31. DNA BARCODING AND PHYLOGEOGRAPHY OF MESO- AND BATHYPELAGIC AMPHIPODS

Charlotte Havermans¹ Evgeny Pakhomov² Brian Hunt² ¹IRScNB ²UBC

Objectives

The objective of this study is to compare the genetic diversity of several pelagic amphipods, belonging to different families using specimens sampled in different parts of their circum-Antarctic distribution. Amphipods are brooders which might limit their dispersal and in the case of benthic species, several circum-Antarctic species appeared to be complexes of species with restricted distributions. This has not been adequately tested for pelagic species. The families selected for this study are the Hyperiidea, Lysianassoidea and Eusiroidea, due to their abundance in the meso- and bathypelagic plankton. Moreover, we will be able to compare the genetic diversity within species with different life-styles, e.g. free-living lysianassoid scavengers (*Eurythenes obesus*) versus hyperiid parasites of medusae (*Chuneola major*).

The second part of this study focuses on the hyperiid *Themisto gaudichaudii*, one of the most abundant pelagic amphipods of the Southern Ocean, occurring in large numbers within the subantarctic and low Antarctic waters (Kane 1966). Previously regarded as a species with a bipolar distribution, in 1986 the Antarctic and the Arctic 'forms' of *T. gaudichaudii* were separated into 2 species using morphological and electrophoretic evidence (Schneppenheim & Weigmann-Haass 1986). The southern hemisphere species, *T. gaudichaudii* is known to have two forms: *compressa* and *bispinosa* (Kane 1966). The genetic divergence between these two forms will be investigated to verify their status as one species or a complex of species.

Work at sea

During the cruise three main regions of the Antarctic Polar Frontal Zone were sampled: a transect between 44°S and 53°S along the 10°E, a long-term station at 51°S 12°W and in the South Georgia Basin at 50°S 37°W. Animals were individually picked from the samples collected using Rectangular Midwater Trawl (RMT-8+1) in either top 300 m during nighttime or during meso- and bathypelagic tows. In total, over 30 shallow (0-300 m) nighttime and 4 mesopelagic (0-1000 m) and 1 bathypelagic (2,500-1,000 m) were carried out. Trawls were towed obliquely and with a speed of 2.5 knots.

Preliminary results

DNA extractions were carried out for 61 specimens caught in shallow and deepwater RMTs: 22 specimens of 4 different lysianassoid species (*Eurythenes obesus*, *Cyphocaris richardi*, *Cyphocaris faurei*, *Cyphocaris bouvieri*) and 39

specimens of 4 different hyperiid species (Themisto gaudichaudii, Vibilia antarctica, Hyperia macrocephala, Chuneola major, Primno macropa) and several specimens of one unidentified Eusiroid species. In the case of *Themisto* gaudichaudii, DNA of 20 specimens of the form compressa and 15 specimens of the form bispinosa has been extracted to investigate whether the genetic divergence between these two morphs is at the level of intra- or interspecific variation. At the RBINS, the mitochondrial COI gene fragment will be amplified using universal primers LCOI1490 and HCOI2198 of Folmer et al. (1994) and the purified PCR products will be sequenced with an ABI3130xl capillary DNA sequencer (Applied Biosystems). To investigate the genetic divergence within the different species and to identify species complexes from sequence variation in COI, (barcoding approach sensu Hebert et al. 2003a,b) a neighbour-joining tree (Saitou and Nei 1987) will be estimated using MEGA 5 (Tamura et al. 2011) and sequence divergence calculated using the Kimura 2-parameter (K2P) distance model (Kimura 1980). Phylogeographic analyses will be conducted with the COI sequence data set, such as the construction of haplotype networks to investigate the relationship between haplotypes and their geographic distribution.

Data management

All data obtained will be prepared for publication and will be made available via PANGAEA.

References

- Folmer, O., Black, M., Hoeh, R., Lutz, R., Vrijenhoek, R. 1994: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3, 294-299.
- Hebert, P.D., Cywinska, A., Ball, S. L., deWaard, J. R. 2003a: Biological identifications through DNA barcodes. Proceedings of the Royal Society of London, Series B: Biological Sciences, 270, 313-321.
- Hebert, P.D., Ratnasingham, S., deWaard, J.R. 2003b: Barcoding animal life: cytochrome c oxidase subunit I divergences among closely related species. Proceedings of the Royal Society of London, Series B: Biological Sciences 270, S96-S99.
- Kane, J. E. 1966: The distribution of *Parathemisto gaudichaudii* (Guer.), with observations on its life-history in the 0° to 20°E sector of the Southern Ocean. Discovery Reports, 34, 134-132.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16, 111-120.
- Schneppenheim, R, Weigmann-Haass, R 1986: Morphological and electrophoretic studies of the genus *Themisto* (Amphipoda, Hyperiidea) from the south and north Atlantic. Polar Biology, 6, 215-225.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution, 28, 2731-2739.