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Josephine S.Y. Ng-Hublin, Grant R. Singleton, Una Ryan

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Molecular characterization of *Cryptosporidium* spp. from wild rats and mice from rural communities in the Philippines

Josephine S.Y. Ng-Hublin[a], Grant R. Singleton[b], Una Ryan[a].

[a]School of Veterinary and Life Sciences, Murdoch University, Murdoch WA, 6150, Australia

[b]International Rice Research Institute, Crop and Environmental Sciences Division, DAPO, Box 7777, Metro Manila, Philippines

*Corresponding author.

Una Ryan

Phone: +61 8 9360 2482

Fax: +61 8 9310 4144

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

ABSTRACT

In order to examine the prevalence of *Cryptosporidium* in wild rodents in the Philippines and understand the role wild rodents play in the transmission of this parasite to humans and livestock, 194 fecal samples from wild rats and mice from Luzon and Mindoro islands were examined. Molecular screening at the 18S and actin gene loci identified an overall prevalence of 25.8% (95%CI: 19.8, 32.5). Sequence and phylogenetic analysis of both loci identified *C. parvum*, *C. muris*, *C. scrofarum*, rat genotypes I-IV and a *C. suis*-like genotype in the rat-derived isolates and is the first report of *C. suis*-like and *C. scrofarum* in rats. Mixed infections were identified in 24% of the *Cryptosporidium* positive isolates. Rat genotypes II, III and IV showed high intragenotypic variation at the 18S gene locus compared to the actin locus.

Keywords: *Cryptosporidium*; rodents; rats; mice; Philippines; 18S; actin gene.

1. Introduction

Cryptosporidium is a ubiquitous protozoan parasite capable of infecting humans and a wide variety of animals. The disease, cryptosporidiosis, usually manifests as self-limiting watery diarrhea, with symptoms ranging in severity and chronicity depending on the age and immunological status of the host. With a low infectious dose, infection with *Cryptosporidium* usually results from ingestion of food or water contaminated with the oocyst stage of the life cycle (Xiao and Ryan, 2004). Currently 26 valid species of *Cryptosporidium* and >50 different genotypes are recognized (Elwin et al., 2012; Kváč et al., 2013; Ren et al., 2012; Xiao, 2010). Molecular data indicates that eight *Cryptosporidium* species/genotypes are responsible for most human cryptosporidiosis cases, including *C. hominis, C. parvum, C. meleagridis, C. felis*, *C. canis, C. ubiquitum*, *C. cuniculus* and *C. viatorum* (Chalmers et al., 2011; Elwin et al., 2012; Xiao, 2010; Xiao and Feng, 2008) with *C. parvum* and *C. hominis* by far, the most common species in humans worldwide (Xiao, 2010).

Earlier studies into the epidemiology of *Cryptosporidium* spp. in rats and mice suggested that rodents may be important reservoir hosts for the parasite (Chalmers et al., 1997; Quy et al., 1999; Torres et al., 2000). Studies of *Cryptosporidium* in rats conducted in Australia, China, Japan, the United Kingdom (UK) and New Zealand (NZ), have reported a prevalence ranging from 2-49%, whereas studies in mice conducted in Australia, China Poland, Spain, the UK, and the United States (US) have reported a prevalence ranging from 1-62% (Chalmers et al., 1997; Chilvers et al., 1998; Foo et al., 2007; Iseki, 1986; Kimura et al., 2007; Klesius et al., 1986; Lv et al., 2009; Miyaji et al., 1989; Paparini et al., 2012; Sinski et al., 1993; Yamura et al., 1990). Most of the earlier epidemiological studies however, were based on morphological identification of *Cryptosporidium* sp. with no molecular data to support the identification. Recent genotyping studies carried out in rats and mice, have

identified the zoonotic *C. parvum*, *C. meleagridis*, *C. muris*, potentially zoonotic *C. tyzzeri*, and host adapted species such as mouse genotype II and rat genotypes I, II, III and IV (Feng et al., 2009; Foo et al., 2007; Kimura et al., 2007; Lv et al., 2009; Paparini et al., 2012).

In the Philippines, rodents cause important agricultural problems in rural communities with damage to rice crops (both pre- and post harvest) causing significant economic losses to the farmers (Singleton et al., 2010). The widespread presence and close proximity of rodents to humans and domestic animals within the rural rice growing communities poses a public and veterinary health risk as these rodents are capable of contaminating large areas including food storage, water sources and domestic and peri-domestic habitats with their fecal droppings, facilitating the dissemination and transmission of *Cryptosporidium* (Meerburg et al., 2009; Singleton, 2003; Singleton et al., 2010). The purpose of the present study is to examine the prevalence of *Cryptosporidium* amongst species of wild rats and mice common in rural communities in two of the main islands of the Philippines and the potential of these rodents as reservoirs for human-infectious *Cryptosporidium*.

2. Materials and Methods

2.1 Sample sites and species trapped

Trapping of wild rats (*Rattus* sp.) and mice (*Mus* sp.) was conducted in two different municipalities (Calauan and Los Baños) located approximately 10 km from each other in Laguna, on the island of Luzon, and in the municipality of San Jose on the island of Mindoro (Figure 1). In Calauan (14°08' N,121°25' E), trapping was carried out in small rice fields (1-3 hectares per household), which were interspersed with houses, shops and fish pens and

located along an access road. In Los Baños, traps were set in a local fresh produce market and on the research farm of the International Rice Research Institute (IRRI) (14°18' N, 121°22' E). In San Jose (12°21' N, 121°05' E), rice farming is more intense and rice fields where trapping was conducted spanned approximately 7 hectares with sparse distribution of houses between each rice field. Morphological measurements were used to confirm the identity of the species as described in Htwe et al., (2012).

There were five species of rodents trapped: the rice-field rat (*Rattues argentiventer*), the pacific rat (*Rattus exulans*), the brown rat (*Rattus norvegicus*), the Asian house rat (*Rattus tanezumi*), and house mouse (*Mus musculus*). All rats and mice were dissected and faecal samples from the rectum or the large intestinal section closest to the rectum were collected and placed in individual 10 mL polyethylene bottles containing 2.5% potassium dichromate and sent to Murdoch University, Western Australia for processing and analysis. A total of 194 faecal samples were collected from wild house mice (n=16), rice-field rats (n=24), a pacific rat (n = 1), brown rats (n=70) and Asian house rats (n = 83) (Table 1).

2.2 Sample processing and DNA extraction

Approximately 400mg of faecal matter from intestinal sections or faeces in 2.5% potassium dichromate were transferred into a 2.0 ml centrifuge tube. All faecal samples were then rinsed with distilled water and centrifuged at 10,000 x*g* and the supernatant removed. This was repeated twice to wash off any residual potassium dichromate. Total DNA was extracted from the faecal samples using a Powersoil DNA Kit (MOBIO, Carlsbad, California, USA), according to the manufacturer's protocol with minor modifications. Briefly, approximately 250 mg of faecal sample was measured into the 2.0 ml tube containing beads provided by the manufacturer and subjected to 5 freeze-thaw cycles (liquid nitrogen/80°C),

followed by 10 minutes of boiling to ensure lysis of the thick-walled *Cryptosporidium* oocysts and release of DNA. The final elution volume was adjusted to 50 µl of Buffer 6 from the kit manufacturer's recommended volume of 200 µl of Buffer 6 in order to increase DNA concentration. DNA was stored at -20°C until required.

2.3 PCR amplification and sequencing

All 194 faecal DNA samples were screened for the presence of *Cryptosporidium* at the 18S rRNA locus and at the actin gene locus using a two-step nested PCR as previously described (Ng et al., 2006; Ryan et al., 2003), with the annealing temperature for the actin locus lowered to 55° C. All PCR amplification was carried out with positive controls (*C. hominis* DNA) and negative controls which contained no DNA. Secondary PCR products were separated by gel electrophoresis and fragments corresponding to the expected length were excised and purified using an MOBIO UltraClean 15 DNA purification kit (MOBIO, Carlsbad, California, USA). Purified PCR products were then sequenced using an ABI Prism Terminator Cycle Sequencing kit (Applied Biosystems, USA) according to manufacturer's protocol with the annealing temperature raised to the respective temperatures of the secondary PCR primers used.

2.4 Sequence and phylogenetic analysis

Nucleotide sequences were analyzed using ChromasPro version v2.3 (http://www.technelysium.com.au) and aligned with reference sequences of *Cryptosporidium* species and genotypes from GenBank using ClustalW (http://www.genome.jp/tools/clustalw). Neighbor joining analysis with evolutionary distances calculated using Tamura-Nei parameters and maximum likelihood analysis were conducted using MEGA version 5.05 (Tamura et al., 2011). A sequence of *Monocystis agilis*

(GenBank accession no. AF457127) was used as an outgroup for the 18S rRNA analysis, whereas a *Plasmodium falciparum* (GenBank accession no. M19146) sequence was used as an outgroup in the analysis of the actin gene. Bootstraping using 1,000 replicates was carried out to assess the reliability of inferred tree topologies. Sequences from a recent rat study in Australia (Paparini et al., 2012) were obtained from GenBank under accession numbers JX294358-JX294376. Representative sequences for each *Cryptosporidium* species/genotype generated from this study have been deposited in GenBank under accession numbers JX485388-JX485418.

3. Results

3.1 Prevalence of Cryptosporidium in rats and mice

PCR screening at the 18S rRNA gene locus and actin locus detected 50 *Cryptosporidium* positives from the 194 faecal DNA samples; a prevalence of 25.8% (95% CI: 19.8, 32.5). No *Cryptosporidium* was detected in the house mice, rice-field rat and the pacific rat. The prevalence in rats was 28.1% (95% CI: 26.1, 35.3) (Table 1). The highest proportion of positive isolates were identified from the Asian house rat (37/50) followed by the brown rat (13/50). The prevalence of *Cryptosporidium* was highest in the municipality of Calauan at 63% (95% CI: 48.7, 75.7) followed by Los Baños at 31.8% (95% CI: 18.6, 47.6). San Jose had the lowest prevalence of *Cryptosporidium* at 2.1% (95% CI: 0.3, 7.3) (Table 1).

3.2 Sequence and phylogenetic analysis of the 18S rRNA gene locus

Of the 50 positives identified at the 18S rRNA gene locus, sequence analysis was successful for 44 of these and 7 different *Cryptosporidium* species and genotypes were identified; *C. muris* (n=3), *C. scrofarum* (n=4), rat genotype I (n=1), rat genotype II (n=6), rat

genotype III (n=19), rat genotype IV (n=6) and *C. suis*-like genotype (n=5) (Table 2). Phylogenetic analysis revealed that rat genotypes I, II and III formed a separate clade from the other intestinal *Cryptosporidium* with rat genotypes II and III grouping closely, exhibiting a genetic similarity of 98.6% and 99.0% respectively with rat genotype I and 99.5% with each other (7 SNPs over 450 bp of sequence) (Figure 2). Intra-genotypic variation was observed within the rat genotype isolates identified in the present study at the 18S locus (Figure 2). Isolates clustering with rat genotype III exhibited genetic similarities ranging between 99.3-100%. Rat genotype IV isolates from the present study along with W19 genotype variants from a previous study (Jiang et al., 2005) formed a cluster, with genetic similarities ranging between 99.0-99.8% (Figure 2). The genetic similarity between rat genotype IV and its closest relative, the *Cryptosporidium* hamster genotype was 98.6%. Genetic variation was also observed within the *C. suis*-like genotype group with 0.2% genetic difference (1 bp difference) between isolates Phi 3, 8, 15, 26 and 68 to *C. suis*-like genotype (Figure 2).

3.3 Sequence and phylogenetic analysis of the actin gene locus

A total of 32 isolates were successfully sequenced at the actin locus and 6 different *Cryptosporidium* species and genotypes were identified; *C. parvum* (n=1), *C. muris* (n=3), rat genotype I (n=2), rat genotype III (n=4), rat genotype IV (n=8) and *C. suis*-like genotype (n=14). Phylogenetic analysis identified some lack of concordance between the two loci and evidence of mixed infections (Table 2). For example, isolate Phi 20 was characterized as *C. scrofarum* at the 18S locus but as *C. suis*-like genotype at the actin locus (Table 2). Similarly isolates Phi 11, Phi 13, Phi 27, Phi 30, Phi 62, Phi 71 and Phi 75 were characterized as rat genotype III at the 18S locus but as *C. suis*-like genotype at the actin locus (Table 2). Isolates Phi 66 and Phi 70 were characterized as rat genotype III and Phi 61 as *C. scrofarum* at the

18S locus but were characterized as rat genotype IV at the actin locus. A mixed infection with *C. parvum* was also identified at the actin locus in isolate Phi 58, which was characterized as rat genotype IV at the 18S locus. Unfortunately despite multiple attempts, representative isolates from rat genotype II could not be amplified and sequenced at the actin locus. Rat genotype I isolates (Phi 36 and 37) formed a separate group from rat genotype III isolates (Phi 12, 18, 69) with 93.7% similarity between the two genotypes and a 93.9% similarity to isolate Phi 19 (Figure 3). Isolate Phi 19, which was characterized at the 18S locus as rat genotype III showed 99.8% similarity to the other rat genotype III isolates (only 1 SNP) at the actin gene locus. Consistent with results from phylogenetic analysis at the 18S rRNA locus, rat genotype IV isolates grouped together forming a separate clade (Figure 3). *C. suis*-like isolates grouped closest to *C. suis* with genetic similarity ranging from 97.9% to 98.1% to *C. suis* and 99.2-99.8% with each other.

4. Discussion

In the Philippines, the Asian house rat (*R. tanezumi),* is the major rodent pest species in agricultural landscapes, particularly on the islands of Luzon and Visayas, and the rice-field rat (*R. argentiventer)* is the major pest species on the islands of Mindoro and Mindanao (Htwe et al., 2012). The other rodent species in this study, the brown rat (*R. norvegicus)*, the pacific rat (*R. exulans)* and the house mouse (*M. musculus)*, occur primarily in urban and peri-urban habitats. In the present study, *Cryptosporidium spp.* were mainly identified in brown rats at a prevalence of 18.6% (13/70) and Asian house rats at a prevalence of 44.6% (37/83). *Cryptosporidium* was not detected in rice field rats, pacific rats or house mice, which may be a result of small sample sizes (Table 1). Earlier studies in the US, UK and NZ, reported a prevalence of 30%, 33% and 24% and 11.8% respectively for *Cryptosporidium* in

the *musculus/domesticus* complex of species (Chalmers et al., 1994; Chilvers et al., 1998; Klesius et al., 1986). The lack of detection of *Cryptosporidium* in mice in the present study, could partially be a result of the small sample size and the fact that the majority of the mice (15/16) examined were trapped in San Jose, Mindoro, where a low prevalence of *Cryptosporidium* was identified (Table 1).

The overall prevalence of *Cryptosporidium* in wild rodents in the Philippines from this study was 25.8% (95% CI: 19.8%, 32.5%) with a prevalence of 28.1% (95% CI: 26.1, 35.3) in rats alone. This study falls within the mid-range of previously reported prevalence's of *Cryptosporidium* in rats from other geographical areas (Chalmers et al., 1997; Chilvers et al., 1998; Iseki, 1986; Kimura et al., 2007; Lv et al., 2009; Miyaji et al., 1989; Paparini et al., 2012; Sinski et al., 1993; Yamura et al., 1990). Of the 3 different municipalities sampled, Calauan had the highest prevalence at 63% (95% CI: 48.7, 75.7), followed by Los Baños at 31.8% (95% CI: 18.6, 47.6), with the lowest prevalence in San Jose, Mindoro, at 2.1% (95% CI: 0.3, 7.3). This large difference in prevalence of *Cryptosporidium* in rats may be due to differences in rodent density and/or the density of the human population in these areas. The latter, however, would not explain the higher prevalence of infection in Calauan versus Los Baños, because the densities of human households are higher in Los Baños, especially around the market place. Another possible influencing factor is that the samples were collected at different times of the year in the different localities. In the rice fields of Calauan and San Jose, there is a marked season variation in Asian house rats and rice-field rats (Htwe et al., 2012). A longitudinal study would be required to determine whether seasonal and/or rodent host densities are important factors influencing the prevalence of the different *Cryptosporidium spp*.

Of the *Cryptosporidium* species and genotypes identified in the rats in the present study, four have been linked to cryptosporidiosis in humans: *C. parvum* (n=1), *C. muris* (n=5), *C. suis*-like genotype (n=14) and *C. scrofarum* (n=4) (Kvac et al., 2009; Ong et al., 2002; Xiao, 2010). The majority of rats that were positive for *Cryptosporidium* however, were identified with host adapted genotypes, with rat genotype III being the most common species identified (38%) followed by rat genotype IV (22%). *C. suis*-like genotype however, was the second most common species identified in 28% of *Cryptosporidium* positive rat isolates. This is the first report of *C. suis*-like genotype and *C. scrofarum* in rats, with *C. suis*-like genotype identified mainly in Asian house rats and *C. scrofarum* isolates identified in both Asian house rats and brown rats, thus expanding its host range. Previously, the *C. suis*-like genotype only has been reported in humans in Canada and cattle in Denmark (Langkjaer et al., 2007; Ong et al., 2002) whilst *C. scrofarum* has previously been identified in pigs, cattle and humans in many geographical areas (Kvac et al., 2009; Langkjaer et al., 2007; Ong et al., 2002). Rats, however, may not be a natural host for *C. suis*-like genotype and *C. scrofarum* and the identification may have been as a result of mechanical transmission as pig entrails and blood are present at the markets and pigs are raised at some of the villages near to the rice fields.

This is the first report of rat genotypes in rats from the Philippines. Rat genotype I was previously identified from a boa constrictor, brown rats in Japan and was detected in raw water samples in China and the UK (Chalmers et al., 2010; Feng et al., 2009; Xiao et al., 2004). Rat genotype II, which has previously been described from Asian house rats in China (Lv et al., 2009) and from wild black rats (*Rattus rattus*) in Northern Australia (Paparini et al., 2012), was identified in both Asian house rats and brown rats in the present study, extending the rat host species range for this genotype. Rat genotype II was also the same parasite previously found in a sheep faecal sample in Australia (Ryan et al., 2005). In

Northern Australia, rat genotype III was identified from wild black rats (Paparini et al., 2012) however, in the present study, similar to the findings in China (Lv et al., 2009), rat genotype III was described from brown rats and Asian house rats. Note that Asian house rats have been reported in Australia but cannot be distinguished morphometrically from black rats (Aplin et al., 2011). Therefore, it is possible that the sample from northern Australia could be from an Asian house rat. Rat genotype IV (previously W19 genotype) has been identified in storm–water in the US (Jiang et al., 2005) and in brown rats in Japan (Kimura et al., 2007) and in the present study was identified in both brown rats and Asian house rats, thus extending the rat host species range for this genotype. In the present study, *C. muris* (which infects a range of rodents, other mammals and humans) was identified from brown rats and Asian house rats. Previous reports of *C. muris* in rats have been limited to morphological description of *C. muris*-like oocysts (RN66) in brown rats in Japan (Iseki, 1986; Iseki et al., 1989).

At the 18S locus, large intragenotypic variation within rat genotypes II, III and IV was observed, consistent with previous observations of rat genotype II and III isolates identified from black rats and rat genotype IV isolates from brown rats and storm water (Jiang et al., 2005; Kimura et al., 2007; Lv et al., 2009). However, at the actin locus, very little intragenotypic variation was seen amongst these rat genotypes. Some intragenotypic variation was observed within *C. suis*-like genotypes at the actin locus with the isolates clustering together, forming a separate clade from *C. suis.* This is the first study to analyse rat genotypes I, III and IV as well as *C. suis*-like genotype at the actin locus. A lack of concordance between the two loci was observed in 12 isolates, suggesting that mixed infections were present. Mixed infections are not uncommon in rats with reports of mixed infection with *C. tyzzeri* and rat genotype III and rat genotype II and rat genotype III in

previous studies (Lv et al., 2009; Paparini et al., 2012). Rat genotype II and rat genotype III appears to be genetically similar, exhibiting a 99.5% similarity at the 18S locus, with 7 SNPs over 450 bp of sequence. This genetic similarity was higher than previously reported (Lv et al., 2009). However, this difference is most likely due to the longer sequences (~ 730 bp) analysed by Lv et al (2009).

Findings from the present study suggest that a high genetic diversity of *Cryptosporidium* spp. exist in wild rodents in the Philippines, indicating high levels of transmission of *Cryptosporidium* amongst these wild rats. The potential of the rat genotypes identified in the present study to cause disease in humans or livestock is unknown. The identification of human infectious zoonotic species of *Cryptosporidium,* however, highlights the role these rodents play as reservoirs of the parasite. To have a better understanding of the extent of host adaptation for these rat genotypes, examination of a larger range of animal and human isolates across larger geographic regions and longitudinal studies are required as this will allow more accurate assessment of the role these rodents play in the transmission of *Cryptosporidium* to humans, particularly when rat and mice population explosions occur in the rural communities in the Philippines.

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Table 1: Prevalence of *Cryptosporidium* in wild rat and mouse samples collected from three different municipalities in the Philippines.

Table 2: *Cryptosporidium* species and genotypes identified in wild rats (*Rattus* sp.) in this present study.

Figure 1: Geographic locations on the islands of Luzon and Mindoro in the Philippines where trapping of wild rodents was conducted.

Figure 2: Phylogenetic inferences of *Cryptosporidium* isolates at the 18S gene locus by neighbor joining analysis of Tamura-Nei distances. Bootstrap test of support (>50%) from distance and maximum likelihood using 1000 replicates is indicated at the left of the supported node (nr = not resolved). Pairwise comparison included sequences from variants of the W19 genotype isolates from Jiang et al. (2005) are denoted by ^ and from rat isolates

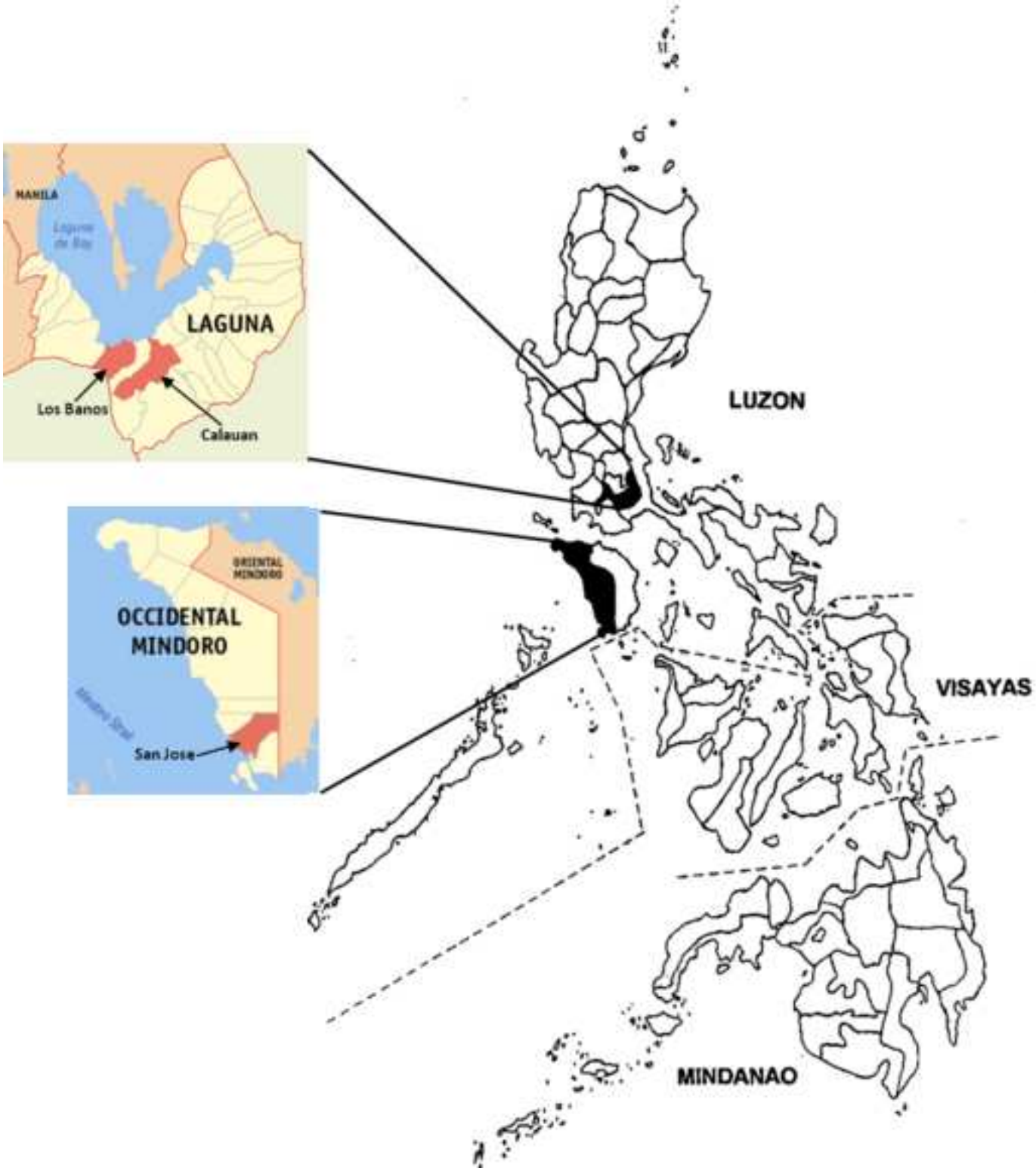
from Paparini et al. (2012) which are denoted by *. Genbank accession no. of sequences used to generate the phylogenetic tree is listed on the right in parentheses.

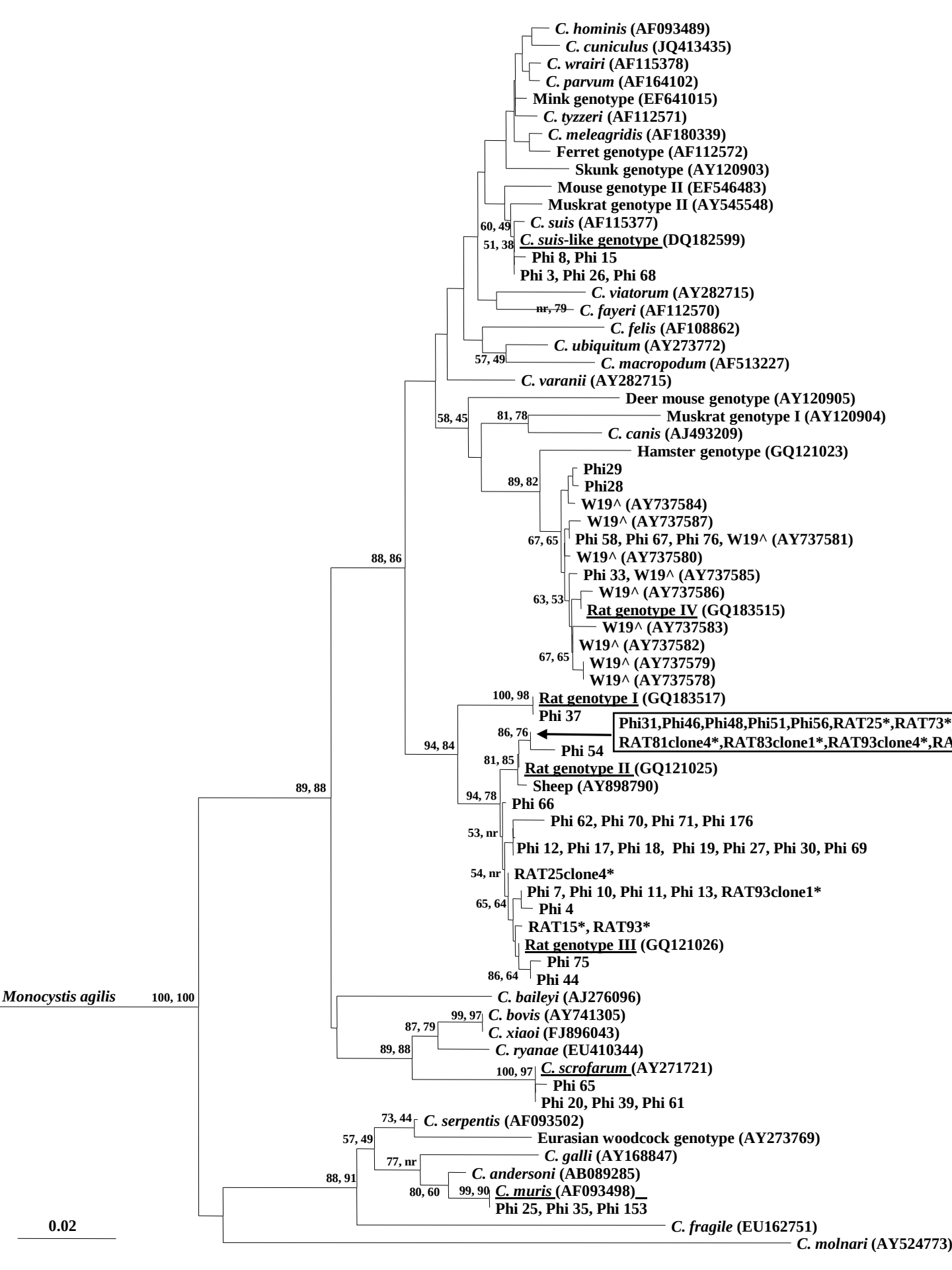
Figure 3: Evolutionary relationships of *Cryptosporidium* isolates at the actin gene locus inferred by neighbor-joining analysis of Tamura-Nei distances. Percentage bootstrap support (>50%) from 1,000 pseudoreplicates of distance and maximum likelihood analyses is indicated at the left of the supported node (nr = not resolved). Pairwise comparisons included DNA sequences from isolates from Paparini et al. (2012) and are denoted by *. Genbank accession no. of sequences used to generate the phylogenetic tree is listed on the right in parentheses.

Location	Species	Latin name	No. collected	No. of positive samples	Prevalence (%)
Los Banos					**31.8% (95% CI: 18.6, 47.6)**
	House mouse	*Mus musculus*	1	0	0
	Pacific rat	*Rattus exulans*	1	0	0
	Brown rat	*Rattus norvegicus*	18	0	0
	Asian house rat	*Rattus tanezumi*	24	14	58.3
Caluan					**63% (95% CI: 48.7, 75.7)**
	Brown rat	*Rattus norvegicus*	24	12	50
	Asian house rat	*Rattus tanezumi*	30	22	73.3
San Jose					**2.1% (95% CI: 0.3, 7.3)**
	House mouse	*Mus musculus*	15	0	0
	Rice-field rat	*Rattus argentiventer*	24	0	0
	Brown rat	*Rattus norvegicus*	28	1	3.6
	Asian house rat	*Rattus tanezumi*	29	1	3.4

Isolate Code	Location	Rat species	Identity at the 18S rRNA gene locus	Identity at the actin gene locus
Phi 1	Caluan	Asian house rat, *Rattus tanezumi*	-	*C. muris*
Phi 3	Caluan	Asian house rat, *Rattus tanezumi*	*C. suis*-like genotype	*C. suis*-like genotype
Phi 4	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	-
Phi 7	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	
Phi 8	Caluan	Asian house rat, *Rattus tanezumi*	*C. suis*-like genotype	*C. suis*-like genotype
Phi 9	Caluan	Asian house rat, *Rattus tanezumi*	-	Rat genotype IV
Phi 10	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	
Phi 11	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	*C. suis*-like genotype
Phi 12	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	Rat genotype III
Phi 13	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	*C. suis*-like genotype
Phi 15	Caluan	Asian house rat, *Rattus tanezumi*	*C. suis*-like genotype	*C. suis*-like genotype
Phi 17	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	
Phi 18	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	Rat genotype III
Phi 19	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	Rat genotype III
Phi 20	Caluan	Asian house rat, *Rattus tanezumi*	*C. scrofarum*	*C. suis*-like genotype
Phi 24	Caluan	Asian house rat, *Rattus tanezumi*	-	*C. suis*-like genotype
Phi 25	Caluan	Asian house rat, *Rattus tanezumi*	*C. muris*	
Phi 26	Caluan	Asian house rat, *Rattus tanezumi*	*C. suis*-like genotype	*C. suis*-like genotype
Phi 27	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	*C. suis*-like genotype
Phi 28	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype IV	
Phi 29	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype IV	Rat genotype IV
Phi 30	Caluan	Asian house rat, *Rattus tanezumi*	Rat genotype III	*C. suis*-like genotype
Phi 31	Caluan	Brown rat, *Rattus norvegicus*	Rat genotype II	
Phi 33	Caluan	Brown rat, *Rattus norvegicus*	Rat genotype IV	

Phi 35	Caluan	Brown rat, *Rattus norvegicus*	*C. muris*	*C. muris*
Phi 36	Caluan	Brown rat, *Rattus norvegicus*	-	Rat genotype I
Phi 37	Caluan	Brown rat, *Rattus norvegicus*	Rat genotype I	Rat genotype I
Phi 39	Caluan	Brown rat, *Rattus norvegicus*	*C. scrofarum*	
Phi 44	Caluan	Brown rat, *Rattus norvegicus*	Rat genotype III	
Phi 46	Caluan	Brown rat, *Rattus norvegicus*	Rat genotype II	
Phi 47	Caluan	Brown rat, *Rattus norvegicus*	-	*C. muris*
Phi 48	Caluan	Brown rat, *Rattus norvegicus*	Rat genotype II	
Phi 51	Caluan	Brown rat, *Rattus norvegicus*	Rat genotype II	
Phi 54	Caluan	Brown rat, *Rattus norvegicus*	Rat genotype II	
Phi 56	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype II	
Phi 58	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype IV	*C. parvum*
Phi 61	Los Banos	Asian house rat, *Rattus tanezumi*	*C. scrofarum*	Rat genotype IV
Phi 62	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype III	*C. suis*-like genotype
Phi 65	Los Banos	Asian house rat, *Rattus tanezumi*	*C. scrofarum*	
Phi 66	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype III	Rat genotype IV
Phi 67	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype IV	Rat genotype IV
Phi 68	Los Banos	Asian house rat, *Rattus tanezumi*	*C. suis*-like genotype	*C. suis*-like genotype
Phi 69	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype III	Rat genotype III
Phi 70	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype III	Rat genotype IV
Phi 71	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype III	*C. suis*-like genotype
Phi 73	Los Banos	Asian house rat, *Rattus tanezumi*	-	Rat genotype IV
Phi 75	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype III	*C. suis*-like genotype
Phi 76	Los Banos	Asian house rat, *Rattus tanezumi*	Rat genotype IV	
Phi 153	San Jose	Brown rat, *Rattus norvegicus*	*C. muris*	-
Phi 176	San Jose	Asian house rat, *Rattus tanezumi*	Rat genotype III	

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C. cuniculus (JQ413435)
C. wrairi (AF115378)
C. parvum (AF164102)
Mink genotype (EF641015)
C. tyzzeri (AF112571)
C. meleagridis (AF180339)
Ferret genotype (AF112572)
Skunk genotype (AY120903)
Mouse genotype II (EF546483)
Muskrat genotype II (AY545548)
60, 49
C. suis (AF115377)
51, 38
C. suis-like genotype (DQ182599)
Phi 8, Phi 15
Phi 3, Phi 26, Phi 68
C. viatorum (AY282715)
nr, 79
C. fayeri (AF112570)
C. felis (AF108862)
C. ubiquitum (AY273772)
57, 49
C. macropodum (AF513227)
C. varanii (AY282715)
Deer mouse genotype (AY120905)
58, 45
81, 78
Muskrat genotype I (AY120904)
C. canis (AJ493209)
Hamster genotype (GQ121023)
89, 82
Phi29
Phi28
W19^ (AY737584)
W19^ (AY737587)
67, 65
Phi 58, Phi 67, Phi 76, W19^ (AY737581)
88, 86
W19^ (AY737580)
Phi 33, W19^ (AY737585)
W19^ (AY737586)
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Rat genotype IV (GQ183515)
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RAT81clone4*,RAT83clone1*,RAT93clone4*,RA
86, 76
94, 84
Phi 54
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Rat genotype II (GQ121025)
Sheep (AY898790)
89, 88
94, 78
Phi 66
Phi 62, Phi 70, Phi 71, Phi 176
53, nr
Phi 12, Phi 17, Phi 18, Phi 19, Phi 27, Phi 30, Phi 69
54, nr
RAT25clone4*
Phi 7, Phi 10, Phi 11, Phi 13, RAT93clone1*
65, 64
Phi 4
RAT15*, RAT93*
Rat genotype III (GQ121026)
Phi 75
86, 64
Phi 44
Monocystis agilis
100, 100
C. baileyi (AJ276096)
99, 97
C. bovis (AY741305)
87, 79
C. xiaoi (FJ896043)
89, 88
C. ryanae (EU410344)
100, 97
C. scrofarum (AY271721)
Phi 65
Phi 20, Phi 39, Phi 61
73, 44
C. serpentis (AF093502)
Eurasian woodcock genotype (AY273769)
57, 49
C. galli (AY168847)
77, nr
C. andersoni (AB089285)
88, 91
80, 60
99, 90
C. muris (AF093498)
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C. fragile (EU162751)
C. molnari (AY524773)
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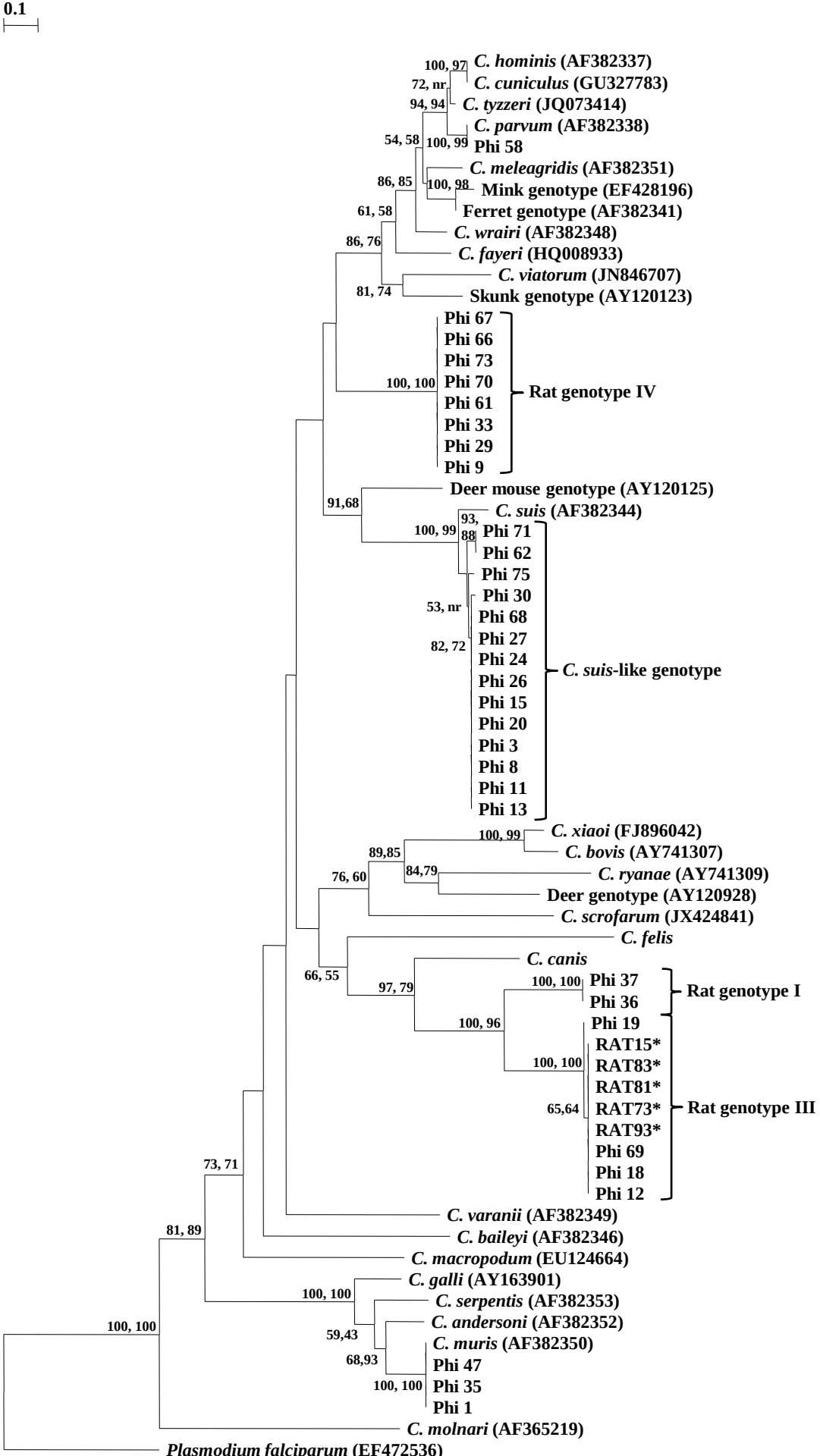
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100, 99
Phi 58
C. meleagridis (AF382351)
86, 85
100, 98
Mink genotype (EF428196)
Ferret genotype (AF382341)
61, 58
C. wrairi (AF382348)
86, 76
C. fayeri (HQ008933)
C. viatorum (JN846707)
81, 74
Skunk genotype (AY120123)
Phi 67
Phi 66
Phi 73
100, 100
Phi 70
Phi 61
Phi 33
Phi 29
Phi 9
Rat genotype IV
Deer mouse genotype (AY120125)
91,68
C. suis (AF382344)
93,
88
100, 99
Phi 71
Phi 62
Phi 75
Phi 30
53, nr
Phi 68
Phi 27
82, 72
Phi 24
Phi 26
Phi 15
Phi 20
Phi 3
Phi 8
Phi 11
Phi 13
C. suis-like genotype
100, 99
C. xiaoi (FJ896042)
89,85
C. bovis (AY741307)
84,79
C. ryanae (AY741309)
76, 60
Deer genotype (AY120928)
C. scrofarum (JX424841)
C. felis
C. canis
66, 55
97, 79
100, 100
Phi 37
Phi 36
Rat genotype I
100, 96
Phi 19
RAT15*
100, 100
RAT83*
RAT81*
65,64
RAT73*
RAT93*
Rat genotype III
Phi 69
Phi 18
Phi 12
73, 71
C. varanii (AF382349)
81, 89
C. baileyi (AF382346)
C. macropodum (EU124664)
C. galli (AY163901)
100, 100
C. serpentis (AF382353)
100, 100
C. andersoni (AF382352)
59,43
C. muris (AF382350)
68,93
Phi 47
100, 100
Phi 35
Phi 1
C. molnari (AF365219)
Plasmodium falciparum (EF472536)

Highlights

- High genetic diversity of *Cryptosporidium* sp. exists in wild rodents in the Philippines
- Human infectious species of *Cryptosporidium* were identified in the wild rodents
- First identification of *C. suis* and *C. scrofarum* in rats
- First study to analyze *Cryptosporidium* rat genotypes I, III and IV and *C. suis*-like genotype at the actin gene locus.
- Large intragenotypic variation at the 18S gene observed compared to the actin gene.