

# New insights into the evolutionary history and the present distribution of the common Southern Ocean pycnogonid *Colossendeis megalonyx*

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## Introduction

The Southern Ocean is a unique ecosystem largely isolated from other oceans and characterized by many endemic taxa, especially among benthic organisms. It is thought to be one of those ecosystems that will be impacted most strongly by global climate change. The history of Antarctic benthos has been strongly influenced by the Pleistocene glaciations, during which the Antarctic shelf was largely covered with ice. Benthic lifeforms could have survived 1) around the Subantarctic islands, 2) in the deep sea, or 3) in temporary ice-free areas. These possibilities would lead to different genetic signatures.

Recent molecular studies have shown that Southern Ocean taxa that have been classified as single species consist of several overlooked species. For example, *Colossendeis megalonyx*, one of the most widespread Southern Ocean sea spiders, has long been considered a species with typical circumpolar eurybathic distribution both in the Antarctic and Subantarctic. A recent study (Krabbe et al. 2010) found that *C. megalonyx* consists of at least six clearly distinct mitochondrial lineages (Fig. 1). Here, we test the distinction of these clades using nuclear gene sequences and morphology and extend the sampling to other areas such as East Antarctica.

## Materials & Methods

The nuclear gene histone 3 (H3) was sequenced for 84 *C. megalonyx* specimens whose COI sequences were already published by Krabbe et al. (2010). Sequences were aligned using MUSCLE and phylogenetic analysis was performed with MrBayes.

For the 59 of these specimens belonging to five of the six clades, the lengths of proboscis, trunk, and all articles of all pairs of extremities were measured. The statistical significance of intraclade differences was tested in Statistica using unifactorial ANOVA with F test and subsequent Fisher's LSD test.

In addition, DNA was extracted from 71 *C. megalonyx* individuals from the East Antarctic (Terre Adélie, George V Land), the Eastern Weddell Sea, and the South Orkney Islands. Partial mitochondrial cytochrome oxidase I (COI) sequences were generated using PCR with standard barcoding primers. Further sequences were retrieved from GenBank. Sequences were aligned using MAFFT. Phylogenetic analysis was performed using MrBayes 3.1.2. A haplotype network for clade E was constructed using HapStar. K2P genetic distances were calculated and visualized using ABGD. For inference of overlooked species within *C. megalonyx*, we used the GMYC approach as implemented within the program Splits. As input, a linearized phylogenetic tree was calculated using BEAST.

ORIGINAL PAPER  
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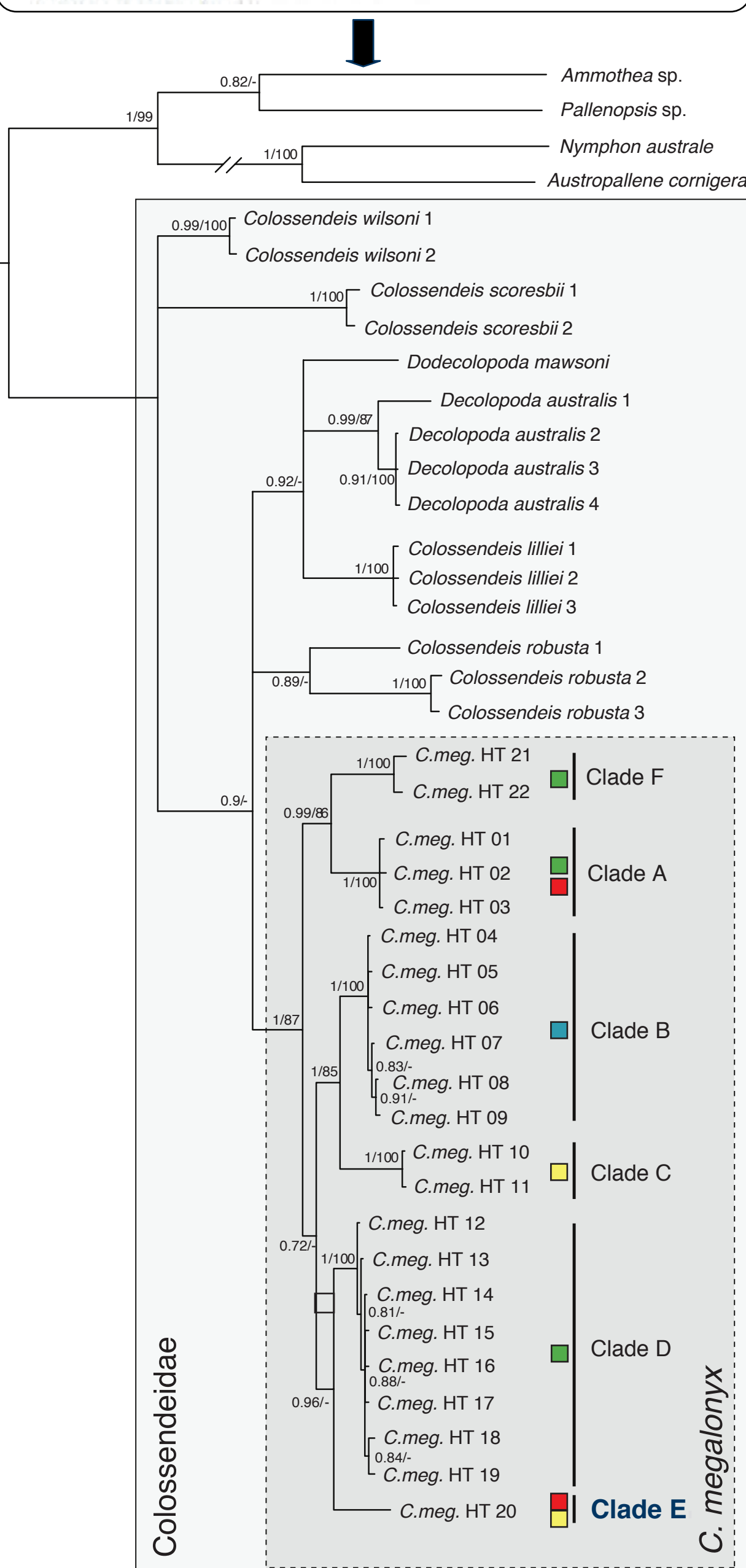


Figure 1: Bayesian tree from Krabbe et al. (2010) based on COI data. Six genetically distinct clades (A-F) were found.

How many more are there and where?

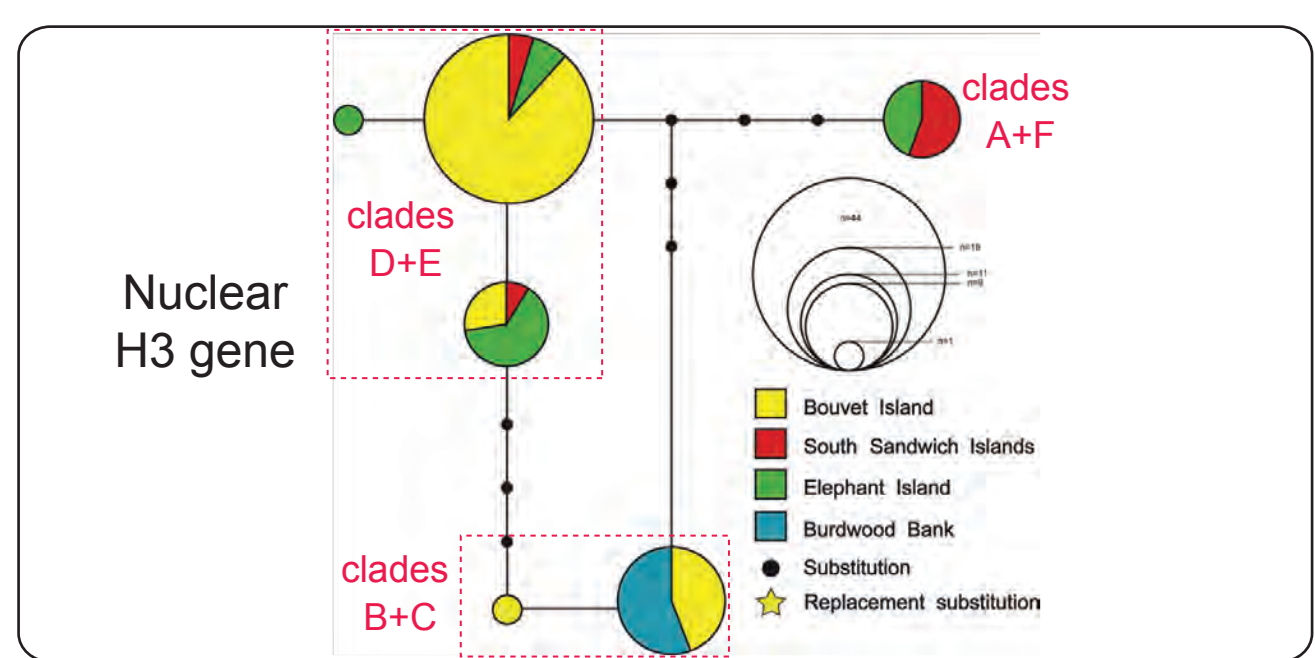


Figure 2: Haplotype network of H3 of the *C. megalonyx* complex. Each H3 haplogroup corresponds to two related clades in the CO1 dataset.

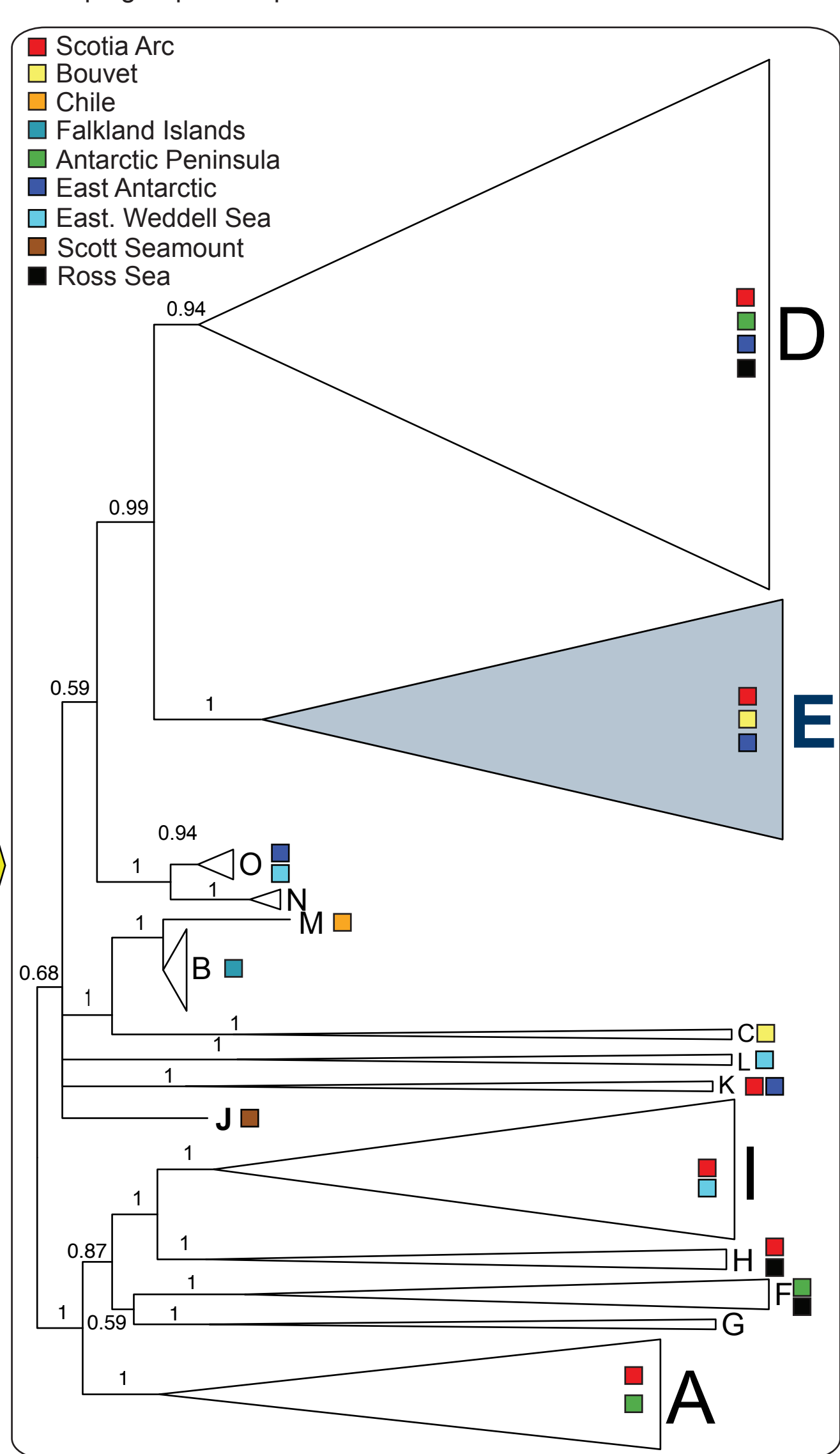


Figure 3: Bayesian phylogenetic tree of CO1 data of the *C. megalonyx* complex. Colours indicate geographic distribution of the different clades.

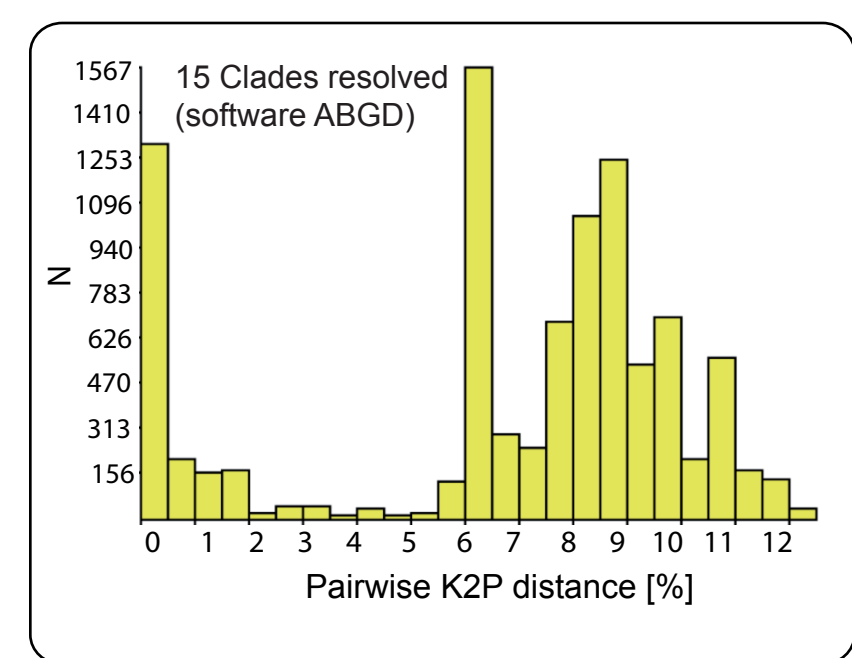


Figure 4: Genetic distances of CO1 within *C. megalonyx* showing a significant barcode gap.

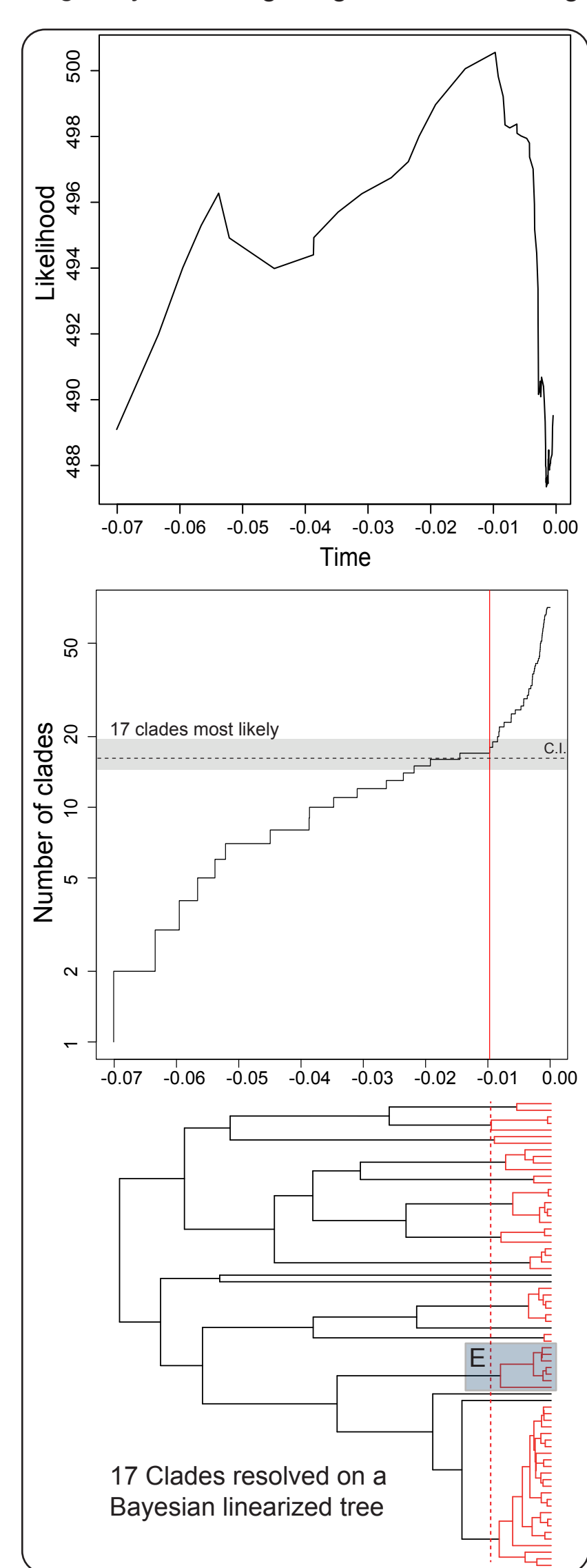
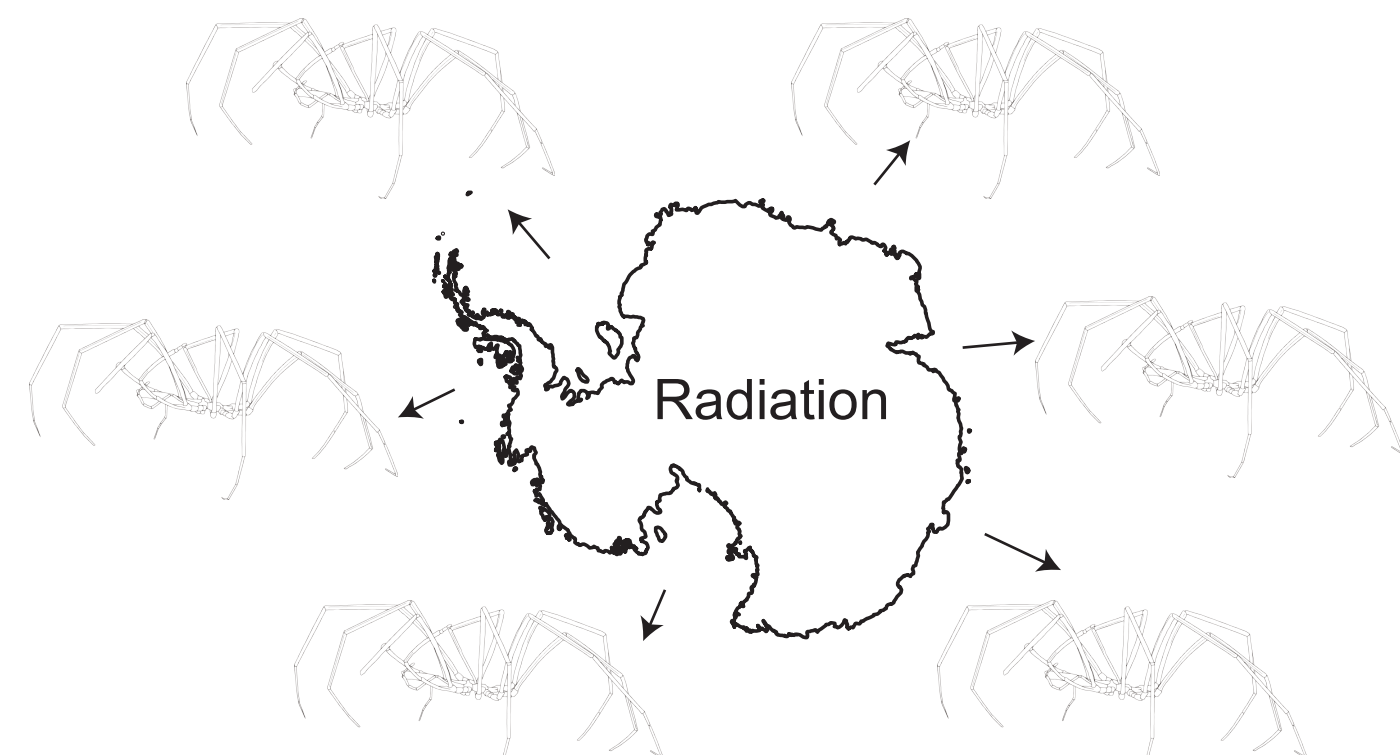


Figure 5: Results of GMYC analysis. Top: Log likelihood through time before present, middle: number of clusters vs. time before present, bottom: haplotype tree used for analysis.



There are very many (≥15)!  
Many have a broad/circumpolar distribution range

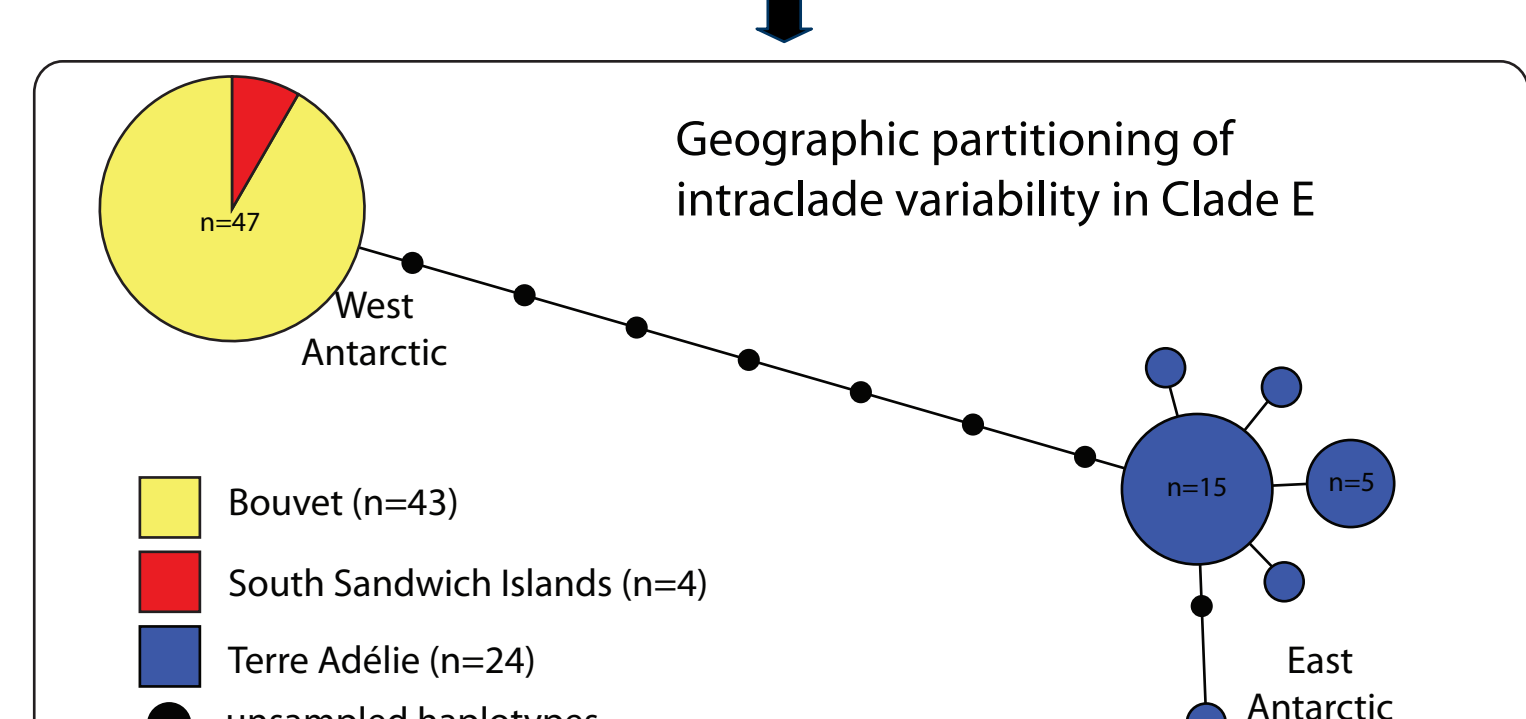


Figure 6: Haplotype network of *C. megalonyx* clade E, showing differentiation between East Antarctic and Scotia Sea/Bouvet haplotypes.

Significant regional differentiation within broadly distributed clades found!  
Low contemporary gene flow

## Results/Discussion

The COI tree shows a differentiation of the *C. megalonyx* complex into at least 15 clearly distinguished clades (Fig. 3), including the six that were previously identified by Krabbe et al. (2010). A typical barcoding gap is present between about 2 and 6 % genetic distance (Fig. 4). GMYC analysis results in 15-20 distinct groupings that agree with the ABGD results (Fig. 5). Several clades are found in both the West and East Antarctic, suggesting that **circumpolar distribution of the different *C. megalonyx* clades is common**. Most clades show a differentiation between haplotypes from those two areas (Fig. 6). In some sampling locations, different clades are found in sympatry. The specimens from the South American shelf and some from Bouvet Island (clades B, C, M) form a strongly supported group, which groups within the Antarctic clades. This suggests that **the Subantarctic was colonized from the Antarctic and not vice versa**.

H3 data partially confirm the clades identified by Krabbe et al. (2010), although the resolution is lower (Fig. 2). Each of the three H3 clades corresponds to two sister clades in the COI tree.

There are statistically significant morphometrical differences between all of the investigated clades. (J. Spaak, M. Sc. thesis). This provides additional evidence that **the mitochondrial clades indeed represent overlooked species**.

Both morphological (morphometrics, ovigeral spine configuration) and molecular data (CO1, H3) indicate that the North Pacific species *C. tenera* is not part of the *C. megalonyx* complex (Dietz et al., under review).

## Future work (2012/2013)

COI sequencing of further samples is planned for the near future. For about 200 specimens from Scotia Arc, DNA has already been extracted. For all samples, a nuclear gene such as H3 will also be sequenced to test the COI clades.

Next generation sequencing techniques will be used to clarify both inter- and intraclade phylogeography. ddRAD-seq (double digest Restriction-site Associated DNA sequencing) libraries of several specimens from different clades are in preparation. This technique allows getting 1000s of homologous loci associated with restriction enzyme cutting sites and therefore achieving much higher resolution than would be possible with single mitochondrial or nuclear genes. Results will be analyzed with various statistical methods used in phylogeography and population genetics to investigate the history of dispersal and colonization of different geographic regions by this species complex. In addition, the new species will be formally described.

