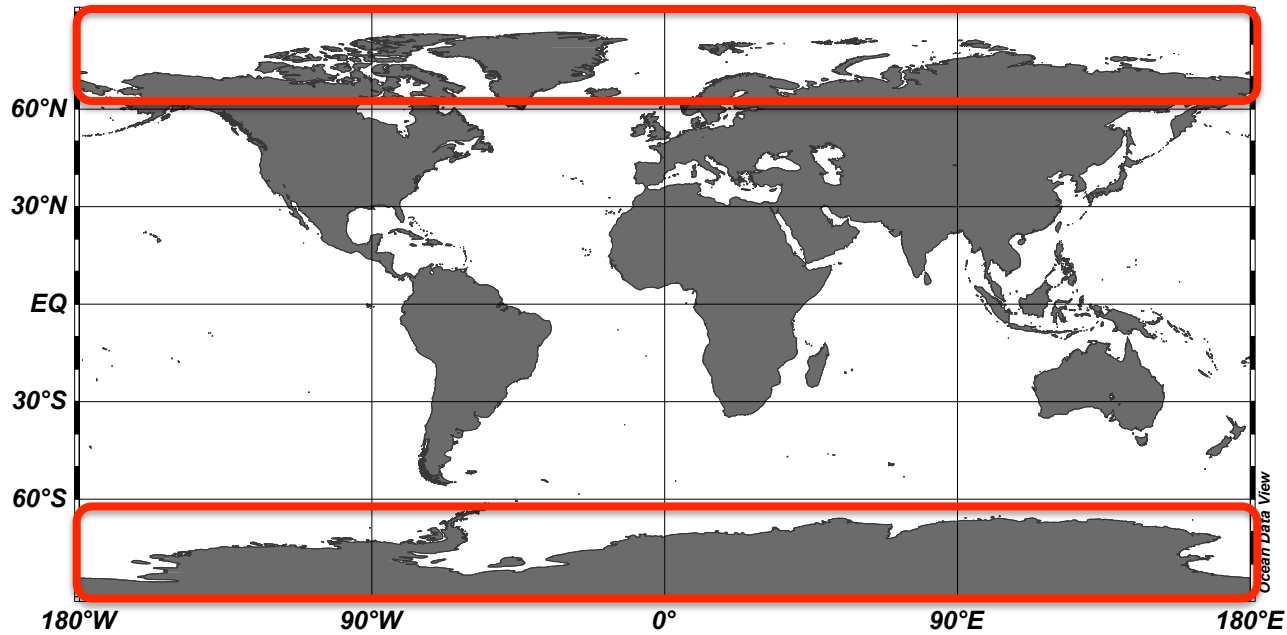


Cosmopolitan, bipolar or endemic? Phylogeography of polar copepod species-groups

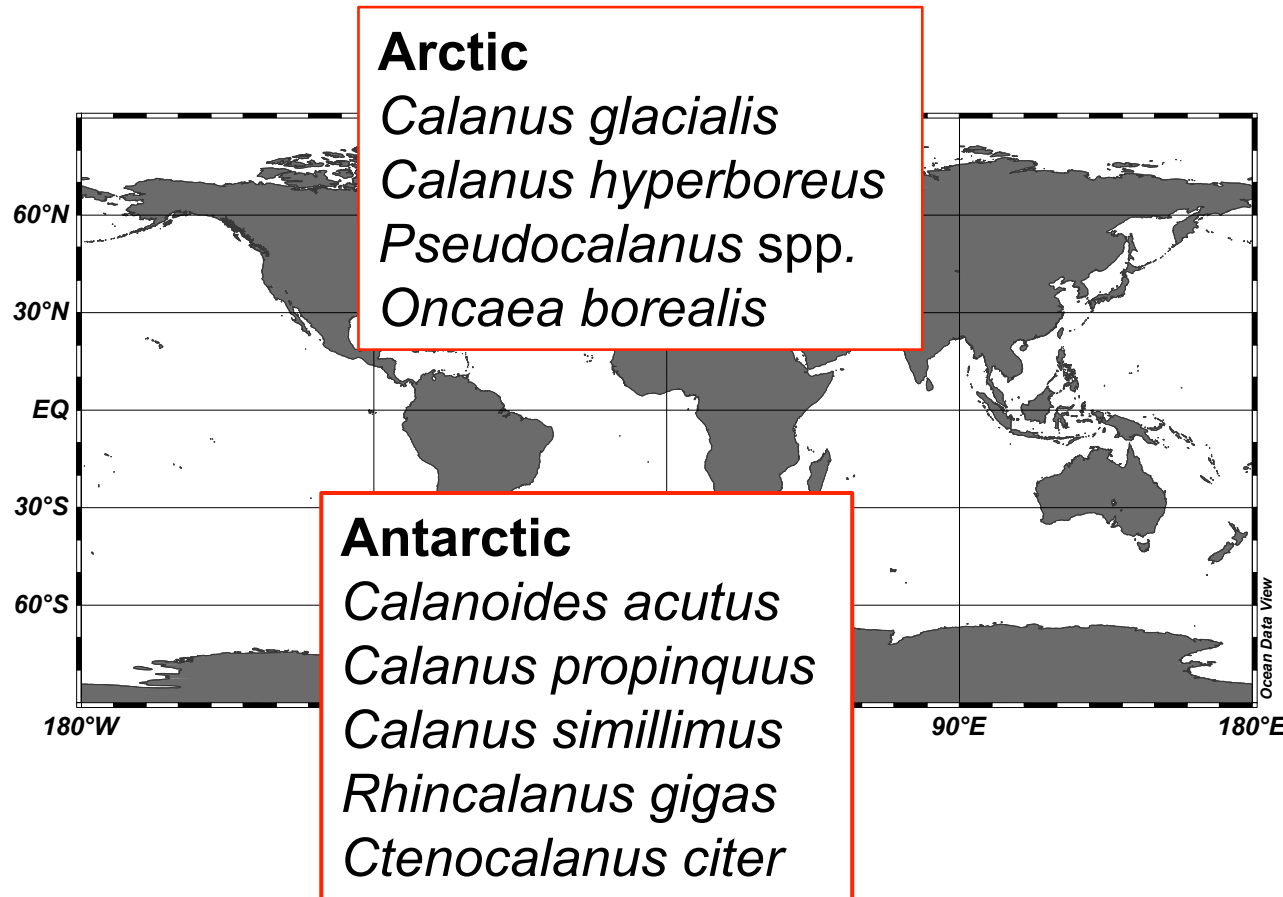
Astrid Cornils, Christoph Held

Alfred Wegener Institute Helmholtz Centre for
Polar and Marine Research

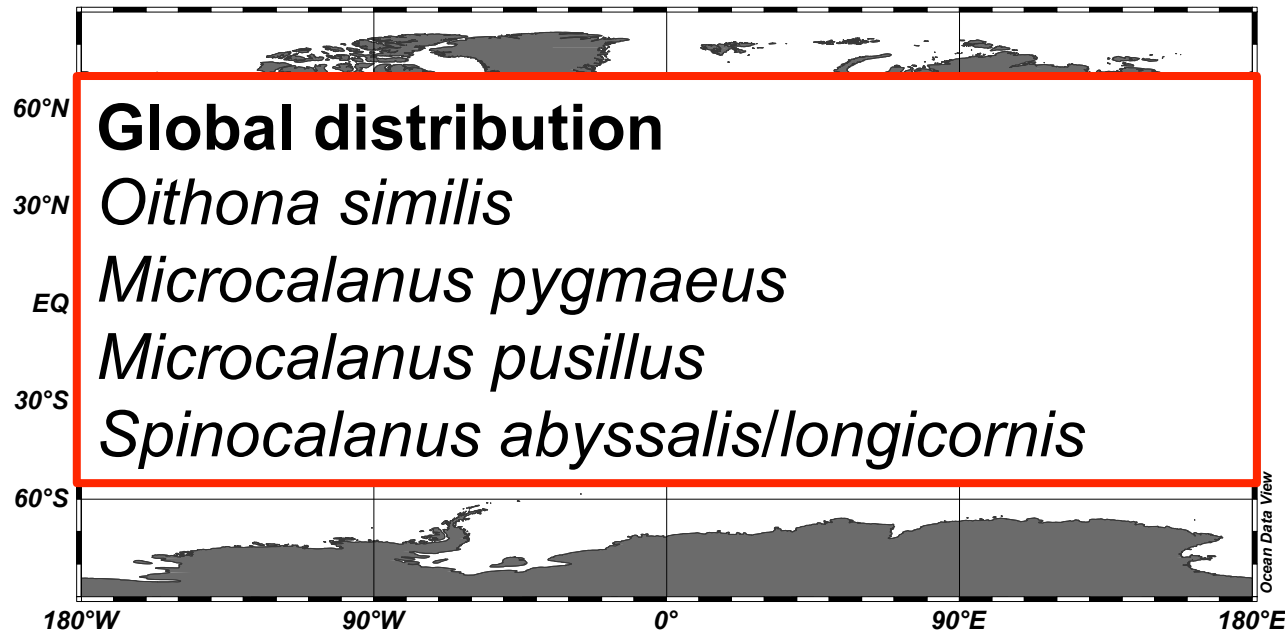
Phylogeography of polar copepods



In the polar oceans most of the abundant copepod species are **restricted to cold water masses of one hemisphere**



In the polar oceans most of the abundant copepod species are **restricted to cold water masses of one hemisphere**



***Oithona similis* s.l.**

- cosmopolitan
- omnivorous
- epipelagic



Microcalanus pygmaeus* and *M. pusillus

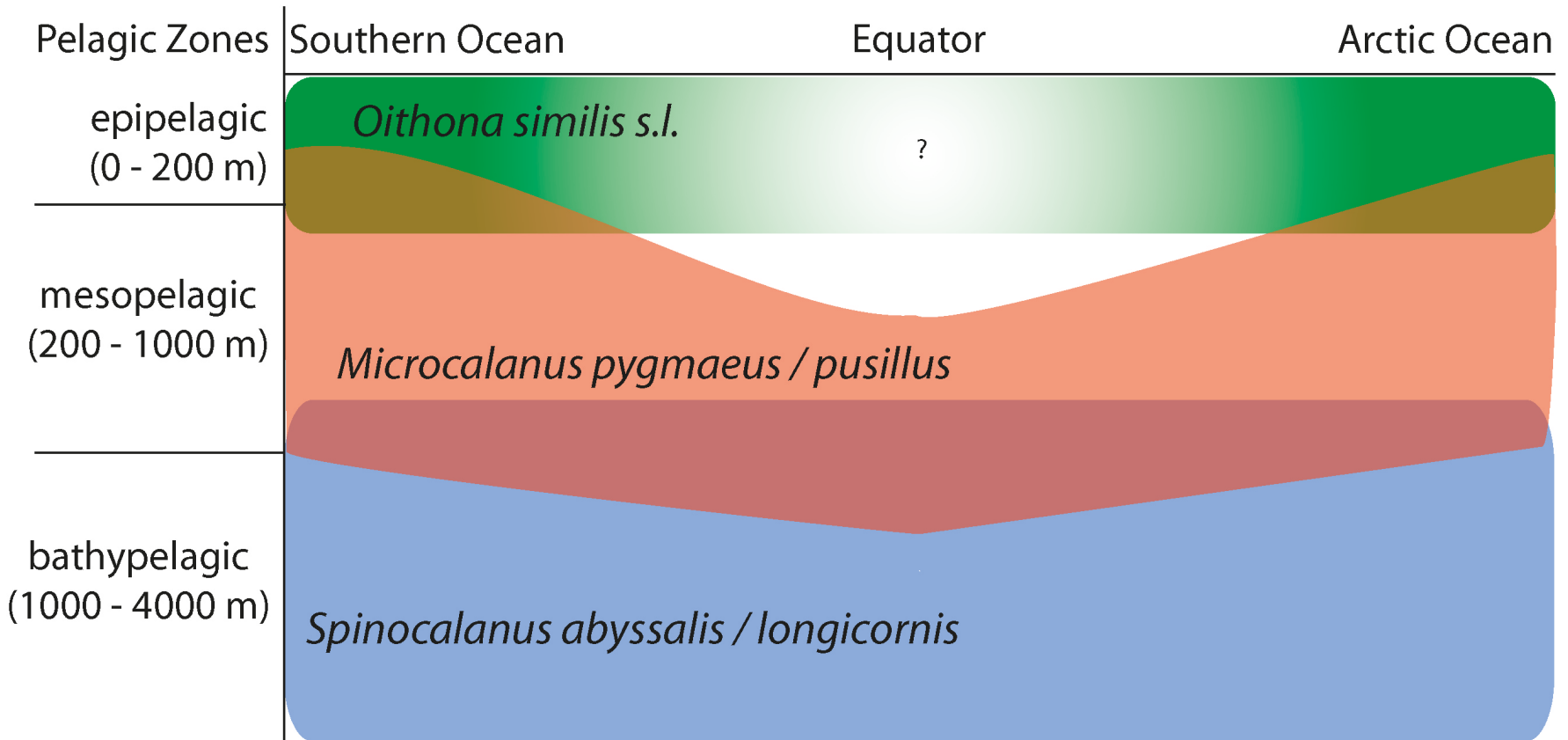
- cosmopolitan / bipolar
- omnivorous
- epi- to mesopelagic



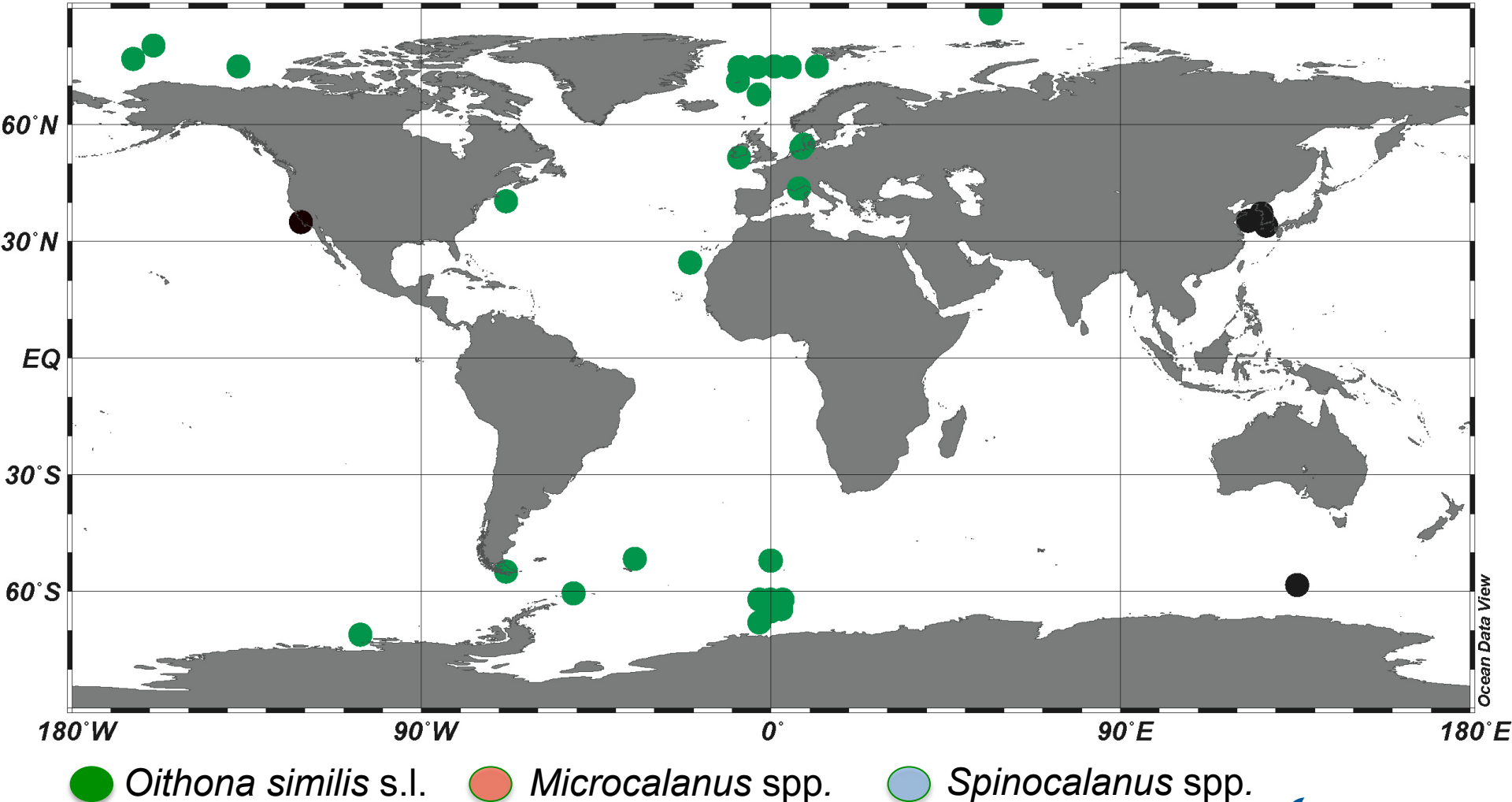
Spinocalanus abyssalis* and *S. longicornis

- cosmopolitan / bipolar
- detritivorous and carnivorous
- bathypelagic

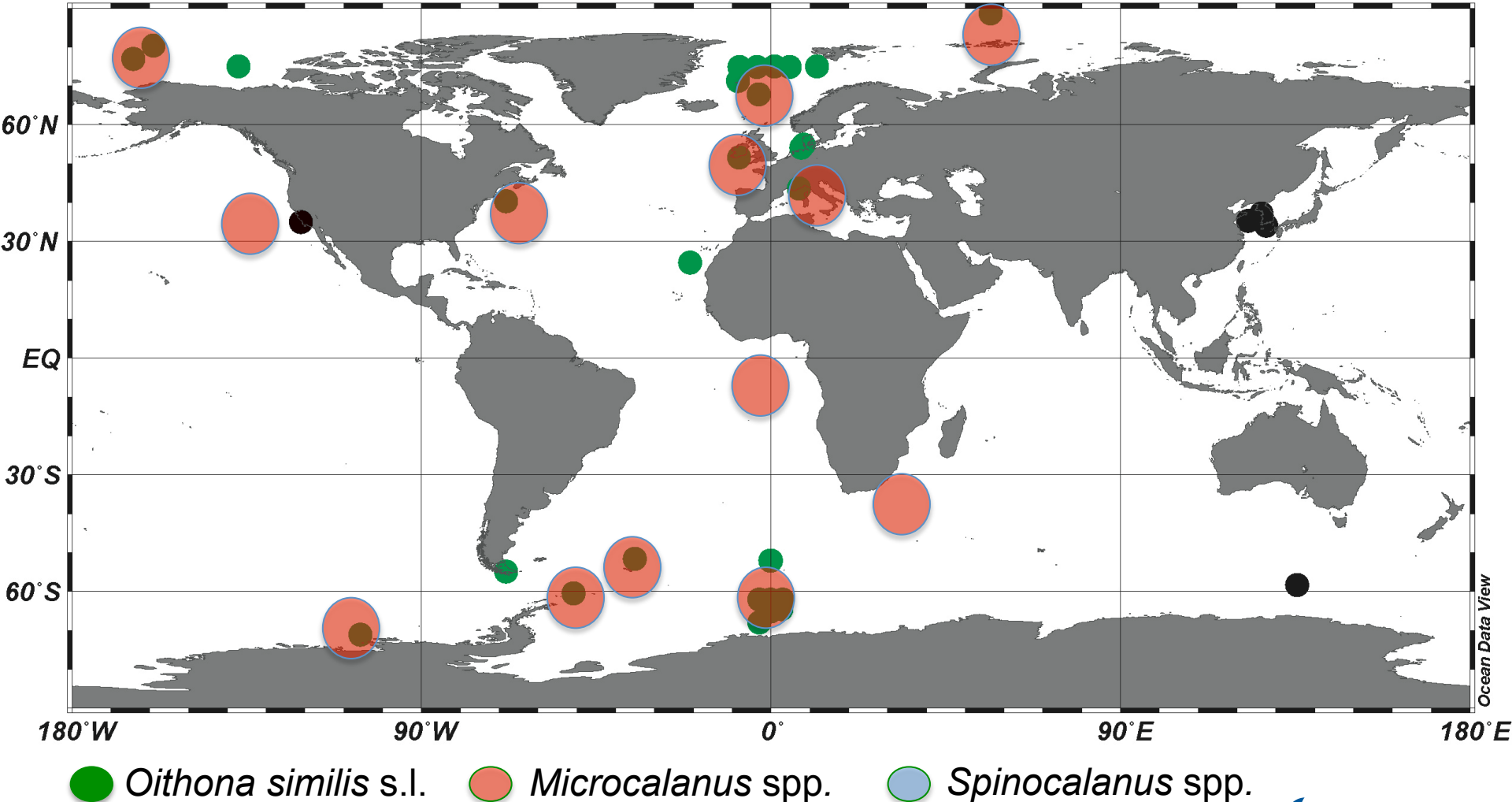




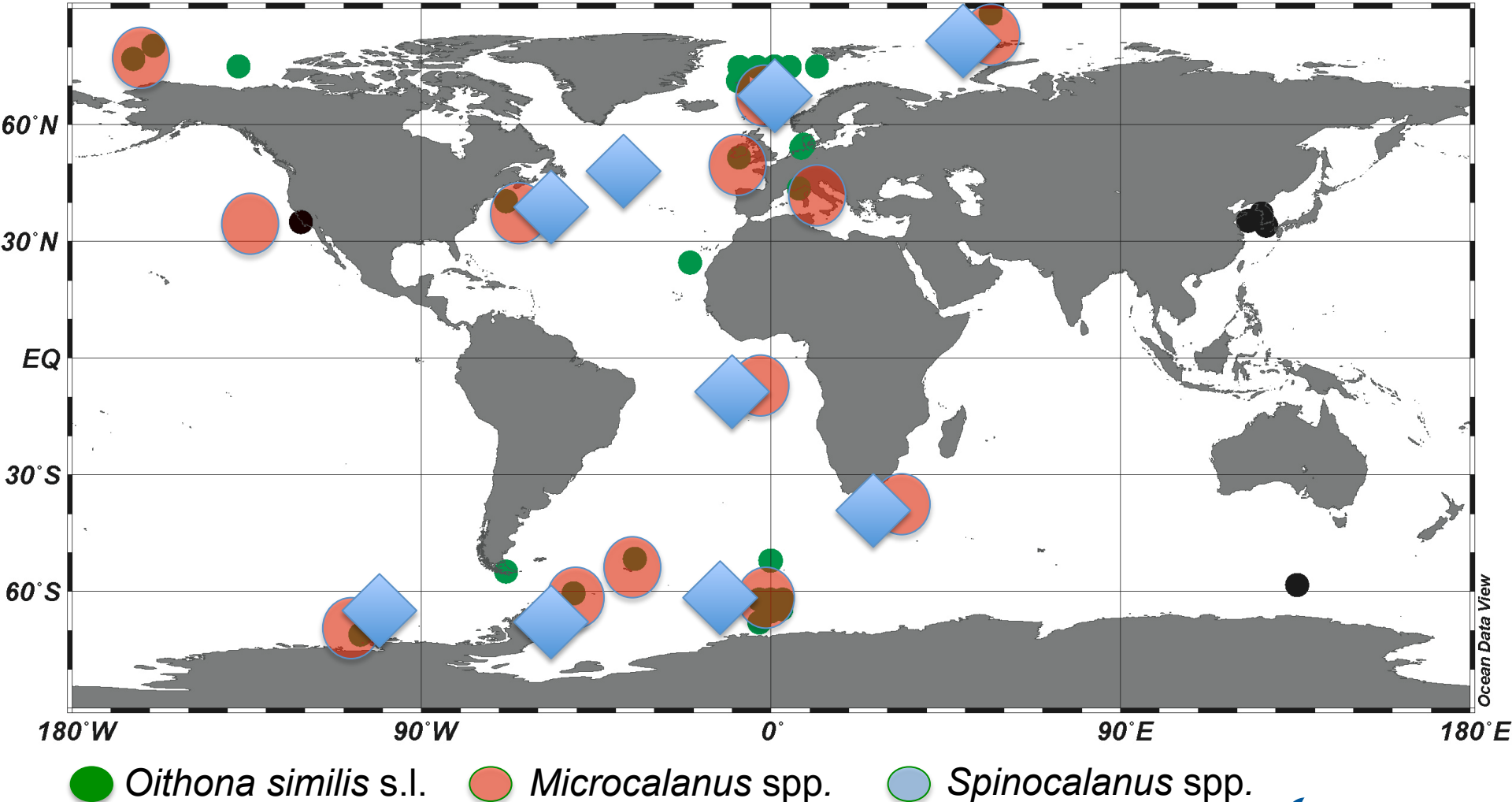
Global distribution of successfully sequenced specimens



Global distribution of successfully sequenced specimens



Global distribution of successfully sequenced specimens



● *Oithona similis* s.l. ● *Microcalanus* spp. ● *Spinocalanus* spp.

***Oithona similis* s.l.**

Collaborators: Britta Wend-Heckmann

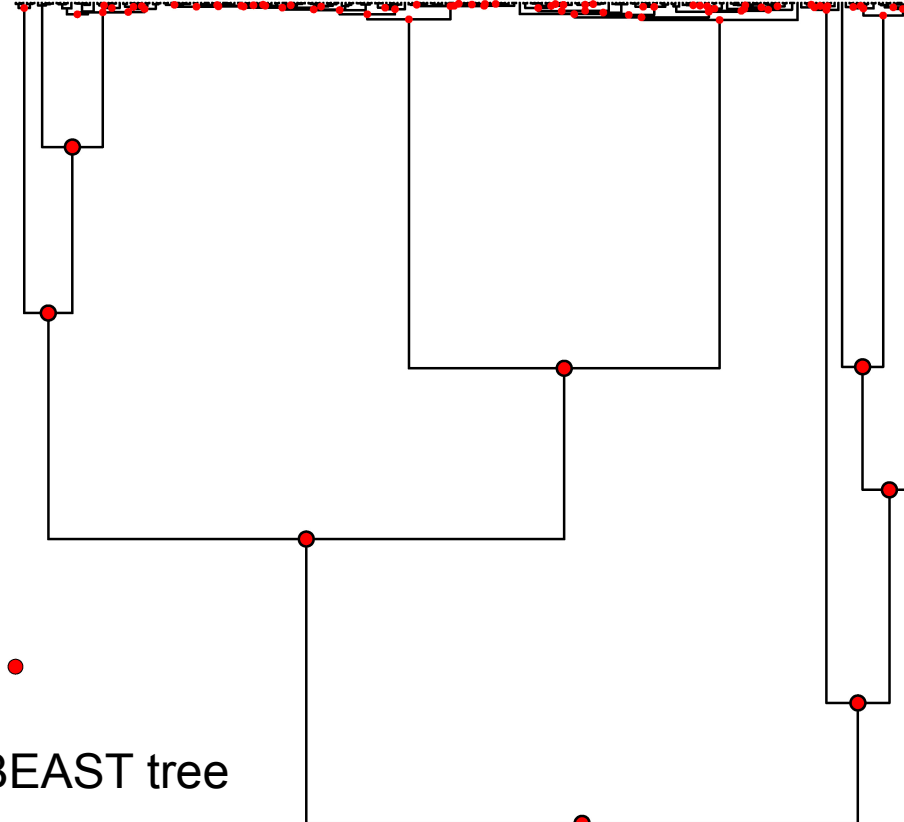
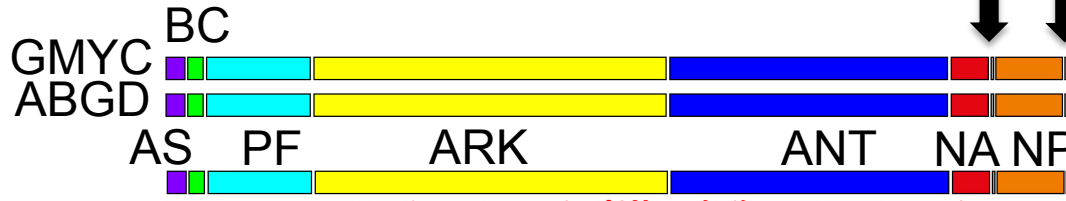


- 244 sequences of cytochrome *c* oxidase subunit I (COI) and 82 sequences of nuclear ribosomal 28S gene
- Species delimitation analyses: Bayesian General mixed Yule coalescent model (bGMYC), Automated Barcoding Gap Discovery (ABGD)
- Morphology: no differences in ornamentation of body parts, but differences in body length, morphometric differences in populations of the northern hemisphere (Shuvalev 1972)



Species discovery in COI

ARK2 ARK3

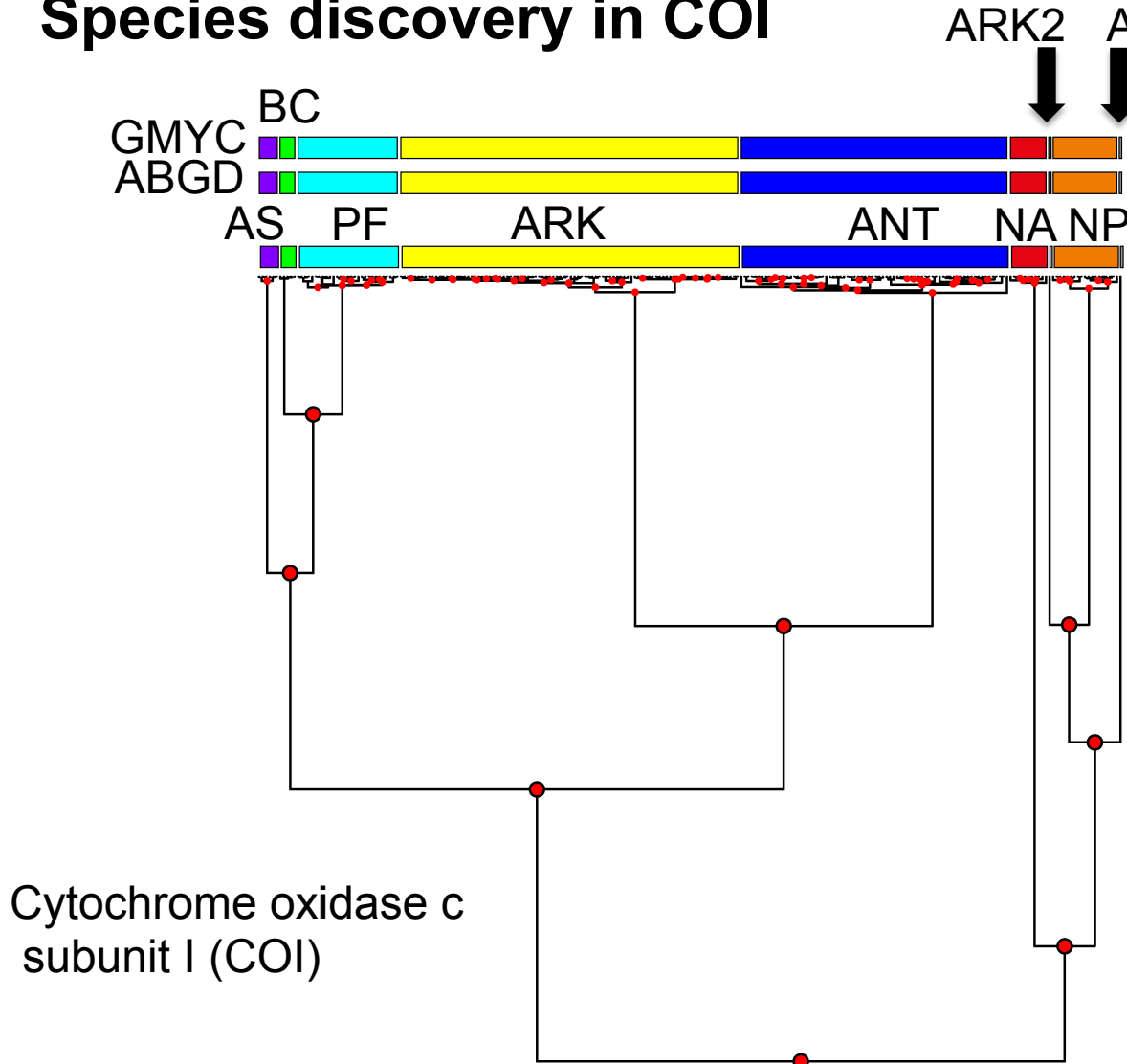


ANT: Southern Ocean
 ARK: Arctic Ocean
 PF: Polar Front
 AS: Amundsen Sea
 BC: Beagle Channel
 NA: North Atlantic
 NP: North Pacific

Ultrametric BEAST tree



Species discovery in COI



Species delimitation methods agree

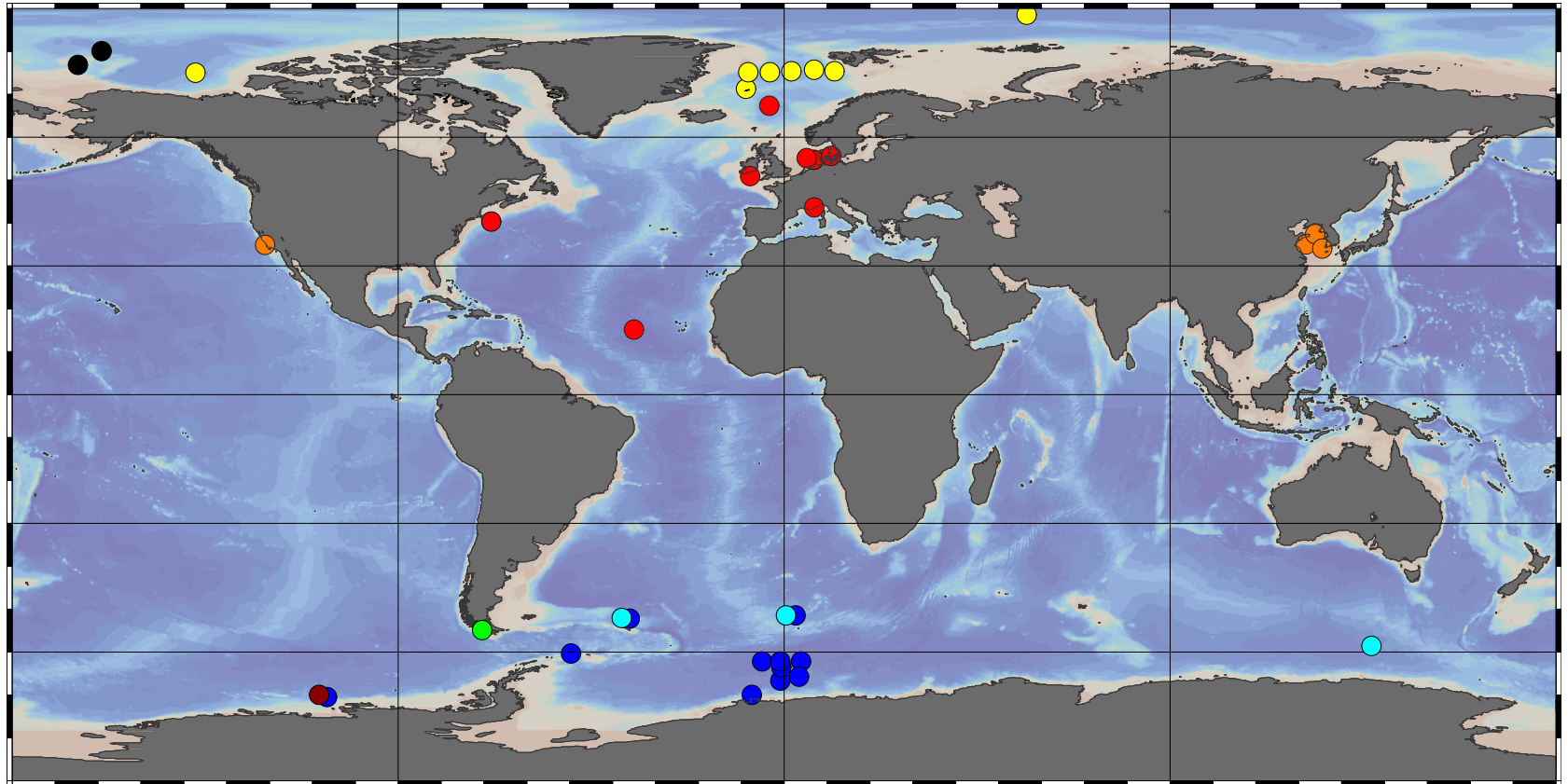
7 putative species, 2 singletons

Uncorrected genetic Distances between Lineages 8.3 – 23,7 %

No cosmopolitan or „bipolar“ lineages

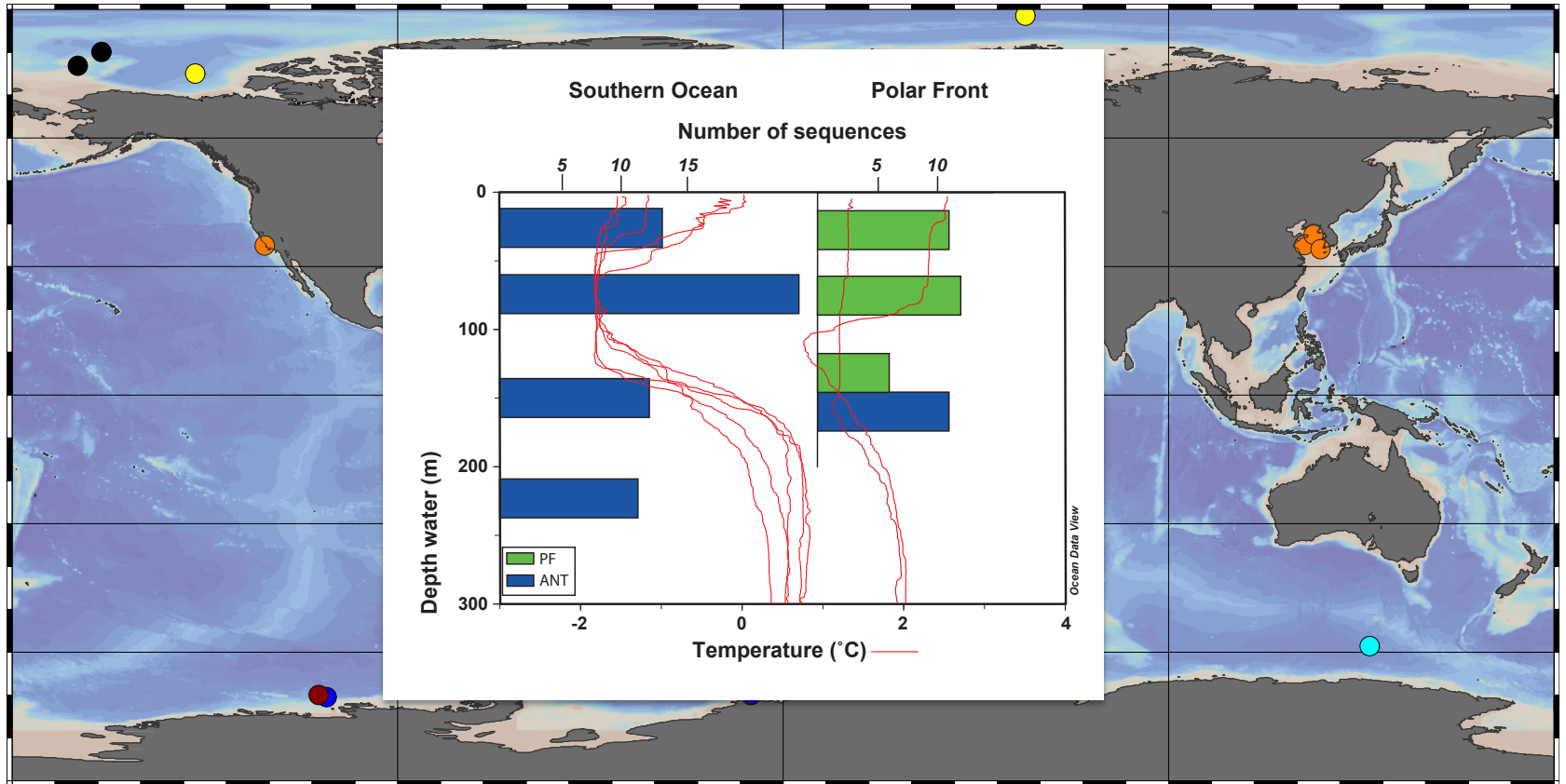


Global distribution of *Oithona similis* s.l. lineages



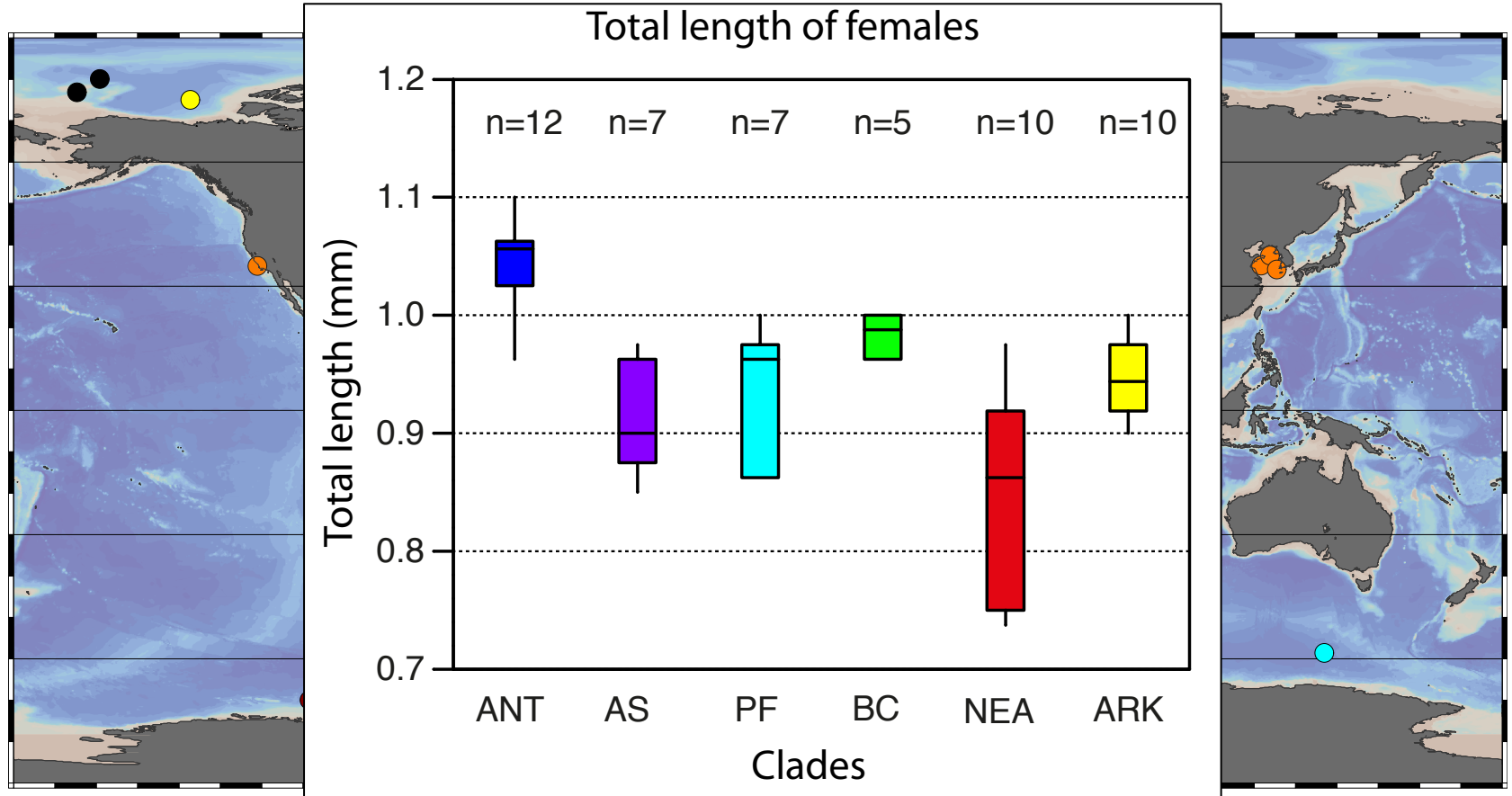


Global distribution of *Oithona similis* s.l.





Global distribution of *Oithona similis* s.l.

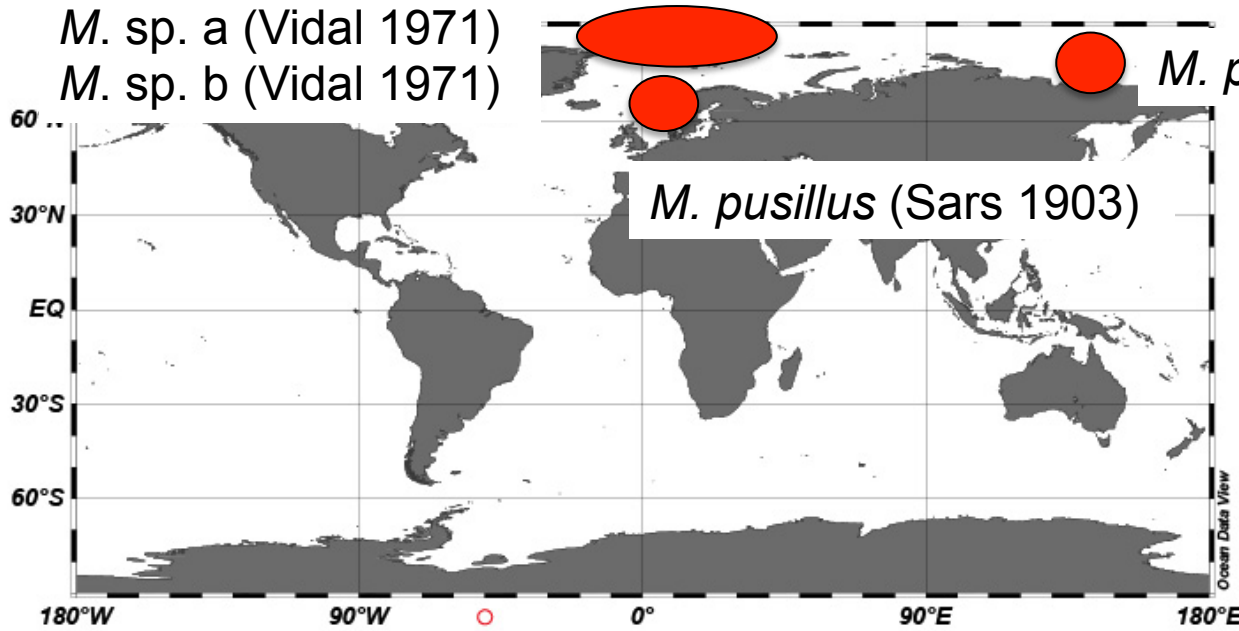


Microcalanus pygmaeus* / *M. pusillus

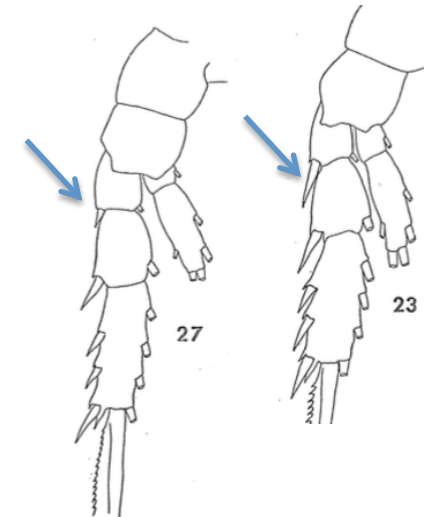


- At present 300 sequences of cytochrome c oxidase subunit I (COI), preliminary sets of 18 sequences of ITS1, 16S, 28S
- Non-destructive DNA extraction (Cornils 2015)
- Morphology:
 - 2 described species (*M. pygmaeus*, *M. pusillus*),
 - 2 unnamed species (Arctic, Vidal 1971).
 - Differences in body parts (e.g. length of antennae, female genital segment) and total length

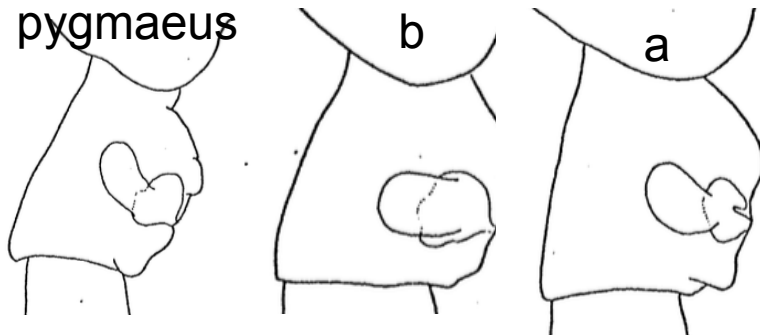
Microcalanus spp.



Swimming leg 2 (P2) (Vidal et al. 1971)



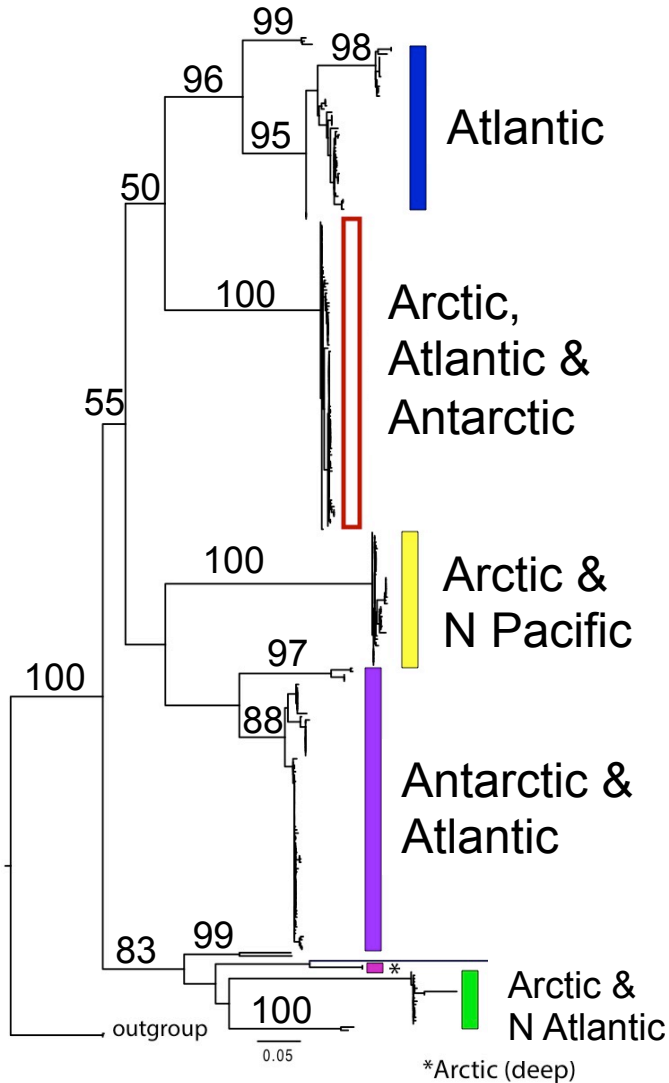
Form of the seminal receptacle (Vidal et al. 1971)



Microcalanus spp.



Maximum Likelihood tree for COI



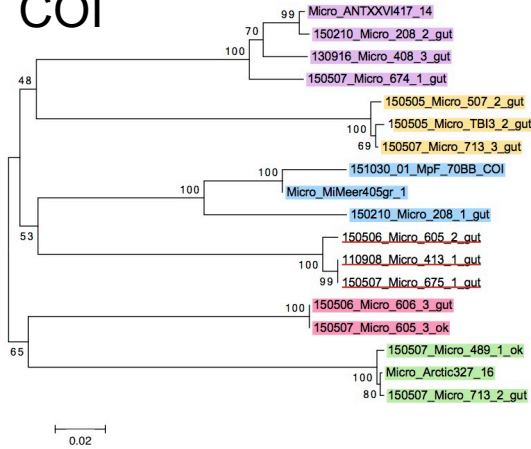
Uncorrected distances between Lineages vary between 7 – 25%

RAxML tree for 300 COI sequences of 657bp length

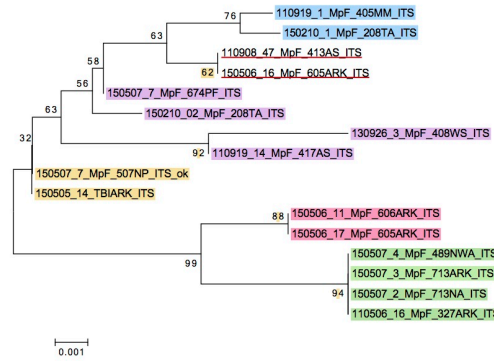
Microcalanus spp.



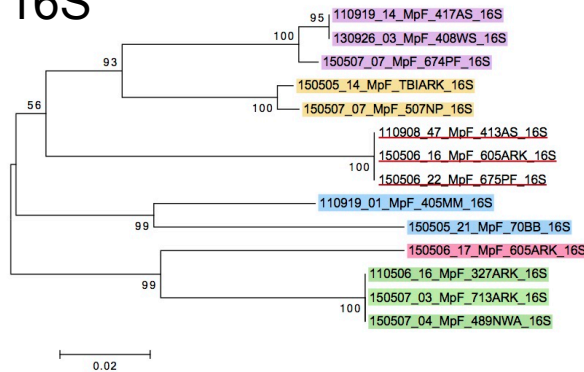
COI



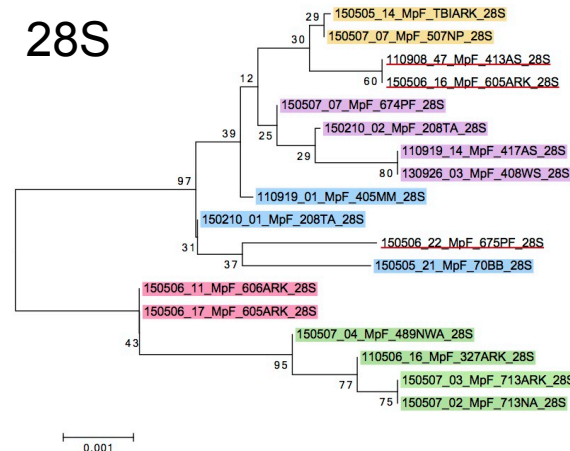
ITS1



16S



28S



Arctic Ocean
 North Atlantic

Arctic Ocean
 North Pacific

Southern Ocean

Atlantic Ocean

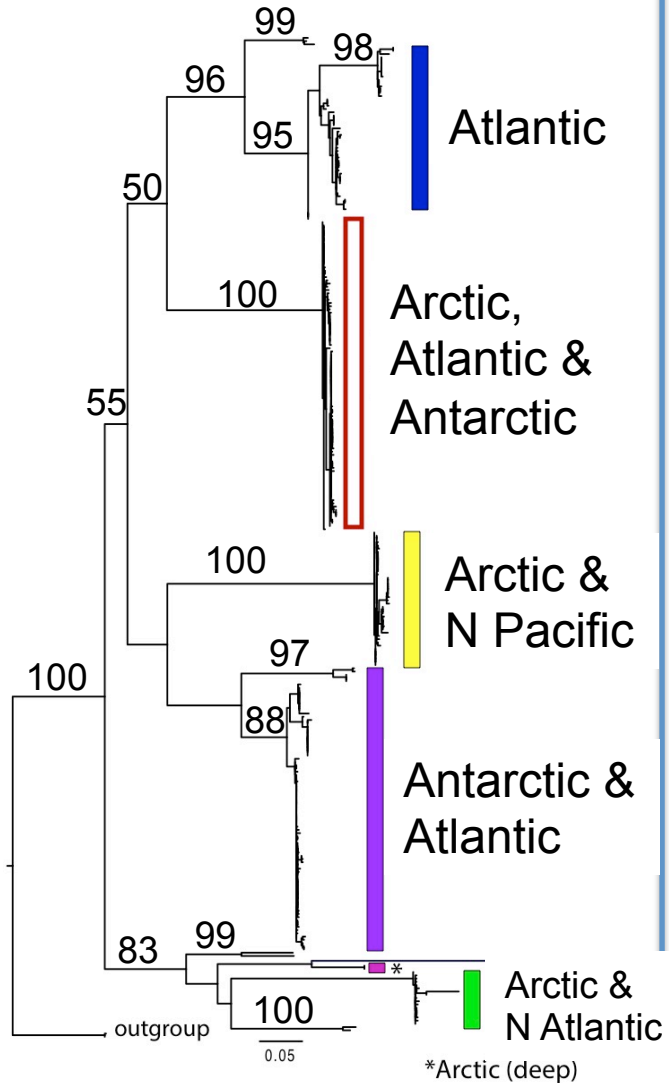
Arctic Ocean
 Southern Ocean

Arctic Ocean

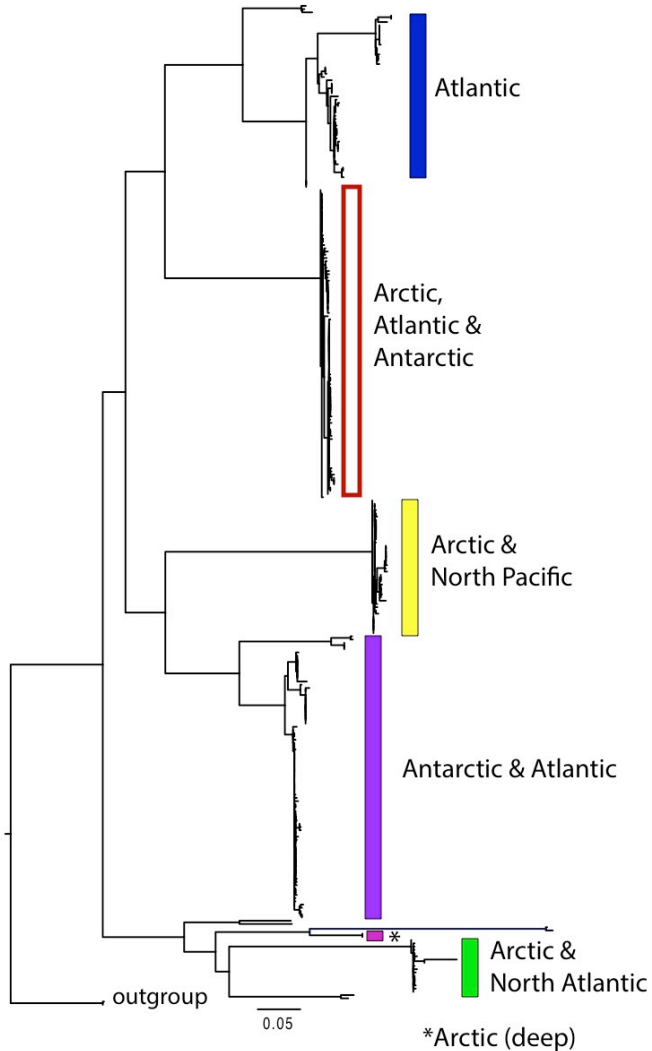
Microcalanus spp.



Maximum Likelihood tree for COI



Prosome length	depth (m)	P2 Exp1 outer spine	Genital segment
0,54 μ m	100 – 1000	long	
0,61 μ m	50 – 2000	long	
0,54 μ m	0 – 200	short	
0,58 μ m	0 – 1500	middle	
0,59 μ m	500 – 1500	short	
0,51 μ m	0 – 200	short	



Putative Species

M. nov. spec. D

M. sp. A (Vidal 1971)

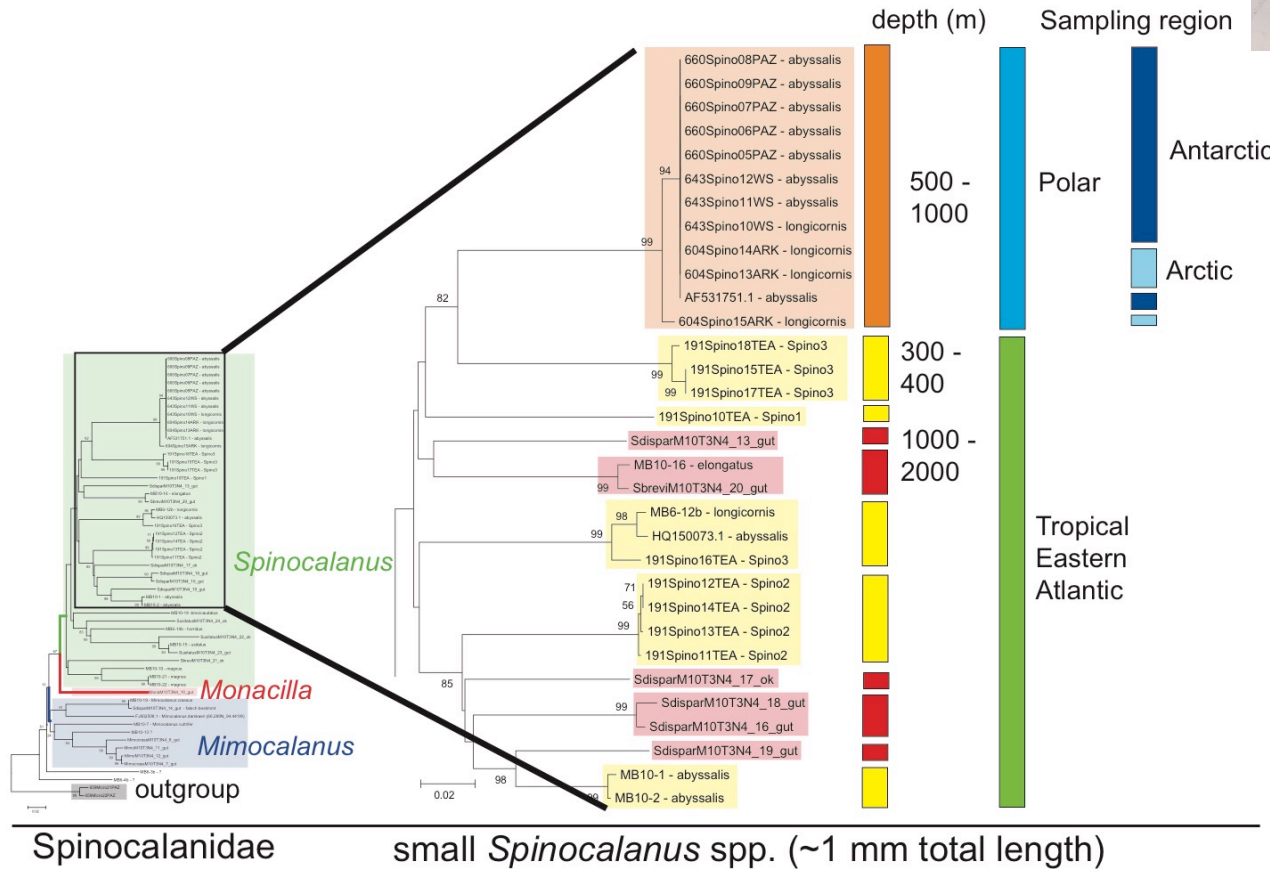
M. pygmaeus (Sars 1900)

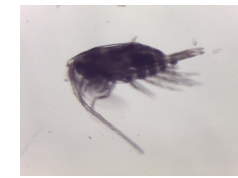
M. nov. spec. C

M. sp. B (Vidal 1971)

M. pusillus (Sars 1900)

Spinocalanus abyssalis/longicornis





MALDI-TOF mass spectrometry as a novel tool for zooplankton biodiversity studies - Copepod distribution throughout the eastern Atlantic Ocean

Maya Bode¹, Patricia Kaiser¹, Holger Auel¹, Wilhelm Hagen¹, Astrid Cornils² and Silke Laakmann³
¹BreMaE, Marine Zoology, University of Bremen, Germany; ²Alfred Wegener Institute, Bremerhaven, Germany; ³German Center for Marine Biodiversity Research (DZMB), Senckenberg Institute, Wilhelmshaven, Germany

BACKGROUND AND OBJECTIVES

Copepods play a key role in marine food webs and the carbon cycle. Their diversity is mainly regulated by environmental parameters and species-specific ecophysiological performances. These are not yet identified in order to predict future changes in marine ecosystems. To understand diversity and distribution patterns we studied copepod communities in the eastern Atlantic. Below 200 m, representatives of the family Spinocalanidae were particularly abundant. Species identification based on morphological characters of both juveniles and adults of this ecologically important, but often little noted deep-sea copepod family, was challenging. Hence, the aim of the study was an integrated species identification approach using morphological and molecular techniques to elucidate their diversity and species- and stage-specific vertical distribution patterns.

METHODS

- 1. Step:** Depth-stratified multinet samples (0 – 2000 m) were taken at six stations along a meridional transect in the eastern Atlantic Ocean (25°N to 21°S, ANTXX) and copepod community structure was analyzed (Fig. 1).
- 2. Step:** Establishment of reference library (Fig. 2).

Spinocalanidae were sorted from a previous transit cruise (ANTXXIV), isolated and identified based on diagnostic morphological characters and halved for the analysis of:

- a) DNA sequences: mitochondrial cytochrome c oxidase subunit I (COI) and nuclear ribosomal 18S rDNA
- b) Proteome fingerprints using the matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS).

- 3. Step:** This reference library was used to identify and quantify specimens in net samples for species- and stage-specific vertical distribution patterns.

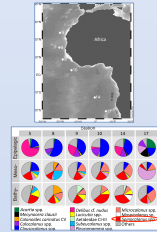


Fig. 1. Station map (ANTXXIX) and proportional community composition of calanoid copepods in terms of abundance at each station represented for size (200-2000 µm) (200-1000 µm) and bathypelagic zones (1000-2000 µm)

RESULTS

28 species could be discriminated based on morphological characters while all molecular approaches revealed in total **40 putative spinocalanid species** (Fig. 3).

We find the same 40 putative species clusters using proteomic fingerprinting and DNA sequence analysis (COI) and thus a comparable species identification success (Fig. 4).

Even putative cryptic species were discriminated based on their species-specific proteome fingerprints. For example, the *Spinocalanus dispar* species complex consisted of eight putative cryptic species (named *S. dispar* 1-8) (Fig. 3).

Species clusters were valid and independent of the developmental stage allowing the analysis of hundreds of specimens for vertical distribution analysis with the result that spinocalanid species (and their juvenile stages) show a pronounced vertical habitat partitioning (Fig. 5).



Fig. 3. Maximum likelihood tree based on COI of 40 Spinocalanus spp. for alignment, bootstrap values >50% shown). Results of the species delimitation method ABGD (Automatic Barcode Gap Discovery) indicated as alternating black and grey boxes.



Fig. 4. Vertical distribution of the most abundant Spinocalanidae at one station in the tropical eastern Atlantic Ocean (st. 7, ANTXXIX). Open circles represent weighted mean depths. Bars indicate the vertical range of the respective species (black dot: only one individual found).

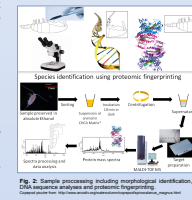


Fig. 2. Sample processing including morphological identification, COI-sequencing and proteomic fingerprinting.

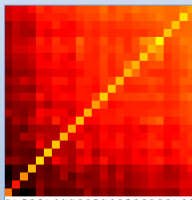


Fig. 5. Heatmap illustrating vertical distribution of species from species between spinocalanid species. Bright colors (yellow) indicate low mean depth (black indicates large distances). 10 Spinocalanus spp. 11: *Spinocalanus* sp. 12: *Spinocalanus* sp. 13: *Spinocalanus* sp. 14: *Spinocalanus* sp. 15: *Spinocalanus* sp. 16: *Spinocalanus* sp. 17: *Spinocalanus* sp. 18: *Spinocalanus* sp. 19: *Spinocalanus* sp. 20: *Spinocalanus* sp. 21: *Spinocalanus* sp. 22: *Spinocalanus* sp. 23: *Spinocalanus* sp. 24: *Spinocalanus* sp. 25: *Spinocalanus* sp. 26: *Spinocalanus* sp. 27: *Spinocalanus* sp. 28: *Spinocalanus* sp. 29: *Spinocalanus* sp. 30: *Spinocalanus* sp. 31: *Spinocalanus* sp. 32: *Spinocalanus* sp. 33: *Spinocalanus* sp. 34: *Spinocalanus* sp. 35: *Spinocalanus* sp. 36: *Spinocalanus* sp. 37: *Spinocalanus* sp. 38: *Spinocalanus* sp. 39: *Spinocalanus* sp. 40: *Spinocalanus* sp.

MALDI-TOF mass spectrometry as a novel tool for zooplankton biodiversity studies – Copepod distribution throughout the eastern Atlantic Ocean

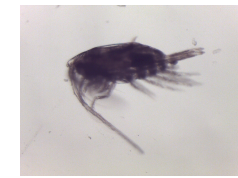
Maya Bode, Patricia Kaiser, Holger Auel, Wilhelm Hagen, Astrid Cornils and Silke Laakmann

CONCLUSIONS

Species richness of Spinocalanidae has been largely underestimated. The status of some species still needs to be clarified.

Proteomic fingerprinting is a cost- and time-efficient tool for rapid species identification and may shed new light on biodiversity, vertical habitat partitioning and phylogeographic patterns, which is particularly promising for taxonomically challenging, closely-related specimens, juveniles and fragile deep-sea species.

To apply proteomic fingerprinting as a routine method in species identification and community analyses, the generation of a reference data base for zooplankton, as already available for microorganisms, will be essential, allowing immediate comparison and analysis of the mass spectra.



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Maya Bode, Patricia Kaiser, Holger Auel, Wilhelm Hagen, Astrid Cornils and Silke Laakmann



7 novel
 species –
 eastern

Summary

- Within these circumglobal copepod the potential of finding cryptic or pseudocryptic lineages is very high
- *Oithona similis* is not a cosmopolitan or „bipolar“ species, but consists of ≥ 7 putative species with distinct biogeographies
- Meso- and bathypelagic species-groups include both widespread and geographically restricted lineages, possibly due to the more homogeneous environment

I would like to thank also

The priority program „Antarctic Research“ (SPP1158) of the German Science Foundation (DFG) for funding this project

Andrea Eschbach for the assistance in the lab

All the colleagues who provided samples:

H. Auel, L. Blanco-Bercial, M. Bode, A. Bucklin, S. Gasparini, R. Hopcroft, G. Lawson, B. Niehoff, J. Questel, U. Tilmann, S. Schnack-Schiel, H. Verheye, B. Wend-Heckmann, S. Wickham and all CMarZ cruise participants of ANTXXIV/1