DNA analysis for identification of a Pinus pollen grain at subsection level found in Belukha Glacier

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We improved our method on DNA analysis for identification of a *Pinus* pollen grain. We presented a method that could identify a *Pinus* pollen grain found in Belukha Glacier, Russian Altai Mountains using polymerase chain reaction (PCR) technique. However the capability of the identification was still at section level and the success rate of PCR was 7.6%. The purpose of the present study was to identify the grain at subsection level and to obtain higher success rate newly using multiplex PCR technique. Fragments of 134-147 bp from five loci of the chloroplast genome in each *Pinus* pollen grain were amplified, and the DNA products were sequenced in order to identify them at subsection level. As a preliminary result, the success rate for sequence amplification in the present study was 35% and exceeded that of our previous study. *Pinus* is a taxon with approximately 111 recognized species in two subgenera, four sections and 11 subsections. From the sequences obtained for the six grains, four pollen grains were identified as belonging to subsection *Pinus*. Trees of *Pinus sylvestris*, in subsection *Pinus*, are currently found surrounding the glacier. The consistency of results for this subsection suggested that these pollen grains originated from the same *Pinus* trees found in the immediate surroundings, which spread also as far as Europe. Interestingly, other two grains were identified as subsection *Australes* that is found in North America, Mexico, Central America and Caribbean.

Keywords: glacier, pollen analysis, DNA, ice core, Russian Altai Mountains