IDENTIFICATION OF EUROPEAN DIPLOZOIDS (MONOGENEA) BY RFLP DIGESTION OF THE ITS2 rDNA

IVETA MATEJUSOVA1, BOZENA KOUBKOVA2, CAREY O. CUNNINGHAM1
1FRS Marine Laboratory, Aberdeen, Scotland, 2Masaryk University, Brno, Czech Republic

INTRODUCTION

- Diplozoids are common gill parasites of cyprinid fish
- The description of the subfamily Diplozoinae is based on the finding of Diplozoon paradoxum Nordmann, 1832, on the gills of Abramis brama
- According to the shape of the posterior part of the body four genera were excluded from Diplozoon: Paradiplozoon Achmerov, 1974; Eudiplozoon Khotenovsky, 1978; Endiplozoon Khotenovsky, 1985 and Sindiplozoon Khotenovsky, 1981
- Around 60 species of diplozoids is described with 18 European restricted species
- Species identification of diplozoids based on morphology is difficult and demands skills and experience as:
  - the measurements of attachment clamp sclerite are variable (dependent on size of host)
  - the structures of taxonomic importance grow gradually

THE AIM OF THE STUDY WAS DESCRIBE MOLECULAR MARKERS, BASED ON RFLP OF THE ITS2 rDNA FOR THE EUROPEAN DIPLOZOIDS

RESULTS - I.

- Differences in the size of PCR product: 800 bp – E. nipponicum
  - 820 bp – Paradiplozoon species
  - 840 bp – D. paradoxum
- Pairwise comparison among the ITS2 sequences: 25 to 177 nucleotides (3.2-22.9%)
- Digestion of the amplified ITS2 with Alu, HinfI, RsaI and Spl revealed in species-specific patterns:
  - Alu: 468, 190, 170, 280, 160, 110, 60, 90 P. megaron
  - HinfI: 628, 120, 80, 520, 200, 120 D. paradoxum
  - RsaI: 628, 200 P. megan
  - Spl: 540, 280 P. nagibinae
- The complete ITS2 alignment spanned 722 position
- The ITS2 sequences were very conserved between diplozoids, with 538bp conserved sites, 379 nucleotides of the primer sequences and 846bp variable sites
- Based on the genetic distances, P. megaron, P. nagibinae and P. sapae were more closely related to D. paradoxum than to the rest of the Paradiplozoa species, such as P. homogen, P. parleski and P. megan. E. nipponicum was the most distant of all diplozoids studied

RESULTS - II.

- Genomic DNA extraction, PCR amplification of ITS2 and RFLPs methods were performed according to Matejusova et al., 2001 (Parasitology 123, 465-474)
- Modelfit v.3.06 selected the HKY+G as a appropriate model of evolution
- Maximum likelihood (ML) analyses were performed using PAUP* v. 4.0b8, following a general time reversible (GTR) model

CONCLUSIONS

- ITS2 region is the powerful marker to distinguish diplozoids on species and generic level
- The close relationship between P. sapae, P. nagibinae, P. megaron and D. paradoxum:
  - Reflection of host-parasite coevolution OR validity of the genera Diplozoon and Paradiplozoa

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