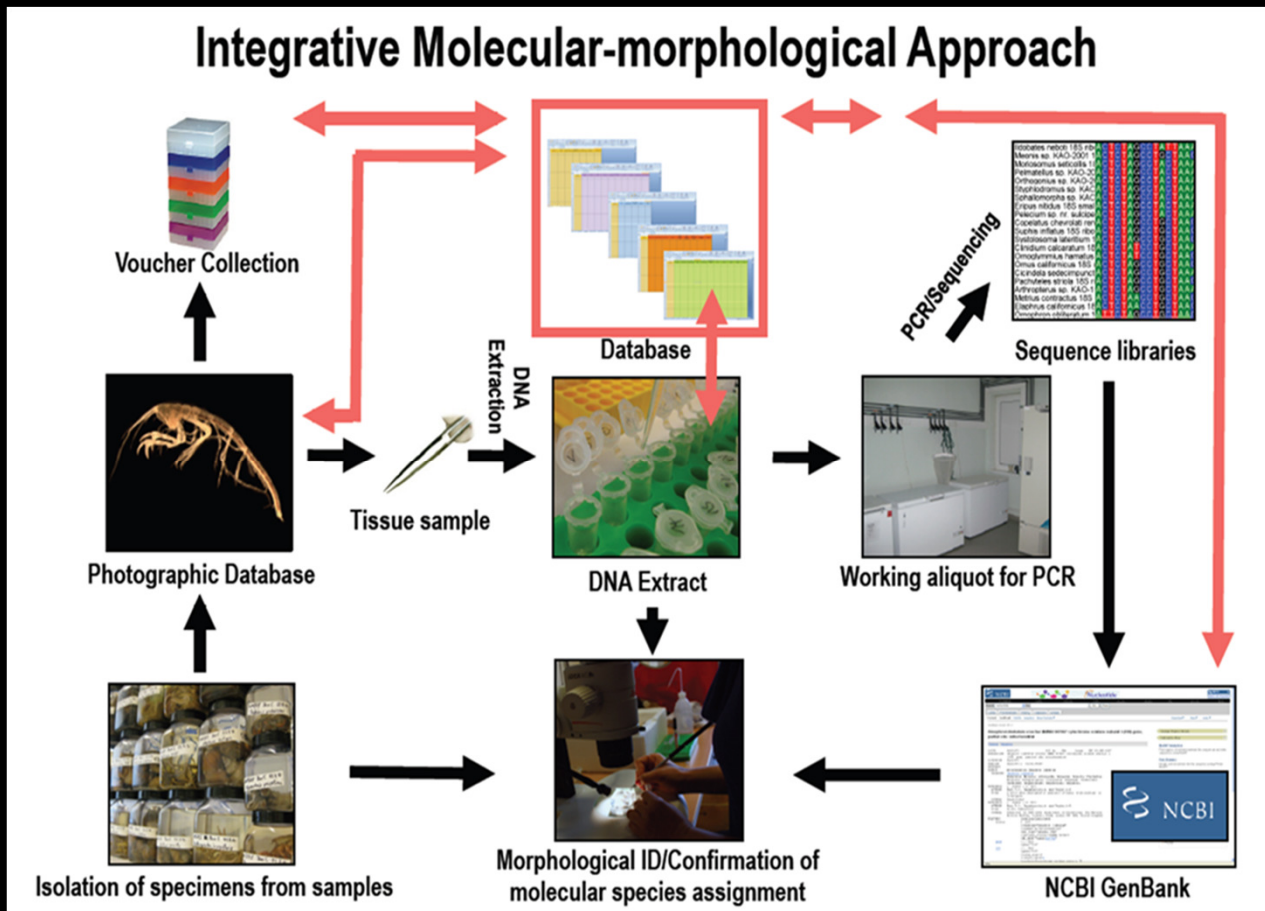
## Reverse taxonomy

- allows for straight-forward allocation of individuals to genotypic clusters
- facilitates comparison, overcoming the time-consuming morphological approach



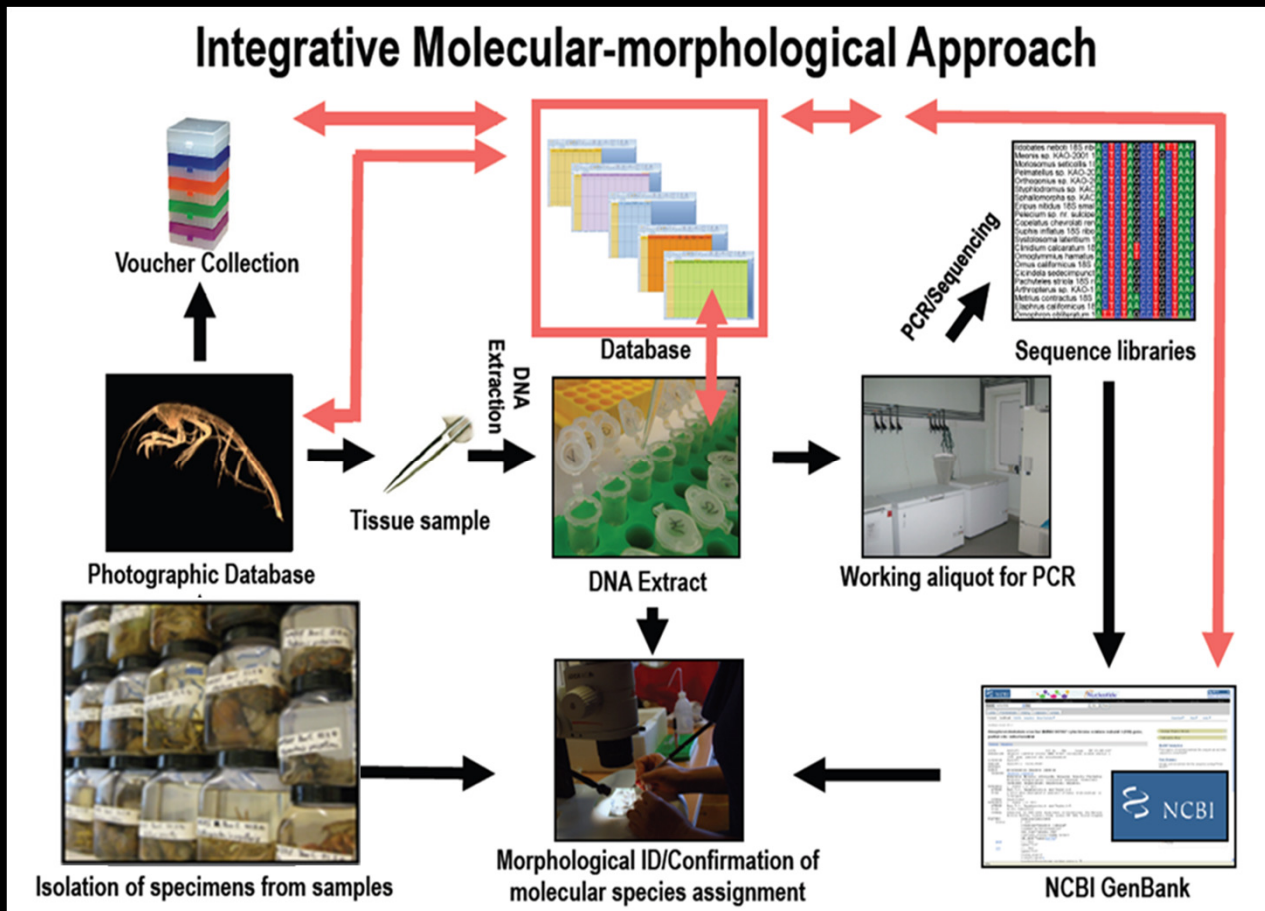
# Material & Methods – treatment of samples

- samples were fixed in pre-cooled undenatured ethanol (96%)
- stored at -20°C for at least 48h (after 24h – replacing with new ethanol)



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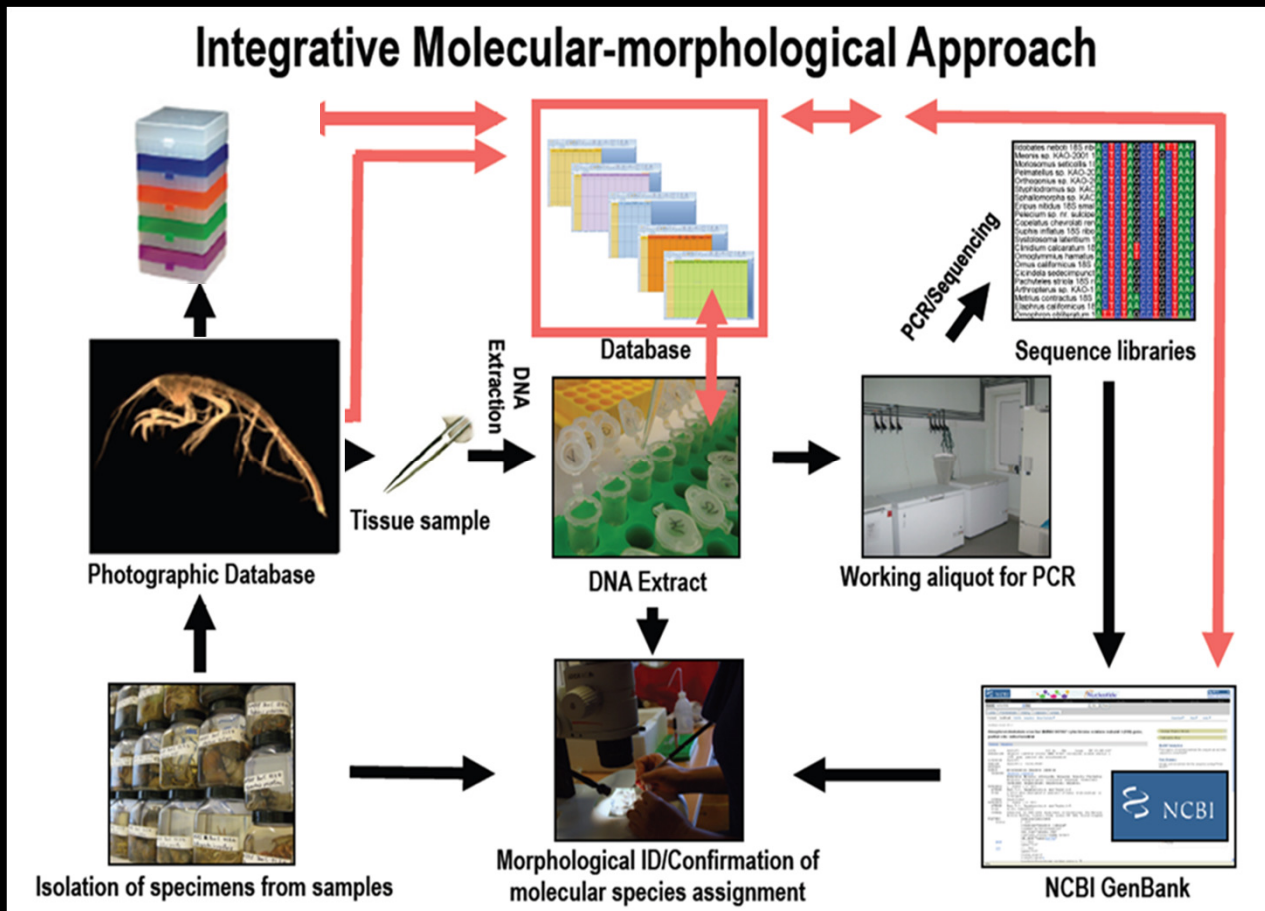
- sorted into separate taxa as soon as possible



- to keep dilution of ethanol low and guarantee preservation of high-quality DNA

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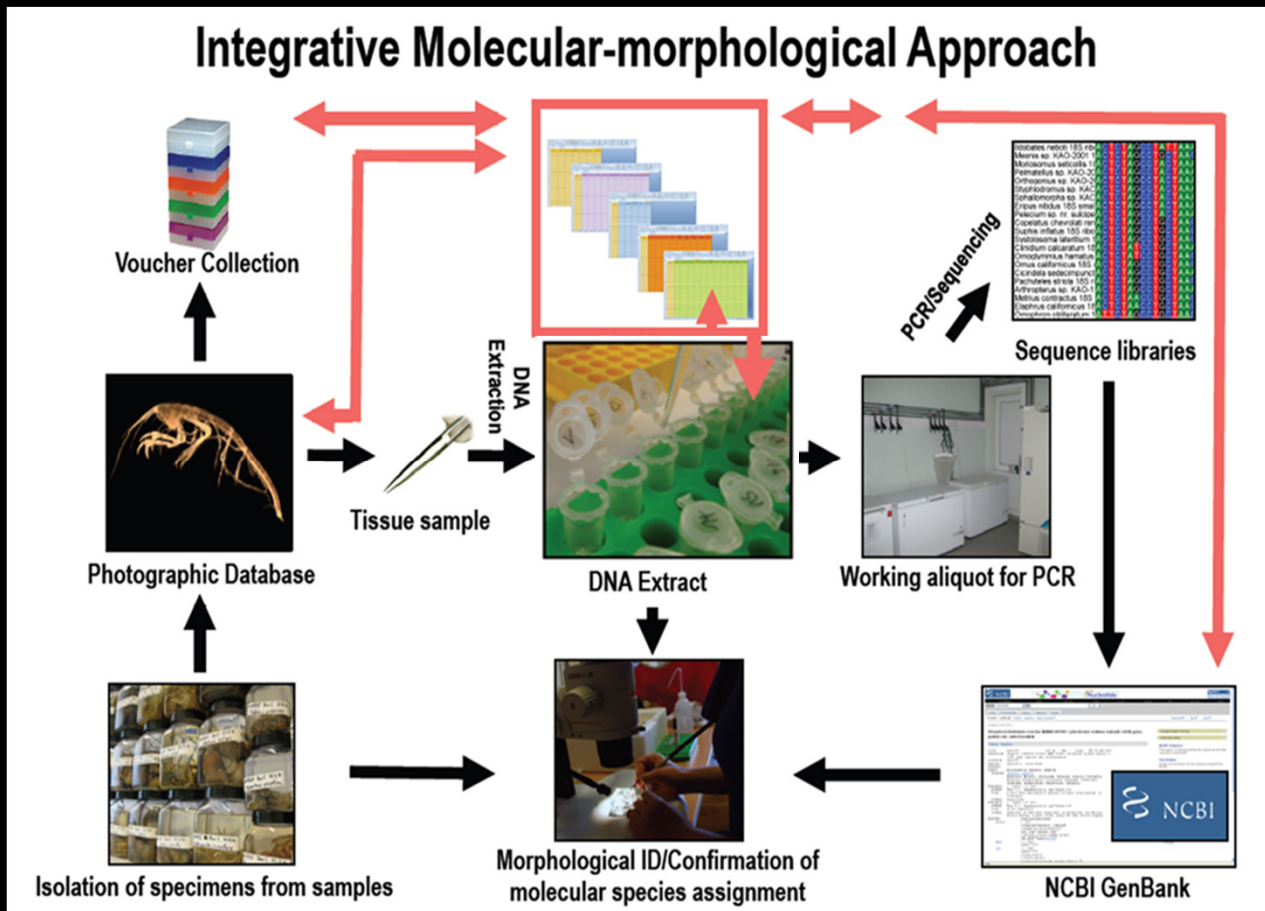


- each specimen was photographed
- tissue dissected
- according to tissue samples voucher were designated collection numbers



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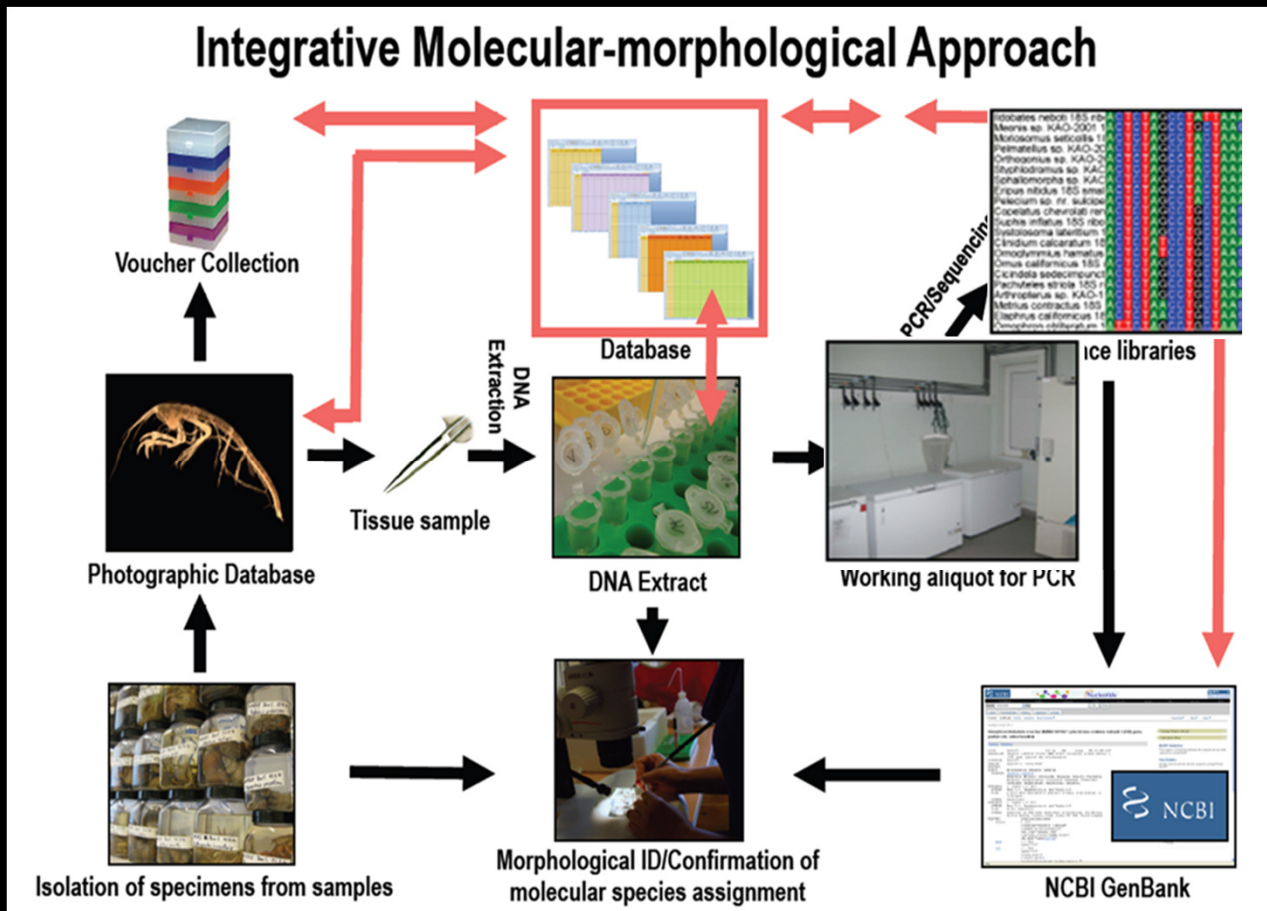
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- Chelex<sup>®</sup> 100 BioRad extraction, quick and dirty
  - extracted DNA not always first quality
  - high amount of DNA
- supernatant was used as template for amplification

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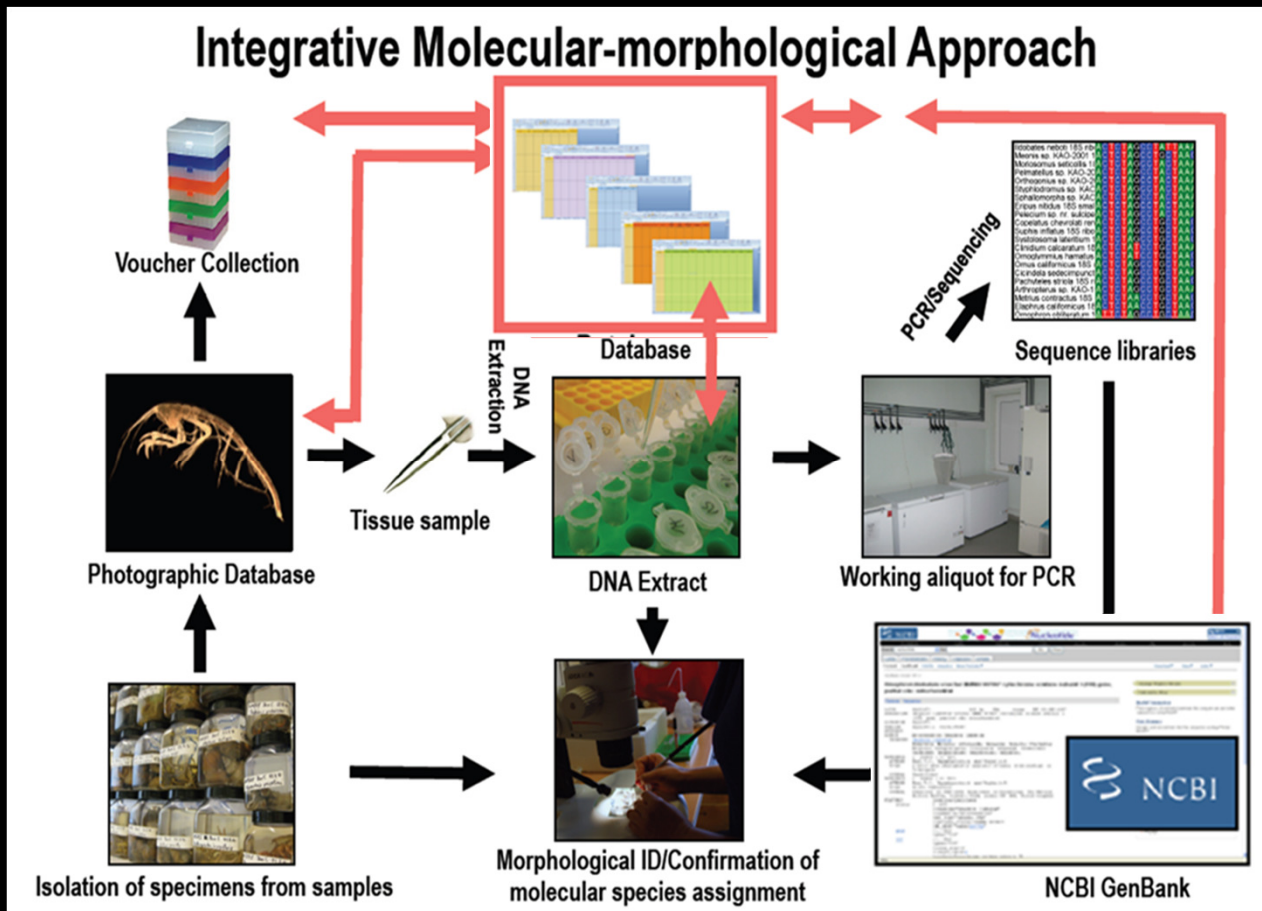
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- mitochondrial COI: universal primers (Folmer 1994) LCO1490, HC02198
- PCR-products were sent for sequencing to MACROGEN

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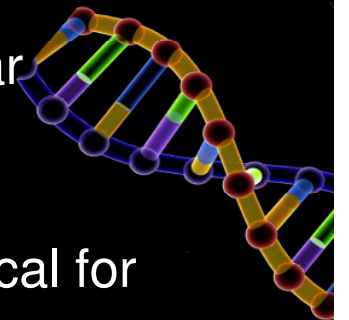


- sequences were blasted in NCBI
- all information collected should be entered in an own database



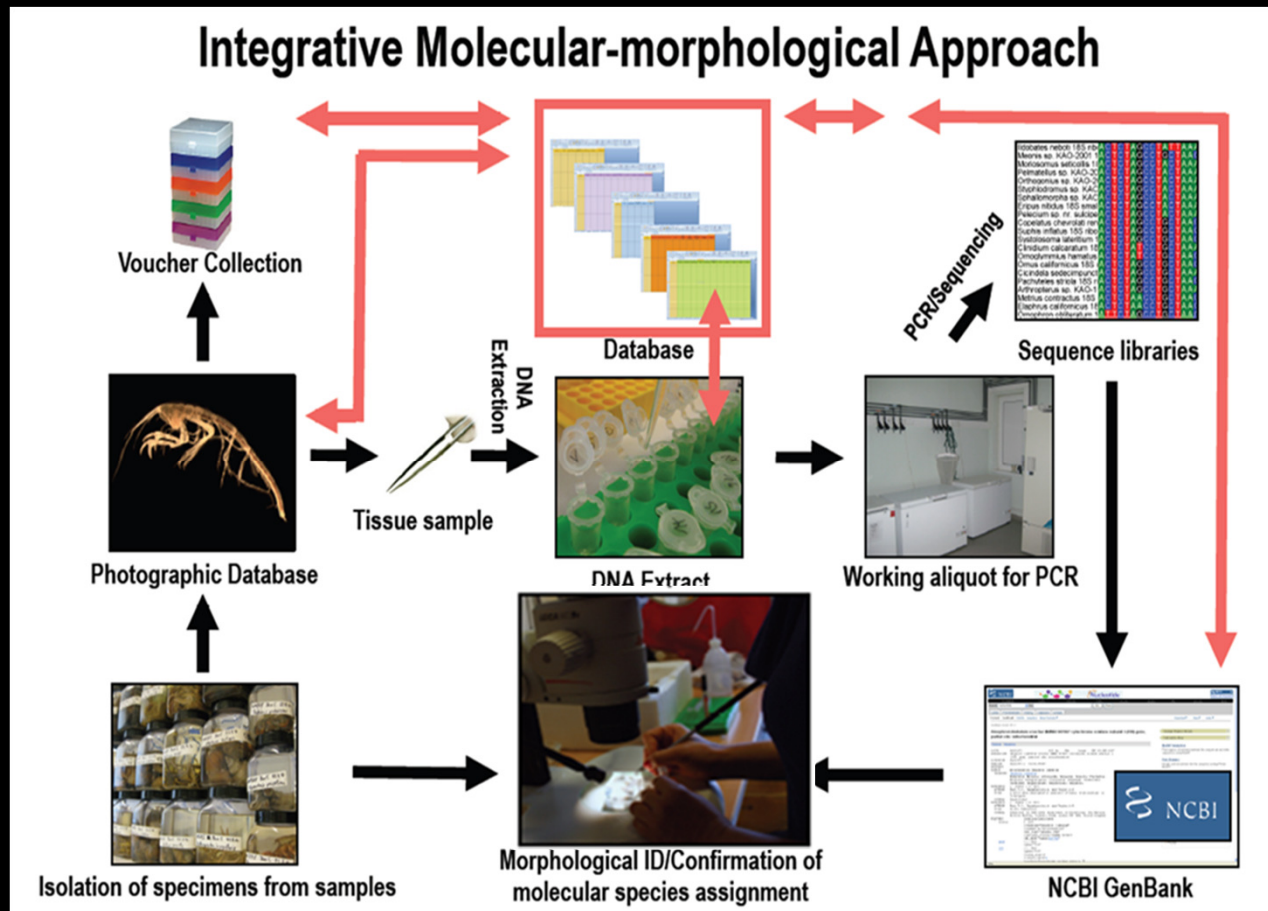
## Material & Methods - treatment of sequences for genetic analyses

- online tool **CD-Hit Suite** (Ying et al. 2010) for identifying MOTUs using pairwise alignment with a defined similarity threshold:
  - sorts sequences in decreasing length order
  - longest sequence → reference of first cluster
  - each sequence is compared pairwise to the reference
  - if sequence similarity exceed the pre-defined threshold → assignment to this cluster
  - if not → a new cluster is defined using this sequence as reference
- thresholds used to define MOTUs is a primary concern for molecular taxonomy, when intraspecific variation is high
  - other approaches (GMYC / ABGD) are not appropriate
    - high number of MOTUs and high number of singletons, typical for deep-sea



# Material & Methods – treatment of samples

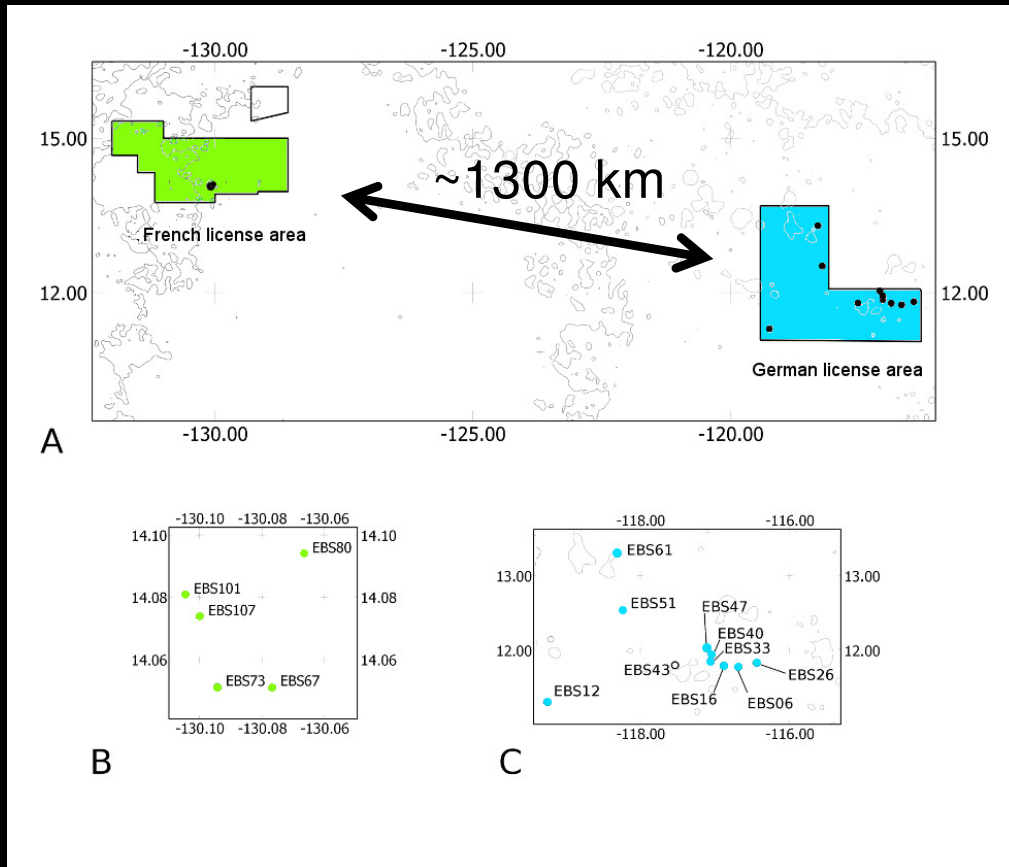
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- identity of MOTUs was analyzed using morphological methods



- merits and pitfalls of DNA taxonomy

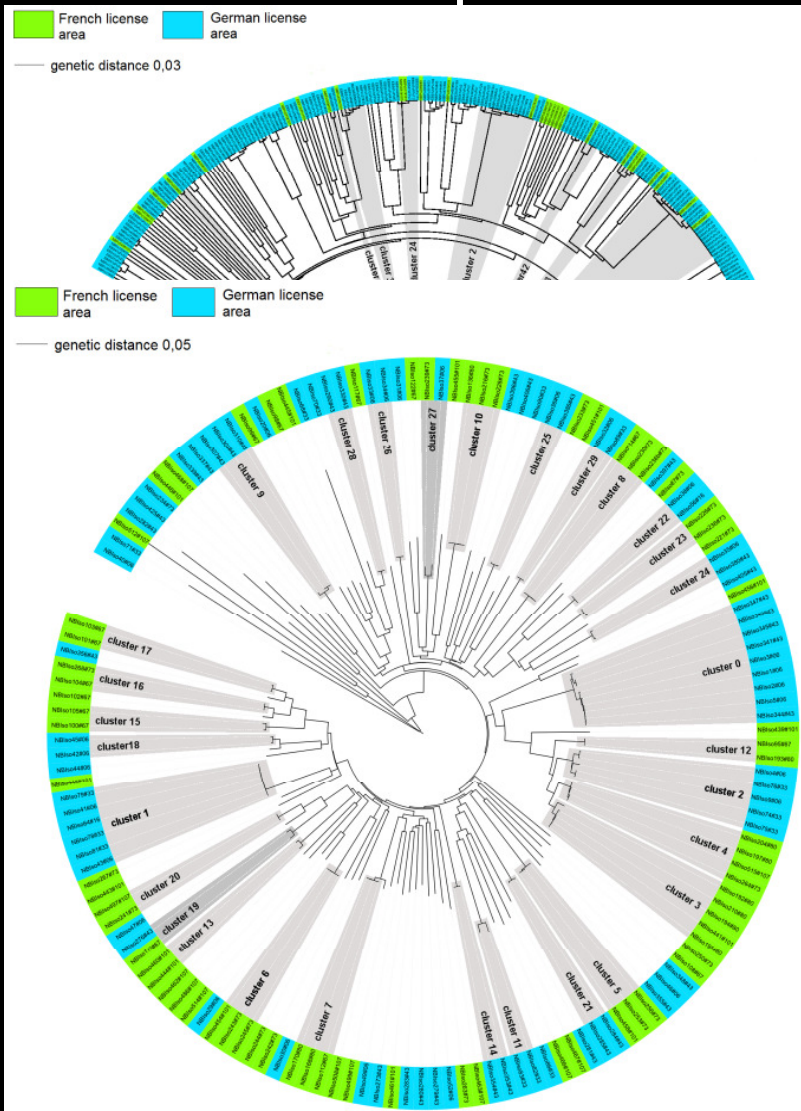


- variation in genetic diversity and distribution ranges of isopods and polychaetes across the CCZ
- faunal assemblages were studied from two mining claims (German and French license areas) being located 1300 km apart

from Janssen et al. submitted



• merits and pitfalls of DNA taxonomy



- most fruitful advantage: suitable programs can be conducted very fast and automated to construct MOTUs

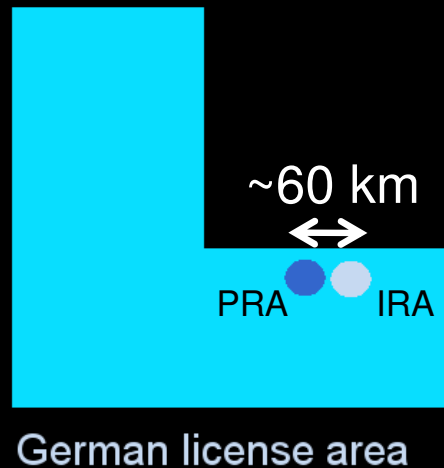
German vs French license area

Polychaeta: 556 sequences - 233 MOTUs  
60% singletons  
12% MOTUs shared

Isopoda: 150 sequences – 95 MOTUs  
70% singletons  
2% MOTUs shared



## merits and pitfalls of DNA taxonomy

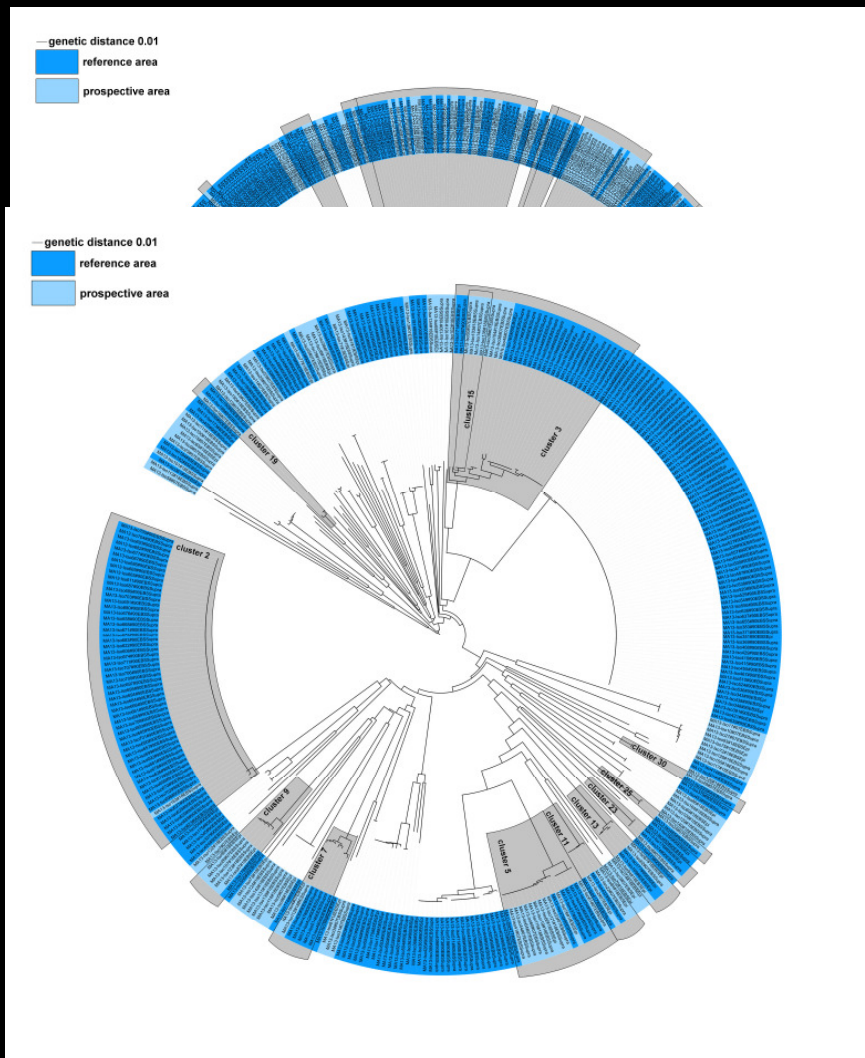


- examined variation in genetic diversity and small-scale connectivity within the German license area
- faunal assemblages were studied from two reference areas
  - IRA impact reference area
    - mining area
  - PRA preservation reference area
    - no mining
- both areas being located 60 km apart.





# merits and pitfalls of DNA taxonomy



preservation vs impact area

Polychaeta: 550 sequences - 147 MOTUs  
 45% singletons  
 27% MOTUs shared

Isopoda: 349 sequences – 83 MOTUs  
 50% singletons  
 13% MOTUs shared

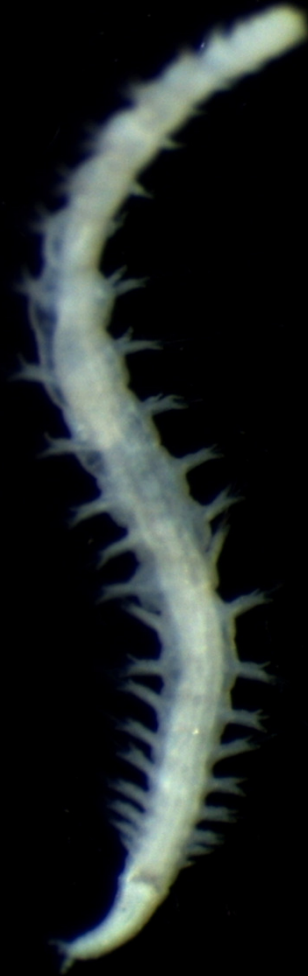


time consuming work of morphology reduced  
 to half of all specimens

- **the disadvantage: morphological identities and ecological functions remain unknown**



## merits and pitfalls of DNA taxonomy



- **the advantage: easy to compare sequences (i.e. GenBank)**

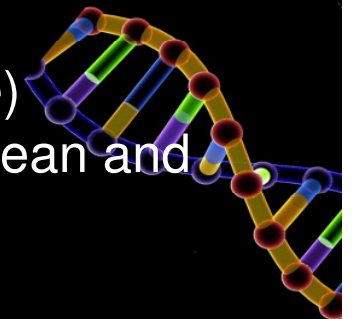
polychaete voucher MA10-Po88 corresponded to GenBank Accession No. GQ426633 with a sequence similarity of 99.2%



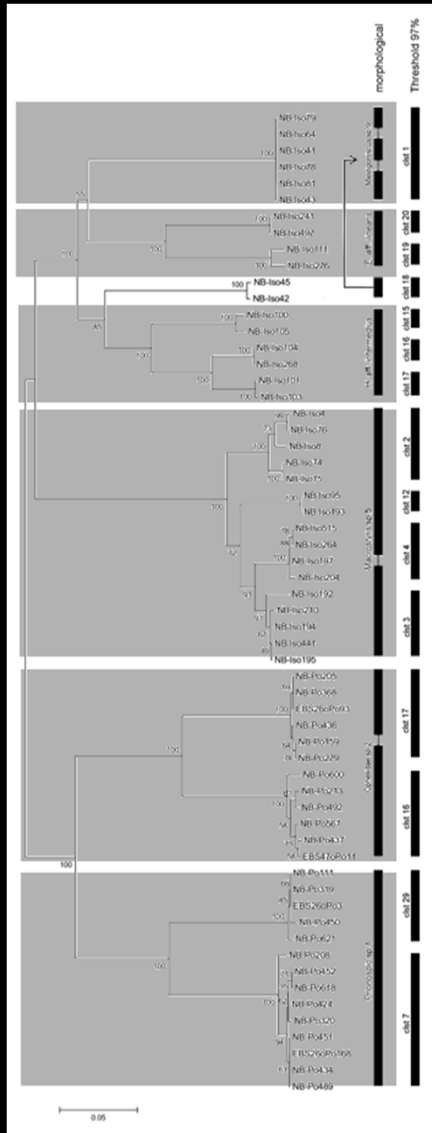
*Bathyglycinde profunda* (Goniadidae)  
-previously recorded : Atlantic Ocean and CCFZ

- **the disadvantage: DNA barcodes can only assign to known species, but not relate them to a species name if unknown**

*Bathyglycinde profunda* (Hartman & Fauchald, 1971)



## merits and pitfalls of DNA taxonomy

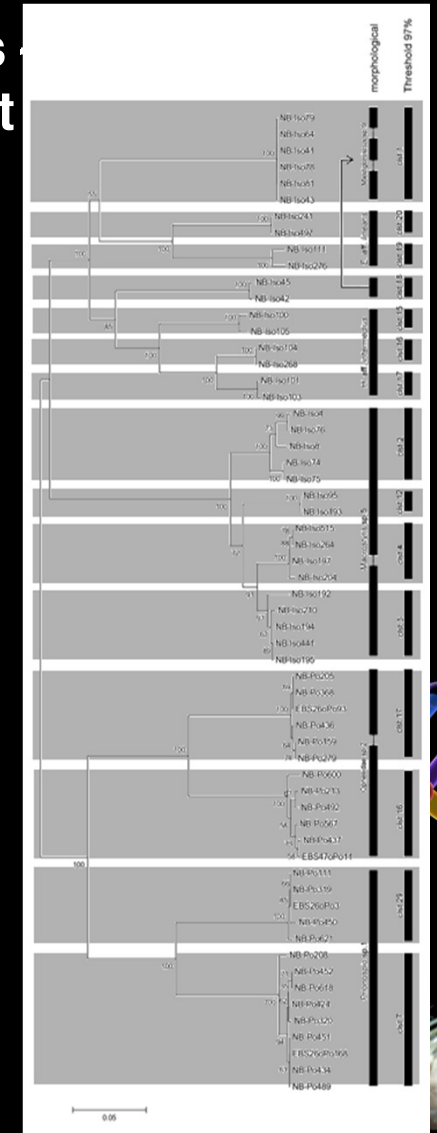


- the advantage: discovery of cryptic species morphologically similar, but genetic distinct

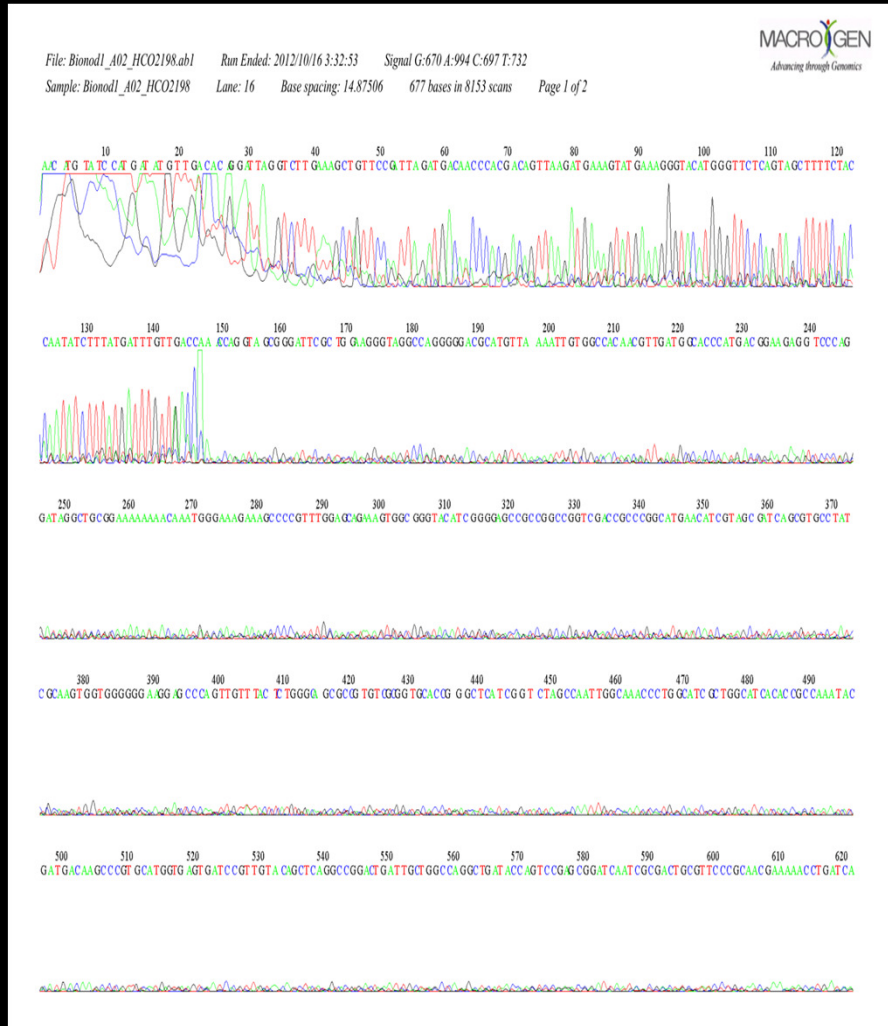
← 6 morpho-species

vs. 15 MOTUs →

- genetic diversity was found to be 2-3 times higher than morphological diversity
- the disadvantage: overestimating the number of species when nuclear mitochondrial pseudogenes (numts) are coamplified  
→ especially problematic by using COI



## merits and pitfalls of DNA taxonomy



- **most serious problem: low sequencing success for abyssal species**
  - success rate ~ 40%-50%
- approach of DNA taxonomy in deep-sea studies is accompanied with a loss of information about a species community
  - analyses need to be adjusted



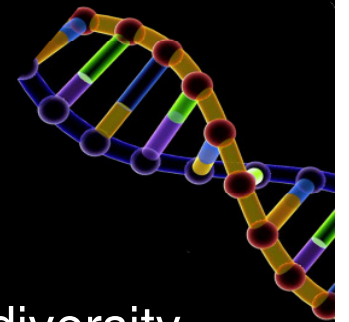
# SENCKENBERG

- integrative molecular-morphological approach (i.e. reverse taxonomy) appears robust and successful for a provisional inventory:
  - MOTUs mostly corresponded to morphologically identified species
  - due to integration of morphological methods → overcome most disadvantages of DNA taxonomy:
    1. morphological identities and ecological functions of MOTUs do not remain unknown
      - time-consuming morphological work can be reduced to MOTUs, which are represented frequently
    2. MOTUs are assigned to morpho-species categories at least
    3. overestimation of species due to pseudogenes can be neglected
      - aminoacid- translation prior to MOTU clustering
      - morphological determination of MOTUs



## conclusions and recommendations

- assessing the mostly undescribed biodiversity of benthic communities in license areas within the CCZ is a challenge
- a chance for joint efforts and unparalleled collections
- barcodes can be easily exchanged between contactors and published in online repositories (GenBank, BOLD)
  - barcodes are of tremendous help and should become standard procedure
  - integrative approach provides the most robust estimates on biodiversity and species ranges so far



# Outlook

to improve the work in future:

- amplification of further mitochondrial and nuclear genes (i.e. to solve cryptic species-complexes, mismatch of MOTUs and morpho-species, higher sequencing success)
- population genetic analyses (i.e. RADtag / Tajima's D) for investigating genetic drift in CCFZ
- establishing a database for barcodes and additional corresponding informations (i.e photos, morphological characters etc.) of specimens from the CCFZ



# THANKS!

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