Intra- and Interspecific Variation in the Ribosomal Internal Transcribed Spacers and 5.8S rDNA among three species of *Pseudodiaptomus* (Calanoida, Pseudodiaptomidae) from Korean Estuarine Waters

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Introduction

Species of the genus *Pseudodiaptomus* are reported as the major constituents in the fresh or brackish waters in the northeastern Asia (Suh et al., 1991; Oka et al., 1991). Of these species, *P. inopinus* (Burckhardt) and *P. poplesia* (Shen) are particularly numerous in spring and autumn (Suh et al., 1991; Shen and Song, 1979). Recently, we found a presence of two types of *Pseudodiaptomus inopinus* during the course of investigation on zooplankton from Korean estuaries. On the basis of morphology of female genital structure, one of them was identified as *P. koreanus* n. sp.

In this study, using rDNA ITS and 5.8S rDNA we address intra- and interspecific phylogenetic relationships between three species of *Pseudodiaptomus*.

Materials and Methods

Materials examined: In Mankyung and Seomjin River estuaries zooplankton collections were monthly made from January to December 2000. All pseudodiaptomids were sorted from the samples. Pseudodiaptomids for morphological taxonomy were dissected and mounted on polyvinyl lactophenol. The bodies and appendages were observed with a differential interference contrast microscope (Zeiss SV6) equipped with a drawing tube. The female genital structures of *Pseudodiaptomus* species were examined with a scanning electron microscope (Hitachi S-4700). The morphological terminology follows Huys and Boxshall (1991).

Sequencing of rDNA ITS regions: Plasmids containing the rDNA ITS fragments were isolated by using a QIAquick plasmid minikit (Qiagene GmbH). Purified plasmids were manually sequenced by using a T7 sequencing kit (Pharmacia Biotech, Inc., Piscataway, N.J.) and the fmol DNA sequencing system (Promega Co.).

Results and Abstract

Three estuarine copepods, *Pseudodiaptomus koreanus* Soh & Suh, 2001, *P. inopinus*, and *P. poplesia*, have been reported to have two types in male fifth legs, finger-like form and shoe-like one (Burckhardt, 1913; Shen and Song, 1979; Soh et al., 2001). In females of *P. poplesia* abnormal individuals, which are bearing a swollen seta on one side only of caudal rami or none at all, are also found (Soh et al., 2001). ITS regions were determined to confirm whether the morphological differences were genetically important. A total of 671 base pairs of ITS regions were sequenced from nine haplotypes of pseudodiaptomiid species from Korean estuaries. In *P. koreanus* the sequence differences between females from Seomjin River Estuary (SRE) and Hyeongsan River Estuary (HRE) were 3.1% and those between finger-like form and shoe-like one of males in SRE were 0.5%. In *P. poplesia* the abnormal females differed normal ones by 2.0% of the base sequences, whereas in its male the finger-like form did the shoe-like one by 1.5%.

The ITS sequence comparisons among the species of *Pseudodiaptomus* revealed sequence differences ranging from 5.8% (between *Pseudodiaptomus* sp. from SRE and *P. inopinus*) to 10.4% (between *Pseudodiaptomus* sp. from HRE and *P. poplesia*). A phylogenetic tree reconstructed by neighbor joining (Saitou and Nei, 1987) on the basis of the molecular data shows that *P. koreanus* and *P. inopinus* were divided from *P. poplesia*.

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