

Complete small ribosomal subunit RNA sequence of the chiton *Acanthopleura japonica* (Lischke, 1873) (Mollusca, Polyplacophora)

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The phylum Mollusca includes such a huge number of species and shows such an overwhelming structural diversity, that its phylogenetic relationships are still very controversial. Consequently, traditional biological characters have led to several competing hypotheses on molluscan phylogeny (e.g. 1, 2, 3, 4). Recently, however, molecular data have been introduced as a new source of phylogenetic information. Particularly, small ribosomal subunit RNA sequences are well suited for resolving relationships among distantly as well as more closely related organisms (e.g. 5, 6). However, controversial results were obtained when different phylogeny inference methods were applied (7, 8, 9, 10) to a data set of partial 18S rRNA sequences from Metazoa including four molluscan representatives viz. the chiton *Cryptochiton stelleri*, the snail *Anisodoris nobilis* and the bivalves *Spisula soldissima* and *Mya arenaria*. Therefore we have tried to assess molluscan phylogeny using only complete or nearly complete 18S rRNA sequences (11). Such sequences were hitherto available only for representatives of two out of the eight existing molluscan classes viz., one gastropod (11) and two bivalves (12, 13). Here we report the complete 18S rRNA sequence of the chiton *Acanthopleura japonica* (Lischke, 1873) (Mollusca, Polyplacophora), determined after gene cloning. We preferred this approach over the faster PCR amplification since it allows sequencing of the entire gene. During PCR amplification, on the contrary, very often the 5' and 3' terminal parts of the gene are chosen as primer annealing sites (e.g. 12, 13), rendering it impossible to determine their sequence.

Specimens of *A. japonica* were collected at Cape d'Aguilar, Hong Kong. 480 µg DNA was extracted (11) from the foot muscle tissue of two individuals. The major part of the sequence, up to an EcoRI site at nucleotide 1591, was determined on a restriction fragment cloned into the plasmid pBluescript SK+ (Stratagene, La Jolla, California, USA). The remaining part was determined on a PCR amplification product obtained by means of primers binding to conserved areas of the rRNA transcription unit (AATTTGACTCAACACGGG, corresponding to nucleotides 1196–1214 of the 18S rDNA, and GCAGTTCGC-TGCGTTCTTC, complementary to nucleotides near the 5'-end of 5.8S rDNA). The latter fragment was cloned in a ddT-tailed vector (14). Sequencing was performed by the dideoxy method (15) by means of primers described previously (11).

The 18S rRNA of *A. japonica* is 1817 bp long. It was aligned manually with the three other complete molluscan 18S rRNA sequences (11, 12, 13) taking into account secondary structure

features (11). The chiton 18S rRNA sequence shows 91.7%, 90.3%, and 92% similarity with the corresponding sequence of respectively the gastropod *Limicolaria kameul* and the bivalves *Crassostrea virginica* and *Placopecten magellanicus*. The similarity with the partial sequence of the chiton *Cryptochiton stelleri* (7) was 97.5% for the 889 nucleotides that could be compared.

A more detailed phylogenetic analysis of Mollusca based on 18S rRNA sequences will have to await the availability of complete sequences for additional molluscan classes.

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