

Disentangling the evolutionary history of the diatoms in Antarctica: the *Pinnularia borealis* species complex as a case study

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Because of their immense diversity, the systematics of protists, and diatoms in particular, is complex and challenging. As species taxonomy has far-reaching consequences for our understanding of diatom evolutionary history, ecology and biogeography, detailed studies into species limits and diversity are needed if we are to advance our understanding of the range dynamics and diversification of diatoms. However, the biogeography and ecology of (pseudo)cryptic diatom species is highly understudied and there are virtually no data available on the timing of lineage splitting within species complexes. This is especially the case for Antarctica for which molecular studies on diatoms are seldom undertaken. In the present study, we used the semiterrestrial diatom morphospecies complex *Pinnularia borealis* as a case study for speciation, evolutionary history and biogeography of terrestrial diatoms, and more generally microscopic eukaryotes, in Antarctica.

During field campaigns on multiple continents, including the entire Antarctic Region, more than 500 samples from (semi)terrestrial habitats were collected. From these, over 900 monoclonal cultures were established for DNA and morphology. Automatic species delimitation based on the cox1 and 28S rDNA genes revealed the presence of at least twelve Antarctic lineages and 93 lineages worldwide. Of all Antarctic lineages, only two were recovered from other continents. A molecular clock analysis on a four marker dataset indicated that the diversification of *P. borealis* started in the late Eocene and strongly increased during the Miocene.

The results indicate a high degree of hidden species diversity in *P. borealis* worldwide and the presence of regional endemics in Antarctica. The topology of the phylogenetic tree and the age of the lineage splitting does not support a radiation of the *P. borealis* species complex in Antarctica. In contrast, it suggests multiple independent colonization events in or out of the Antarctic Region during the past 20 million years. Together with the high levels of genetic diversity, these findings indicate that although the *P. borealis* complex is relatively successful in dispersal, this is not sufficient to prevent speciation. Altogether, our data provide evidence for the moderate endemicity hypothesis for micro-organisms and suggest that, whereas Antarctica is the most remote and hostile continent on the world, it has been reached and successfully colonized multiple times by terrestrial diatoms.