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1
2 Identification of novel and zoonotic *Cryptosporidium* species in fish from Papua New Guinea

3
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17
18 **Abstract**

19 There is still limited information on the distribution of *Cryptosporidium* species and genotypes
20 in fish. The present study investigated the prevalence of *Cryptosporidium* species in cultured
21 freshwater ($n=132$), wild freshwater ($n=206$) and wild marine ($n=276$) fish in Papua New
22 Guinea (PNG) by PCR screening at the 18S rRNA locus. A total of seven fish (2 cultured
23 freshwater, 1 wild freshwater and 4 wild marine fish) were identified as positive for
24 *Cryptosporidium*. Specifically, *Cryptosporidium* was found in four different host species (Nile
25 tilapia, *Oreochromis niloticus*; silver barb, *Puntius gonionotus*; mackerel scad, *Decapterus*
26 *maracellus* and oblong silver biddy, *Gerres oblongus*), giving an overall prevalence of 1.14 %
27 (95 % CI: 0.3 % - 2 %, $n=7/614$). Of the seven positive isolates, five were identified as *C.*
28 *parvum* and two were a novel piscine genotype, which we have named piscine genotype 8.
29 Piscine genotype 8 was identified in two marine oblong silver biddies and exhibited 4.3 %
30 genetic distance from piscine genotype 3 at the 18S locus. Further subtyping of *C. parvum*
31 isolates at the 60 kDa glycoprotein (*gp60*) locus identified 3 *C. parvum* subtypes (IIaA14G2R1,
32 IIaA15G2R1 and IIaA19G4R1) all of which are zoonotic and a *C. hominis* subtype (IdA15G1).
33 The zoonotic *Cryptosporidium* were identified in fish samples from all three groups; cultured
34 and wild freshwater and wild marine fish. Detection of *Cryptosporidium* among aquaculture
35 fingerlings warrants further research to gain a better understanding of the epidemiology of
36 *Cryptosporidium* infection in cultured fish. The identification of zoonotic *Cryptosporidium*
37 genotypes in fish from PNG has important public health implications and should be investigated
38 further.

39 **Keywords:** *Cryptosporidium*, fish, 18S rRNA, *gp60*, novel genotype, zoonotic.

40 **1 Introduction**

41 The apicomplexan protozoan parasite *Cryptosporidium* infects a wide range of mammals, birds,
42 reptiles and fish, primarily causing diarrhoea in mammals, diarrhoea and/or catarrhal respiratory
43 signs in birds and gastritis in reptiles and possibly fish (O'Donoghue, 1995; Ryan, 2010).

44 *Cryptosporidium* has been described in more than 17 species of both fresh and salt water fish
45 with parasitic stages located deep within and on the surface of the stomach or intestinal
46 epithelium (Alvarez-Pellitero and Sitja-Bobadilla, 2002; Alvarez-Pellitero et al., 2004; Ryan et
47 al., 2004; Murphy et al., 2009; Reid et al., 2010; Zanguee et al., 2010; Morine et al., 2012). In
48 fish, *Cryptosporidium* can cause high morbidity with clinical signs including variable levels of
49 emaciation, poor growth rates, swollen coelomic cavities, anorexia, listlessness and increased
50 mortality (Murphy et al., 2009).

51 Currently the only recognised species infecting fish is *Cryptosporidium molnari*, which was
52 identified in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*)
53 (Alvarez-Pellitero and Sitja-Bobadilla, 2002) and was characterised genetically in 2010
54 (Palenzuela et al. 2010). *Cryptosporidium molnari* primarily infects the epithelium of the
55 stomach and seldom the intestine (Alvarez-Pellitero and Sitja-Bobadilla 2002). In 2004, *C.*
56 *scophthalmi* was described in turbot (*Psetta maxima*. syn. *Scophthalmus maximus*) (Alvarez-
57 Pellitero and Sitja-Bobadilla, 2002; Alvarez-Pellitero et al., 2004). However, no genetic
58 sequences are available for *C. scophthalmi* and it can thus not be considered a valid species due
59 to the high genetic heterogeneity and morphological similarity among *Cryptosporidium* species
60 in fish. In addition to *C. molnari*, a total of 3 species and 10 genotypes have been characterised
61 genetically in fish (Table 1).

62 Fish are an important part of the diet and a source of income especially for people living on the
63 coast and along the rivers in Papua New Guinea (PNG). Freshwater fish farming began in the
64 1960s with the introduction of carp and trout species; however, difficulties due to lack of
65 knowledge among farmers impeded farming progress and the spread of its nutritional and
66 financial benefits to rural communities (Smith, 2007). Since 1995, the number of inland
67 aquaculture operations has increased in PNG due to international programs that involve the
68 expansion of hatcheries, training of farmers and the introduction of new fish species (Smith,
69 2007). To date, very little is known about the prevalence and genotypes of *Cryptosporidium* in
70 fish or other animals in PNG (Owen, 2005; Koinari et al., 2012; Koinari et al., 2013). The
71 present study represents a detailed investigation of the prevalence and genetic characterisation of
72 *Cryptosporidium* in cultured freshwater, wild freshwater and wild marine fish sampled from a
73 number of different locations throughout PNG. It is therefore the first comprehensive study
74 describing the distribution of *Cryptosporidium* species in PNG.

76 **2 Materials and Methods**

77 *2.1 Sample collection*

78 A total of 614 fish from cultured freshwater, wild freshwater and wild marine environments
79 were collected in PNG between February and August 2011 (Fig. 1). Cultured fish ($n=133$)
80 included three species, which were collected from four smallholder fish ponds in Kundiawa,
81 Asaro, Mumeng and Bathem (Table 2). Wild freshwater fish ($n=205$) included six species and
82 were collected from the Ramu and Sepik Rivers, while 276 wild marine fish consisting of 16
83 species were bought from local fishermen in Bilbil, Madang, Tavana and Pilapila (Table 2). On
84 average, time lag between collection or purchasing and processing of the fish was up to 4 h. All
85 sampling was conducted under Murdoch University Animal Ethics permit R2369/10.

86 The fish were weighed, measured (length and weight) and dissected. Sections of intestine and
87 stomach were cut using a sterile scalpel blade for each fish, placed in 2 mL Eppendorf tubes and
88 preserved in 70 % ethanol for molecular screening. The remaining stomach and intestine were
89 fixed in 10 % buffered formalin for histological analysis. All samples were stored at 4 °C in

90 PNG until sample collection was completed. The samples were then transported to Murdoch
91 University, Perth, Australia and stored at 4 °C until analysis.

92

93 2.2 DNA isolation

94 The preserved intestines and stomachs were washed 5 times with water to remove ethanol and
95 the epithelial layers were scraped using a sterile scalpel blade for each fish. DNA was extracted
96 from 25 mg of intestinal and stomach scrapings using a PowerSoil® DNA Isolation Kit (MO
97 BIO laboratories, Carlsbad, California, USA) according to the manufacturer's instructions and
98 incorporating five freeze-thaw cycles as described previously (Ng et al., 2006) to break open the
99 *Cryptosporidium* oocysts. DNA was eluted in 50 µL of elution buffer. All extracted samples
100 were stored at -20 °C until required for screening.

101

102 2.3 *Cryptosporidium* genotyping and subtyping

103 All 614 samples from fish were screened for the presence of *Cryptosporidium* at the 18S rRNA
104 locus as previously described (Morgan et al., 1999). Prevalences were expressed as a percentage
105 of positive samples; with 95 % confidence intervals calculated assuming a binomial distribution,
106 using the software Quantitative Parasitology 3.0 (Rozsa et al., 2000). Isolates that were positive
107 at the 18S locus were subtyped at the 60 kDa glycoprotein (*gp60*) locus using primers which
108 produce an 850 bp product (Alves et al., 2003)., however, amplification was unsuccessful and a
109 shorter (400 bp) product was amplified as described by Sulaiman et al. (2005).

110 Positive isolates were also amplified at the actin locus. New primers were designed specifically
111 based on actin gene sequences of piscine-derived *Cryptosporidium* and a semi-nested PCR
112 protocol was used. For the primary PCR, a PCR product of ~392 bp was amplified using the
113 forward primer ActinallF1 (5'-GTAAATATACAGGCAGTT-3') and reverse primer ActinallR1
114 (5'-GGTTGGAACAATGCTTC-3'). Each PCR was performed in a reaction volume of 25 µL
115 using 1 µL of DNA, 1 x PCR buffer (Kapa Biosystems, Cape Town, South Africa), 2.0 mM
116 MgCl₂, 200 µM (each) dNTP (Fisher Biotech, Perth, Australia), 12.5 pmol of forward and
117 reverse primers and 0.5 U of *kapa* Taq DNA polymerase (Kapa Biosystems, Cape Town, South
118 Africa). Forty-five PCR cycles (95 °C for 30 s, 46 °C for 30 s, 72 °C for 30 s) were performed
119 using a Perkin Elmer Gene Amp PCR 2400 thermocycler with an initial hot start (95 °C for 4
120 min) and a final extension (72 °C for 7 min). For the secondary PCR, a fragment of ~278 bp was
121 amplified using 1 µL of primary PCR product with forward primer ActinallF2 (5'-
122 CCTCATGCTATAATGAG-3') and reverse primer ActinallR1. The conditions used for the
123 secondary PCR were identical to those for the primary PCR.

124 Secondary PCR products were separated by gel electrophoresis and purified using a simple tip
125 elution method. Briefly, the PCR product was excised from the gel using a scalpel blade and
126 purified using an in house filter tip method and used for sequencing without any further
127 purification as previously described (Yang et al., 2013). DNA sequencing was performed using
128 the ABI Prism BigDye® terminator cycle sequencing kit (Applied Biosystems, Foster City, CA,
129 USA) on an Applied Biosystems 3730 DNA Analyser instrument. Nucleotide sequences were
130 analysed using FinchTV 1.4.0 (Geospiza, Inc., Seattle, WA, USA; <http://www.geospiza.com>)
131 and aligned with reference genotypes retrieved from GenBank using Clustal Omega
132 (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).

133 Phylogenetic trees were constructed using additional sequences retrieved from GenBank.
134 Distance estimation was conducted using MEGA5 (Tamura et al., 2011) based on evolutionary
135 distances calculated using the *p* distance model (Nei and Zhang, 2006) and grouped using
136 neighbour-joining. Parsimony and maximum likelihood analyses were also conducted using the
137 MEGA5 software. Reliabilities for the trees were tested using 1000 bootstrap replications
138 (Felsenstein, 1985) and bootstrap values exceeding 70 were considered well supported (Hills and
139 Bull, 1993).

140

141

2.4 Microscopy

142 Sections of intestinal and stomach tissues fixed in 10 % formalin were embedded in paraffin.

143 Histological sections were cut at 5 µm thicknesses, stained with hematoxylin and eosin and

144 examined with an Olympus BX50 light microscope at 400 and 1000 fold magnification.

145

146

3 Results

147

148

3.1 Prevalence of *Cryptosporidium* in fish hosts

149 Of the 614 fish sampled, seven (1.14 %, 95 % CI 0.3 % - 2 %) were positive by PCR, of which,

150 two (0.33 %, 95 % CI 0.09 % - 1.2 %) were cultured fingerlings, one (0.16 %, 95 % CI 0.03 % -

151 0.9 %) was a wild freshwater species and four (0.65 %, 95 % CI 0.25 % - 1.66 %) were wild

152 marine species. Among the fish hosts infected were: two Nile tilapias (*Oreochromis niloticus*)

153 with a prevalence of 2.4 % (95 % CI = 0 % - 5.7 %; 2/83) from fish ponds in Kundiawa and

154 Mumeng; one silver barb (*Puntius gonionotus*) from Sepik River with a prevalence of 1.9 % (95155 % CI = 0 % - 5.7 %; 1/52); two oblong silver biddies (*Gerres oblongus*) from Bilbil and Tavana156 with a prevalence of 3.6 % (95 % CI = 0 % - 8.5 %; 2/55) and two mackerel scads (*Decapterus*157 *macarellus*) from Pilapila with a prevalence of 6.9 % (95 % CI = 0 % - 16.1 %; 2/29) (Fig.1).

158

159

3.2 Sequence and phylogenetic analysis of the 18S rDNA, *gp60* and *actin* genes

160 Sequence analysis identified *C. parvum* (5 isolates: Nile tilapia ON36 and ON68, mackerel scad

161 DM17 and DM18, and silver barb PG37) and a novel piscine genotype (2 isolates: silver biddy

162 GO18 and GO55), hereafter referred to as piscine genotype 8 at the 18S rRNA locus (Table 3).

163 The two piscine genotype 8 isolates from silver biddies were genetically identical to each other,

164 but distinct from all isolates previously characterised at the 18S rRNA locus (Table 4).

165 Neighbour-joining, parsimony and maximum likelihood analysis produced similar results and

166 indicated that piscine 8 genotype clustered most closely with the piscine 3 genotype from a sea

167 mullet, while the other five isolates regularly clustered with *C. parvum* (85 % bootstrap support)

168 (Fig. 2). Sequences were also obtained for the two piscine genotype 8 isolates at the actin locus.

169 At this locus, sequence information was only available for *C. molnari* and piscine genotype 1170 and piscine genotype 8 grouped more closely with *C. molnari* genotypes (7.3 % - 8.5 % genetic

171 differences) (Fig. 3 and Table 4).

172 At the *gp60* locus, three subtypes belonging to *C. parvum* (family IIa: IIaA14G2R1,173 IIaA15G2R1, IIaA19G4R1) and a *C. hominis* subtype (family Id: IdA15G1) were identified

174 (Table 3).

175

176

3.3 Microscopy

177 No parasites were observed during microscopic examination of the intestinal or stomach tissues

178 due to substantial autolysis of tissues.

179

180

3.4 Nucleotide sequence accession numbers

181 The unique partial 18S rRNA and actin sequences of piscine genotype 8 from silver biddies were

182 deposited in the GenBank database under the accession numbers KC807985 to KC807988.

183

184

4. Discussion

185 In the present study, the overall prevalence of *Cryptosporidium* sp. was low (1.14 %, 7/614).

186 Previous studies have also reported low prevalences in similar groups of fish (0.8 %, 6/709)

187 (Reid et al., 2010) and in ornamental fish (3.5 %, 6/171) (Morine et al., 2012) while others have

188 reported higher prevalences (10 - 100 %) mostly among juvenile fish (Alvarez-Pellitero et al.,

189 2004; Sitja-Bobadilla et al., 2005; Murphy et al., 2009; Zanguee et al., 2010).

190 This study identified three new fish hosts for *Cryptosporidium*; silver barb (*P. gonionotus*),
191 mackerel scad (*D. maracellus*) and oblong silver biddy (*G. oblongus*). The fourth host Nile
192 tilapia (*O. niloticus*) could also be a new host since previous studies have detected
193 *Cryptosporidium* in the same genus but did not identified the species (Landsberg and Paperna,
194 1986; Paperna and Vilenkin, 1996).

195 No oocysts or life cycle stages were observed in the infected fish hosts in the present study due
196 to substantial autolysis of tissues, which has been reported as an issue for *Cryptosporidium*
197 detection in piscine hosts (Zanguee et al., 2010). Fish are known to have a very rapid rate of
198 tissue autolysis compared to homeotherms (Roberts, 2012) and many of the fish were dead for
199 up to 4 hours prior to being processed which contributed to the problem. Previous studies have
200 provided histological and electron microscopic evidence of considerable cellular damage
201 associated with several *Cryptosporidium* species/genotypes that infect fish; *C. molnari* in the
202 stomach of fingerlings and juveniles of gilt-head sea bream (Alvarez-Pellitero and Sitja-
203 Bobadilla, 2002), piscine genotype 1 in the stomach of a guppy (Ryan et al., 2004) and piscine
204 genotype 3 in the intestine of a mullet (Reid et al., 2010). Piscine genotype 2 was associated with
205 gastric infections in angelfish, with the greatest morbidity and mortality seen in larval and
206 juvenile fish (Murphy et al., 2009). Whether the *C. parvum* and *C. hominis* identified in the
207 present study represents actual or mechanical infections remains to be determined as the oocysts
208 may have been passing through rather than infecting these fish and further research using
209 histological analysis of rapidly preserved tissue specimen is required to confirm this.

210 The zoonotic *Cryptosporidium* genotypes identified in this study are of significance to public
211 health. *Cryptosporidium parvum* subtypes IIaA14G2R1, IIaA15G2R1 and IIaA19G4R1 were
212 found in cultured freshwater (Nile tilapia), wild freshwater (silver barb) and a marine (mackerel
213 scad) fish. The zoonotic *C. parvum* IIa subtype family has predominantly been found in calves
214 and in humans in North America, Europe and Australia (Xiao, 2010). Only one study has
215 previously detected zoonotic *C. parvum* (subtype IIaA18G3R1) in a marine fish (Reid et al.,
216 2010). The presence of IIa subtypes in the fish samples from the present study could be due to
217 waterborne contamination with human and animal waste. Human sewage management systems
218 in PNG villages typically involve dugout toilets near the homes and toilets built over the rivers
219 or seas. No cattle farms were seen in close vicinity to where the samples were collected, whereas
220 dugout toilets, companion animals and/or domesticated poultry and pigs were seen in the
221 environment. Fish ponds, rivers and seas could be contaminated from rainwater runoff and from
222 humans and/or animals bathing in them. A previous study has detected antibodies against
223 *Cryptosporidium* among children from PNG (Groves et al., 1994), however, no molecular work
224 has been done to confirm the species or genotypes present.

225 One marine fish (mackerel scad DM18) was identified as *C. parvum* at the 18S locus while at the
226 *gp60* locus it was subtyped as *C. hominis* IdA15G1R1, indicating that a mixed *C. parvum/C.*
227 *hominis* infection was present. This is the first report of *C. hominis* in fish. The only other
228 marine organism in which *C. hominis* has been reported was a dugong (*Dugong dugon*) (Morgan
229 et al., 2000), and its presence probably reflects human sewage contamination of the water.

230 The novel piscine genotype 8 was identified in two marine silver biddies. At the 18S locus,
231 piscine genotype 8 exhibited 4.3 % genetic difference with piscine genotype 3 and 13.8 % - 17.7
232 % genetic difference with other *Cryptosporidium* spp. (Table 4). At the actin locus, piscine
233 genotype 8 exhibited 7.3 % genetic difference with *C. molnari* 2 and 19.0 % - 21.4 % with other
234 *Cryptosporidium* spp. (Table 4). Based on the differences in the genetic sequences, piscine
235 genotype 8 is unique and may represent a new species; however, further research is required to
236 confirm this.

237 The present study identified zoonotic *C. parvum* subtypes in fish species, which are frequently
238 eaten in PNG. Previous studies have not identified conclusive evidence for transmission of
239 *Cryptosporidium* from fish to humans but one study reported that urban anglers are at a risk for

240 contracting cryptosporidiosis from exposures received while fishing and consuming caught fish
241 (mean probability of infection was nearly one) (Roberts et al., 2007). It is therefore essential that
242 fish for human consumption are handled appropriately to avoid contamination. In addition, a
243 novel *Cryptosporidium* sp. (piscine genotype 8) was identified based on molecular data but lacks
244 histological details of infection or morphological features of the oocysts, thus future work is
245 required to establish this genotype as a species.

246

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254

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334 **Table captions**335 Table 1: Species/genotypes of *Cryptosporidium* reported in fish in previous studies.336 Table 2: Cultured freshwater, wild freshwater and marine fish species collected in the present
337 study.338 Table 3: Species, genotypes and subtypes of *Cryptosporidium* identified in fish in the present
339 study. Sample DM18 was typed as *C. parvum* at the 18S locus but at *gp60* locus it was typed as
340 *C. hominis* (IdA15G1) indicating the presence of mixed *C. parvum*/*C. hominis* in this sample.

341 Table 4: Percentage of genetic differences between piscine genotype 8 and other

342 *Cryptosporidium* species/genotypes at the 18S rRNA and actin loci.

343 **Figure Legends**

344 **Figure 1. Map of the study sites in Papua New Guinea.** Cultured freshwater fish were
345 obtained from Bathem, Kundiawa, Asaro and Mumeng; wild freshwater fish were collected in
346 the Ramu River near Sausi and the Sepik River near Pagwi and marine fish were collected from
347 Bilbil, Madang, Pilapila and Tavana.

348 **Figure 2. Evolutionary relationships of *Cryptosporidium* piscine-derived isolates inferred
349 by neighbour-joining analysis of *p* distances calculated from pairwise comparison of 18S
350 rRNA sequences.** The percentage of replicate trees in which associated taxa clustered together
351 in the bootstrap test (1000 replicates), are shown at the internal nodes (>50% only) for distance,
352 ML and parsimony (n.s. = not supported). Accession numbers are given in parentheses. Isolates
353 from the present study are marked with asterisk (*).

354 **Figure 3. Phylogenetic relationships of *Cryptosporidium* isolates inferred by neighbour-
355 joining analysis of the actin gene based on genetic distances calculated by the *p* distance
356 model.** The percentage of replicate trees in which associated taxa clustered together in the
357 bootstrap test (1000 replicates), are shown at the internal nodes (>50% only) for distance, ML
358 and parsimony (n.s. = not supported). Accession numbers are given in parentheses. Isolates from
359 the present study are marked with asterisk (*).

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360 **Table 1:** Species/genotypes of *Cryptosporidium* reported in fish in previous studies (N = the
 361 number of specimens in which each species/genotype of *Cryptosporidium* was identified.

Species/genotype	N	Fish Host	Reference
<i>C. molnari</i>	>100	Gilthead sea bream and European seabass	Alvarez-Pellitero and Sitja-Bobadilla, 2002; Palenzuela et al., 2010
<i>C. scophthalmi</i>	49	Turbot	Alvarez-Pellitero and Sitja-Bobadilla, 2002; Alvarez-Pellitero et al., 2004
<i>C. molnari</i> -like	7	Butter bream, madder seaperch, bristle tooth tang, upsidedown catfish, wedgetailed blue tang and green chromas, golden algae eater	Zanguee et al., 2010
Piscine genotype 1	2	Guppy and neon tetra	Ryan et al., 2004; Zanguee et al., 2010
Piscine genotype 2	>5	Angelfish, neon tetra and Oscar fish	Murphy et al., 2009; Zanguee et al., 2010
Piscine genotype 3	2	Sea mullet	Reid et al., 2010
Piscine genotype 4	4	Golden algae eater, kupang damsel, Oscar fish and neon tetra	Zanguee et al., 2010; Morine et al., 2012
Piscine genotype 5	3	Angelfish, butter bream and golden algae eater	Zanguee et al., 2010
Piscine genotype 6	1	Guppy	Zanguee et al., 2010
Piscine genotype 6-like	1	Gold gourami	Morine et al., 2012
Piscine genotype 7	3	Red-eye tetra	Morine et al., 2012
Rat genotype 3-like		Goldfish	Morine et al., 2012
<i>C. scrofarum</i>	2	School whiting	Reid et al., 2010
<i>C. parvum</i>	1	School whiting	Reid et al., 2010
<i>C. xiaoi</i>	1	School whiting	Reid et al., 2010

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363 **Table 2:** Cultured freshwater, wild freshwater and marine fish species collected in the present
 364 study.
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Cultured freshwater fish	Locations				
	Kundiawa	Asaro	Mumeng	Bathem	Total
Nile tilapia (<i>Oreochromis niloticus</i>)	36	20	27	-	83
Common carp (<i>Cyprinus carpio</i>)	-	11	32	-	43
Mozambique tilapia (<i>Oreochromis massambic</i>)	-	-	-	7	7
Total	36	31	59	7	133
Wild freshwater fish	Ramu River	Sepik River	Total		
Silver barb (<i>Puntius gonionotus</i>)	13	39	52		
Highfin catfish (<i>Neoarius berneyi</i>)	-	20	20		
Mozambique tilapia (<i>Oreochromis massambic</i>)	-	15	15		
Pacu (<i>Colossoma bidens</i>)	-	34	34		
Indo-Pacific trapon (<i>Megalops cyprinoides</i>)	-	70	70		
Redtail catfish (<i>Phractocephalus hemiliopterus</i>)	-	14	14		
Total	12	192	205		
Wild marine fish	Bilbil	Madang	Pilapila	Tavana	Total
Bigeye scad (<i>Selar crumenophthalmus</i>)	46	-	60	-	106
Bigeye trevally (<i>Caranx sexfasciatus</i>)	4	-	-	-	4
Blackfin barracuda (<i>Sphyræna qenie</i>)	9	-	-	-	9
Coachwhip trevally (<i>Carangoides oblongus</i>)	-	-	-	1	1
Indo-Pacific trapon (<i>Megalops cyprinoides</i>)	4	-	-	-	4
Mackerel scad (<i>Decapterus macarellus</i>)	5	-	24	-	29
Oblong silver biddy (<i>Gerres oblongus</i>)	1	-	-	54	55
Oriental bonito (<i>Sarda orientalis</i>)	-	4	-	-	4
Pinjalo snapper (<i>Pinjalo pinjalo</i>)	-	-	1	-	1
Rainbow runner (<i>Elagatis bipinnulatus</i>)	-	5	-	-	5
Reef needlefish (<i>Strongylura incisa</i>)	-	4	-	-	4
Slender pinjalo (<i>Pinjalo lewisi</i>)	-	-	-	14	14
Spanish mackerel (<i>Scomberomous maculatus</i>)	-	3	-	-	3
Talang queenfish (<i>Scomberoides commersonianus</i>)	-	-	2	-	2
Wahoo (<i>Acanthocybium solandri</i>)	-	1	-	-	1
Yellow fin tuna (<i>Thunnus albacares</i>)	-	34	-	-	34
Total	69	51	87	69	276

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367 **Table 3:** Species, genotypes and subtypes of *Cryptosporidium* identified in fish in the present
 368 study. Sample DM18 was typed as *C. parvum* at the 18S locus but at *gp60* locus it was typed as
 369 *C. hominis* (IdA15G1) indicating the presence of mixed *C. parvum*/*C. hominis* in this sample.
 370

Sample	Host species	Group	18S	<i>gp60</i>	Actin
ON36	Nile tilapia	Cultured	<i>C. parvum</i>	IlaA19G4R1	-
ON68	Nile tilapia	Cultured	<i>C. parvum</i>	IlaA14G2R1	-
PG37	Silver barb	Wild freshwater	<i>C. parvum</i>	IlaA19G4R1	-
DM17	Mackerel scad	Wild marine	<i>C. parvum</i>	IlaA15G2R1	-
DM18	Mackerel scad	Wild marine	<i>C. parvum</i> ,	IdA15G1	-
GO18	Silver biddy	Wild marine	Piscine genotype 8	-	Piscine genotype 8
GO55	Silver biddy	Wild marine	Piscine genotype 8	-	Piscine genotype 8

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372 **Table 4:** Percentage of genetic differences between piscine genotype 8 and other
 373 *Cryptosporidium* species/genotypes at the 18S rRNA and actin loci.

	18S locus	Actin locus
Piscine genotype 1	13.0	17.4
Piscine genotype 2	5.1	Not analysed*
Piscine genotype 3	4.3	Not analysed*
Piscine genotype 4	6.3	Not analysed*
Piscine genotype 5	5.5	Not analysed*
Piscine genotype 6	13.0	Not analysed*
Piscine genotype 7	11.8	Not analysed*
<i>C. molnari</i>	10.8	7.3-8.5
<i>C. parvum</i>	15.4	21.4
<i>C. hominis</i>	15.4	21.4
<i>C. bovis</i>	17.7	19.0
<i>C. baileyi</i>	14.7	19.4
<i>C. muris</i>	15.0	19.4
<i>C. andersoni</i>	13.8	20.1

374 *Actin sequences were not available for these genotypes.
 375





