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Comparative analysis of *Tritrichomonas foetus* (Riedmüller, 1928) cat genotype, *T. foetus* (Riedmüller, 1928) cattle genotype and *Tritrichomonas suis* (Davaine, 1875) at 10 DNA loci ☆

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ABSTRACT

The parasitic protists in the genus *Tritrichomonas* cause significant disease in domestic cattle and cats. To assess the genetic diversity of feline and bovine isolates of *Tritrichomonas foetus* (Riedmüller, 1928) Wenrich and Emmerson, 1933, we used 10 different genetic regions, namely the protein coding genes of cysteine proteases 1, 2 and 4–9 (CP1, 2, 4–9) involved in the pathogenesis of the disease caused by the parasite. The cytosolic malate dehydrogenase 1 (MDH1) and internal transcribed spacer region 2 of the rDNA unit (ITS2) were included as additional markers. The gene sequences were compared with those of *Tritrichomonas suis* (Davaine, 1875) Morgan and Hawkins, 1948 and *Tritrichomonas mobilensis* Culberson et al., 1986. The study revealed 100% identity for all 10 genes among all feline isolates (=*T. foetus* cat genotype), 100% identity among all bovine isolates (=*T. foetus* cattle genotype) and a genetic distinctness of 1% between the cat and cattle genotypes of *T. foetus*. The cattle genotype of *T. foetus* was 100% identical to *T. suis* at nine loci (CP1, 2, 4–8, ITS2, MDH1). At CP9, three out of four *T. suis* isolates were identical to the *T. foetus* cattle genotype, while the *T. suis* isolate SUI-H3B sequence contained a single unique nucleotide substitution. *Tritrichomonas mobilensis* was 0.4% and 0.7% distinct from the cat and cattle genotypes of *T. foetus*, respectively. The genetic differences resulted in amino acid changes in the CP genes, most pronouncedly in CP2, potentially providing a platform for elucidation of genotype-specific host-pathogen interactions of *T. foetus*. On the basis of this data we judge *T. suis* and *T. foetus* to be subjective synonyms. For the first time, on objective nomenclatural grounds, the authority of *T. suis* is given to Davaine, 1875, rather than the commonly cited Gruby and Delafond, 1843. To maintain prevailing usage of *T. foetus*, we are suppressing the senior synomym *T. suis* Davaine, 1875 according to Article 23.9, because it has never been used as a valid name after 1899 and *T. foetus* is widely discussed as the cause of bovine trichomonosis. Thus bovine, feline and porcine isolates should all be given the name *T. foetus*. This promotes the stability of *T. foetus* for the veterinary and economically significant venereal parasite causing bovine trichomonosis.

1. Introduction

Flagellated protists in the genus *Tritrichomonas* Kofoid, 1920 are traditionally considered as mucus-dwelling parasites characterised by three anterior flagella (BonDurant and Honigberg, 1994). The best-studied member of the group is *Tritrichomonas foetus* (Riedmüller, 1928), a parasite of cattle. This parasite causes a sexually transmitted disease in cattle that can result in early embryonic death, infertility and abortion, even permanent sterility, which causes significant economic losses worldwide (Yule et al., 1989; BonDurant, 1997). Artificial insemination has successfully been used to control the disease, but in many parts of the world the parasite is still prevalent (Campero and Gottstein, 2007). In countries that have successfully eradicated the parasite, strict regulations to prevent a reintroduction are in place. The disease, termed bovine trichomonosis, remains notifiable in many countries around the world (including Australia, USA, Argentina, Switzerland, New Zealand and the Czech Republic).

☆ Nucleotide sequence data reported in this paper are available in GenBank under Accession numbers: JX187000–JX187133 and JX648146–JX648175.

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Recently, *T. foetus* was recognised as the causative agent of feline enteric trichomonosis (Levy et al., 2003; Gookin et al., 2004; Hale et al., 2009; Bell et al., 2010). In contrast to the bovine disease, infection in cats manifests as large-bowel disease with signs varying from asymptomatic carriage to intractable, chronic diarrhoea which may be continuous, or more commonly, follow a waxing and waning time course (Gookin et al., 2001; Foster et al., 2004). Feline trichomonosis is prevalent in typically young, densely housed populations of both pure and mixed breeds, with no sex predilection reported (Holliday et al., 2009; Stockdale et al., 2009). The disease has been recognised worldwide with the prevalence of *T. foetus* estimated to be as high as 30% (reviewed in Frey and Müller, 2012). The increased incidence of infection in multi-cat environments is believed to be due to the reliance on close, if not direct, contact between individuals (Gookin et al., 2004). *Tritrichomonas foetus* can survive up to 7 days in cat faeces, suggesting that grooming and faecal contamination of the environment may play an equal role in the epidemiology of feline enteric trichomonosis, especially in the multi-cat environment (Hale et al., 2009; Van der Saag et al., 2011). A treatment for feline enteric trichomonosis is available, i.e. ronidazole (Gookin et al., 2006; LeVine et al., 2011). However, the practise of treating only clinically affected animals, coupled with a lack of knowledge on disease epidemiology, has negative consequences for mitigating the spread of the pathogen. Despite the improvement in diagnosis and management of trichomonosis, little has been learned about the pathogenesis of the disease in cats.

At the cellular level, results by Stockdale et al. (2007, 2008) clearly demonstrate that significant differences exist in both infectivity and pathogenicity for feline *T. foetus* and bovine *T. foetus* isolates in experimentally infected cats and cattle. These combined results indicate that there are key differences between isolates influencing parasite-host species range. Recent reports show small but consistently detectable genetic differences between the cattle and cat isolates, suggesting the presence of two host-adapted genotypes of *T. foetus* (Šlapeta et al., 2010; Reinmann et al., 2012; Sun et al., 2012). The nucleotide and amino acid differences in the cysteine protease 8 between the cattle and cat genotypes of *T. foetus* offered the first insight into possible adaptation to its preferred host (Sun et al., 2012).

Soon after bovine trichomonosis was recognised and *T. foetus* named as the agent causing the disease, parasitologists debated the apparent similarity of *T. foetus* with the porcine trichomonad – *Tritrichomonas suis* – isolated from the nasal cavity, caecum and stomach of pigs. In fact the apparent similarity between *T. foetus* and *T. suis* became one of the parasitological enigmas. Hibler et al. (1960) reviewed the pig trichomonads isolated from nasal cavities and caeca of pigs, and concluded that if tritrichomonads from pigs and cattle were to be placed in a single species, the name *T. suis* would have priority over *T. foetus*. Levine (1973) stated that "... it might still be worthwhile to retain both names simply as matter of convenience" and Honigberg (1978) concluded that "... because much confusion would be created among parasitologists and veterinarians if the taxonomic status of especially *T. foetus* were changed, it seems advisable to postpone the implementation of such changes". However, none of these and more recent articles suggested any practical solution based on objective data (Tachezy et al., 2002; Lun et al., 2005; Frey and Müller, 2012).

The aim of this study was to use an array of protein coding genes to establish genetic conservation of the cattle and cat genotypes of *T. foetus* (Riedmüller, 1928) and *T. suis* (Davaine, 1875) from pigs, in particular, using the multigene cysteine proteases. To evaluate the genetic diversity these genotypes were compared with the most closely related taxon, *Tritrichomonas mobilensis* Culberson et al., 1986, from squirrel monkeys. Using 10 independent genetic loci, we confirmed genetic distinctness of the cattle and cat genotypes of *T. foetus*, and demonstrated identity of the cattle genotype of *T. foetus* with *T. suis*, which has prompted us to re-evaluate the nomenclature and taxonomy of the latter species.

2. Material and methods

2.1. Feline and bovine isolates of T. foetus, T. suis and T. mobilensis

Twenty isolates of *Tritrichomonas* spp. were obtained for this study (Table 1). Seven feline *T. foetus* isolates were included in this study (Hale et al., 2009; Šlapeta et al., 2010; Reinmann et al., 2012; Sun et al., 2012). Eight bovine *T. foetus* isolates were included in this study: one Australian reference strain YVL-W (Department of Agriculture, Fisheries and Forestry, Queensland), one reference strain from the Czech Republic (Department of Parasitology, Charles University, Prague), and six Argentinean isolates (Tachezy et al., 2002; McMillen and Lew, 2006; Reinmann et al., 2012). Four porcine *T. suis* were used to investigate their relationship to the bovine and feline isolates (Tachezy et al., 2002). In addition *T. mobilensis* was used in this study (Culberson et al., 1986). DNA dissolved in water was stored at −20 °C prior to amplification.

2.2. Primer design and PCR amplification

Primers were designed for the *Tritrichomonas* cysteine proteases (CP1, 2, 4–9) and cytosolic malate dehydrogenase 1 (MDH1) using sequences available from GenBank (Table 2). Primers were designed using CLC Main Workbench 6.2 (CLC bio, Denmark). Internal transcribed spacer (ITS) rDNA was amplified by PCR using primers TFR3 (5′-CGG GTC TTC CTA TAT GAG ACA GAA CC-3′) and TFR4 (5′-CCT GCC GTT GGA TCA GTT TCG TTA A-3′) according to Felleisen et al. (1998) and produced a 348 bp amplicon. All primers were synthesized by Macrogen Ltd. (Seoul, Korea).

All PCR amplifications were performed with MyTaq™ Red Mix (BioLine, Australia). Primers were added at a concentration of 0.25 μM each. The PCR was run using the following cycling conditions: 95 °C for 15 s, 55 °C for 15 s and 72 °C for 30 s for 35 cycles (40 cycles resulted in no difference in sequencing outcome, data not shown). All reactions were initiated at 95 °C for 5 min and concluded at 72 °C for 5 min. PCRs were amplified in the Eppendorf Mastercycler Personel or Eppendorf Mastercycler Gradient. Each PCR mix (40 μl) contained 2 μl of the sample DNA. All PCRs were run with negative controls (distilled water). Resulting products were resolved in 2% (w/v) agarose. All PCRs yielded unambiguous single bands of expected sizes (see Table 2). CP9 was further amplified using the MyTaq™ HS Mix (BioLine) and KAPA2G™ Fast HotStart ReadyMix (KAPA BioSystems, MA, USA). All sequences were directly and bidirectionally sequenced using amplification primers at Macrogen Ltd. Sequences were assembled, aligned with related sequences and analysed using CLC Main Workbench 6.2 and deposited in GenBank (National Center for Biotechnology Information, NCBI) under the Accession Numbers: JX187000–JX187133, JX648146–JX648175. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011). Multiple sequences alignments were appended and absent gene sequences coded as missing data. A phylogenetic tree was inferred using maximum likelihood and the bootstrap support inferred from 100 replicates.

3. Results

3.1. Tritrichomonas foetus cat genotype is distinct from T. foetus cattle genotype at 10 different loci

All 10 gene loci were successfully amplified using DNA of *T. foetus* from cats and *T. foetus* from cattle. PCR amplicons were

Table 1
Summary of *Tritrichomonas* spp. and strains used in this study.

Species	Strain	Host localisation	Origin	Isolation
Tritrichomonas foetus	Sydney-08/I	*Felis catus* rectum	Sydney, Australia	Šlapeta, McDonell 2008
Tritrichomonas foetus	Sydney-08/II	*Felis catus* rectum	Sydney, Australia	Šlapeta, McDonell 2008
Tritrichomonas foetus	Sydney-10/I	*Felis catus* rectum	Sydney, Australia	Šlapeta, McDonell 2008
Tritrichomonas foetus	IPA-Berne 2145	*Felis catus* rectum	Berne, Switzerland	Frey, Müller 2009
Tritrichomonas foetus	IPA-Berne 2458	*Felis catus* rectum	Berne, Switzerland	Frey, Müller 2009
Tritrichomonas foetus	IPA-Berne 360	*Felis catus* rectum	Berne, Switzerland	Frey, Müller 2010
Tritrichomonas foetus	FLI-Wusterhausen 64	*Felis catus* rectum	Wusterhausen, Germany	Henning, 2009
Tritrichomonas foetus	YVL-W	*Bos taurus* prepucium	Queensland, Australia	–
Tritrichomonas foetus	kV-1	*Bos taurus* prepucium	Žalmanov, Czech Republic	Lípová, Kulda 1957
Tritrichomonas foetus	INTA Balcarce 04796	*Bos taurus* foetal abomasal fluid	Villa Mercedes, Argentina	Campero, Fort 2004
Tritrichomonas foetus	INTA LP16	*Bos taurus* prepucium	Cuchillo Co, Argentina	Campero, Fort 2009
Tritrichomonas foetus	INTA Balcarce B1	*Bos taurus* pyometra	Balcarce, Argentina	Campero, Fort 1994
Tritrichomonas foetus	INTA Balcarce 04417	*Bos taurus* foetal abomasal fluid	Maipú, Argentina	Campero, Fort 2004
Tritrichomonas foetus	INTA LP 4	*Bos taurus* prepucium	Hucal, Argentina	Campero, Fort 2009
Tritrichomonas foetus	INTA LP115	*Bos taurus* prepucium	San Rafael, Argentina	Campero, Fort 2008
Tritrichomonas suis	1/N (ATCC 30167)	*Sus scrofa* nasal cavity	Ames, IA, USA	Buttrey, 1956
Tritrichomonas suis	11/S (ATCC 30168)	*Sus scrofa* stomach	Ames, IA, USA	Buttrey, 1956
Tritrichomonas suis	C19F (ATCC 30169)	*Sus scrofa* caecum	Logan, UT, USA	Hibler, 1959
Tritrichomonas suis	SUI-H3B	*Sus scrofa* caecum	Halle, Germany	Tachezy, 1988
Tritrichomonas mobilensis	M776 (ATCC 50116)	*Saimiri boliviensis boliviensis* rectum	Bolivia	Pindak, 1984

Table 2
Oligonucleotide sequences used to amplify protein coding genes of *Tritrichomonas* spp.

Gene	Acc. No.	Forward primer (5'-3')	Reverse primer (5'-3')	PCR size (bp)
CP1	U13153	S0293 (ggg gaa tga aga aac cca at)	S0294 (cat tgt ctg agc caa atc ca)	543
CP2	U13154	S0295 (cga aag gtc acg gat aca ca)	S0296 (ccc cat gag ttt ctc acg at)	709
CP4	X87777	S0305 (tca acg ccc ttc gtt tta ag)	S0306 (gat gca tca atg caa aca gc)	313
CP5	X87778	S0303 (ttt ccg agc aga atc tcg tt)	S0304 (gtc ttg ccc ttc agc act tc)	401
CP6	X87779	S0301 (cca agc cat cga atc agt tt)	S0302 (tcc tcc cca gta gag ttg ga)	358
CP7	X87780	S0299 (ccg aat cag cca atg cta tc)	S0300 (ata acc gac aca gcc gac ac)	413
CP8	EF610628, X87781	S0250 (atg ttt gca gtt ctt gct tcc c)	S0249 (tta ggc aac tgg gac gca g)	948
CP9	X87782	S0297 (tca gtg gca ttt tcc atg tc)	S0298 (ctt tgt tcc agc ctc agc tc)	329
MDH1	AF307994	S0307 (ccc acg taa gga tgg tat gg)	S0308 (cgc atg gga atg aga aaa ct)	599

CP, *Tritrichomonas* sp. cysteine protease; MDH, malate dehydrogenase.

DNA sequenced yielding 4,552 nucleotides (nt) for 11 of the studied isolates (six *T. foetus* from cats in Australia, Switzerland and Germany; five *T. foetus* from cattle in Australia, Czech Republic and Argentina). In total 50,072 nt were sequenced across 11 *T. foetus* isolates. Comparison of the DNA sequences confirmed 100% identity of cat isolates of *T. foetus* (=cat genotype) and 100% identity of cattle isolates of *T. foetus* (=cattle genotype). Comparison of DNA sequences between the cat and cattle genotype of *T. foetus* identified 1.03% (47/4,552) nt dissimilarity (Table 3). The two genotypes differed at 1.19% (44/3,693) of the nt sequence from the eight cysteine proteases (CP) and at 0.36% (2/562) of the nt sequence from the cytosolic MDH1. These nt changes coded for the total of 18 amino acid changes.

The most divergent gene was CP2 with 22 (3.29%) nt changes and 13 amino acids changes between the cattle and cat genotypes of *T. foetus* (Fig. 1A, Supplementary data 1). Although the cat and cattle genotypes differ by 13 amino acids it should be noted that the catalytic triad (Cys^{25}, His^{159} and Asn^{175}, papain numbering system) typical of the papain family are conserved between the genotypes. In addition, a single amino acid change was observed in CP4 (cattle > cat: Asp > Asn), CP5 (Asp > Asn) and CP6 (Ile > Phe), respectively (Fig. 1B). CP8 had two amino acid changes (Ser > Arg, Pro > Ala) (Fig. 1B). Moreover, there was a single nt mutation (GGA > TGA) in CP5 of *T. foetus* cat genotype coding for a Stop codon (TGA) in the canonical translation, while *T. foetus* cattle genotype GGA codes for the amino acid Glycine (Fig. 1B). In addition, there were two synonymous nt changes in MDH1. Sequencing of the ITS region confirmed the single nt difference at the ITS2, the original difference between the cat and cattle genotype (Šlapeta et al., 2010).

Table 3
Summary of nucleotide and amino acid differences between *Tritrichomonas foetus* cat genotype and *T. foetus* cattle genotype[a].

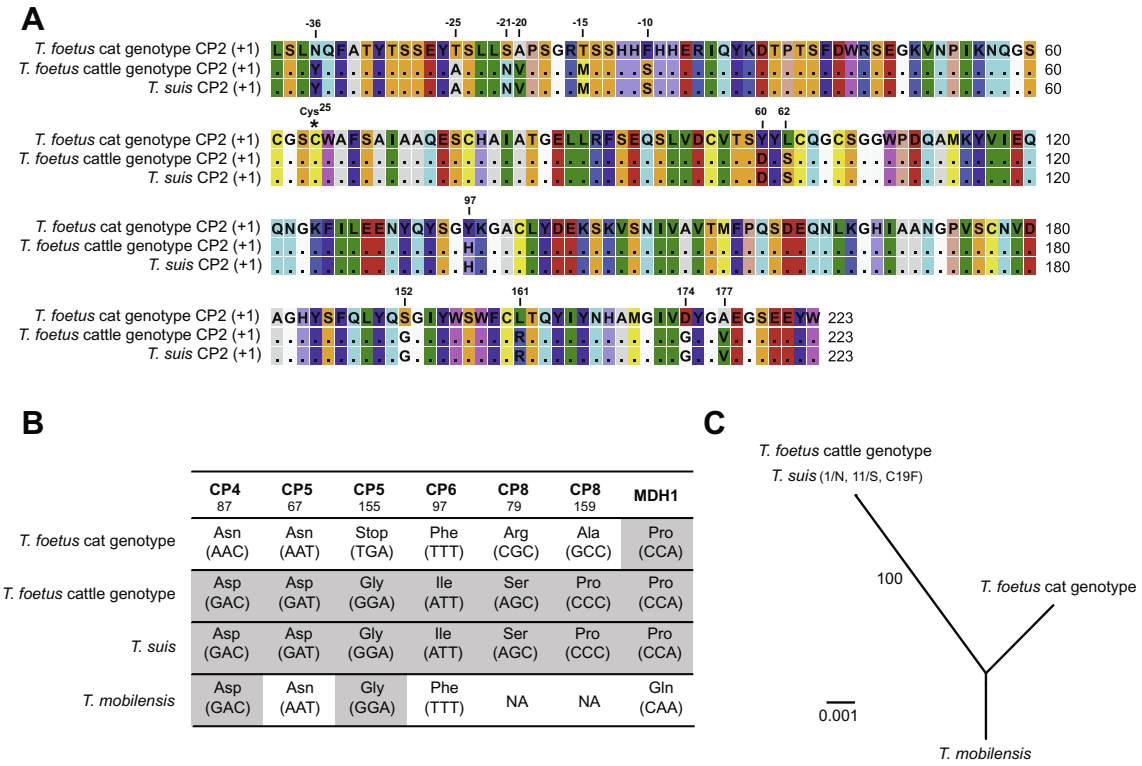
Gene	Sequence[b] (nt)	Nucleotide difference (%)	Amino acid difference
CP1	503	3 (0.60%)	0
CP2	669	22 (3.29%)	13
CP4	273	2 (0.73%)	1
CP5	361	3 (0.83%)	1 + Stop
CP6	318	6 (1.89%)	1
CP7	373	1 (0.27%)	0
CP8	907	5 (0.55%)	2
CP9	289	2 (0.69%)	0
MDH1	562	2 (0.36%)	0
ITS1 + 2	297	1 (0.34%)	NA
TOTAL	4,552	47 (1.03%)	18 + Stop

CP, *Tritrichomonas* sp. cysteine protease; nt, nucleotide; MDH, malate dehydrogenase; ITS, internal transcribed spacer.

[a] *Tritrichomonas foetus* cattle genotype is identical to *Tritrichomonas suis* 1/N (ATCC 30167), 11/S (ATCC 30168) and C19F (ATCC 30169); note SUI-H3B single autapomorphic residue, a derived substitution, in CP9 (see Section 3.2).

[b] PCR amplified and sequenced DNA without PCR primers.

We then focused our attention to CP2 and CP5 due to the high amino acid divergence and the presence of a Stop codon, respectively. We confirmed the polymorphism at these two loci for additional four isolates (one *T. foetus* from a cat in Australia; three *T. foetus* from cattle in Argentina). PCR amplicons were DNA sequenced yielding 1,030 nt for each isolate. In total an additional 4,120 nt were sequenced across four *T. foetus* isolates. Comparison of the DNA sequences confirmed 100% identity of cat isolates of *T.*



	CP4 87	CP5 67	CP5 155	CP6 97	CP8 79	CP8 159	MDH1
T. foetus cat genotype	Asn (AAC)	Asn (AAT)	Stop (TGA)	Phe (TTT)	Arg (CGC)	Ala (GCC)	Pro (CCA)
T. foetus cattle genotype	Asp (GAC)	Asp (GAT)	Gly (GGA)	Ile (ATT)	Ser (AGC)	Pro (CCC)	Pro (CCA)
T. suis	Asp (GAC)	Asp (GAT)	Gly (GGA)	Ile (ATT)	Ser (AGC)	Pro (CCC)	Pro (CCA)
T. mobilensis	Asp (GAC)	Asn (AAT)	Gly (GGA)	Phe (TTT)	NA	NA	Gln (CAA)

Fig. 1. Amino acid differences between *Tritrichomonas foetus* cat genotype, *T. foetus* cattle genotype, *Tritrichomonas suis* and *Tritrichomonas mobilensis*. (A) Multiple sequence alignment at cysteine protease 2 (CP2) of *T. foetus* and *T. suis*. CP2 was not amplified for *T. mobilensis*. (B) Variable residues of the cysteine proteases (CP4, CP5, CP6, CP8) and malate dehydrogenase (MDH)1 of *T. foetus, T. suis* (1/N, 11/S, C19F) and *T. mobilensis*. Codon sequences are indicated for each amino acid and Stop codon. Amino acids of cysteine proteases are numbered according to CP2 coordinated by the active cysteine (*) – Cys^{25} (papain numbering system) and then all residues in the mature peptide are positive numbers and those in the propepetide are negative numbers. CP8 was not amplified for *T. mobilensis* (NA). (C) Maximum Likelihood phylogenetic analysis (Tamura-Nei model) using all 10 loci and 4,552 alignment positions in the final dataset. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The number on the branch is the bootstrap support out of 100 replicates.

foetus (=cat genotype) and 100% identity of cattle isolates of *T. foetus* (=cattle genotype).

3.2. Tritrichomonas foetus cattle genotype is identical to T. suis

All 10 gene loci were successfully amplified using four isolates of *T. suis* DNA and subjected to DNA sequencing. Both the nasal cavity (1/N) and stomach (11/S) isolates of *T. suis* were 100% identical to *T. foetus* cattle genotype at all 4,552 nt of *T. suis* (Fig. 1, Tables 3 and 4). Two caecal isolates (C19F, SUI-H3B) of *T. suis* were analysed at 10 loci. The C19F was 100% identical to the cattle genotype of *T. foetus* and *T. suis* (isolates 1/N, 11/S). The SUI-H3B was 100% identical to the cattle genotype of *T. foetus* and *T. suis* (isolates 1/N, 11/S, C19F) at nine loci (CP1, 2, 4–8, ITS2, MDH1). At CP9, *T. suis* isolate SUI-H3B sequence contained a single nt substitution, a derived substitution that is unique to SUI-H3B isolate (Fig. 2), that changes the codon from GGT (Gly^{109}) in all other genotyped *Tritrichomonas* spp. isolates in this study to TGT (Cys^{109}). The substitution has been confirmed by CP9 amplification using three different DNA polymerases and bidirectional sequencing. Therefore, the nt distance of SUI-H3B isolate from *T. foetus* cattle genotype/*T. suis* is 0.02% (1/4,552).

3.3. Tritrichomonas mobilensis is closely related to T. foetus at six different loci

In addition to the previously published ITS primers, CP4–6, 9 and MDH1 primer sets (see Table 2) yielded positive PCR amplicons using *T. mobilensis* DNA. Amplicons were subjected to DNA sequencing. Across 2,100 nt sequence from the six loci, *T. mobilensis* differed at nine (0.43%) and 15 (0.71%) nts from *T. foetus* cat genotype and *T. foetus* cattle genotype/*T. suis*, respectively (Table 4, Fig. 1C). At the amino acid level, there were five amino acid changes between the studied *Tritrichomonas* spp. isolates. One amino acid change was in the MDH1, two in the CP5 and one in the CP4 and CP6 (Fig. 1B). CP9 was identical at the amino acid level for all isolates studied. Importantly, the Stop codon (TGA) identified in *T. foetus* cat genotype is not present in *T. mobilensis* CP5, but is identical to GGA (Gly) of *T. foetus* cattle genotype/*T. suis* CP5. Only one amino acid change was unique for *T. mobilensis* and confined to MDH1.

Table 4
Summary of nucleotide differences between *Tritrichomonas mobilensis* and *Tritrichomonas foetus*.

T. mobilensis	Nucleotide differences (%)	
	T. foetus cat genotype	*T. foetus* cattle genotype[a]
CP4	1 (0.37%)	1 (0.37%)
CP5	2 (0.55%)	3 (0.83%)
CP6	1 (0.31%)	5 (1.57%)
CP9	0 (0.00%)	2 (0.69%)
MDH1	3 (0.53%)	3 (0.53%)
ITS1 + 2	2 (0.67%)	1 (0.34%)

CP, *Tritrichomonas* sp. cysteine protease; MDH, malate dehydrogenase; ITS, internal transcribed spacer.

[a] *Tritrichomonas foetus* cattle genotype is identical to *Tritrichomonas suis* 1/N (ATCC 30167), 11/S (ATCC 30168) and C19F (ATCC 30169); note SUI-H3B single autapomorphic residue in CP9 (see Section 3.2, Fig. 2).

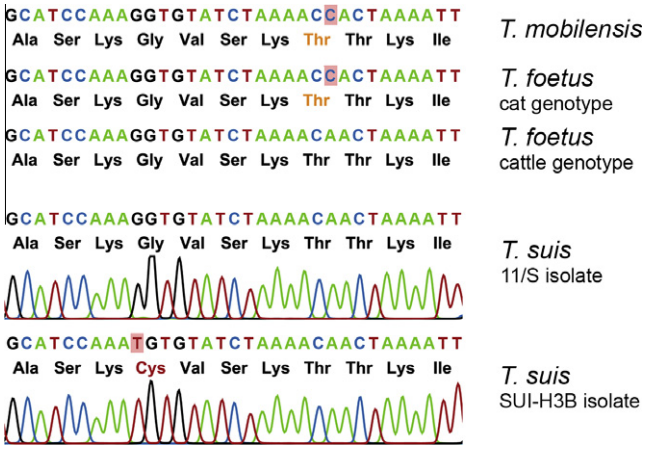


Fig. 2. Sequence alignment of *Tritrichomonas* sp. cysteine protease 9 (CP9). Sequence chromatograph of *Tritrichomonas suis* isolate SUI-H3B and 11/S is shown (primer S0297, Table 2). The sequences of *T. suis* isolate SUI-H3B contains a non-synonymous substitution coding for cysteine – Cys^{109}. Reverse primer sequence (S0297) confirmed (G > T) polymorphism. *Tritrichomonas suis* isolate 1/N and C19F had identical sequence to 11/S (chromatographs not shown).

4. Discussion

We have confirmed genetic conservation within each of the cattle and cat genotypes of *T. foetus* (Riedmüller, 1928) using a multigene family of cysteine proteases. A number of these enzymes are secreted by the parasite and as such represent key virulence factors. The extended presence of these proteases results in a series of cytotoxic effects on host cells and tissue structure (Singh et al., 2004, 2005; Lucas et al., 2008). Cysteine proteases have been detected in the cervical-vaginal mucus of cows infected with *T. foetus* (Yule et al., 1989). *Tritrichomonas foetus* cysteine proteases are reported to be multiform, lysosomal and are able to induce apoptosis of cultured bovine vaginal epithelial and uterine cells (Singh et al., 2004, 2005). The importance of this activity has been conclusively demonstrated using the cysteine protease inhibitor E-64, which inhibited the cytotoxicity and apoptosis in treated cells (Lucas et al., 2008). As with other protist parasites, these enzymes play a vital role in a number host-parasite interactions such as virulence, adherence, acquisition of nutrition as well as causing apoptosis of cells of the reproductive tract (Mallinson et al., 1995; Singh et al., 2004; Lucas et al., 2008). In the absence of the genome of *T. foetus* being available, it is predicted that the diversity of this protease family in *T. foetus* is large because 117 were identified in the genome of closely related human parasite *Trichomonas vaginalis* (Mallinson et al., 1995; Carlton et al., 2007). Our results, together with the initial CP8 results (Sun et al., 2012), demonstrate differences between *T. foetus* strains from cats and cattle, suggesting that these genes may play a role in adaptation to their preferred host or niche within the host. An intriguing finding is the Stop codon (TGA) in CP5 in the cat genotype of *T. foetus* that is an unusual nt substitution from GGA coding for glycine (Gly) in the cattle genotype. This Stop codon was found in all eight feline isolates tested and will require further elucidation.

We have used a collection of *T. foetus* isolates of bovine and feline origin. Each of the feline and bovine collections of isolates were 100% identical within themselves, which is a strong indication that each of these two global lineages has had a distinct, non-interbreeding evolutionary trajectory. Compared with existing knowledge on diversity of other significant intestinal parasites in cattle, such conservation of *T. foetus* genotypes at all 10 different loci is remarkable, because genotyping of *Giardia duodenalis* assemblages A and B at four independent loci could not unambiguously resolve assemblage B (Cacciò et al., 2008). Similarly, high gene flow is documented for the nematodes *Haemonchus contrortus* and *Haemonchus pacei* which have sheep and cattle as their preferred hosts, respectively (Brasil et al., 2012). The finding that all cat genotypes and all cattle genotypes were isolated from cats and cows is a strong indicator that under current conditions these two genotypes have their own distinct epidemiology. We therefore conclude that *T. foetus* genotypes are host-specific under natural circumstances and although experimental infections with the non-homologous genotype are possible under laboratory conditions, they do not result in the same disease outcome (Stockdale et al., 2007, 2008). Our results suggest that, based on the fact that cysteine proteases are directly linked to host-parasite interactions, the differences in the histopathological appearance in the experimental infection with bovine and feline isolates by Stockdale et al. (2007, 2008) may be related to the cysteine protease gene variation between the two genotypes of *T. foetus* isolates. Therefore, elucidating the genetic and functional diversity of cysteine proteases will improve our understanding of the pathogenesis of the feline enteric trichomonosis.

A further outcome of our study was to resolve the subjective synonymy of *T. foetus* and *T. suis*. The identity has previously been proposed based on morphological, biochemical, antigenic and genetic characteristics (Tachezy et al., 2002; Lun et al., 2005). For this purpose an array of all 10 loci was used to compare *T. foetus* with a reference strain of *T. suis*. For three strains of *T. suis*, we confirmed perfect identity at each locus with the cattle genotype of *T. foetus*. A single *T. suis* isolate contained a derived substitution in CP9 that is not present in any of the studied trichomonads. While the question as to why cysteine proteases are identical between bovine and porcine isolates remains to be explained, the identity of *T. suis* and *T. foetus* cattle genotypes led us to re-evaluate the nomenclature of *T. suis* and *T. foetus*. Here, we reveal that the correct name is *T. suis* (Davaine, 1875) (see Supplementary data 2). The organism now referred to as *T. suis* was noted by Gruby and Delafond (1843) in pig stomach, but their report did not introduce any name. For the first time we disclose that Davaine (1875) rather than Davaine (1877) formally introduced the name, and that the correct authority was never applied (Davaine, 1877; Dimock, 1921; Switzer, 1951; Hibler et al., 1960; BonDurant and Honigberg, 1994; Tachezy et al., 2002; Marquardt, 1954). Subsequent authors linked the name and the reference to the description, leading to a common misconception that the correct name should be *T. suis* (Gruby and Delafond, 1843) or *T. suis* (Davaine, 1877). However, reassessment of the published records leads us to argue that neither *T. suis* Gruby and Delafond, 1843 nor *T. suis* Davaine, 1877 are correct names. Our genotyping results (and those of Tachezy et al., 2002; Lun et al., 2005), imply that *T. suis* and (bovine) *T. foetus* are the same thing (i.e. taxonomic synonyms). To maintain the overwhelming prevailing usage of *T. foetus*, detailed in Supplementary data 2, we recommend suppressing the senior synomym *Trichomonas suis* Davaine, 1875 according to Article 23.9 (ICZN, 1999) because it has never been used as a valid name after 1899; and conserving the junior synonym *T. foetus*, which is the established name for the organism causing bovine trichomonosis. The name *T. foetus* was first applied by Riedmüller in 1928 while he was describing the bovine disease and the name remains in use in all texts for veterinary parasitologists, clinicians or international and national quarantine and disease organisations including the World Organisation for Animal Health (Riedmüller, 1928; Radostits and Arundel, 2000; Ortega-Mora et al., 2007 and references within; http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2012/). In such cases as *T. foetus*, we are justified in applying Article 23.9 to protect the name *T. foetus*, while *T. suis* becomes a nomen oblitum, and an unavailable name where synonymy is not in doubt (ICZN, 1999). In other words bovine, feline and porcine isolates should all be referred to as *T. foetus*.

Both cattle and cat genotypes of *T. foetus* are closely related to *T. mobilensis*. In its natural host, the Bolivian squirrel monkey, *T. mobilensis* is common but its potential pathogenicity remains unclear (Scimeca et al., 1989; Felleisen, 1997; Tachezy et al., 2002; Reinmann et al., 2012). The close relationships of the genotypes and taxa examined does not allow reliable resolution, because at the amino acid level (i.e. CP4, CP5, CP6, CP9, MDH1) *T. mobilensis* differs in only three amino acids from the *T. foetus* cattle genotype and in three amino acids from the *T. foetus* cat genotype (Fig. 1B). Across the same regions (CP4, CP5, CP6, CP9, MDH1) there are four amino acid differences between the two genotypes of *T. foetus*. These results demonstrate that the two genotypes of *T. foetus* are as distant from each other as they are from *T. mobilensis*. On the other hand, the further nt distance of *T. mobilensis* is supported by our inability to amplify CP1, CP2, CP7 and CP8 with the primers that successfully amplified these loci in both *T. foetus* genotypes.

In conclusion, using a 10 molecular marker approach we have genetically distinguished host adapted genotypes of *T. foetus,* confirming and considerably expanding previous findings based on one or two markers (Šlapeta et al., 2010; Reinmann et al., 2012; Sun et al., 2012). This study lays the foundation for elucidation of host specific pathogenesis using amino acid diversity in the family of cysteine proteases between the two genotypes of *T. foetus*. Furthermore, the study shows that *T. mobilensis* is closely related to, but distinct from, the cattle and cat genotypes of *T. foetus*. One of the practical applied outcomes is the suitability of using CP2 for genotyping isolates of *T. foetus.* The genetic difference in CP2 serves as the best single marker with 22 differences across an easily PCR-amplifiable 669 nt compared with single or very few nt difference in ITS2 or protein coding genes (Šlapeta et al., 2010; Reinmann et al., 2012). The implications which the 13 amino acid substitutions found in CP2 may have on host-parasite compatibility and host species range remain to be investigated. Last but not least, we recommend protecting the name *T. foetus* (Riedmüller, 1928). Such treatment promotes the stability and usage of *T. foetus* for the veterinary and economically significant venereal parasite causing bovine trichomonosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2012.10.004.

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