

## Species identification of Spumellaria (Radiolaria) on the basis of the small subunit and internal transcribed spacer region of ribosomal DNA

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Radiolaria are one of the best candidates for time- and environmental-indicators in the modern and past, because their tests have been preserved and the diversity of these morphological species has reflected the water ecosystem in the long Earth history. Although the significance of radiolarian ecology and evolution is well-known issue in paleontology and paleoceanography, the biological studies of radiolarians have been poorly studied so far, especially for the genetics. For the better understanding of the ecology and evolution, the diversity and distribution on the basis of biological species is essential data. However, there is no criterion to identify the biological species of radiolarians by molecular approach. The motivation for this study is setting the delimitation to identify the biological species of radiolarians by molecular data, for the future ecological study.

Identification of the biological species by molecular technique is different among the organisms in consideration of the various types of the reproduction systems: sex and hybridization. Some species identification by genetics are based on molecular distance of the small subunit ribosomal DNA (SSU rDNA) or the secondary structure of the internal transcribed spacer region of ribosomal DNA (ITS1 and 2). In the present study, 232 individuals of five morphological species of spumellarian radiolarians were collected from various environments. I amplified the three gene regions (SSU, ITS1, and ITS2 rDNA) from all collected individuals and examined whether molecular distance of rDNA or secondary structure of ITS2 sequences is useful to identify species in radiolarians. Comprehensive SSU and ITS rDNAs survey clearly show that molecular distance of ITS rDNA is good to identify the species, though it is difficult to set the common delimitation to identify the biological species, among all the spumellarian radiolarians. On the other hand, the secondary structure of ITS2 rDNA have the common threshold for the species identification: below 4 hemi-CBCs (HCBCs: base changes occurring on one side of a double-stranded portion) in a single biological species. The results show that the secondary structure of ITS 2 rDNA could be the criterion for radiolarian species identification.

Keywords: Radiolaria, Internal Transcribed Spacer region, biological species