A new avian Cryptosporidium *genotype in a 1-month-old caged brown wood owl (*Strix leptogrammica*) with severe dehydration and diarrhea*

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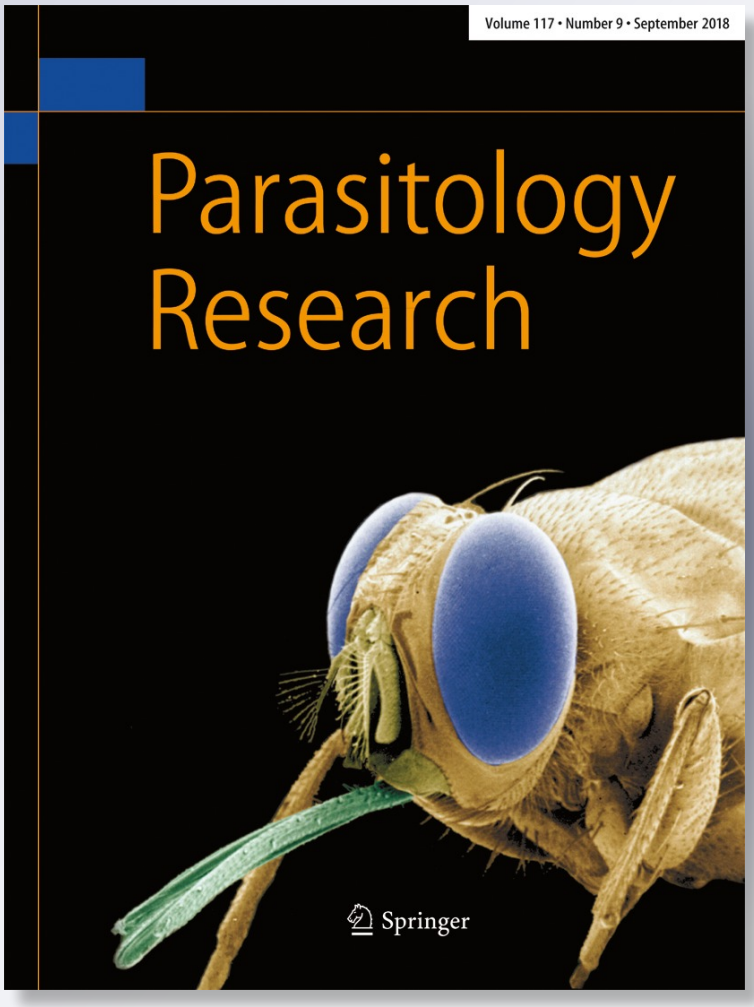

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SHORT COMMUNICATION

A new avian *Cryptosporidium* genotype in a 1-month-old caged brown wood owl (*Strix leptogrammica*) with severe dehydration and diarrhea

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Abstract

A 1-month-old brown wood owlet (*Strix leptogrammica*) purchased from a wholesaler and housed as a companion bird by an individual owner in Japan showed severe dehydration and anorexia following a week of vomiting and severe diarrhea. A great number of approximately 5 × 4-μm-sized *Cryptosporidium* oocysts were found in the feces by microscopy. The owlet was administered subcutaneous fluid and intragastric tube feeding for 2 weeks, resulting in improvement of the condition with a decreased number of oocysts in the feces. At days 51 and 119, no oocysts were found in the feces by microscope and PCR detection. These results suggested that this parasite was a possible agent of severe diarrhea in the affected bird. Molecular analysis of DNA extracted from oocysts based on the 18SrRNA loci identified *C. avium*; however, analysis of actin and *hsp* (heat shock protein) genes identified a novel genotype indicating a mixed infection with *C. avium* and a novel genotype.

Keywords *Cryptosporidium* · *Strix leptogrammica* · New genotype · Clinical signs

Introduction

Many avian *Cryptosporidium* spp. isolated from caged birds have been reported worldwide, including four species (*C. meleagridis*, *C. baileyi*, *C. galli*, and *C. avium*) and 12 genotypes (Ng et al. 2006; Nakamura et al. 2009, 2014; Nakamura & Meireles 2015; Holubová et al. 2016; Chelladurai et al. 2016). In Japan, *C. meleagridis*, *C. galli*., *C. baileyi*, *C. avium*, and avian genotype III have been identified by using multilocus sequence analysis of the isolates from caged birds such as cockatiels (*Nymphicus hollandicus*), peach-faced lovebirds (*Agapornis roseicollis*), budgerigars (*Melopsittacus undulatus*), Pacific parrotlets (*Forpus coelestis*), masked lovebirds (*Agapornis personata*), and Java sparrows (*Padda oryzivora*) (Abe & Iseki 2004; Abe & Makino 2010; Abe et al. 2015, 2016; Makino et al. 2010). Some avian *Cryptosporidium* species and genotypes are recognized to be pathogenic and cause clinical signs such as diarrhea, weight loss, chronic vomiting, emaciation, chronic weight loss, and lethargy (Abe et al. 2010, 2015, 2016; Makino et al. 2010), and *C. meleagridis* is known to be zoonotic (Ryan 2010).

In owls (family Strigidae), only two cases of cryptosporidiosis have been reported: *C. baileyi* in a snowy owl (*Bubo scandiacus*) with proventriculitis in a zoo in Japan (Nakagun et al. 2017) and *C. baileyi* in 16 young otus owls (*Otus scops*) with ocular and respiratory diseases in a raptor rehabilitation center in Spain (Molina-Lopez et al. 2010). In the present study, we detected *Cryptosporidium* oocysts in the feces of a privately owned caged domestic owlet, which exhibited gastrointestinal symptoms and performed an anti-parasitic treatment. During the treatment, we observed a reduction and eventual disappearance of oocysts in fecal samples and via genetic detection. To determine possible infection routes, we also examined fecal samples from 19 owls purchased from the same wholesaler.

Clinical history and therapy

A 1-month-old brown wood owlet was brought to the Fujisawa Avian Clinic in Kanagawa Prefecture, Japan. The owlet showed severe dehydration and anorexia following a week of vomiting and severe diarrhea on April 3, 2016. The

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owlet had been hatched in an artificial incubator on a raptor breeding farm in Japan with other owl species and was subsequently moved to an owl's wholesaler with other owlets and raised until the age of 3 weeks. A few days after the owl was sold to a private individual, the owlet's appetite gradually decreased, and it began to vomit. The owlet had been fed defrosted adult Japanese quails (with the gastrointestinal tract removed), which were stored in the −4 °C freezer, with multivitamins and calcium twice a day, and offered tap water ad libitum while at the wholesaler. After the owlet was admitted to the clinic, it received supportive care including 10–20 ml fluids (Ringer's solution) via subcutaneous injection and was treated via an intragastric tube with a high-calorie nutrient paste, including berberine chloride as an antidiarrheal agent. The number of oocysts per gram (OPG) was counted as previously described (Abbassi et al. 2000) and the body weight was also measured during each treatment visit to the clinic.

Progress after treatment

At the initial physical examination, the owlet had a body weight of 251 g and sunken eyes and ataxia were observed. Numerous oocysts were identified as bright pink structures by Sheather's sucrose flotation method (specific gravity of 1.2) in the feces under light microscopy, showing the mean size of 20 oocysts as 5.4 × 4.13 μm. The owlet's body weight and numbers of OPG were continuously examined until day 119 of treatment. On day 3, the owlet's condition noticeably improved, and its body weight increased. Thirteen days after treatment was completed, no sign of diarrhea was observed, and the owlet was discharged. After treatment, the owlet showed signs of improvement of the observed clinical signs and weight gain with a reduction in numbers of OPG. On day 51, no oocysts were found in the feces microscopically and *Cryptosporidium* DNA was not amplified (Fig. 1).

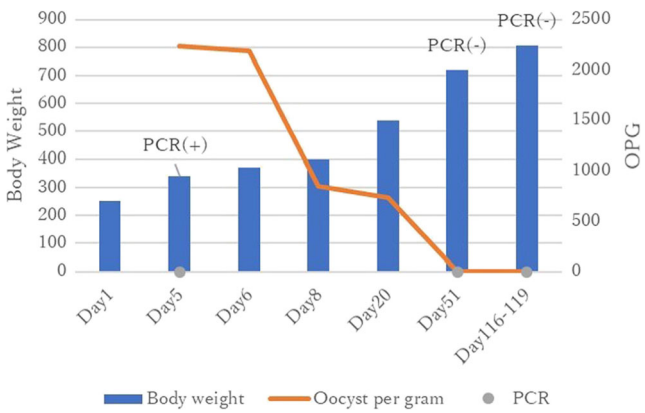

Fig. 1 Changes in body weight gain and oocysts per gram over time

Identification of a new *Cryptosporidium* genotype in the brown wood owlet

Oocysts from the brown wood owlet were analyzed by molecular biological methods as described previously (Sulaiman et al. 2002; Abe et al. 2004, 2010, 2015, 2016). Briefly, extracted DNA was designated for amplification of the 18S rRNA, *hsp* (heat shock protein), and actin gene regions of *Cryptosporidium* and amplified fragments were sequenced by using the ABI 3130 automated sequencer (Applied Biosystems, Foster, CA, USA). A phylogenetic tree was constructed using the neighbor-joining method, based on evolutionary distances calculated from Tamura-Nei distance estimates, with 1000 bootstrap sampling and drawn by NJ plot software (http://pbil.univ-lyon1.fr/software/njplot.html). The obtained partial 18S rRNA, actin gene, and *hsp* sequences were deposited in the International Nucleotide Sequence Database (GenBank/DDBJ/EMBL) under accession numbers LC310795, LC310796, and LC310797, respectively.

Partial sequences of the 18S, actin, and *hsp* loci were successfully amplified. Phylogenetic analysis of the 18S locus identified the isolate as *C. avium* (Fig. 2); however, at the actin and *hsp* locus, a novel genotype was identified that grouped separately from *C. avium* and avian genotype II (Figs. 3 and 4).

Identification of the new *Cryptosporidium* genotype in a spotted wood owl

Fecal samples from another 19 owls kept by the same wholesaler (from which the studied brown wood owlet had been purchased) were analyzed by PCR. These included an adult great gray owl (*Strix nebulosi*), a spotted wood owl (*Strix seloputo*), a barn owl (*Tyto alba*), a brown wood owl, a young Malay eagle owl (*Bubo sumatranus*), a cape eagle owl (*Bubo capensis*), five Eurasian eagle owls (*Bubo bubo*), two rock eagle owls (*Bubo bengalensis*), and six spotted eagle owls (*Bubo africanus*). Adult owls stayed on individual perches and owlets were placed into separated containers. The only other birds kept there were raptors. They were also fed defrosted quails.

Cryptosporidium was detected in the fecal sample of a 3-month-old spotted wood owl by PCR and sequence analysis revealed that it was identical to that from the studied brown wood owlet. However, the 3-month-old spotted wood owl exhibited no clinical signs.

Discussion

In the present case, we detected the same *Cryptosporidium* genotype analyzed at three loci from the both the original sample from the 1-month-old brown wood owlet and from a

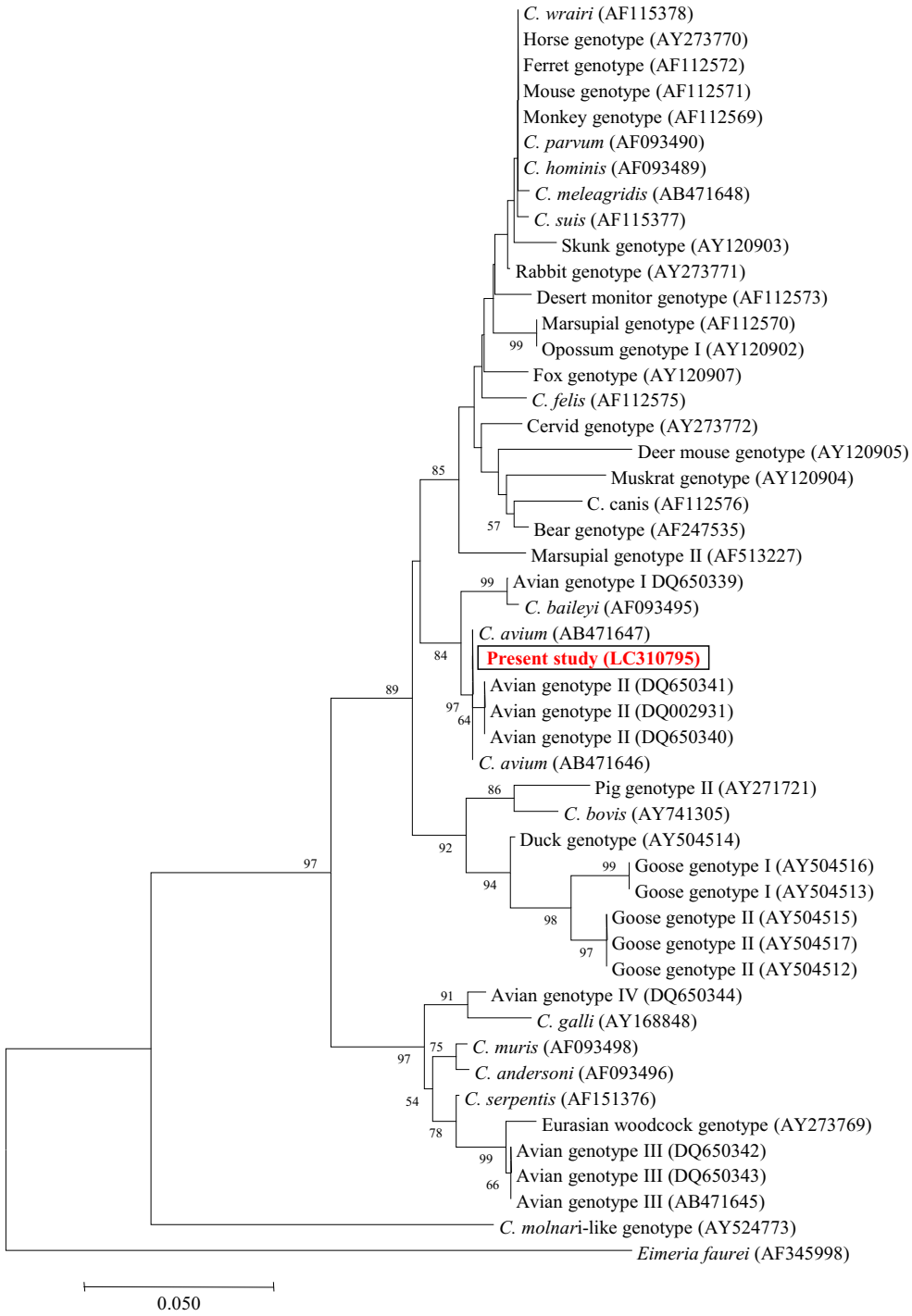

Fig. 2 Phylogenetic analysis of the detected lineage of the isolate obtained from the brown wood owlet in this study, constructed from 18SrRNA

3-month-old spotted wood owl from the same wholesaler, suggesting that both owlets were infected at the breeder or the wholesaler. Both were artificially hatched at the breeders and then moved to the wholesaler. The brown wood owlet was then sold to an individual household, where no domestic animals were kept and no contact could occur with wild birds. Clinical signs were not observed in the 3-month-old spotted wood owl kept by the same wholesaler, which may have been due to its more mature immune system. Avian cryptosporidiosis has been reported in chicks or young birds in Japan (Abe et al. 2004, 2010, 2015, 2016) except in birds infected with avian genotype III which may be a possible agent of chronic infections (Makino et al. 2010). Given that keeping owls as companion birds has become popular in Japan, cases of avian cryptosporidiosis may increase in the future.

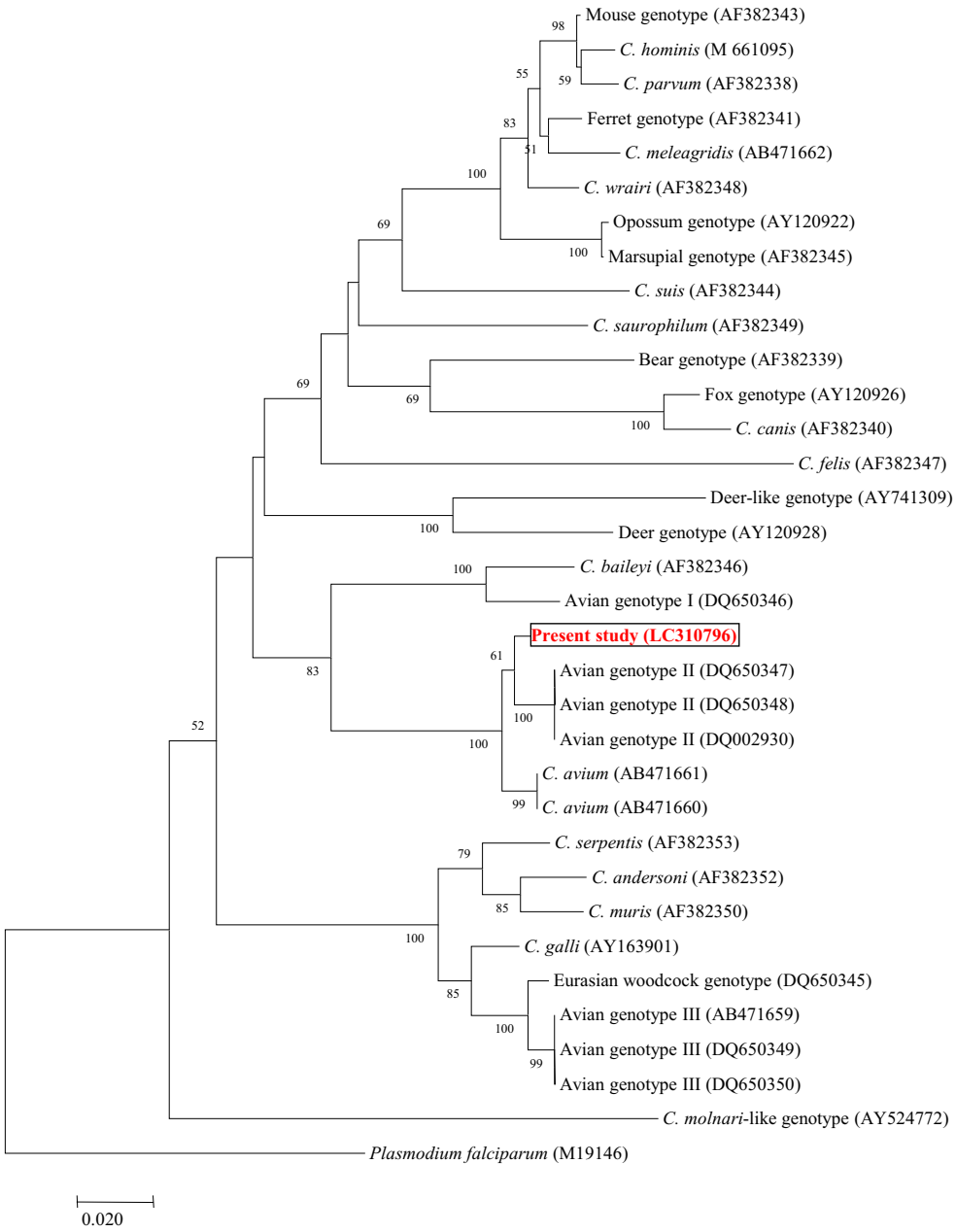

Fig. 3 Phylogenetic analysis of the detected lineage of the isolate obtained from the brown wood owlet in this study, constructed from actin gene

In addition to diarrhea, enteric *Cryptosporidium* infection in birds causes mucosal thickening, excessive mucus production, and a lymphoplasmacytic and histiocytic inflammatory response (Schmidt et al. 2015). Co-infections with other pathogens such as *Candida* spp., *Escherichia coli*, *Salmonella* spp., and many viruses may exacerbate signs of diarrhea. Identification of probable etiological agents in this clinical case was difficult because we were unable to examine potential agents of diarrhea. Sequence and phylogenetic analysis from both owls the 18S locus identified *C. avium*; however, analysis of actin and *hsp* loci identified a novel genotype that grouped with avian genotype II but was genetically distinct. Further studies are required to better characterize the novel genotype. Next-generation sequencing because of its ability to sequence all reads in an amplicon would be useful for clarifying the extent of the mixed infection. Some *Cryptosporidium* species associated with clinical signs and are zoonotic (Qi et al. 2011; Ryan et al. 2014); however, it is still unclear whether the novel genotype can infect humans and other animals but it did group with other intestinal *Cryptosporidium* species. Further studies are necessary to clarify the zoonotic and pathogenic potential of the novel *Cryptosporidium* genotype. The original source or reservoir of this avian intestinal protozoan in Japan is unknown. It is therefore important to determine the distribution of avian *Cryptosporidium* among caged companion birds, especially

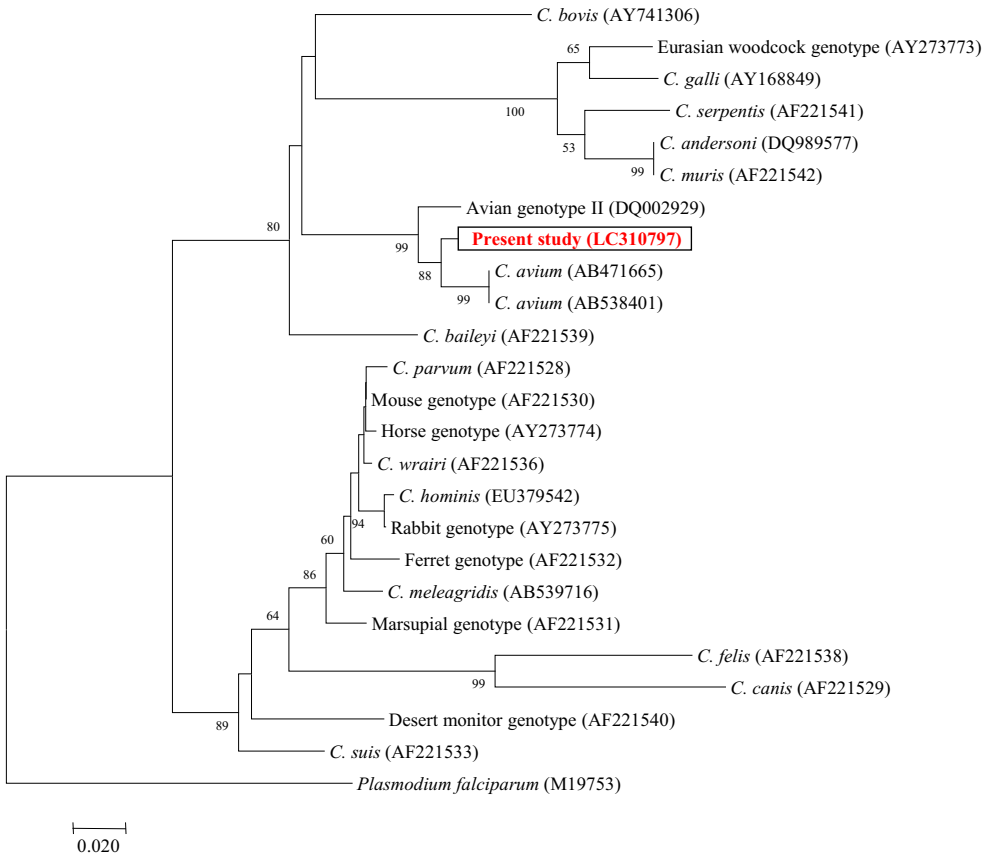

Fig. 4 Phylogenetic analysis of the detected lineage of the isolate obtained from the brown wood owlet in this study, constructed from *hsp* gene

those kept by wholesalers in Japan, to prevent the dispersal of pathogenic protozoa.

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