

New Insight into the Phylogeny of Mesozoa: Evidence from the 18S and 28S rRNA Genes

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Abstract—The phylogenetic relationships of two Mesozoa groups were studied by the comparative analysis of complete sequences of 18S and 28S rRNA. Two groups of Mesozoa were found to form a statistically supported clade in phylogenetic trees. The results of the analysis placed Mesozoa in the Spiralia group of Lophotrochozoa and showed the tendency of Mesozoa to converge with one of the Annelida groups.

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Two small groups of simply organized invertebrates: Dicyemida (Rhombozoa) and Orthonectida, are included in Mesozoa. Animals of both groups consist of a small number of cells that form an outer ciliary layer and inner reproductive mono- (Dicyemida) or multilayer (Orthonectida). They do not have the tissues, organs, or organ systems that are common to other animals, which could be evidence that these animals are an intermediate between unicellular eukaryotes and multicellular animals, as their name indicates. All representatives of this group are endoparasites of invertebrates and are characterized by a complicated and not completely understood life cycle with a change of asexual and sexual generations but without change of host.

The phylogenetic relationships between Mesozoa and other groups of invertebrates are not clear yet due to their scarcity of morphological features. The analysis of 18S rRNA and Hox genes [1, 2, 3] and recently obtained molecular [4] and morphological data [5] have not completely solved this problem and the question of the close relationship of the two Mesozoa groups. For a better understanding of phylogenetic relations of Mesozoa, a comprehensive analysis of an extended set of joint 18S and 28S rRNA gene sequences of main animal phyla was performed.

Almost complete nucleotide sequences of the 28S rRNA genes of two Orthonectida species (*Rhopalura litorali* and *Intoshia linei*) and one Dicyemida species (*Dicyema sp.*), as well as the 18S rRNA gene of *Intoshia linei* were obtained by PCR [6] with appropriate primer kits [7, 8] followed by direct sequencing of amplification products in an automatic analyzer in the Genom collective use center (Institute of Molecular Biology of Russian Academy of Sciences). The

obtained sequences were aligned with sequences of representatives of main phyla of multicellular animals, including an extended sequence set of 82 Annelida species. Initial alignment with length of 4564 nucleotides included sequences of 101 bilaterally symmetrical species.

Bayes analysis was performed using the general time reversed model of nucleotide sequence evolution taking the heterogeneity of evolution rates and percentage of invariant positions (GTR + H + I) into account. The parameters of evolution were calculated by the mrbayes program [9]. During four independent analyses, from 2 to 6 million generations by four to eight Markov chains were made. 10000 generated trees were chosen, and then 6000 of them were considered as chains that did not reach stationary state and cast out; and the remaining 4000 were used for making a consensus tree.

The schematic representation of a tree constructed from the initial alignment is shown in Figure 1. On this tree the Ecdysozoa group is represented by Cephalorhyncha (Priapulida + Kinorhyncha), Nematoda, Nematomorpha, and Arthropoda phyla. Spiralia includes a large group of trochophore animals united into Lophotrochozoa clade (Mollusca, Nemertea and Annelida, Brachiopoda, Echiura and Sipuncula) and also the Syndermata subgroup (Rotifera + Acanthocephala) and flatworms (Platyhelminthes). Three Mesozoa species (two Orthonectida and one Dicyemida species) form a well supported clade, which is placed in the group of lophotrochophore animals (Mollusca, Brachiopoda, Annelida, Sipuncula and Echiura). Considering the tendency of Mesozoa to converge with Annelida and its allied groups (Echiura and Pogonophora) and particularly with the

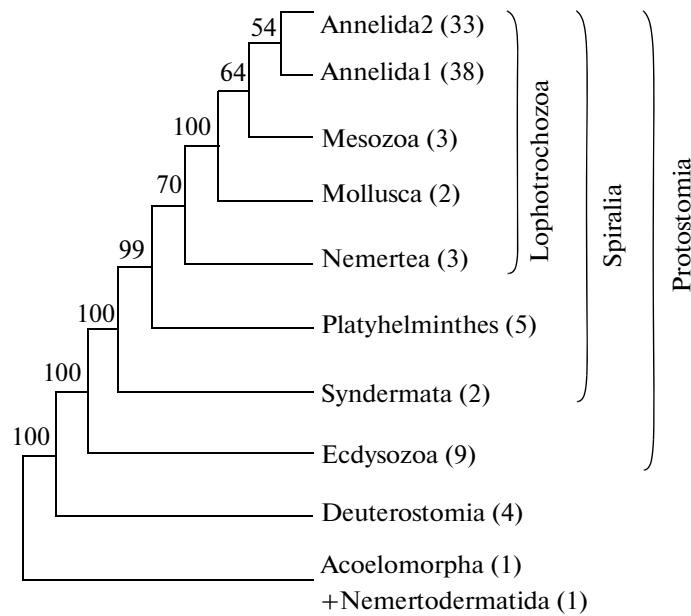


Fig. 1. A schematic representation of Bayes phylogenetic tree of main groups of bilaterally symmetrical animals. The analyzed set included 101 sequences of 18 + 28S rRNA genes (4564 nucleotide positions), 71 of them belonged to Annelida groups and related species (Sipuncula, Echiura, and Pogonophora). The numbers on the branches represent the statistical support, % of posterior probability. The parenthetical numbers indicate the number of sequences.

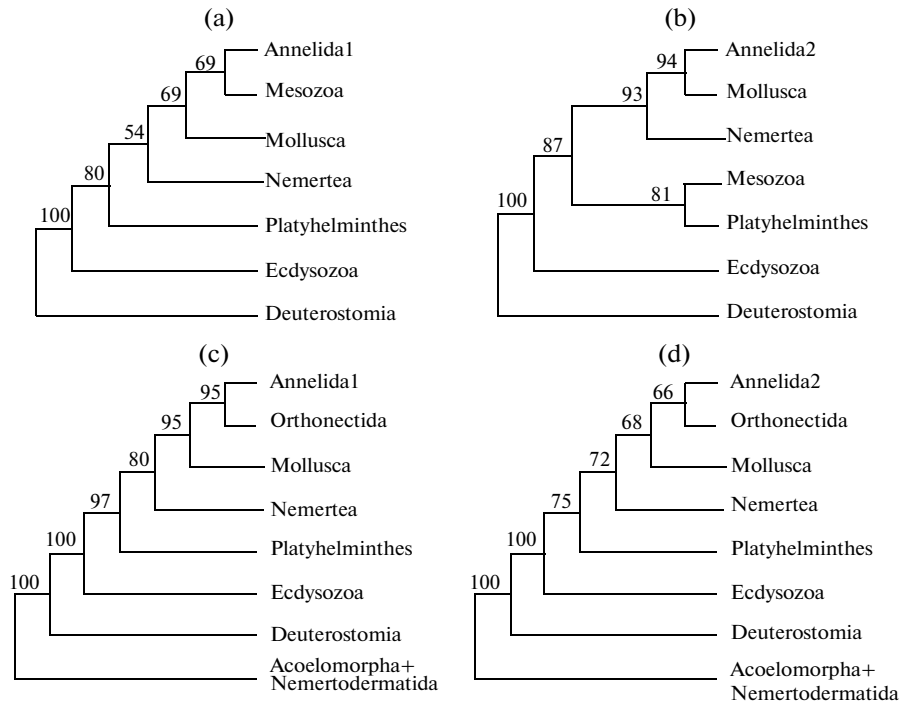


Fig. 2. Schematic representation of the Bayes phylogenetic tree of main groups of bilaterally symmetrical animals. The analyzed set included various numbers of 18 + 28S rRNA gene sequences (4564 nucleotide positions). In the (a) and (b) panels Mesozoa are represented by two groups (Orthonectida and Dicyemida); in (c) and (d) Mesozoa are represented only by Orthonectida. Numbers on branches represent the statistical support, % of posterior probability.

group that includes Capitellida, Echiura, Sipuncula, and Oligochaeta, we tried to define the Mesozoa place in the scheme more accurately by dividing Annelida and related species sequences into two groups. The first group (Annelida1) included Capitellida, Echiura,

Sipuncula, and Oligochaeta inter alia, the second group (Annelida2) included the remaining Annelida groups in our analysis.

The results of the analysis of Annelida1 groups are shown in Figure 2a. The monophyly of Mesozoa had

100% support and it was also supported by eight molecular synapomorphies; Mesozoa directly bordered with Annelida1 group with 69% support. Although such support is considered moderate for Bayes trees [10], it exceeds the support of groups within the Annelida1 clade (35–60%). The position of Mesozoa on the tree obtained by analysis of the second set of sequences, which included the Annelida2 group, is shown in Figure 2b. On this tree Mesozoa does not border with the Annelida2 group and takes a more basal position bordering with flatworms (Platyhelminthes); however in this case a Mesozoan position in the Spiralia group is 87% supported.

Mesozoa (especially Dicyemida) branches on all trees were much longer than other branches, which is the evidence of high rate of evolution of their rRNA genes. Apparently the Mesozoan position was influenced by the long-branch attraction effect [11], which shifted this group towards the base of the tree. To check this observation, Orthonectida and Dicyemida were analyzed separately in sets that included Annelida1 and Annelida2. A single sequence of Dicyemida (trees are not shown) was united with flatworm sequences, at that Annelida1 and Annelida2 sets provided this united group with different support, 89% and 80%, respectively. Inclusion of only Orthonectida into sequence sets led to their unification with Mollusca and Annelida, and Annelida1 and Annelida2 sets supported this union by 95 and 66%, respectively (Figs. 2c and 2d).

Together, these results demonstrate that joining of Orthonectida and Dicyemida into one monophyletic Mesozoa group is supported by molecular analysis results. The position of Mesozoa on 18 + 28S rRNA trees is influenced by the long-branch attraction effect and the greatest impact is made by Dicyemida sequences. We can also claim that Mesozoa are included in the Spiralia group, possibly together with lophotrochochore animals, and tended to converge with the Annelida group which includes Capitellida, Sipunculida, Echiurida, and Oligochaeta.

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