



Integrated North Sea zooplankton identification: A combination of morphology, DNA and proteome analyses towards a cost and time-effective application

Silke Laakmann, Thomas Knebelsberger, Sabine Holst

German Center for Marine Biodiversity Research (DZMB), Senckenberg Institute, Germany

MOTIVATION

Accurate species identification as essential step in various studies

Challenges in morphological species identification:

- Young developmental stages (Holo- and Meroplankton, i.e. eggs, larvae)
- Distinct life-history forms (i.e. metagenic species)
- Phenotypic variations
- Sibling / cryptic species (co-occurrence)

Ensure species identification by using integrated morphological & molecular taxonomic approaches

MOTIVATION

Accurate species identification as essential step in various studies

Challenges in morphological species identification:

- Young developmental stages (Holo- and Meroplankton, i.e. eggs, larvae)
- Distinct life-history forms (i.e. metagenic species)
- Phenotypic variations
- Sibling / cryptic species (co-occurrence)

Ensure species identification by using integrated morphological & molecular taxonomic approaches

→ Need to establish a valid Reference Library due to increasing interests in molecular-based diversity approaches (i.e. high-throughput DNA sequencing techniques)

METHODOLOGY

1. Sampling and sorting

(Calcofi 300, 500 μm , Apstein 150 μm mesh size)

2. Morphological identification

(Specimens with diagnostic morphological characters)

3. Molecular analysis

a) DNA sequence analyses

- mitochondrial cytochrome c oxidase subunit I (COI)
- nuclear ribosomal 18S rDNA

b) Proteomic Fingerprinting

- MALDI-TOF Mass Spectrometry

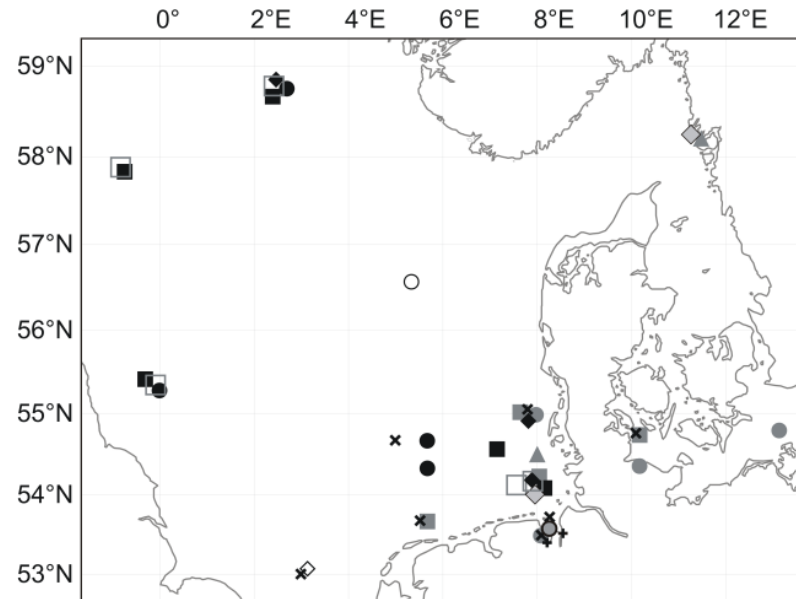
4. Establishment of Reference Library by the comparison / integration of identifications



Calanoid Copepods

Morphological identifications (333 specimens)

→ 12 species based on morphology

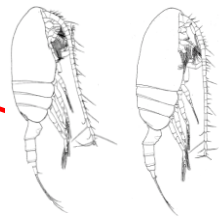
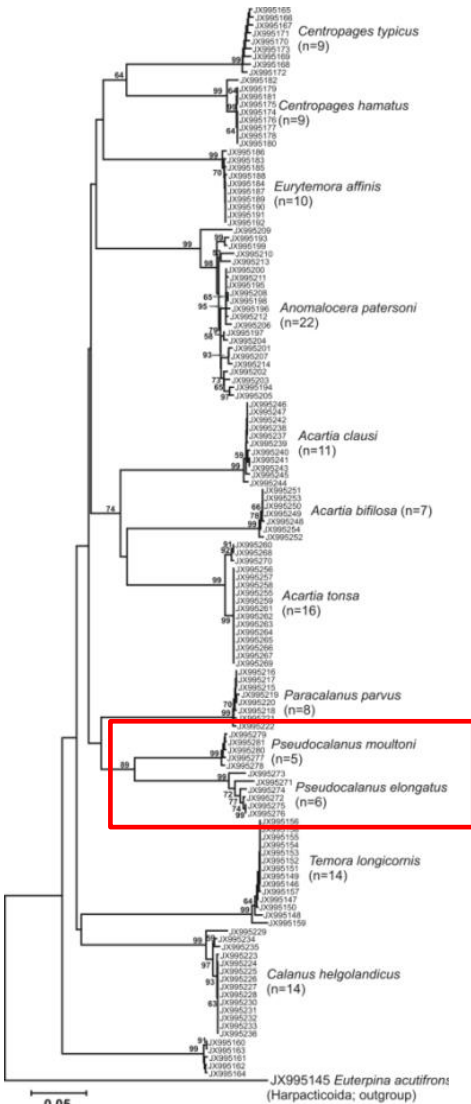


Acartia clausi
Acartia bifilosa
Acartia tonsa
Anomalocera patersoni
Calanus helgolandicus
Centropages hamatus
Centropages typicus
Eurytemora affinis
Isias clavipes
Paracalanus parvus
Pseudocalanus elongatus.
Temora longicornis

Calanoid Copepods

Molecular identifications – Mitochondrial COI (136 specimens CVI F,M; 657bp)

→ 13 species based on COI (≥11.7%)



Siblings

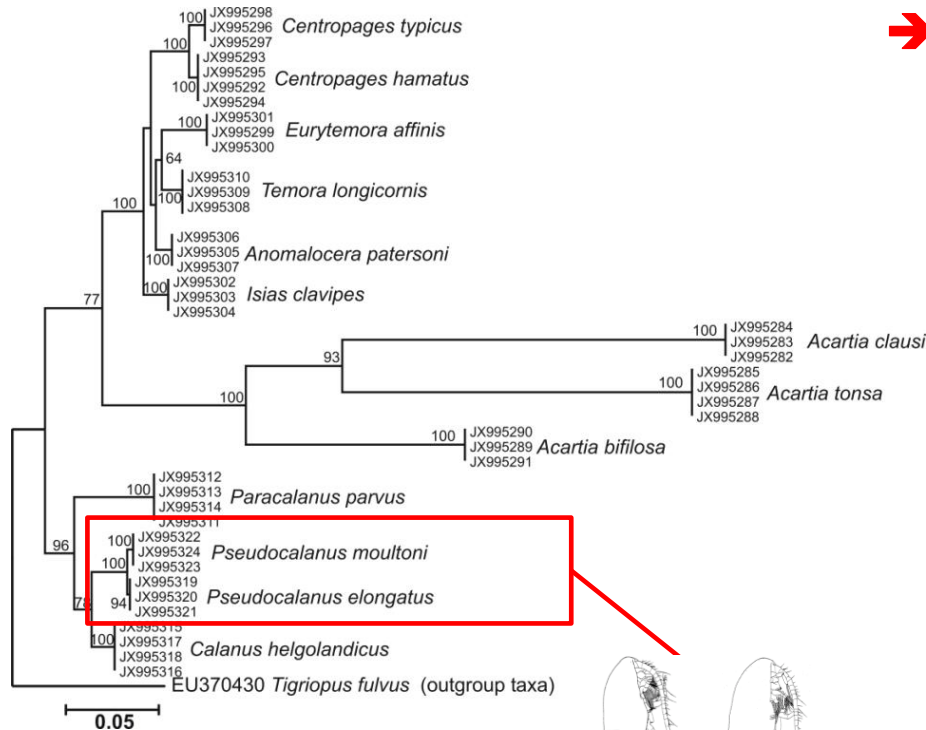
- Acartia clausi*
- Acartia bifilosa*
- Acartia tonsa*
- Anomalocera patersoni*
- Calanus helgolandicus*
- Centropages hamatus*
- Centropages typicus*
- Eurytemora affinis*
- Isias clavipes*
- Paracalanus parvus*
- Pseudocalanus elongatus*
- Pseudocalanus moultoni*
- Temora longicornis*

P. moultoni: New in the NE Atlantic or always there but not identified?

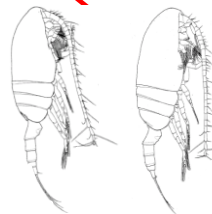
Calanoid Copepods

Molecular identifications – Nuclear 18S rDNA (43 specimens CVI F,M; 1844 bp)

→ 13 species based on 18S rDNA ($\geq 0.5\%$)



- Acartia clausi*
- Acartia bifilosa*
- Acartia tonsa*
- Anomalocera patersoni*
- Calanus helgolandicus*
- Centropages hamatus*
- Centropages typicus*
- Eurytemora affinis*
- Isias clavipes*
- Paracalanus parvus*
- Pseudocalanus elongatus***
- Pseudocalanus moultoni***
- Temora longicornis*

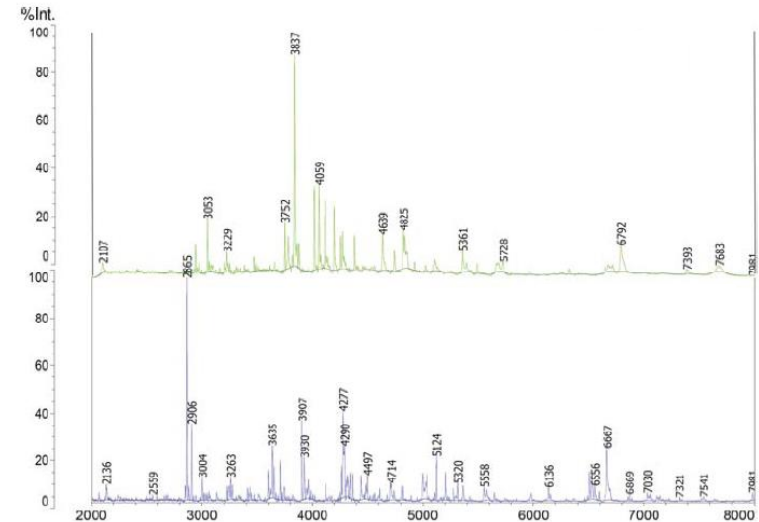


Siblings

Proteomic Fingerprinting: MALDI-TOF MS

(Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry)

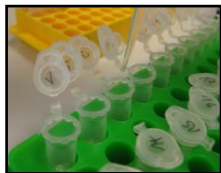
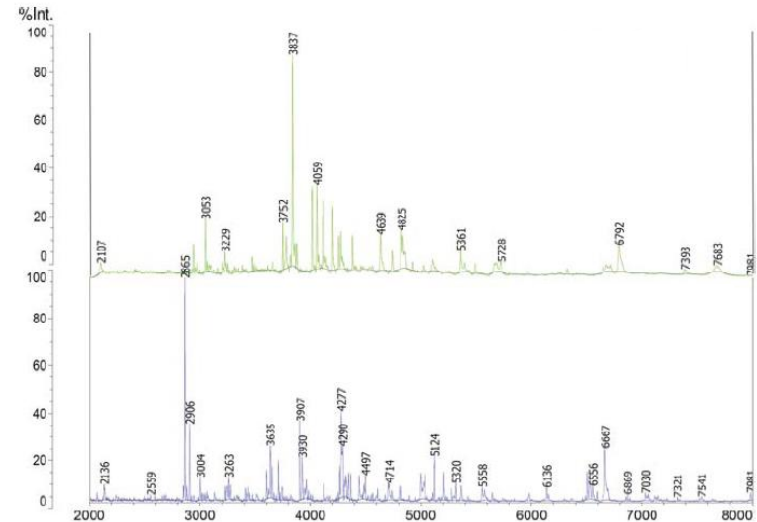
- Established in microbiology for routine identification of prokaryotic species & strains (e.g. industry, hospitals)
- Comparison of species-specific mass spectra (2 – 20 kDa)



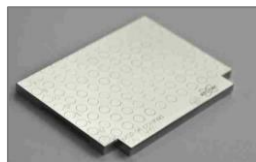
Proteomic Fingerprinting: MALDI-TOF MS

(Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry)

- Established in microbiology for routine identification of prokaryotic species & strains (e.g. industry, hospitals)
- Comparison of species-specific mass spectra (2 – 20 kDa)



Protein extraction:
10 min in matrix
(cinnamic acid)



Extract spotted on target
plate & evaporates for
several minutes



Sample analysis:
96 samples ~ 1.5 h



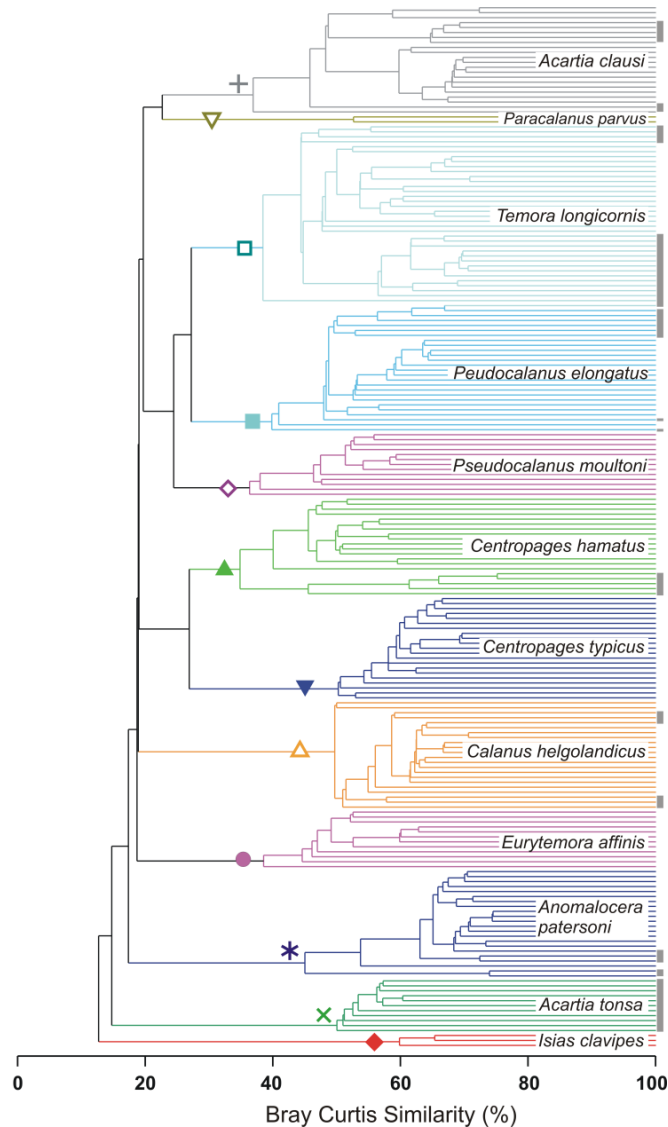
Comparative analyses:

- MALDI Biotyper (directly)
- Excel-based analyses, Primer6
- R packages

➔ **Benefit: Time- and cost-efficient!!!**

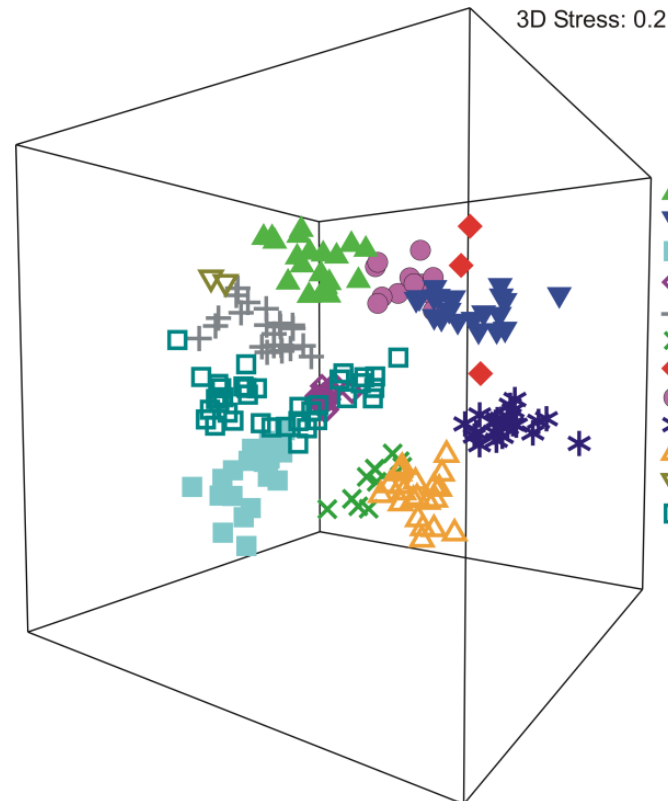
Calanoid Copepods

Molecular identifications – Proteomic Fingerprinting (210 specimens: N, CI-VIF, M)



→ **12 species** (*Acartia bifilosa* was not analysed)

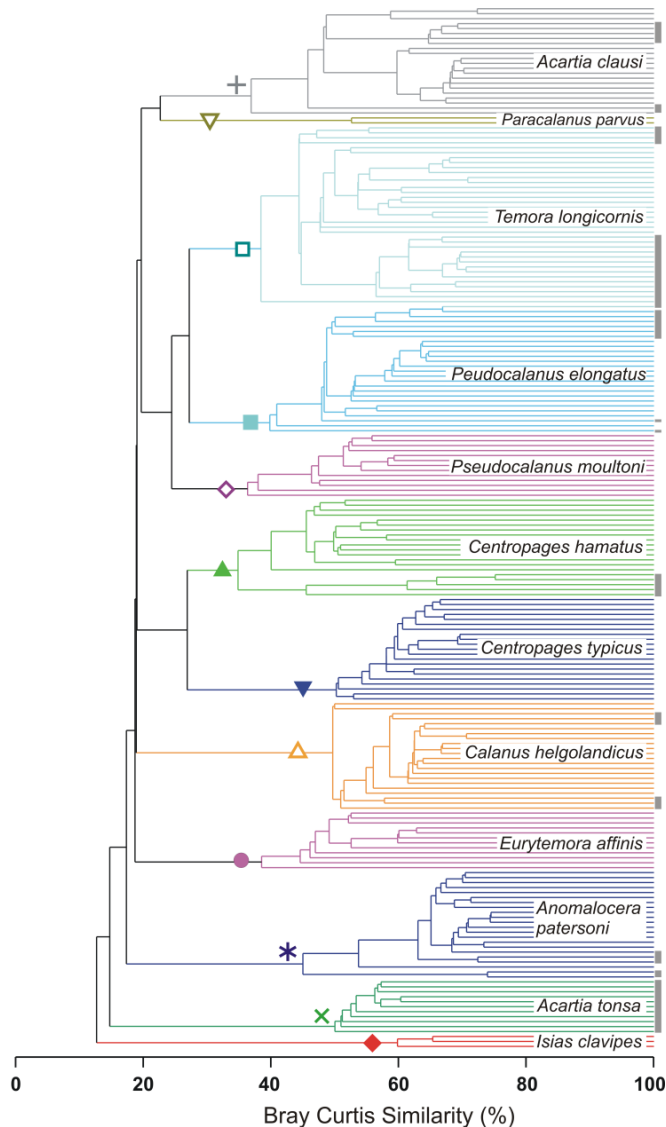
Nauplii, CI-CV



- ▲ *Centropages hamatus*
- ▼ *Centropages typicus*
- *Pseudocalanus elongatus*
- ◇ *Pseudocalanus moultoni*
- +
- × *Acartia tonsa*
- ◆ *Isias clavipes*
- *Eurytemora affinis*
- * *Anomalocera patersoni*
- △ *Calanus helgolandicus*
- ▽ *Paracalanus parvus*
- *Temora longicornis*

Calanoid Copepods

Molecular identifications – Proteomic Fingerprinting (210 specimens: N, CI-VIF, M)

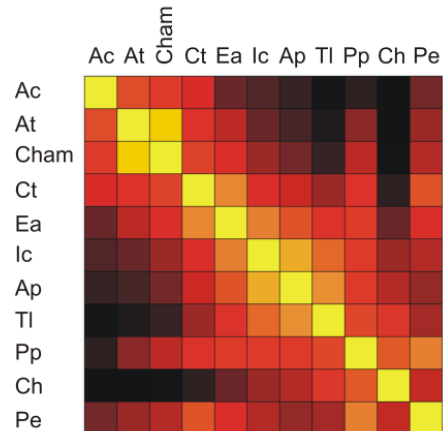


→ **12 species** (*Acartia bifilosa* was not analysed)

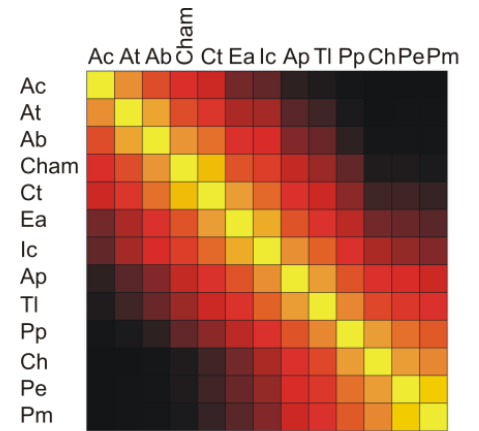
Nauplii, CI-CV



→ **Comparable species identifications**



Proteome Fingerprints



COI sequence analysis

Morphological identifications (167 specimens)

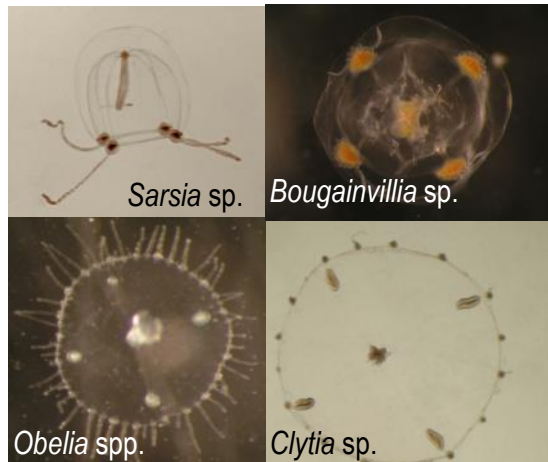
08/2010 – 08/2012
Helgoland Roads
Jade & Elbe Estuary



→ 18 species & 4 to genus only
based on morphology

- Aglantha digitale*
- Codonium proliferum*
- Corymorpha nutans*
- Ectopleura dumortierii*
- Eirene viridula*
- Eucheilota maculata*
- Eutima gegenbauri*
- Eutima gracilis*
- Eutonina indicans*
- Helgicirrha schulzei*
- Lizzia blondina*
- Leuckartiara octona*
- Melicertum octocostatum*
- Nemopsis bachei*
- Rathkea octopunctata*
- Sarsia tubulosa*
- Stauridiosarsia gemmifera*
- Tiaropsis multicirrata*

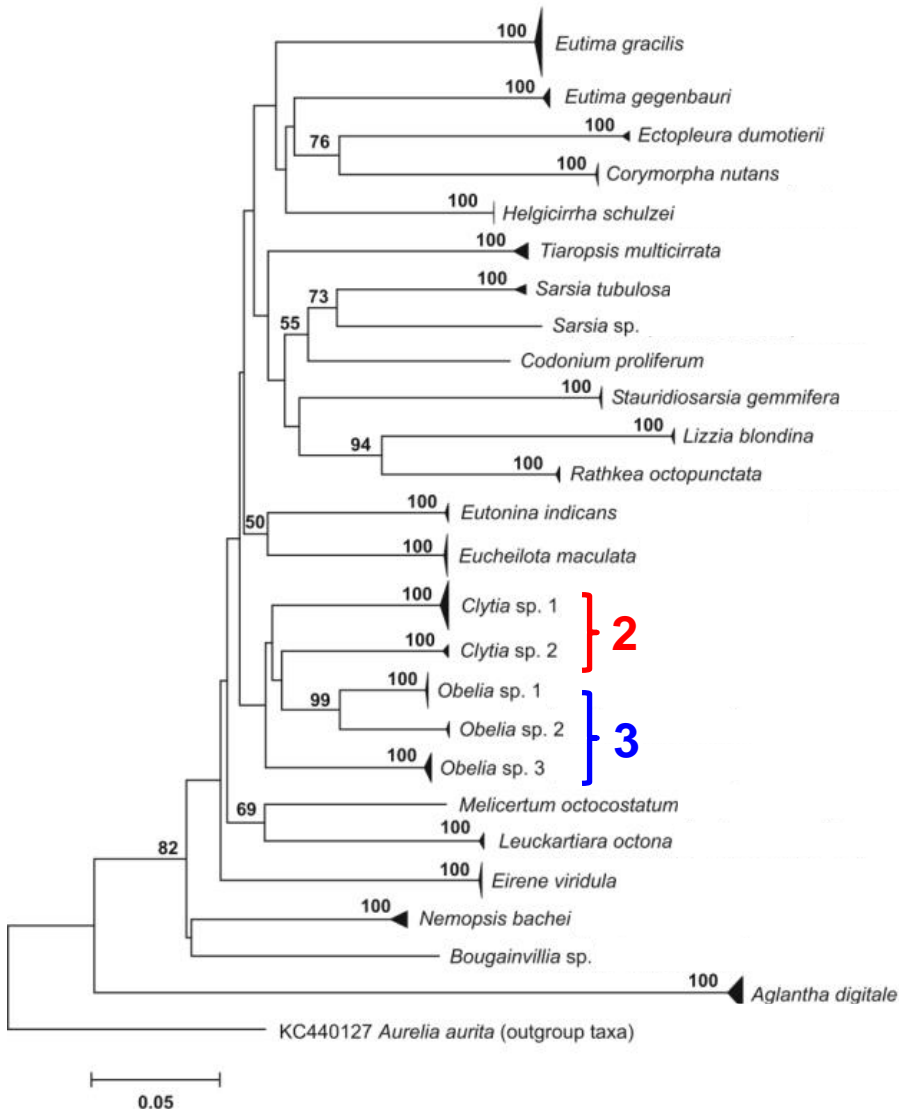
- Bougainvillia* sp.
- Sarsia* sp.
- Clytia* sp.
- Obelia* sp.



SENCKENBERG

Hydromedusae

Molecular identifications – Mitochondrial COI (167 specimens, 658 bp)



→ 25 species based on COI (≥5.4%)

✓ 20 species

Aglantha digitale
Codonium proliferum
Corymorpha nutans
Ectopleura dumortierii
Eirene viridula
Eucheilota maculata
Eutima gegenbauri
Eutima gracilis
Eutonina indicans
Helgicirra schulzei
Lizzia blondina
Leuckartiara octona
Melicertum octocostatum
Nemopsis bachei
Rathkea octopunctata
Sarsia tubulosa
Stauridiosarsia gemmifera
Tiaropsis multicirrata

Bougainvillia sp.
Sarsia sp.
Clytia sp. 1
Clytia sp. 2

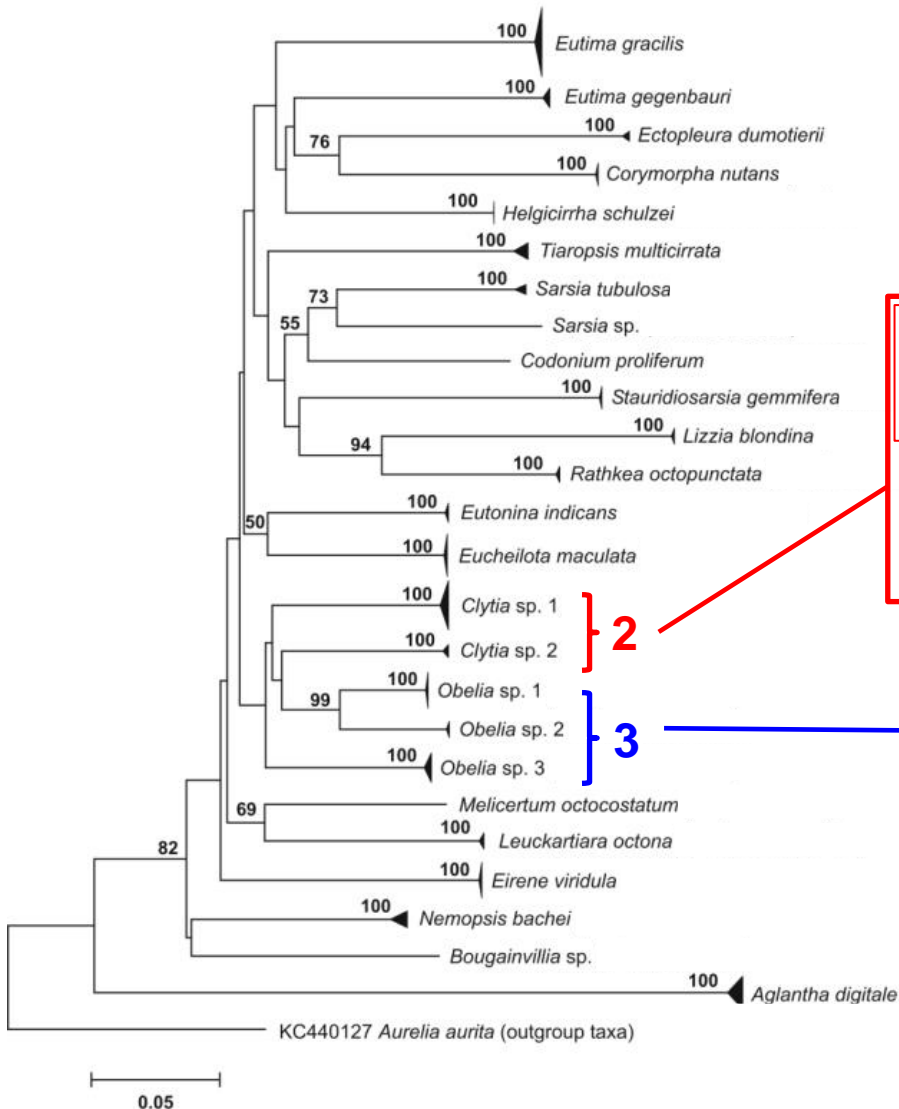
Obelia sp. 1
Obelia sp. 2
Obelia sp. 3

SENCKENBERG

Hydromedusae

Molecular identifications – Mitochondrial COI (167 specimens, 658 bp)


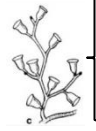
→ 25 species based on COI (≥5.4%)



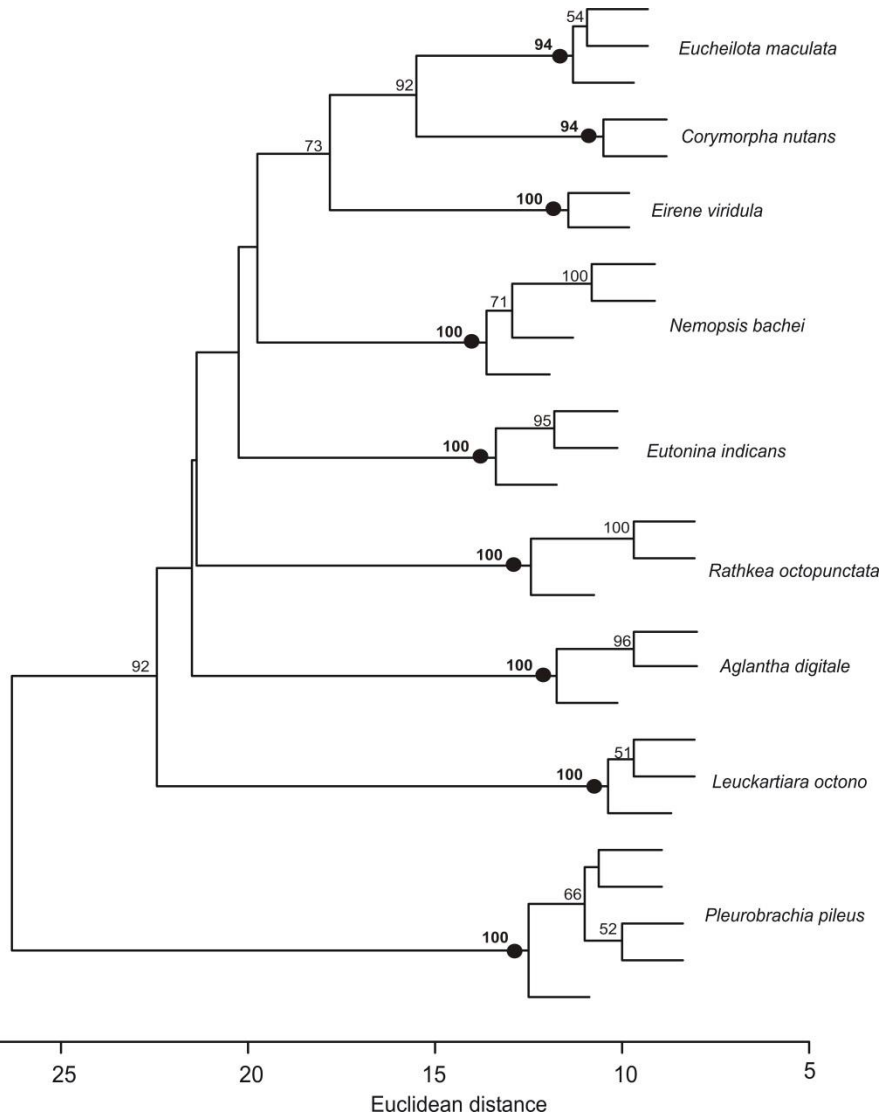
C. languida: New in the NE Atlantic or always there but not identified?

 &  ***Clytia hemisphaerica***
Clytia languida

In European and West-African waters *hemisphaericum*.
In American waters *languidum*.
Kramp 1959

 &  ***Obelia dichotoma***
Obelia geniculata
Obelia longissima

Molecular identifications – Proteomic Fingerprinting (28 specimens)



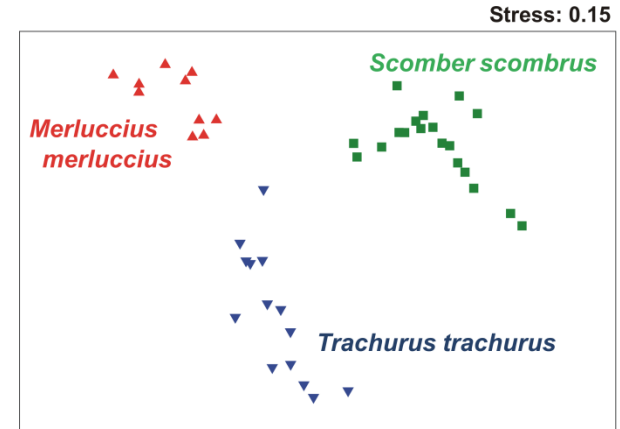
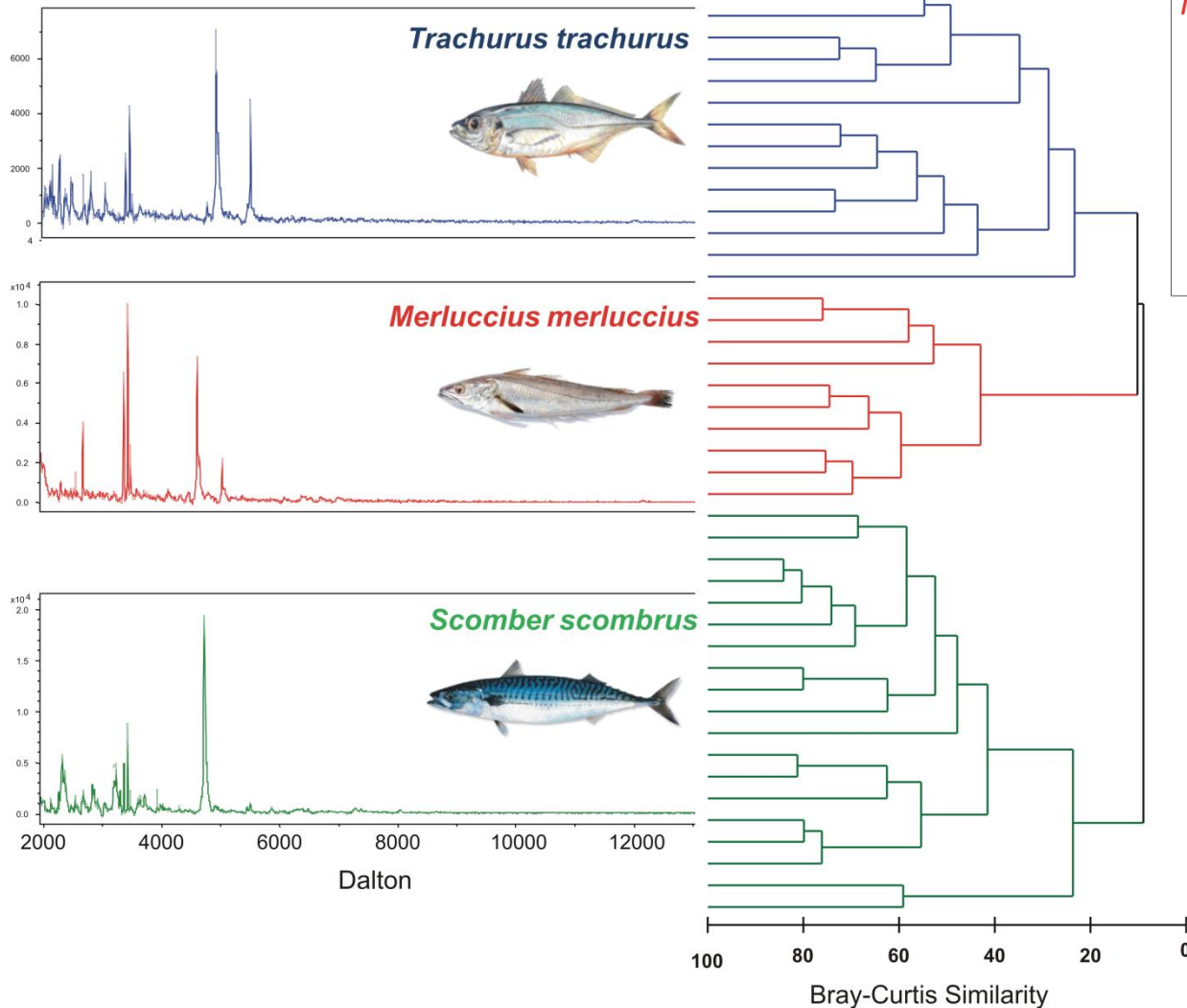
→ 9 species

- Lower intra- than interspecific variability
- High bootstrap values

Aglantha digitale
Corymorpha nutans
Eirene viridula
Eucheilota maculata
Eutonina indicans
Leuckartiara octona
Nemopsis bachei
Rathkea octopunctata
Pleurobrachia pileus

Fish eggs

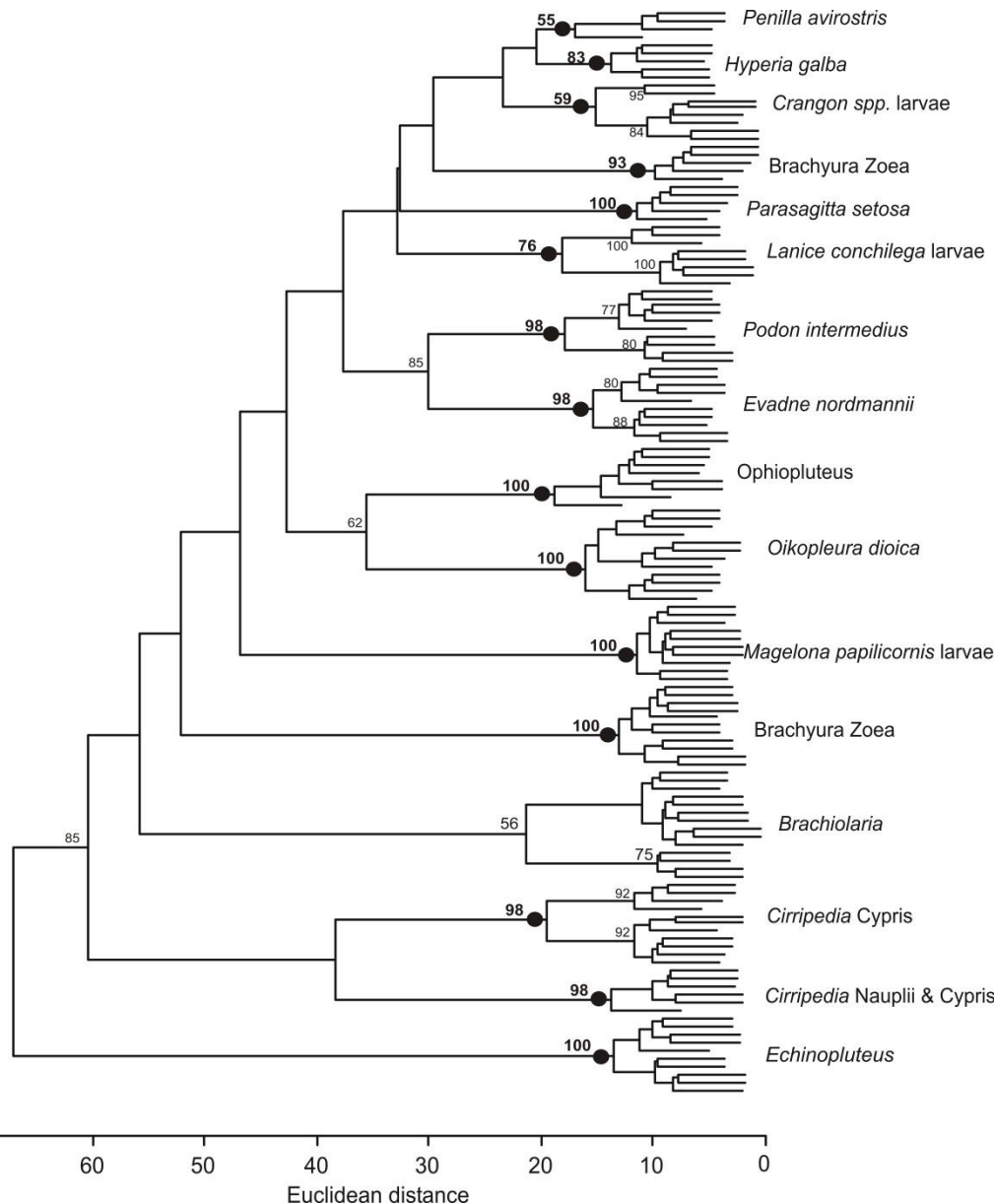
Proteomic Fingerprinting



Species-specific proteome spectra for the 3 different fish species eggs

SENCKENBERG

Mixed Zooplankton

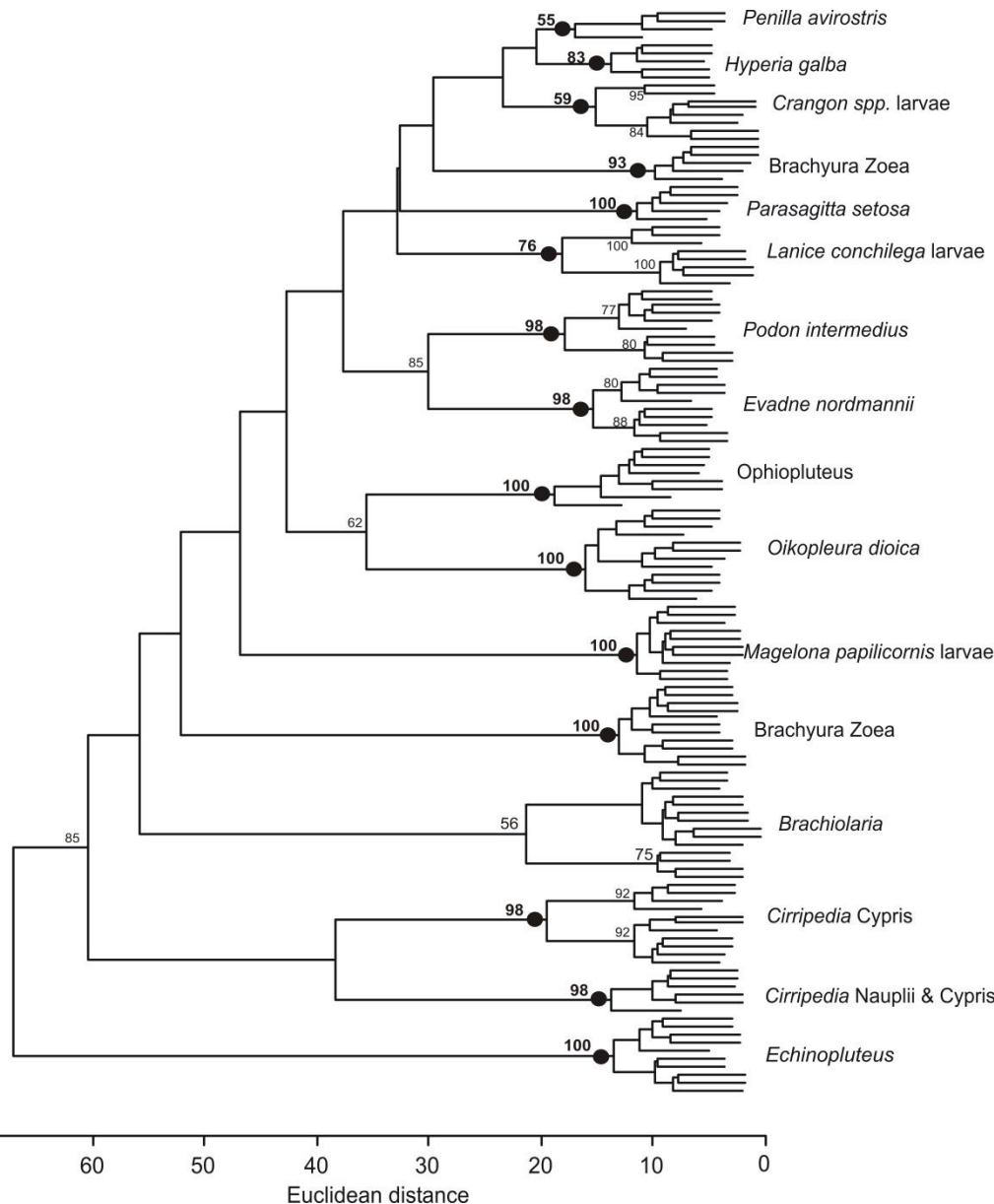


Proteome Fingerprints for diverse Holo- & Meroplankton
(i.e. Crustacea, Echinoderms, Polychaets, Appendicularians, Chaetognaths)

- Lower intra- than interspecific variability
- High Bootstrap values

SENCKENBERG

Mixed Zooplankton



Proteome Fingerprints for diverse Holo- & Meroplankton
(i.e. Crustacea, Echinoderms, Polychaets, Appendicularians, Chaetognaths)

- Lower intra- than interspecific variability
- High Bootstrap values

Possible Applications:

- ➔ Monitoring
- ➔ Detection of introduced species (e.g. rapid alert system)
- ➔ Biodiversity studies
- ➔ Ecological studies (i.e. species- & stage-specific vertical distribution)

CONCLUSIONS on Integrated North Sea zooplankton identification

Integrated morphological & molecular taxonomic approaches

- Consensus of morphological & molecular species status for most species
- New insights in diversity by uncovering sibling species

CONCLUSIONS on Integrated North Sea zooplankton identification

Integrated morphological & molecular taxonomic approaches

- Consensus of morphological & molecular species status for most species
- New insights in diversity by uncovering sibling species

Reference data base ensures future North Sea species identifications

- Identify specimens with challenging morphological identification (i.e. young stages, different life-history forms, phenotypic variations, siblings)
- Analyse data generated by high-throughput DNA sequencing techniques

CONCLUSIONS on Integrated North Sea zooplankton identification

Integrated morphological & molecular taxonomic approaches

- Consensus of morphological & molecular species status for most species
- New insights in diversity by uncovering sibling species

Reference data base ensures future North Sea species identifications

- Identify specimens with challenging morphological identification (i.e. young stages, different life-history forms, phenotypic variations, siblings)
- Analyse data generated by high-throughput DNA sequencing techniques

Proteomic Fingerprinting as time- and cost-efficient species identification tool

- Monitoring (Biodiversity), Rapid alert system

Thank you for your attention

Thanks to:

- Rebekka Schüller, Sven Hoffmann, Jasmin Renz-Gehnke, Viola Siegler, Sabine Gollner, Inga Mohrbeck, Peggy Weist, Pedro Martínez Arbizu (DZMB, Senckenberg Institute)
- Elena Markhaseva (Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia)
- Gunnar Gerdts, René Erler, Astrid Cornils, Antje Wichels, Maarten Boersma (AWI)
- Maya Bode (Bremen University)
- Dominik Gloe, Klas Ove Möller, Bettina Walter, Janna Peters (IHF, Hamburg University)

Funding

This work is supported by the Federal Ministry of Education and Research (BMBF, Grant No. 03F0499A) and the Land Niedersachsen.



Bundesministerium
für Bildung
und Forschung

