Genetic diversity of *Loma acerinae* (Microsporidia: Glugeida) from different fish hosts and localities – Short communication

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ABSTRACT

*Loma acerinae* is a xenoma-forming fish microsporidium described from common ruffe *Gymnocephalus cernua* (Perciformes: Percidae) and also found in Ponto-Caspian gobies (Gobiiformes: Gobiidae). This casts doubt on the strict host specificity of this parasite. The largest subunit RNA polymerase II (*rpb1*) was used as a genetic marker of the parasite isolated from six host species of Perciformes (*G. cernua* from the Baltic Sea), Atheriniformes (*Atherina boyeri* from the Azov Sea) and Gobiiformes (*Neogobius* spp. and *Zosterisessor ophiocephalus* from the Black Sea and *Ponticola kessleri* from the Caspian Sea basin). Two major *rpb1* haplogroups were found with 98.5% identity between the groups. Notably, Haplogroup I was associated with *Neogobius* spp. samples (*n* = 6) only, whereas Haplogroup II included the samples from other host species (*n* = 7). These findings confirm the broad distribution and host range of *L. acerinae*, but also indicate that certain patterns of host-driven intraspecific polymorphism may exist. Furthermore, the study revealed low similarity between the ribosomal RNA gene sequences of *L. acerinae* and the type species, *Loma morhua* (as well as other species of the genus). This suggests loose genetic association within the genus, and may raise the need for the taxonomic revision of *L. acerinae*.

KEYWORDS

microsporidia, fish parasites, *rpb1*, genetic polymorphism, host specificity

*Loma acerinae* is a xenoma-forming fish microsporidium that was originally described from the intestine of the common ruffe *Gymnocephalus cernua* (Perciformes: Percidae) (Lom and Pekkarinen, 1999). A recent morphological and molecular genetic study has confirmed that the microsporidium species found in Ponto-Caspian gobies (Gobiiformes: Gobiidae) and big-scale sand smelt *Atherina boyeri* (synonym *Atherina mohon pontica*) (Gobiiformes: Atherinidae) was *L. acerinae* (Ovcharenko et al., 2017). Due to the wide host range of *L. acerinae* including phylogenetically distant hosts, and the divergence in the sampling sites of gobies and the sand smelt (the Black Sea) vs. those of common ruffe (the Czech Republic and Finland), it is of great interest to elucidate the intraspecific genetic polymorphism of the parasite.

In the present paper, we used small subunit rRNA gene (SSU rDNA) and the largest subunit RNA polymerase II (*rpb1*) as genetic markers to compare *L. acerinae* samples. Thirteen samples of *L. acerinae* isolated from six species of fish hosts were examined: *G. cernua* (number of examined samples *n* = 2) from Finnish Bay of the Baltic Sea (60°06′N
29°55′E), monkey goby Neogobius fluviatilis (n = 4), round goby Neogobius melanostomus (n = 2) and grass goby Zosterisessor ophiopcephalus (n = 3) from Karkinit Bay of the Black Sea (45°52′N 33°28′E), A. boyeri (n = 1) from Sivash Bay of the Azov Sea (45°25′N 35°10′E) and bighedead bighead goby Ponticola kessleri (n = 1) from the Volga River Delta in the Caspian Sea (46°10′N 49°13′E) (Fig. 1).

Adult fishes were caught by a fishing net, transported to the laboratory in cooled plastic bags, chilled on ice, then euthanised by quick and accurate decapitation (Jenkins et al., 2014). Then the adult fishes were dissected and their inner organs were examined visually for the presence of parasite xenomas (Lom and Dykova, 1992). Xenomas were excised and fixed with 95% ethanol for molecular genetic analysis. For DNA extraction, the ethanol was removed, the tissue sample was dried until remnant ethanol evaporated, then 100 µL of lysis buffer (2% CTAB, 1.4 M NaCl, 100 mM EDTA, 100 mM Tris-Cl, pH 8.0) (VWR Life Science AMRESCO) was added. The samples were homogenised with a plastic pestle adapted for a 1.5-mL microcentrifuge tube (SSIbio), followed by incubation at 65 °C for 2 h in 500 µL lysis buffer as above with the addition of 0.2% of β-mercaptoethanol (VWR Life Science AMRESCO). Genomic DNA was extracted by a phenol/chloroform extraction method, isopropanol sedimentation and 70% ethanol washing (Malysh et al., 2019).

To amplify a fragment of SSU rDNA, the universal SSU rDNA primers fl18f (5′-GGTATTCTGGCTTGACG-3′) and 1047r (5′-ACGGCGATCATCAG-3′) were used (Weiss and Vossbrinck, 1999). For rpb1, novel degenerate primers lgtrPB1F (5′-CCKAGRTGGATGATCATMAGTG-3′) and lgtrPBI (5′-ATCTARAARTCVCRTTGTACG-3′) were designed on the basis of the respective sequences of L. acerinae (Genbank accession # AJ278951), Glugea anomala (# AJ278952), Trachipleistophora hominis (# AJ278945), and Vavraia culcis (#AJ278956), aligned in BioEdit software version 7.2.5 (Hall, 1999). The primer sequences were selected manually and checked for specificity using Primer-BLAST online utility (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and for compatibility using PerlPrimer software version 1.1.21 (Marshall, 2004).

The PCR was applied using a protocol including the initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 1 min, the annealing at 54 °C for 1 min, the elongation at 72 °C for 1 min, and the final elongation step at 72 °C for 5 min (Malysh et al., 2020). The PCR mixture contained 1× DreamTaq Green PCR Mastermix (Thermo Fisher Scientific) and 10 nM of primers. The amplicons were visualised using electrophoresis in 1% agarose gels. Then gel fragments containing the specific amplicons were excised, melted with 3 M guanidine isothiocyanate (Thermo Fisher Scientific), absorbed using silicon dioxide (Sigma-Aldrich) and eluted with deionised molecular grade water (Vogelstein and Gillespie, 1979). The purified DNA fragments were sequenced in both directions by Sanger dideoxymethod using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) with respective forward and reverse primers as above, and detected on ABI Prism 3500 Genetic Analyzer. The raw reads with no ambiguous sites (double peaks etc.) were aligned using Clustal W multiple alignments in BioEdit. The sequence identities and genetic distances were calculated using BioEdit. Phylogenetic reconstruction was performed in MEGA 7 (Kumar et al., 2016). The maximum likelihood method using the Tamura 3-parameter model with a proportion of invariable sites and gamma distribution of rate categories was applied. G. anomala was used as an outgroup. The rpb1 sequence of L. acerinae available in Genbank (#AJ278951) was excluded from the analysis due to uncertainties in the nucleotide sequence and respective amino acid translation.

Partial sequences of SSU rDNA (950 bp in length) were obtained for 13 samples of microsporidia from Percidae, Gobiidae, and Atherinidae, showing 100% identity to each other. When compared to Genbank entries, the sequences showed 100% identity to L. acerinae from G. cernua (#AF356224) and big-scale sand smelt A. boyeri (#KT934810) and 99.6% identity to another isolate from G. cernua (#AJ252951). However, SSU rDNA sequence identity of L. acerinae ranged between 85.1 and 91.6% when compared to Loma morhua (#KX084449) from Atlantic cod Gadus morhua (Gadiformes: Gadidae), and other species attributed to the genus Loma, as well as representatives of other fish-infecting microsporidia from the genera Glugea, Dasyatispora, Heterosporis, Ichtyosporidium and Pleistophora (Table 1).

The PCR assay using the primers designed for the amplification of the rpb1 gene fragment was successful, and the obtained 686-bp-long sequences showed 98.6–100% sequence identity among the samples examined (Table 2). In particular, rpb1 sequence of L. acerinae from G. cernua (# MN335641) was 100% identical to those from A. boyeri.
Table 1. Nucleotide sequence identity and genetic distance of small subunit rRNA gene (SSU rDNA) of Loma acerinae isolates and related microsporidium species

polymorphism of the parasite may exist. Further studies are needed to elucidate the biological meaning of the genetic divergence between Neogobius-associated samples and those from other host species.

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Table 2. Nucleotide sequence identity and genetic distance of RNA-polymerase II largest subunit gene (rpB1) of Loma acerinae isolated from different hosts and localities. Haplogroup I: #8–13; Haplogroup II: #1–7

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<th>#</th>
<th>Host</th>
<th>Locality</th>
<th>Genbank accession</th>
<th>Sequence identity (%) left-lower cells</th>
<th>Genetic distance (%) right-upper cells</th>
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<td>99.8 99.8 99.8 99.8 99.8 99.8 99.8</td>
</tr>
</tbody>
</table>

*Numbers indicate microsporidium sequences from individual fish samples.

**ID – identical entries

Fig. 2. Phylogenetic reconstruction of Loma acerinae samples based on the 686-bp-long alignment of the largest subunit RNA polymerase II gene (rpB1). Values at branches indicate bootstrap support. Glugea anomala was used as an outgroup. Scale bar = 0.01 expected changes per site.

REFERENCES


