IS THE OCTOMACRIDAE THE SISTER FAMILY OF THE DIPLOZOIDAE?

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**Summary:**
Diplozoidae and Octomacridae are usually considered as sister families. Essentially this is because they are the only polyopisthocotyleans parasitising primary freshwater teleosts. Because of the lack of phylogenetically informative morphological characters to explore the pattern of colonisation of the primary continental freshwater teleosts and in order to understand the appearance of the "natural parabiosis" of Diplozoidae, a molecular phylogeny was inferred by comparing newly obtained partial 28S and 18S rDNA gene sequences of *Eudiplozoon nipponicum* and *Diplozoon konion* with other already available sequences. The phylogenetic analysis seems to show that Diplozoidae and Octomacridae are not sister groups. Thus, the colonisation of primary freshwater teleosts by these two families could be independent.

**Key Words:** Diplozoidae, Octomacridae, Cyprinidae, Catostomidae, freshwater fishes, molecular phylogeny.

Polyopisthocotyleans reach their greatest diversification mainly on marine teleost fishes. Nevertheless, in this subclass, the Diplozoidae and Octomacridae are the only families parasitising primary freshwater teleosts. The Diplozoidae with more of 50 species actually described are really diversified on Cyprinidae and Characidae, the Octomacridae with only five species described on Catostomidae and Cyprinidae are less diversified (Khothenovsky, 1985). These two parasite families along with the Discocotylidae constitute the suborder Discocotylina (Boeger & Kritsky, 1993; Lebedev, 1995; Boeger & Kritsky, 1997; Boeger & Kritsky, 2001). The few morphological characters in favour of the grouping of these three families in this suborder are not very strong and this decision is debatable. Boeger & Kritsky (1993) claimed that two morphological characters support the monophyly of the Discocotylina, namely the absence of spines on the male copulatory organ and the absence of anchors at all stages of development. But a phylogenetic analysis based on partial D2 sequences of 28S rDNA showed that monophyly of the Discocotylinae is questionable because the Discocotylidae appeared to be only distantly related to the Diplozoidae (Jovelin & Justine, 2001). A divergence of the Discocotylidae is likely since they are essentially parasites of Salmonidae. This fish family contains many anadromous fishes and is not a primary freshwater family, unlike the Cyprinidae, Characidae and Catostomidae. On this basis, Khothenovsky (1985) has proposed the association of Diplozoidae and Octomacridae in a suborder, the Octomacrinae. Since Diplozoidae and Octomacridae are the only polyopisthocotyleans diversified on continental freshwater fishes, it is possible that they have diverged from a recent common ancestor already present on freshwater teleosts.

The present-day diversity is thus the result of co-speciation and switch between fishes and parasites, the freshwater habitat being a simple shared phylogenetic character (synapomorphy). So, a detailed examination of the Discocotylinae, namely the absence of spines on the male copulatory organ and the absence of anchors at all stages of development. But a phylogenetic analysis based on partial D2 sequences of 28S rDNA showed that monophyly of the Discocotylinae is questionable because the Discocotylidae appeared to be only distantly related to the Diplozoidae (Jovelin & Justine, 2001). A divergence of the Discocotylidae is likely since they are essentially parasites of Salmonidae. This fish family contains many anadromous fishes and is not a primary freshwater family, unlike the Cyprinidae, Characidae and Catostomidae. On this basis, Khothenovsky (1985) has proposed the association of Diplozoidae and Octomacridae in a suborder, the Octomacrinae. Since Diplozoidae and Octomacridae are the only polyopisthocotyleans diversified on continental freshwater fishes, it is possible that they have diverged from a recent common ancestor already present on freshwater teleosts.

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of their phylogenetic relationship could reveal whether the colonisation of primary freshwater teleosts by poly-
pisthocotyleans took place once by their common ancestor or several times in independent events of
colonisation.
The exploration of the phylogenetic relationship be-
tween these two families could also provide us with
the opportunity to address another intriguing question:
the Diplozoidae exhibits one of the most striking
distances between the others families: Axinidae, Microco-
tylidae, Diplozoidae and Octomacridae that are closed
in the cladistic morphological analysis (Boeger &
Kritsky, 1993).
The DNA of members of the Diplozoidae was obtained
with a CTAB buffer and amplified as previously described
by Sicard et al. (2001). The 28S rDNA was amplified with
the primers cer58S2249: 5'-GCTGACGTAGATGAAGAGG-
and cer28S3116: 5'TCGCTATCGGACTCGTGC'; the 18S
rDNA with the primer cer18S386: 5'ACGGCTACCACTC-
CAAAGG- and reverse primer cer18S1585: 5'GACGG-
GACGTATTGCAGACA- (the numbers in the name of
the primer refer to the number position in the Coe-
norbabditis elegans sequences). PCR products
were purified with the GeneClean kit (Bio 101) and sequen-
ced with the same primers as for PCR with the
ThermoSequenase kit (Aptibitech). The electropho-
resis was performed in an ALFred (Aptibitech) automatic
sequencer. The sequences were first aligned
automatically on the Multalin server (Corpet, 1988)
(www.toulouse.inra.fr/multalin) and manually revised
using the Software Genedoc (www.psc.edu.biomed/
genedoc). The partial sequence of 18S rDNA and partial
sequence of 28S rDNA (full domain C1, full D1 and
partial D2) were used. The phylogenetic analyses
were performed by Phylowin (Galtier et al., 1996). Trees
were constructed with the bio-Neighbour-joining
(bioNJ), the maximum likelihood (ML) and the
maximum parsimony (MP) methods. Bootstrap values
were calculated for bioNJ, ML and MP with 500 repli-
cates and likelihood of the topologies was tested with
Phylowin.

RESULTS AND DISCUSSION

When we compiled the 18S rDNA and the 28S
rDNA in the same phylogenetic analysis, we obtain the most strongly supported topo-
logy (Fig. 1). The relative rate of evolution of internal
branches was estimated with RRTree (Robinson et al.,
1998) to detect a potential “long-branch” effect. In our
analysis of the tree, only the Mazocraeidae shows a
significantly faster evolutionary rate compared to the
other polyopisthocotyleans using Neopolystoma as refe-
rence. This probably explains why the values of the
bootstrap of the branch between Kubnia and the
others are so low.

The phylogenetic tree obtained modulates the idea of
close phylogenetic relationship between Diplozoidae
and Octomacridae, and at the same time the monophy-
ly of the Discocotylinae obtained from morpholo-

MATERIALS AND METHODS

W
ere sequenced 640 pb of the 3' end of the 18S
rDNA and 250 pb of the 5' end of the 28S
rDNA region D1 of two Diplozoidae: Diplo-
zoon bomoion from Rutilus rutilus and Eudiplozoon
nipponicum from Cyprinus carpio collected in southern
France. Those two partial sequences correspond to the
molecular information already available for the Octo-
macridae and other Polyopistocotylea (Liddlewood et al.,
1998; Littlewood et al., 1999; Mallaret et al., 1997;
Mallaret et al., 2000). This molecular information is
available in Genbank data base for: Octomacrum lancea-
tum (Octomacridae) parasitisling Catostomus cata-
ostomus, O. mexicanum (Octomacridae) parasitis-
ing Catostomus sp., Neopolyzota sprattii (Polyostomatidae)
parasitisling Chelodina longicollis, Zeuxapta seriolae
(Axinidae) parasitisling Seriola hippos, Bivagina pagro-
somi (Microcotylidae) parasitisling Chrysophrys aurata;
Kubnia scombri (Mazocraeidae) parasitisling Scomber
scombrus. The tree was rooted with Polyostomatidae
that are tetrapod parasites. Mazocraeidae divergent
from other teleost's parasites (Mollaret et al., 2000)
drewn in order to evaluate the phylogenetic dis-
tance between the others families: Axinidae, Microco-
tylidae, Diplozoidae and Octomacridae that are closed
in the cladistic morphological analysis (Boeger &
Kritsky, 1993).

The tree was rooted with Polystomatidae
and manually revised
Fig. 1. — Molecular Phylogenetic relationship between Diplozoidae, Octomacridae and other teleost’s Polyopisthocotylea inferred from partial 18S rDNA sequences and partial 28S rDNA sequences. We have compiled the 18S rDNA and the 28S rDNA sequences in the same phylogenetic analysis and we obtained a most strongly supported topology than the separate analysis. Numbers on branching are the bootstrap proportion calculated with 500 iterations respectively with bio neighbour-joining method, MP and ML. The likelihood for other imposed topology was tested with Phylowin and this tree still the best one. The relative rate of evolution of internal branches was estimated with RRtree to detect a potential “long-branch” effect. Only the Mazocraeidae show a significantly faster evolutionary rate compared to the other Polyopisthocotyleans using Neo-polystoma as reference.

REFERENCES


Reçu le 15 octobre 2001
Accepté le 6 décembre 2001