

**INFECTION OF TWO DISTINCT *TRICHURIS* SP. GENOTYPES
WITHIN AND AMONG BABOON (*PAPIO URSINUS*) TROOPS ON
THE CAPE PENINSULA, SOUTH AFRICA**

by

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Abstract

The chacma baboon (*Papio ursinus*) population on the Cape Peninsula, South Africa is divided into 16 geographically isolated troops, 14 of which are classified as being commensal with humans. Regular contact with humans in urban and agricultural settings may have increased the risk of transmission of the different parasite species identified within this population. The aim of the study was to identify whether two previously-identified genotypes of the whipworm, *Trichuris* sp., infect the same individual baboon simultaneously and whether both genotypes infect baboons of different troops on the Peninsula. Genomic DNA was extracted from adult *Trichuris* worms extracted from the gastrointestinal tract of six baboons from five different troops on the Peninsula. Two sets of primers were designed to amplify different sized products of the ITS1-5.8S-ITS2 region of the ribosomal DNA through PCR. Diagnostic PCR revealed the DG genotype among two *Trichuris* sp. specimens in a baboon from an unknown troop, while the CP-GOB genotype was observed among five specimens within a baboon from the Groot Olifantsbos troop. Sequence data confirmed the presence of a single genotype in each troop. This study suggests that the genotypes are specific to baboon troops but the potential for both genotypes to infect baboons within troops on the Peninsula cannot be ruled out. Knowledge of the specificity of the *Trichuris* genotypes to baboon hosts of different troops may inform our understanding of the evolution of diversity within this genus. Future research into the transmission of both genotypes within and between troops may also highlight the potential for two distinct species of *Trichuris* to exist among the baboons. Considering the close contact between baboons and humans on the Peninsula, clarification on host specificity of either genotype will also be important for managing zoonoses and preventing break-outs of infectious diseases between the species.

Introduction

The chacma baboon (*Papio ursinus*) population on the Cape Peninsula, South Africa forms 16 troops that are free-ranging (Hoffman & O’Riain 2012b). Fourteen of these troops are actively managed to reduce the spatial overlap between baboons and the mosaic of anthropogenically-disturbed habitats occupied by humans (Kaplan *et al.* 2011; Hoffman & O’Riain 2012a). Human population growth, urban sprawl and agriculture have led to a reduction in suitable habitat available to the troops, forcing them into frequent and close contact with humans in densely populated suburban areas, rural settlements and protected areas frequented by foreign tourists (Hoffman & O’Riain 2011; Kaplan *et al.* 2011; Drewe *et al.* 2012; Hoffman & O’Riain 2012a).

Habitat disturbance and interspecies cohabitation have negative implications for intrinsic troop factors, including feeding and ranging patterns (Gillespie 2006). These have downstream effects on the susceptibility of the baboons to infection by a number of parasites, especially helminthic nematodes (Hahn *et al.* 2003; Legesse & Erko 2004). Natural primate host-parasite dynamics, especially parasite transmission rates and virulence, have been altered dramatically in response to anthropogenic effects on the habitat and human encroachment (Hope *et al.* 2004; Gillespie *et al.* 2004). Logging and habitat fragmentation in African equatorial forests, for example, account for the higher parasite infection rates observed among the wild primate populations on forest edges than in the forest interior, as these activities promote contact between humans and non-human primates (Chapman *et al.* 2006; Gillespie 2006). Novel transmissions of the helminthic whipworm (*Trichuris* sp.) have been recently observed between humans and baboons (Ravasi 2009), and this has been attributed to their increasing contact on the Peninsula. The human-infecting species *Trichuris trichiura* displays 51% prevalence among humans, especially children, in informal settlements on the Peninsula (Adams *et al.* 2005) and 66% among the baboon troops (Ravasi *et al.* 2012a), suggesting a high risk of zoonotic or anthroponotic infection between populations (Drewe *et al.* 2012).

Trichuris sp. display a direct life cycle with eggs in soil and water infecting hosts via the faecal-oral route and adult worms developing within the gastrointestinal tract of the primary host (Freeland 1979; Lorenzetti *et al.* 2003; Kalema-Zikusoka *et al.* 2005). Direct effects on hosts, not including compromised competition for resources and mates, nutrition, reproduction and dispersal (Gillespie 2006), are associated with the inflammation of the mucosal lining and obstruction of the gut, particularly the large intestine, due to intense worm infestations, known as trichuriasis

(Lorenzetti *et al.* 2003; Liu *et al.* 2012a). This condition is often characterized by chronic anaemia and tissue damage, bloody diarrhoea, rectal prolapse and death (Lorenzetti *et al.* 2003). Infection is asymptomatic in low-intensity incidences but clinical signs of severe parasitosis, whether pathological or reflected in host condition, are generally similar between hosts (Martinez *et al.* 2003; Gillespie 2006; Gillespie & Chapman 2008).

Trichuris sp. are known to display host specificity (Muriuki *et al.* 1998; Liu *et al.* 2012a), despite the identification of porcine-derived *T. suis* and “heterozygote” worms with mixed sequence types among humans (Nissen *et al.* 2012) (a finding suggested by Ravasi *et al.* (2012b) to be the result of cross-contamination of samples). *Trichuris trichiura* is the whipworm species that is traditionally assumed to infect both humans and both wild and captive non-human primates on the Cape Peninsula, throughout Africa, Asia and America (Ooi *et al.* 1993; Kalema-Zikusoka *et al.* 2005; Kringel & Roepstorff 2006; Petrasova *et al.* 2012). The presence of *T. trichiura* in both of these hosts is suggested to have resulted from ancient cross-infection (Ravasi *et al.* 2012a), an event facilitated by the close phylogenetic relationship between humans and primates (Muriuki *et al.* 1998; Nunn *et al.* 2003; Trueba & Dunthorn 2012). Such examples highlight the threat of zoonotic (and anthroponotic) cross-transmission between non-human primates and humans (Hope *et al.* 2004; Drewe *et al.* 2012) and has raised the question of whether *T. trichiura* is host specific (Petrasova *et al.* 2012). Addressing this question may have important implications for our understanding of the low diversity of the *Trichuris* sp. assemblages observed among humans and simian primates in contrast with other mammalian host species, which is potentially the result of an underestimation of whipworm species richness in primate hosts (Petrasova *et al.* 2012).

Since the *Trichuris* genus was first identified and described, there has been considerable controversy around the differentiation and diagnosis of the species (Cutillas *et al.* 2009). Historical methods of identification that used the geographic (Grove 1990) or host species origin (Beer 1976), mode of transmission and pathological effects to define different whipworm species displayed poor distinguishing power and are likely responsible for this persistent controversy today (Gasser 2001; Liu *et al.* 2012a; Petrasova *et al.* 2012). Light and scanning electron microscopy approaches have also been used for differentiation among trichurids (Spakulova 1994; Lanfredi *et al.* 1995; Robles *et al.* 2006; Cutillas *et al.* 2009), following from the identification of several morphological criteria that were distinguishable among them (Schwartz 1926; Chandler 1930). Today, only a limited number of morphological and biometric features among adult worms, especially spicule length and presence of the spicular sheath, are considered reliable and are extensively adopted to describe *Trichuris* species

(Ooi *et al.* 1993; Oliveros *et al.* 2000; Cutillas *et al.* 2009; Robles 2011). The pericloacal papillae are often suggested to be the most informative feature for *Trichuris* species identification (Cutillas *et al.* 2007; Liu *et al.* 2012a).

Despite the use of these primary morphological traits to define *Trichuris* species (Oliveros *et al.* 2000), this approach is challenging for reliable species diagnosis however, as species often closely resemble each other due to overlapping character ranges and the sharing of common traits (Olivier *et al.* 2001; Cutillas *et al.* 2002; Robles 2011). Even among species that are accepted as biologically-distinct today, such as swine-derived *T. suis* and human- and primate-derived *T. trichiura* (Beer 1976; Kalema-Zikusoka *et al.* 2005; Cutillas *et al.* 2009), there is significant overlap in the ranges of the morphometric traits among female adult worms (Schwartz 1926; Ooi *et al.* 1993; Nissen *et al.* 2012). Furthermore, these traits are also expected to be phenotypic adaptations to structural or physiological characteristics of the host, including nutrition and levels of intra- and inter-specific competition among other parasites within the host (Oliveros *et al.* 2000; Nissen *et al.* 2012). These difficulties render morphological traits relatively uninformative in the unequivocal delimitation of *Trichuris* sp. and have likely contributed to gross misdiagnosis and underestimation of diversity within this genus, especially among non-human primate host species (Criscione *et al.* 2005; Petrasova *et al.* 2012; Guardone *et al.* 2013).

While morphological approaches have had limited success in distinguishing between *T. suis* and human- and simian primate-derived *Trichuris* sp. (Ooi *et al.* 1993; Cutillas *et al.* 2007), recent molecular evidence confirms that they are in fact two different species (Cutillas *et al.* 2009; Liu *et al.* 2012a; Nissen *et al.* 2012). In addition, the previous suggestion that *T. trichuria* derived from humans and non-human primates was the same species based on microscopy analysis of the pericloacal papillae (Ooi *et al.* 1993; Cutillas *et al.* 2009) has also since been addressed using biomolecular tools (Cutillas *et al.* 2002; Liu *et al.* 2012a, Liu *et al.* 2012b; Ravasi *et al.* 2012b). These tools, in combination with genetic data are increasingly used to replace these unsatisfactory traditional approaches to screen hosts, improve diagnostics and for the establishment of reliable parasite phylogenies (Olivier *et al.* 2001; Criscione *et al.* 2005; Guardone *et al.* 2013). Furthermore, molecular techniques present avenues to study parasite transmission, speciation and evolution of host specificity (Criscione *et al.* 2005; Jex *et al.* 2010).

Polymerase chain reaction (PCR)-based techniques can be used to identify different species, or genotypes within the same species of *Trichuris* by taking advantage of the genetic variability and

similarity within and between species (Martinez *et al.* 2003; Jex *et al.* 2010). PCR approaches are powerful tools for identification of trichurid species as their high sensitivity allows them to selectively amplify target gene sequences from the small amounts of DNA typically extractable from nematodes (Gasser 2001; Martinez *et al.* 2003). The target sequences that display considerably lower sequence variability within species than between species are suitable genetic markers for species identification (Gasser 2001). For identification of conspecific strains, however, substantially higher levels of intraspecific heterogeneity are required (Gasser 2001). These target sequences, often regions of nuclear ribosomal DNA (rDNA) or mitochondrial DNA (mtDNA) are then amplified using species-specific primers during PCR (Callejon *et al.* 2009; Cutillas *et al.* 2009; Jex *et al.* 2010).

The rDNA genetic material is a complex of multiple gene sequences that are relatively conserved within a species but display considerable interspecific heterogeneity in response to concerted evolutionary pressure (Gasser 1996; Cutillas *et al.* 2009). The internal transcribed spacer region (ITS1-5.8S-ITS2) of the rDNA in particular displays high interspecific sequence heterogeneity due to the weaker evolutionary constraint that its component ITS1 and ITS2 regions experience relative to the flanking 18S and 28S rRNA genes (and component 5.8S gene), which display high levels of sequence homogeneity (Gasser 2001; Olivier *et al.* 2001) (see Fig. 1). In addition, the ITS region is known to display very little, if any, variability in its sequence within a species (Oliveros *et al.* 2000), with 0.2 - 1.7% sequence variation reported for ITS-1 and 0-1.3% for ITS-2 in *T. trichiura* (Liu *et al.* 2012b). As a result, this region is commonly used as an accurate genetic marker in phylogenetic studies of closely related organisms (sister species) or genotypes and species strains (Olivier *et al.* 2001; Liu *et al.* 2007; Liu *et al.* 2012b; Ravasi *et al.* 2012b). The region has been used as a reliable marker in previous studies to distinguish between morphologically identical but genetically distinct species, including *T. suis* from wild boar and domestic pigs (Cutillas *et al.* 2007; Liu *et al.* 2012b), *T. vulpis* from dogs (Cutillas *et al.* 2007), *T. muris* and *T. arvicolae* from rodents (Cutillas *et al.* 2002), *T. trichiura* from humans (Liu *et al.* 2012b; Nissen *et al.* 2012; Ravasi *et al.* 2012b) and from non-human primates (*Colobus guereza kikuyensis* and *Nomascus gabriellae*) (Cutillas *et al.* 2009).

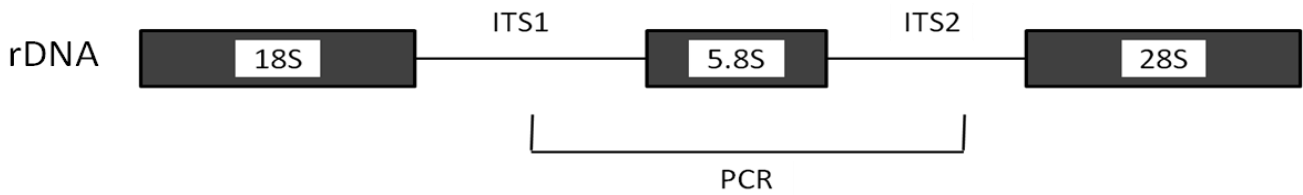


Figure 1. Schematic diagram of the internal transcribed region (ITS1-5.8S-ITS2) and flanking conserved 18S and 28S regions (rRNA genes) of the nuclear ribosomal DNA (rDNA). Shaded blocks represent gene subunits that are evolutionarily conserved with low sequence variability between species/genotypes. Lines represent non-coding, intergenic transcribed spacer regions that are not evolutionarily constrained and display high sequence variability between species/genotypes. The unscaled, generalized partial ITS1-5.8S-ITS2 region considered in this study is designated by “PCR”.

The ITS region was used by Ravasi *et al.* (2012b) to investigate whether a single species, *Trichuris trichiura*, infects both humans and baboons on the Cape Peninsula. The study revealed that *T. trichiura* displayed two distinct genotypes among both humans and non-human primates that are only 54% similar in their ITS sequences. One genotype was observed among whipworms from baboons of the Da Gama Park (DG) troop, as well as from humans in China, Thailand and the Czech Republic. *Trichuris* sp. individuals displaying this specific genotype cluster together to form the genetically distinct “DG clade” in the *Trichuris* phylogeny. The second genotype was observed among *Trichuris* sp. individuals derived from baboons from the Cape Peninsula (CP) and Groot Olifantsbos (GOB) troops. This genotype displayed similarity with *T. trichiura* specimens from humans in Cameroon, Uganda and Jamaica and from hamadryas baboons (*Papio hamadryas*), vervet monkeys (*Chlorocebus aethiops*) and chimpanzees (*Pan troglodytes*) in a Spanish zoo. These individuals cluster together into the “CP-GOB clade”, which shows stronger similarity (79%) to porcine-derived *T. suis* than the DG clade. From these conclusions, it was confirmed that *Trichuris* sp. from both clades are capable of infecting non-human primates and humans.

This study was aimed at further exploring the prevalence of infection with the two established *Trichuris* genotypes among baboons from five different troops on the Cape Peninsula. Through use of PCR-based techniques and the partial ITS1-5.8S-ITS2 region of rDNA (Fig. 1), we first investigated whether *T. trichiura* individuals of both the DG and CP-GOB clades cause multiple simultaneous infections (polyparasitism) in a single baboon host. Secondly, we investigated whether individuals from different troops on the Peninsula were infected by *T. trichiura* from either the DG

or CP-GOB clades only, or both clades. The study has implications for understanding whether cross-infection can occur among baboons within and among the different troops and, since all baboon troops come into contact with humans, whether there is zoonotic potential for either of the *T. trichiura* genotypes. We predict that if both genotypes have the potential to infect individuals of a single troop, contact among the troops will result in baboons throughout the Peninsula being infected by both genotypes. Alternatively, the prevalence of both genotypes will likely be higher within than between troops. Building on the ecological knowledge of parasitism on the Peninsula will also inform management decisions involving baboons and humans, especially if parasite diversity is associated with increasing cohabitation, to control outbreaks and the spread of infectious diseases by restricting the spatial overlap between populations on the Cape Peninsula.

Methods and Materials

Study site and host species

Our study took place on the Cape Peninsula, the most south-western portion of South Africa (33°55' - 34°21' S 18°25' - 18°28' E). This region extends from the densely-populated city of Cape Town to the Cape of Good Hope section of the Table Mountain National Park (TMNP) which is a major attraction to foreign tourists (MacDonald & Cowling 1996). The region is characterized by a Mediterranean climate with wet and cool winters, and mild, dry summers (Cowling *et al.* 1996). Forming an integral part of the Cape Floristic region, the study site supports a rich diversity of vegetation and invertebrates, despite the nutrient-poor soils and marked seasonal variation in annual rainfall (Cowling *et al.* 1996; Picker & Samways 1996). The region is generally undeveloped, indigenous fynbos but land has been modified for agriculture, residential and informal settlements and recreational demands (Richardson *et al.* 1996; Trinder-Smith *et al.* 1996).

The diversity and abundance of higher vertebrates is low in the region (Picker & Samways 1996), with chacma baboons (*Papio ursinus*) existing as the largest mammals and the only non-human primate species (Fraser 1994). The 16 troops, together comprising ca.470 individuals (Drewe *et al.* 2012) with an average of 34 individuals per troop (Hoffman & O'Riain 2011), are devoid of predators and are geographically isolated from other baboon populations in South Africa by human development, especially on the Cape Flats (Kansky & Gaynor 2000). Historically, it is unknown whether baboons came into contact with other mammalian or primate species on the Peninsula as this fauna was extirpated before accurate inventories were compiled (Skead 1980). The population is

broadly divided into a northern (Tokai) and southern subpopulation with limited movement of migrating males between the two (Beamish 2010).

Whipworms (*Trichuris* sp.) are ubiquitous parasites known to infect the gastrointestinal tract of chacma baboons. Co-infection by *Trichuris* and other helminthic nematodes, including individuals of the genera *Oesophagostomum*, *Trichostrongylus*, *Physaloptera* and *Ascaris*, has also been reported among the baboons (Ravasi 2009). The propensity for transmission of *Trichuris* is known to be high, especially among populations living in sympatry and sharing feeding and sleeping sites, and travel routes (Hahn *et al.* 2003; Hope *et al.* 2004).

Specimen collection

We obtained adult *Trichuris* sp. worms from routine necropsies on dead baboons delivered to the University of Cape Town, South Africa by conservation authorities between 2012 and 2013. The stomach and caecum, and randomly selected sections of the small intestine and large intestine were cut open and thoroughly inspected for adult *Trichuris* sp., identifiable by their thin, thread-like tail (Liu *et al.* 2012a).

We obtained worms from six different baboons from four troops, including Da Gama Park, Plateau Road, Tokai, and Groot Olifantsbos. We also obtained worms from a single baboon whose troop of origin on the Peninsula was unknown. All worms collected from the same individual were placed together into containers containing 70% ethanol and were stored at room temperature prior to use in this study.

DNA extraction

DNA was extracted from 17 adult *Trichuris* sp. worms. Two of the worms were from a single individual of the unknown troop (UNK_A and UNK_B), ten were from an individual of the Groot Olifantsbos troop (GOB_A – J), two were from the Tokai troop (TK_A and TK_B), and the remaining two were from the Da Gama park (DG_A) and Plateau Road (PR_A) troops.

Prior to the extraction process, all worms were separately rinsed in 0.9% saline solution. Each worm was then placed in lysis suspension, consisting of 180µl ATL lysis buffer (Qiagen, Venlo, Netherlands) and 20µl Proteinase K (Qiagen, Venlo, Netherlands), overnight at 56°C. Two steel balls were then added to each solution (*sensu* Ravasi *et al.* 2012b) and pulse-vortexed for 15s using a vortex mixer to ensure complete homogenization of the worms. Genomic DNA was then extracted

from each worm using a QIAamp DNA mini kit (Qiagen, Venlo, Netherlands) according to the manufacturer's Tissue Protocol, with the following modifications: 50µl of AE buffer was used to elute the DNA from the spin columns and elution was performed twice with an initial 20 min incubation period and 20 min interval between elutions. After the final centrifugation, the DNA was quantified at 260nm using a Nanodrop Spectrophotometer (NanoDrop Technologies, USA). Samples were also run on 2% (w/v) agarose gel stained with Ethidium Bromide (EtBr) (0.2µg/ml) at 100V for 30 mins and then visualized under UV using a G:Box BioImaging System (Syngene, Cambridge, UK). All genomic DNA samples were stored at -20°C until required.

Primer design and preliminary PCR

Primers NC2 and NC5, designed by Cutillas *et al.* (2002) and modified by Ravasi *et al.* (2012), were used to amplify the partial ITS1-5.8S-ITS2 region of the genomic DNA isolated from the *Trichuris* worms. Corresponding to the conserved 5' and 3' ends of the ITS1-5.8S-ITS2 region, these primers (here further referred to as the NC primers) are non-specific and amplify this region across all *Trichuris* species and genotypes (Gasser *et al.* 1996). We also designed unique primer pairs to amplify the partial ITS1-5.8S-ITS2 region of sequences within 1) the DG clade and 2) the CP-GOB clade using alignment sequences of this region for both genotypes of *Trichuris* sp. from humans and non-human primates obtained by Ravasi *et al.* (2012), as well as pigs (Table 1).

The specificity of the primers was tested in a preliminary PCR using the partial ITS1-5.8S-ITS2 sequences for DG and CP-GOB *Trichuris* cloned in pGEM-T plasmids from a previous study (Ravasi *et al.* 2012) as templates. The plasmids were isolated from archived *Escherichia coli* colonies using a Promega pGEM-T Easy Miniprep Kit (Promega, Madison, USA). A negative (no template) control was included in the PCR reaction for each set of primers. The PCR reaction mix used to amplify the partial ITS1-5.8S-ITS2 region was prepared using 2x KAPA Taq ReadyMix (KAPA Biosystems, Boston, USA), according to the manufacturer's protocol. 1µl of plasmid suspension (20.7 ng/µl DG plasmids; 21.3ng/µl CP-GOB plasmids) was used as the template in each reaction. The PCR reaction was performed using a GeneAmp 9700 Thermocycler (Applied Biosystems, California). The following conditions were applied: 95°C for 3 min (denaturing), 35 cycles at 95°C for 30 sec (denaturing), 55°C for 30 sec (annealing), and 72°C for 1 min (primer extension), followed by a single 1-min step at 72°C. PCR products were electrophoresed on 2%

(w/v) agarose gels stained with EtBr and visualized under UV. Negative (no template) controls were also run for each primer set.

Table 1. Genbank Accession Numbers for partial ITS1-5.8S-ITS2 sequences of *Trichuris* sp. extracted from humans, non-human primates and pigs used to design primers specific to sequences within the DG and CP-GOB clades in this study. An asterisk (*) indicates sequences identified by Ravasi *et al.* (2012).

Genbank Accession No.	Species	Host Species	Clade
AM992981	<i>Trichuris trichiura</i>	<i>Homo sapiens</i>	DG
GQ301553*	<i>Trichuris</i> sp.	<i>Papio ursinus</i> _DGI	DG
GQ301552*	<i>Trichuris</i> sp.	<i>Papio ursinus</i> _DGII	DG
GQ301551*	<i>Trichuris</i> sp.	<i>Papio ursinus</i> _DGIII	DG
GQ301555*	<i>Trichuris trichiura</i>	<i>Homo sapiens</i>	CP-GOB
GQ301554*	<i>Trichuris</i> sp.	<i>Papio ursinus</i>	CP-GOB
FM991956	<i>Trichuris trichiura</i>	<i>Colobus guereza</i>	CP-GOB
FM991955	<i>Trichuris trichiura</i>	<i>Nomascus gabriellae</i>	CP-GOB
AM993016	<i>Trichuris suis</i>	<i>Sus scrofa</i>	CP-GOB
AM993012	<i>Trichuris suis</i>	<i>Sus scrofa</i>	CP-GOB

Cloning and sequence analysis

Amplicons of each *Trichuris* sp. specimen produced by PCR with the NC5 and NC2 primers were then selected to be sequenced. Reactions that produced a single band (1300 – 1440 bp in size) on the electrophoresis gel were targeted. The amplified products of these specimens were then purified from the PCR reaction mixtures by centrifugation using the Wizard SV Gel and PCR Clean-up System (Promega, Madison, USA). The amplicons were then ligated into pGEM-T plasmid vectors overnight at 4°C according to the pGEM-T Easy Vector System protocol (Promega, Madison, USA). Following the same protocol, 5µl of each vector was then cloned into high-efficiency competent *Escherichia coli* (DH5α) cells and 100µl of each transformation culture was incubated overnight on LB/Amp/X-gal/IPTG plates at 37°C. Where possible, ten recombinant colonies (clones) were then re-plated and incubated overnight at 37°C.

Ten UNK-A and UNK-B clones and a range of three to five clones of each GOB specimen were screened for the partial ITS1-5.8S-ITS2 insert. The screening was performed by colony PCR using KAPA Taq Ready mix (KAPA Biosystems, Boston, USA) following the manufacturer's protocol. Clones which contained the correct insert were then incubated with shaking overnight in LB medium with 100mg/ml ampicillin at 37°C.

Plasmids containing the partial ITS1-5.8S-ITS2 insert were then isolated from the bacterial cells using the PureYield Plasmid Mini and Midi Prep Systems (Promega, Madison, USA) to obtain plasmid concentrations of minimum 100ng/μl for sequencing. These plasmids were then sequenced by the Central Analytical Facility at the University of Stellenbosch, South Africa using the following universal pGEM-T primers: Forward primer (M13F): 5'-GTAAAACGACGGCCAG-3' and reverse primer (M13R): 5'-CAGGAAACAGCTATGA-3'. Sequencing was performed from both directions using the dye terminator sequencing method. Sequences of the partial ITS1-5.8S-ITS2 inserts were then edited using BioEdit (version 7.0.9.0). The identity of the sequences was determined by comparison with those of archived *Trichuris* sp. specimens extracted from humans and non-human primates in a public database (GenBank, NCBI).

Results

Primer design

Two unique sets of primers were designed to discriminately amplify the partial ITS1-5.8S-ITS2 region of rDNA of the DG and CP-GOB genotypes (DG and CP-GOB primers, respectively) (Table 2). Both the DG and CP-GOB reverse primers had single G nucleotides added to their 5' ends to improve downstream cloning efficiency of the PCR amplicons into the pGEM-T vector. The 5' G of primer CP-GOB_F was exchanged with an A residue to eliminate the self-dimer-forming property of this primer. The sequences that the DG and CP-GOB primer pairs amplify in PCR are both nested within the region amplified by the conserved NC5 and NC2 primers. The primers were designed to sequences of the target region that were conserved within each clade (DG and CP-GOB) but highly variable between the clades (Fig. 2). Primers specific to the DG clade were not specific to any region of the sequences included in the CP-GOB clade, and vice versa. Additionally, the CP-GOB primers were also designed to distinguish between the CP-GOB genotype and *T. suis* derived from pigs (*Sus scrofa*) (Fig. 2), which cluster closely together in the CP-GOB clade and display high sequence similarity in the partial ITS1-5.8S-ITS2 region.

Table 2. Sequences of the forward (F) and reverse (R) primers used to amplify different regions of the partial ITS1-5.8S-ITS2 from genomic DNA extracted from *Trichuris* sp. The specific genotype targeted by the primers and size of the amplified sequence product following PCR with each primer set is also presented. Lower case residues represent G nucleotides added to improve primer amplicon cloning quality. NC5 and NC2 primers are universal primers for this region across all *Trichuris* species and have been adapted from Cutillas *et al.* (2002) according to Ravasi *et al.* (2012).

Primer	Target genotype	Sequence (5' – 3')	Amplicon Size
NC5 (F)	Universal	GTA GGT GAA CCT GCG GAA GGA	1300 – 1440
NC2 (R)		TCA TT gg TTA GTT TCT TTT CCT CCG CT	
DG_F	DG	ACA AAG CGG CCG TAG CTG	800
DG_R		gAA CCT CAA CCT GTA GCC TGA G	
CP-GOB_F	CP-GOB	ACG CCT GCC AAC AAC GAT	282
CP-GOB_R		gT CAG CGA AGC GCA GCT TA	


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                                160      170      180      190      200
                                .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
T trichiura_H sapiens AM992981 -----AGC
Trichuris_sp_P ursinus DGI GQ3 -----AGC
Trichurs_sp_P ursinus DGII GQ3 -----AGC
Trichuris_sp_P ursinus DGIII G -----AGC

T trichiura_H sapiens GQ301555 -----C
Trichuris_sp_P ursinus CP-GOB -----C
T trichiura_C guereza FM991956 -----GGC
T trichiura_N gabriellae FM991 -----GGC

T suis_S scrofa AM993016 -----AGC
T suis_S scrofa AM993012 -----AGC

                                210      220      230      240      250
                                .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
T trichiura_H sapiens AM992981 AGCAGCAGGCGCTGGT -----GTGGCGTTTG
Trichuris_sp_P ursinus DGI GQ3 AGCAGCAGGCGCTGGT -----GTGGCGTTTG
Trichurs_sp_P ursinus DGII GQ3 AGCAGCAGGCGCTGGT -----GTGGCGTTTG
Trichuris_sp_P ursinus DGIII G AGCAGCAGGCGCTGGT -----GTGGCGTTTG

T trichiura_H sapiens GQ301555 ATCATCGGGTGCTGGT --GACGCTCGTTGCTTCGGCAGTGTGGCGTTCA
Trichuris_sp_P ursinus CP-GOB ATCATCGGGTGCTGGT --GACGCTCGTTGCTTCAGCAGTGTGGCGTTCCG
T trichiura_C guereza FM991956 GGCACAGGGTGCCGGT --GACGCTCGTTTCTACGGCGGCGGACGT---
T trichiura_N gabriellae FM991 GGCACAGGGTGCCGGT --GACGCCGTTTCTACGGCGGCGGACGT---

T suis_S scrofa AM993016 AGCGTCAGGTGCTGGTGACGACGCTCGTTCTCCGGCATTGTGGCGTGT
T suis_S scrofa AM993012 AGCGTCAGGTGCTGGTGACGACGCTCGTTCTCCGGCATTGTGGCGTGT
                                * * *
                                +

                                260      270      280      290      300
                                .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
T trichiura_H sapiens AM992981 CTCGGT-----
Trichuris_sp_P ursinus DGI GQ3 CTCGGT-----
Trichurs_sp_P ursinus DGII GQ3 CTCGGT-----
Trichuris_sp_P ursinus DGIII G CTCGGT-----

T trichiura_H sapiens GQ301555 --CAGTCGGCCGCGTA-----CTTTGGCTCGACATGAGCGCGCG
Trichuris_sp_P ursinus CP-GOB --CAGTCGGCCGCGTA-----CTTCCGGCTCGACACGAGCGCGCG
T trichiura_C guereza FM991956 -----GAGCGCGCG
T trichiura_N gabriellae FM991 -----GAGCGCGCG

T suis_S scrofa AM993016 CGCAGTTGGCCGCCCC-----GTACTTGGGCGCGACGTGAGCGCGCG
T suis_S scrofa AM993012 CGCAGTTGGCCGCCCC-----GTACTTGGGCGCGACGTGAGCGCGCG
                                *

                                310      320      330      340      350
                                .....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
T trichiura_H sapiens AM992981 -----CCGATGCGAGC-----AACGTCCGCCGGCTTA
Trichuris_sp_P ursinus DGI GQ3 -----CCGATGCGAGC-----AACGTCCGCCGGCTTA
Trichurs_sp_P ursinus DGII GQ3 -----CCGATGCGAGC-----AACGTCCGCCGGCTTA
Trichuris_sp_P ursinus DGIII G -----CCGATGCGAGC-----AACGTCCGCCGGCTTA

T trichiura_H sapiens GQ301555 TTGCCGCTGCCGAGGAGAAC-----GGCGTGACCGTTGCA
Trichuris_sp_P ursinus CP-GOB TTGCCGCTGCCGAGGAGAAC-----AGCGTGACCGTTGCA
T trichiura_C guereza FM991956 TTGCCACTGCC--GAGAGC-----AGCGTCCACCGTTGCA
T trichiura_N gabriellae FM991 TTGCCACTGCC--GAGAGC-----AGCGTCCACCGTTGCA

T suis_S scrofa AM993016 TTGCCACTGCC--GTGAGC-----AGCGTCCACCGTTGCA
T suis_S scrofa AM993012 TTGCCACTGCC--GTGAGC-----AGCGTCCACCGTTGCA
                                * * * * *
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Figure 2 continued

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          360          370          380          390          400
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 C-----AGTTCCAGGCTGAA--GAGCGCAGGCGGGCGGC--
Trichuris_sp_P ursinus DGI GQ3 C-----AGTTCCAGGCTGAA--GAGCGCAGGCGGGCGGC--
Trichuris_sp_P ursinus DGII GQ3 C-----AGTTCCAGGCTGAA--GAGCGCAGGCGGGCGGC--
Trichuris_sp_P ursinus DGIII G C-----AGTTCCAGGCTGAA--GAGCGCAGGCGGGCGGC--

T trichiura_H sapiens GQ301555 GCACCAGC----ACTTCCAGATCCGAA--GAGCA----GGCCGCGC
Trichuris_sp_P ursinus CP-GOB CCAGC-----ACTTCCAGATCCGAA--GAGCA----GGCCGCGC
T trichiura_C guereza FM991956 CCAGC-----AGTTCCAGATCCGAA--GAGCA----GGTCGCGC
T trichiura_N gabriellae FM991 CCAGC-----AGTTCCAGATCCGAA--GAGCA----GGTCGCGC

T suis_S scrofa AM993016 CCACC-----ACTTCCAGATCCGAA--GCGCA----GGCCGCGC
T suis_S scrofa AM993012 CCACC-----ACTTCCAGATCCGAA--GCGCA----GGCCGCGC
                                     * * * *

          410          420          430          440          450
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 -----TCGTTCCG--CCCGGCGGGCGAGTCGTCGATCGTCGCGTGT--
Trichuris_sp_P ursinus DGI GQ3 -----GGCTCGTTCCG--CCCGGCGGGCGAGTCGTCGATCGTCGCGGT--
Trichuris_sp_P ursinus DGII GQ3 -----TCGTTCCG--CCCGGCGGGCGAGTCGTCATCGTCGCGGT--
Trichuris_sp_P ursinus DGIII G -----TCGTTCCG--CCCGGCGGGCGAGTCGTCGATCGTCGCGGT--

T trichiura_H sapiens GQ301555 TGGCTACGGTTCGTCGCCGCGTGTCTCGACGACTACGTGGCTACCGCCAGTA
Trichuris_sp_P ursinus CP-GOB TTGCTACGGTTCGTCGCCGCGTGTCTCGACGACTACGCGGTACCGCCAGT--
T trichiura_C guereza FM991956 TGACCACGGTTCGACCTG--CGCCGCTCGACACGGTGAACACCAGCAGTA
T trichiura_N gabriellae FM991 TGACCACGGTTCGACCTG--CGCCGCTCGACACGGTGAACACCAGCAGTA

T suis_S scrofa AM993016 TGGCTACGGTTCGTCCTG--CGCCGCTTGACTCGGTGATCACCACCAGTA
T suis_S scrofa AM993012 TGGCTACGGTTCCTCCTGCGCCGCGCTTGACTCGGTGATCACCACCAGTA
                                     * * * * * * * *
                                     +

          460          470          480          490          500
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 --CACCGCCTCGATCAGGCAGC-----AGCAATTTTCA
Trichuris_sp_P ursinus DGI GQ3 --CACCGCCTCGATCAGGCAGC-----AGCGGTTTTCA
Trichuris_sp_P ursinus DGII GQ3 --CACCGCCTCGATCAGGCAGC-----AGCAATTTTCA
Trichuris_sp_P ursinus DGIII G --CACCGCCTCGATCAGGCAGC-----AGCAATTTTCA

T trichiura_H sapiens GQ301555 CGACCCGCTGCAGACGGACGACTGCTGCTGCGTAGCTCAGTCAGCTAGCA
Trichuris_sp_P ursinus CP-GOB CGACCCGCTGCAGACGAACTGCGTA--GTAGCTCAGGCAGCTAGCA
T trichiura_C guereza FM991956 CCGCCGCTGCAGACGGACGACTGC----GCGGCTGAGTCAGCCAGCA
T trichiura_N gabriellae FM991 CCGCCGCTGCAGACGGACGACTGC----GCGGCTGAGTCAGCCAGCA

T suis_S scrofa AM993016 CGACCCGCTGCAGACGGACGACTGC----GTGGCTGAGTCAGTCGGCA
T suis_S scrofa AM993012 CGACCCGCTGCAGACGGACGACTGC----GTGGCTGAGTCAGTCGGCA
                                     ** * ** ** * * *
                                     * * **
                                     +

          510          520          530          540          550
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 -----CTTGCTGCTCGCCGCTGCGCG--
Trichuris_sp_P ursinus DGI GQ3 -----CTTGCTGCTCGCCGCTGCGCG--
Trichuris_sp_P ursinus DGII GQ3 -----CTTGCTGCTCGCCGCTGCGCG--
Trichuris_sp_P ursinus DGIII G -----CTTGCTGCTCGCCGCTGCGCG--

T trichiura_H sapiens GQ301555 CCAGCACCCGGCAGCCGCTAGACGTCGGTGCCTCGCGCGCCCTGCCAACAA
Trichuris_sp_P ursinus CP-GOB CCAGCA-----GCCGCTAGGCGTCCGTGCT--CGCGCGCTGCCAACAA
T trichiura_C guereza FM991956 TCGTTCG--AGCGGCCGGACGTCGGTGT--CCCGCGCTGCCAACAA
T trichiura_N gabriellae FM991 TCGTTCG--AGCGGCCGGACGTCGGTGT--CCCGCGCTGCCAACAA
                                     CP-GOB_F
T suis_S scrofa AM993016 TCGTTCG-----CGGGACGTCGGTGT--TGCTCGCTGCCAA--
T suis_S scrofa AM993012 TCGTTCG-----CGGGACGTCGGTGT--TGCTCGCTGCCAA--
                                     * * * *
                                     + +

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Figure 2 continued

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                    560       570       580       590       600
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 CAGTCACCGGTACCTGTTCCGTTGTGG--CCTCCGTGGCTGCGGCAGTT
Trichuris_sp_P ursinus DGI GQ3 CAGTCACCGGTACCTGTTCCGTTGTGG--CCTCCGTGGCTGCGGCAGTT
Trichuris_sp_P ursinus DGII GQ3 CAGTCACCGGTACCTGTTCCGTTGTGG--CCTCCGTGGCTGCGGCAGTT
Trichuris_sp_P ursinus DGIII G CAGTCACCGGTACCTGTTCCGTTGTGG--CCTCCGTGGCTGCGGCAGTT

T trichiura_H sapiens GQ301555 CGATGACCGGTACCTGTTCCGTTGGG--CCTCAGTAGCTGCGGCAGTG
Trichuris_sp_P ursinus CP-GOB CGATGACCGGTACCTGTTCCGTTGGG--CCTCAGTAGCTGCGGCAGTG
T trichiura_C guereza FM991956 CGATGACCGGTACCTGTTCCGTTGGG--CCTCAGTAGCTGCGGCAGTG
T trichiura_N gabriellae FM991 CGATGACCGGTACCTGTTCCGTTGGG--CCTCAGTAGCTGCGGCAGTG
CP-GOB_F →
T suis_S scrofa AM993016 CAACGACCGGTACCTGTTCCGTTGGG--CCTCAGTGGCTGCGGCAGTG
T suis_S scrofa AM993012 CAACGACCGGTACCTGTTCCGTTGGG--CCTCAGTGGCTGCGGCAGTG
* * * * *
+

                    610       620       630       640       650
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 TGGATCTGGCTGTCGTTAGCGCCGCGTTGCAGTCGACCGTGCTGC---
Trichuris_sp_P ursinus DGI GQ3 TGGATCTGGCTGTCGTTAGCGCCGCGTTGCAGTCGACCGTGCTGC---
Trichuris_sp_P ursinus DGII GQ3 TGGATCTGGCTGTCGTTAGCGCCGCGTTGCAGTCGACCGTGCTGC---
Trichuris_sp_P ursinus DGIII G TGGATCTGGCTGTCGTTAGCGCCGCGTTGCAGTCGACCGTGCTGC---

T trichiura_H sapiens GQ301555 TGGATCTGGCTGCCGTCAGCGCCGCGTTGCAACCGACCGTGCTGCTGC
Trichuris_sp_P ursinus CP-GOB TGGATCTGGCTGCCGTCAGCGCCGCGTTGCAACCGACCTGTGTCTGCTGC
T trichiura_C guereza FM991956 TGGATCTGGCTGCCGTCAGCGCCGCGTTGCAGCCGACCGTGCCGTCGC
T trichiura_N gabriellae FM991 TGGATCTGGCTGCCGTCAGCGCCGCGTTGCAGCCGACCGTGCCGTCGC

T suis_S scrofa AM993016 TGGATCTGGCTGCCGTTAGCGCCGCGTTGCAGCCGACCGTGCTGCTGC
T suis_S scrofa AM993012 TGGATCTGGCTGCCGTTAGCGCCGCGTTGCAGCCGACCGTGCTGCTGC
* * *
+

                    660       670       680       690       700
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 -----CCGCCAGTAGTTGCACTGGCGCTGAAGAAGCAGCG
Trichuris_sp_P ursinus DGI GQ3 -----CCGCCAGTAGTTGCACTGGCGCTGAAGAAGCAGCG
Trichuris_sp_P ursinus DGII GQ3 -----CCGCCAGTAGTTGCACTGGCGCTGAAGAAGCAGCG
Trichuris_sp_P ursinus DGIII G -----CCGCCAGTAGTTGCACTGGCGCTGAAGAAGCAGCG

T trichiura_H sapiens GQ301555 GGGCTGTCATCACGTCGCCGACCGCCCCCGGTGGCGTTGATGATGCGGCA
Trichuris_sp_P ursinus CP-GOB GGGCTGTCATCACGTCGCCGACCGCCCCCGGTGGCGTTGATGATGCGGCA
T trichiura_C guereza FM991956 GAGCTGTCATCACGTCGCCGACCGCTCCCGGTGGCGTTGATGAAGCGGCG
T trichiura_N gabriellae FM991 GAGCTGTCATCACGTCGCCGACCGCTCCCGGTGGCGTTGATGAAGCGGCG

T suis_S scrofa AM993016 GGGTTTTCATCACGTCGCCGAGC--CCCCCGGTGGCGTCGATGAAGCGGCA
T suis_S scrofa AM993012 GGGTTTTCATCACGTCGCCGAGC--CCCCCGGTGGCGTCGATGAAGCGGCA
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+

                    710       720       730       740       750
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 GCTAGCG-----TCGACGAGGTTCAAA
Trichuris_sp_P ursinus DGI GQ3 GCTAGCG-----TCGACGAGGTTCAAA
Trichuris_sp_P ursinus DGII GQ3 GCTAGCG-----TCGACGAGGTTCAAA
Trichuris_sp_P ursinus DGIII G GCTAGCG-----TCGACGAGGTTCAAA

T trichiura_H sapiens GQ301555 --TGCTATG-----TCGGTGAGGTTTAAA
Trichuris_sp_P ursinus CP-GOB --GTATG-----TCGGTGAGGTTTAAA
T trichiura_C guereza FM991956 GCACGACACG-----TCGGTGAGGTGTAAG
T trichiura_N gabriellae FM991 GCACGACACG-----TCGGTGAGGTGTAAG

T suis_S scrofa AM993016 --TGCTATG-----TCGGTGAGGTTTAAA
T suis_S scrofa AM993012 --TGCTATG-----TCGGTGAGGTTTAAA
* * * * *

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Figure 2 continued

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          760          770          780          790          800
...|...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 GAACAGCCGTT---CG---ACCTCGAGCATCGAGG---ACGGCT
Trichuris_sp_P ursinus DGI GQ3 GAACAGCCGTT---CG---ACCTCGAGCATCGAGG---ACGGCT
Trichuris_sp_P ursinus DGII GQ3 GAACAGCCGTT---CG---ACCTCGAGCATCGAGG---ACGGCT
Trichuris_sp_P ursinus DGIII G GAACAGCCGTT---CG---ACCTCGAGCATCGAGG---ACGGCT

T trichiura_H sapiens GQ301555 GAACGGCCGTT---CGCCTCCGTGAGCGAATCGGGGAAGG---ACGGCT
Trichuris_sp_P ursinus CP-GOB GAACGGCCGTT---CGCCTCCATGAGCGAATCGGGGAAGG---ACGGCT
T trichiura_C guereza FM991956 GAACGGCCGTT---CGCCACCTTGAGCGCAC---AAGAAAGCAACGGCT
T trichiura_N gabriellae FM991 GAACGGCCGTT---CGCCACCTTGAGCGCAC---AAGAAAGCAACGGCT

T suis_S scrofa AM993016 GAACGGCCGTT-----CGAACTCGAGCGCATCGAGGAACGCGACGGCT
T suis_S scrofa AM993012 GAACGGCCGTT-----CGAACTCGAGCGCATCGAGGAACGCGACGGCT
          *                *****
          +

          810          820          830          840          850
...|...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 CGTCGCCGGTTGGAAAAAGAAAAAGCAGCTTCA---CCAGTGC
Trichuris_sp_P ursinus DGI GQ3 CGTCGCCGGTTGGAAAAAGAAAAAGCAGCTTCA---CCAGTGC
Trichuris_sp_P ursinus DGII GQ3 CGTCGCCGGTTGGAAAAAGAAAAAGCAGCTTCA---CCAGTGC
Trichuris_sp_P ursinus DGIII G CGTCGCCGGTTGGAAAAAGAAAAAGCAGCTTCA---CCAGTGC

T trichiura_H sapiens GQ301555 CGTCGCCGGTTGG-----ATCTTCC---TAAATGACCAATGC
Trichuris_sp_P ursinus CP-GOB CGTCGCCGGTTGG-----ATCTTCG---TTCATGACCAATGC
T trichiura_C guereza FM991956 CGTCGCCGGTTGG-----ATCTCCG---TTTACGACCAATGC
T trichiura_N gabriellae FM991 CGTCGCCGGTTGG-----ATCTCCG---TTTACGACCAATGC

T suis_S scrofa AM993016 CGTCGCCGGTTGG-----ATCTTCGTTATTAAACGACCAATGC
T suis_S scrofa AM993012 CGTCGCCGGTTGG-----ATCTTCGTTATTAAACGACCAATGC
          * * *

          860          870          880          890          900
...|...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 ACAAGGCGTCGTCCTACTACGCA-CGCCGATGTGCCTTTCGACACGCAGAC
Trichuris_sp_P ursinus DGI GQ3 ACAAGGCGTCGTCCTACTACGCA-CGCCGATGTGCCTTTCGACACGCAGAC
Trichuris_sp_P ursinus DGII GQ3 ACAAGGCGTCGTCCTACTACGCA-CGCCGATGTGCCTTTCGACACGCAGAC
Trichuris_sp_P ursinus DGIII G ACAAGGCGTCGTCCTACTACGCA-CGCCGATGTGCCTTTCGACACGCAGAC

T trichiura_H sapiens GQ301555 AG-----AT-----AAGCTGCGCTTCGCTGAAGCGTAGG-
Trichuris_sp_P ursinus CP-GOB AG-----AT-----AAGCTGCGCTTCGCTGAAGCGACGCA
T trichiura_C guereza FM991956 AG-----AT-----AAGCTGCGCTTCGCTGAAGCGCAGGA
T trichiura_N gabriellae FM991 AG-----AT-----AAGCTGCGCTTCGCTGAAGCGCAGGA
          ← CP-GOB_R

T suis_S scrofa AM993016 AG-----AT-----AAGCTGCGCTTCGCTGAAGTGCAG-
T suis_S scrofa AM993012 AG-----AT-----AAGCTGCGCTTCGCTGAAGTGCAG-
          * * * * * * * *
          ++

          910          920          930          940          950
...|...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 ACTGCTGTCGGCGCAATGAGCGCTAGTAGCA-----
Trichuris_sp_P ursinus DGI GQ3 ACTGCTGTCGGCGCAATGAGCGCTAGTAGCA-----
Trichuris_sp_P ursinus DGII GQ3 ACTGCTGTCGGCGCAATGAGCGCTAGTAGCA-----
Trichuris_sp_P ursinus DGIII G ACTGCTGTCGGCGCAATGAGCGCTAGTAGCA-----

T trichiura_H sapiens GQ301555 -----
Trichuris_sp_P ursinus CP-GOB GG-----
T trichiura_C guereza FM991956 ACGAACTGTTGAGCGATGATGACCCCGGTACCTGTTCCGTCCGGGCCCTC
T trichiura_N gabriellae FM991 ACGAACTGTTGAGCGATGATGACCCCGGTACCTGTTCCGTCCGGGCCCTC

T suis_S scrofa AM993016 -----
T suis_S scrofa AM993012 -----

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Figure 2 continued

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          960          970          980          990          1000
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 -----
Trichuris_sp_P ursinus DGI GQ3 -----
Trichurs_sp_P ursinus DGII GQ3 -----
Trichuris_sp_P ursinus DGIII G -----

T trichiura_H sapiens GQ301555 -----
Trichuris_sp_P ursinus CP-GOB -----
T trichiura_C guereza FM991956 AGCGGCTGCGGCAGCGTGGATCAGGCTGCCGTTAGCGCCGCCGGTTGCAG
T trichiura_N gabriellae FM991 AGCGGCTGCGGCAGCGTGGATCAGGCTGCCGTTAGCGCCGCCGGTTGCAG

T suis_S scrofa AM993016 -----
T suis_S scrofa AM993012 -----

          1010          1020          1030          1040          1050
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 -----
Trichuris_sp_P ursinus DGI GQ3 -----
Trichurs_sp_P ursinus DGII GQ3 -----
Trichuris_sp_P ursinus DGIII G -----

T trichiura_H sapiens GQ301555 -----
Trichuris_sp_P ursinus CP-GOB -----
T trichiura_C guereza FM991956 CCGACCGTGCCGTCGCGAGCTGTTCATCACGTGCCGACCGCTCCCGGTGG
T trichiura_N gabriellae FM991 CCGACCGTGCCGTCGCGAGCTGTTCATCACGTGCCGACCGCTCCCGGTGG

T suis_S scrofa AM993016 -----
T suis_S scrofa AM993012 -----

          1060          1070          1080          1090          1100
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 -----
Trichuris_sp_P ursinus DGI GQ3 -----
Trichurs_sp_P ursinus DGII GQ3 -----
Trichuris_sp_P ursinus DGIII G -----

T trichiura_H sapiens GQ301555 -----
Trichuris_sp_P ursinus CP-GOB -----
T trichiura_C guereza FM991956 CGTTGATGCTGCGTCGTGCTATGTCGGTGAGGTGTAAGAAGCGTCCGTTTC
T trichiura_N gabriellae FM991 CGTTGATGCTGCGTCGTGCTATGTCGGTGAGGTGTAAGAAGCGTCCGTTTC

T suis_S scrofa AM993016 -----
T suis_S scrofa AM993012 -----

          1110          1120          1130          1140          1150
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 -----
Trichuris_sp_P ursinus DGI GQ3 -----
Trichurs_sp_P ursinus DGII GQ3 -----
Trichuris_sp_P ursinus DGIII G -----

T trichiura_H sapiens GQ301555 -----
Trichuris_sp_P ursinus CP-GOB -----
T trichiura_C guereza FM991956 GTCTCTTGAGCGCACCGAGGAGAGACGGCTCGTCGCCGGTTGGATCTTCG
T trichiura_N gabriellae FM991 GTCTCTTGAGCGCACCGAGGAGAGACGGCTCGTCGCCGGTTGGATCTTCG

T suis_S scrofa AM993016 -----
T suis_S scrofa AM993012 -----

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Figure 2 continued

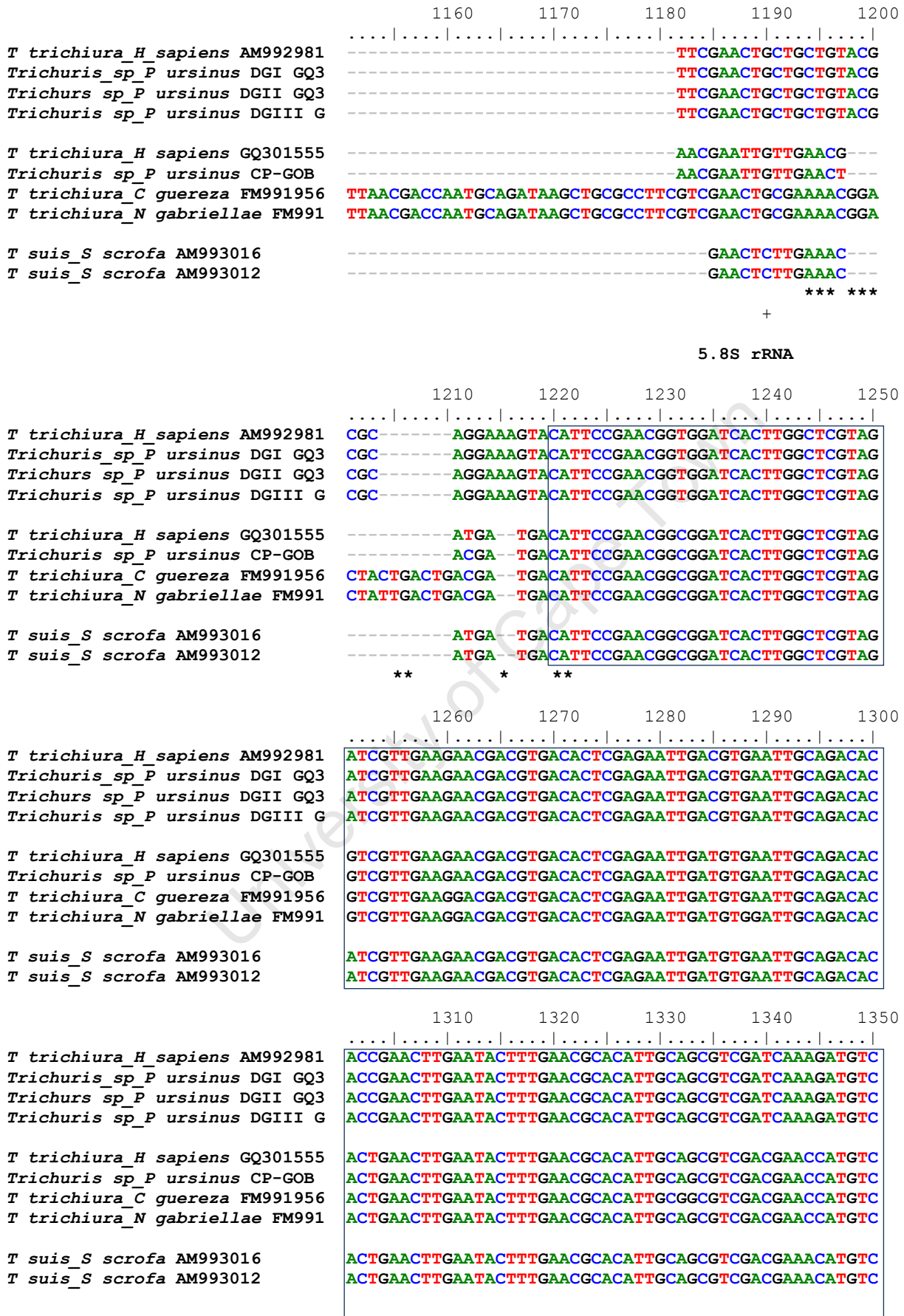


Figure 2 continued


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          1560      1570      1580      1590      1600
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 GATGGCAACTGGT-AGGCCGG-----AGCAGCGGAG
Trichuris_sp_P ursinus DGI GQ3 GATGGCAACTGGT-AGGCCGG-----AGCAGCGGAG
Trichuris_sp_P ursinus DGII GQ3 GATGGCAACTGGT-AGGCCGG-----AGCAGCGGAG
Trichuris_sp_P ursinus DGIII G GATGGCAACTGGT-AGGCCGG-----AGCAGCGGAG

T trichiura_H sapiens GQ301555 CGTCATCGCTGAC-AGGCAGCCG-----CAGCTTCTGCGGAG
Trichuris_sp_P ursinus CP-GOB CGTCATCGCTGAC-AGGCAGCCG-----GAGCTGCGGAG
T trichiura_C guereza FM991956 CGCTATCGCTGCC-AGGCAGACG-----GACCAGCGGAG
T trichiura_N gabriellae FM991 CGCTATCGCTGCC-AGGCAGACG-----GACCAGCGGAG

T suis_S scrofa AM993016 CGTCATCGCTGGC-AGGCAGCCG-----GAGCTGCGGAG
T suis_S scrofa AM993012 CGTCATCGCTGGC-AGGCAGCCG-----GAGCTGCGGAG
** ***** * *

          1610      1620      1630      1640      1650
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 AGCGGCCAAGTCAGCGTAGGGCGAAGACTACCCGACTTGGCTAC-CGGCC
Trichuris_sp_P ursinus DGI GQ3 AGCGGCCAAGTCAGCGTAGGGCGAAGACTACCCGACTTGGCTAC-CGGCC
Trichuris_sp_P ursinus DGII GQ3 AGCGGCCAAGTCAGCGTAGGGCGAAGACTACCCGACTTGGCTAC-CGGCC
Trichuris_sp_P ursinus DGIII G AGCGGCCAAGTCAGCGTAGGGCGAAGACTACCCGACTTGGCTAC-CGGCC

T trichiura_H sapiens GQ301555 AGCGGCTAACTCAGCGCAGTACGGAAGCTGCCCGAGTTGGCTATGTCGCT
Trichuris_sp_P ursinus CP-GOB AGCGGCCAACTCAGCGCAGTACGGAAGCTGCCCGAGTTGGCTATGTCGCT
T trichiura_C guereza FM991956 AGCGGCTAACTCAGCGCAGCAGGGAAGCTGCCCGAGTCCGCTACGTCGCC
T trichiura_N gabriellae FM991 AGCGGCTAACTCAGCGCAGCAGGGAAGCTGCCCGAGTCCGCTACGTCGCC

T suis_S scrofa AM993016 AGCGGCTAACTCAGCGCAGTACGGAAGCTGCCCGAGTTGGCTACGTCGCT
T suis_S scrofa AM993012 AGCGGCTAACTCAGCGCAGTACGGAAGCTGCCCGAGTTGGCTACGTCGCT
* * ** * ** * * **

          1660      1670      1680      1690      1700
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 GCG-CCGTCCG--GCGTACAGC-----AGTTG----AGCAGGG
Trichuris_sp_P ursinus DGI GQ3 GCG-CCGTCCG--GCGTACAGC-----AGTTG----AGCAGGG
Trichuris_sp_P ursinus DGII GQ3 GCG-CCGTCCG--GCGTACAGC-----AGTTG----AGCAGGG
Trichuris_sp_P ursinus DGIII G GCG-CCGTCCG--GCGTACAGC-----AGTTG----AGCAGGG

T trichiura_H sapiens GQ301555 ACA-TCGTCA--GCGTAAAGCCGGCGAACGACCGTTG----ACCACCG
Trichuris_sp_P ursinus CP-GOB ACA-TCGTCA--GCGTAAAGCCGGCGAACGACCGTTG----ACCACCG
T trichiura_C guereza FM991956 GCG-TCGTCCG-----GC-GCGACCGTCCGTTG----ACCACCG
T trichiura_N gabriellae FM991 GCG-TCGTCCG-----GC-GCGACCGTCCGTTG----ACCACCG

T suis_S scrofa AM993016 ACA-TCGTCCGTACCGTACAGC--GCGACTG--AGTTGTTTTACCACCG
T suis_S scrofa AM993012 ACA-TCGTCCGTACCGTACAGC--GCGACTG--AGTTGTTTTACCACCG
* * * * *
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          1710      1720      1730      1740      1750
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 AGC--GGTGACCGCACGCGCT-----CGTAGCAGCAAGTGTTCCGTCCG
Trichuris_sp_P ursinus DGI GQ3 AGC--GGTGACCGCACGCGCT-----CGTAGCAGCAAGTGTTCCGTCCG
Trichuris_sp_P ursinus DGII GQ3 AGC--GGTGACCGCACGCGCT-----CGTAGCAGCAAGTGTTCCGTCCG
Trichuris_sp_P ursinus DGIII G AGC--GGTGACCGCACGCGCT-----CGTAGCAGCAAGTGTTCCGTCCG

T trichiura_H sapiens GQ301555 AGC--GACCACTGCGGGCGCGAG-----CGCAGTCGTCCGTCTTCGTCCG
Trichuris_sp_P ursinus CP-GOB AGC--AACCATCGCGTCCGCGAG-----CGCAGTCGTCCGTCTTCGTCCG
T trichiura_C guereza FM991956 AGC--GACCACTCGCAATCGAG-----CGCAGTCGTCCGTCTTCGTCCG
T trichiura_N gabriellae FM991 AGC--GACCACTCGCAATCGAG-----CGCAGTCGTCCGTCTTCGTCCG

T suis_S scrofa AM993016 AGC--GACCACCCCGCGCGCGAC---CGTAGTCGTCCGTCTTCGTCA
T suis_S scrofa AM993012 AGC--GACCACCCCGCGCGCGAC---CGTAGTCGTCCGTCTTCGTCA
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Figure 2 continued

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          1760      1770      1780      1790      1800
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 TCGTTGCACGGCAGCAGCAGCAG--CAGCAGCAGTCGACGACGACGACG
Trichuris_sp_P ursinus DGI GQ3 TAGTTGCACGGCAGCAGCAGCAGCGGCAGCAGCAGTCGACGACGACGACG
Trichuris_sp_P ursinus DGII GQ3 TAGTTGCACGGCAGCAGCAGCAGCGGCAGCAGCAGTCGACGACGACGACG
Trichuris_sp_P ursinus DGIII G TAGTTGCACGGCAGCAGCAGCAGCGGCAGCAGCAGTCGACGAC--GACC

T trichiura_H sapiens GQ301555 CCGC-CCCCTAGATCGACG-----GCAGCGGCAGTCGACTAGAG-GACC
Trichuris_sp_P ursinus CP-GOB CCGC-CCCCTAGATCGACG-----GCAGCGGCAGTCGACTAGAA-GACC
T trichiura_C guereza FM991956 CCGC-TCCCTAGATCGACG-----GCAGTGGCAGTCGACTAGTA-GACC
T trichiura_N gabriellae FM991 CCGC-TCCCTAGATCGACG-----GCAGTGGCAGTCGACTAGTA-GACC

T suis_S scrofa AM993016 CCGG-CCCCTAGATCGACG-----GCAGCAGCAGTCGACTAGTA-GACC
T suis_S scrofa AM993012 CCGGCCCCCTAGATCGACG-----GCAGCAGCAGTCGACTAGTA-GACC
*  *** *  *** *  **  ***** * *
+ +

          1810      1820      1830      1840      1850
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 ACGAAACCGTTCGCCTTGCTGCG---GCGGCGGGCACCCTCGACCGAC
Trichuris_sp_P ursinus DGI GQ3 ACGAAACCGTTCGCCTTGCTGCG---GCGGCGGGCACCCTCGACCGAC
Trichuris_sp_P ursinus DGII GQ3 ACGAAACCGTTCGCCTTGCTGCG---GCGGCGGGCACCCTCGACCGAC
Trichuris_sp_P ursinus DGIII G ACGAAACCGTTCGCCTTGCTGCG---GCGGCGGGCACCCTCGACCGAC

T trichiura_H sapiens GQ301555 ACGACGGCGTCTGCTGCTACGCGCTCTGCGAGCGCGATCTCG-----GC
Trichuris_sp_P ursinus CP-GOB ACGACGGCGTCTGCTGCTACGCGCTCTGCGGCGGGCAGCGATCTCGGC
T trichiura_C guereza FM991956 ACGACGACG-----GCTGCTGCTGCTACGCGATCCGC
T trichiura_N gabriellae FM991 ACGACGACG-----GCTGCTGCTGCTACGCGATCCGC

T suis_S scrofa AM993016 ACGACG-----GCTGCTACGCGCTCCGCGGGCGGTCTGGCT-----GC
T suis_S scrofa AM993012 ATGACG-----GCTGCTACGCGCTCCGCGGGCGGTCTGGCT-----GC
*** ***** * * *
+ +

          1860      1870      1880      1890      1900
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 GACGGCGGCAAACGTTGCTGACACACTG--CTACCGGTCAGGCGGGCAG
Trichuris_sp_P ursinus DGI GQ3 GACGGCGGCAAACGTTGCTGACACACTG--CTACCGGTCAG--GCGAG
Trichuris_sp_P ursinus DGII GQ3 GACGGCGGCAAACGTTGCTGACACACTG--CTACCGGTCAG--GCGAG
Trichuris_sp_P ursinus DGIII G GACGGCGGCAAACGTTGCTGACACACTG--CTACCGGTCAG--GCGAG

T trichiura_H sapiens GQ301555 TGGCAGGGCAAACGTTGCTGACACACCGCTGCTACCCTTGTGCTGCTG
Trichuris_sp_P ursinus CP-GOB TGGCAGGGCAAACGTTGCTGACACACCGTTACTACCCTTGT-----G
T trichiura_C guereza FM991956 GGCGGGCAAACGTTGCTGACACACCGCTACTACCCTTGT-----G
T trichiura_N gabriellae FM991 GGCGGGCAAACGTTGCTGACACACCGCTACTACCCTTGT-----G

T suis_S scrofa AM993016 GCCGGCGGCAAACGTTGCTGACACTCCGCTACTACCCTG-----G
T suis_S scrofa AM993012 GCCGGCGGCAAACGTTGCTGACACTCCGCTACTACCCTG-----G
* * * * *
+ +

          1910      1920      1930      1940      1950
...|...|...|...|...|...|...|...|...|...|
T trichiura_H sapiens AM992981 CTGCCGGTA--GCGGCGTCTTTGCCGTTGTC--AACTAG-----CG
Trichuris_sp_P ursinus DGI GQ3 CTGCCGGTA--GCGGCGTCTTTGCCGTTGTC--AACTAG-----CG
Trichuris_sp_P ursinus DGII GQ3 CTGCCGGTA--GCGGCGTCTTTGCCGTTGTC--AACTAG-----CG
Trichuris_sp_P ursinus DGIII G CTGCCGGTA--GCGGCGTCTTTGCCGTTGTC--AACTAG-----CG

T trichiura_H sapiens GQ301555 CTGCTGGTAGCGGGCGGCTCTTTGCCGTACGCTGGTCAGTCA-----CG
Trichuris_sp_P ursinus CP-GOB CTGCTGGTAGCAGCGGCGTCTTTGCCGTACGCTGGTCACCGT-----CG
T trichiura_C guereza FM991956 CTGCTGGTAGCGGGCGGCTCTTTGCCGTACGCTGGTCACTCT-----CG
T trichiura_N gabriellae FM991 CTGCTGGTAGCGGGCGGCTCTTTGCCGTACGCTGGTCACTCT-----CG

T suis_S scrofa AM993016 CTGCTGGTAGCGGGCGGCTCTTTGCCGTACAGTGGTGACTCT-----CG
T suis_S scrofa AM993012 CTGCTGGTAGCGGGCGGCTCTTTGCCGTACAGTGGTGACTCT-----CA
* * * * *
++ +

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Figure 2 continued

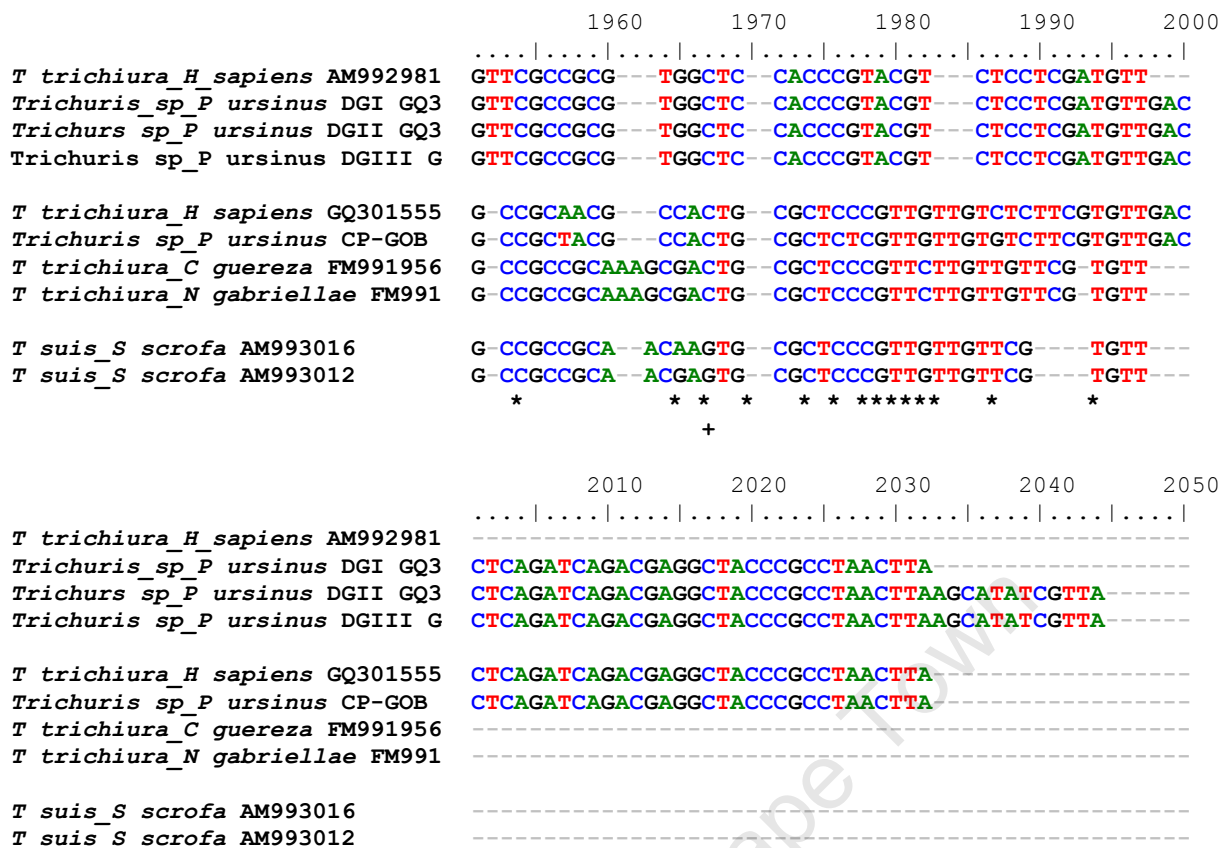


Figure 2 continued

Testing primers using preliminary PCR

Plasmids containing the partial ITS1-5.8S-ITS2 region of *Trichuris* sp. specimens extracted from baboons in the Cape Peninsula troop (GQ301554) (CP-GOB plasmids) and the Da Gama troop (GQ301553) (DG plasmids) (from Ravasi *et al.* 2012) were purified to yield concentrations of 21.3ng/μl and 20.7ng/μl, respectively. A preliminary PCR was performed to establish whether the NC primers were working and whether the DG and CP-GOB primers designed in this study were specific to the DG and CP-GOB genotype sequences, respectively. The three negative controls of the preliminary PCR, each containing a single set of primers and no template (plasmids), produced no bands on the electrophoresis gel (Fig. 3). The reactions containing the NC primers were also included as positive controls, since these primers are specific to the conserved ends of the ITS1-5.8S-ITS2 region across all *Trichuris* species (and genotypes). For these reactions, a band corresponding to the expected amplicon size of ca. 1400bp was produced when the DG plasmids, but not the CP-GOB plasmids, were used as the PCR template (Fig. 3).

As expected, the reaction containing the CP-GOB plasmids as the template and the CP-GOB primers produced a band (ca. 300bp) corresponding to the amplicon size of 282bp expected from this

reaction. No band was observed for the reaction where the DG plasmids were used as the template for the CP-GOB primers (Fig. 3). This confirms that the CP-GOB primers will amplify the CP-GOB genotype but not the DG genotype.

The DG primers produced a band corresponding to the expected amplicon size of 800 bp in the reaction containing the DG plasmids as the template (Fig. 3). However, a band, again corresponding to an amplicon size of 800bp was also produced when the CP-GOB plasmids were used as the template and no band was expected to be observed. Though the DG primers amplify the target region on DG plasmids, amplification of the CP-GOB genotype suggests that these primers may not be genotype-specific.



Figure 3. Diagnostic PCR using plasmids containing the partial ITS1-5.8S-ITS2 region from *Trichuris* sp. specimens with the DG genotype and CP-GOB genotype as templates and three different sets of primers. Lanes 1-3 are negative control reactions containing no template (plasmid) and the NC primers, DG primers and CP-GOB primers, respectively. Lane 1 is the GeneRuler 10,000bp DNA Ladder (Thermo Scientific, Bremen, Germany). Lanes 4-6 use DG plasmids as the template in reactions containing the NC primers, DG primers and CP-GOB primers, respectively. Lanes 7-9 use CP-GOB plasmids as the template in reactions containing NC primers, DG primers and CP-GOB primers, respectively.

The preliminary PCR was repeated using the CP-GOB plasmids to confirm that the DG primers were non-specifically targeting the CP-GOB genotype, in addition to its specific DG genotype target sequence. Again, the negative control produced no band. Additionally, the NC primers of the positive control did not amplify its target region and did not produce a band corresponding to an amplicon 1300-1440bp in size (Fig. 4). Again, the CP-GOB primers amplified their target region on the CP-GOB template, producing a band (ca. 300bp) corresponding to the amplicon size of 282bp expected from this reaction. A band corresponding to an 800bp product was also observed for the reaction containing DG primers. However, the intensity of this band was considerably lower than that produced by the CP-GOB primers (Fig. 4). Therefore, the band produced by the DG primers may be the result of upstream contamination of the CP-GOB plasmid stock with DG plasmids, or the incorrect ligation of the DG sequence into colonies containing CP-GOB sequence clones during cloning. These results, in addition to those above (Fig. 3), suggest that the DG and CP-GOB primers are specific to the DG and CP-GOB genotype sequences, respectively.

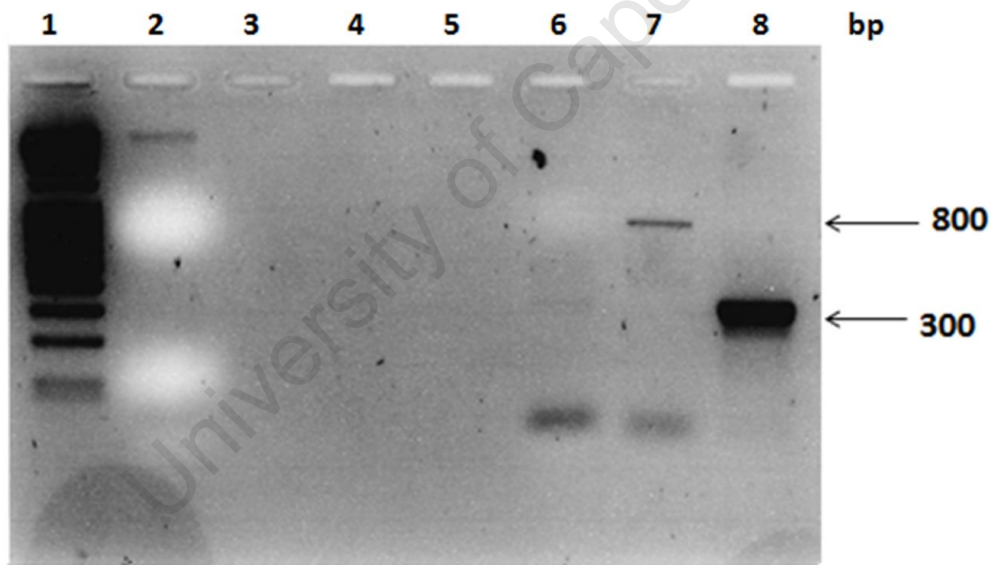


Figure 4. Preliminary PCR using plasmids containing the partial ITS1-5.8S-ITS2 region from *Trichuris* sp. with the CP-GOB genotype and three sets of primers. Lane 1 is the GeneRuler 10,000bp DNA Ladder (Thermo Scientific, Bremen, Germany). Lanes 2-5 are results not included in this study. Lanes 6-8 are reactions containing the CP-GOB plasmids as templates and NC primers, DG primers and CP-GOB primers, respectively.

Genomic DNA extractions

Genomic DNA was efficiently extracted from 17 *Trichuris* sp. specimens extracted from six different baboons from four troops and one unknown troop on the Cape Peninsula. The DNA samples were highly variable in concentration among the worms (Table 3). The NC primers amplified the ITS1-5.8S-ITS2 region of seven of the 17 DNA samples, corresponding to a 41.2% success rate of the positive controls. Not all samples amplified by the NC primers were amplified by the DG or CP-GOB primers. Two samples were amplified by the DG primers, while five were amplified by the CP-GOB primers, corresponding to a 41.2% of the primers in identifying the genotype of the specimens.

The quality of the genomic DNA of four of the *Trichuris* sp. specimens randomly selected from a baboon of the Groot Olifantsbos (GOB) troop was low, as indicated by the smeared bands produced following electrophoresis on a gel (Fig. 5a). In addition, the absence of distinct, intact bands produced for the genomic DNA from the *Trichuris* sp. extracted from baboons of the Da Gama park (DG), Tokai (TK) and Plateau Road (PR) troops on the electrophoresis gel suggests that the DNA is of very low quality (Fig. 5b). The genomic DNA from the *Trichuris* sp. specimens isolated from the unknown troop was of high quality (results not displayed).

Table 3. Summary of genomic DNA samples extracted from *Trichuris* sp. specimens from baboon hosts of four known and one unknown troops on the Cape Peninsula. The results of the PCR amplification of the ITS1-5.8S-ITS2 region using the NC primers, DG primers and CP-GOB primers are also presented. An X sign indicates positive PCR amplification by each primer set and a dash (-) represents unsuccessful PCR amplification.

<i>Trichuris</i> sp. sample	Troop	Purified DNA concentration (ng/μl)	PCR amplification with primers		
			NC	DG	CP-GOB
UNK_A	Unknown	233.2	X	X	-
UNK_B		177.6	X	X	-
GOB_A		52.8	-	-	-
GOB_B		107.6	X	-	X
GOB_C		69.1	X	-	-
GOB_D		57.7	-	-	X
GOB_E	Groot	90.5	-	-	-
GOB_F	Olifantsbos	48.3	X	-	X
GOB_G		95.0	X	-	-
GOB_H		90.1	X	-	X
GOB_I		73.4	-	-	-
GOB_J		41.6	-	-	X
DG_A	Da Gama Park	117.4	-	-	-
TK_A		11.5	-	-	-
TK_BI	Tokai	12.6	-	-	-
TK_BII		18.1	-	-	-
PR_A	Plateau Road	153.8	-	-	-

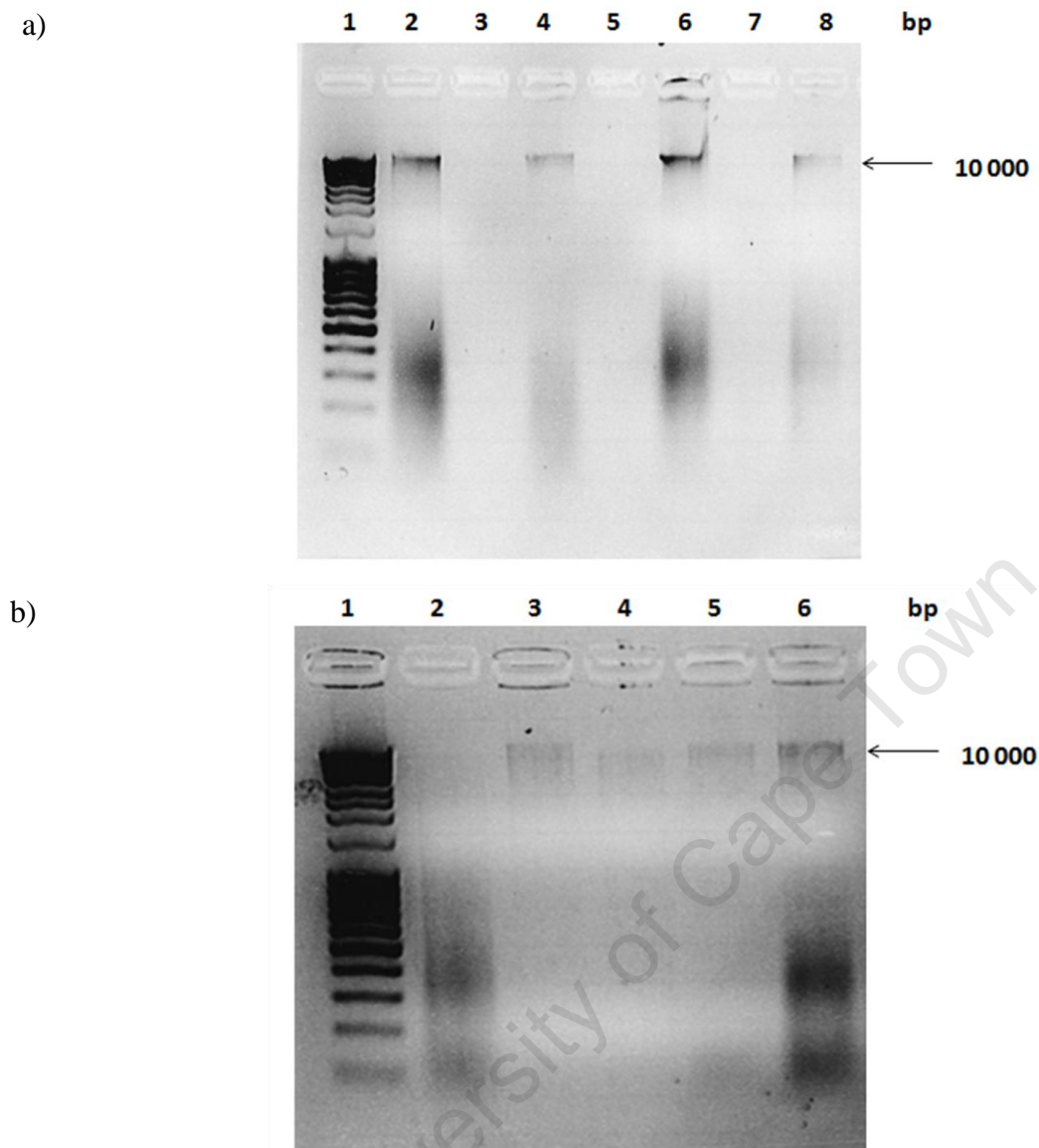


Figure 5. Quality of genomic DNA extracted from the following *Trichuris* sp. specimens: a) four worms isolated from a baboon of the Groot Olifantsbos (GOB) troop and b) five worms from baboons of five troops on the Cape Peninsula. Lane 1 (a and b) is the GeneRuler 10, 000bp DNA Ruler (Thermo Scientific, Bremen, Germany). a) Lane 2, 4, 6 and 8 is the genomic DNA for *Trichuris* sp. specimens GOB_B, GOB_C, GOB_F and GOB_H, respectively. Lanes 3, 5 and 7 contain no genomic DNA. b) Lanes 2-6 are the genomic DNA of *Trichuris* sp. specimens DG_A, TK_A, TK_BI, TK_BII and PR_A, respectively.

Genotype identifications by diagnostic PCR

We were able to identify the genotype of the two *Trichuris* sp. specimens isolated from baboons of the unknown troop (UNK_A and UNK_B) using a diagnostic PCR with the three sets of primers. Absence of bands on the electrophoresis gels for the negative (no template) controls confirms that samples were not contaminated and primers amplified their target regions only on the DNA templates included in each reaction (Fig. 6). The NC primers amplified the ITS1-5.8S-ITS2 region on both the UNK_A and UNK_B templates (Fig. 6). The band produced by this reaction corresponds to 1400 bp, which is within the expected amplicon size range (1300 – 1440bp) for these primers. The success of these positive control reactions suggests that the primers included in this diagnostic PCR are capable of amplifying their target region on the intact genomic DNA of these samples (the DNA is amplifiable).

The reaction including the genomic DNA of *Trichuris* sp. specimen UNK_A and the DG primers produced a band corresponding to an amplicon 800bp in size (Fig. 6). The band size corresponds to the PCR product size expected from a reaction including the DG primers. No band was produced for the reaction containing UNK_A and the CP-GOB primers (Fig. 6). The specificity of the DG primers to the ITS1-5.8S-ITS2 region of UNK_A genomic DNA and lack of product by the CP-GOB primers suggests that this specimen displays the DG genotype rather than the CP-GOB genotype.

Similar results were obtained for *Trichuris* specimen UNK_B, with a band produced for the reaction containing the DG primers, but not for that containing the CP-GOB primers (Fig. 6). The band produced by the reaction containing the DG primers corresponded to a PCR amplicon size of 800bp which, again, is the size of the product expected from a PCR reaction with the DG primers. The amplification of the ITS1-5.8S-ITS2 region of the genomic DNA of UNK_B by the DG primers but not the CP-GOB primers suggests that this *Trichuris* sp. specimen also displays the DG genotype. Therefore, both *Trichuris* sp. specimens UNK_A and UNK_B isolated from the same baboon of the unknown troop on the Cape Peninsula share the same genotype and both fall within the DG clade.

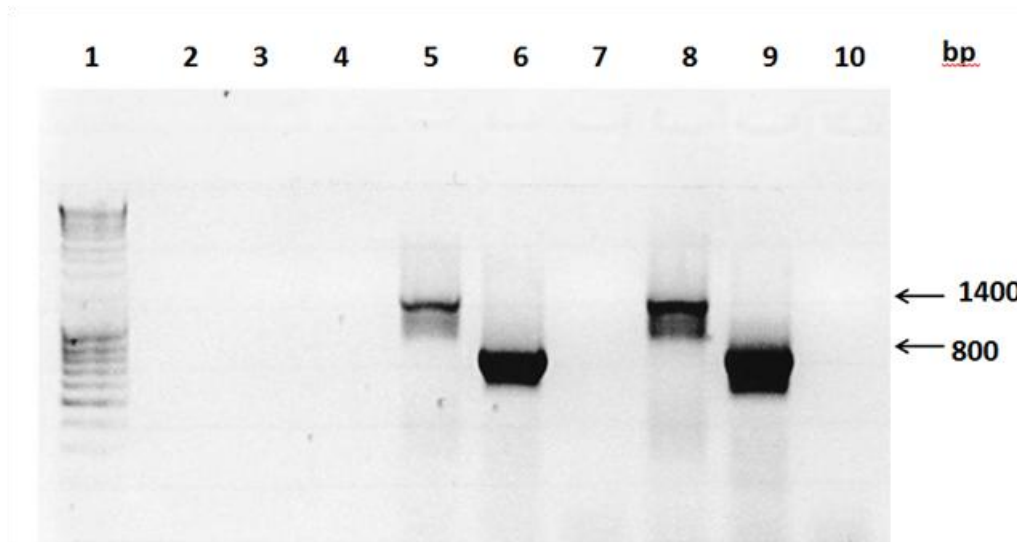


Figure 6. Diagnostic PCR using genomic DNA from two *Trichuris* sp. specimens (UNK_A and UNK_B) extracted from a single baboon of an unknown troop on the Cape Peninsula. Lane 1 is the GeneRuler 10, 000bp DNA Ladder (Thermo Scientific, Bremen, Germany). Lanes 2-4 are the negative (no template) control reactions with NC primers, DG primers and CP-GOB primers, respectively. Lanes 5-7 are the reactions containing genomic DNA of UNK_A and NC primers, DG primers and CP-GOB primers, respectively. Lanes 8-10 are the reactions containing genomic DNA of UNK_B and NC primers, DG primers and CP-GOB primers, respectively.

We were able to identify the genotype of five of the ten *Trichuris* sp. specimens isolated from a single baboon of the Groot Olifantsbos troop on the Cape Peninsula in another diagnostic PCR reaction using the three primer sets. The NC primers successfully amplified the ITS1-5.8S-ITS2 region from the genomic DNA of five of the ten *Trichuris* sp. specimens, namely GOB_B, GOB_C, GOB_F, GOB_G and GOB_H (Fig. 7), corresponding to a 50% primer amplification success rate among the GOB samples. The size of the products were within the size range of 1300-1440bp, corresponding to the product size expected when NC primers are used in the reaction. The 50% amplification success rate in these positive control reactions suggests that the diagnostic primer pairs should also only be able to identify the genotypes of 50% of the *Trichuris* sp. specimens (only 50% of the DNA samples are amplifiable).

In the diagnostic PCR, the negative control (no template) reactions produced no bands (Fig. 8), suggesting that the reactions were clear of contamination and that the primers only amplified the ITS1-5.8S-ITS2 region of the template DNA present in each reaction. For reactions containing the DG primers, no bands were produced on the electrophoresis gel following PCR for any of the

Trichuris sp. specimens (Fig. 8). However, for reactions containing the CP-GOB primers, 50% of the samples produced distinct bands corresponding to an expected amplicon size of ca. 300bp (282bp). The samples amplified by the CP-GOB primers were GOB_B, GOB_D, GOB_F, GOB_H and GOB_J. Only three of these samples (GOB_B, GOB_F and GOB_H) were amplified by the NC primers. The remaining five *Trichuris* sp. samples failed to produce a band for all three primer sets. Therefore, among the ten *Trichuris* sp. specimens extracted from the GOB baboon, five specimens (50%) displayed the CP-GOB genotype. No definitive conclusion can be made about the genetic identity of the remaining 50% of the *Trichuris* sp. specimens.

The poor quality of the genomic DNA of the five *Trichuris* sp. specimens isolated from the five baboons of the Da Gama park, Tokai and Plateau Road troops resulted in failure of the three primers to amplify the ITS1-5.8S-ITS2 region. No conclusion about the genotypic identity of these samples can be made as a result.

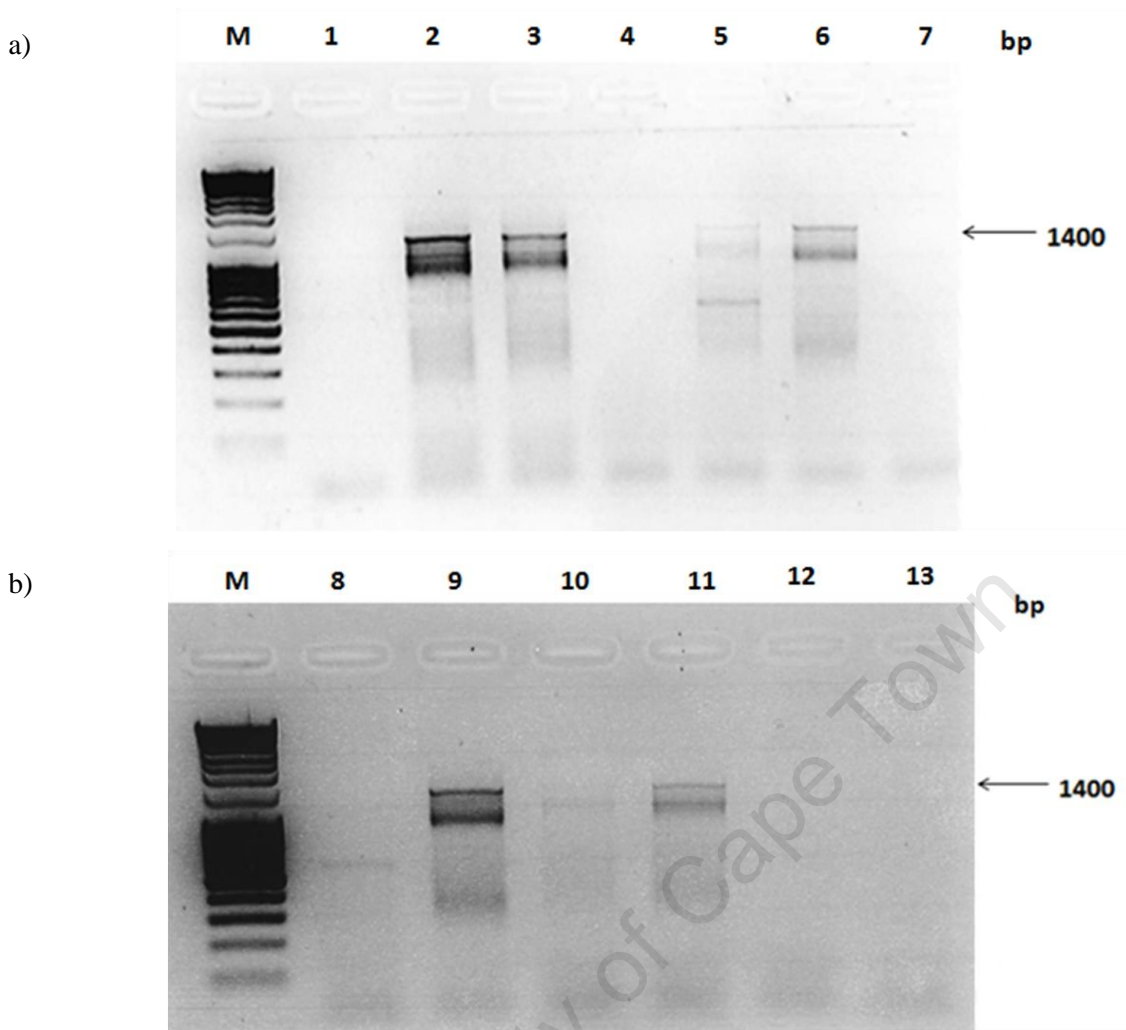


Figure 7. Positive control PCR with the NC primers and the genomic DNA extracted from the ten *Trichuris* sp. specimens (GOB_A – GOB_J) isolated from a single baboon of the Groot Olifantsbos troop on the Cape Peninsula. Lane M (a and b) is the GeneRuler 10,000bp DNA Ladder (Thermo Scientific, Bremen, Germany). Lane 1 is the negative control (no template) reaction. Lanes 2 and 3 are positive controls containing the genomic DNA of samples UNK_A and UNK_B, respectively. Lanes 4 – 13 contain the genomic DNA of GOB_A, GOB_B, GOB_C, GOB_D, GOB_E, GOB_E, GOB_F, GOB_H, GOB_I and GOB_J, respectively.

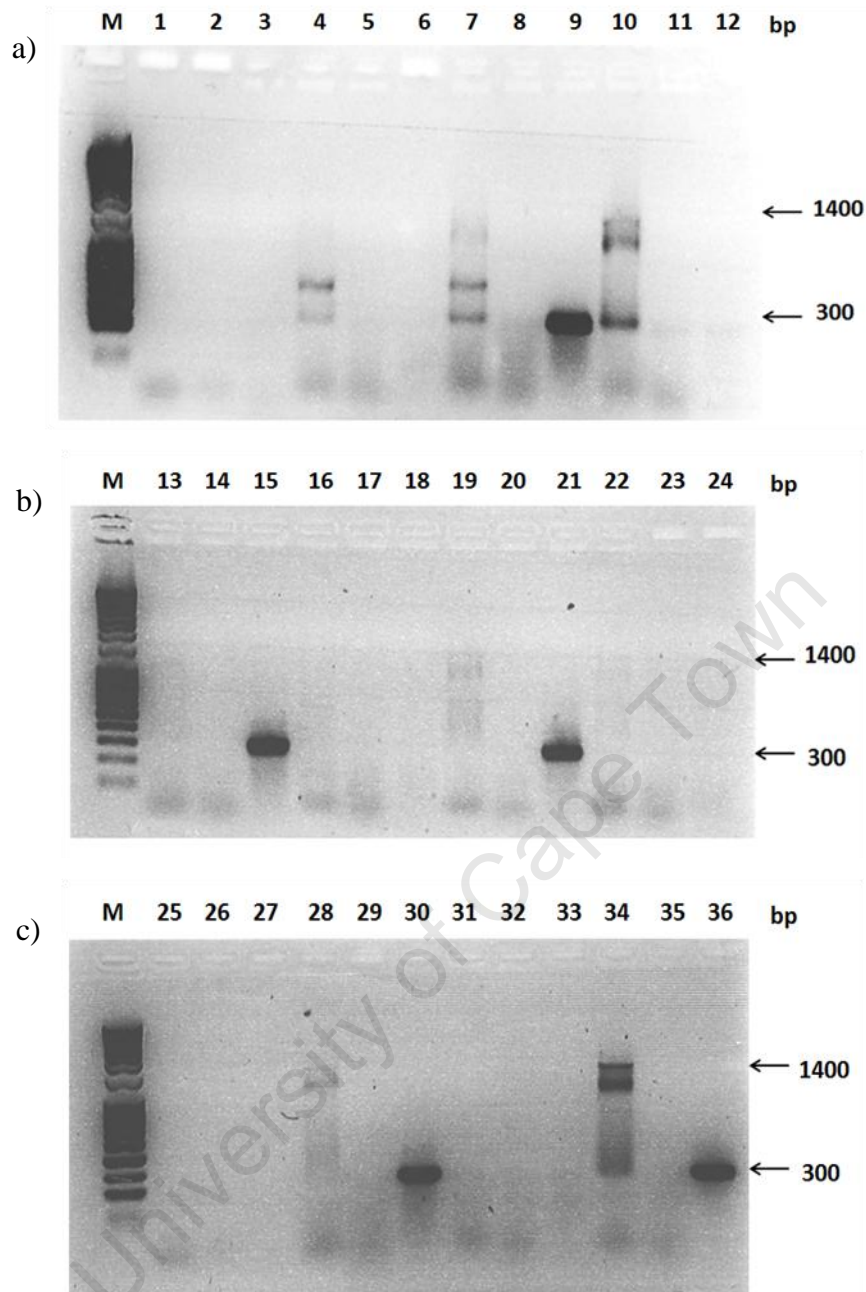


Figure 8. Diagnostic PCR using ten *Trichuris* sp. specimens (GOB_A – GOB_B) extracted from a single baboon from the Groot Olifantsbos troop on the Cape Peninsula as the template with NC primers, DG primers and CP-GOB primers. The order of these primers is maintained in the PCR reactions involving the following *Trichuris* specimens: (4-6): GOB_A; (7-9):GOB_B; (10-12): GOB_C; (13-15): GOB_D; (16-18): GOB_E; (19-21): GOB_F; (22-24): GOB_G; (28-30): GOB_H; (31-33): GOB_I; (34-36): GOB_J. Negative (no template) controls were also run with the NC primers (1 and 25), DG primers (2 and 26) and CP-GOB primers (3 and 27). M is the GeneRuler 10,000bp DNA Ladder (Thermo Scientific, Bremen, Germany) and amplicon size has been displayed.

UNK and GOB specimen sequencing

Two randomly selected recombinant clones (I and II) of the *Trichuris* sp. specimens UNK_A and UNK_B were selected for sequencing. Forward sequencing (with M13F primer) was successful for clones UNK_AI, UNK_BI and UNK_BII. UNK_A, UNK_BI and UNK_BII specimens display high percent identity (97, 85 and 94%) in their partial ITS1-5.8S-ITS2 region with three *Trichuris* sp. specimens isolated from three baboons of the Da Gama park troop on the Cape Peninsula by Ravasi *et al.* (2012) (Table 4). The sequencing results therefore confirm that the two *Trichuris* sp. specimens extracted from the same baboon of the unknown troop display the same genotype and both fall within the DG clade.

Among the GOB colonies, only GOB_F was found to contain the partial ITS1-5.8S-ITS2 amplicon and four of these clones (I-IV) were randomly selected for sequencing. Sequencing was successful using both M13F and M13R primers for all four clones. All of the clones displayed high % identity in their ITS1-5.8S-ITS2 regions with *Trichuris* sp. isolated from baboons of the Groot Olifantsbos and Cape Peninsula troops (92-95%) (Table 4). However, two clones also displayed high identity with *T. trichiura* isolated from a human from Cameroon (86-91%) and the other two with *Trichuris* sp. isolated from *Chlorocebus sabaenus* in captivity in Czech Republic (91-92%) (Table 4). All *Trichuris* sp. isolated from the baboons of the Groot Olifantsbos and Cape Peninsula troops, the human in Cameroon and *Chlorocebus sabaenus* in Czech Republic display the CP-GOB genotype and fall within the CP-GOB clade (Ravasi *et al.* 2012). The close identity of GOB_F with these specimens therefore suggests that this *Trichuris* sp. specimen also displays the CP-GOB genotype. This specimen will therefore cluster within the CP-GOB clade with the three *Trichuris* sp. with which it displays high % identity.

Table 4. GenBank accession numbers for the *Trichuris* sp. sequences with which the partial ITS1-5.8S-ITS2 region of clones of three *Trichuris* sp. specimens from two baboon (*Papio ursinus*) troops on the Cape Peninsula show highest % identity. The suggested genotype of each *Trichuris* sp. specimen, according to the % identity, is also presented.

Worm Origin	GenBank ID	% identity	Trichuris species	Host species	Country	Genotype
UNK_AI	GQ301552	97	<i>Trichuris</i> sp.	<i>P. ursinus</i> , DGII	South Africa	DG
	GQ301551	97	<i>Trichuris</i> sp.	<i>P. ursinus</i> , DGIII	South Africa	
	GQ301553	97	<i>Trichuris</i> sp.	<i>P. ursinus</i> , DGI	South Africa	
UNK_BI	GQ301552	85	<i>Trichuris</i> sp.	<i>P. ursinus</i> , DGII	South Africa	DG
	GQ301553	85	<i>Trichuris</i> sp.	<i>P. ursinus</i> , DGI	South Africa	
	GQ301551	85	<i>Trichuris</i> sp.	<i>P. ursinus</i> , DGIII	South Africa	
UNK_BII	GQ301551	94	<i>Trichuris</i> sp.	<i>P. ursinus</i> , DGIII	South Africa	DG
	GQ301552	94	<i>Trichuris</i> sp.	<i>P. ursinus</i> , DGII	South Africa	
GOB-FI	GQ301554	95	<i>Trichuris</i> sp.	<i>P. ursinus</i> , CP_GOB	South Africa	CP-GOB
	GQ301555	91	<i>T. trichiura</i>	<i>Homo sapiens</i>	Cameroon	
GOB_FII	GQ301554	93	<i>Trichuris</i> sp.	<i>P. ursinus</i> , CP_GOB	South Africa	CP-GOB
	JF690944	91	<i>Trichuris</i> sp.	<i>Chlorocebus sabaeus</i>	Czech Republic	
GOB_FIII	GQ301554	92	<i>Trichuris</i> sp.	<i>P. ursinus</i> , CP_GOB	South Africa	CP-GOB
	GQ301555	86	<i>T. trichiura</i>	<i>Homo sapiens</i>	Cameroon	
GOB_FIV	GQ301554	95	<i>Trichuris</i> sp.	<i>P. ursinus</i> , CP_GOB	South Africa	CP-GOB
	JF690944	92	<i>Trichuris</i> sp.	<i>Chlorocebus sabaeus</i>	Czech Republic	

Discussion

To our knowledge, this is the first study to investigate the use of molecular evidence to determine whether two genotypes of *Trichuris* sp. can infect baboons within and between troops on the Cape Peninsula, South Africa. Our results suggest that a single baboon may be infected by two distinct *Trichuris* sp. genotypes previously identified on the Peninsula (Ravasi *et al.* 2012). Among ten *Trichuris* sp. specimens from a single baboon in the Groot Olifantsbos (GOB) troop, the amplification of the ITS1-5.8S-ITS2 region of rDNA by three sets of primers was variable. In the diagnostic PCR, the CP-GOB primers amplified their target sequence of the ITS1-5.8S-ITS2 region among 50% of the GOB *Trichuris* sp. specimens. In addition, the sequence of the NC primer amplicon of one of these specimens (GOB_F) displayed high identity with *Trichuris* sequences from humans and non-human primates displaying the CP-GOB genotype, namely *Papio ursinus* of the Groot Olifantsbos and Cape Peninsula (92-95%), a human from Cameroon (86%) and *Chlorocebus sabaues* in Czech Republic (91-92%). These results suggest that the five GOB *Trichuris* sp. specimens amplified by the CP-GOB primers display the CP-GOB genotype. These five specimens will therefore cluster with *Trichuris* sp. specimens extracted from baboons in the Groot Olifantsbos and Cape Peninsula troops, other non-human primates (*Papio hamadryas* and *Chlorocebus aethiops*) in captivity in Spain and Czech Republic, and humans in Cameroon, Uganda and Jamaica within the CP-GOB clade.

While the results confirm that five of the *Trichuris* sp. specimens from baboons in the Groot Olifantsbos troop display the CP-GOB genotype, the genotype of the remaining five specimens cannot be confirmed. In fact, these specimens have the potential to display either the CP-GOB or DG genotype. We cannot draw any conclusions about the identity of these specimens because factors other than the genotype sequence of each may have contributed to the lack of amplification by any primer sets.

The NC primers, specific to the conserved ends of the ITS1-5.8S rRNA-ITS2 region, are used as a positive control to confirm that the DNA is capable of amplification. It is expected that DNA amplified by these primers will be amplifiable by the genotype-specific primers (DG and CP-GOB primers). However, two GOB specimens positively amplified by the NC primers produced no bands in the diagnostic PCR (GOB_C and GOB_G) and two specimens that were amplified by the CP-GOB primers were not amplified by the NC primers (GOB_D and GOB_J). The failure of the DG primers in this diagnostic PCR to amplify any specimens may be associated with the conditions that led to these unexpected results. The amplification anomalies are not related to the concentration of

the isolated DNA since amplification by two sets of primers was possible for samples with concentrations ranging from 48.3ng/μl to 107.6ng/μl. This was confirmed by results of the preliminary PCR in which 1:100 plasmid dilutions were amplified by all three sets of primers, similarly to the concentrated plasmid stocks (results not shown). Rather, the results may be attributed to the poor quality of the DNA observed on an electrophoretic gel. This may be the result of long term storage of the *Trichuris* sp. specimens at room temperature prior to this study, which may have led to DNA degradation.

While our results confirm that *Trichuris* sp. with the CP-GOB genotype are observed in the baboon from the Groot Olifantsbos troop, we cannot conclude that this is the only genotype to infect baboons in the troop. Our results from the GOB *Trichuris* sp. specimens in combination with findings from Ravasi *et al.* (2012) confirm that the CP-GOB genotype is observed within this troop. However, this does not eliminate the potential for the DG genotype within this troop. Therefore, this study highlights the need for further investigation into the cross-infection of *Trichuris* sp. specimens with both DG and CP-GOB genotypes within the same troop.

In addition, no conclusive statement can be made about whether both genotypes may infect the same baboon individual. A higher success rate of ligation of the NC primer products from the GOB_A – J individuals into cloning vectors would have allowed for more of these specimens to be unequivocally genotyped through sequencing in this study where PCR failed. Though diagnostic PCR methods have been suggested as favourable alternatives to sequencing in the past (Gasser 1996), the complete absence of bands among some individuals during the PCR reaction, even for reactions where the conserved NC primers were used, suggests that sequencing should provide a more reliable result for species identification. With this in mind, future research should consider focussing on the use of sequencing to identify whether both genotypes of *Trichuris* sp. infect the same individual. This study again suggests that there is still the potential for both genotypes to co-exist within a single baboon host.

Both *Trichuris* sp. specimens extracted from the baboon of the unknown troop displayed the DG genotype. This identity was confirmed using both the diagnostic PCR method and through sequencing of the NC partial ITS1-5.8S-ITS2 amplicons. Since the two specimens displayed high sequence identity (85-97%) with *Trichuris* sp. isolated from the Da Gama Park troop baboons (Ravasi *et al.* 2012) suggests that these sequences will cluster within the DG clade. The results may

even suggest that the unknown baboon sampled in this study originates from the Da Gama troop, but this conclusion would be unreliable since no definitive conclusion about whether worms displaying the DG genotype are found in troops other than the Da Gama, troop has been made. Again, this result supports Ravasi *et al.*'s (2012) finding that the DG genotype was distinct in the baboon in the Da Gama Park troop. Interestingly, however, only baboons of the Da Gama Park and humans occur within the DG clade (Ravasi *et al.* 2012). This, in conjunction with our findings, may suggest that baboons of the Da Gama Park troop are the only non-human primate to support *Trichuris* sp. with the DG genotype, but this is may be contradicted through sampling of more *Trichuris* sp. from troops throughout the Peninsula in the future.

Parasite diversity (or the presence of multiple *Trichuris* species in a single host) is often limited among non-human primates other than baboons due to interspecific parasite competition for nutrition and space within the host (Bradley & Altizier 2006). This may support the suggestion that only single species (or genotypes) are found within particular host groups. According to Freeland (1979), primate social groups exist as “biological islands” and create isolated habitats for their specific parasites through social barriers. A parasite will infect all members of a population only when a network for transmission is created through social interactions (Nunn *et al.* 2003). In this way, parasite transmission determined by contact among hosts results in higher parasite prevalence within troops, populations or species than between them (Freeland 1979). Though this host specificity is commonly observed for many trichurid species (Muriuki *et al.* 1998; Cutillas *et al.* 2002), this does not appear to be the case for the two *Trichuris* sp. genotypes investigated in this study. This is particularly evident for the CP-GOB genotype which infects other non-human primates (*Papio hamadryas* and *Chlorocebus aethiops*) in addition to *Papio ursinus* on the Cape Peninsula (Ravasi *et al.* 2012).

It is unlikely that the genotypes will be specific to certain troops on the Peninsula if the spatial range of the troops overlaps. Though not known to be completely nomadic (Brain & Bohrmann 1992), baboon males may migrate between troops and increase the frequency of troop interactions (Freeland 1979). This has the potential to increase the risk of parasite transmission between troops through the faecal-oral route or direct contact, resulting in homogeneity of intergroup parasite communities (Ezenwa 2003; Nunn *et al.* 2003). In addition, the increasing contact of baboon troops with humans in urban areas may also enhance parasite transmission between troops which indirectly come into contact through human refuse and food items (Muller-Graf 1997; Bell & Burt 1991; Bradley & Altizier 2006). However, supplemental feeding may improve the immune defences

of the baboons and increased immunity to trichurid infections may limit their diversity in the host (Coop & Kyriakis 2001; Bradley & Altizier 2006). A wider array of ecological, social and demographic factors, including season (Huffman *et al.* 1997), social hierarchy (Freeland 1979), host body size and population density (Poulin 1995; Poulin & Morand 2000), influence parasite diversity among baboons and will have an effect on the presence of the two *Trichuris* sp. genotypes within and between the troops. This study does not represent the only case where the host specificity of *Trichuris* has been questioned (Petrasova *et al.* 2012) and parasite-host dynamics of the genus remain highly controversial today.

Another challenge presented in this study is the questionable reliability of the ITS1-5.8S-ITS2 region. The identification of multiple genetic variants or nuclear paralogues within species of *Cyclospora* (Olivier *et al.* 2001) and *T. trichiura* (Nissen *et al.* 2012) was achieved using the region in previous studies. The occurrence of different ITS sequence types were also observed within single parasite specimens in both of these studies (though the finding was suggested to result from sample contamination by Ravasi *et al.* (2012)). In fact, molecular studies on several gastrointestinal helminths, including *Ascaris* and *Ligula intestinalis*, have shown that the ITS region exists in heterozygous forms, possibly as a result of gene duplications within the genome or recent hybridization events among the helminths (Petrasova *et al.* 2012). This may suggest that the ITS region has the potential to lead to incorrect species identification and over-estimation of the diversity of helminths (Oliveros 2000). Mitochondrial studies may be more informative for this purpose and should be considered in future research to address the questions raised in this study.

Furthermore, despite the problems associated with biometric studies of *Trichuris*, the usefulness of morphological analysis cannot be completely overlooked, as indicated by the recent identification and validation of the new *Trichuris* species, *T. navonae* n sp. from the *Akodon montensis sigmodontine* rodent based on SEM analysis (Robles 2011). In the future, statistical differences in morphological features should be used in conjunction with molecular approaches to confirm differences in species or genotypes (Chapman *et al.* 2006; Petrasova *et al.* 2012; Ravasi *et al.* 2012). Cutillas *et al.* (2009) suggest the use of new differential criteria in morphological studies. This is of particular importance if studies of *Trichuris* cross-transmission between species, especially humans and non-human primates, are to be undertaken (Chapman *et al.* 2006; Ravasi 2009).

Many new avenues for future research around the infection and transmission dynamics of *Trichuris* sp. among *Papio ursinus* on the Peninsula have been identified. Having observed the

potential for cross-infection of two genotypes of *Trichuris* among troops, research may now be directed toward identifying whether co-existence of the genotypes occurs within the same primate host. Knowledge of host-parasite systems on the Peninsula will not only be significant for informing management policy of the baboon troops in the future, but also for controlling outbreaks of infectious diseases caused by helminthic parasites, especially *Trichuris*, among humans and baboons (Bradley & Altizier 2006). In addition, insight into the occurrence of the different genotypes and species will be important for evolutionary studies of parasite emergence among animals and humans. This then will also inform our understanding of zoonoses and the potential spread of disease and parasite between species and especially to humans as overlap of the species increases. Overall, knowledge of parasite dynamics not only allows us to better manage and conserve our biodiversity, especially of threatened or endangered primate species, but to ensure the health and safety of human populations in close proximity with these animals in the future.

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