



RESEARCH REPOSITORY

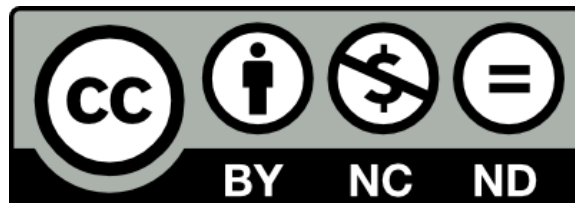
This is the author's final version of the work, as accepted for publication following peer review but without the publisher's layout or pagination.

The definitive version is available at:

<https://doi.org/10.1016/j.vetpar.2017.08.014>

Zahedi, A., Durmic, Z., Gofton, A.W., Kueh, S., Austen, J., Lawson, M., Callahan, L., Jardine, J. and Ryan, U. (2017) *Cryptosporidium homai* n. sp. (Apicomplexa: Cryptosporididae) from the guinea pig (*Cavia porcellus*). *Veterinary Parasitology*, 245. pp. 92-101.

<http://researchrepository.murdoch.edu.au/38537/>



Copyright © 2017 Elsevier B.V.

Accepted Manuscript

Title: *Cryptosporidium homai* n. sp. (Apicomplexa: Cryptosporidii) from the guinea pig (*Cavia porcellus*)

Authors: Alireza Zahedi, Zoey Durmic, Alexander W. Gofton, Susan Kueh, Jill Austen, Malcolm Lawson, Lauren Callahan, John Jardine, Una Ryan



PII: S0304-4017(17)30363-1
DOI: <http://dx.doi.org/10.1016/j.vetpar.2017.08.014>
Reference: VETPAR 8446

To appear in: *Veterinary Parasitology*

Received date: 19-7-2017
Revised date: 17-8-2017
Accepted date: 18-8-2017

Please cite this article as: Zahedi, Alireza, Durmic, Zoey, Gofton, Alexander W., Kueh, Susan, Austen, Jill, Lawson, Malcolm, Callahan, Lauren, Jardine, John, Ryan, Una, *Cryptosporidium homai* n.sp.(Apicomplexa: Cryptosporidii) from the guinea pig (*Cavia porcellus*). *Veterinary Parasitology* <http://dx.doi.org/10.1016/j.vetpar.2017.08.014>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

***Cryptosporidium homai* n. sp. (Apicomplexa: Cryptosporididae) from the guinea pig (*Cavia porcellus*)**

Alireza Zahedi^a, Zoey Durmic^b, Alexander W. Gofton^a, Susan Kueh^a, Jill Austen^a, Malcolm Lawson^c, Lauren Callahan^c, John Jardine^d, Una Ryan^{a*}.

^aSchool of Veterinary and Life Sciences, Murdoch University, Perth, Australia

^bSchool of Agriculture and Environment, The University of Western Australia, Perth, Australia

^cAnimal Care Services, The University of Western Australia, Perth, Australia

^dVetpath Laboratory Services, Perth, Australia

*Corresponding author: Una Ryan

Phone: 08 9360 2482

Fax: 08 9310 4144

E-mail: Una.Ryan@murdoch.edu.au

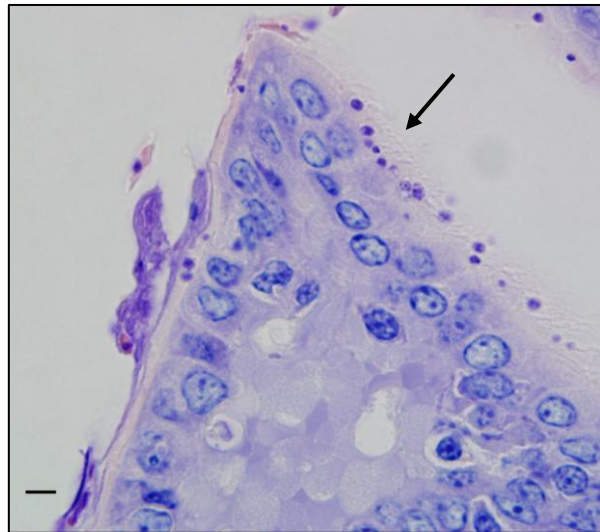
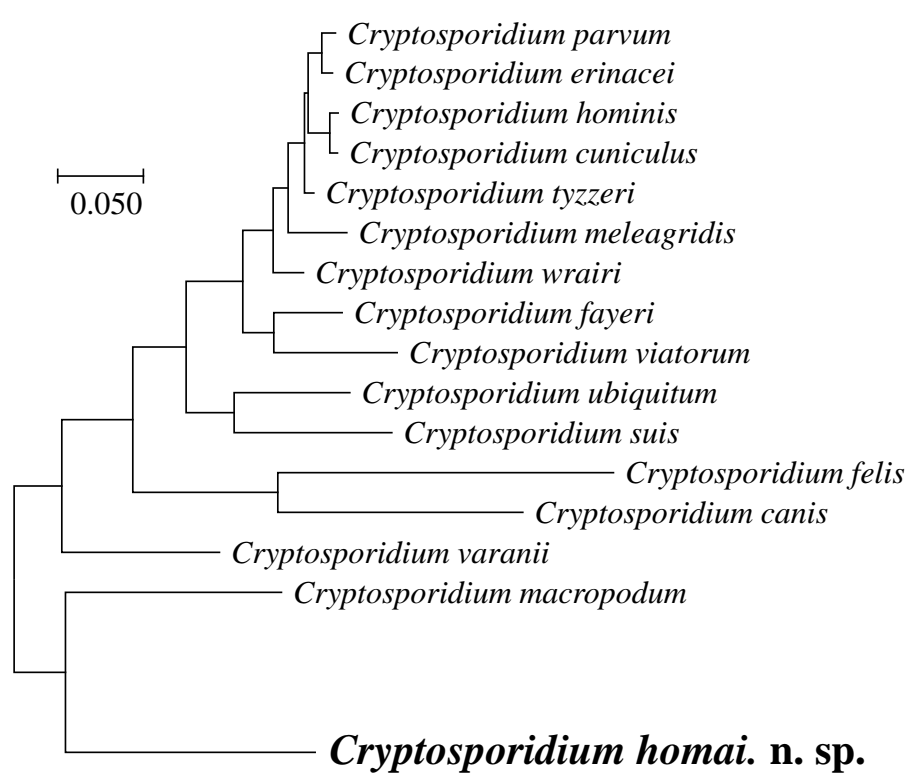
Highlights:

- Morphological and molecular characterisation of a new *Cryptosporidium* species.
- Phylogenetic relationships of *C. homai* n. sp.
- Histological analysis of the small intestine of the infected host.

ABSTRACT

The morphological, biological, and molecular characterisation of a new *Cryptosporidium* species from the guinea pig (*Cavia porcellus*) are described, and the species name *Cryptosporidium homai* n. sp. is proposed. Histological analysis conducted on a *post-mortem* sample from a guinea pig euthanised due to respiratory distress, identified developmental stages of *C. homai* n. sp. (trophozoites and meronts) along the intestinal epithelium. Molecular analysis at 18S rRNA (18S), actin and *hsp70* loci was then conducted on faeces from an additional 7 guinea pigs positive for *C. homai* n. sp. At the 18S, actin and *hsp70* loci, *C. homai* n. sp. exhibited genetic distances ranging from 3.1% to 14.3%, 14.4% to 24.5%, and 6.6% to 20.9% from other *Cryptosporidium* spp., respectively. At the 18S locus, *C.*

Graphical Abstract



homai n. sp. shared 99.1% similarity with a previously described *Cryptosporidium* genotype in guinea pigs from Brazil and it is likely that they are the same species, however this cannot be confirmed as actin and *hsp70* sequences from the Brazilian guinea pig genotype are not available. Phylogenetic analysis of concatenated 18S, actin and *hsp70* sequences showed that *C. homai* n. sp. exhibited 9.1% to 17.3% genetic distance from all other *Cryptosporidium* spp. This clearly supports the validity of *C. homai* n. sp. as a separate species.

Keywords: *Cryptosporidium homai*; guinea pig, 18S; actin; *hsp70*

1. Introduction

Cryptosporidium spp. are protozoan parasites responsible for gastroenteritis in a wide range of vertebrates including humans, domestic and wild animals and are a common cause of waterborne outbreaks worldwide (Zahedi et al., 2016; Ryan et al., 2016; Efstratiou et al., 2017). The parasite is transmitted via the faecal-oral route with both zoonotic and anthroponotic transmission cycles (Ryan et al., 2014). Currently relatively little is known about the molecular characteristics, host specificity, pathogenicity and zoonotic importance of *Cryptosporidium* spp. in wild and domestic rodents (Appelbee et al., 2005; Ziegler et al., 2007a, b; Ryan et al., 2014; Kváč et al., 2016; Li et al., 2016). To date, of the 33 recognised *Cryptosporidium* spp. (cf. Ryan et al. 2016; Jezkova et al., 2016), 11 species including *C. proliferans*, *C. meleagridis*, *C. tyzzeri*, *C. andersoni*, *C. ubiquitum*, *C. wrairi*, *C. parvum*, *C. suis*, *C. meleagridis*, *C. muris* and *C. rubeyi* and over 20 genotypes of unknown species status have been reported in rodents with a prevalence ranging from 1% to 63% (Table 1) (Qi et al., 2015; Song et al., 2015; Stenger et al., 2015; Zahedi et al., 2016; Li et al., 2016).

The guinea pig (*Cavia porcellus*) is one of eight species in the genus *Cavia* (Rodentia: Caviidae), and is endemic to South America. Based on available archaeological and molecular data, it has been living in the region since the Miocene-Pliocene boundary, and it has been suggested that *C. porcellus* was initially derived from *Cavia tschudii*, when the

Amerindia peoples of Peru started to domesticate guinea pigs 4500 to 7000 years ago. Eventually, the utility of the domesticated form of guinea pig as a food source or pet and laboratory animal, has resulted in its worldwide distribution including Australia (Dunnum and Salazar-Bravo, 2009).

Currently, *C. wairi* is the only valid *Cryptosporidium* spp. described in guinea pigs (*Cavia porcellus*), with strong host specificity and no reports of human infection (Vetterling et al., 1971; Chrisp et al., 1990; Spano et al., 1997; Lv et al., 2009, Gressler et al., 2010; Smith et al., 2010). Previous experimental infections indicated that *C. wairi* was infective to mice, lambs and calves, causing a sparse infection in ruminants, however as genotyping was not conducted, this cannot be confirmed (Angus et al., 1985; Chrisp et al., 1992).

The present study examined the morphological, biological and molecular characteristics of a *Cryptosporidium* sp. detected in the gastrointestinal tract and faeces of guinea pigs. Based on the collective data from the present study, the *Cryptosporidium* spp. detected in these guinea pigs is genetically and biologically distinct from all species of *Cryptosporidium* described previously, and we propose the species name *Cryptosporidium homai* n. sp. For clarity, we herein refer to this novel species by its proposed name.

2. Materials and methods

2.1. Source of sample and sample processing

A guinea pig, which was part of a group of experimental animals held at the University of Western Australia Animal Care Services, Perth, Australia, presented with audible respiratory distress (rattled breathing sounds, difficulty in breathing and chin coated in saliva) and as a result was euthanised. *Post-mortem* examination was performed and intestinal and lung sections were sent to a specialist veterinary laboratory for further histopathological examination, and during routine handling, individual faecal sample were collected and stored at 4°C until required. Further to the initial histopathology and molecular analysis, additional

faecal samples (n=28) were collected either from individual animals or pooled from animals kept in the same enclosure for further molecular analysis (Table 2).

2.2. Histopathology

Sections of intestinal tissue were fixed in 10mg/100 mL phosphate buffered formalin for at least 24 hrs, dehydrated in an ethanol-xylene series and embedded in paraffin wax. Two micrometer tissue sections were dewaxed in xylene, rehydrated in an ethanol series and stained by haematoxylin & eosin (H&E) or Giemsa. Giemsa stock solutions were made up with 0.75 g Giemsa powder, 65 ml methanol and 65 ml glycerol, and diluted 1:10 with tap water immediately prior to use. Giemsa stained tissue sections were acidified with 10% acetic acid for 15 sec, and placed in freshly diluted Giemsa stain, preheated in a microwave (Kambrook model 686LE, 1150W) on medium for 30 sec, followed by microwaving on low for 30 sec. Slides were rinsed in tap water followed by absolute ethanol, before permanent mounting in DPX (Dako).

2.3. DNA isolation

Following five cycles of freeze-thaw, genomic DNA was extracted from 250 mg of each faecal sample (n=29), using a Power Soil Kit (MO BIO, Carlsbad, California, USA) in accordance to the manufacturer's instructions. An extraction blank (no faecal sample) was used in each extraction group. Purified DNA was stored in -20°C prior to molecular analyses. DNA extraction and post-DNA extraction procedures were performed in separate dedicated laboratories.

2.4. PCR amplification

A nested PCR approach was used to amplify an approximately 825 bp 18S rRNA fragment using the primers SSU-F2 (5'-TTCTAGAGCTAATACATGCG-3') and SSU-R2 (5'-CCCATTTTCCTTCGAAACAGGA-3') for the primary PCR and SSU-F3 (5'-GGAAGGGTTGTATTTATTAGATAAAG-3') and SSU-R4 (5'-

AAGGAGTAAGGAACAACCTCCA-3') for the nested PCR (Xiao et al., 1999). Each 25 µl PCR mixture contained 1 µl of genomic DNA, 1x Go Taq PCR buffer (KAPA Biosystems, South Africa), 3.75 mM MgCl₂, 400 µM of each dNTPs, 0.4 µM of forward and reverse primers and 1 U Kapa DNA polymerase (Kapa Biosystems, South Africa). The PCR cycling conditions consisted of an initial denaturation step at 94°C for 3 min followed by 40 cycles of 94°C for 45 sec, 58°C for 90 sec, and 72°C for 1 min, followed by a final extension step at 72°C for 7 min. An approx. ~818bp fragment of the actin gene was amplified as previously described (Ng et al., 2006), with the following modifications; denaturation time was increased from 30 sec to 45 sec, annealing time from 20 sec to 30 sec and extension time from 40 sec to 1 min. PCR amplification of an approximately 325 bp fragment of the *hsp70* gene was performed using a nested PCR as previously described (Hong et al., 2014).

No-template and extraction reagent blank controls were included in every PCR run. Positive control DNA (*C. macropodum*) was also added to every run to validate the PCRs. PCR setup and DNA handling procedure were performed in separate physically contained PCR-hoods, and post-PCR procedures were performed in a separate laboratory.

2.5. Sequence and phylogenetic analysis

Nested PCR products were electrophoresed through 1% agarose gels, and DNA fragments of the expected size (bp) for the 18S, actin and *hsp70* assays were excised from the gels and purified for Sanger sequencing using an in-house filter tip method (Yang et al., 2013). Purified PCR products from all three assays, were sequenced independently in both directions using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instructions at 58°C, 58°C and 56°C annealing temperature for the 18S rRNA, actin and *hsp70* loci, respectively. Sanger sequencing chromatogram files were imported into Geneious Pro 8.1.6 (Kearse et al., 2012), and the nucleotide sequences of each gene was curated, analysed and aligned with reference

sequences from GenBank using Clustal W (<http://www.clustalw.genome.jp>). The most suitable nucleotide substitution model was assessed in MEGA7 (Kumar et al., 2015). Distance, Parsimony and Maximum Likelihood (ML) trees were constructed using MEGA version 7 (Kumar et al., 2015)). Bootstrap support for branching was based on 1000 replications. Sequences have been deposited in GenBank under the accession numbers MF499131- MF499151.

3. Results

3.1. Prevalence and histological analysis

In the present study, *C. homai* n. sp. was detected in 24.1% (7/29 -95% CI: 10.3% - 43.5%) of faecal samples collected from guinea pig enclosures by PCR and sequencing at 3 loci. Histological analysis of the small intestine of one guinea pig (isolate E89), indicated moderate to heavy epicellular infection of the intestinal epithelium by *Cryptosporidium*, associated with a minimal host inflammatory response. Trophozoites and meronts generally measured $< 5 \mu\text{m}$, as is typical for *Cryptosporidium*. There was a predominance of merogony with both type I and type II meronts present (Fig 1). The lamina propria was expanded by mild to moderate predominantly lymphocytic-plasmocytic inflammatory infiltrate with the occasional neutrophils, eosinophils and necrotic cells. An average of 1-2 and up to 3 mitotic figures were observed per intestinal crypt in 40-50% of crypts per high power field (40x objective). Mildly tortuous intestinal glands or crypts and the prominence of mitotic figures are suggestive of intestinal epithelial hyperplasia.

3.2. Sequence and phylogenetic analysis *C. homai* n. sp. at the 18S, actin and *hsp70* loci

Phylogenetic relationships were inferred by Distance, Parsimony and Maximum Likelihood (ML) analyses at 18S, actin and *hsp70* loci, based on 825, 818 and 325 bp of nucleotide sequences, respectively, and produced trees with mostly similar topologies with

some exceptions (Fig 2, 3, 4). An ML tree was also inferred from concatenated 18S, actin and *hsp70* sequences (Fig 5).

At the 18S locus, all *C. homai* n. sp. (n=7, which included 6 faecal samples and the intestinal sample, E89) were identical and grouped in a separate clade, sharing 99.1% identity with a novel genotype of *Cryptosporidium* spp. reported in guinea pigs (*C. procellus*) from Brazil (7 and 6 SNPs difference over 676 bp of submissions DQ885337 and DQ885338, respectively) (Huber et al., 2007). *C. homai* n. sp exhibited 3.1% genetic distance from the closest species, *C. felis*, 3.6% genetic distance from *C. wrairi*, and 3.2% (*C. suis*) to 15.4% (*C. scophthalmi*) genetic distance from all other *Cryptosporidium* spp.

At the actin locus, *C. homai* n. sp. again grouped separately and exhibited 14.4% genetic distance from the closest species, *C. varanii*, 18.4% genetic distance from *C. wrairi* and genetic distances ranging from 15.7% (*C. suis*) to 24.5% (*C. scophthalmi*) from all other *Cryptosporidium* spp.

Phylogenetic analysis of the *hsp70* gene, also confirmed the genetic distinctness of *C. homai* n. sp., where it exhibited 6.6% genetic distance from the closest species, *C. suis*, 7% genetic distance from *C. wrairi*, and 7.1% (*C. varanii*) to 20.9% (*C. serpentis*) genetic distance from all other *Cryptosporidium* spp.

An ML tree inferred from concatenated 18S, actin and *hsp70* sequences grouped *C. homai* n. sp. with *C. varanii* and *C. macropodum*, with 9.1% and 10% genetic distance respectively. Based on phylogenetic analysis using concatenated sequences, *C. homai* n. sp. exhibited 10.8% genetic distance from *C. wrairi*, the only valid *Cryptosporidium* sp. described in guinea pigs, and exhibited between 17.3% (*C. proliferans*) and 9.7 % (*C. suis*) genetic distance from all other *Cryptosporidium* spp.

3.3. Taxonomic summary and species description

Order: Cryptogregarida (Cavalier-Smith, 2014).

Family: Cryptosporidiidae

Species name: *C. homai* n. sp.

Type host: Guinea pigs (*Cavia porcellus*)

Other natural hosts: Unknown

Type locality: Perth, Western Australia

Site of infection: Intestine

Prepatent period: Unknown

Patent period: Unknown

Material deposited: partial sequences of 18S, actin and *hsp70* genes were submitted to GenBank under accession numbers MF499131-MF499151.

Etymology: This species is named *C. homai* n. sp. in honor of my late aunt, Ms. Homa Hoorfar.

4. Discussion

In the present study, post-mortem analysis of a guinea pig euthanised due to respiratory distress, identified an intestinal infection with a *Cryptosporidium* species, which on the basis of molecular analysis is a new species, named *C. homai* n. sp. The new species was detected in 24.1% of faecal samples from guinea pigs held at an experimental animal facility. The exact prevalence is difficult to determine as pooled faecal samples were obtained from enclosures, however the high prevalence is likely due to the close proximity of animals to each other, which would facilitate transmission. Very little is known about *Cryptosporidium* in guinea pigs. Surveys of pet guinea pigs in Italy (n=80) and Ecuador (n=40) failed to detect *Cryptosporidium* (d'Ovidio et al., 2015; Vasco et al., 2016). Another study in Brazilian guinea pigs (*Cavia aperea aperea*), detected *Cryptosporidium* in 3 of 5 faecal samples by microscopy, and *Cryptosporidium* was also detected in one guinea pig on a farm in the UK, but no genotyping was conducted in either study (Gressler et al., 2010; Smith et al, 2010).

Other studies have identified *C. wrairi* in guinea pigs (Lv et al., 2009; Feng et al., 2011), and until recently this was the only *Cryptosporidium* spp. identified in guinea pigs.

A previous study identified a novel *Cryptosporidium* genotype in guinea pigs (*C. procellus*) obtained from an indoor public market, in Rio de Janeiro, Brazil (Huber et al., 2007), which shared 99.1% similarity with *C. homai* n. sp. at the 18S locus (7 and 6 SNPs difference over 676 bp of submissions DQ885337 and DQ885338, respectively).

Phylogenetic analysis grouped them in a clade together with high bootstrap support, suggesting that they are likely the same species. Unfortunately, sequences at the actin and *hsp70* loci were unavailable for this genotype to confirm this.

Trophozoites and meronts of *C. homai* n. sp. measured $< 5 \mu\text{m}$, but oocysts were not observed. However, it is widely accepted that morphology is not a useful criterion for delimiting *Cryptosporidium* spp. (Fall et al., 2003). Phylogenetic analysis at the 18S, actin and *hsp70* loci confirmed the genetic distinctness of *C. homai* n. sp. which exhibited genetic distances ranging from 3.1% to 15.4%, 14.4% to 24.5%, and 6.6% to 20.9% from all other *Cryptosporidium* spp., respectively. Phylogenetic analysis of concatenated 18S, actin and *hsp70* sequences also exhibited 9.1% to 17.3% genetic distances between *C. homai* n. sp. and other *Cryptosporidium* spp. This clearly supports the species status of *C. homai* n. sp., as these differences are greater than between many currently accepted species. For example, the genetic distance at the 18S and actin loci between *C. hominis* and *C. cuniculus* is 0.4% and 1.6%, respectively (Kvác et al., 2014), and the genetic distance between *C. muris* and *C. andersoni* at the 18S, actin, *hsp70* loci is 0.7%, 3.5% and 2.2%, respectively (Holubová et al., 2016).

In the present study, *C. homai* n. sp. did not group with *C. wrairi* (the only currently valid species in guinea pigs) and exhibited 3.6%, 18.4% and 7.0% genetic distance from this species at 18S, actin and *hsp70* loci, respectively and is clearly a separate species from *C.*

wrairi. The phylogenetic relationship of *C. homai* n. sp. to other *Cryptosporidium* spp. is however still ambiguous; at the 18S locus, it was most closely related to *C. felis*, while at the actin locus, it was closest to *C. varanii* (14.4% genetic distance), at the *hsp70* locus, it grouped most closely with *C. suis* (6.6%), and a concatenated analysis of all 3 loci, grouped it most closely with *C. varanii* (9.1%). Analysis at additional loci or whole genome analysis will shed more light on the evolutionary relationships between *C. homai* n. sp. and other *Cryptosporidium* spp.

The pathogenic potential of *C. homai* n. sp. is unknown. Histopathological analysis indicated minimal host inflammatory responses, with the lamina propria expanded by mild predominantly lymphocytic-plasmocytic inflammatory infiltrate with the occasional neutrophils, eosinophils and necrotic cells. Intestinal mucosal cells are usually replaced from germinal cells in the crypts, as the older epithelial cells are sloughed at the tips of villi. The occasional mitotic figure is expected in the normal healthy animal as renewal of cells. But large numbers indicate a response to the infection. Histopathological analysis of the infected guinea pig in the present study revealed large numbers of mitotic figures which suggest increased replacement of intestinal cells from hyperplasia. More structured studies are required to clearly define the clinical signs (if any) caused by *C. homai* n. sp.

The host range of *C. homai* n. sp. and its zoonotic potential are also currently unknown, but it has not been previously reported in any other host, suggesting that it may be host specific, however, further analysis is required to determine this.

Acknowledgements

This study was financially supported by an Australian Research Council Linkage Grant number LP130100035.

References

- Adamu, H., Petros, B., Zhang, G., Kassa, H., Amer, S., Ye, J., Feng, Y., Xiao, L., 2014. Distribution and clinical manifestations of *Cryptosporidium* species and subtypes in HIV/AIDS patients in Ethiopia. PLoS Negl. Trop. Dis. 8, e2831.
- Agholi, M., Hatam, G.M., Motazedian, M.H., 2013. HIV/AIDS-Associated Opportunistic Protozoal Diarrhea. AIDS Res. Hum. Retroviruses. 29, 35-41.
- Al Brikan, F.A., Salem, H.S., Beeching, N., Hilal, N., 2008. Multilocus genetic analysis of *Cryptosporidium* isolates from Saudi Arabia. J. Egypt. Soc. Parasitol. 38, 645-658.
- Angus, K.W., Hutchison, G., Munro, H.M., 1985. Infectivity of a strain of *Cryptosporidium* found in the guinea-pig (*Cavia porcellus*) for guinea-pigs, mice and lambs. J. Comp. Pathol. 95, 151-165.
- ANOFEL *Cryptosporidium* National Network. 2010. Laboratory based surveillance for *Cryptosporidium* in France, 2006-2009. Euro Surveill. 15, 19642.
<http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19642>.
- Appelbee, A.J., Thompson, R.C.A., Olson, M.E., 2005. *Giardia* and *Cryptosporidium* in mammalian wildlife - current status and future needs. Trends. Parasitol. 21, 339-341.
- Atwill, E.R., Phillips, R., Pereira, M.D., Li, X., McCowan, B., 2004. Seasonal shedding of multiple *Cryptosporidium* genotypes in California ground squirrels (*Spermophilus beecheyi*). Appl. Environ. Microbiol. 70, 6748-6752.
- Azami, M., Moghaddam, D.D., Salehi, R., Salehi, M., 2007. The identification of *Cryptosporidium* species (protozoa) in Ifsahan, Iran by PCR RFLP analysis of the 18S rRNA gene. Mol. Biol. 41, 934-939.
- Bajer, A., Cacciò, S., Bednarska, M., Behnke, J.M., Pieniasek, N.J., Sinski, E., 2003. Preliminary molecular characterization of *Cryptosporidium parvum* isolates of wildlife rodents from Poland. J. Parasitol. 89, 1053-1055.

- Berrilli, F., D'Alfonso, R., Giangaspero, A., Marangi, M., Brandonisio, O., Kaboré, Y., Glé, C., Cianfanelli, C., Lauro, R., Di Cave, D., 2012. *Giardia duodenalis* genotypes and *Cryptosporidium* species in humans and domestic animals in Côte d'Ivoire: occurrence and evidence for environmental contamination. *Trans. R. Soc. Trop. Med. Hyg.* 106, 191-195.
- Bodager, J.R., Parsons, M.B., Wright, P.C., Rasambainarivo, F., Roellig, D., Xiao, L., Gillespie, T.R., 2015. Complex epidemiology and zoonotic potential for *Cryptosporidium suis* in rural Madagascar. *Vet. Parasitol.* 207, 140-143.
- Cama, V.A., Bern, C., Sulaiman, I.M., Gilman, R.H., Ticona, E., Vivar, A., Kawai, V., Vargas, D., Zhou, L., Xiao, L., 2003. *Cryptosporidium* species and genotypes in HIV positive patients in Lima, Peru. *J. Eukaryot. Microbiol.* 50, 531-533.
- Cama, V.A., Ross, J.M., Crawford, S., Kawai, V., Chavez Valdez, R., Vargas, D., Vivar, A., Ticona, A., Ñavincopa, M., Williamson, J., Ortega, Y., Gilman, R.H., Bern, C., Xiao, L., 2007. Differences in Clinical Manifestations among *Cryptosporidium* Species and Subtypes in HIV Infected Persons. *J. Infect. Dis.* 196, 684-691.
- Cavalier-Smith, T., 2014. Gregarine site-heterogeneous 18S rDNA trees, revision of gregarine higher classification, and the evolutionary diversification of Sporozoa. *Eur. J. Protistol.* 50, 472-95.
- Chalmers, R.M., Sturdee, A.P., Bull, S.A., Miller, A., Wright, S.E., 1997. The prevalence of *Cryptosporidium parvum* and *C. Muris* in *Mus domesticus*, *Apodemus sylvaticus* and *Clethrionomys glareolus* in an agricultural system. *Parasitol. Res.* 83,478-482.
- Chalmers, R.M., Robinson, G., Elwin, K., Hadfield, S.J., Xiao, L., Ryan, U., Modha, D., Mallaghan, C., 2009. *Cryptosporidium* sp. rabbit genotype, a newly identified human Pathogen. *Emerg. Infect. Dis.* 15, 829- 830.

- Chalmers, R.M., Robinson, G., Elwin, K., Hadfield, S.J., Thomas, E., Watkins, J., Casemore, D., Kay, D., 2010. Detection of *Cryptosporidium* species and sources of contamination with *Cryptosporidium hominis* during a waterborne outbreak in north west Wales. *J. Water Health*. 8, 311-325.
- Chalmers, R.M., Smith, R., Elwin, K., Clifton-Hadley, F.A., Giles, M., 2011. Epidemiology of anthroponotic and zoonotic human cryptosporidiosis in England and Wales, 2004-2006. *Epidem. Infect.* 139, 700-712.
- Chrisp, C.E., Reid, W.C., Rush, H.G., Suckow, M.A., Bush, A., Thomann, M.J., 1990. Cryptosporidiosis in guinea pigs: an animal model. *Infect. Immun.* 58, 674-679.
- Chrisp, C.E., Suckow, M.A., Fayer, R., Arrowood, M.J., Healey, M.C., Sterling, C.R., 1992. Comparison of the host ranges and antigenicity of *Cryptosporidium parvum* and *Cryptosporidium wrairi* from guinea pigs *J. Protozool.* 39, 406-409.
- d'Ovidio, D., Noviello, E., Ianniello, D., Cringoli, G., Rinaldi, L., 2015. Survey of endoparasites in pet guinea pigs in Italy. *Parasitol. Res.* 114(3), 1213-1206.
- Dunnum, J.L., Salazar-Bravo, J., 2009. Molecular systematics, taxonomy and biogeography of the genus *Cavia* (Rodentia: Caviidae). *J. Zool. Syst. Evol. Res.* 48, 376-388.
- Ebner, J., Koehler, A.V., Robertson, G., Bradbury, R.S., Haydon, S.R., Stevens, M.A., Norton, R., Joachim, A., Gasser, R.B., 2015. Genetic analysis of *Giardia* and *Cryptosporidium* from people in Northern Australia using PCR based tools. *Infect. Genet. Evol.* 36, 389-395.
- Efstratiou, A., Ongerth, J.E., Karanis, P., 2017. Waterborne transmission of protozoan parasites: Review of worldwide outbreaks - An update 2011-2016. *Water Res.* 114, 14-22.

- Elwin, K., Hadfield, S.J., Robinson, G., Chalmers, R.M., 2012. The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000-2008. *Epidemiol. Infect.* 140, 673-683.
- Fall, A., Thompson, R.C.A., Hobbs, R.P., Morgan-Ryan, U.M., 2003. Morphology is not a reliable tool for delineating species within *Cryptosporidium*. *J. Parasitol.* 89, 399-402.
- Fayer, R., Santín, M., Macarisin, D., 2010. *Cryptosporidium ubiquitum* n. sp. in animals and humans. *Vet. Parasitol.* 172, 23-32.
- Feltus, D.C., Giddings, C.W., Schneck, B.L., Monson, T., Warshauer, D., McEvoy, J.M., 2006. Evidence supporting zoonotic transmission of *Cryptosporidium* spp. in Wisconsin. *J. Clin. Microbiol.* 44, 4303-4308.
- Feng, Y., Alderisio, K.A., Yang, W., Blancero, L.A., Kuhne, W.G., Ndareski, C.A., Reid, M., Xiao, L., 2007. *Cryptosporidium* genotypes in wildlife from a New York watershed. *Appl Environ Microbiol.* 73, 6475-6483.
- Feng, Y., Lal, A.A., Xiao, L., 2011. Subtypes of *Cryptosporidium* spp. in mice and other small mammals. *Exp. Parasitol.* 127, 238-242.
- Foo, C., Farrell, J., Boxell, A., Robertson, I., Ryan, U.M., 2007. Novel *Cryptosporidium* genotype in wild Australian mice (*Mus domesticus*). *Appl. Environ. Microbiol.* 73, 7693-7696.
- Gatei, W., Wamae, C.N., Mbae, C., Waruru, A., Mulinge, E., 2006. Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. *Am. J. Trop. Med. Hyg.* 75, 78-82.
- Ghaffari, S., Kalantari, N., 2014. A multi locus study of *cryptosporidium* parasites isolated from patients living in Iran, Malawi, Nigeria, the United Kingdom, and Vietnam. *Iran. J. Parasitol.* 9, 79-89.

- Gressler, L.T., da Silva, A.S., da Silva, M.K., Tonin, A.A., Monteiro, S.G., 2010. Gastrointestinal parasites of cavy (*Cavia aperea aperea*) in southern Brazil. Res. Vet. Sci. 89, 206-208.
- Guo, Y., Cebelinski, E., Matusевич, C., Alderisio, K.A., Lebbad, M., McEvoy, J., Roellig, D.M., Yang, C., Feng, Y., Xiao, L., 2015. Subtyping novel zoonotic pathogen *Cryptosporidium* chipmunk genotype I. J. Clin. Microbiol. 53, 1648-1654.
- Hasajová, A., Valenčáková, A., Malčėková, B., Danišová, O., Halán, M., Goldová, M., Sak, B., Květoňová, D., Kváč, M., Halánová, M., 2014. Significantly higher occurrence of *Cryptosporidium* infection in Roma children compared with non-Roma children in Slovakia. Eur. J. Clin. Microbiol. Infect. Dis. 33, 1401-1406.
- Hikosaka, K., Nakai, Y., 2005. A novel genotype of *Cryptosporidium muris* from large Japanese field mice, *Apodemus speciosus*. Parasitol Res. 97, 373-379.
- Holubová, N., Sak, B., Horčíčková, M., Hlásková, L., Květoňová, D., Menchaca, S., McEvoy, J., Kváč, M., 2016. *Cryptosporidium avium* n. sp. (Apicomplexa: Cryptosporidiidae) in birds. Parasitol Res. 115, 2243-2251.
- Hong, S.H., Anu, D., Jeong, Y.I., Abmed, D., Cho, S.H., Lee, W.J., Lee, S.E., 2014. Molecular Characterization of *Giardia duodenalis* and *Cryptosporidium parvum* in Fecal Samples of Individuals in Mongolia. Am. J. Trop. Med. Hyg. 90, 43-47.
- Huber, F., da Silva, S., Bomfim, T.C., Teixeira, K.R., Bello, A.R., 2007. Genotypic characterization and phylogenetic analysis of *Cryptosporidium* sp. from domestic animals in Brazil. Vet. Parasitol. 150(1-2), 65-74.
- Hurkova, L., Hajdusek, O., Modry, D., 2003. Natural infection of *Cryptosporidium muris* (Apicomplexa: Cryptosporidiidae) in Siberian chipmunks. J. Wildl. Dis. 39, 441-444.

- Insulander, M., Silverlas, C., Lebbad, M., Karlsson, L., Mattsson, J.G., Svenungsson, B., 2013. Molecular epidemiology and clinical manifestations of human cryptosporidiosis in Sweden. *Epidemiol. Infect.* 141, 1009-1020.
- Jezkova, J., Horcickova, M., Hlaskova, L., Sak, B., Kvetonova, D., Novak, J., Hofmannova, L., McEvoy, J., Kvac, M., 2016. *Cryptosporidium testudinis* sp. n., *Cryptosporidium ducismarci* Traversa, 2010 and *Cryptosporidium* tortoise genotype III (Apicomplexa: Cryptosporidiidae) in tortoises. *Folia Parasitol (Praha)*. 63. pii: 2016.035.
- Jiang, Y., Ren, J., Yuan, Z., Liu, A., Zhao, H., Liu, H., Chu, L., Pan, W., Cao, J., Lin, Y., Shen, Y., 2014. *Cryptosporidium andersoni* as a novel predominant *Cryptosporidium* species in outpatients with diarrhea in Jiangsu Province, China. *BMC. Infect. Dis.* 14, 555.
- Karanis, P., Plutzer, J., Halim, N.A., Igori, K., Nagasawa, H., Ongerth, J., Liqing, M., 2007. Molecular characterization of *Cryptosporidium* from animal sources in Qinghai province of China. *Parasitol. Res.* 101,1575-1580.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*. 28,1647-1649.
- Kimura, A., Edagawa, A., Okada, K., Takimoto, A., Yonesho, S., Karanis, P., 2007. Detection and genotyping of *Cryptosporidium* from brown rats (*Rattus norvegicus*) captured in an urban area of Japan. *Parasitol. Res.*100, 1417-1420
- Koehler, A.V., Whipp, M.J., Haydon, S.R., Gasser, R.B., 2014. *Cryptosporidium cuniculus* new records in human and kangaroo in Australia. *Parasit. Vectors.* 7, 492.
- Kumar, S., Stecher, G., Tamura, K., 2015. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Bio. Evol.* 33, 1870-1874.

- Kurniawan, A., Dwintasari, S.W., Connelly, L., Nichols, R.A., Yunihastuti, E., Karyadi, T., Djauzi, S., 2013. *Cryptosporidium* species from human immunodeficiency infected patients with chronic diarrhea in Jakarta, Indonesia. *Ann. Epidemiol.* 23, 720-723.
- Kváč, M., Hofmannova, L., Bertolino, S., Wauters, L., Tosi, G., Modry, D., 2008. Natural infection with two genotypes of *Cryptosporidium* in red squirrels (*Sciurus vulgaris*) in Italy. *Folia Parasitologica.* 55, 95-99.
- Kváč, M., Hanzlíková, D., Sak, B., Květoňová, D., 2009a. Prevalence and age-related infection of *Cryptosporidium suis*, *C. muris* and *Cryptosporidium* pig genotype II in pigs on a farm complex in the Czech Republic. *Vet. Parasitol.* 160, 319-322.
- Kváč, M., Květoňová, D., Sak, B., Ditrich, O., 2009b. *Cryptosporidium* Pig Genotype II in Immunocompetent Man. *Emerg. Infect. Dis.* 15, 982-983.
- Kváč, M., McEvoy, J., Loudová, M., Stenger, B., Sak, B., Květoňová, D., Ditrich, O., Rašková, V., Moriarty, E., Rost, M., Macholán, M., Piálek, J., 2013. Coevolution of *Cryptosporidium tyzzeri* and the house mouse (*Mus musculus*). *Int. J. Parasitol.* 43, 805-817.
- Kváč, M., Havrdová, N., Hlásková, L., Daňková, T., Kanděra, J., Ježková, J., Vítovec, J., Sak, B., Ortega, Y., Xiao, L., Modrý, D., Chelladurai, J.R.J.J., Prantlová, V., McEvoy, J., 2016. *Cryptosporidium proliferans* n. sp. (Apicomplexa: Cryptosporidiidae): Molecular and biological evidence of cryptic species within gastric *Cryptosporidium* of mammals. *PLoS ONE.* 11(1), e0147090.
- Lebbad, M., Beser, J., Insulander, M., Karlsson, L., Mattsson, J.G., Svenungsson, B., Axen, C., 2013. Unusual cryptosporidiosis cases in Swedish patients: extended molecular characterization of *Cryptosporidium viatorum* and *Cryptosporidium* chipmunk genotype I. *J. Parasitol.* 140, 1735-1740.

- Leoni, F., Amar, C., Nichols, G., Pedraza Díaz, S., McLauchlin, J., 2006. Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhoea in England between 1985 and 2000. *J. Med. Microbiol.* 5, 703-707.
- Li, N., Xiao, L., Alderisio, K., Elwin, K., Cebelinski, E., Chalmers, R., Santin, M., Fayer, R., Kváč, M., Ryan, U., Sak, B., Stanko, M., Guo, Y., Wang, L., Zhang, L., Cai, J., Roellig, D., Feng, Y., 2014. Subtyping *Cryptosporidium ubiquitum*, a zoonotic pathogen emerging in humans. *Emerg. Infect. Dis.* 20, 217-224.
- Li, X., Pereira, Md., Larsen, R. Xiao, C., Phillips, R., Striby, K., McCowan, B., Atwill, E.R., 2015. *Cryptosporidium rubeyi* n. sp. (Apicomplexa: Cryptosporidiidae) in multiple *Spermophilus* ground squirrel species. *Int. J. Parasitol. Parasites Wildl.* 4, 343-350.
- Li, Q., Li, Lu., Tao, W., Jiang, Y., Wan, Q., Lin, Y., Li, W., 2016. Molecular investigation of *Cryptosporidium* in small caged pets in northeast China: host specificity and zoonotic implications. *Parasitol Res.* 115, 2905-2911.
- Liu, X., Zhou, X., Zhong, Z., Chen, W., Deng, J., Niu, L., Wang, Q., Peng, G., 2014. New subtype of *Cryptosporidium cuniculus* isolated from rabbits by sequencing the gp60 gene. *J. Parasitol.* 100, 532-536.
- Lv, C., Zhang, L., Wang, R., Jian, F., Zhang, S., Ning, C., Wang, H., Feng, C., Wang, X., Ren, X., Qi, M., Xiao, L., 2009. *Cryptosporidium* spp. in wild, laboratory, and pet rodents in china: prevalence and molecular characterization. *Appl. Environ. Microbiol.* 75, 7692-7699.
- Matsui, T., Fujino, T., Kajima, J., Tsuji, M., 2000. Infectivity to experimental rodents of *Cryptosporidium parvum* oocysts from Siberian chipmunks (*Tamias sibiricus*) originated in the People's Republic of China. *J. Vet. Med. Sci.* 62, 487-489.

- Molloy, S.F., Smith, H.V., Kirwan, P., Nichols, R.A., Asaolu, S.O., Connelly, L., Holland, C.V., 2010. Identification of a high diversity of *Cryptosporidium* species genotypes and subtypes in a pediatric population in Nigeria. *J. Trop. Med. Hyg.* 82, 608-613.
- Morgan, U.M, Sturdee, A.P, Singleton, G., Gomez, M.S., Gracenea, M., Torres, J., Hamilton, S.G., Woodside, D.P., Thompson, R.C.A., 1999. The *Cryptosporidium* “mouse” genotype is conserved across geographic areas. *J. Clin. Microbiol.* 37,1302-1305.
- Morgan, U.M., Xiao, L., Limor, J., Gelis, S., Raidal, S.R., Fayer, R., Lal, A., Elliot, A., Thompson, R.C.A., 2000. *Cryptosporidium meleagridis* in an Indian ring necked parrot (*Psittacula krameri*). *Aus. Vet. J.* 78, 182-183.
- Morse, T.D., Nichols, R.A., Grimason, A.M., Campbell, B.M., Tembo, K.C., 2007. Incidence of cryptosporidiosis species in paediatric patients in Malawi. *Epidemiol. Infect.* 135, 1307-1315.
- Murakoshi, F., Fukuda, Y., Matsubara, R., Kato, Y., Sato, R., Sasaki, T., Tada, C., Nakai, Y., 2013. Detection and genotyping of *Cryptosporidium* spp. in large Japanese field mice, *Apodemus speciosus*. *Vet. Parasitol.* 196, 184-188.
- Muthusamy, D., Rao, S.S., Ramani, S., Monica, B., Banerjee, I., Abraham, O.C., Mathai, D. C., Primrose, B., Muliyl, J., Wanke, C.A., Ward, H.D., Kang, G., 2006. Multilocus genotyping of *Cryptosporidium* sp. isolates from human immunodeficiency virus infected individuals in South India. *J. Clin. Microbiol.* 44, 632-634.
- Neira, O.P., Muñoz, S.N., Wilson, L.G., Barthel, M.M.E., Rosales, L.M.J., Henríquez, R.C., 2012. *Cryptosporidium* species in immunodeficient and immunocompetent patients of Valparaíso: a descriptive study. *Rev. Chilena. Infectol.* 29, 63-71.
- Ng, J., Pavlasek, I., Ryan, U., 2006. Identification of novel *Cryptosporidium* genotypes from avian hosts. *Appl. Environ. Microbiol.* 72. 7548-7553.

- Ng-Hublin, J.S., Singleton, G.R., Ryan, U., 2013. Molecular characterization of *Cryptosporidium* spp. from wild rats and mice from rural communities in the Philippines. *Infect. Genet. Evol.* 16, 5-12.
- Pereira, M.G., Li, X., McCowan, B. Phillips, R.L., Atwill, E.R., 2010. Multiple unique *Cryptosporidium* isolates from three species of ground squirrels (*Spermophilus beecheyi*, *S. beldingi*, and *S. lateralis*) in California. *Appl. Environ. Microbiol.* 76, 8269-8276.
- Perez, J.F., Le Blancq, S.M., 2001. *Cryptosporidium parvum* infection involving novel genotypes in wildlife from lower New York State. *Appl. Environ. Microbiol.* 67, 1154-1162.
- Petrincová, A., Valenčáková, A., Luptáková, L., Ondriska, F., Kalinová, J., Halánová, M., Danišová, O., Jarčuška, P., 2015. Molecular characterization and first report of *Cryptosporidium* genotypes in human population in the Slovak Republic. *Electrophor.* 36, 2925-2930.
- Qi, M., Luo, N., Wang, H., Yu, F., Wang, R., Huang, J., Zhang, L., 2015. Zoonotic *Cryptosporidium* spp. and *Enterocystozoon* in pet chinchillas (*Chinchilla lanigera*) in China. *Parasitol. Int.* 64, 339-341.
- Rahmouni, I., Essid, R., Aoun, K., Bouratbine, A., 2014. Glycoprotein 60 diversity in *Cryptosporidium parvum* causing human and cattle cryptosporidiosis in the rural region of Northern Tunisia. *Am. J. Trop. Med. Hyg.* 90, 346-350.
- Rasková, V., Kvetonová, D., Sak, B., McEvoy, J., Edwinston, A., Stenger, B., Kvac, M., 2013. Human cryptosporidiosis caused by *Cryptosporidium tyzzeri* and *C. parvum* isolates presumably transmitted from wild mice. *J. Clin. Microbiol.* 51, 360-362.

- Ren, X, Zhao, J. , Zhang, L., Ning, C., Jian, F., Wang, R., Lv, C., Wang, Q., Arrowood, M. J., Xiao, L., 2012. *Cryptosporidium tyzzeri* n. sp. (Apicomplexa: Cryptosporidiidae) in domestic mice (*Mus musculus*). *Exp. Parasitol.* 130, 274-281.
- Robinson, G., Elwin, K., Chalmers, R.M., 2008. Unusual *Cryptosporidium* genotypes in human cases of diarrhea. *Emerg. Infect. Dis.* 14, 1800-1802.
- Robinson, G., Chalmers, R.M., Stapleton, C., Palmer, S.R., Watkins, J., Francis, C., Kay, D., 2011. A whole water catchment approach to investigating the origin and distribution of *Cryptosporidium* species. *J. Appl. Microbiol.* 111, 717-730.
- Ryan, U., Xiao, L., 2014. Taxonomy and molecular taxonomy. In: Caccio, S.M., Widmer, G. (Ed.), *Cryptosporidium: parasite and disease*. Springer, New York, pp, 28.
- Ryan, U., Fayer, R., Xiao, L., 2014. *Cryptosporidium* species in humans and animals: current understanding and research needs. *Parasitol.* 141, 1667-1685.
- Ryan, U., Paparini, A., Monis, P., Hijjawi, N., 2016. It's official - *Cryptosporidium* is a gregarine: What are the implications for the water industry? *Water Res.* 105, 305-313.
- Sharma, P., Sharma, A., Sehgal, R., Malla, N., Khurana, S., 2013. Genetic diversity of *Cryptosporidium* isolates from patients in North India. *Int. J. Infect. Dis.* 17, e601-605.
- Silva, S.O.S., Richtzenhain, L.J., Barros, I.N., Gomes, A.M., Silva, A.V., Kozerski, N.D., de Araújo-Ceranto, J.B., Keid, L.B., Soares, R.M., 2013. A new set of primers directed to 18S rRNA gene for molecular identification of *Cryptosporidium* spp. and their performance in the detection and differentiation of oocysts shed by synanthropic rodents. *Exp Parasitol.* 135, 551-557.
- Silverlås, C., Mattsson, J.G., Insulander, M., Lebbad, M., 2012. Zoonotic transmission of *Cryptosporidium meleagridis* on an organic Swedish farm. *Int. J. Parasitol.* 42, 963-967.

- Smith, R.P., Chalmers, R.M., Mueller-Doblies, D., Clifton-Hadley, F.A., Elwin, K., Watkins, J., Paiba, G.A., Hadfield, S.J., Giles, M., 2010. Investigation of farms linked to human patients with cryptosporidiosis in England and Wales. *Prev. Vet. Med.* 94(1-2), 9-17.
- Song, J., Kim, C.Y., Chang, S.N., Abdelkader, T.S., Han, J., Kim, T.H., Oh, H., Lee, J.M., Kim, D.S., Kim, J.T., Oh, H.S., Hur, M., Suh, J.H., Park, J.H., 2015. Detection and molecular characterization of *Cryptosporidium* spp. from wild rodents and insectivores in South Korea. *Korean J. Parasitol.* 56, 737-743.
- Spanakos, G., Biba, A., Mavridou, A., Karanis, P., 2015. Occurrence of *Cryptosporidium* and *Giardia* in recycled waters used for irrigation and first description of *Cryptosporidium parvum* and *C. muris* in Greece. *Parasitol. Res.* 114, 1803-1810.
- Spano, F., Putignani, L., McLauchlin, J., Casemore, D.P., Crisanti, A., 1997. PCR-RFLP analysis of the *Cryptosporidium* oocyst wall protein (COWP) gene discriminates between *C. wrairi* and *C. parvum* and between *C. parvum* isolates of human and animal origin. *FEMS Microbiol. Lett.* 150, 209-217.
- Stenger, B.L.S., Clark, M.E., Kváč, M., Khan, E., Giddings, C.W., Prediger, J., McEvoy, J.M., 2015. North American tree squirrels and ground squirrels with overlapping ranges host different *Cryptosporidium* species and genotypes. *Infect. Genet. Evol.* 36, 287-293.
- Stensvold, C.R., Beser, J., Axén, C., Lebbad, M., 2014. High Applicability of a Novel Method for gp60 Based Subtyping of *Cryptosporidium meleagridis*. *J. Clin. Microbiol.* 52, 2311-2319.
- Stensvold, C.R., Ethelberg, S., Hansen, L., Sahar, S., Voldstedlund, M., Kemp, M., Hartmeyer, G.N., Otte, E., Engsbro, A.L., Nielsen, H.V., Mølbak, K., 2015. *Cryptosporidium* infections in Denmark, 2010-2014. *Dan. Med. J.* 62, A5086.

- Torres, J., Gracenea, M., Gomez, M.S., Arrizabalaga, A., Gonzalez-Moreno, O., 2000. The occurrence of *Cryptosporidium parvum* and *C. muris* in wild rodents and insectivores in Spain. *Vet. Parasitol.* 92, 253-260.
- Vasco, K., Graham, J.P., Trueba, G., 2016. Detection of Zoonotic Enteropathogens in Children and Domestic Animals in a Semirural Community in Ecuador. *Appl. Environ. Microbiol.* 82, 4218-4224.
- Vetterling, J.M., Jervis, H.R., Merrill, T.G., Sprinz, H., 1971. *Cryptosporidium wrairi* sp. n. from the guinea pig *Cavia porcellus*, with an emendation of the genus. *J. Protozool.* 18, 243-247.
- Waldron, L.S., Dimeski, B., Beggs, P.J., Ferrari, B.C. Power, M.L., 2011. Molecular Epidemiology, Spatiotemporal Analysis, and Ecology of Sporadic Human Cryptosporidiosis in Australia. *Appl. Environ. Microbiol.* 77, 7757-7765.
- Wang, R., Jian, F., Zhang, L., Ning, C., Liu, A., Zhao, J., Feng, Y., Qi, M., Wang, H., Lv, C., Zhao, G., Xiao, L., 2012. Multilocus sequence subtyping and genetic structure of *Cryptosporidium muris* and *Cryptosporidium andersoni*. *PLoS One.* 7(8), e43782.
- Wang, L., Zhang, H., Zhao, X., Zhang, L., Zhang, G., Guo, M., Liu, L., Feng, Y., Xiao, L., 2013. Zoonotic *Cryptosporidium* Species and *Enterocytozoon bienersi* Genotypes in HIV-Positive Patients on Antiretroviral Therapy. *Clinic. Microbiol.* 2, 557-563.
- Wang, Y., Yang, W., Cama, V., Wang, L., Cabrera, L., Ortega, Y., Bern, C., Feng, Y., Gilman, R., Xiao, L., 2014. Population genetics of *Cryptosporidium meleagridis* in humans and birds: evidence for cross species transmission. *Int. J. Parasitol.* 44, 515-521.
- Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A.A., Montali, R.J., Fayer, R., Lal, A.A., 1999. Phylogenetic Analysis of *Cryptosporidium* Parasites Based on the Small-Subunit rRNA Gene Locus. *Appl Environ Microbiol.* 65, 1578-1583.

- Xiao, L., Bern, C., Arrowood, M., Sulaiman, I., Zhou, L., Kawai, V., Vivar, A., Lal, A.A., Gilman, R.H., 2002. Identification of the *Cryptosporidium* pig genotype in a human patient. *J. Infect. Dis.* 185, 1846-1848.
- Yang, R., Murphy, C., Song, Y., Ng-Hublin, J., Estcourt, A., Hijjawi, N., Chalmers, R., Hadfield, S., Bath, A., Gordon, C., Ryan, U., 2013. Specific and quantitative detection and identification of *Cryptosporidium hominis* and *C. parvum* in clinical and environmental samples. *Exp. Parasitol.* 135, 142-147.
- Zahedi, A., Papparini, A., Jian, F., Robertson, I., Ryan, U., 2016. Public health significance of zoonotic *Cryptosporidium* species in wildlife: critical insights into better drinking water management. *Int. J. Parasitol: Parasit. Wildl.* 5, 88-109.
- Zhao, Z., Wang, R., Zhao, W., Qi, M., Zhao, J., Zhang, L., Li, J., Liu, A., 2015. Genotyping and subtyping of *Giardia* and *Cryptosporidium* isolates from commensal rodents in China. *Parasitol.* 142, 800-806.
- Zhou, L., Fayer, R., Trout, J.M., Ryan, U.M., Schaefer, F.W., Xiao, L., 2004. Genotypes of *Cryptosporidium* species infecting fur bearing mammals differ from those of species infecting humans. *Appl. Environ. Microbiol.* 70, 7574-7577.
- Ziegler, P.E., Wade, S.E., Schaaf, S.L., Chang, Y.F., Mohammed, H.O., 2007a. *Cryptosporidium spp.* From small mammals in the New York City watershed. *J Wildl Dis.* 43: 586-596.
- Ziegler, P.E., Wade, S.E., Schaaf, S.L., Stern, D.A., Nadareski, C.A., Mohammed, H.O., 2007b. Prevalence of *Cryptosporidium* species in wildlife populations within a watershed landscape in southeastern New York State. *Vet. Parasitol.* 147, 176-184.

Fig 1. (A-D) Giemsa-stained section of intestinal epithelium showing moderate to heavy epicellular infection by *Cryptosporidium*, associated with minimal host inflammatory response (isolate E89). Trophozoites (T) and meronts (Me) generally measured less than five micrometres as is typical for *Cryptosporidium*. There was a predominance of merogony with both type I (MeI) and type II meronts (MeII) present. Scale bar: 5 μ m.

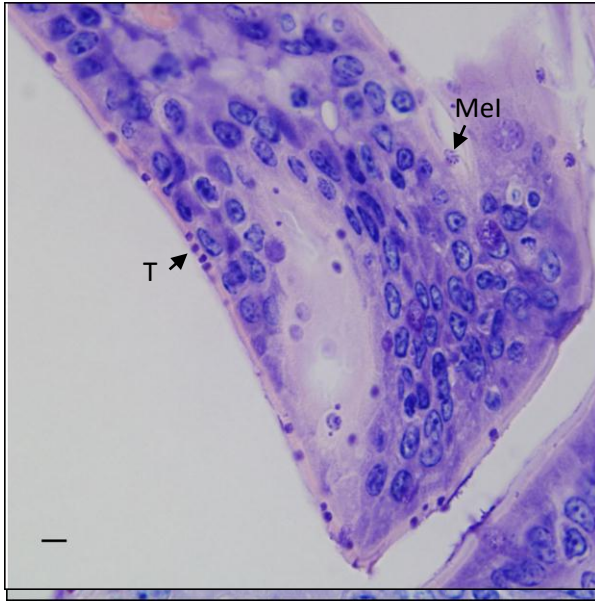
Fig 2. Evolutionary phylogenetic relationship between *C. homai* n. sp. and *Cryptosporidium* species described to date as inferred by maximum likelihood (ML) analysis of 18S rRNA locus. Percentage support (>50%) from 1000 pseudoreplicates from ML analyses is indicated at the left of the supported node. Scale bars indicate the number of substitutions per nucleotide position.

Fig 3. Phylogenetic relationships between *C. homai* n. sp. and other *Cryptosporidium* species inferred by ML analysis of actin gene. Percentage support (>50%) from 1000 pseudoreplicates from ML analyses is indicated at the left of the supported node. Scale bars indicate the number of substitutions per nucleotide position.

Fig 4. Phylogenetic relationships between *C. homai* n. sp. and other *Cryptosporidium* species inferred by ML analysis of partial *hsp70* gene sequences. Percentage support (>50%) from 1000 pseudoreplicates from ML analyses is indicated at the left of the supported node. Scale bars indicate the number of substitutions per nucleotide position.

Fig 5. Phylogenetic relationships between *C. homai* n. sp. and other *Cryptosporidium* species inferred ML analysis of concatenated sequences constructed from partial DNA sequences of 18S, actin and *hsp70* loci. Percentage support (>50%) from 1000 pseudoreplicates from ML analyses is indicated at the left of the supported node. Scale bars indicate the number of substitutions per nucleotide position.

Figr-1A



B

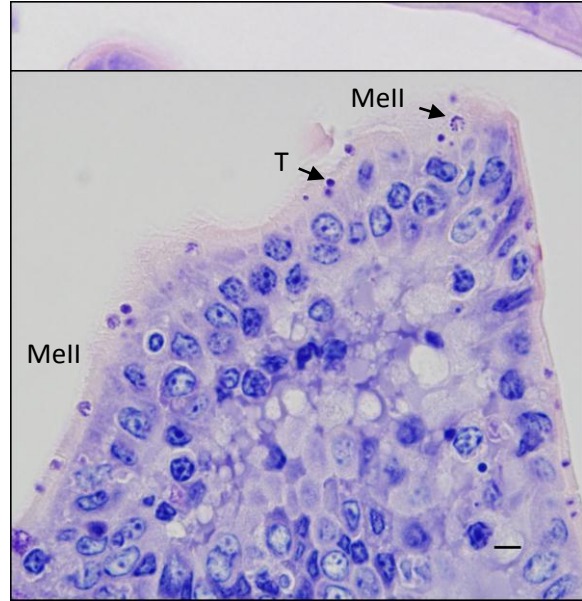


Table 1. *Cryptosporidium* species and genotypes reported in rodents.

Species/genotype	Major host	Report in rodents	References	Reports in humans
<i>C. proliferans</i>	Rodents	Spiny mouse (<i>Acomys cahirinus</i>), Tristram's jird (<i>Meriones tristrami</i>), Lesser gerbil (<i>Gerbilus gerbilus</i>), Mongolian gerbil (<i>Meriones unguiculatus</i>), Bushy-tailed jird (<i>Sekeetamys calurus</i>), Southern multimammate mouse (<i>Mastomys coucha</i>), Natal multimammate mouse (<i>Mastomys natalensi</i>), Brandt's voles (<i>Microtus brandti</i>), House mouse (<i>Mus spp.</i>), Eastern gray squirrel (<i>Sciurus carolinensis</i>), East African mole-rat (<i>Tachyoryctes splendens</i>)	Kváč et al., 2016	No reports in humans to date
<i>C. rubeyi</i>	Rodents	Golden-mantled ground squirrels (<i>Callospermophilus lateralis</i>), Belding's ground squirrels (<i>Urocitellus beldingi</i>), California ground squirrels (<i>Otospermophilus beecheyi</i>), Black-tailed prairie dogs (<i>Cynomys ludovicianus</i>)	Atwill et al., 2004; Pereira et al., 2010; Li et al., 2015; Stenger et al., 2015	No reports in humans to date
<i>C. scrofarum</i>	Pigs	Asian house rat (<i>Rattus tanezumi</i>), Brown rat (<i>Rattus norvegicus</i>)	Ng-Hublin et al., 2013	Occasionally reported in humans. Kváč et al., 2009a; Kváč et al., 2009b
<i>C. tyzzeri</i>	Rodents	Mice (<i>Mus musculus</i>), Brown rats (<i>Rattus norvegicus</i>), Large-footed bat (<i>Myotis adversus</i>), Yellow-necked mouse (<i>Apodemus flavicollis</i>), Bank vole (<i>Myodes glareolus</i>), Common vole (<i>Microtus arvalis</i>)	Morgan et al., 1999; badjer et al., 2003; Karanis et al., 2007; Lv et al., 2009; Ren et al., 2012; Kváč et al., 2013	Occasionally reported in humans. Rasková et al., 2013
<i>C. ubiquitum</i>	Ruminants, rodents, primates	Deer mouse (<i>Peromyscus</i>), Eastern grey squirrels (<i>Sciurus carolinensis</i>), Red squirrel (<i>Sciurus vulgaris</i>), Eastern chipmunk (<i>Tamias striatus</i>), Large Japanese field mouse (<i>Apodemus speciosus</i>), Prehensile-tailed porcupines (<i>Coendou prehensilis</i>), Wood chuck (<i>Marmota monax</i>)	Perz and Le Blancq, 2001; Feng et al., 2007; Ziegler et al., 2007; Fayer et al., 2010; Murakoshi et al., 2013; Li et al., 2014; Song et al., 2015; Stenger et al., 2015; Qi et al., 2015; Li et al., 2016	Commonly reported. Gatei et al., 2002; Tiangtip and Jongwutiwes 2002; Gatei et al., 2003; Palmer et al., 2003; Gatei et al., 2006; Leoni et al., 2006; Muthusamy et al., 2006; Azami et al., 2007; Al-Brikan et al., 2008; Neira et al., 2012;

<i>C. suis</i>	Pigs	Rodents	Bodager et al., 2015	Hasajová et al., 2014; Li et al., 2014; Chappell et al., 2015; Petrincová et al., 2015; Spanakos et al., 2015. Occasionally reported in humans. Xiao et al., 2002; Leoni et al., 2006; Cama et al., 2007; Wang et al., 2013; Bodager et al., 2015
<i>C. suis</i> -like	Pigs	Asian house rat (<i>Rattus tanezumi</i>)	Ng-Hublin et al., 2013	No reports in humans to date.
<i>C. andersoni</i>	Cattle	Marmots (<i>Marmota monax</i>), Campbell's dwarf hamster (<i>Phodopus campbelli</i>), Golden hamster (<i>Mesocricetus auratus</i>)	Lv et al., 2009; Wang et al., 2012	Numerous reports. Leoni et al., 2006; Morse et al., 2007; Waldron et al., 2011; Agholi et al., 2013; Jiang et al., 2014; Liu et al., 2014; Hussain et al., 2017
<i>C. parvum</i>	Ruminants	Eastern grey squirrel (<i>Sciurus carolinensis</i>), Ground Squirrels (<i>Spermophilus beecheyi</i>), Siberian chipmunk (<i>Tamias sibiricus</i>), Wood mice (<i>Apodemus sylvaticus</i>), White-footed mouse (<i>Peromyscus leucopus</i>), Capybara (<i>Hydrochoerus hydrochaeris</i>), Yellow-bellied marmot (<i>Marmota flaviventris</i>), Bamboo rats (<i>Rhizomys sinensis</i>), Campbell's dwarf hamster (<i>Phodopus campbelli</i>), Golden hamster (<i>Mesocricetus auratus</i>)	Chalmers et al., 1997; Matsui et al., 2000; Torres et al., 2000; Perz and Le Blancq, 2001; Bajer et al., 2003; Lv et al., 2009; Ng-Hublin et al., 2013; Qi et al., 2105; Zhao et al., 2015; Li et al., 2016	Commonly reported in humans
<i>C. meleagridis</i>	Birds and humans	Deer mouse (<i>Peromyscus</i>)	Feng et al., 2007; Bodager et al., 2015	Commonly reported in humans. Morgan et al., 2000; Cama et al., 2003; Gatei et al., 2006; Muthusamy et al., 2006; Leoni et al., 2006; Berrilli et al., 2012; Elwin et al., 2012; Neira et al., 2012; Silverlås et al., 2012; Kurniawan et al., 2013; Sharma

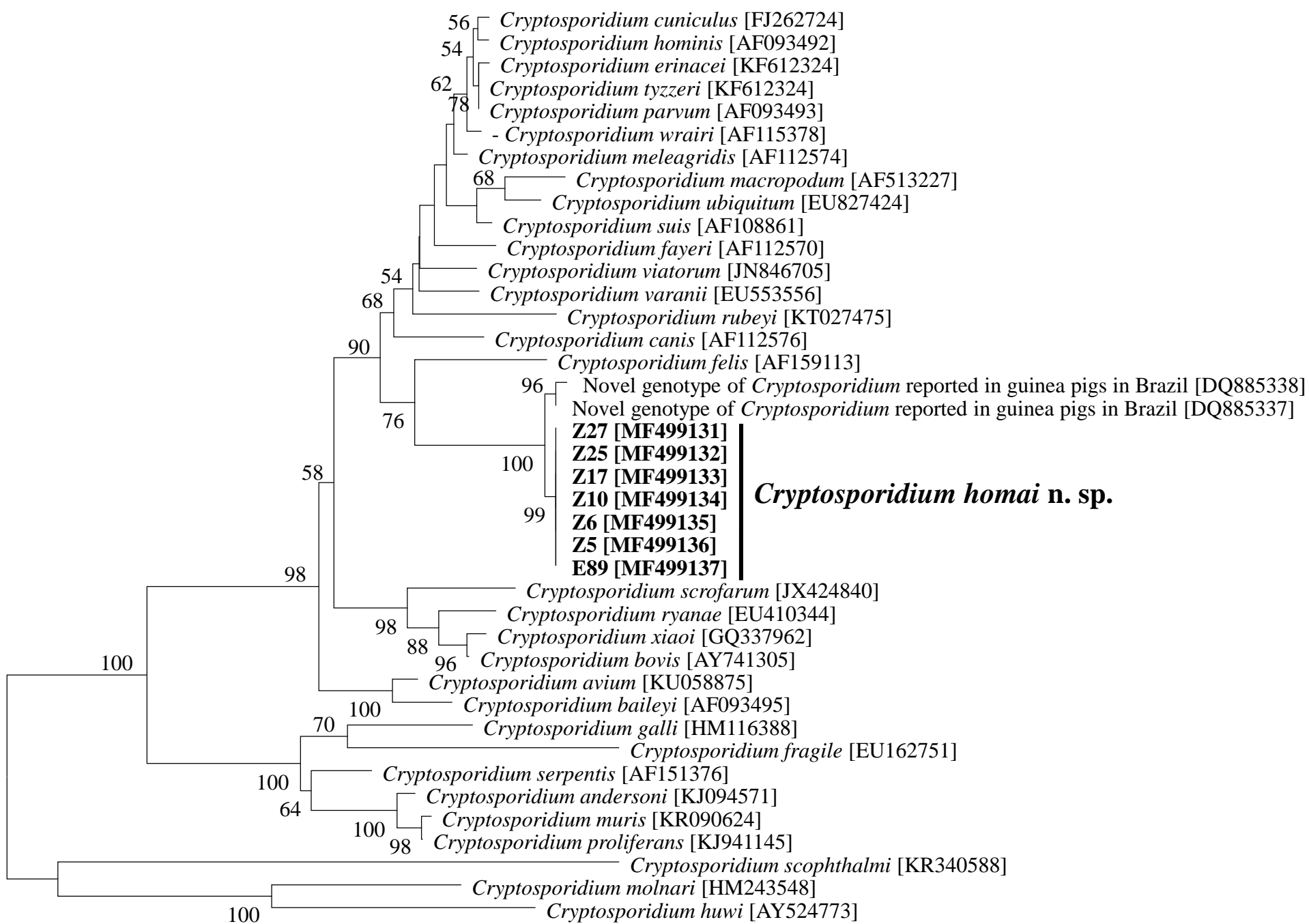
				et al., 2013; Wang et al., 2014; Adamu et al., 2014; Ghaffari and Kalantari, 2014; Ryan and Xiao, 2014; Ghaffari and Kalantari, 2014; Rahmouni et al., 2014; Wang et al., 2014; Stensvold et al., 2014; Stensvold et al., 2015)
<i>C. wrairi</i>	Guinea pigs	Guinea pig (<i>Cavia porcellus</i>), California ground squirrels (<i>Spermophilus beecheyi</i>)	Vetterling et al., 1971; Chrisp et al., 1990; Spano et al., 1997; Lv et al., 2009	No reports in humans to date
<i>C. muris</i>	Rodents	Wild rats (<i>Rattus</i> sp.), Mice (<i>Mus</i> sp.), Girrafes house mice (<i>Mus musculus</i>), Eastern grey squirrel (<i>Sciurus carolinensis</i>), Golden hamster (<i>Mesocricetus auratus</i>), Japanese field mouse (<i>Apodemus argenteus</i>), Bank voles (<i>Clethrionomys glareolus</i>), Campbell hamster (<i>Phodopus campbelli</i>), Siberian hamster (<i>Phodopus sungorus</i>), Golden hamster (<i>Mesocricetus auratus</i>), East African mole rat (<i>Tachyoryctes splendens</i>), Large Japanese field mouse (<i>Apodemus speciosus</i>)	Chalmers et al., 1997; Torres et al., 2000; Hurkova et al., 2003; Hikosoka et al., 2005; Ziegler et al., 2007; Kváč et al., 2008; Lv et al., 2009; Wang et al., 2012; Murakoshi et al., 2013; Ng-Hublin et al., 2013; Song et al., 2015; Zhao et al., 2015	Many reports - Gatei et al., 2006; Leoni et al., 2006; Muthusamy et al., 2006; Azami et al., 2007; Al-Brikan et al., 2008; Neira et al., 2012; Hasajová et al., 2014; Petrinová et al., 2015; Spanakos et al., 2015
Beaver genotype	Rodents	North American beaver (<i>Castor canadensis</i>)	Feng et al., 2007	No reports in humans to date
Chipmunk genotype I	Rodents	Chipmunk sp. (<i>Tamias</i> sp.), Eastern grey squirrel (<i>Sciurus carolinensis</i>), Deer mice (<i>Peromyscus maniculatus</i>)	Jiang et al., 2005; Feltus et al., 2006; Feng et al., 2007; Kváč et al., 2008; Insulander et al., 2013; Lebbad et al., 2013; Guo et al., 2015; Song et al., 2015	Emerging human pathogen Feltus et al., 2006; ANOFEL, 2010; Lebbad et al., 2013; Guo et al., 2015

Chipmunk genotype II	Rodents	Eastern chipmunk (<i>Tamias striatus</i>)	Feng et al., 2007; Stenger et al., 2015	No reports in humans to date
Chipmunk genotype III	Rodents	Siberian chipmunk (<i>Tamias sibiricus</i>)	Lv et al., 2009	No reports in humans to date
Deer mouse genotype I	Rodents	Deer mouse (<i>Peromyscus</i>)	Feng et al., 2007	No reports in humans to date
Deer mouse genotype II	Rodents	Deer mouse (<i>Peromyscus</i>)	Feng et al., 2007	No reports in humans to date
Deer mouse genotype III	Rodents	Deer mouse (<i>Peromyscus</i>)	Feng et al., 2007	No reports in humans to date
Deer mouse genotype IV	Rodents	Deer mouse (<i>Peromyscus</i>)	Feng et al., 2007	No reports in humans to date
Ferret genotype	Rodents	Siberian chipmunk (<i>Tamias sibiricus</i>), River otters (<i>Lontra canadensis</i>), Red squirrel (<i>Sciurus vulgaris</i>), Guinea pig (<i>Cavia porcellus</i>), Hamster (<i>Phodopus sungorus</i>)	Kváč et al., 2008; Lv et al., 2009; Feng et al., 2011; Li et al., 2016	No reports in humans to date
Ground squirrel genotype I	Rodents	Thirteen-lined ground squirrel (<i>Ictidomys tridecemlineatus</i>)	Stenger et al., 2015	No reports in humans to date
Ground squirrel genotype II	Rodents	Black-tailed prairie dog (<i>Cynomys ludovicianus</i>)	Stenger et al., 2015	No reports in humans to date
Ground squirrel genotype III	Rodents	Thirteen-lined ground squirrel (<i>Ictidomys tridecemlineatus</i>)	Stenger et al., 2015	No reports in humans to date
Hamster genotype	Rodents	Siberian hamster (<i>Phodopus sungorus</i>)	Lv et al., 2009	No reports in humans to date
Mouse genotype II	Rodents	House mouse (<i>Mus musculus</i>)	Foo et al., 2007; Silva et al., 2013	No reports in humans to date
Mouse genotype III	Rodents	House mouse (<i>Mus musculus</i>)	Silva et al., 2013	No reports in humans to date
Muskrat genotype I	Rodents	Muskrat (<i>Ondatra zibethicus</i>), Boreal red-backed vole (<i>Myodes rutilus</i>).	Zhou et al., 2004; Feng et al., 2007	No reports in humans to date
Muskrat genotype II	Rodents	Muskrat (<i>Ondatra zibethicus</i>), Deer mouse (<i>Peromyscus maniculatus</i>), Meadow vole (<i>Microtus pennsylvanicus</i>)	Ziegler et al., 2007; Robinson et al., 2011	No reports in humans to date
Naruko genotype	Rodents	Large Japanese field mouse (<i>Apodemus speciosus</i>)	Murakoshi et al., 2013	No reports in humans to date
Rat genotype I	Rodents	Brown rat (<i>Rattus norvegicus</i>)	Kimura et al., 2007; Chalmers et al., 2010; Ng-Hublin et al., 2013	No reports in humans to date

Rat genotype II	Rodents	Brown rat (<i>Rattus tanezum</i>), Wild black rat (<i>Rattus rattus</i>), Brown rat (<i>Rattus norvegicus</i>)	Lv et al., 2009; Ng-Hublin et al., 2013; Silva et al., 2013	No reports in humans to date
Rat genotype III	Rodents	Asian house rat (<i>Rattus tanezumi</i>), Wild black rat (<i>Rattus rattus</i>)	Lv et al., 2009; Ng-Hublin et al., 2013; Silva et al., 2013	No reports in humans to date
Rat genotype IV	Rodents	Tanezumi rat (<i>Rattus tanezumi</i>), Asian house rat (<i>Rattus tanezumi</i>), Brown rat (<i>Rattus norvegicus</i>)	Ng-Hublin et al., 2013	No reports in humans to date
Skunk/ skunk-like genotype	Skunk	Eastern grey squirrel (<i>Sciurus carolinensis</i>), American red squirrels (<i>Tamiasciurus hudsonicus</i>)	Feng et al., 2007; Ziegler et al., 2007	Several reports. Zhou et al., 2004; Feng et al., 2007; Ziegler et al., 2007; Robinson et al., 2008; Feng et al., 2011; Elwin et al., 2012
Vole genotype	Rodents	Meadow vole (<i>Microtus pennsylvanicus</i>)	Feng et al., 2007	No reports in humans to date
Novel genotype	Guinea pigs	Guinea pig (<i>Cavia porcellus</i>)	Huber et al., 2007	No reports in humans to date

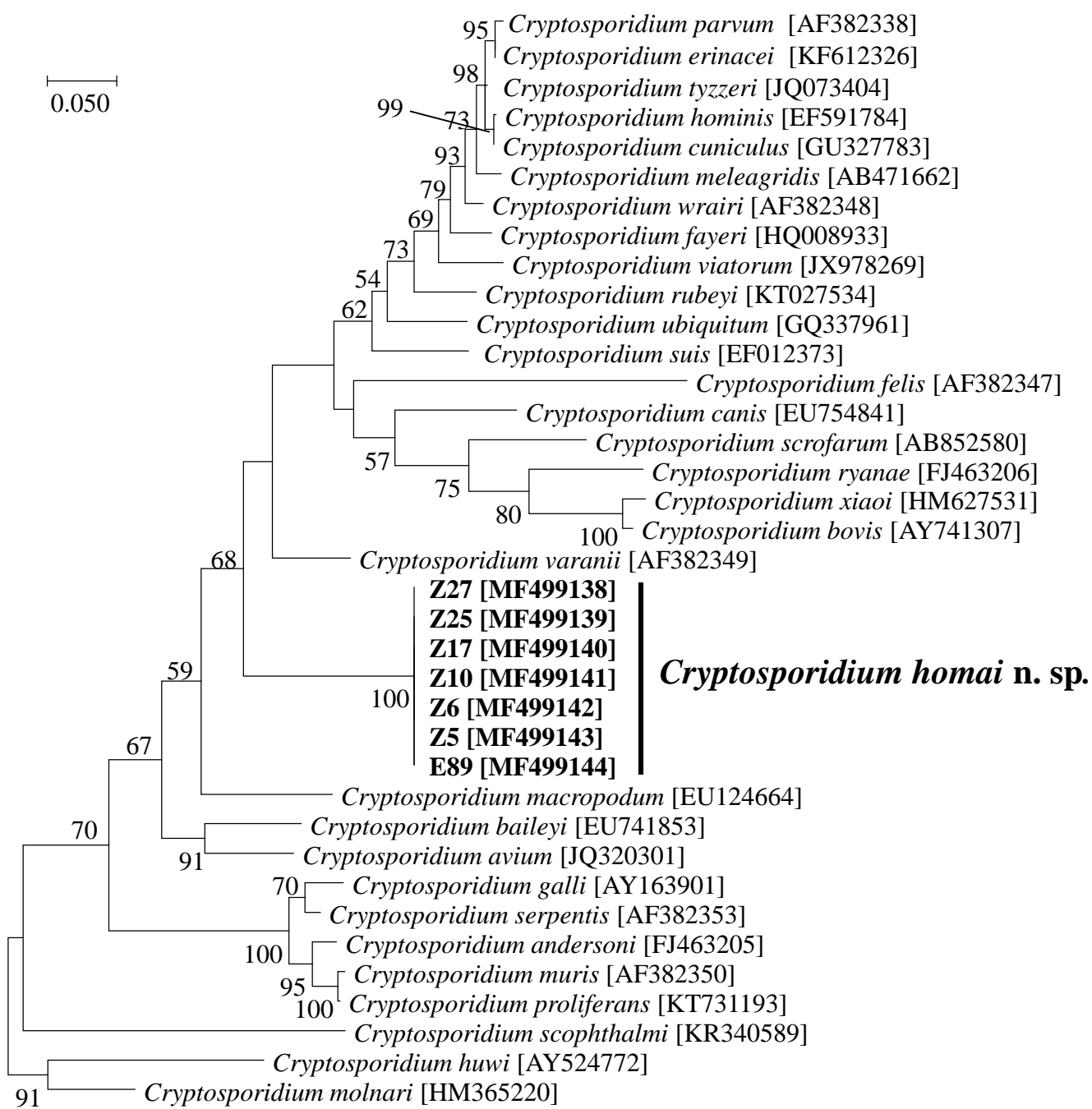
Table 2. List of faecal samples collected for this study, from guinea pigs, held at the University of Western Australia Animal Care Services, Perth, Australia.

Sample ID	Date	Type of sample
Z1	12/12/2016	Faeces – Pooled
Z2	12/12/2016	Faeces - Individual
Z3	12/12/2016	Faeces - Individual
Z4	12/12/2016	Faeces – Pooled
Z5	12/12/2016	Faeces – Pooled
Z6	12/12/2016	Faeces – Pooled
Z7	12/12/2016	Faeces – Pooled
Z8	12/12/2016	Faeces – Pooled
Z9	12/12/2016	Faeces – Pooled
Z10	12/12/2016	Faeces – Pooled
Z11	12/12/2016	Faeces – Pooled
Z12	12/12/2016	Faeces – Pooled
Z13	12/12/2016	Faeces – Pooled
Z14	12/12/2016	Faeces - Individual
Z15	12/12/2016	Faeces – Pooled
Z16	12/12/2016	Faeces – Pooled
Z17	12/12/2016	Faeces – Pooled
Z18	12/12/2016	Faeces - Individual
Z19	12/12/2016	Faeces – Pooled
Z20	12/12/2016	Faeces – Pooled
Z21	12/12/2016	Faeces – Pooled
Z22	12/12/2016	Faeces – Pooled
Z23	12/12/2016	Faeces – Pooled
Z24	12/12/2016	Faeces – Pooled
Z25	12/12/2016	Faeces – Pooled
Z26	12/12/2016	Faeces – Pooled
Z27	12/12/2016	Faeces – Pooled
Z28	12/12/2016	Faeces - Individual
E89	01/11/2016	Faeces - Individual



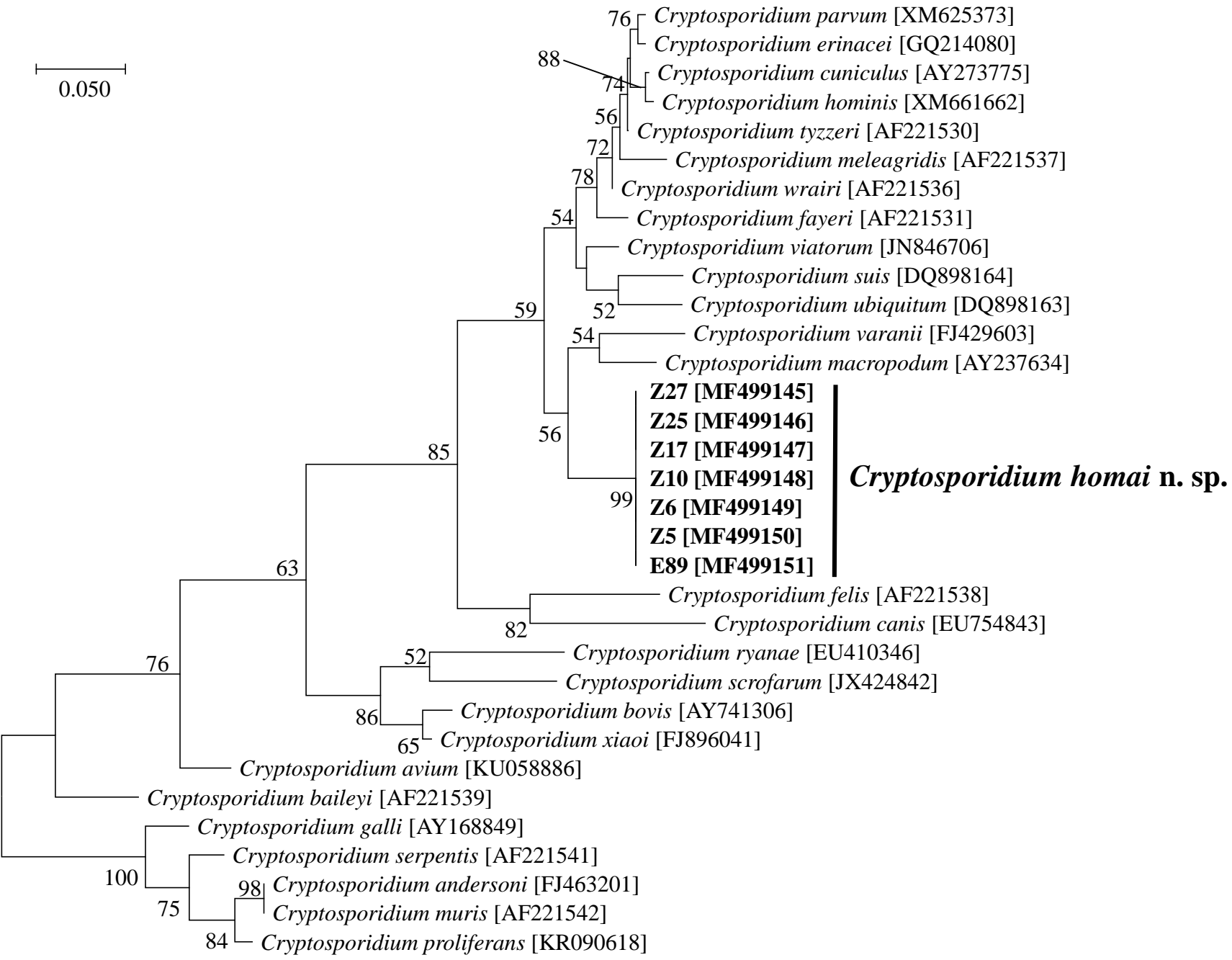
0.050

0.050

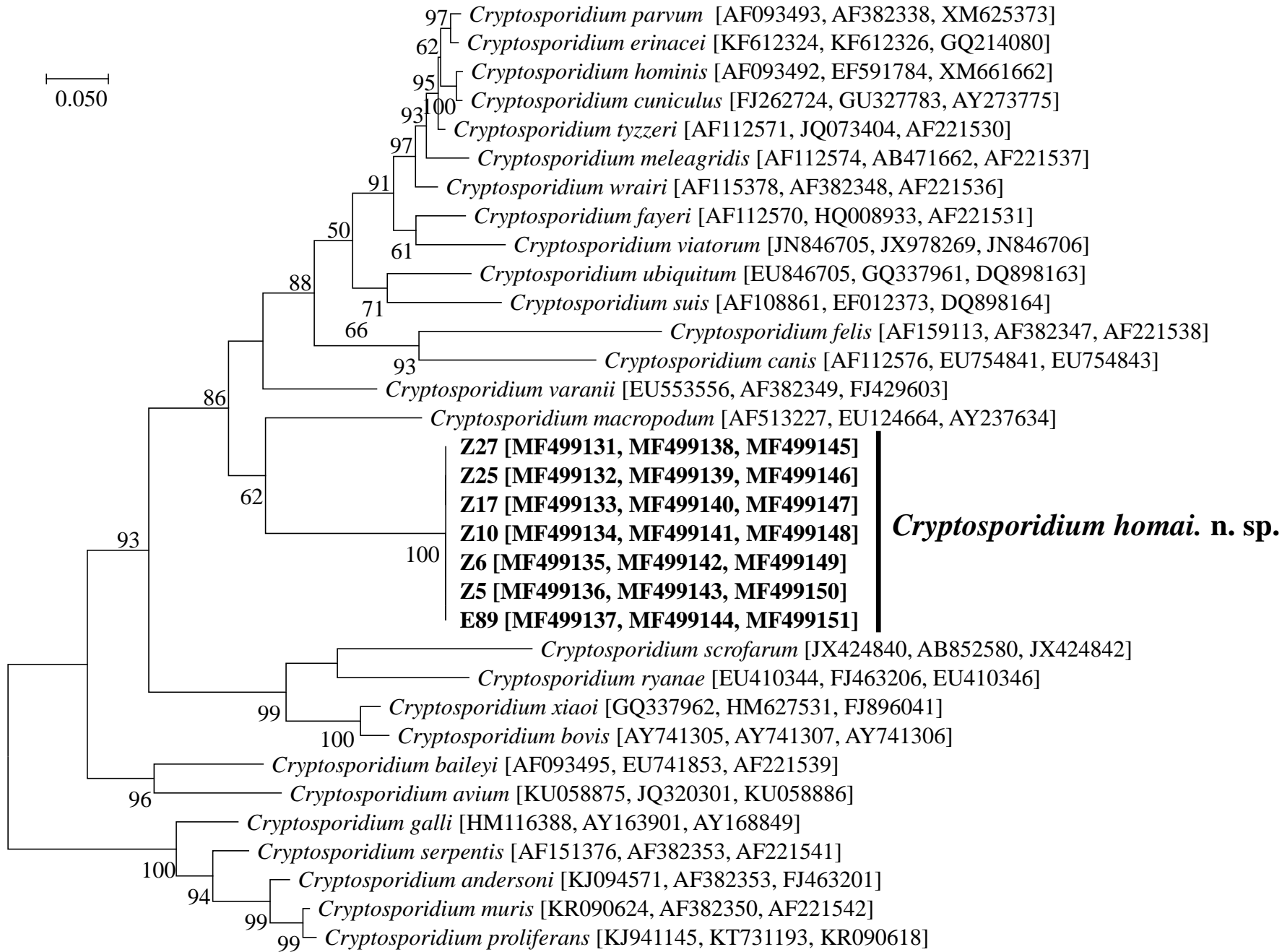


Cryptosporidium homai n. sp.

0.050



0.050



***Cryptosporidium homai*. n. sp.**