TAXONOMIC REEXAMINATION OF JAPANESE CHARALES (CHAROPHYCEAE, CHLOROPHYTA): A COMBINED ANALYSIS OF SEM OBSERVATION OF THE OOSPORE ORNAMENTATION AND MOLECULAR PHYLOGENETIC ANALYSIS BASED ON \textit{rbcL} GENE SEQUENCES

Hidetoshi Sakayama\textsuperscript{1}, Hisayoshi Nozaki\textsuperscript{1}, Hideo Kasaki\textsuperscript{2} and Yoshiaki Hara\textsuperscript{3}

1: Department of Biological Science, University of Tokyo, Tokyo 113-0033, Japan
2: Department of Biology, Tokyo Metropolitan University, Tokyo 192-0364, Japan
3: Department of Biology, Yamagata University, Yamagata 990-8560, Japan

Seventy-four species/infraspecific taxa belonging to four genera (\textit{Chara}, \textit{Nitella}, \textit{Nitellopsis} and \textit{Lamprothamnium}) of the Charales have been recorded from Japan, and 35 of them are Japanese or eastern Asian endemic species. Previous studies of SEM oospore morphology have shown its taxonomic significance for delineating charalean species (e.g. John & Moore 1987, Phycologia 26: 334-355; John et al. 1990, Br. phycol. J. 25: 1-24; Casanava 1991, Phycologia 30: 237-242). Moreover, molecular phylogenetic analyses based on \textit{rbcL} or 18S ribosomal RNA gene sequences from European, Australian and North American taxa of the Charales have been carried out to resolve phylogenetic relationships at species or genus level (e.g. McCourt et al. 1996, Am. J. Bot. 83: 125-131; McCourt et al. 1999, Aust. J. Bot. 47: 361-369; Meiers et al. 1999, Aust. J. Bot. 47: 347-360). However, Japanese charalean taxa have not been examined by these modern methods, except for \textit{Nitella gracilens} Morioka (Nozaki et al. 1998, Eur. J. Phycol. 33: 357-359).

In this study thirteen species belonging to \textit{Chara} and \textit{Nitella} collected from the central and northern areas of Japan were investigated based on SEM oospore morphology and \textit{rbcL} gene phylogeny. SEM oospore observations were carried out for the first time in \textit{Nitella pulchella} T. F. Allen, \textit{N. furcata} (Roxburgh ex Bruzelius) Agardh, \textit{N. inversa} Imahori, \textit{N. spiciformis} Morioka, \textit{N. moriokae} Wood and \textit{N. axilliformis} Imahori. The \textit{rbcL} gene sequence data showed that the morphologically delineated taxa of Japanese charalean algae are essentially consistent with those of Europe, Australia and North America. In addition, oospore morphology of three Japanese or eastern Asian endemic species of \textit{Nitella}, \textit{N. gracilens}, \textit{N. spiciformis} and \textit{N. axilliformis}, was distinctly different from that of the species (\textit{N. furcata}, \textit{N. gracilis} and \textit{N. translucens}, respectively) to which Wood (1965, Monograph of the Characeae, J. Cramer Weinheim) assigned as infraspecific taxa. Furthermore, the present sequence data clearly demonstrated that \textit{N. gracilens} or \textit{N. axilliformis} was separated from \textit{N. furcata} or \textit{N. translucens}, respectively. This is the first integration of SEM oospore morphology and molecular phylogenetics in the charalean taxonomy, demonstrating the efficiency of both clues to address problems at lower taxonomic levels.