

# Report COMNAP fellowship 2013/14

## The impact of environmental changes on a key component of pelagic food webs in the Southern Ocean: the amphipod *Themisto gaudichaudii* (Crustacea: Hyperiidea)

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### **Background & Rationale**

For most of the past decades, almost the entire research effort concerning the pelagic ecosystem of the Southern Ocean was directed towards krill (*Euphausia*). As a consequence, krill served as the icon of polar pelagic metazoans whereas other major players have received disproportionately little attention. Other trophic pathways are both regionally and seasonally important, comprising planktonic herbivores (salps, copepods) and carnivores (amphipods, ctenophores, cnidarians) (Murphy et al. 2007, Mackey et al. 2012), but their functioning and roles are currently much less understood (Smetacek & Nicol 2005, Murphy et al. 2007). *Themisto gaudichaudii*, the most common pelagic amphipod, plays a similar role to that of Antarctic krill (*Euphausia superba*) further south in Antarctic waters and has therefore been referred to as “krill of the north” (Bocher et al. 2001, Padovani et al. 2012). Similarly, it forms dense swarms with densities reaching up to several hundreds of individuals per cubic metre (Froneman et al. 2000) and is both a key consumer of mesozooplankton (e.g. Watts & Tarling 2012) and an important food source for fish, squids, birds and whales (e.g. Nemoto & Yoo 1970, Bocher et al. 2001, Ivanovic & Brunetti 1994, Padovani et al. 2012). However, unlike krill that is more closely associated with polar waters, *T. gaudichaudii* occupies a much broader temperature range occupying waters around the continent (subzero temperatures) to the subtropics (17°C) (Kane 1966, Auel & Ekau 2009). Hence, this predatory amphipod should therefore be better equipped to cope with ongoing and future environmental changes and its abundance has been predicted to increase southwards in the warming Southwest Atlantic region of the Southern Ocean (Mackey et al. 2012).

Previous studies on *T. compressa*, formerly synonymized with *T. gaudichaudii* under *Parathemisto gaudichaudii*, have shown a substantial phenotypic variability, distinguishing three different ‘forms’: compressa, bispinosa and intermedia, which seemed to develop according to the environmental conditions in which the individuals were raised (Shearer 1975). Similar forms have been identified within *T. gaudichaudii* from the Southern Ocean (Schneppenheim & Weigmann-Haass 1986). However, this study based on electrophoretic evidence supported the hypothesis of a single interbreeding population despite these morphological variations as well as a substantial geographic distance separating sampling sites in the Scotia Sea (Schneppenheim & Weignmann-Haass 1986). Nonetheless, so far, genetic connectivity between populations of *T. gaudichaudii* has never been investigated on a large geographical scale, nor with recent molecular techniques. This is in sharp contrast with the abundance of molecular studies carried out on Antarctic krill over the last decades, from pioneering allozyme studies (Fevolden & Schneppenheim 1988) to the application of Next-Generation Sequencing techniques (e.g. Johansson et al. 2012).

As opposed to benthic amphipods, in which a high level of cryptic speciation has been observed within presumed circum-Antarctic species (e.g. Havermans et al. 2011), crustaceans with one or more pelagic life stages are expected to be characterized by a certain degree of genetic homogeneity around the continent (e.g. decapods, Raupach et al. 2010; krill, Bortolotto et al. 2011). The dominant circumpolar current systems around Antarctica certainly ease the passive dispersal of pelagic organisms in the

Southern Ocean whilst on the other hand, oceanic gyres might be responsible for retention of larval stages, limiting gene flow and promoting separate interbreeding stocks (as hypothesized for krill, Zane et al. 1998).

*T. gaudichaudii*'s status of a circum-Antarctic species has recently been questioned: when comparing specimens from Prydz Bay and the Pacific sector of the Southern Ocean, a substantial morphological variation was reported, suggesting the possibility of (at least) two distinct lineages or species within the Southern Ocean (Zeidler & De Broyer 2014). Hence, the biogeographic limits of the species need to be re-evaluated with both morphological and molecular methods (Zeidler & De Broyer 2014). Finally, a better knowledge on its genetic connectivity and distribution is urgently required for meaningful forecasts of the Southern Ocean pelagic ecosystems' responses to ongoing and future environmental changes, e.g. evaluating potential distributional or ecological shifts. Hence, I aimed to test whether *T. gaudichaudii* comprises genetically differentiated strains throughout its distribution area.

## Objectives

The hyperiid amphipod *Themisto gaudichaudii* represents a currently understudied key component of the Southern Ocean pelagic realm. Being characterized by a circum-Antarctic distribution extending to south of the sub-Tropical Front (Kane 1966, Auel & Ekau 2009), populations of *T. gaudichaudii* are found along a latitudinal gradient of environmental conditions, covering three distinct zones: temperate, sub-Antarctic and high Antarctic. To the best of our knowledge, no recent molecular studies have been presented on *T. gaudichaudii* despite its abundance and ecological importance. This project addresses this knowledge gap for *Themisto gaudichaudii* by characterizing its spatial genetic structure using phylogeographic methods with as aim to determine whether *T. gaudichaudii* represents a single, circum-Antarctic species or identify potential genetically differentiated strains throughout its distribution area. The prediction of the hypothesized range extension and associated ecological shifts fully depends on the outcome of these molecular analyses. The scenario in which *Themisto* may be responsible for outcompeting or controlling the standing stock of other key pelagic players such as Antarctic krill and salps further south in the warming parts of the Southern Ocean might only hold true if it represents a single, panmictic population across its wide geographic and associated temperature range.

## Methods

Specimens of *Themisto gaudichaudii* were collected during expeditions of RV *Polarstern* with midwater trawls, Agassiz/bottom trawls, Rauschert dredge, handnets, multinet and Bongo nets. Samples were collected by myself or colleagues in the Weddell Sea between Dresher Inlet and Atka Bay (Austasen area) during the ANT-XXI/2 expedition (2003), in the Scotia Sea (Shag Rocks) during the ANT-XXVII/3 expedition (2011), in the Polar Frontal zone from East (10°) to West (South Georgia) during the ANT-XXVIII/3 expedition (2012). Additional samples have been obtained in the course of the fellowship from collaborators, originating from the ANT-XXIX/3 and ANT-XXIX/7 expeditions in the Scotia Sea. New samples were obtained during the period of the fellowship on the expedition ANT-XXIX/9 to the Filchner area in the Weddell Sea (2013-2014), in which I participated. Details on the sampling sites and specimens used for analyses are listed in Table 1.

Specimens were classified into the *bispinosa* or *compressa* form either on board before preservation or in the laboratory before DNA extraction. For a total of 259 individuals, genomic DNA was isolated from pereopod 6 using the Nucleospin Tissue kit (Macherey-Nagel) according to the manufacturer's protocol. PCR amplifications of a fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) gene was carried out for a subset of the DNA extracts using the LCO1490 and HCO2198 primers (Folmer et al. 1994). The 25µl PCR reactions consisted of 0.02U/µl Hotmaster Taq (5Prime GmbH), 0.2mM dNTPs, 0.5 µM of forward and reverse primers, 1x PCR-buffer and 1µl (about 30ng) of template DNA. PCR conditions were: initial denaturation at 94°C for 2 min, followed by 36 cycles of 94°C for 20 s,

annealing at 42°C for 20 s, extension at 65°C for 1 min and a final extension at 65°C for 15 min. Amplified products were purified using the Exo-SAP-IT kit (Affymetrix, Santa Clara, Canada). Both forward and reverse strands of the gene were sequenced for 95 individuals on an ABI 3130xl sequencer after cycle sequencing with the BigDye Terminator Kit (Applied Biosystems, Foster City, Canada).

Sequences were checked for ambiguities and aligned using the software CodonCode Aligner v.3.7.1.1. (CodonCode Corporation, Deham, USA). In order to prevent inclusion of pseudogenes in the analyses, electropherograms were checked for ambiguous base calls and sequences were translated into amino acids and checked for stop codons. A neighbour-joining tree (Saitou & Nei 1987) was estimated using MEGA 5 (Tamura et al. 2011) and sequence divergences calculated using the Kimura-2-parameter (K2P) distance model (Kimura 1980). Relationships between COI haplotypes and their geographic distribution were investigated by generating haplotype networks using TCS 1.21 (Clement et al. 2000), with gaps considered as a fifth state and a 95% probability threshold.

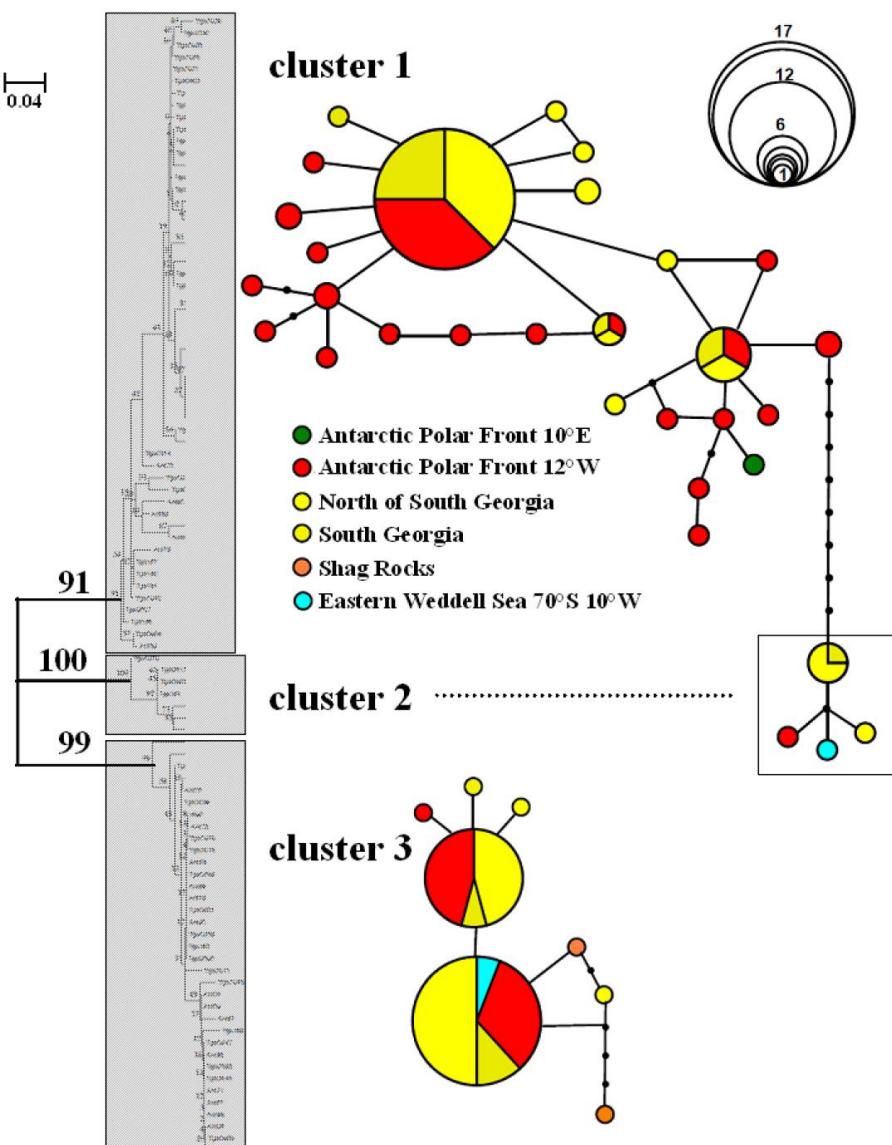
**Table 1.** Sampling details of *Themisto gaudichaudii* specimens processed for this study. Abbreviations: N = Number of specimens used for molecular analyses, AGT= Agassiz Trawl, HN = handnet, MN = Multinet, RMT = rectangular midwater trawl, BT = bottom trawl, RD = Rauschert Dredge. In grey the sample localities for which DNA sequences have been obtained from sampled specimens. The number of specimens for which DNA has been isolated are indicated (N). For the non-coloured sample localities, DNA has been isolated for the number of specimens indicated (N) but not yet sequenced.

Expedition	Station	Locality	Coordinates	Depth (m)	N
RV Polarstern ANT XXVIII/3	084-26 AGT	10°E Antarctic Polar Front	52°59'S 10°02'E	0-4118	8
RV Polarstern ANT XXVIII/3	085-16 AGT	7°W Antarctic Polar Front	52°00'S 07°59'W	0-2752	2
RV Polarstern ANT XXVIII/3	086-21 AGT	12°W Antarctic Polar Front	52°00'S 12°01'W	0-3951	19
RV Polarstern ANT XXVIII/3	086-22 AGT	12°W Antarctic Polar Front	51°59'S 12°00'W	0-3964	6
RV Polarstern ANT XXVIII/3	086-23 AGT	12°W Antarctic Polar Front	51°59'S 12°04'W	0-3990	4
RV Polarstern ANT XXVIII/3	140-3 HN	12°W Antarctic Polar Front	51°12'S 12°39'W	0-50	1
RV Polarstern ANT XXVIII/3	140-4 MN	12°W Antarctic Polar Front	51°12'S 12°29'W	0-1000	1
RV Polarstern ANT XXVIII/3	140-8 RMT	12°W Antarctic Polar Front	51°12'S 12°41'W	0-250	4
RV Polarstern ANT XXVIII/3	140-9 RMT	12°W Antarctic Polar Front	51°10'S 12°48'W	0-250	4
RV Polarstern ANT XXVIII/3	141-7 AGT	12°W Antarctic Polar Front	51°15'S 12°37'W	0-4110	10
RV Polarstern ANT XXVIII/3	141-8 AGT	12°W Antarctic Polar Front	51°16'S 12°37'W	0-4112	8
RV Polarstern ANT XXVIII/3	146-3 RMT	N of South Georgia	50°01'S 36°58'W	0-250	7
RV Polarstern ANT XXVIII/3	152-3 RMT	N of South Georgia	49°37'S 37°37'W	0-250	11
RV Polarstern ANT XXVIII/3	163-4 RMT	N of South Georgia	50°24'S 39°21'W	0-250	1
RV Polarstern ANT XXVIII/3	172-3 RMT	N of South Georgia	49°12'S 38°12'W	0-250	8
RV Polarstern ANT XXVIII/3	175-3 RD	N of South Georgia	50°46'S 39°24'W	0-4164	1
RV Polarstern ANT XXVIII/3	175-3 AGT	N of South Georgia	50°46'S 39°24'W	0-4164	6
RV Polarstern ANT XXVIII/3	174-11 RMT	N of South Georgia	49°38'S 38°18'W	0-250	3
RV Polarstern ANT XXVIII/3	175-4 RD	N of South Georgia	50°47'S 39°24'W	0-4167	3
RV Polarstern ANT XXVIII/3	175-10 RMT	N of South Georgia	50°46'S 39°25'W	0-250	8
RV Polarstern ANT XXVIII/3	176-1 RMT	N of South Georgia	52°15'S 40°29'W	0-250	13
RV Polarstern ANT XXVII/3	211-7 BT	Shag Rocks	53°24'S 42°40'W	0-309	2
RV Polarstern ANT XXI/2	145 AGT	Austasen Weddell Sea	70°56'S 10°48'W	0-402	2
RV Polarstern ANT XXIX/3	131-1 RMT	N of Elephant Island	60°50'S 55°00'W	0-200	14
RV Polarstern ANT XXIX/3	234-2 RMT	NW of Livingston Island	62°17'S 61°15'W	0-200	10
RV Polarstern ANT XXIX/3	238-1 RMT	NW of Livingston Island	62°20'S 61°17'W	0-387	2
RV Polarstern ANT XXIX/7	538-1 RMT	NW of South Georgia	53°30'S 40°12'W	0-500	40
RV Polarstern ANT XXIX/7	540-1 RMT	W of South Georgia	54°26'S 40°36'W	0-500	20
RV Polarstern ANT XXIX/7	541-1 RMT	W of South Georgia	55°00'S 40°42'W	0-500	20
RV Polarstern ANT XXIX/7	548-1 RMT	NE of South Orkney Islands	59°59'S 42°24'W	0-500	6
RV Polarstern ANT XXIX/9	003-1 RMT	Kapp Norvegia, Weddell Sea	73°44'S 25°46'W	0-500	1
RV Polarstern ANT XXIX/9	042-1 RMT	NE Filchner area	76°04'S 15°59'W	0-450	1
RV Polarstern ANT XXIX/9	083-1 RMT	SE Filchner area	77°01'S 33°41'W	0-400	1
RV Polarstern ANT XXIX/9	107-1 RMT	Southern Filchner Trench	77°55'S 38°02'W	0-850	3
RV Polarstern ANT XXIX/9	117-1 RMT	Southern Filchner Trench	77°36'S 38°55'W	0-500	7
RV Polarstern ANT XXIX/9	199-1 RMT	NW Filchner area	74°35'S 36°21'W	0-500	1
RV Polarstern ANT XXIX/9	227-1 RMT	NW Filchner area	74°19'S 37°40'W	0-500	1

## Preliminary results

DNA was extracted for a total of 259 individuals from 37 different stations in the Atlantic sector of the Southern Ocean (Weddell and Scotia seas, Polar Frontal zone). After optimization of the protocol, PCR amplifications and sequencing of the COI gene were successfully carried out for the first set of 95 DNA extractions, as much as the budget for the fellowship allowed.

Analyses were based on a COI dataset of 95 sequences. Results show that, throughout the sampling localities, *Themisto gaudichaudii* consists of three well-supported genetic lineages (see Figure 1). Cluster 1, 2 and 3 comprised sequences of 53, 7 and 35 specimens, respectively, and were separated by pairwise K2P distances of minimum 2.7 %. Within-cluster variation did not exceed 0.5 %. Cluster 1 and 2 appeared to be separated by 9 mutational steps, whilst cluster 3 was recovered as an unconnected network for the statistical parsimony network analysis (95% probability threshold). Specimens of the different forms (*bispinosa* vs. *compressa*) were recovered in all three clusters. Hence, neither morphological variation nor geographical distribution can explain the observed genetic structure.



**Fig. 1** Neighbour-joining tree with bootstrap values indicated next to each cluster (nreps = 2000) showing the three well-supported clades of *T. gaudichaudii*. At the right, statistical parsimony haplotype networks are presented (95% probability threshold). The area of each circle of the networks is proportional to the frequency of the haplotype. Each line represents a single substitution, nodes represent hypothetical haplotypes and colours refer to the sampling localities indicated in the legend.

COI sequences indicate that *T. gaudichaudii* comprises distinct genetic lineages occurring in sympatry and which are independent of the morphological forms. The latter observation confirms previous molecular results (Schneppenheim & Weigmann-Haass 1986) and experimental observations showing that individuals can either develop towards the *compressa* or the *bispinosa* form depending on the conditions which they experience during growth, such as nutrition and temperature (Sheader 1975). Even though *T. gaudichaudii* does not seem to represent a single, panmictic population across its distribution range, the different genetic lineage do cover a wide geographical range. A genetic homogeneity was found for specimens from a zone close to the continent (Austasen iceberg resting area in Weddell Sea) and specimens from the Polar Frontal zone, as well as from waters around South Georgia, across distinct water masses and associated temperature regimes. Hence, the *Themisto* lineages might be able to cope with warming and have a great potential of southward expansion in the Southwest Atlantic sector if the hypothesis for panmixia between different geographic localities within a single lineage would hold true.

## Future work

The presented work will be continued under a research project on *Themisto gaudichaudii* funded by the German Science Foundation (DFG) that will be running from October 2015 to September 2018 at the University of Bremen. Current work has shown that the three well-supported lineages might all occur over an identically wide geographic range, however, whether this holds true for the entire distributional range needs to be further investigated with the additional samples obtained during this study and supplementary material from other sectors of the Southern Ocean. To do so, COI amplifications will be carried out for the remaining 164 specimens of which the DNA has been extracted during this study. Furthermore, more samples will be collected via collaborators from various regions of the Southern Ocean. The planned molecular analysis of samples from the East Antarctic will shed light on the nature of the morphological variation observed between specimens of Prydz Bay and the other sectors (Zeidler & De Broyer 2014). Other standard markers will be amplified and sequenced for the material collected here, in order to cover both nuclear and mitochondrial sequence variation. Finally, the foreseen population genetic analyses based on fast-evolving markers such as SNPs or microsatellites, developed based on Next-Generation Sequencing data, will allow me to evaluate the level of gene flow between different populations of *T. gaudichaudii*.

## Outcomes

### *Talks*

Havermans C (2014). Genetic connectivity of Southern Ocean amphipods: circumpolarity, eurybathy, bipolarity and (pseudo)cryptic speciation. **Oral communication** at the SCAR Open Science Conference, Auckland, New Zealand, August 2014.

Havermans C (2014). Phylogeography of Southern Ocean amphipods shows evidence for circumpolar, eurybathic, bipolar and (pseudo)cryptic species. Double slot **oral communication** at the 21<sup>st</sup> Benelux Congress of Zoology, Liège, Belgium, December 2014.

### *Publication*

Havermans C et al. (*in prep*). On the phylogeography of the pelagic amphipod *Themisto gaudichaudii* (Crustacea: Hyperiidea) in the Atlantic sector of the Southern Ocean.

## Budget expenses

Expenses provided by the COMNAP fellowship covered the costs of an 8-month stay in Bremerhaven (Germany), divided in two periods: Oct-Dec 2013, April-Sept 2014 and an expedition with R/V *Polarstern* from December 2013 until March 2014. No remaining money was left at the end of this

period with the budget being spent mainly in accommodation, lab costs, expedition costs and travel costs. For the SCAR Open Science Conference, supplementary funding for travel costs was obtained via a SCAR-AnT-ERA (Antarctic Thresholds – Ecosystem Resilience and Adaptation) fellowship.

Transport Brussels-Bremerhaven.....	400 EUR
Stay at Havenhostel BHVN for one night (Aug 2013; packing for expedition).....	70 EUR
Rent apartment in Bremerhaven for 8 months (250EUR/month).....	2000 EUR
Flights Brussels-Cape Town-Brussels.....	1450 EUR
Insurance Antarctic field work.....	350 EUR
Hotel nights in Cape Town before and after expedition.....	300 EUR
SCAR OSC 2014 Auckland Registration costs.....	440 EUR
Costs for products for laboratory work (extraction, PCR, sequencing).....	4284 EUR
<b>TOTAL.....</b>	<b>9294 EUR</b>
	<b>(12500 USD)</b>

## Acknowledgements

I will be ever grateful to COMNAP for supporting this project. Not only had it laid the foundation of my current research career path, but helped me to carry on researching during the kind of period that almost every scientist encounters at least once during their career – the one of many dreams but no funding. Thanks to COMNAP, I was able to seize one of my dreams and building upon its results I will now initiate a longer-term project on this topic.

Secondly, I thank the AWI and my host Dr. Christoph Held for his support and guidance throughout the years of my coming and going in the AWI.

I also want to thank the respective chief scientists, captain and crew and fellow scientists of the expeditions ANT-XXI/2, ANT-XXVII/3, ANT-XXVIII/3, ANT-XXIX/3, ANT-XXIX/7 and ANT-XXIX/9. Special thanks go to Dr. Angelika Brandt for granting me a berth in the SYSTCOLI-cruise (ANT-XXVIII/3) during which this *Themisto* story was initiated.

My thanks also go to the SCAR's program AnT-ERA for according me a 'mini-grant' to travel to Auckland and present my results at the Open Science Conference.

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