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An Evaluation of the Use of the LSU rRNA D1-D5 Domain for DNA-based Taxonomy of Eukaryotic Protists

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Diagnostic signature DNA sequences are important tools for the identification of species. There is an active debate in the literature on the choice of the best markers applicable for a broad range of organisms. Protists have seldom been included in these evaluations. Mitochondrial gene sequences are inappropriate for protists since several groups do not possess mitochondria. Here we studied the application of the large subunit (LSU) rRNA gene fragments (D1-D5) regarding their usefulness to discriminate between a wide range of heterotrophic nanoflagellates. Phylogenetic analyses based on the LSU rRNA fragments showed similar results compared to phylogenetic trees based on the small subunit (SSU) rRNA. The data set indicates the power of the use of the D1-D5 region as a marker for a DNA-based taxonomy. Our results, together with the available sequences in Genbank, form a comprehensive database for unicellular eukaryotes, especially heterotrophic flagellates. It is now possible to assign new sequences to the different groups of heterotrophic flagellates which we have tested for different closely related *Cercomonas* and *Paracercomonas* strains from groundwater.

Key words: Eukaryotes; protists; heterotrophic flagellates; DNA-taxonomy; ribosomal genes; D1-D5 domain of LSU rRNA; groundwater.

Introduction

The different approaches to DNA-based taxonomy and the choice of genes useful for discrimination of closely related species were intensively discussed in literature (for review see Frézal and Leblois 2008). While Hebert et al. (2004) proposed mitochondrial genes such as cytochrome c oxidase subunit I (CO1) for use in DNA based taxonomy, Tautz and colleagues (e.g. Markmann and Tautz 2005; Tautz et al. 2002) were in favour of nuclear ribosomal genes especially fast evolving fragments of the LSU rRNA. Both proposals have been critically discussed by several authors

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(e.g. Blaxter et al. 2004; Moritz and Cicero 2004). There are recent studies which evaluated these proposed gene regions for their application in the discrimination of different taxa. Most of these studies included only metazoan taxa (e.g. Hebert et al. 2004; Johnson and Cicero 2002; Markmann and Tautz 2005; Sonnenberg et al. 2007; Vences et al. 2005). Up to now, the unicellular eukaryotes (protists) were hardly taken into consideration. It seems to be important to include protists into the current debate of barcoding genes since protists comprise many different phylogenetic lineages with extraordinary molecular heterogeneity. The evolutionary rates differ significantly between groups of protists and also within different genes. For instance, the foraminiferans and euglenozoans have remarkably high evolutionary rates concerning the ribosomal genes compared to most other eukaryotes (e.g. Busse and Preisfeld 2002; Pawlowski et al. 1997). CO1 has been proven to be a suitable gene for species identification in some groups of protists (Barth et al. 2006; Evans et al. 2007; Lynn and Strüder-Kypke 2006; Robba et al. 2006), however, due to the absence of typical mitochondria in several unicellular taxa (e.g. pelobionts, entamoebae, diplomonads) this gene is not suitable for an overall comparison of protists. Furthermore, ribosomal genes have the advantage that they are already pre-amplified in the cell which is especially useful for ribosomal RNA-based detection approaches (e.g. fluorescence-in-situ-hybridization, microarray technology, see also Ki and Han 2006). Conserved and divergent regions which are more extensive than the homologous regions in prokaryotes (expansions segments, Gerbi 1986) alternate with each other in the ribosomal genes. The SSU rRNA contains seven variable regions (termed V1-V7). Twelve of such divergent regions named D1-D12 exist in the LSU rRNA gene, of which D2 and D8 show the highest variability in length and base composition (Hassouna et al. 1984). Fragments of ribosomal genes are easy to amplify with universal primers which bind on the conservative regions between the highly different domains. However, the problem of the existence of paralogous genes in the genomes for phylogenetic reconstructions is not yet solved. It is known that multiple independent copies could be present for ribosomal genes. There are only a few reports about this phenomenon in SSU rRNA (e.g. Carranza et al. 1996; Schlegel et al. 1996). Such independent rRNA copies were found to be transcribed in different life stages (Gunderson et al. 1987) or may be considered as pseudogenes (Scholin et al. 1993). The problem of the occurrence of intra-specific polymorphisms seems to be more common in the internal transcribed spacer regions (e.g. Aanen et al. 2001; Wenner et al. 2002; Windsor et al. 2006). But not a single polymorphism could be found in the rDNA repeat of the recently sequenced genome of a marine alveolate (Massana et al. 2008).

New groups of protists have been found via small subunit ribosomal RNA (SSU rRNA) gene libraries (e.g. López-García et al. 2001; Massana et al. 2004; Vandenkoornhuyse et al. 2002). A high unexpected diversity of protists (e.g. Habura et al. 2004; Rappé et al. 1998) as well as bacteria (e.g. Brown and Bowman 2001) and metazoans (e.g. Blaxter et al. 2004) have been detected using the conservative SSU rRNA gene. The available data base concerning the large subunit (LSU) rRNA gene is much smaller for unicellular eukaryotes.

We have studied here whether the D1-D5 domains of LSU rRNA which were proposed for DNA based taxonomy in metazoan taxa (Markmann and Tautz 2005; Sonnenberg et al. 2007) offer a suitable tool for the discrimination of heterotrophic flagellate taxa.

Diagnostic sequences are of special interest for the analysis of those habitats where extremely low abundances and specific ecological requirements of eukaryotic microbes limit the application of traditional cultivation and counting techniques (e.g. in groundwater). We have created a sequence data set of heterotrophic nanoflagellates and compared it with the SSU rRNA data set. In addition, we checked for the possibility to differentiate closely related strains using the D1-D5 region of the LSU rRNA gene.

Results

A total of 64 new partial LSU rRNA sequences of heterotrophic nanoflagellates from different groundwater and surface water habitats could be acquired in this study (Table 1). The branching order of new partial eukaryotic LSU rRNA sequences (Fig. 1) was based on an alignment of 1142 characters excluding the completely variable D2 region (701 characters were parsimony informative). Our partial LSU tree shows monophyletic branches for the opisthokonts, apusomonads, cercozoans, cryptophytes, alveolates, kinetopastids, and the stramenopiles with the various groups. The bootstrap values were only moderate for the cercozoans, opisthokonts, kinetoplastids, apusomonads and stramenopiles.