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SHORT COMMUNICATION

# Comparison of 18S ribosomal RNA gene sequences of *Eurytrema coelmaticum* and *Eurytrema pancreaticum*

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**Abstract** The partial 18S rRNA sequences of *E. coelmaticum* and *E. pancreaticum* were amplified using conserved primers and an evolutionary tree was constructed using Neighbor-Joining. The percent identity of *Eurytrema* species with other Dicrocoeliidae varied from 97.5 to 98.2, while the percent identity between the two *Eurytrema* species was up to 99.3. The tree showed that *E. coelmaticum* and *E. pancreaticum* were not situated in the same position, and they formed one cluster with *L. collurioni*. These results support a confirmation with molecular data that *E. coelomaticum* and *E. pancreaticum* are different species which apparently were not seriously questioned in the past.

*Eurytrema* spp. is a pancreatic parasite found in many areas worldwide, which harbors in pancreatic ducts and, rarely, bile ducts in ruminant animals such as cattle, buffaloes, deer, camels, pigs, sheep, goats, and human beings. There are at least seven species in China, including *E. coelmaticum*, *E. pancreaticum*, *E. cladorchis*, *E. fukienensis*, *E. hydropotes*, *E. minutum*, and *E. sphaeriorchis*. *Eurytrema* species are mainly distributed in many regions in southern and northern China, such as Fujian, Hunan, Guangdong, and Inner Mongolia (Zhan 2005). Previous studies on *E. coelmaticum* and *E. pancreaticum* were greatly focused on their morphology, life history, main organs and their functions, and epidemic investigation (Vykhrestyuk and Yarygina 1982; Pinheiro and Amato 1995; Dorny et al. 1996. However, no molecular data on *E. coelmaticum* and *E. pancreaticum* are available at present, and their relationship is not determined based on molecular data, which have successfully been used in the analysis of phylogeny and in the identification of trematodes (Attwood et al. 2002; Park et al. 2003; Olson et al. 2003). Until recently, only three partial 18S rRNA sequences of three genera, *Brachylecithum*, *Lyperosomum*, and *Dicrocoelium*, are available and two of them have been used in molecular phylogenetic analyses (Olson et al. 2003). This study will provide some molecular information on *E. coelmaticum* and *E. pancreaticum*.

Adult worms of *E. coelmaticum* and *E. pancreaticum* were generously donated by Prof. Zong, which were both dissected from the pancreatic ducts of slaughtered cattle in Fujian, China.

After having been washed in autoclaved normal saline water three times, the adult worms were gently homogenized in liquid nitrogen and genomic DNAs were extracted according to the method previously recorded, with a few modifications (Davis et al. 1988). The powdered tissue was incubated overnight at 65°C in 100 mM Tris (pH 7.4), 1 mM EDTA, 0.5% SDS, and 3 mg/ml Proteinase K. The DNA was then purified by phenol extraction followed by isopropanol precipitation. The precipitate containing genomic DNA was resuspended in TE (pH 8.0) with the final concentration of 1 μg/ml RNase and incubated at 37°C for 5 min and then at 65°C for another 5 min.

Using a polymerase chain reaction (PCR) protocol of 40 s at 94°C, 30 s at 55°C, and 2 min at 72°C for 35 cycles,

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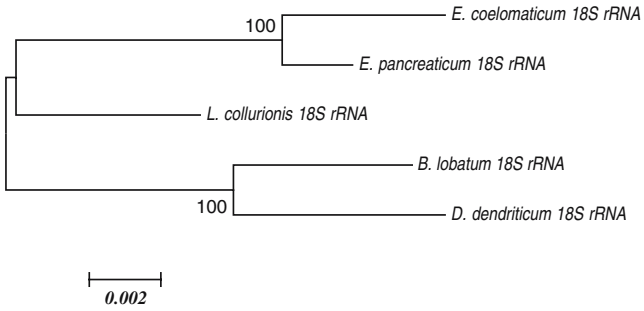


**Fig. 1** A Neighbor-joining tree of *E. coelmaticum* and *E. pancreaticum* inferred from 18S rRNA sequences. The *scale bar* is shown below. *Numbers* at nodes are bootstrap values

the partial 18S rRNAs were amplified by PCR with primers E-18S-F (5′-GGC TCA TTA AAT CAG CTA TGG TT-3′) and E-18S-R (5′-ACG ACT TTT ACT TCC TCT AAA T-3′), directed to conserved 5′- and 3′-sequences of 18S rRNA genes in other trematodes. The PCR products were purified with DNA Purification Kit (U-gene), subcloned into pGEM-T easy vector (Promega). Plasmid DNAs of transformants were isolated as described by (Sambrook and Russell 2001) and the positives were identified by PCR and by restriction endonuclease *Eco*RI. The positive clones were sequenced using ABI PRISM™377XL DNA sequencer (TaKaRa).

Alignment of *E. coelmaticum* (DQ401035) and *E. pancreaticum* (DQ401034) was conducted with three Dicrocoeliidae species, *Brachylecithum lobatum* (AY222144), *Lyperosomum collurionis* (AY222143) and *Dicrocoelium dendriticum* (Y11236) using 'Clustal W' with default parameters (DNAStar version 4.01, Madison, WI, USA). A Neighbor-Joining tree was generated using MEGA version 3.1 (Kumar et al. 2004). In the process of tree construction, the gaps and missing sites were completely deleted and the Kimura two-parameter method was used as substitution model. In bootstrap analysis, 100 replications were performed with random seed 37901.

The partial 18S rRNA sequences were 1,857 bp in length in both *Eurytrema* species with 99.3% similarity and there were 13 variable sites in total between them. One insertion of 2 bp (AC) was found in both 18S rRNA sequences of *E. coelmaticum* and *E. pancreaticum*, compared with the ones of other Dicrocoeliidae species. The percent similarity of *E. coelmaticum* and *E. pancreaticum* to other Dicrocoeliidae varied from 97.5 to 98.2. Among them, the percent identity of *E. pancreaticum* with *L. collurionis* was 98.2, while the percent identity of *E. coelmaticum* with *B. lobatum* and *D. dendriticum* was 97.5.

A NJ tree was produced based on the partial 18S rRNA sequences, including the 18S rRNA sequences of three different genera that were retrieved from GenBank and all are more than 1,857 bp (Fig. 1). The tree showed that *E. coelmaticum* and *E. pancreaticum* situated at different positions with a high bootstrap value of 100 and, together with *L.collurionis*, formed one cluster, and that *B. lobatum* and *D. dendriticum* formed the other. These indicated that the evolutionary relationship of *Eurytrema* species might be closer to *L.collurionis* than to *B. lobatum* and *D. dendriticum*, and that *B. lobatum* and *D. dendriticum* might diverge early before the *Eurytrema* spp.

Together with the above results, it is confirmed that at a molecular level *E. coelmaticum* and *E. pancreaticum* are different species.

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## References

Attwood SW, Upatham ES, Meng XH, Qiu DC, Southgate VR (2002) The phylogeography of Asian *Schistosoma* (Trematoda: Schistosomatidae). Parasitology 125:99–112

Davis RE, Davis AH, Carroll SM, Rajkovic A, Rottman FM (1988) Tandemly repeated exons encode 81-base repeats in multiple, developmentally regulated *Schistosoma mansoni* transcripts. Mol Cell Biol 8(11):4745–4755

Dorny P, Batubara A, Iskander M, Pandey VS (1996) Helminth infections of sheep in North Sumatra, Indonesia. Vet Parasitol 61 (3–4):353–358

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). Int J Parasitol 33:733–755

Park BK, Kim MJ, Kim EH, Kim MS, Na DG, Chae JS (2003) Identification of trematode cercariae carrying *Neorickettsia risticii* in freshwater stream snails. Ann N Y Acad Sci 990:239–247

Pinheiro J, Amato SB (1995) *Eurytrema coelomaticum*: influence of the infection on the reproduction and nucleic acids contents in the albumen gland and ovotestis of *Bradybaena similaris*. Mem Inst Oswaldo Cruz 90(5):635–638

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual (3rd edn). Cold Spring Harbor Laboratory, New York

Vykhrestyuk NP, Yarygina GV (1982) Preliminary studies of lipids of the trematodes *Eurytrema pancreaticum*, *Calicophoron erschowi* and the turbellarian *Penecurva sibirica*. Mol Biochem Parasitol 5 (4):221–229

Zhan XM (2005) Parasitology on humans (in Chinese). People's Medical Publishing House, Beijing