

BLANDENIER Quentin (2015): Design and evaluation of new mitochondrial marker for Arcellinida (Amoebozoa)

Abstract

Barcoding is an indispensable tool for assessing phylogenetic relationships among protists. The most commonly used marker is the 18S (or SSU) ribosomal RNA gene, a highly conserved gene that is present in many copies in the nuclear genome of all organisms from the three domains of life. However, this marker cannot resolve all phylogenies, as it is either too slow-evolving for young groups, or presents important differences in evolutionary pace. Here, we designed Amoebozoaspecific primers to amplify a region that includes partial sequences of two subunits of mitochondrial nicotinamide adenine dinucleotide dehydrogenase gene (NAD9/NAD7). We applied them to Arcellinids belonging to distantly related genera (*Arcella*, elongated *Diffugia*, and *Bullinularia*). We also applied it to an environmental sample (*Sphagnum*) to obtain additional Arcellinid sequences. Monophyly of Arcellinida was recovered and robustly supported, as was genus *Diffugia*. *Diffugia nodosa* yielded two different sequences, which suggested hidden diversity within this morphospecies; this was corroborated with differences in morphology (shell size). Gene arrangement took three different configurations between NAD9 and NAD7: (1) a small intergenic sequence, (2) both genes are placed end-to-end, or (3) an overlap depending on the considered species. Variations in the genetic code as well as putative RNA editing are discussed. In this study, we developed and applied to Arcellinida the first primers for a mitochondrial gene other than the cytochrome oxidase subunit 1 gene (COX). We present also the first molecular sequences for two morphospecies of the still little studied but highly diverse genus *Diffugia* (*D. nodosa*, *D. pyriformis*).