

Molecular and Biochemical Parasitology 84 (1997) 263-266



Short communication

Phylogenetic analysis of a fish tapeworm, *Proteocephalus* exiguus, based on the small subunit rRNA gene¹

Ivica Král'ová^{2,a}, Yves Van de Peer^b, Milan Jirků^a, Marc Van Ranst^c, Tomáš Scholz^a, Julius Lukeš^{a,d,*}

^aInstitute of Parasitology, Academy of Sciences of the Czech Republic, Branišovská 31, České Budějovice, Czech Republic ^bDepartment of Biochemistry, University of Antwerp, Antwerp, Belgium ^cRega Institute, University of Leuven, Leuven, Belgium ^dFaculty of Biology, University of South Bohemia, České Budějovice, Czech Republic

Received 13 August 1996; revised 26 November 1996; accepted 29 November 1996

Keywords: Proteocephalus; Cestoda; Platyhelminthes; SSU ribosomal RNA; Phylogeny

Within Platyhelminthes, phylogenetic trees based on the total small subunit ribosomal RNA gene (SSU rRNA) sequences have been constructed only for the aspidogastrean *Lobatostoma manteri*, the monogenean *Gyrodactylus salaris* and some digenetic trematodes, mainly schistosomes [1–5]. Besides these data, partial sequences of SSU rRNA genes of some other helminths have been described. However, no complete sequences of the rRNA gene of any cestode have been published and only that of the cyclophyllidean *Echinococcus granulosus* is available in the Gen-BankTM (Picon et al., unpublished results). Herein, the complete sequence of SSU rRNA of *Proteocephalus exiguus* La Rue, 1911 (Cestoda: Proteocephalidae) is presented and the phylogenetic position of this parasite is determined.

The SSU rRNA sequence of *P. exiguus* was aligned with the other eukaryotic SSU rRNA sequences [6] using the DCSE sequence editor [7]. An evolutionary tree was constructed by the neighbor-joining method and bootstrap analysis, involving the computation of 500 trees from resampled data, was performed using the software package TREECON for Windows [8]. Maximum likelihood [9] and maximum parsimony [10] methods were used to construct alternative trees.

The evolutionary trees based on the neighborjoining and maximum likelihood methods (Figs. 1

^{*} Corresponding author. Tel.: + 42 38 817, ext. 428; fax: + 42 38 47743; e-mail: jula@paru.cas.cz

¹ Note: Nucleotide sequence data reported in this paper will appear in the EMBL, GenBank[™] and DDBJ Nucleotide Sequence Databases under the accession number X99976.

²Permanent address: Institute of Parasitology, Slovak Academy of Sciences, Hlinkova 3, 040 01, Košice, Slovakia.



Fig. 1. Evolutionary tree constructed by neighbor-joining method. The ascomycete *Saccharomyces cerevisiae* was used to root the tree. The evolutionary distance between two organisms is obtained by summing the length of the connecting branches along the horizontal axis, using the scale on top. Bootstrap values above 50% are shown at the internodes. The tapeworm *Proteocephalus exiguus*, a widely distributed parasite of salmonid and coregonid fishes in the Holarctic Region [15], was isolated from the intestine of rainbow trout (*Oncorhynchus mykiss* (Walbaum)) from Dobšiná water reservoir (Slovakia) and DNA was prepared from homogenized worm tissue. The SSU rRNA gene was amplified via polymerase chain reaction (PCR) using primers 18e (5'-CTG-GTTGATCCTGCCAGT-3') and modified 18g (5'-GCGACGGGCGGTGTGTACAAAGG-3') of Hillis and Dixon [16] and cloned into the pT7blue vector (Novagen). A set of overlapping subclones was constructed and the sequence of the DNA determined for both strands. The SSU rRNA of *P. exiguus* is 1934 bp long, with a GC content of 52%.

and 2) show that both cestodes, the proteocephalan *P. exiguus* and *E. granulosus*, are grouped in one clade with trematodes comprising digeneans and the aspidogastrean *L. manteri* as a sister group. The monoopisthocotylean monogenean *Gyrodactylus salaris* represents a sister group of both Cestoda and Trematoda.

In the neighbor-joining trees, both nematodes, for which complete SSU rRNA sequences are available, constitute a monophyletic group, which is a sister group to all Platyhelminthes (Fig. 1), in agreement with the current systems of classification of these parasitic animals [11,12]. However, with the maximum likelihood and maximum parsimony (data not shown), they branch off at the base of the trees. This clustering is apparently artificial, most likely due to the increased evolutionary rate of the nematode SSU rRNA sequences. It is a well known phenomenon that 'long' branches are pulled towards the base of the tree [13] or artificially clustered with other long branches [14].

The phylogenetic position of cestodes and monogeneans, based on the total SSU rRNA sequences reported here, casts doubts upon the validity of the Cercomeromorphae and is controversial with respect to the present classification of parasitic Platyhelminthes. All the methods used



Fig. 2. Maximum likelihood tree constructed with fastDNAml method [8] using the options of randomized sequence input, and global rearrangements.

(neighbor-joining, maximum likelihood, maximum parsimony) support the paraphyly of the Cercomeromorphae. It should be, however, noted that in maximum parsimony trees (data not shown) the mutual topology of cestodes and monogeneans is unstable, with only 45% of bootstrap replicates showing a topology identical to the neighbor-joining and maximum likelihood trees (Figs. 1 and 2). More sequence data are obviously needed to disprove or support the current branching order. The same applies to the phylogenetic position of monopisthocotylean and polyopisthocotylean monogeneans.

Acknowledgements

This work was supported in part by grants of the Grant Agency of the Czech Republic (No. 508/95/294), Grant Agency of the Czech Academy of Sciences (No. A6022503), and Grant Agency of the Slovak Academy of Sciences (No. 2/1364/96). Y.V. de Peer is a Research Assistant of the National Fund for Scientific Research (Belgium).

References

- Ali, P.O., Simpson, A.J.G., Allen, R., Waters, A.P., Humphries, C.J., Johnston, D.A. and Rollinson, D. (1991) Sequence of a small subunit rRNA gene of *Schistosoma mansoni* and its use in phylogenetic analysis. Mol. Biochem. Parasitol. 46, 201–208.
- [2] Barker, S.C., Blair, D., Cribb T.H. and Tonion, K. (1993) Phylogenetic position of *Heronimus mollis* (Digenea): evidence from 18S ribosomal RNA. Int. J. Parasitol. 23, 533–536.
- [3] Blair, D. and Barker, S.C. (1993) Affinities of the Gyliauchenidae: Utility of the 18S rRNA gene for phylogenetic inference in the Digenea (Platyhelminthes). Int. J. Parasitol. 23, 527–532.
- [4] Johnston, D.A., Kane, R.A. and Rollinson, D. (1993) Small subunit (18S) ribosomal RNA gene divergence in the genus *Schistosoma*. Parasitology 107, 147–156.

- [5] Cunnigham, C.O., McGillivray, D.M. and MacKenzie, K. (1995) Phylogenetic analysis of *Gyrodactylus salaris* Malmberg, 1957 based on the small subunit (18) ribosomal RNA gene. Mol. Biochem. Parasitol. 71, 139–142.
- [6] Van de Peer, Y., Nicolai, S., De Rijk, P. and De Wachter, R. (1996) Database on the structure of small ribosomal subunit RNA. Nucleic Acids Res. 24, 86–91.
- [7] De Rijk, P. and De Wachter, R. (1993) DCSE, an interactive tool for sequence alignment and secondary structure research. Comput. Appl. Biosci. 9, 735–740.
- [8] Van de Peer, Y. and De Wachter, R. (1994) TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Appl. Biosci. 10, 569–570.
- [9] Olsen, G.J., Matsuda, H., Hagstrom, R. and Overbeek, R. (1994) FastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Comput. Appl. Biosci. 10, 41–48.
- [10] Felsenstein, J. (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics. University of Washington, Seattle.

- [11] Ehlers, U. (1985) Das phylogenetische System der Plathelminthes. Gustav Fischer, Stuttgart.
- [12] Rohde, K. (1990) Phylogeny of Platyhelminthes, with special reference to parasitic groups. Int. J. Parasitol. 20, 979–1007.
- [13] Olsen, G.J. (1987) Earliest phylogenetic branchings: comparing rRNA-based evolutionary trees inferred with various techniques. Cold Spring Harbor Symposia on Quantitative Biology, Vol. LII, 825–837.
- [14] Van de Peer, Y., Van de Auwera, G. and De Wachter, R. (1996): The evolution of stramenopiles and alveolates as derived by 'substitution rate calibration' of small ribosomal subunit RNA. J. Mol. Evol. 42, 201–210.
- [15] Hanzelová, V., Scholz, T. and Fagerholm, H-P. (1995) Synonymy of *Proteocephalus neglectus* La Rue, 1911, with *P. exiguus* La Rue, 1911, two fish cestodes from the Holarctic Region. Syst. Parasitol. 30, 173–185.
- [16] Hillis, D.M. and Dixon, M.T. (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66, 411–453.