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Lophophorate Phylogeny Inferred from Complete Mitochondrial Genome Information

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Bryozoans (= ectoprocts), or "moss animals," are aquatic organisms, living for the most part in colonies of interconnected individuals. Usually they encrust rocky surfaces, shells, or algae. There are about 5000 living species, with several times that number of fossil forms known. Together with the Brachiopoda and Phoronida they have been classified as Lophophorata because they possess a similar suspension feeding apparatus, the lophophore, which is a horseshoe-shaped structure surrounding the mouth with ciliated tentacles arranged on it. Although, among the lophophorates, complete mitochondrial genomes have been reported from 3 brachiopod and 1 phoronid species, no complete mitochondrial genome from the third lophophorate phylum Bryozoa has been sequenced so far. In this study, we determined the complete mitochondrial genome (15,433bp) of a bryozoan *Bugula neritina*, which is the first report from the phylum Bryozoa and thus has great value as the 13th phylum that mitochondrial genome was completely sequenced in 35 metazoan animal phyla. The bryozoan mitochondrial genome contained the same set of 37 genes (2 ribosomal RNA, 22 transfer RNA, and 13 protein-coding genes) as found in other metazoan animals, but its gene arrangement is not identical to those of any other metazoans reported so far. Even compared with those of other lophophorates such as Phoronida and Brachiopoda, almost all genes of the *B. neritina* mitochondrial genome placed in different positions and their orientations between either of these taxa were inverted in many cases. Thirty-one genes excluding 5 tRNA genes among 37 genes were encoded from only one strand with the same orientation. Such a one-strand dependant transcription is a relatively uncommon state found only in 3 articulate brachiopods, 2 annelids, 1 mollusk, 2 nematodes, and 1 cnidarian. Among 13 noncoding regions longer than 10 bp, the largest one considered as a control region was 517 bp in total length, excluding tRNALys gene (69 bp) inserted into the middle part of the control region probably by a recent translocation event. Moreover, positions and structures of inferred tRNA genes are much more variable than those of the other metazoan tRNA genes. To elucidate lophophorate phylogeny, here, Bayesian analysis was done with complete lophotrochozoan mitochondrial genomes with those of three kinds of lophophorates. The result showed that lophophorates including bryozoans, phoronids, and brachiopods are not a monophyletic group but members of the lophotrochozoan group (1.00 posterior probability) including mollusks and annelids and interestingly that Bryozoa is closely related with Brachiopoda (0.97) and the clade of Bryozoa/Brachiopoda with Annelida (0.97), supported with high degree of posterior probability, which indicated the level of confidence for each node. The two reference groups, deuterostomes and arthropods of ecdysozoans, formed successfully monophyletic groups. Arthropoda was a sister group of lophotrochozoans (1.00) supporting monophyly of protostomes as expected on the basis of the recent animal molecular phylogenetic studies. The present result with the previous viewpoints based on the molecular data made it much more evident that the long-held view related with deuterostome affinity of lophophorates inferred from morphological data must be wrong. Unfortunately, in the resultant phylogenetic tree, we did not recover mollusk monophyly because a gastropod *Loligo bleekeri* separated from a polyplacophoran mollusk Katharina. The same result was obtained by Tomita et al. (2002) based on the mitochondrial proteom phylogeny. Even when we included *Pupa strigosa* and *Albinaria caerulea* for the analysis (data not shown), mollusks were not clustered into a monophyletic group. The result of the phylogenetic analysis was coincident with the comparative analysis of gene arrangement patterns because gene arrangements of Bryozoa/Brachiopoda and Phoronida/Katharina were very similar than any others of metazoan mitochondrial genomes, respectively, as shown in the phylogenetic tree. Also, Bryozoa, Brachiopoda, and Annelida shared uncommon feature of one-strand dependency for gene transcription although 5 inverted tRNA genes were found in the bryozoan species. In mitochondrial proteom phylogeny, we can also find the clade of Bryozoa and Brachiopoda and the clade formed a monophyletic group with Annelida. Through further study related with lophophorate phylogeny problem, it is necessary to get much more complete mitochondrial genome sequences from lophophorates and a number of lophotrochozoans. It could give an important clue to elucidate lophotrochozoan phylogeny as well as lophophorate phylogeny.