Genetic diversity of Microsporidia in the circulatory system of endemic amphipods from different locations and depths of ancient Lake Baikal

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Endemic amphipods (Amphipoda, Crustacea) of the most ancient and large freshwater Lake Baikal (Siberia, Russia) are a highly diverse group comprising >15 % of all known species of continental amphipods. The extensive endemic biodiversity of Baikal amphipods provides the unique opportunity to study interactions and possible coevolution of this group and their parasites, such as Microsporidia. In this study, we investigated microsporidian diversity in the circulatory system of 22 endemic species of amphipods inhabiting littoral, sublittoral and deep-water zones in all three basins of Lake Baikal. Using molecular genetic techniques, we found microsporidian DNA in two littoral (*Eulimnogammarus verrucosus*, *E. cyaneus*), two littoral/sublittoral (*Pallasea cancellus*, *E. marituji*) and two sublittoral/deep-water (*Acanthogammarus lappaceus longispinus*, *A. victorii maculosus*) endemic species. Twenty sequences of the small subunit ribosomal (SSU) rDNA were obtained from the haemolymph of the six endemic amphipod species sampled from 0–60 m depths at the Southern Lake Baikal's basin (only the Western shore) and at the Central Baikal. They form clusters with similarity to *Enterocytospora*, *Cucumispora*, *Dictyocoela*, and several unassigned Microsporidia sequences, respectively. Our sequence data show similarity to previously identified microsporidian DNA from inhabitants of both Lake Baikal and other water reservoirs. The results of our study suggest that the genetic diversity of Microsporidia in haemolymph of endemic amphipods from Lake Baikal does not correlate with host species, geographic location or depth factors but is homogeneously diverse.

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19   **Abstract**

20   Endemic amphipods (Amphipoda, Crustacea) of the most ancient and large freshwater

21 Lake Baikal (Siberia, Russia) are a highly diverse group comprising >15 % of all known species

22 of continental amphipods. The extensive endemic biodiversity of Baikal amphipods provides the

23 unique opportunity to study interactions and possible coevolution of this group and their

24 parasites, such as Microsporidia. In this study, we investigated microsporidian diversity in the

25 circulatory system of 22 endemic species of amphipods inhabiting littoral, sublittoral and deep-

26 water zones in all three basins of Lake Baikal. Using molecular genetic techniques, we found

27 microsporidian DNA in two littoral (*Eulimnogammarus verrucosus*, *E. cyaneus*), two

28 littoral/sublittoral (*Pallasea cancellus*, *E. marituji*) and two sublittoral/deep-water

29 (*Acanthogammarus lappaceus longispinus*, *A. victorii maculosus*) endemic species. Twenty

30 sequences of the small subunit ribosomal (SSU) rDNA were obtained from the haemolymph of

31 the six endemic amphipod species sampled from 0–60 m depths at the Southern Lake Baikal's

32 basin (only the Western shore) and at the Central Baikal. They form clusters with similarity to

33 *Enterocytospora*, *Cucumispora*, *Dictyocoela*, and several unassigned Microsporidia sequences,

34 respectively. Our sequence data show similarity to previously identified microsporidian DNA

35 from inhabitants of both Lake Baikal and other water reservoirs. The results of our study suggest

36 that the genetic diversity of Microsporidia in haemolymph of endemic amphipods from Lake

37 Baikal does not correlate with host species, geographic location or depth factors but is

38 homogeneously diverse.

39

40   **Introduction**

41   Lake Baikal is the deepest (1643 m) and oldest lake in the world (25–30 million years); it

42   is the largest (by volume; 23,000 km^3) reservoir of clean fresh water, containing approximately

43   20 % of the available world's liquid fresh water (Martens, 1997; Yoshii, 1999). Lake Baikal has

44   specific abiotic characteristics that distinguish it from all the other freshwater bodies in the world:

45   high oxygen content throughout the entire water column, stable low water temperatures with

46   long seasonal ice coverage on the lake's surface, and super-oligotrophic conditions. The

47   environmental conditions of the lake's open-water and deep-water zones have remained close to

48   their current state for the last 2–4 million years (Kozhova and Izmest′eva, 1998). As a unique

49   ecosystem with exceptionally high degrees of biological diversity and endemism, Lake Baikal

50   was designated a UNESCO World Heritage Site. To date, 2595 animal species from Lake Baikal

51   have been described, approximately 80 % of which are endemic (Timoshkin, 2001).

52   Among macroinvertebrates, the highest number of species in the lake is presented by

53   amphipods. The diversity of amphipods in Lake Baikal is very high: currently, 276 species and

54   78 subspecies (over 10 % of the total species diversity of fauna of Lake Baikal; over 15 % of all

55   recognized amphipod species from fresh or inland waters world-wide), grouped in 7 families and

56   41 genera, have been found in the lake (Väinölä et al., 2008; Takhteev, 2015). Amphipods

57   inhabit all depths of Baikal, including littoral (0-20 m), sublittoral (20-70 m), supra-abyssal (70-

58   250 m) and abyssal (250-1640 m); the last two depths are typically grouped as a deep-water zone

59   (Kozhov, 1962). The age and geographical isolation of this group create excellent opportunities

60   for exploring the diversity and evolution of host-parasite relationships.

61   Microsporidia are a diverse phylum of eukaryotic parasites, which are a sister clade to the

62   Fungi kingdom and infect a wide range of hosts from invertebrates to humans (Keeling and Fast,

63   2002; Smith, 2009; Issi et al., 2012; Gismondi et al., 2012; Stentiford et al., 2013; Bojko et al.,

64   2016). The number of described species of Microsporidia is more than 1300, and the various

65   species belong to approximately 187 genera, 50 of which are found in aquatic arthropods

66   (Stentiford et al. 2013; Weiss and Becnel, 2014). Some microsporidian species may influence the

67   sex ratio of arthropod populations (Terry et al., 1998; Ironside et al., 2003; Ironside and

68   Alexander, 2015), the behaviour of the host, and the host population dynamics (Dunn and Smith,

69   2001). For example, a selective sexual behaviour is described for amphipod species *Gammarus*

70   *duebeni* Liljeborg, 1852. The males of this species infected by Microsporidia are only able to

71   hold down infected females for breeding (Dunn et al., 2006). Microsporidia can be transmitted

72   horizontally, vertically or both ways (Terry et al., 2004; Smith, 2009). They have been found to

73   infect muscles, gonads, intestinal walls, hepatopancreas, haemocytes, and other organs (Kelly

74   and Anthony, 1979; Weiss and Becnel, 2014).

75   Despite the fact that amphipods endemic to Lake Baikal offer great possibility to study

76   the basic principles of coevolution of the host-parasite system and distribution of parasites

77   among evolutionary close species, only a few papers have been published on the microsporidian

78   diversity in the lake's fauna. The study of Microsporidia in Baikal amphipods began in 1967

79   when *Nosema kozhovi* was found in intestinal epithelium of *Brandtia latissima lata* (Dyb., 1874)

80   (Lipa, 1967). The first molecular genetic studies of Microsporidia were performed in the 21st

81   century. Six endemic and one cosmopolitan microsporidian species (*Dictyocoela duebenum*)

82   were detected in the Baikal amphipod *Gmelinoides fasciatus* (Stebbing, 1899; Kuzmenkova et al.,

83   2008), and 100 new gene isolates of Microsporidia in 31 species of amphipods of Lake Baikal

84   were discovered by molecular methods (Smith et al., 2008). A recent study provided the first

85   glimpse at horizontal distribution and exchange of Microsporidia between Baikal littoral and

86   non-Baikal amphipods, which indicated frequent introductions of the parasites into Lake Baikal

87 ecosystem and high homogeneity of Microsporidia between species of endemic hosts in the

88 coastal zone (Ironside and Wilkinson, 2017).

89 However, most previous studies utilized the whole individuals or soft amphipod tissues

90 for amplification of microsporidian DNA, which does not rule out the possibility of

91 contamination. To avoid this problem, our group recently conducted a study using molecular

92 genetic techniques (SSU rDNA sequencing) to detect Microsporidia in circulatory system of

93 several endemic amphipod species from Lake Baikal (Madyarova et al., 2015). Searching

94 directly in the haemolymph minimizes the possibility of identifying SSU rDNA of Microsporidia

95 located on exoskeleton, in gut lumen or inside parasites of the amphipods and can guarantee that

96 the found Microsporidia are parasitic directly to the amphipod species studied. Moreover, using

97 haemolymph provides a unified way of treating amphipods of very different sizes, including

98 small species and young individuals.

99 Spatial variations, especially vertical distribution, in biodiversity of Microsporidia in

100 Lake Baikal amphipods remain poorly investigated. Thus, the aim of the current study was to

101 search for diversity of Microsporidia in the haemolymph of endemic amphipods inhabiting

102 littoral, sublittoral and deep-water zones of the lake in all three basins of Baikal: Southern,

103 Central and Northern.

104

105 **Materials and Methods**

106 *Sampling and location*

107 To study microsporidian biodiversity, the haemolymph of 22 amphipod species from 11

108   points of Lake Baikal was used (Table S1). The following seven species were collected at depths

109   of 0-1 m using a hand net in the period from 2011 to 2016: *Eulimnogammarus verrucosus*, *E.*

110   *cyaneus*, *E. marituji*, *E. vitatus*, *E. maaki, E. viridis olivaceus, Pallasea cancelus*. The other

111   amphipod species were sampled from depths of 20-200 m: *Acanthogammarus victorii maculosus,*

112   *A. flavus*, *A. lappaceus longispinus*, *A. godlewskii*, *A. brevispinus*, *A. reicherii*, *Carinurus belkini*,

113   *Garjajewia   cabanisi*,   *Propachygammarus   maximus*,   *Ommatogammarus   carneolus*

114   *melanophthalmus*, *Parapallasea lagovskii*, *P. puzyllii*, *P. brandtia*, *P. kesslerii*, *P. grubii*. The

115   latter group of species was collected during the expeditions in 2014 and 2016 using a trawl,

116   dredge and deep-water traps with rotten fish installed at a depth of 200 m. In this work, only

117   benthic amphipod species were studied. The chosen amphipod species are not endangered or

118   protected.

119   Species determination was performed according to the identification key (Bazikalova,

120   1945; Takhteev and Didorenko, 2015).

121   The collections cover all three of Lake Baikal's basins: the Southern (Listvyanka, Bolshie

122   Koty, Port Baikal), the Central (Olkhon Island, Kharauz, Ushkany Islands) and the Northern

123   (Kotelnikovskiy Cap), including both the Western (Listvyanka, Bolshie Koty, Port Baikal) and

124   Eastern (Kharauz) shores (Fig. 1). Around Olkhon Island, five sampling points were screened:

125   Burkhan Cape, Cape Umysh-Tame, Olkhon Gate, Kharantsy Island and Peschanka Bay. The

126   Lake Baikal's basins and the Angara River act as natural geographic barriers for benthic

127   amphipods (Mashiko, 2000; Gomanenko et al., 2005). No specific permissions were required for

128   sampling of amphipods in these locations.

129

130 *Haemolymph extraction*

131   Haemolymph extraction was performed from live amphipods in a laboratory using a glass

132 capillary. Each sample subsequently used for DNA extraction (volumes ranging from 50 to 100

133 μl) contained haemolymph collected from one or pooled from several individuals of the same

134 species. The number of individuals per sample depended on the size of the amphipods (1–50

135 individuals; see Table S1 for details). Isolated haemolymph samples were stored in liquid

136 nitrogen.

137

138 *DNA extraction and PCR*

139   Total DNA extraction from individual or pooled haemolymph was performed using the

140 "Riboprep" reagent kit (AmpliSens, Russia). The concentration and purity of the isolated DNA

141 was determined using the UV spectrophotometer UNICO 2802 (UNICO, USA).

142   The small subunit of ribosomal DNA (SSU rDNA) of the Microsporidia was selected as a

143 molecular phylogenetic marker. Amplification was performed in the nested PCR with universal

144 primers for Microsporidia: V1f: 5'-CACCAGGTTGATTCTGCCTGAC-3' (Weiss et al., 1994);

145 1342r: 5'-ACGGGCGGTGTGTACAAAGAACAG-3' (McClymont et al., 2005); 18sf: 5'-

146 GTTGATTCTGCCTGACGT-3' (Baker et al., 1995); and 981r: 5'-

147 TGGTAAGCTGTCCCGCGTTGAGTC-3' (MacNeil et al., 2003). Each PCR was performed in a

148 Gradient Thermocycler (Biometra, Germany) in a volume of 25 μl and contained 1X PCR buffer,

149 6.25 pmol of each dNTP, 2.5 U SynTaq DNA polymerase, 6.25 pmol $MgCl_2$, 10 pmol primers, 3

150 μl of DNA template, and deionized water. The conditions for the first round were 95°C for 5 min,

151 followed by 40 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 90 sec, and then 72°C

152   for 7 min. Conditions for the second round of PCR were 95°C for 5 min, followed by 35 cycles

153   of 95°C for 30 sec, 52°C for 30 sec, and 72°C for 1 min, followed by 72°C for 7 min.

154   The PCR products of the second round were visualized in 1 % agarose gels, and products

155   of the expected size were excised from the gel and purified by ethanol and sodium acetate

156   precipitation (Sambrook et al., 1989). Sequencing of the amplified DNA fragments was

157   performed using Genetic Analyzer 3500 xL (Applied Biosystems, Japan) with the BigDye

158   Terminator Cycle Sequencing kit v.3.1 (Applied Biosystems, USA).

159

160   *Nucleotide sequence analysis, phylogenetic reconstruction and statistical analysis*

161   The obtained sequences were identified with NCBI nucleotide BLAST with default

162   parameters against the nt database (last accessed 2018/05/17). The best B hit belonging to a

163   sample identified in other studies was chosen to describe the samples (Table S2).

164   The sequences were aligned with the MAFFT 7.397 E-INS-i strategy (Katoh et al., 2009)

165   and then trimmed with Gblocks (Castresana et al., 2000) accessed through the Phylogeny.fr web

166   interface (Dereeper et al., 2008) with all options for less stringent selection checked. The

167   resulting alignment (Alignment S1) contained 514 bases. Nucleotide substitution models were

168   evaluated with jModelTest 2.1.10 (Guindon and Gascuel, 2003; Darriba et al., 2012). The best

169   model according to Bayesian information criterion (BIC) score was TrN+G with GTR+G falling

170   very close behind. As the formed model was not available in RAxML, GTR+G was used for all

171   calculations. The phylogenetic tree was reconstructed with BEAST 2.5 (Bouckaert et al., 2014)

172   for 10,000,000 generations with sampling every 1,000th tree. After this run, ESS value was close

173   to 150, indicating acceptable convergence. In addition, we reconstructed the tree with RAxML

174   8.2.11 (Stamatakis, 2014) under the GTRGAMMA model and 5,000 rapid boostrap generations.

175   RAxML bootstrap results were reanalysed with the tree obtained with BEAST to get comparable

176   support rates for the nodes. Raw trees obtained with BEAST and RAxML are shown in Tree S1

177   and   Tree   S2,   respectively.   The   results   were   visualized   in   FigTree   1.4.2

178   (http://tree.bio.ed.ac.uk/software/figtree/) and Dendroscope, and Fig. 2 was prepared with the

179   ggtree package (Yu et al., 2017) for R (R Core team, 2017) and Inkscape (inkscape.org).

180   Simpson's diversity index was calculated using the diversity function (option *index* set to

181   *invsimpson*) of the vegan package (Dixon, 2003) for R. Fisher's exact test was implemented with

182   the stats package for R.

183

184   **Results**

185   We screened the haemolymph of 22 species (Table S1) of endemic amphipods from Lake

186   Baikal and found microsporidian DNA in two littoral (*E. verrucosus*, *E. cyaneus*), two

187   littoral/sublittoral (*P. cancellus*, *E. marituji*) and two sublittoral/deep-water species (*A. lappaceus*

188   *longispinus*, *A. victorii maculosus*). A total of over 1,000 individual amphipods were analysed

189   (Table S1). Twenty nucleotide sequences of SSU rDNA belonging to the *Cucumispora*,

190   *Dictyocoela, Enterocytospora*-like groups and unassigned Microsporidia were obtained from the

191   haemolymph of the six endemic amphipod species sampled from depths of 0-60 m at Southern

192   Lake Baikal's basin (only the Western shore) and at Central Baikal (Table S2). Only one

193   microsporidian isolate has been identified in each positive pooled haemolymph sample. The

194   phylogenetic tree (Fig. 2) reconstructed using these and some other published microsporidian

195   sequences (Alignment S1) shows clustering of the DNA identified in the current study to four

196   groups.

197   Three microsporidian DNA sequences were found in the haemolymph of *E. cyaneus*

198   (sampled at 0-1 m in the Southern Baikal), and one was found in *A. victorii maculosus* (sampled

199   at 53-60 m in the Central Baikal). These four sequences are presented as a single cluster on the

200   phylogenetic tree marked as *Cucumispora*-like (C) and are similar to taxonomically defined

201   *Cucumispora* isolates (Ovcharenko et al., 2010; Bojko et al., 2015, 2017b) as well as

202   microsporidian sequences found earlier in some other endemic amphipods of Lake Baikal, such

203   as *P. cancellus* (KM977842, KM977843, KM977844, KM977845, KM977846; Madyarova et al.,

204   2015), *Acanthogammarus victorii* (FJ756173), *Garjajewia cabanisii* (FJ755959) and

205   *Pallaseopsis kessleri* (FJ756022).

206   The other microsporidian DNA from one haemolymph sample of *P. cancellus*, three

207   samples of *E. verrucosus* (both sampled at 0-1 m in the Southern Baikal) and two of *A.*

208   *lappaceus longispinus* (sampled at 25-35 m in the Central Baikal) were clustered with those of

209   Microsporidia of the recently defined (Bacela-Spychalska et al., 2018) genus *Dictyocoela* (D),

210   namely *D. duebenum* (Grabner et al., 2015) and *D. berillonum*. Representatives of *Dictyocoela*

211   were found earlier in an endemic Baikal amphipod *A. lappaceus* (KM977839; Madyarova et al.,

212   2015).

213   Two more sequences amplified from *Eulimnogammarus verrucosus* haemolymph

214   clustered with other isolates of the *Enterocytospora*-like clade (E), such as *Enterocytospora*

215   *artemiae* (JX915760; Rode et al., 2013a), *Globulispora mitoportans* (KT762153; Vávra et al.,

216   2016) and recently described *Parahepatospora carcini* (KX757849; Bojko et al., 2017a). A

217   similar sequence was also found in an endemic Baikal amphipod *Micruropus wahlii* (FJ756186).

218   Finally, some isolates detected in the littoral species *E. verrucosus* (sampled at 0–1 m)

219   from four different habitats, Listvyanka, Bolshie Koty, Port Baikal (Southern Baikal) and

220   Burkhan Cape (Central Baikal), clustered with other microsporidian sequences previously

221   amplified from Baikal endemic amphipods (FJ756111 from *Dorogostaiskia parasitica* and

222   FJ755992 from *Odontogammarus calcaratus*). This cluster is designated as unclassified

223   Microsporidia (uM).

224   As we have data for seven different locations, we have calculated Simpson's diversity

225   index for the groups of amphipods defined according to the phylogenetic tree and these locations

226   (Fig. S1). Our samples are not big enough to draw any conclusions, as we quite predictably saw

227   higher diversity in locations with higher numbers of analysed pools, but further studies may help

228   clarify this issue.

229

230   **Discussion**

231   The microsporidian DNA sequences found in the haemolymph of amphipods from Lake

232   Baikal during this study belong to four clusters: *Cucumispora*-, *Dictyocoela*-, *Enterocytospora*-

233   like, as well as some unclassified Microsporidia. Some other sequences belonging to the

234   *Cucumispora*-like and *Dictyocoela*-like clusters, as well as to unclassified Microsporidia were

235   also identified in our previous work (Madyarova et al., 2015). Apart from these similarities, there

236   were also some differences, as in the earlier work we also identified representatives of the genus

237   *Nosema* (N) in Baikal amphipods *E. verrucosus* from Listvyanka (Madyarova et al., 2015), but in

238   the current study, these microsporidian species were not found at all.

239   Moreover, in winter 2013-2014 (Table S1) *E. verrucosus* from Listvyanka showed

240 relatively high infection (~30 % of pools) by Microsporidia of groups *Nosema/Vairimorpha*-

241 (NV), *Dictyocoela*- (D), *Enterocytospora*-like (E) and unclassified Microsporidia (uM), while in

242 summer 2016 only one infected pool (~3 %) was identified in this location and corresponded to

243 an unclassified Microsporidia (uM). We have compared the largest samples with exact Fisher's

244 test (5 out of 18 *vs.* 1 out of 33) and found that the difference was statistically significant (p =

245 0.017). Sampling of Microsporidia during summer and autumn (2015 and 2016) at other points

246 (Port Baikal, Bolshie Koty and Burkhan Cape) also demonstrated relatively low infection (no

247 more than 13 % of pools) only by unclassified Microsporidia. It is interesting to note that two

248 highest infection rates belonged to pools sampled in winter, the reproduction season of *E.*

249 *verrucosus* (Bazikalova, 1941). Generally, by comparing the percentage of infected haemolymph

250 pools of different amphipod species from chosen sampling points (Table S1), we can conclude

251 that the proportion of infected individuals can vary significantly.

252   The species diversity of the obtained samples is also interesting in the context of

253 worldwide diversity of microsporidian parasites of amphipods. Microsporidian SSU rDNA

254 nucleotide sequences similar to the C group in our analysis (Fig. 2) were also found in

255 *Gammarus chevreuxi* Sexton, 1913 from the Avon River in the UK (AJ438962) (Terry et al.,

256 2004). The identified sequences of the D group are similar to the *Dictyocoela duebenum* isolate

257 775 (FN434091), which was previously found in *Gammarus duebeni* from Iceland (Krebes et al.,

258 2010), and the *Dictyocoela muelleri* isolate (AJ438955) found in *Gammarus duebeni celticus*

259 from Ireland (Terry et al., 2004). The *Enterocytospora*-like sequences are similar to the

260 sequences of *Enterocytospora artemiae* found in *Artemia franciscana monica* from Mono Lake,

261 USA (Rode et. al., 2013b). These results may suggest that microsporidian distribution in Baikal

262 amphipods is similar to that in amphipods and other crustaceans from other water bodies.

263   Concerning the spatial and host distribution of Microsporidia in Baikal amphipods, we

264   should note that similar and identical sequences of microsporidian DNA were found in

265   amphipods inhabiting different depths and basins of the lake. Two microsporidian sequences

266   from *P. cancellus* (KM977844 and KM977846) were identical to sequences from completely

267   different amphipod species *G. cabanisi* (FJ755959) and *D. parasitica* (FJ756113) (Table S2; Fig.

268   2). Almost identical DNA of Microsporidia that belong to the group C were found at sampling

269   depths 0-1 m of Southern Baikal (KT956054, KR080135, KT956053 from *E. cyaneus* and

270   KM977844 from *P. cancellus*) and depths of 54-60 m of Central Baikal (KT956063 from *A.*

271   *victorii maculosus*). Similar sequences of Microsporidia of the group D are found at depths of 0-

272   1 m (*E. marituji*, *E. verrucosus* and *P. cancellus* from the Southern Baikal) and at 25-35 m (*A.*

273   *lappaceus longispinus* from the Central Baikal). Additionally, amphipods of species *E.*

274   *verrucosus* from Southern and Central Baikal were infected by microsporidia with almost

275   identical SSU rDNA belonging to some unclassified Microsporidia (uM). So, similar

276   microsporidian isolates infect different hosts at different depths and locations. There are several

277   possible explanations for this fact. Amphipods are omnivorous animals and can feed on other

278   amphipods of different or the same species. Moreover, Baikal amphipods are characterized by

279   high vertical and horizontal mobility: a significant part of the analysed species (*E. marituji*, *P.*

280   *cancellus*, *A. lappaceus longispinus* and others) can be found in a range of depths of at least

281   several dozen meters and can participate in nocturnal vertical migrations observed for many

282   benthic animals in Baikal (Bazikalova, 1945; Karnaukhov et al., 2016); also, in Lake Baikal

283   there are eurybathic scavenger amphipods (like *Ommatogammarus flavus* and *O. albinus*), found

284   in depth ranges of several hundred meters (Bazikalova, 1945), which may serve as additional

285   "carriers" of infections in the benthic community.

286   Thus, the results of our study do not suggest any link between the genetic diversity of

287   Microsporidia found in circulatory system of endemic amphipods from Lake Baikal and host

288   species, geographic location or depth. This result corroborates previous studies (Madyarova et al.,

289   2015; Ironside & Wilkinson, 2017).

290

291   **Conclusions**

292   The sequence isolates that we have identified during our study belong to four clusters and

293   show similarity to previously identified microsporidian DNA from inhabitants of both Lake

294   Baikal and other water reservoirs. No specificity in distribution of Microsporidia was identified

295   depending on host amphipod species and their spatial relations, which may indicate high

296   connectivity of individuals within species and between species of Lake Baikal amphipods.

297   However, search for Microsporidia in amphipods at greater depths (200-1600 m) is still

298   necessary to confirm the observed homogeneity in distribution of these parasites.

299

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306

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458

459   **Figure legends**

460

461   **Figure 1**. Sampling sites for amphipod collection in Lake Baikal. Grey dots correspond to sites

462   where no infected amphipods were found, while black dots mark places where microsporidian

463   DNA was identified in amphipod haemolymph. The bathymetric map of Lake Baikal was taken

464   from http://users.ugent.be/~mdbatist/intas/intas.htm and modified with CorelDRAW.

465   **Figure 2**. Bayesian phylogenetic tree of Microsporidia identified in amphipods of Lake Baikal

466   and   some   other   crustaceans.   The   tree   contains   the   following   clusters:

467   Enterocytozoonidae&Hepatosporidae-like (EH), *Enterocytospora-* (E), *Cucumispora-* (C),

468   *Dictyocoela-* (D), *Nosema-Vairimorpha*-like (NV), and some unclassified Microsporidia (uM),

469   *Nosema* (N). Branch lengths are drawn to scale. Grey semi-circles mark nodes with bootstrap

470   support >70%, and red semi-circles mark those with posterior probability >0.7. Baikal shapes

471   mark microsporidian sequences amplified from Lake Baikal amphipods, and samples obtained in

472   this work are in blue font. The alignment used to build this tree is presented in Alignment S1.

473

474   **Supplementary materials**

475   **Supplemental Table S1**. All analysed samples of haemolymph of amphipods collected in Lake

476   Baikal.

477   **Supplemental Table S2**. All microsporidian DNA identified in haemolymph of amphipods

478   collected in Lake Baikal. It contains sequences obtained both in the current study and in

479   (Madyarova et al., 2015). Columns A-H describe samples obtained in our work, while columns

480   H-M describe their closest their closest hits in the NCBI database. E-values equal to 0 represent

481   values lower than 10^{-200}.

482   **Supplemental Alignment S1**. Raw alignment of microsporidian DNA sequences used for

483   construction of the phylogenetic tree on Fig. 2.

484   **Supplemental Tree S1.** Bayesian tree obtained from the Alignment S1.

485   **Supplemental Tree S2.** Maximum likelihood bootstrap support for the tree obtained from the

486   Alignment S1.

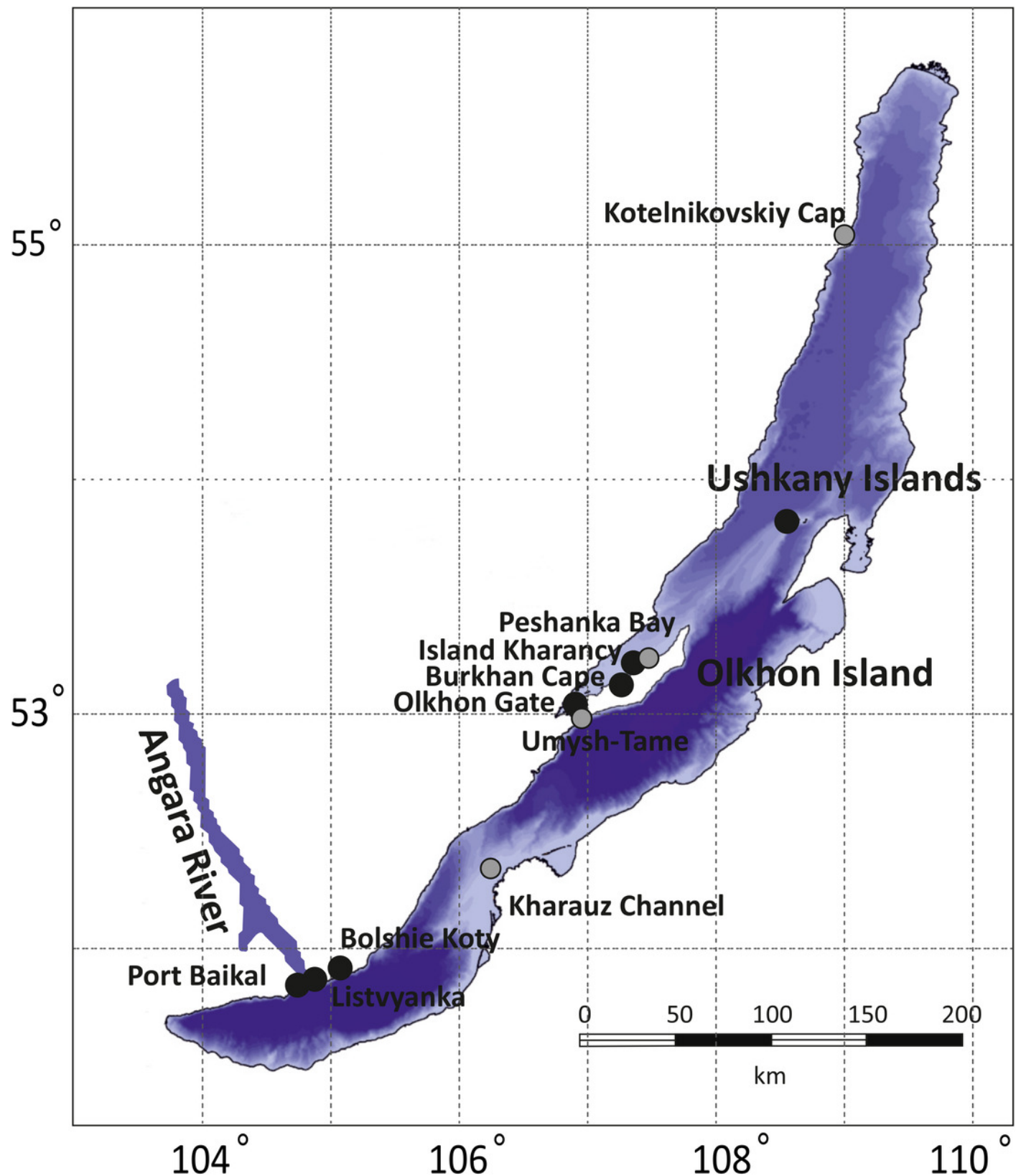
487   **Supplemental Figure S1.** Inverse Simpson's diversity index for each location and number of

488   samples for each location where microsporidian DNA was detected. The vertical axis features

489   the number of microsporidian isolates.

Figure 1

Sampling sites for amphipod collection in Lake Baikal.

Grey dots correspond to sites where no infected amphipods were found, while black dots mark places where microsporidian DNA was identified in amphipod haemolymph. The bathymetric map of Lake Baikal was taken from

http://users.ugent.be/~mdbatist/intas/intas.htm and modified with CorelDRAW.

Kotelnikovskiy Cap

Ushkany Islands

Peshanka Bay
Island Kharancy
Burkhan Cape
Olkhon Gate
Umysh-Tame

Olkhon Island

Angara River

Kharauz Channel

Bolshie Koty
Port Baikal
Listvyanka

55°

53°

104°   106°   108°   110°

0   50   100   150   200
km

Bayesian phylogenetic tree of Microsporidia identified in amphipods of Lake Baikal and some other crustaceans.

The tree contains the following clusters: Enterocytozoonidae&Hepatosporidae-like (EH), *Enterocytospora-* (E), *Cucumispora-* (C), *Dictyocoela-* (D), *Nosema-Vairimorpha*-like (NV), and some unclassified Microsporidia (uM), *Nosema* (N). Branch lengths are drawn to scale. Grey semi-circles mark nodes with bootstrap support >70%, and red semi-circles mark those with posterior probability >0.7. Baikal shapes mark microsporidian sequences amplified from Lake Baikal amphipods, and samples obtained in this work are in blue font. The alignment used to build this tree is presented in Alignment S1.