PREVALENCE AND GENOTYPING OF AVIAN TRICHOMONIASIS IN URBAN AND EXURBAN AREAS OF SAN ANGELO, TOM GREEN COUNTY, TEXAS

A Thesis

Presented to the

Faculty of the College of Graduate Studies and Research

Angelo State University

In Partial Fulfillment of the

Requirements for the Degree

MASTER OF SCIENCE

by

KELLY ANN PERSINGER

December 2018

Major: Biology

PREVALENCE AND GENOTYPING OF AVIAN TRICHOMONIASIS IN URBAN AND EXURBAN AREAS OF SAN ANGELO, TOM GREEN COUNTY, TEXAS

by

KELLY ANN PERSINGER

APPROVED:

Dr. Ben Skipper

Dr. Nicholas Negovetich

Dr. Loren Ammerman

Dr. Twyla Tasker

November 7, 2018

APPROVED:

Dr. Susan E. Keith
Dean, College of Graduate Studies and Research

DEDICATION

I dedicate this thesis to my parents for their unwavering support throughout the years.

ACKNOWLEDGMENTS

I would like to thank Dr. Ben R. Skipper for his continued guidance and support through my graduate career at Angelo State University. Without him, none of this would have been possible. I would like to thank Dr. Nicholas Negovetich for his patience and support throughout this project. I would like to thank Dr. Loren Ammerman for her assistance in completing this project.

This thesis would not have been possible without the samples of extracted DNA so graciously gifted to me by Dr. John Yabsely at the College of Veterinary Medicine at the University of Georgia and Dr. Richard Gerhold of the College of Veterinary Medicine at the University of Tennessee. I would also like to thank Dr. Gerhold for his guidance and assistance in PCR methods and interpreting phylogenetic data of *T. gallinae*.

I would also like to thank everyone who helped me along the way with advice, field work and sample collection – Mary Jones, Kalin Skinner, Katie Kuzdak, Katrina Keith, Kerri Bynum, Alex Shaffer and Roxy Pourshoushtari. I would especially like to thank Jody Casares for being an incredible help and friend throughout this entire process.

I would like to extend a special thank you to the landowners that were gracious enough to allow us to collect on their property.

Additionally, I am grateful for all funding that was received that helped make this project happen – Angelo State Graduate Research Fellowship and Head-of-the-River-Ranch travel and research grants.

ABSTRACT

Avian trichomoniasis is caused by an infection of the protozoan *Trichomonas gallinae*, a cosmopolitan parasite of columbiform birds. Infection is characterized by the necrotic ulceration of the mouth, esophagus, crop and proventriculus. These lesions can obstruct the upper respiratory and digestive tracts, frequently resulting in death. The objectives of this study were to determine the prevalence and genetic lineage of avian trichomoniasis in four species of columbiforms in San Angelo, Texas. Samples were cultured and analyzed using InPouch TF diagnostic pouches. A total avian trichomoniasis prevalence of 66% (n=94 total), and evidence of the presence of multiple lineages among collected samples were found in this study. Lineages found in my study were responsible for outbreaks of trichomoniasis that resulted in high mortalities, indicating the possibility for such an epizootic in San Angelo, Tom Green County, Texas.

TABLE OF CONTENTS

Page

DEDICATION ... iii

ACKNOWLEDGMENTS ... iv

ABSTRACT ... v

LIST OF TABLES ... viii

LIST OF FIGURES ... ix

INTRODUCTION ... 1

METHODS ... 8

Sample Collection ... 8

pH Measurement. ... 9

Analysis of Diagnostic Pouches ... 9

Statistical Analysis ... 9

DNA Extraction ... 11

ITS1/5.8s rRNA/ITS2 Genetic Analysis ... 11

Constructing Phylogenetic Trees ... 13

RESULTS ... 16

pH Measurements ... 20

Sequence Analysis of ITS1/5.8s rRNA/ITS2 ... 20

Phylogenetic Analysis of ITS1/5.8s rRNA/ITS2 region ... 22

Sequence Analysis of Fe-hydrogenase ... 26

Phylogenetic Analysis of Fe-hydrogenase region ... 26

DISCUSSION ... 30

Phylogenetic Analysis.. 35
LITERATURE CITED 42

APPENDIX I.. 51

APPENDIX II.. 52

APPENDIX III.. 53

APPENDIX IV.. 54

APPENDIX V 60

APPENDIX VI.. 78

APPENDIX VII………………………………………………………………….. 80

LIST OF TABLES

Table 1. Prevalence of *Trichomonas gallinae* among doves sampled at urban and exurban locations, listed by species.. 17

Table 2. Overall comparison of prevalence of *T. gallinae* infection between species in both urban and exurban sites combined.. 18

Table 3. Comparison of intraspecific prevalence of *T. gallinae* between urban and exurban locations.. 19

Table 4. Summarized NCBI BLASTn results of sequences of the ITS1/5.8s/ITS2 region from samples collected in the current study.. 21

Table 5. Groups of identical ITS1/5.8s/ITS2 sequences collected from samples, listed by the avian species they were collected from... 25

Table 6. Samples that amplified the Fe-hydrogenase region successfully and were used for sequence and phylogenetic analysis.. 27

Table 7. Known subtypes of the Fe-hydrogenase region of the *Trichomonas gallinae* genome, as reported in the literature... 28

Table 8. Known sequence groups of the ITS1/5.8s rRNA/ITS2 region of *T. gallinae* as reported in the literature.. 37

LIST OF FIGURES

Figure 1. Maps of urban and exurban trapping locations.. 10

Figure 2. Molecular phylogenetic analysis of the ITS1/5.8s rRNA/ITS2 region of *Trichomonas* collected in this study.……………………………………...……......…......... 23

Figure 3. Molecular phylogenetic analysis of the Fe-hydrogenase gene region of *Trichomonas* collected in this study ……………………….……………..….................. 29

Figure 4. Accumulation curve of samples collected from white-winged doves in chronological order of collection with the prevalence of *T. gallinae*.................................... 33

INTRODUCTION

Avian trichomoniasis is caused by an infection of the flagellated protozoan *Trichomonas gallinae*, a cosmopolitan parasite of columbiform birds (Stabler and Herman 1951; Schulz et al. 2005). It has also been reported other avian species including house finches (*Haemorhous mexicanus*) and corvids (Family Corvidae) (Anderson et al. 2009; Neimanis et al. 2010; Chi et al. 2013; Marx et al. 2017).

Current classification lists *T. gallinae* under the phylum Metamonada, class Parabasalia, order Trichomonadida and family Trichomonadidae (Cavalier-Smith 2002). *T. gallinae* was first described by Rivolta (1878) when he isolated organisms from the caseaous lesions of the upper digestive tract of a common pigeon (*Columba livia*; [Stabler 1939]). Rivolta initially identified the organism as a cercomonad, then naming it *Cercomonas gallinae*; it was later correctly identified as a trichomonad and renamed *Trichomonas gallinae* (Stabler 1939, 1954).

Infections by *T. gallinae* are characterized by the necrotic erosion and ulceration of the epithelial lining of mouth, esophagus, crop and proventriculus that it causes in birds (Stabler 1947). Infection with the milder strains result in lesions in the oral or upper esophageal regions that resolve after a few days (Stabler 1947). In contrast, infection with one of the more virulent strains can result in an earlier onset of symptoms, lesions which prevent feeding and inhibit breathing through the obstruction of the upper respiratory and digestive tracts, frequently resulting in death by starvation or suffocation (Sansano-Maestre

____

Journal of Wildlife Diseases
Epidemiology

et al. 2009; Bunbury et al. 2005). Less commonly, the liver, air sacs and part of the cranium can be affected by these caseaous lesions (Stabler 1954). Initial lesions appear on the oral mucosa after approximately 7 days, with death following on day 10 (Stabler 1947). Other research has noted that the species of the host, as well as strain of the parasite, may influence the severity of infection and presence of symptoms (Boal et al. 1998; Hedlund 1998; Sansano-Maestre et al. 2009).

Historically, the primary hosts of *T. gallinae* are members of the family Columbidae, which, when symptomatic, is termed "canker" in these species (Stabler 1947). Columbids can also be asymptomatic and act as reservoir hosts for this parasite, although the "nature of this immunity is not understood" (Stabler 1954). Trichomoniasis affects not only members of the order Columbiformes but also a variety of non-columbids, including species from orders Anseriformes, Accipitriformes, Falconiformes, Galliformes, Passeriformes, and Strigiformes (Stabler and Herman 1951; Samour et al. 1995; Anderson et al. 2009; Rogers et al. 2016; Grunenwald et al. 2018). However, resistance to *T. gallinae* has not been observed outside of Columbidae (Kocan and Amend 1972). Transmission has been associated with artificial feeding and watering sources in urban and exurban areas where infected birds may pick up and drop seed or grain or contaminate communal drinking water with saliva (Stabler and Herman 1951; Kocan 1969). Cross species infections can occur at this point as artificial feed sources negate typical foraging habits that would limit availability of feed contaminated with columbiform saliva in favor of a communal feeding arrangement (Kocan 1969; Krone et al. 2005; Anderson et al. 2009). Adult doves and pigeons that frequent these sources may then transmit the infection to squabs when feeding crop milk (Stabler 1954; Kocan 1969; El-

Khatam et al. 2016). Several studies found infection in various raptor species, including the peregrine falcon (*Falco peregrinis*), barn owl (*Tyto alba*), Cooper's hawk (*Accipiter cooperii*), sparrowhawk (*A. nisus*), bald eagle (*Haliaeetus leucocephalus*), American kestrel (*Falco sparverius*), northern goshawk (*A. gentilis*), and the endangered Bonelli's eagle [(*Hieraaetus fasciatus*); (Stabler 1948; Stone and Janes 1969; Stone and Nye 1981; Work and Hale 1996; Boal et al. 1998; Real et al. 2000; Krone et al. 2005; Bunbury et al. 2007; Ecco et al. 2012)]. Trophic spillover to ornithophagous raptors occurs when infected prey such as doves, or even bird carcasses, are utilized as primary food sources (Work and Hale 1996; Boal et al. 1998; Erwin et al. 2000; Krone et al. 2005; Rogers et al. 2016). Nestlings could then become infected indirectly when they are fed the infected prey, or directly from infected parents (Forrester and Foster 2008). An example of the impact of this disease on birds of prey is the Cooper's hawk, where approximately 80% of urban nestling mortalities in Arizona were attributed to avian trichomoniasis while no rural nestling mortalities were caused by avian trichomoniasis (Boal 1997). The diet of urban Cooper's hawks is comprised of 83% dove while the diet of its exurban counterpart is comprised of 10% dove (Rogers et al. 2016). Further, several studies found that nestlings have a higher prevalence compared to breeding age Cooper's hawks (Boal et al. 1998; Rosenfield et al. 2002; Krone et al. 2005; Dudek et al. 2018). Two possible explanations are suggested for this. The first is breeding age hawks acquire immunity through initial infection as nestlings if they survive the infection (Urban and Mannan 2014). Second is the result of physiological changes that occur in the oral cavity during avian development, including the oral pH (Boal et al. 1998; Urban and Mannan 2014). Urban and Mannan (2014) found that the average oral pH of breeding age

(pH=6.12 [SE] 0.059) and fledgling (pH= 6.05 [SE] 0.066) Cooper's Hawks were seven times more acidic than that of nestlings (pH= 6.83 [SE] 0.033). In general, trichomonads are sensitive to environmental pH, and *T. gallinae* specifically prospers at pH levels between 6.5 and 7.5, with its optimal pH being 7.2 (Read 1957; Urban and Mannan 2014).

Transmission success, proliferation and the virulence of the disease is also dependent on the genetic lineage of *T. gallinae* (Stabler 1948; Honigberg 1961; Gerhold et al. 2008; Sansano-Maestre et al. 2009; Marx et al. 2017). The ITS1-5.8-ITS2 ribosomal and Fe-hydrogenase gene regions of the *T. gallinae* genome (Kleina et al. 2004) have been effective in differentiating between lineages, and specific lineages have been identified that are linked to increased virulence through the observation of clinical disease (Gerhold et al. 2008; Sansano-Maestre et al. 2009; Girard et al. 2014a; Marx et al. 2017).

As a recent study described, understanding the prevalence of both virulent and less-virulent lineages in columbids can be relevant to estimating the potential impact of the disease in a given area (Marx et al. 2017). Exposure of birds of prey to various strains of this parasite has been increasing as human encroachment and subsequent habitat loss forces them to nest in urban areas and traditional prey items are replaced by urban columbids (Boal et al. 1998; Sansano-Maestre et al. 2009). Further understanding could also be beneficial to future human health issues, as some lineages isolated from columbids have a higher genetic similarity to *T. vaginalis*, the causative agent of the human sexually transmitted infection trichomoniasis, than to *T. gallinae* (Gerhold et al. 2008; Girard et al. 2014a). One review suggests that zoonotic transfer of trichomonad parasites between human and bird may have already occurred (Maritz et al. 2014).

Several surveys of avian trichomoniasis have been conducted across North America and found prevalences vary greatly across species. Published prevalences in white-winged doves (*Zenaida asiatica*) range from 96.7-100% (Stabler 1961; Conti and Forrester 1981; Glass et al. 2001); incidence in mourning doves (*Z. macroura*) ranges from 1.1-47% in various U.S. states (Haugen 1952; Kocan and Amend 1972; Greiner and Baxter 1974; Conti and Forrester 1981; Ostrand et al. 1995; Schulz et al. 2005). Hedlund (1998) reported incidence in Inca doves (*Columbina inca*) ranging from 47-60% across two consecutive years (1994-95) in Tucson, Arizona, USA. No species-specific surveys of avian trichomoniasis in Eurasian collared doves (*Streptopelia decaocto*) have been conducted in North America. This is likely due to their status as an invasive species, with colonies first appearing in Florida in the early 1980s, and disjunct populations occurring in various locations in the 1990s, including Texas (Romagosa and Labisky 2000). Furthermore, only two surveys in white-winged doves have been conducted in Texas (Stabler 1961; Glass et al. 2001), and none have been conducted on the other three target species (Inca dove, mourning dove and Eurasian collared-dove) in the current study, although infections in these species have been reported in Texas (Gerhold et al. 2008).

Stabler (1961) sampled 51 white-winged doves in Hidalgo and Cameron counties and all 51 birds were infected with *T. gallinae*; however none displayed clinical signs of trichomoniasis. In 2001, Glass et al. reported 170 of 171 White-winged doves sampled in eastern Texas tested positive for *T. gallinae* using InPouch^{TM} TF diagnostic pouches. However, none of the individuals sampled displayed any clinical signs of trichomoniasis, and

neither Stabler (1961) nor Glass et al. (2001) characterized the collected samples molecularly.

While multiple studies exist in the literature detailing the prevalences of various avian species, the literature on prevalences in sympatric species is limited. Conti and Forrester (1981) found that prevalences in mourning doves sympatric with white-winged doves in southern Florida had a 17% prevalence, while mourning doves in northern Florida where there are no white-winged doves, had a 1.1% prevalence. This suggests the same would be true of additional species of columbids sympatric with white-winged doves, but no literature on this comparison could be found.

The objectives of this study were to determine the (i) pH of infected versus uninfected sympatric species of columbids (ii) prevalence and (iii) genetic lineage (and ultimately the virulence) of avian trichomoniasis through the detection of the protozoan *T. gallinae* in four sympatric avian species in the urban and the exurban areas of San Angelo, Tom Green County, Texas. These species include the Inca dove, white-winged dove, mourning dove, and Eurasian collared-dove. I hypothesized that the pH of the oral cavities of birds sampled would be more acidic in those that are culture negative for *T. gallinae* infection compared to those that are positive (Urban and Mannan 2014). I hypothesized that I would find a higher occurrence of *T. gallinae* infection in birds sampled in urban areas than in exurban areas, as infection and population densities of columbids have been found to increase in residential areas (Stabler 1954; Boal et al. 1998). I also expected to find a higher prevalence in white-winged doves than in the other four species listed, as this species is noted to be the most abundant columbid in these residential areas (Conti and Forrester 1981; Boal et al. 1998;

Gerhold et al. 2008). Lastly, I expected to find more than one unique genetic lineage of *T. gallinae* among collected samples, as many lineages have been reported and are found to differ by avian host species (Gerhold et al. 2008; Girard et al. 2014a; Martínez-Herrero et al. 2014).

METHODS

Sample collection

Beginning in Spring 2017 and continuing through Summer 2018, white-winged, Inca, mourning, and Eurasian collared-doves were sampled at various urban and exurban sites for avian trichomoniasis. All samples were collected within in Tom Green County (Texas, USA) at urban and exurban locations (Figure 1). Urban and exurban designations were determined by observations of density of residences. All four species were captured using walk-in funnel traps or mist nets in and around San Angelo. After being removed from the mist nets or funnel traps, birds were held in cloth bags until sampling, not exceeding 15 minutes. Each bird was then tested for *T. gallinae* by sampling the oral cavity and oropharynx using an algonite tipped swab and inserting it in an InPouch™ TF diagnostic pouch (BioMed Diagnostics, White City, Oregon, USA) and marked with an identifying leg band. These methods were utilized successfully by Erwin et al. (2000). Pouches were stored at room temperature and out of sunlight until use. Cover et al. (1994) found the InPouch™ TF diagnostic pouches to possess practical advantages over in vitro Diamond's medium while being as accurate in detecting *T. gallinae*. The samples collected using the InPouch™ TF diagnostic pouches were transported to the laboratory within 24-hours for incubation and analysis. The Angelo State University IACUC (protocol 17-01) approved all capture and sampling techniques and banding was done under USGS BBL permit number 22801 and TPWD permit number 1215-258.

pH Measurement

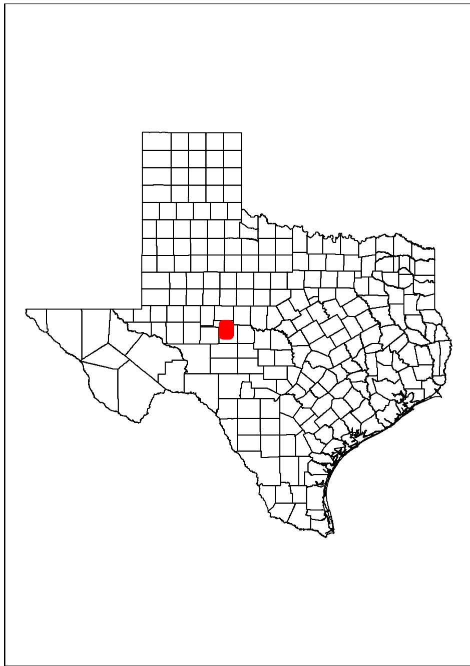
The pH was measured by holding a microelectrode (HALO® Wireless pH Meter with Microbulb, HANNA lnstruments, Woonsocket, Rhode Island, USA) in the oral cavity of each dove until the reading on the HANNA Lab App (HANNA Instruments) stabilized (approximately 15-30 sec). The microelectrode was stored in Electrode Storage Solution (HANNA Instruments) and rinsed with distilled water between samples and before returning to storage. The microprobe was calibrated to 3 points (pH=4.01, 7.01, 10.01) every day before sampling.

Analysis of Diagnostic Pouches

The InPouch™ TF diagnostic pouches were viewed under a microscope at 100-400x magnification for 15 minutes or until *T. gallinae* was observed. If, after 15 minutes, no *T. gallinae* was observed, the diagnostic pouch was incubated at 37°C and reevaluated at 24, 48 and 72 hours. If, after 72 hours, no *T. gallinae* was present, the sample was considered negative for *T. gallinae* (Boal et al. 1998; Erwin et al. 2000). Samples found to be positive were transferred to cryovials using disposable pipettes and stored at -80°C until DNA extraction.

Statistical Analysis

Fisher's exact and Chi-squared tests were used to compare prevalences following the methods of Boal et al. (1997). Analysis of variance (ANOVA) was used to compare average oral pH measurements following the methods of Urban et al. (2013). P-values that required adjustment were adjusted using Bonferroni correction.



esent trapping locations
considered urban, crosshatching
represents trapping locations
considered exurban.

Figure 1 — Maps of urban and exurban trapping locations

DNA Extraction

Stored specimens were removed from freezer and thawed by rolling between palms. Thawed specimens were then centrifuged at 1000x *g* for 5 minutes, supernatant discarded, and the pellet was re-suspended in 1 ml of phosphate-buffered saline (PBS). The specimens were centrifuged again at 1000x *g* for 5 min, the supernatant again discarded. One-half of the resultant gelatinous-pellet was aliquotted using a transfer pipet to a new 1.5 ml tube for use in DNA extraction. The pellet remnant was resuspended in 100 µl of PBS and returned to -80°C storage. This method was adapted from Marx et al. (2017), who found it effective for preparation of *T. gallinae* samples for DNA extraction.

DNA from previously prepared pellets was extracted using the DNeasy blood and tissue extraction kit (Qiagen, Valencia, California, USA) according to the manufacturer's animal tissues and cells protocol. Samples underwent a final elution with 50 µl of Buffer AE to increase resultant DNA concentrations. Concentrations were quantified using a Qubit™ 3 Fluorometer and Qubit™ dsDNA HS Assay kit (Invitrogen, Carlsbad, California, USA). Extracted DNA was stored at -20°C until use.

ITS1/5.8S rRNA/ITS2 Genetic Analysis

I chose to sequence the highly conserved ITS1/5.8s/ITS2 ribosomal region due to its successful use in several genetic studies of parasitic trichomonads (Kleina et al. 2004; Gaspar da Silva et al. 2007; Gerhold et al. 2008; Sansano-Maestre et al. 2009, 2016; Grabensteiner et al. 2010; Lawson et al. 2011; Kelly-Clark et al. 2013). This region contains internal transcribed spacers (ITS) that have a higher rate of nucleotide variation in comparison to the 5.8s rRNA sequence and are used for phylogenetic analysis among species within the same

genus (Hillis and Dixon 1991; Kleina et al. 2004; del Carmen Martínez Herrero 2016). Amplification protocol of this region was adapted from Cepicka et al. (2005) and Gerhold et al. (2008). Trichomonad specific primers, ITSF (5'-TTCAGTTCAGCGGGTCTTCC-3') and ITSR (5'-GTAGGTGAACCTGCCGTTGG-3'), were obtained from AlphaDNA (Montreal, Quebec, Canada) and used to perform polymerase chain reactions (PCR) (Cepicka et al. 2005). Amplification protocol of the Fe-hydrogenase gene was adapted from Ganas et al. (2014) and Lawson et al. (2011). Published primers for this gene, TrichhydFOR (5'-GTTTGGGATGGCCTCAGAAT-3') and TrichhydREV (5'-AGCCGAAGATGTTGTCGAAT-3'), were also obtained from AlphaDNA and used to perform PCR. All PCR reactions were conducted using AmpliTaq Gold® Fast PCR Mastermix (2X) (Applied Biosystems, Foster City, CA, USA). The mastermix contained AmpliTaq Gold® DNA Polymerase, UP, PCR buffer, dNTPs, $MgCl_2$ and stabilizers. A total reaction volume of 25 µl was used and consisted of 1X AmpliTaq Gold™ Fast PCR Mastermix, 0.2 µM of each primer, and approximately 2 ng extracted DNA for all reactions. All reactions were conducted on a Bio-Rad MyCycler™ thermal cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The following cycling conditions were used for all ITSF/5.8s/ITSR PCR reactions: polymerase activation and initial denaturation at 95°C for 10 minutes, followed by 35 cycles of denaturation at 96°C for 3 seconds, annealing at 52°C for 3 seconds and extension at 68°C for 5 seconds. Final extension was set to 72°C for 10 seconds. The following cycling conditions were used for all Fe-hydrogenase PCR reactions: polymerase activation and initial denaturation at 95°C for 10 minutes, followed by 35 cycles of denaturation at 96°C for 5 seconds, annealing at 53°C for 3 seconds and extension at 68°C

for 15 seconds. Final extension was set to 72°C for 10 seconds. Samples were then held at 10°C until removed. All PCR reactions were run with a negative control using autoclaved diH_2O. Two samples of known, extracted *T. gallinae* DNA were obtained from Dr. Richard Gerhold at University of Tennessee and used as positive controls. This DNA was extracted from samples obtained from a white-winged dove and a Cooper's hawk. PCR products were evaluated for bands of appropriate size using FastRuler Middle Range DNA Ruler and 6x MassRuler LD (Thermo Scientific ™, Waltham, MA, USA) on a 1.5% agarose gel, stained with ethidium bromide, in 1X TAE (Tris-Acetic Acid-EDTA) buffer and then observed under UV light.

Amplified PCR products were treated with ExoSAP-IT Express™ PCR Product Cleanup Reagent (Applied Biosystems, Foster City, CA, USA) following the manufacturers protocol. Sequencing was carried out using Sanger sequencing (Genomics Core Lab, Texas A & M University at Corpus Christi, Corpus Christi, Texas).

Constructing phylogenetic trees

Chromatograms of the ITS1/5.8s rRNA/ITS2 region and Fe-hydrogenase gene were manually checked in both directions (forward and reverse) and assembled using Sequencher™ 5.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Assembled sequences were aligned using the MUSCLE algorithm in MEGAX: Molecular Evolutionary Genetics Analysis version 10.0 for bigger datasets (Kumar et al. 2018). There were a total of 365 positions in the final alignment for ITS1/5.8s rRNA/ITS2 region and 884 positions for the Fe-hydrogenase locus. A pairwise distance test was conducted to determine similarities between sequences. Pairwise distances were calculated using nucleotide substitutions in a

Kimura 2-parameter model (Kimura 1980). Substitutions included were transitions and transversions and codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. Identical sequences were grouped and condensed to a single representative sequence and a representative sequence from each group was selected for use in further analysis. Each unique sequence and the representative sequences from respective groups were queried on NCBI GenBank using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and sequences with $\geq$98% query cover, $\leq 1E^{-3}$ expectation values and $\geq$98% identity to the queried sequence were recorded. Previously published sequence types of the ITS1/5.8s rRNA/ITS2 region of *T. gallinae* were used as reference sequences. The "Find Best DNA/Protein Models (ML)" tool in MEGAX was used to determine the best-fit substitution model for the ITS1/5.8s rRNA/ITS2 region, which indicated the model with the lowest AIC, BIC and highest lnL was the Tamura 3-parameter model with Gamma distribution (Tamura 1992). The evolutionary history of this region was inferred by using the neighbor-joining method based on the Tamura 3-parameter model with bootstrap test (10,000 replicates). A gamma distribution was used to model evolutionary rate differences among sites (5 categories shape parameter = 1). All ambiguous positions were removed for each sequence pair. There were 365 positions in the final dataset and the resultant phylogenetic tree was edited in MEGAX.

For the Fe-hydrogenase locus, the "Find Best DNA/Protein Models (ML)" tool indicated the Kimura 2-parameter model with gamma distribution had the lowest AIC, BIC, and highest lnL for this region (Kimura 1980). The evolutionary history was inferred by using the neighbor-joining method based on the Kimura 2-parameter model with bootstrap

test (10,000). A gamma distribution was used to model evolutionary rate differences among sites (5 categories, shape parameter = 0.45). All ambiguous positions were removed for each sequence pair. There was a total of 884 positions in the final dataset and the resultant phylogenetic tree was edited in MEGAX (Kumar et al. 2018).

RESULTS

A total of 94 oral swabs were collected from both urban and exurban locations (Table 1). Samples were obtained from 19 Inca doves, 6 Eurasian collared-doves, 66 white-winged and 3 mourning doves. Three of the white-winged doves were double-sampled as controls to ensure accuracy of culture pouches.

Mourning and Eurasian collared-doves were not included in all comparisons due to small sample sizes (Table 1). Comparing prevalence between species regardless of location (Table 2) revealed a significant difference between white-winged (80.3%) and Inca doves (31.6%) only (Fisher's exact test: $P_{adj}<0.001$). There was no significant difference between urban and exurban prevalence (Fisher's exact test: $P_{adj}=1.000$). However, there was a significant difference between prevalence in urban white-winged (77.8%) and urban Inca doves (40%) (Fisher's exact test: $P_{adj}<0.031380$) as well as exurban white-winged (85.3%) and exurban Inca doves (0%) (Fisher's exact test: $P_{adj}=0.008$). Lastly, prevalence within avian species between locations were not significantly different (Table 3)

Table 1 — Prevalence of *Trichomonas gallinae* among doves sampled at urban and exurban locations, listed by species. Sampling was conducted between January 2017 and July 2018 in urban and exurban locations of San Angelo, Texas. Oral swabs were cultured in InPouch™ TF diagnostic pouches and observed via light microscopy; the presence of ≥1 motile trichomonad was considered culture positive. *Streptopelia decaocto*: Eurasian collared-dove; *Columbina inca*: Inca dove; *Zenaida macroura*: mourning dove; *Zenaida asiatica*: white-winged dove.

	Urban		Exurban		N_{Total}
Species	Positive	Negative	Positive	Negative	
Streptopelia decaocto	0	0	3	3	6
Columbina inca	6	9	0	4	19
Zenaida macroura	0	2	0	1	3
Zenaida asiatica	35	10	18	3	66
Total	41	21	21	11	94

Table 2 — Overall comparison of prevalence of *T. gallinae* infection between species in both urban and exurban sites combined. Fisher's exact test for count data was used to compare prevalences between sampled avian species, regardless of location. P-values have been adjusted using Bonferroni adjustment. *Streptopelia decaocto*: Eurasian collared-dove; *Columbina inca*: Inca dove; *Zenaida macroura*: mourning dove; *Zenaida asiatica*: white-winged dove.

		Fisher's exact test	
Species comparisons		P	P_{adj}
Z. asiatica	*C. inca*	< 0.001	<0.001
Z. asiatica	*S. decaocto*	0.119	0.713
C. inca	*S. decaocto*	0.63	1
Z. asiatica	*Z. macroura*	0.011	0.064
S. decaocto	*Z. macroura*	0.464	1
C. inca	*Z. macroura*	0.533	1

Table 3 — Comparison of intraspecific prevalence of *T. gallinae* between urban and exurban locations. Fisher's Exact test for count data was used to compare intraspecific prevalences at urban and exurban locations. P-values have been adjusted using Bonferroni adjustment. *Streptopelia decaocto*: Eurasian collared-dove; *Columbina inca*: Inca dove; *Zenaida macroura*: mourning dove; *Zenaida asiatica*: white-winged dove.

	Fisher's exact test	
Intraspecific comparisons	P	P_{adj}
Z. asiatica	0.519	1
C. inca	0.255	0.766
S. decaocto	1	1

pH measurements

The pH of columbids sampled in this study (n=27) was not influenced by infection (F=1.985, df=1, P=0.171). The average pH of infected individuals was 7.07 ± 0.081 [SE] and the average pH of uninfected individuals was 6.87± 0.120 [SE]. The pH measurements of sampled columbids were then compared by location, which was an influencing factor (F=9.318, df=1, P=0.005). The average pH of urban individuals was 7.16± 0.074 [SE] and the average pH of exurban individuals was 6.79± 0.099 [SE].

Sequence Analysis of ITS1/5.8s rRNA/ITS2 region

Of the 62 positive samples, 57 successfully amplified the ITS1/5.8s/ITS2 region and were sequenced. Two positive samples were used for staining purposes and were not processed for DNA extraction and sequencing, two additional samples did not successfully amplify and one sample (10 ITS WWDO) was omitted from alignment and further analysis due to poor quality sequence product. Summarized NCBI BLASTn results of sample sequences are in Table 4. Detailed NCBI BLASTn results are in Appendix IV (Table 12). All 48 sequences from samples collected from white-winged doves had higher sequence similarities to a reference sequence for *T. vaginalis* (n=35) or a *Trichomonas* genus (n=13) specific sequence than to reference sequences for *T. gallinae* (Table 4). Four sequences collected from Inca doves also had a higher sequence similarity to *T. vaginalis* than to *T. gallinae*. All sequences from samples collected from Eurasian collared-doves and 1 Inca dove had higher sequence similarities to different reference sequences of *T. gallinae* than to any other organism (Table 4).

Table 4 — Summarized NCBI BLASTn results of 57 sequences of the ITS1/5.8s/ITS2 region from samples collected in the current study. Samples: number of samples that matched and the avian species they were collected from; Acc. n.: GenBank Accession number

Database Sequence Organism	Samples, Avian Species Collected From	Identity	GenBank Acc. #, Author
T. vaginalis	4 – Inca, 35 – white-winged dove	≥98%	U86613.1; Felleisen 2001
Trichomonas sp.	13 – all white-winged dove	≥ 98%	EU215361.1; Gerhold 2008
T. gallinae	3 – all Eurasian collared-dove	99%	KX459505.1; Marx 2017
T. gallinae	1 – Inca dove	99%	KC215387.1; Girard 2014

Phylogenetic analysis of ITS1/5.8s rRNA/ITS2

Phylogenetic alignment of the ITS1/5.8s rRNA/ITS2 region included a representative sequence from each unique group, sequences from individually unique samples, representative sequences from each known ITS1/5.8s rRNA/ITS2 sequence group of *T. gallinae*, as well as *Tritrichomonas foetus* (Acc. n: AF466749.1) and *Tetratrichomonas gallinarum* (Acc. n.: AY245126.1) as outgroups. Samples from this study clustered into two groups (Figure 2); *T. gallinae*-like and *T. vaginalis*-like. These lineages are weakly supported by maximum likelihood bootstrap values of 54% (*T. gallinae*-like clade) and 69% (*T. vaginalis*-like clade).

One group of samples clustered with *T. vaginalis* and known reference sequences of *T. gallinae* that are more similar to a reference sequence of *T. vaginalis* than to other reference sequences of *T. gallinae*. The other group of samples clustered with known reference sequences of *T. gallinae* that are more similar to other reference sequences of *T. gallinae* than to *T. vaginalis* (Figure 2). There is no significant difference in the sequence groups found at either location type (Fisher's exact test: $P_{adj}=0.216$). However, sample size and species sampled should be considered when interpreting these results. These groups were then compared by avian host species (Table 5). A significant difference was found between groups found in avian species (Fisher's exact test: $P_{adj}<0.001$). However, species composition and sample size should be taken into account when interpreting these results.

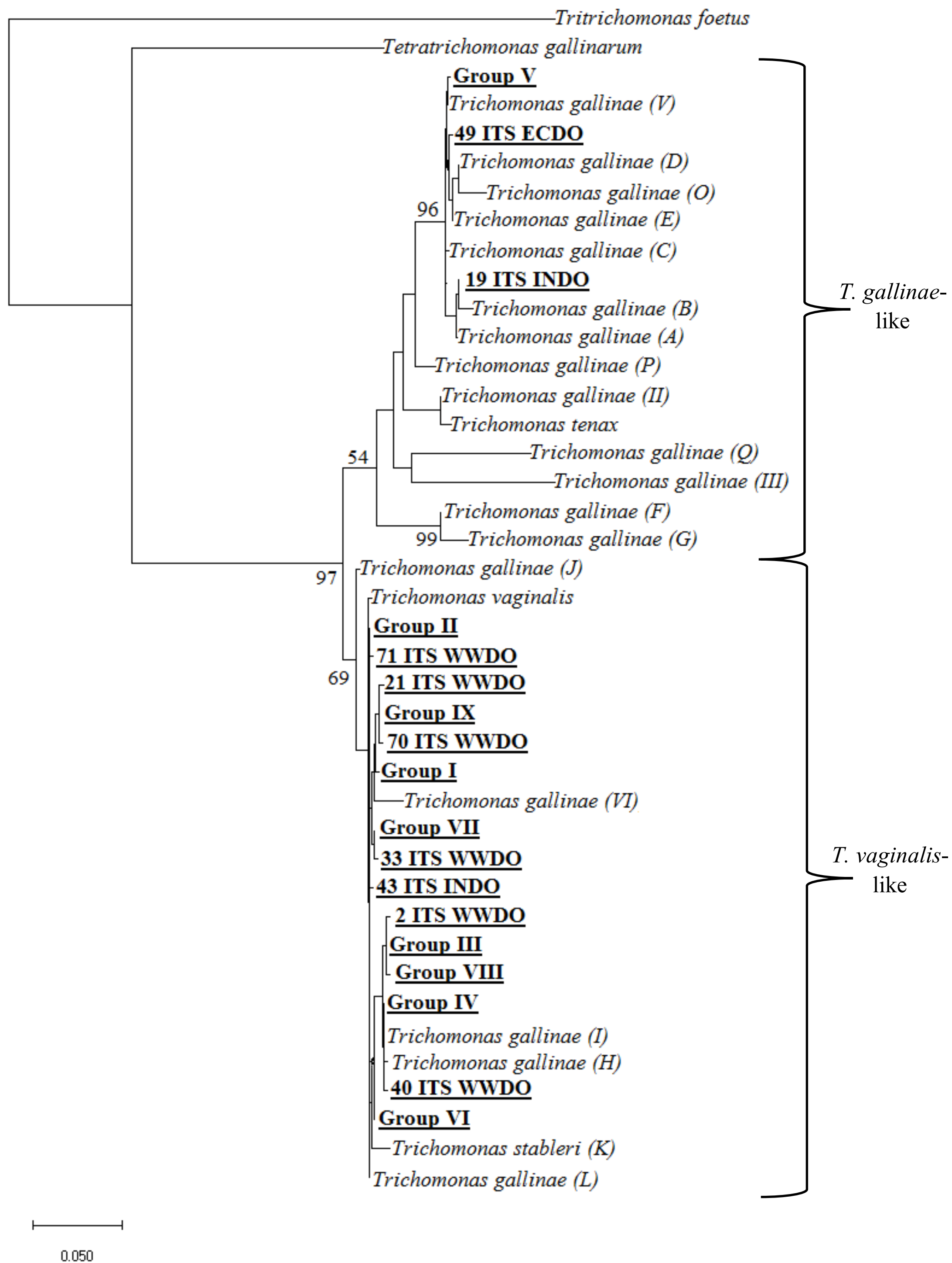


Figure 2— Molecular phylogenetic analysis of the ITS1/5.8s rRNA/ITS2 region of *Trichomonas* collected in this study.

Figure 2 — Phylogenetic analysis of *Trichomonas gallinae* samples collected from columbiforms based on the sequencing of ITS1/5.8s/ITS2 region. The tree was constructed using 365-bp aligned nucleotide positions using a neighbor-joining algorithm with 10,000 bootstrap replications. The DNA substitution model was a Tamura 3-parameter model with gamma distribution, and *Tritrichomonas foetus* and *Tetratrichomonas gallinarum* were used as outgroups. Study-specific identification (numeric, sequence identifier, avian species sample was collected from) or identical sequence group ("Group" followed by roman numeral) represent sequences collected in my study and are in bold type. WWDO: white-winged dove; ECDO: Eurasian collared-dove; INDO: Inca dove. Reference sequences for known sequence types of *T. gallinae* are in italics. The numbers adjacent to nodes are the branch support values for the branch connecting to that node. The scale bar represents evolutionary distance and is in the units of number of base substitutions per site. The bracketed groups "*T. vaginalis* - like" and "*T. gallinae* - like" indicate the clustering pattern seen in this analysis.

Table 5 — Groups of identical ITS1/5.8s/ITS2 sequences collected from samples of *Trichomonas*, listed by the avian species they were collected from.

Species	Sequence lineage	
	T. gallinae-like	*T. vaginalis*-like
White-winged dove	0	47
Inca dove	1	5
Eurasian collared-dove	3	0

Sequence Analysis of Fe-hydrogenase

Of the 58 samples that successfully amplified the ITS1/5.8s/ITS2 region, only 4 successfully amplified the Fe-hydrogenase region. Sample 55 FeHyd ECDO and 50 FeHyd ECDO had a ≥ 99% identity to *T. gallinae* subtype C2, sample 49 FeHyd ECDO had a 99% identity to both *T. gallinae* subtypes C2 and C5, and sample 19 FeHyd INDO had a 100% identity to *T. gallinae* subtype A1 (Table 6).

Phylogenetic analysis of Fe-hydrogenase

Phylogenetic alignment of the Fe-hydrogenase region included 4 sequenced samples (Table 6), representative sequences from each known Fe-hydrogenase subtype of *T. gallinae* (Table 7), a known sequence of this region for *T. stableri* (Acc. n.: KC660123), and a known sequence of this region for *T. vaginalis* (Acc. n.: XM001310179) as an out-group. Samples 55 FeHyd ECDO and 59 FeHyd ECDO clustered with a representative sequence (KP900031) of *T. gallinae* subtype C2, supported by a bootstrap value for neighbor-joining value of 89% (Figure 3). Sample 19 FeHyd INDO clustered with a representative sequence of *T. gallinae* subtype A1, supported by a bootstrap value for neighbor-joining value of 89% (Figure 3).

Table 6 —BLAST results and sequence identity values for the Fe-hydrogenase gene region from *Trichomonas* samples are listed with the first match from a published study; Acc. n.: GenBank Accession number. Four letters at the end of the study-specific sample ID indicate the species the oral swab was collected from; INDO: Inca dove; ECDO: Eurasian collared-dove.

		Sequence Identity
Study-specific Sample ID	Organism-subtype, Acc. n.	% BLAST Identity, E-value
19 FeHy INDO	*T. gallinae*-A1 (KC244201; Girard et al. 2014)	99%, 0.00
49 FeHy ECDO	*T. gallinae*-C5 (KP900040; Sansano-Maestre et al. 2016)	100%, 0.00
	T. gallinae-C2 (KP900032; Sansano-Maestre et al. 2016)	100%, 0.00
55 FeHy ECDO	*T. gallinae*-C2 (KP900031; Sansano-Maestre et al. 2016)	99%, 0.00
59 FeHy ECDO	*T. gallinae*-C2 (KP900031; Sansano-Maestre et al. 2016)	100%, 0.00

Table 7 — Known subtypes of the Fe-hydrogenase region of the *Trichomonas gallinae* genome, as reported in the literature.

Acc. n.: Accession number.

Known Subtype	Species	Host	GenBank Acc. n.	Origin	Author
A1[a]	*T. gallinae*	Band-tailed pigeon *Patagioenas fasciata*	KC244201	California	Girard et al. 2014
A2	*T. gallinae*	Rock dove *Columba livia*	KP900030	Spain	Sansano-Maestre et al. 2016
C1	*T. gallinae*	*Not available*	AF446077	Not Available	Voncken et al 2002
C2 [a]	*T. gallinae*	Eurasian collared dove *Streptopelia decaocto*	KP900031	Spain	Sansano-Maestre et al. 2016
C4	*T. gallinae*	Rock dove *Columba livia*	KP900037	Spain	Sansano-Maestre et al. 2016
C5 [a]	*T. gallinae.*	Rock dove *Columba livia*	KP900040	Spain	Sansano-Maestre et al. 2016
C6	*T. gallinae*	Booted eagle *Aquila pennata*	KP900041	Spain	Sansano-Maestre et al. 2016
C7	*T. gallinae*	Eurasian collared dove *Streptopelia decaocto*	KP900034	Spain	Sansano-Maestre et al. 2016

[a] Indicates subtypes found in the current study.

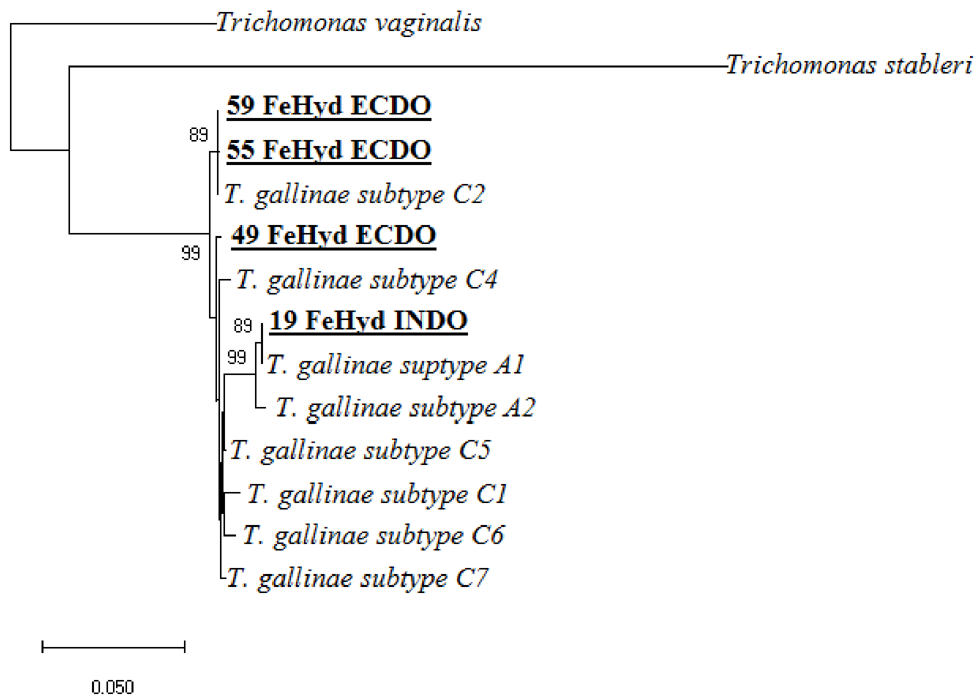


Figure 3. — Molecular phylogenetic analysis of the Fe-hydrogenase gene region of *Trichomonas* collected in this study. The tree was constructed using 884-bp aligned nucleotide positions using a neighbor-joining algorithm with 10,000 bootstrap replications in a Kimura 2-parameter model with gamma distribution, and *T. vaginalis* and *T. stableri* as outgroups. Sequences collected in the current study are represented by their study-specific identification (numeric, sequence identifier, avian species sample was collected from) in bold. WWDO: white-winged dove; ECDO: Eurasian collared-dove; INDO: Inca dove. Reference sequences for known sequence types of *T. gallinae* are in italics. The numbers adjacent to nodes are the branch support values for the branch connecting to that node. The scale bar represents evolutionary distance and is in the units of number of base substitutions per site.

DISCUSSION

My first hypothesis was that the pH of the oral cavities of birds sampled would be more acidic in those that are culture negative for *T. gallinae* infection compared to those that are positive (Urban and Mannan 2014). I measured the oral pH of 27 of the columbids sampled in this study and the average pH of infected individuals sampled was slightly less acidic (7.07 ± 0.081 SE) than the average of those not infected (6.87± 0.120 SE), and not statistically different, and did not support my hypothesis. However, this is consistent with the optimal pH of 7.2 reported for *T. gallinae* proliferation (Read 1957). Read (1957) also noted that viability and potential for *T. gallinae* decrease as the environment becomes more acidic. Urban et al. (2013) found that the average oral pH of urban nestling Cooper's Hawks (6.8) was less acidic than the average pH in breeding-aged individuals (6.1). In this same population 85% of nestlings were infected with *T. gallinae*, while only 1% of breeding individuals were infected. Although the results in my study are not statistically different, they do follow the pattern seen in the Urban et al. (2013) study. Additionally, the differences in pH range between Cooper's hawks and columbids sampled in my study could be an explanatory factor of columbids being a well-known host species for *T. gallinae*. There was a difference in pH between urban and exurban locations in my study which could be attributed to water quality or treatment, or simply an artifact of the small sample size. Due to the survival capabilities of *T. gallinae* in artificial water sources, it would be interesting to conduct a similar, long-term study comparing incidence of *T. gallinae* in drought and wet years, especially in urban areas where drought induced residential water restrictions may reduce availability of artificial water sources.

My second hypothesis was that I would find a higher prevalence of *T. gallinae* infection in birds sampled in urban areas than in exurban areas, as infection and population densities of columbids have been found to increase in residential areas (Stabler 1954; Boal et al. 1998). My results did not support this hypothesis. I found a 66% incidence of *T. gallinae* in urban areas and a 66% incidence in exurban areas. The total sample size and species composition of the samples may have played a role in this outcome (Table 1). Further, the general foraging behavior of doves could explain the similar values as they are known to fly up to 5 to 20 km from roosting sites to foraging sites and the urban and exurban trapping locations were well within that range of one another (Cottam and Trefethen 1968). It's likely the birds sampled in exurban locations frequented urban locations as foraging grounds and vice versa, impacting *T. gallinae* prevalence. Moreover, the factors that increase opportunistic disease transmission in urban areas (artificial feed sources that negate typical feeding habits) only provide for comparably higher prevalences if similar feed sources are not available in exurban locations as well. The exurban locations used for trapping included feedlots and cropland, possibly providing a type of artificial feed source and source of disease transmission. The majority of samples were collected from white-winged doves (Table 1), which is a well-documented host of *T. gallinae* (Stabler 1947, 1961). Additionally, none of the birds sampled in this study exhibited obvious clinical signs of trichomoniasis, although thorough examinations were not conducted in an effort to reduce stress on each individual. This is consistent with the results of other researchers in regards to the absence of lesions associated with *T. gallinae* infection in white-winged doves and mourning doves,

although no culture positive mourning doves were found in my study (Stabler 1951, 1961; Hedlund 1998).

My third hypothesis was that I would find a higher prevalence of *T. gallinae* in white-winged doves than in the other four species listed, as white-winged dove is noted to be the most abundant columbid in these residential areas (Conti and Forrester 1981; Boal et al. 1998; Gerhold et al. 2008). In this study, white-winged doves presented a higher prevalence (80.3%) of *T. gallinae*, irrespective of location, than other species sampled; which supported my hypothesis. The combination of white-winged doves having a higher density compared to other columbid species in San Angelo, the colonial nesting behaviors of this species (Goodwin 1967) and ease of transmission of the disease (Cottam and Trefethen 1968) is likely responsible for the higher incidence my study found. If the other species sampled in my study had similar population densities in this area to white-winged doves, I would expect to find similarly high incidence of *T. gallinae*. However, the prevalence in white-winged doves found in my study is slightly lower than other reported infection rates in white-winged doves, which range from 97 to 100% (Stabler 1961; Hedlund 1998; Glass et al. 2001). To rule out this result being an artifact of small sample size, I graphed the white winged dove samples on an accumulation curve (Figure 4). The prevalence of infection on this curve remains relatively constant at approximately 80%, with no indication of increasing with the addition of samples. The accumulation curve indicates that the low prevalence (80%) in white-winged doves compared to previous studies (97-100%) is not an artifact of low sample size.

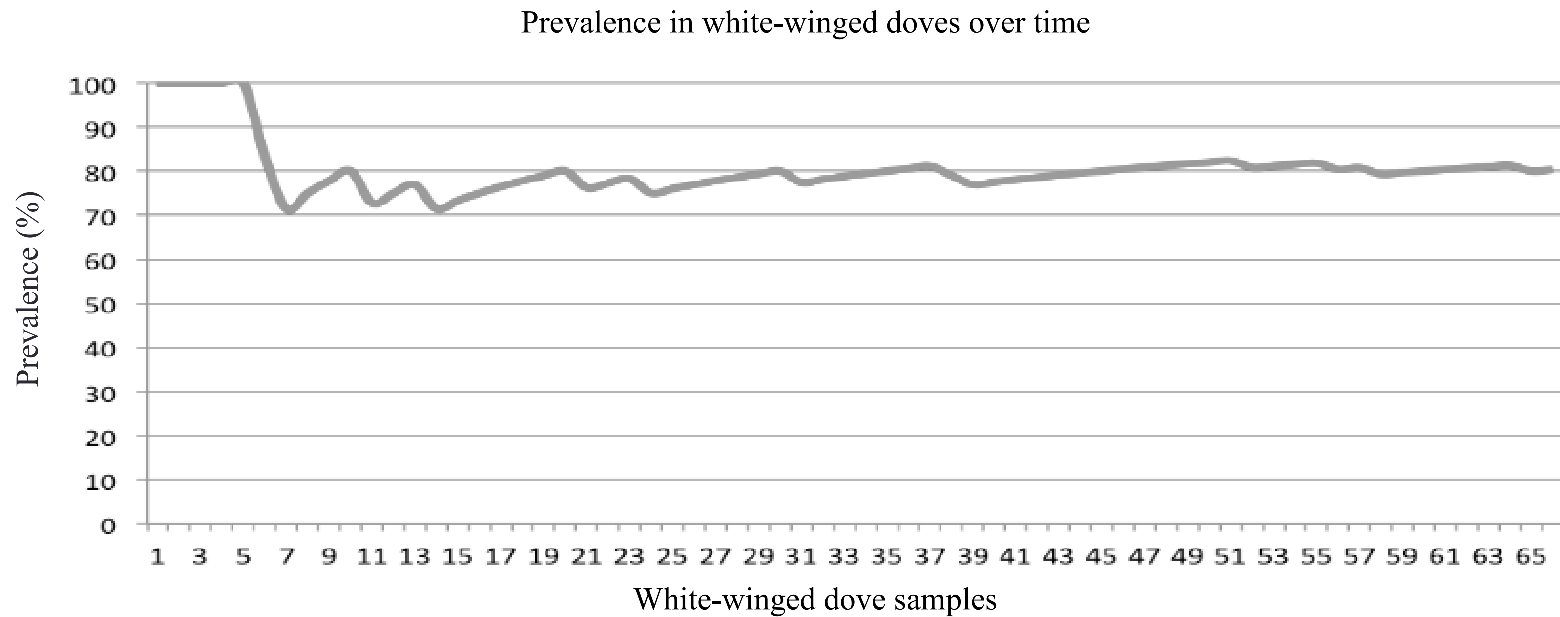


Figure 4 — Accumulation curve of samples collected from white-winged doves in chronological order of collection with the prevalence of *T. gallinae*. Samples were collected beginning in January 2017 and concluding in June 2018

Additionally, the annually cyclic behavior of avian trichomoniasis in columbids, where latent infection is lost and then reacquired, could also explain the below average incidence (Schulz et al. 2005; Urban and Mannan 2014). Another explanatory factor could be the comparatively small sample sizes; acquiring equal sample sizes from all species groups may have yielded different results. Several studies in the United States note a susceptibility to *T. gallinae* infection in mourning doves, especially those populations sympatric with white-winged doves (Stabler and Herman 1951; Haugen 1952; Conti and Forrester 1981; Glass et al. 2001; Schulz et al. 2005). Additionally, expanding my sample locations to include more rural areas might have decreased my estimate of exurban prevalence.

In contrast with the available literature regarding *T. gallinae* in white-winged doves, the literature on incidence in Inca doves is lacking. Hedlund (1998) conducted a survey of *T. gallinae* in avian populations in urban Tucson, Arizona over two consecutive years and reported incidences of 47% (n=83) and 60% (n=25) in Inca doves. This is consistent with the urban incidence found in this study (66%, n=9), however the difference in sample sizes should be taken into account. Mourning doves had the lowest sample size in this study; three were collected at urban locations and none were collected at exurban locations, making drawing reliable conclusions from these data extremely difficult. Other researchers report that low asymptomatic infection rates in mourning dove populations is common and those populations that are sympatric with white-winged dove populations or share similar habitats in wintering areas have an increase in incidence as well as trichomoniasis-related mortalities (Conti and Forrester 1981; Glass et al. 2001; Schulz et al. 2005). A substantial decrease in

mourning dove populations in Alabama was attributed to an epizootic of trichomoniasis (Haugen 1952).

Eurasian collared-doves had an exurban incidence of 50% (n=6); due to the small sample size and the fact that none were collected in urban areas, it is difficult to draw reliable conclusions. Similar to the Inca dove, other than noting that *T. gallinae* infection can occur in Eurasian collared-doves, literature concerning the incidence of this parasite in Eurasian collared-doves in the United States is lacking. However, several studies in Europe have found similar or higher infection rates in Eurasian collared doves, with one study in Spain reporting only 1% of the infected birds (n=167) exhibiting lesions (Lennon et al. 2013; del Carmen Martínez Herrero 2016). The lack of lesions coupled with high prevalences demonstrates the enzootic character of this infection in columbids, serving as reservoir hosts for this parasite.

Phylogenetic Analysis

My fourth hypothesis was that I would find more than one unique genetic lineage of *T. gallinae* among collected samples, as many lineages have been reported and are found to differ by avian host species (Gerhold et al. 2008; Girard et al. 2014a; Martínez-Herrero et al. 2014). Phylogenetic analysis of the ITS1/5.8s rRNA/ITS2 region revealed the presence of more than one sequence group among my samples, which supports my hypothesis. This could be attributed to the unique manner in which pigeons and doves consume water; unlike most birds that scoop water in their bill and tilt their heads back, members of the family Columbidae drink by dipping bills into the water and sucking up the liquid. This provides ample exposure time of various strains of *T. gallinae* to the water which can then survive up

to 60 minutes in water sources contaminated with organic detritus (Kocan 1969; Purple et al. 2015). The group foraging and watering behaviors of columbids provide adequate opportunities for transmission between individuals to occur within the survival window of *T. gallinae* in water sources (Kocan 1969; Gerhold et al. 2013; Purple et al. 2015).

Comparison of the sequence groups by location revealed no preference of specific sequence groups to a location; this is consistent with known foraging behavior of doves, as they are known to fly up to 5 to 20 km from roosting sites to foraging sites which supports the easy transmission of multiple strains between my urban and exurban trapping locations (Cottam and Trefethen 1968). Similarly, several studies found that samples collected from columbids clustered into at least two clades; *T. gallinae*-like and *T. vaginalis*-like (Gaspar da Silva et al. 2007; Gerhold et al. 2008; Girard et al. 2014a, 2014b). Gerhold et al. (2008) found similar host-parasite relationships to the one observed in the clustering patterns of my samples (Figure 2) (white-winged doves - *T. vaginalis*-like; Eurasian collared-doves - *T. gallinae*-like). This may indicate a strain-specific host-parasite dynamic. Girard et al. (2014a) described a new species of trichomonad, *Trichomonas stableri*, based on genetic information from ITS1/5.8s rRNA/ITS2 and Fe-Hydrogenase regions as well as morphological characteristics. This proposed new species was found in band-tailed pigeons (*P. fasciata*) in California, and is also sequence group K, as described by Gerhold et al. (2008). Known sequence groups for the ITS1/5.8s rRNA/ITS2 region are compiled in Table 8. Sequence groups H, I and J were also found in white-winged doves in Texas by another study, which

Table 8 — Known sequence groups of the ITS1/5.8s rRNA/ITS2 region of *T. gallinae* as reported in the literature. Acc. n.: Accession number; *T. gallinae* : *Trichomonas gallinae*; *T. sp*: *Trichomonas species*; Author: author that reported sequence in Genbank.

Known Sequence Group	Species	Host	GenBank Acc. n.	Origin	Author
A[a]	*T. gallinae*	Mourning dove *Zenaida macroura*	EU215369	Arizona	Gerhold et al. (2008)
B	*T. gallinae*	Broad-winged hawk *Buteo platypterus*	EU215368	Florida	Gerhold et al. (2008)
C[ba]	*T. gallinae*	Rock pigeon *Columba livia*	EU215362	Colorado	Gerhold et al. (2008)
D[b]	*T. gallinae*	Eurasian collared-dove *Streptopelia decaocto*	EU215364	Texas	Gerhold et al. (2008)
E[b]	*T. gallinae*	Eurasian collared-dove *Streptopelia decaocto*	EU215363	Texas	Gerhold et al. (2008)
F	*T. sp.*	Common ground dove *Columbina passerina*	EU215358	Texas	Gerhold et al. (2008)
G	*T. sp.*	Common ground dove *Columbina passerina*	EU215359	Texas	Gerhold et al. (2008)
H	*T. sp.*	White-winged dove *Zenaida asiatica*	EU215360	Texas	Gerhold et al. (2008)
I	*T. sp.*	White-winged dove *Zenaida asiatica*	EU215361	Texas	Gerhold et al. (2008)

[b] Indicates sequence groups that are identical but differ slightly in length.
[a] Indicates sequence groups found in the current study

Table 8 (Continued)

Known Sequence Group	Species	Host	GenBank Acc. n.	Origin	Author
J	*T. sp.*	Mourning dove *Zenaida macroura*	EU215365	Texas	Gerhold et al. (2008)
K	*T. stableri*	Band-tailed pigeon *Patagioenas fasciata*	EU215367	California	Gerhold et al. (2008)
L	*T. sp.*	Cooper's hawk *Accipiter cooperii*	EU215366	Arizona	Gerhold et al. (2008)
O	*T. gallinae*	Stock dove *Columba oenas*	KX459442	Germany	Marx et al. (2017)
P	*T. sp.*	Turtle dove *Streptopelia turtur*	KF993705	Italy	Marinez-Herrero (2017)
Q	*T. sp.*	Turtle dove *Streptopelia turtur*	KX459510	Spain	Marx et al. (2017)
II	*T. sp.*	Racing pigeon *Columba livia domestica*	FN433474	Austria	Grabensteiner et al. (2010)
III	*T. gallinae*	Nicobar pigeon *Caloenas nicobarica*	KC529665	India	Chi et al. (2013)
V	*T. gallinae*	Canary bird *Serinus canaria domestica*	FN433477	Austria	Grabensteiner et al. (2010)
VI	*T. sp.*	Bearded vulture *Gypaetus barbatus*	FN433478	Czech Republic	Grabensteiner et al. (2010)

may suggest a geographic component to the already known host- parasite relationship between this species and these sequence groups (Gerhold et al. 2008).

An epizootic in Tucson, Arizona, involving both mourning doves and Cooper's hawks was attributed to isolates found to cluster with the *T. vaginalis*-like clade and the *T. gallinae*-like clade, specifically ITS sequence groups L and A, respectively (Gerhold et al. 2008). This suggests that white-winged doves played a role in the spread of this disease (Gerhold et al. 2008). A similar epizootic could occur in the situation of a colonization event in San Angelo, as urban Cooper's hawks diets consist of more columbids than their rural counterparts and sequence group A was found in at least one sample in my study (Boal 1997; Boal et al. 1998; Estes and Mannan 2003; Mannan et al. 2008).

ITS1/5.8s/ITS2 sequence groups C, D and E (which are identical, but differ slightly in length), were also reported in Eurasian collared-doves from Texas by Gerhold et al. (2008). All samples collected from Eurasian collared-doves in my study also belonged to sequence group C. Additional literature regarding these sequence groups in the United States is lacking. Chi et al. (2013) reported ITS sequence group A is responsible for an epizootic of trichomoniasis producing a large number of mortalities in European finches. Sequence group A was also found in one Inca dove sample in my study. Researchers reported that individuals infected with ITS sequence group A were more likely to exhibit clinical symptoms than those infected with ITS sequence group C, regardless of host species (Chi et al. 2013). It should be noted in that study feral pigeons exhibited no sign of disease regardless of infecting strain. This could indicate weak virulence, however further research including histological studies is needed to draw reliable conclusions, especially in the United States.

The iron-hydrogenase region of the trichomonad genome is known to have separation capabilities in addition to those provided by the ITS1/5.8s rRNA/ITS2 region (Voncken et al. 2002; Lawson et al. 2011; Girard et al. 2014a; Sansano-Maestre et al. 2016). Successful amplification and sequencing of the Fe-hydrogenase region in these samples may have shed more light on the genetic lineages and sequence groups of *Trichomonas* found in these samples. Future studies that include molecular characterization of both of these regions of *T. gallinae* in white-winged doves in Texas should elaborate on this. Additionally, it is possible that the Fe-hydrogenase primers I used are not suitable for amplifying the *T. vaginalis*-like lineage. Since most of my samples fell into the *T. vaginalis*-like lineage based on the ITS sequence types, this may explain the lack of PCR amplification in those samples.

There are several known subtypes of the iron-hydrogenase region of the *T. gallinae* genome (Table 7). Literature involving the study and reporting of these subtypes in the United States is lacking. Samples 55 FeHyd ECDO and 59 FeHyd ECDO (Group V) clustered with *T. gallinae* subtype C2, further elucidating their ITS sequence group as C. Sample 49 FeHyd ECDO clustered with the previously described clade, and had a 99% identity with both *T. gallinae* subtypes C2 and C5, indicating it also falls in ITS sequence group C. Another survey of *T. gallinae* in avian populations using the iron hydrogenase loci found that all infected Eurasian collared-doves were one of the C-subtypes, and inferred as all belonging to ITS sequence group C (Sansano-Maestre et al. 2016). This could indicate a strain specific host-parasite relationship between ITS sequence group C and Eurasian collared-doves. However, the study referenced took place in Spain and additional research would be needed at different geographic locations (especially in the United States) to confirm

this. Sample 19 FeHyd INDO clustered with *T. gallinae* subtype A1 and has been reported in mortality events related to epizootic trichomoniasis in band tailed pigeons in California and is responsible for the trichomoniasis epidemic that lead to significant declines in breeding populations of European finches (Lawson et al. 2011; Chi et al. 2013; Girard et al. 2014b; Rogers et al. 2016). Further research is needed to determine the extent of infection of this subtype at different geographical regions across the United States, and sample species should be expanded to include passerines.

This study has demonstrated that incidence of *T. gallinae* in columbids may be influenced more by the species of columbid than by habitat type. Additionally, I show that oral pH of columbids infected and not infected with *T. gallinae* mimics the pattern demonstrated in in Cooper's hawks (Urban et al. 2013). I also show additional support for host-parasite strain relationships previously suggested in earlier literature, and that there is the potential for an epizootic event resulting in high mortalities of different avian species including passerines, columbids or protected birds of prey in and around the city of San Angelo, Tom Green County, Texas.

LITERATURE CITED

Amin A, Bilic I, Liebhart D, Hess M. 2014. Trichomonads in birds-a review. *Parasitology* 141:733–747.

Anderson NL, Grahn RA, Van Hoosear K, BonDurant RH. 2009. Studies of trichomonad protozoa in free ranging songbirds: Prevalence of *Trichomonas gallinae* in house finches (*Carpodacus mexicanus*) and corvids and a novel trichomonad in mockingbirds *(Mimus polyglottos*). *Vet Parasitol* 161:178–186.

Boal CW. 1997. *An urban environment as an ecological trap for Cooper's hawks*. Ph.D. Dissertation, Renewable Natural Resources, University of Arizona, Tuscon, Arizona, United States of America, 85 pp.

Boal CW, Mannan RW, Hudelson SK. 1998. Trichomoniasis in Cooper's hawks from Arizona. *J Wildl Dis* 34:590–593.

Bunbury N, Jones CG, Greenwood AG, Bell DJ. 2007. *Trichomonas gallinae* in Mauritian columbids: Implications for an endangered endemic. *J Wildl Dis* 43:399–407.

del Carmen Martínez Herrero M. 2016. *Characterization of oropharyngeal trichomonads in wild birds. Description of new variants, virulence assays and differential proteomic analysis using clonal cultures*. Ph.D. Thesis, Animal Production, Animal and Public Health, Food Technology and Science, CEU Cedenal Herrera University, Valencia, Spain, 257 pp.

Cavalier-Smith T. 2002. The phagotrophic origin of eukaryotes and phylogenetic classification on protozoa. *Int J Syst Evol Microbiol* 52:297–354.

Cepicka I, Kutišová K, Tachezy J, Kulda J, Flegr J. 2005. Cryptic species within the *Tetratrichomonas gallinarum* species complex revealed by molecular polymorphism. *Vet Parasitol* 128:11–21.

Chi JF, Lawson B, Durrant C, Beckmann K, John S, Alrefaei AF, Kirkbride K, Bell DJ, Cunningham AA, Tyler KM. 2013. The finch epidemic strain of *Trichomonas gallinae* is predominant in British non-passerines. *Parasitology* 140:1234–1245.

Conti JA, Forrester DJ. 1981. Interrelationships of parasites of white-winged doves and mourning doves in Florida. *J Wildl Dis* 17:529–536.

Cottam C, Trefethen J. 1968. *Whitewings - The life history, status, and management of the whitewinged dove.* N. J., Van Nostrand Reinhold Inc, Princeton, New Jersey, United States of America. 320 pp.

Dudek BM, Kochert MN, Barnes JG, Bloom PH, Joseph PM, Gerhold RW, Purple KE, Jacobson KV, Preston CR, Vennum CR, Watson JW, Heath JA. 2018. Prevalence and risk factors of Trichomonas gallinae and trichomonosis in golden eagle (*Aquila chrysaetos*) nestlings in western north america. *J Wildl Dis* 54:1–11.

Ecco R, Preis IS, Vilela DAR, Luppi MM, Malta MCC, Beckstead RB, Stimmelmayer R, Gerhold RW. 2012. Molecular confirmation of *Trichomonas gallinae* and other parabasalids from Brazil using the 5.8S and ITS-1 rRNA regions. *Vet Parasitol* 190:36–42.

El-Khatam AO, AbouLaila MR, Ibrahim M, AbdEl-Gaber MM. 2016. *Trichomonas gallinae*: Prevalence and molecular characterization from pigeons in Minoufiya governorate, Egypt. *Exp Parasitol* 170:161–167

Erwin KG, Kloss C, Lyles J, Felderhoff J, Fedynich AM, Henke SE, Roberson JA. 2000. Survival of *Trichomonas gallinae* in white-winged dove carcasses. *J Wildl Dis* 36:551–4.

Estes WA, Mannan RW. 2003. Feeding behavior of Cooper's hawks at urban and rural nests in southeastern Arizona. *Condor* 105:107–116.

Gaspar da Silva D, Barton E, Bunbury N, Lunness P, Bell DJ, Tyler KM. 2007. Molecular identity and heterogeneity of trichomonad parasites in a closed avian population. *Infect Genet Evol* 7:433–440.

Gerhold RW, Maestas LP, Harnage PM. 2013. Persistence of two *Trichomonas gallinae* isolates in chlorinated and distilled water with or without organic material. *Avian Dis* 57:681–683

Gerhold RW, Yabsley MJ, Smith AJ, Ostergaard E, Mannan W, Cann JD, Fischer JR. 2008. Molecular characterization of the *Trichomonas gallinae* morphologic complex in the United States. *J Parasitol* 94:1335–1341.

Girard YA, Rogers KH, Gerhold R, Land KM, Lenaghan SC, Woods LW, Haberkern N, Hopper M, Cann JD, Johnson CK. 2014a. *Trichomonas stableri* n. sp., an agent of trichomonosis in Pacific Coast band-tailed pigeons (*Patagioenas fasciata monilis*). *Int J Parasitol Parasites Wildl* 3:32–40.

Girard YA, Rogers KH, Woods LW, Chouicha N, Miller WA, Johnson CK. 2014b. Dual-pathogen etiology of avian trichomonosis in a declining band-tailed pigeon population. *Infect Genet Evol* 24:146–156.

Glass JW, Fedynich AM, Small MF, Benn SJ. 2001. *Trichomonas gallinae* in an expanding population of white-winged doves from Texas. *Southwest Nat* 46:234–237.

Goodwin D. 1967. *Pigeons and doves of the world.* British Museum (Natural History), London, England. 1967. 363 pp.

Grabensteiner E, Bilic I, Kolbe T, Hess M. 2010. Molecular analysis of clonal trichomonad isolates indicate the existence of heterogenic species present in different birds and within the same host. *Vet Parasitol* 172:53–64.

Greiner E, Baxter W. 1974. A localized epizootic of trichomoniasis in mourning doves. *J Wildl Dis* 10:104–106.

Grunenwald C, Sidor I, Mickley R, Dwyer C, Gerhold R. 2018. *Tetratrichomonas* and *Trichomonas* spp -associated disease in free-ranging common eiders (*Somateria mollissima*) from Wellfleet Bay, MA and description of ITS1 region genotypes. *Avian Dis* 62:117–123.

Haugen AO. 1952. Trichomoniasis in Alabama mourning doves. *J Wildl Manage* 16:164–169.

Hedlund CA. 1998. Trichomonas gallinae *in avian populations in urban Tucson, Arizona*. M.S. Thesis, Renewable Natural Resources, University of Arizona, Tuscon, Arizona, United States of America. 89 pp.

Hillis DM, Dixon MT. 1991. Ribosomal DNA : Molecular Evolution and Phylogenetic Inference. *Q Rev Biol* 66:411–453.

Honigberg BM. 1961. Comparative pathogenicity of *Trichomonas vaginalis* and *Trichomonas gallinae* to mice . I. Gross pathology, quantitative evaluation of virulence, and some factors affecting pathogenicity. *J Parasitol* 47:545–571.

Kelly-Clark WK, McBurney S, Forzán MJ, Desmarchelier M, Greenwood SJ. 2013. Detection and characterization of a *Trichomonas* isolate from a rehabilitated bald eagle (*Halliaeetus leucocephalus*). *J Zoo Wildl Med* 44:1123–1126.

Kimura M. 1980. Evolutionary Rates models. *J Mol Evol* 16:111–120.

Kleina P, Bettim-Bandinelli J, Bonatto SL, Benchimol M, Bogo MR. 2004. Molecular phylogeny of Trichomonadidae family inferred from ITS-1, 5.8S rRNA and ITS-2 sequences. *Int J Parasitol* 34:963–970.

Kocan RM. 1969. Various grains and liquid as potential vehicles of transmission for *Trichomonas gallinae*. *Wildl Dis Assoc* 5:148–149.

Kocan RM, Amend SR. 1972. Immunologic status of mourning doves following an epizootic of trichomoniasis. *J Wildl Dis* 8:176–180.

Krone O, Altenkamp R, Kenntner N. 2005. Prevalence of *Trichomonas gallinae* in northern goshawks from the Berlin area of northeastern Germany. *J Wildl Dis* 41:304–309.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549.

Lawson B, Cunningham AA, Chantrey J, Hughes LA, John SK, Bunbury N, Bell DJ, Tyler KM. 2011. A clonal strain of *Trichomonas gallinae* is the aetiologic agent of an emerging avian epidemic disease. *Infect Genet Evol* 11:1638–1645.

Lennon R, Dunn J, Stockdale J, Goodman SJ, Morris AJ, Hamer KC. 2013. Trichomonad parasite infection in four species of Columbidae in the UK. *Parasitology* 140:1368–1376.

Mannan RW, Steidl RJ, Boal CW. 2008. Identifying habitat sinks: A case study of Cooper's hawks in an urban environment. *Urban Ecosyst* 11:141–148.

Maritz JM, Land KM, Carlton JM, Hirt RP. 2014. What is the importance of zoonotic trichomonads for human health? *Trends Parasitol* 30:333–341.

Martínez-Herrero MC, Sansano-Maestre J, López Márquez I, Obón E, Ponce C, González J, Garijo-Toledo MM, Gómez-Muñoz MT. 2014. Genetic characterization of oropharyngeal trichomonad isolates from wild birds indicates that genotype is associated with host species, diet and presence of pathognomonic lesions. *Avian Pathol* 43:535–546.

Marx M, Reiner G, Willems H, Rocha G, Hillerich K, Masello JF, Mayr SL, Moussa S, Dunn JC, Thomas RC, et al. 2017. High prevalence of *Trichomonas gallinae* in wild columbids across western and southern Europe. *Parasites and Vectors* 10:1–11.

Neimanis AS, Handeland K, Isomursu M, Ågren E, Mattsson R, Hamnes IS, Bergsjø B, Hirvelä-Koski V. 2010. First report of epizootic trichomoniasis in wild finches (Family Fringillidae) in southern Fennoscandia. *Avian Dis Dig* 5:e41–e43.

Ostrand WD, Bissonette JA, Conover MR. 1995. Trichomoniasis as a factor in mourning dove population decline in Fillmore, Utah. *J Wildl Dis* 31:87–89.

Purple KE, Humm JM, Kirby RB, Saidak CG, Gerhold R. 2015. *Trichomonas gallinae* persistence in four water treatments. *J Wildl Dis* 51:739–742.

Read CP. 1957. Comparative studies on the physiology of trichomonad protozoa. *J Parasitol* 43:385–394.

Real J, Mañosa S, Muñoz E. 2000. Trichomoniasis in a Bonelli's eagle population in Spain. *J Wildl Dis* 36:64–70.

Rogers KH, Girard YA, Woods L, Johnson CK. 2016. Avian trichomonosis in spotted owls (*Strix occidentalis*): Indication of opportunistic spillover from prey. *Int J Parasitol Parasites Wildl* 5:305–311.

Romagosa CM, Labisky RF. 2000. Establishment and dispersal of the Eurasian collared-dove in Florida. *J F Ornithol* 71:159–166.

Rosenfield RN, Bielefeldt J, Rosenfield LJ, Taft SJ, Murphy RK, Stewart AC. 2002. Prevalence of *Trichomonas gallinae* in nestling Cooper's hawks among three North American populations. *Wilson Bull* 114:145–147.

Samour JH, Bailey TA, Cooper JE. 1995. Trichomoniasis in birds of prey (order Falconiformes) in Bahrain. *Vet Rec* 136:358 LP-362.

Sansano-Maestre J, Garijo-Toledo MM, Gómez-Muñoz MT. 2009. Prevalence and genotyping of *Trichomonas gallinae* in pigeons and birds of prey. *Avian Pathol* 38:201–207.

Sansano-Maestre J, Martínez-Herrero MdelC, Garijo-Toledo MM, Gómez-Muñoz MT. 2016. RAPD analysis and sequencing of ITS1/5.8S rRNA/ITS2 and Fe-hydrogenase as tools for genetic classification of potentially pathogenic isolates of *Trichomonas gallinae*. *Res Vet Sci* 107:182–189.

Schulz JH, Bermudez AJ, Millspaugh JJ. 2005. Monitoring presence and annual variation of trichomoniasis in mourning doves. *Avian Dis* 49:387–389.

Stabler RM. 1939. "*Trichomonas gallinae*" (Rivolta, 1878). The correct name for the flagellate in the mouth, crop and liver of the pigeon *J Parasitol* 24:553–554.

———. 1947. *Trichomonas gallinae*, pathogenic trichomonad of birds. *J Parasitol* 33:207–213.

———. 1948. Variations in virulence of strains of *Trichomonas gallinae* in pigeons. *J Parasitol* 34:147–149.

———. 1951. Effect of *Trichomonas gallinae* from diseased mourning doves on clean domestic pigeons. *J Parasitol* 37:473–478.

———. 1954. *Trichomonas gallinae*: A Review. *Exp Parasitol* 3:368–402.

———. 1961. A parasitological survey of fifty-one eastern white-winged doves. *J Parasitol* 47:309–311.

Stabler RM, Herman CM. 1951. Upper digestive tract trichomoniasis in mourning doves and other birds. *Trans North Am Wildl Conf* 16:145–162.

Stone WB, Janes DE. 1969. Trichomoniasis in captive sparrow hawks. *Bull Wildl Dis Assoc* 5:147.

Stone WB, Nye PE. 1981. Trichomoniasis in bald eagles. *Wilson Bull* 93:109.

Tamura K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Mol Biol Evol* 9:678–687.

Urban EH, Mannan RW. 2014. The potential role of oral pH in the persistance of *Trichomoinas gallinae* in Cooper's hawks (*Accipiter cooperii*). *J Wildl Dis* 50:50–55.

Voncken FGJ, Boxma B, van Hoek AHAM, Akhmanova AS, Vogels GD, Huynen M, Veenhuis M, Hackstein JHP. 2002. A hydrogenosomal [Fe] -hydrogenase from the anaerobic chytrid. *Gene* 284:103–112.

Work TM, Hale J. 1996. Causes of owl mortality in Hawaii, 1992 to 1994. *J Wildl Dis* 32:266–273.

APPENDIX I

Sequences of groups of identical ITS1/5.8sITS2 regions from collected samples, which will be submitted to NCBI GenBank.

Sample ID	Sequence
Group I	GCGGGTTTTCCTGGGTATGGCAGANTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATAAAAGATGTAGTA CTGTCACACCCATGCT--TCTCGACC--GAGATCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAAC ACATTATGCCACGTTCTT CATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TG GTTTTNG--ATTAATAAAANGAAGTT GGTGTTAGTTANTTAAAACTAGAACTGATCCAACGG
Group II	GCGGGTCTTCCTGCGTATGGCAGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTA CTGTCACACCCATGCT--TCTCGACC--GAGATCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAAC ACATTATGCCACGTTCT TCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TG GTTTTTG--ATTAATAAAANGAAGT TGGTGTTAGTTANTTAAAACTAGAACTGATCCAACGG
Group III	G-GGGTCTTCCTGGGTATGGCAGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTAC TGTCACACCCATGCT--TCTCGGCC--GAGACCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACA CATTATGCCACGTTCTTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TGG TTTTG--ATAAATAAAANGAAGTTGGTGTTAGTTANTTAAAACTAGAA
Group IV	GCGGGTCTTCCTGCGTATGGCAGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTA CTGTCACACCCATGCT--TCTCGGCC--GAGACCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAAC ACATTATGCCACGTTCTTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TG GTTTTTG--ATAAATAAAANGAAGTTGGTGTTAGTTANTTAAAACTAGAA
Group V	GNGGGTCTTCCTGCGTATAGCAGACAAAGTATTGTTTGT-CTTATAATTTATTTGCTTATTCGCGTAGAATAAGAAT----TATTATA-AATTATGTTGTA CTGTTACACGCATGCT--CCTCGACC--GAAGTCGNGGTAAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAAC ACATTATGCCACGTTCTTCATCGTGTGAGGAGCCAAGACATCCGTTGCTTAGAGACTTGA-TT-TTTGATGAAG-TTAGTTATTT-AAGTTTGTA-T---TG GTTTTTG--ATTAATAAAAAGAAGTTGGTATTAGTTAATTATAACTAGAA
Group VI	--GGGTCTTCCTGCGTATGGCAGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTAC TGTCACACCCATGCT--TCTCGACC--GAGACCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACA CATTATGCCACGTTCTTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TGG TTTTTG--ATTAATAAAANGAAGTTGGTGTTAGTTANTTAAAACTAGAA
Group VII	GCGGGTCTTCCTGCGTATGGCGGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTA CTGTCACACCCATGCT--TCTCGACC--GAGATCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAAC ACATTATGCCACGTTCTTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TG GTTTTTG--ATTAATAAAANGAAGTTGGTGTTAGTTANTTAAAACTAGAA
Group VIII	GCGGGTTTTCCTGGGTATGGCAGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTA CTGTCACACCCATGCT--TCTCGGCC--GAGACCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAAC ACATTATGCCACGTTCTTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TG GTTTTTG--ATAAATAAAANGAAGTTGGTGTTAGTTANTTAAAACTAGAACTGATCCAACGG
Group IX	GCGGGTCTTCCTGGGTATGGCGGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTA CTGTCACACCCATGCT--TCTCGACC--GAGATCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAAC ACATTATGCCACGTTCTTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TG GTTTTTG--ATTAATAAAANGAAGTTGGTGTTAGTTANTTAAAACTAGAACTGATCCAACGG

APPENDIX II

Sequences of individual ITS1/5.8sITS2 regions from collected samples, which will be submitted to NCBI GenBank.

Sample ID	Sequence
2 ITS WWDO	GCGGGTCTTCCTGGGTATGGCAGANTACGTGTTGTTTTT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTACTG TCACA CCCATGCT--TCTCGGCC--GAGACCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACACATT ATGCCACGTTC TTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGA-TTGG-TT-TTTGATGAAG-TTAGTTNTTT-AA-TTTGTA-T---TGGTTTTTG-- ATAAATAAAANGAAGTT GGTGTTAGTTANTTAAAACTAGAACTGATCCAACGG
19 ITS INDO	GCGGGTTTTCCTGCGTATAGCAGACAACGTATTGTTTGT-CTTATAATTTATTTGCTTATTCGCGTAGAATAAGAAT----TATTATA-AATTATGTTGTACTG TTACA CGCATGCT--CCTCGGCC--GAAGCCGNGGTAAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACACATT ATGCCACGTTC TTCATCGTGTGAGGAGCCAAGACATCCGTTGCTTAGAGACTTGA-TT-TTTGATGAAG-TTAGTTATTT-AAGTTTGTA-T---TGGTTTTTG-- ATTAGTAAAAAGAAGT TGGTATTAGTTAATTATAACTAGAACTGATCCAACGG
21 ITS WWDO	GCGGGTCTTCCTGGGTATGGCGGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTACTG TCACA CCCCTGCT--TCTCGACC--GAGATCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACACATTA TGCCACGTTC TTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TGGTTTTTG— ATTAATAAAANGAAGT TGGTGTTAGTTANTTAAAACTAGAACTGATCCAAC--
33 ITS WWDO	GCGGGTCTTCCTGCGTTTGGCGGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTACTG TCACA CCCATGCT--TCTCGACC--GAGATCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACACATT ATGCCACGTTC TTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TGGTTTTTG-- ATTAATAAAANGAAGT TGGTGTTAGTTANTTAAAACTAGAACTGATCCNACGG
40 ITS WWDO	NGGGGTCTTCCTGCGTATGGCAGACTACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTACT GTCACA CCCATGCT--TCTCGGCC--GAGACCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACACA TTATGCCACGTTC TTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TGGTTTT TG—ATAAATAAAANGAAGT TGGTGTTAGTTANTTAAAACTAGAACTGATCCAACGG
43 ITS INDO	GCGGGTCTTCCTGCGTATGGCAGACTACGGGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTACTG TCACACCCATGCT--TCTCGACC--GAGATCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACACATT ATGCCACGTTC TTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TGGTTTTTG-- ATTAATAAAANGAAGT TGGTGTTAGTTANTTAAAACTAGAACTGATCCAACGG
49 ITS ECDO	GCGGGTTTTCCTGGGTATAGCAGACAACGTATTGTTTGT-CTTATAATTTATTTGCTTATTCGCGTAGAATAAGAAT----TATTATA-AATTATGTTGTAC TGTTACA CGCATGCT--CCTCGACC--GAAGTCGNGGTAAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACACA TTATGCCACGTTC TTCATCGTGTGAGGAGCCAAGACATCCGTTGCTTAGAGACTTGA-TT-TTTGATGAAG-TTAGTTATTT-AAGTTTGTA-T---TGGTTTT TG—ATTAATAAAAAGAAG TTGGTATTAGTTAATTATAACTAGAACTGATCCAACGG
70 ITS WWDO	GCGGGTCTTCCTGGGTATGGCGGAATACGTGTTGTTTGT-CTTATATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTACT GTCACACCCATGCT--TCTCGACC--GAGATCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACACATT ATGCCACGTTC TTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TGGTTTTTG-- ATTAATAAAANGAAGT TGGTGTTAGTTANTTAAAACTAGAACTGATCCAACGG
71 ITS WWDO	G-GGGTCTTCCTGCGTATGGCAGACTACGTGTTGTTTGT-CTTAAATATTATTTACTTATTCGCTTAGAATAAGAAT----TATTATA-AAAGATGTAGTACTG TCACACCCATGCT--TCTCGACC--GAGATCGAGTTTAGCGCAATTTGCATTCAAAGATT-AACCTGTCATGATGTTTGCAACTCCGGTTACTTAACACATTA TGCCACGTTC TTCATCGTGTGAGGAGCCAAGACATCCATTGCTTAGAGACTTGG-TT-TTTGATGAAG-TTAGTTNTTT-AATTTTGTA-T---TGGTTTTTG-- ATTAATAAAANGAAGT TGGTGTTAGTTANTTAAAACTAGAACTGATCCAACGG

APPENDIX III

Sequences of individual Fe-hydrogenase regions from collected samples, which will be submitted to NCBI GenBank.

Sample ID	Sequence
19 FeHyd INDO	AACTCCTTGACTGCCACGATGAAACATGCTCTTCCTGCGTTGCTAACCACAGATGCCAGTTCAGAGACATGAACGTCGCCTACTCCGTTAAGGCTGACAC CAAGGAAATCTGCTCTGAAGAGGGCATCGATGAGTCAACACATGCCATCAGACTCGACACATCCAAATGCGTCCTTTGCGGCCGTTGCATCCGCGCTTGC GAGGAAGTTGCTGGCACATCCGCCATCATCTTCGGCAACCGTGCTAAGCACATGAGAATCCAGCCAACATTCGGTGGCACACTTCAGGAGACTTCCTGCA TCAAGTGCGGCCAATGCACACTCTATTGCCCAGTCGGTGCCATCACAGAGAAGTCCCAGGTTAAGGAGGCCCTCGACATCCTTGCTAACAAGGGCAAGA AGGTCACAGTCGTCCAGGTCGCTCCAGCTGTCCGTGTTGCTCTCTCCGAGGCTTTTGGCTACAAGGAGGGTACAGTCACAACAGGCAAGATGGTTTCCGC CCTCAAGGCTCTCGGCTTCGACTTAGTTTACGACACAAACTATGGTGCTGACCTTACAATCTGCGAAGAGGCCGGTGAACTCGTCAACCGCCTTAAGGAT CCAAAGGCTGTCTTCCCAATGTTCACATCCTGCTGCCCAGCTTGGGTTAACTACGTTGAGCAGTCCGCCCCAGACTTCATTCCAAACCTTTCCTCCTGCCGC TCACCACAGGGCATGCTTTCCTCCCTCATCAAGAACTACCTTCCAAAGCTCCTCGGCATCAAGCAGGAAGAAGTCATGAACTTCTCCATCATGCCATGCAC AGCTAAGAAGGACGAAATCGAGCGCCCAGAGCTCCAGACAAAGACTGGCCTCAAGGAGACAGATATGGTTCTCACAGTTCGC
49 FeHyd ECDO	AACTCCTTGACTGCCACGATGAAACATGCTCATCCTGCGTTGCTAACCACAGATGCCAGTTCAGAGACATGAACGTCGCCTACTCCGTTAAGGCTGACAC CAAGGAAATCTGCTCTGAAGAGGGCATCGATGAGTCAACACACGCCATCAGACTCGACACATCCAAGTGCGTCCTTTGCGGCCGTTGCATCCGCGCTTGC GAGGAAGTTGCTGGCACATCCGCCATCATCTTCGGCAACCGTGCTAAGCACATGAGAATCCAGCCAACATTCGGTGGCACACTTCAGGAGACAGCCTGCA TCAAGTGCGGCCAATGCACACTCTACTGCCCAGTCGGTGCCATCACAGAGAAGTCCCAGGTTAAGGAGGCCCTCGACATCCTTGCTAACAAGGGCAAGA AGGTCACAGTCGTCCAGGTCGCTCCAGCCGTCCGTGTTGCTCTCTCCGAGGCTTTCGGCTACAAGGAGGGTACAGTCACAACAGGCAAGATGGTTTCCGC CCTCAAGGCCCTCGGCTTCGACTTAGTTTACGACACAAACTATGGTGCTGACCTTACAATCTGCGAAGAGGCTGGCGAACTCGTCAACCGCCTTAAGGAT CCAAAGGCTGTCTTCCCAATGTTCACATCCTGCTGCCCGGCTTGGGTTAACTACGTTGAGCAGTCCGCCCCAGACTTCATTCCAAACCTTTCCTCCTGCCGC TCACCACAGGGCATGCTTTCCTCCCTCATCAAGAACTACCTTCCAAAGCTCCTCGGCATCAAGCAGGAAGAAGTCATGAACTTCTCCATCATGCCATGCAC AGCTAAGAAGGACGAAATCGAGCGCCCAGAGCTCCAGACAAAGACAGGCCTCAAGGAGACAGACATGGTCCTCACAGTTCGC
55 FeHyd ECDO	AACTCCTTGACTGCCACGATGAAACATGCTCATTCTGCGTTGCTAACCACAGATGCCAGTTCAGAGACATGAACGTCGCCTACTCCGTTAAGGCTGACAC CAAGGAAATCTGCTCTGAAGAGGGCATCGATGAGTCAACACACGCCATCAGACTCGACACTTCCAAGTGCGTCCTTTGCGGCCGTTGCATCCGCGCTTGC GAGGAAGTTGCTGGCACATCTGCCATCATCTTCGGCAACCGTGCTAAGCACATGAGAATCCAGCCAACATTCGGTGGCACACTTCAGGAGACAGCCTGCA TCAAGTGCGGCCAATGCACACTCTACTGCCCAGTCGGTGCCATCACAGAGAAGTCCCAGGTTAAGGAGGCCCTCGACATCCTTGCTAACAAGGGCAAGA AGGTCACAGTCGTCCAGGTCGCTCCAGCCGTCCGTGTTGCTCTCTCCGAGGCTTTCGGCTACAAGGAAGGTACAGTCACAACAGGCAAGATGGTTTCCGC CCTCAAGGCCCTCGGCTTCGACTTAGTTTACGACACAAACTATGGTGCTGACCTTACAATCTGCGAAGAGGCTGGCGAACTCGTCAACCGCCTTAAGGAT CCAAAGGCTGTCTTCCCAATGTTCACATCCTGCTGCCCAGCTTGGGTTAACTATGTTGAGCAGTCCGCCCCAGACTTCATTCCAAACCTTTCCTCCTGCCGC TCACCACAGGGCATGCTTTCCTCCCTCATCAAGAACTACCTTCCAAAGCTCCTCGGCATCAAGCAGGAAGAAGTCATGAACTTCTCCATCATGCCATGCAC AGCTAAGAAGGACGAAATCGAGCGCCCAGAGCTCCAGACAAAGACAGGCCTCAAGGAGACAGACATGGTCCTCACAGTTCGC
59 FeHyd ECDO	AACTCCTTGACTGCCACGATGAAACATGCTCATCCTGCGTTGCTAACCACAGATGCCAGTTCAGAGACATGAACGTCGCCTACTCCGTTAAGGCTGACAC CAAGGAAATCTGCTCTGAAGAGGGCATCGATGAGTCAACACACGCCATCAGACTCGACACTTCCAAGTGCGTCCTTTGCGGCCGTTGCATCCGCGCTTGC GAGGAAGTTGCTGGCACATCTGCCATCATCTTCGGCAACCGTGCTAAGCACATGAGAATCCAGCCAACATTCGGTGGCACACTTCAGGAGACAGCCTGCA TCAAGTGCGGCCAATGCACACTCTACTGCCCAGTCGGTGCCATCACAGAGAAGTCCCAGGTTAAGGAGGCCCTCGACATCCTTGCTAACAAGGGCAAGA AGGTCACAGTCGTCCAGGTCGCTCCAGCCGTCCGTGTTGCTCTCTCCGAGGCTTTCGGCTACAAGGAAGGTACAGTCACAACAGGCAAGATGGTTTCCGC CCTCAAGGCCCTCGGCTTCGACTTAGTTTACGACACAAACTATGGTGCTGACCTTACAATCTGCGAAGAGGCTGGCGAACTCGTCAACCGCCTTAAGGAT CCAAAGGCTGTCTTCCCAATGTTCACATCCTGCTGCCCAGCTTGGGTTAACTATGTTGAGCAGTCCGCCCCAGACTTCATTCCAAACCTTTCCTCCTGCCGC TCACCACAGGGCATGCTTTCCTCCCTCATCAAGAACTACCTTCCAAAGCTCCTCGGCATCAAGCAGGAAGAAGTCATGAACTTCTCCATCATGCCATGCAC AGCTAAGAAGGACGAAATCGAGCGCCCAGAGCTCCAGACAAAGACAGGCCTCAAGGAGACAGACATGGTCCTCACAGTTCGC

APPENDIX IV

BLAST results and sequence identity values for ITS1/5.8s rRNA/ITS2 region from *Trichomonas* samples are listed with the first match from a published study. Identical sequences are grouped into assigned sequence groups, with the study-specific sample ID of the representative sequence used italicized. Samples with unique sequences are listed below the groups; Acc. n.: GenBank Accession number. Four letters at the end of the study-specific sample ID indicate the species the oral swab was collected from; WWDO: white winged-dove; INDO: Inca dove; ECDO: Eurasian collared-dove.

			Sequence Identity
Study-specific Sample ID	Assigned Sequence Group	Organism, Acc. n.	% BLAST Identitiy, E-value
1 ITS WWDO	Group I	*T. vaginalis* (U86613.1; Felleisen 2001)	98%, 4.00E-165
4 ITS WWDO			
5 ITS WWDO			
14 ITS INDO			
22 ITS WWDO			

APPENDIX IV (CONTINUED)

Study-specific Sample ID	Assigned Sequence Group	Organism, Acc. n.	Sequence Identity % BLAST Identity, E-value
24 ITS WWDO	Group I	*T. vaginalis* (U86613.1; Felleisen 2001)	98%, 4.00E-165
27 ITS WWDO			
34 ITS WWDO			
37 ITS WWDO			
54 ITS WWDO			
8 ITS INDO	Group II	*T. vaginalis* (U86613.1; Felleisen 2001)	99%, 1.00E-170
11 ITS WWDO			
12 ITS WWDO			
13 ITS WWDO			
15 ITS WWDO			

APPENDIX IV (CONTINUED)

Study-specific Sample ID	Assigned Sequence Group	Organism, Acc. n.	Sequence Identity
			% BLAST Identity, E-value
18 ITS INDO	Group II	*T. vaginalis* (U86613.1; Felleisen 2001)	99%, 1.00E-170
20 ITS WWDO			
28 ITS WWDO			
29 ITS WWDO			
31 ITS WWDO			
32 ITS WWDO			
38 ITS WWDO			
39 ITS WWDO			
47 ITS WWDO			
64 ITS WWDO			
65 ITS WWDO			

APPENDIX IV (CONTINUED)

Study-specific Sample ID	Assigned Sequence Group	Organism, Acc. n.	Sequence Identity % BLAST Identity, E-value
68 ITS WWDO	Group II		
72 ITS WWDO			
16 ITS WWDO	Group III	*T. sp* (EU215361.1; Gerhold 2008)	99%, 1.00E-169
17 ITS WWDO			
35 ITS WWDO			
3 ITS WWDO	Group IV	*T. sp* (EU215361.1; Gerhold 2008)	99%, 2.00E172
23 ITS WWDO			
58 ITS WWDO			
30 ITS WWDO			
55 ITS ECDO	Group V	*T. gallinae* (KX459505.1; Marx 2017)	99%, 2.00E-173

APPENDIX IV (CONTINUED)

Study-specific Sample ID	Assigned Sequence Group	Organism, Acc. n.	Sequence Identity
			% BLAST Identity, E-value
59 ITS ECDO	Group V	*T. gallinae* (KX459505.1; Marx 2017)	99%, 2.00E-173
9 ITS WWDO	Group VI	*T. sp* (EU215361.1; Gerhold 2008)	99%, 6.00E-168
56 ITS WWDO			
60 ITS WWDO	Group VII	*T. vaginalis* (U86613.1; Felleisen 2001)	99%, 5.00E-169
62 ITS WWDO			
67 ITS WWDO			
69 ITS WWDO			
7 ITS WWDO	Group VIII	*T. sp* (EU215361.1; Gerhold 2008)	99%, 5.00E-169
25 ITS WWDO			
61 ITS WWDO	Group IX	*T. vaginalis* (U86613.1; Felleisen 2001)	98%, 2.00E-167

APPENDIX IV (CONTINUED)

Study-specific Sample ID	Assigned Sequence Group	Organism, Acc. n.	Sequence Identity % BLAST Identity, E-value
66 ITS WWDO	Group IX	*T. gallinae* (KX459498; Marx 2017)	92%, 6.00E-133
2 ITS WWDO	NA	*T. sp* (EU215361.1; Gerhold 2008)	98%, 5.00E-164
10 ITS WWDO	NA	*T. vaginalis* (U86613.1; Felleisen 1998)	99%, 6.00E-122
19 ITS INDO	NA	*T. gallinae* (KC215387.1; Girard 2014)	99%, 2.00E-173
21 ITS WWDO	NA	*T. vaginalis* (U86613.1; Felleisen 1998)	98%, 1.00E-164
33 ITS WWDO	NA	*T. vaginalis* (U86613.1; Felleisen 1998)	98%, 3.00E-166
40 ITS WWDO	NA	*T. sp* (EU215361.1; Gerhold 2008)	99%, 3.00E-171
43 ITS INDO	NA	*T. vaginalis* (U86613.1; Felleisen 1998)	99%, 5.00E-169
49 ITS ECDO	NA	*T. gallinae* (KX459498.1; Marx 2017)	99%, 6.00E-173
70 ITS WWDO	NA	*T. vaginalis* (U86613.1; Felleisen 1998)	98%, 1.00E-165
71 ITS WWDO	NA	*T. vaginalis* (U86613.1; Felleisen 1998)	99%, 6.00E-168

APPENDIX V

Pairwise distances of ITS1/5.8s/ITS2 sequences from this study and known reference sequences from the literature. Pairwise distances were calculated using the number of differences method and nucleotide substitutions. Substitutions included transitions and transversions. Sample/Group: sample from current study; Comparison: sequence compared to sample; No. of differences: number of differences between the two sequences being compared.

Sample/Group	Comparison	No. of Differences
Group I	2 ITS WWDO	5
	19 ITS INDO	26
	21 ITS WWDO	3
	33 ITS WWDO	4
	40 ITS WWDO	6
	43 ITS INDO	3
	49 ITS ECDO	22
	70 ITS WWDO	2
	71 ITS WWDO	3
	Group II	2
	Group III	4
	Group IV	5
	Group V	25
	Group VI	3
	Group VII	3
	Group VIII	3
	Group IX	2
	T. gallinae (A)	24
	T. gallinae (B)	27
	T. gallinae (C)	25
	T. gallinae (D)	21
	T. gallinae (E)	17
	T. gallinae (F)	17
	T. gallinae (G)	28
	T. gallinae (H)	6
	T. gallinae (I)	5
	T. gallinae (J)	5
	T. stableri (K)	6
	T. gallinae (L)	2
	T. gallinae (O)	31
	T. gallinae (P)	24
	T. gallinae (Q)	37

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
Group I	*T. gallinae* (II)	20
	T. gallinae (V)	19
	T. gallinae (VI)	6
	T. vaginalis	3
	T. tenax	27
	Tetratrichomonas gallinarum	62
	Tritrichomonas foetus	92
Group II	2 ITS WWDO	5
	19 ITS INDO	26
	21 ITS WWDO	3
	33 ITS WWDO	2
	40 ITS WWDO	4
	43 ITS INDO	1
	49 ITS ECDO	24
	70 ITS WWDO	3
	71 ITS WWDO	1
	Group III	4
	Group IV	3
	Group V	23
	Group VI	1
	Group VII	1
	Group VIII	5
	Group IX	2
	T. gallinae (A)	22
	T. gallinae (B)	27
	T. gallinae (C)	23
	T. gallinae (D)	21
	T. gallinae (E)	17
	T. gallinae (F)	17
	T. gallinae (G)	26
	T. gallinae (H)	4
	T. gallinae (I)	3
	T. gallinae (J)	3
	T. stableri (K)	4
	T. gallinae (L)	0
	T. gallinae (O)	29
	T. gallinae (P)	22
	T. gallinae (Q)	35
	T. gallinae (II)	20
	T. gallinae (V)	19
	T. gallinae (VI)	6
	T. vaginalis	1
	T. tenax	25
	Tetratrichomonas gallinarum	63

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
Group II	*Tritrichomonas foetus*	92
Group III	Group IV	1
	Group V	27
	Group VI	3
	Group VII	5
	Group VIII	1
	Group IX	4
	2 ITS WWDO	1
	19 ITS INDO	26
	21 ITS WWDO	5
	33 ITS WWDO	6
	40 ITS WWDO	1
	43 ITS INDO	5
	49 ITS ECDO	26
	70 ITS WWDO	5
	71 ITS WWDO	5
	T. gallinae (A)	21
	T. gallinae (B)	26
	T. gallinae (C)	27
	T. gallinae (D)	24
	T. gallinae (E)	19
	T. gallinae (F)	17
	T. gallinae (G)	28
	T. gallinae (H)	2
	T. gallinae (I)	1
	T. gallinae (J)	7
	T. stableri (K)	6
	T. gallinae (L)	4
	T. gallinae (O)	31
	T. gallinae (P)	22
	T. gallinae (Q)	36
	T. gallinae (II)	19
	T. gallinae (V)	22
	T. gallinae (VI)	9
	T. vaginalis	5
	T. tenax	25
	Tetratrichomonas gallinarum	65
	Tritrichomonas foetus	93

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
Group IV	Group V	26
	Group VI	2
	Group VII	4
	Group VIII	2
	Group IX	5
	2 ITS WWDO	2
	19 ITS INDO	25
	21 ITS WWDO	6
	33 ITS WWDO	5
	40 ITS WWDO	1
	43 ITS INDO	4
	49 ITS ECDO	27
	70 ITS WWDO	6
	71 ITS WWDO	4
	T. gallinae (A)	20
	T. gallinae (B)	26
	T. gallinae (C)	26
	T. gallinae (D)	24
	T. gallinae (E)	19
	T. gallinae (F)	17
	T. gallinae (G)	27
	T. gallinae (H)	1
	T. gallinae (I)	0
	T. gallinae (J)	6
	T. stableri (K)	5
	T. gallinae (L)	3
	T. gallinae (O)	30
	T. gallinae (P)	21
	T. gallinae (Q)	36
	T. gallinae (II)	19
	T. gallinae (V)	22
	T. gallinae (VI)	9
	T. vaginalis	4
	T. tenax	24
	Tetratrichomonas gallinarum	65
	Tritrichomonas foetus	93

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
Group V	2 ITS WWDO	27
	19 ITS INDO	5
	21 ITS WWDO	26
	33 ITS WWDO	25
	40 ITS WWDO	26
	43 ITS INDO	24
	49 ITS ECDO	3
	70 ITS WWDO	26
	71 ITS WWDO	24
	Group VI	24
	Group VII	24
	Group VIII	28
	Group IX	25
	T. gallinae (A)	3
	T. gallinae (B)	7
	T. gallinae (C)	2
	T. gallinae (D)	1
	T. gallinae (E)	0
	T. gallinae (F)	19
	T. gallinae (G)	28
	T. gallinae (H)	28
	T. gallinae (I)	26
	T. gallinae (J)	21
	T. stableri (K)	22
	T. gallinae (L)	23
	T. gallinae (O)	10
	T. gallinae (P)	12
	T. gallinae (Q)	31
	T. gallinae (II)	16
	T. gallinae (V)	0
	T. gallinae (VI)	19
	T. vaginalis	24
	T. tenax	19
	Tetratrichomonas gallinarum	62
	Tritrichomonas foetus	99

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
Group VI	2 ITS WWDO	4
	19 ITS INDO	25
	21 ITS WWDO	4
	33 ITS WWDO	3
	40 ITS WWDO	2
	43 ITS INDO	2
	49 ITS ECDO	25
	70 ITS WWDO	4
	71 ITS WWDO	2
	Group VII	2
	Group VIII	4
	Group IX	3
	T. gallinae (A)	21
	T. gallinae (B)	26
	T. gallinae (C)	24
	T. gallinae (D)	22
	T. gallinae (E)	18
	T. gallinae (F)	18
	T. gallinae (G)	27
	T. gallinae (H)	3
	T. gallinae (I)	2
	T. gallinae (J)	4
	T. stableri (K)	5
	T. gallinae (L)	1
	T. gallinae (O)	28
	T. gallinae (P)	21
	T. gallinae (Q)	34
	T. gallinae (II)	19
	T. gallinae (V)	20
	T. gallinae (VI)	7
	T. vaginalis	2
	T. tenax	24
	Tetratrichomonas gallinarum	64
	Tritrichomonas foetus	93

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
Group VII	2 ITS WWDO	6
	19 ITS INDO	27
	21 ITS WWDO	2
	33 ITS WWDO	1
	40 ITS WWDO	5
	43 ITS INDO	2
	49 ITS ECDO	25
	70 ITS WWDO	2
	71 ITS WWDO	2
	Group VIII	6
	Group IX	1
	T. gallinae (A)	23
	T. gallinae (B)	27
	T. gallinae (C)	24
	T. gallinae (D)	22
	T. gallinae (E)	17
	T. gallinae (F)	17
	T. gallinae (G)	27
	T. gallinae (H)	5
	T. gallinae (I)	4
	T. gallinae (J)	4
	T. stableri (K)	5
	T. gallinae (L)	1
	T. gallinae (O)	30
	T. gallinae (P)	23
	T. gallinae (Q)	36
	T. gallinae (II)	20
	T. gallinae (V)	19
	T. gallinae (VI)	6
	T. vaginalis	2
	T. tenax	26
	Tetratrichomonas gallinarum	64
	Tritrichomonas foetus	93

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
Group VIII	2 ITS WWDO	2
	19 ITS INDO	25
	21 ITS WWDO	6
	33 ITS WWDO	7
	40 ITS WWDO	3
	43 ITS INDO	6
	49 ITS ECDO	25
	70 ITS WWDO	6
	71 ITS WWDO	6
	Group IX	5
	T. gallinae (A)	22
	T. gallinae (B)	26
	T. gallinae (C)	28
	T. gallinae (D)	24
	T. gallinae (E)	19
	T. gallinae (F)	17
	T. gallinae (G)	29
	T. gallinae (H)	3
	T. gallinae (I)	2
	T. gallinae (J)	8
	T. stableri (K)	7
	T. gallinae (L)	5
	T. gallinae (O)	32
	T. gallinae (P)	23
	T. gallinae (Q)	38
	T. gallinae (II)	19
	T. gallinae (V)	22
	T. gallinae (VI)	9
	T. vaginalis	6
	T. tenax	26
	Tetratrichomonas gallinarum	65
	Tritrichomonas foetus	94

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
Group IX	2 ITS WWDO	5
	19 ITS INDO	28
	21 ITS WWDO	1
	33 ITS WWDO	2
	40 ITS WWDO	6
	43 ITS INDO	3
	49 ITS ECDO	24
	70 ITS WWDO	1
	71 ITS WWDO	3
	T. gallinae (A)	24
	T. gallinae (B)	27
	T. gallinae (C)	25
	T. gallinae (D)	22
	T. gallinae (E)	17
	T. gallinae (F)	17
	T. gallinae (G)	28
	T. gallinae (H)	6
	T. gallinae (I)	5
	T. gallinae (J)	5
	T. stableri (K)	6
	T. gallinae (L)	2
	T. gallinae (O)	31
	T. gallinae (P)	24
	T. gallinae (Q)	37
	T. gallinae (II)	20
	T. gallinae (V)	19
	T. gallinae (VI)	6
	T. vaginalis	3
	T. tenax	27
	Tetratrichomonas gallinarum	64
	Tritrichomonas foetus	93

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
2 ITS WWDO	19 ITS INDO	26
	21 ITS WWDO	6
	49 ITS ECDO	26
	33 ITS WWDO	7
	40 ITS WWDO	3
	43 ITS INDO	6
	70 ITS WWDO	5
	71 ITS WWDO	6
	T. gallinae (A)	21
	T. gallinae (B)	26
	T. gallinae (C)	27
	T. gallinae (D)	24
	T. gallinae (E)	19
	T. gallinae (F)	17
	T. gallinae (G)	28
	T. gallinae (H)	3
	T. gallinae (I)	2
	T. gallinae (J)	7
	T. stableri (K)	6
	T. gallinae (L)	5
	T. gallinae (O)	29
	T. gallinae (P)	22
	T. gallinae (Q)	37
	T. gallinae (II)	19
	T. gallinae (V)	22
	T. gallinae (VI)	10
	T. vaginalis	6
	T. tenax	25
	Tetratrichomonas gallinarum	66
	Tritrichomonas foetus	93

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
19 ITS INDO	21 ITS WWDO	29
	33 ITS WWDO	28
	40 ITS WWDO	26
	43 ITS INDO	27
	49 ITS ECDO	4
	70 ITS WWDO	29
	71 ITS WWDO	27
	T. gallinae (A)	1
	T. gallinae (B)	3
	T. gallinae (C)	5
	T. gallinae (D)	3
	T. gallinae (E)	2
	T. gallinae (F)	18
	T. gallinae (G)	29
	T. gallinae (H)	27
	T. gallinae (I)	25
	T. gallinae (J)	24
	T. stableri (K)	25
	T. gallinae (L)	26
	T. gallinae (O)	11
	T. gallinae (P)	11
	T. gallinae (Q)	33
	T. gallinae (II)	15
	T. gallinae (V)	3
	T. gallinae (VI)	22
	T. vaginalis	27
	T. tenax	18
	Tetratrichomonas gallinarum	63
	Tritrichomonas foetus	100

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
21 ITS WWDO	33 ITS WWDO	3
	40 ITS WWDO	7
	43 ITS INDO	4
	49 ITS ECDO	25
	70 ITS WWDO	2
	71 ITS WWDO	4
	T. gallinae (A)	25
	T. gallinae (B)	28
	T. gallinae (C)	26
	T. gallinae (D)	23
	T. gallinae (E)	18
	T. gallinae (F)	18
	T. gallinae (G)	29
	T. gallinae (H)	7
	T. gallinae (I)	6
	T. gallinae (J)	6
	T. stableri (K)	7
	T. gallinae (L)	3
	T. gallinae (O)	32
	T. gallinae (P)	25
	T. gallinae (Q)	38
	T. gallinae (II)	21
	T. gallinae (V)	20
	T. gallinae (VI)	7
	T. vaginalis	4
	T. tenax	28
	Tetratrichomonas gallinarum	65
	Tritrichomonas foetus	94

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
33 ITS WWDO	40 ITS WWDO	6
	43 ITS INDO	3
	49 ITS ECDO	26
	70 ITS WWDO	3
	71 ITS WWDO	3
	T. gallinae (A)	24
	T. gallinae (B)	27
	T. gallinae (C)	25
	T. gallinae (D)	22
	T. gallinae (E)	17
	T. gallinae (F)	17
	T. gallinae (G)	28
	T. gallinae (H)	6
	T. gallinae (I)	5
	T. gallinae (J)	5
	T. stableri (K)	6
	T. gallinae (L)	2
	T. gallinae (O)	31
	T. gallinae (P)	24
	T. gallinae (Q)	37
	T. gallinae (II)	20
	T. gallinae (V)	19
	T. gallinae (VI)	6
	T. vaginalis	3
	T. tenax	27
	Tetratrichomonas gallinarum	64
	Tritrichomonas foetus	93

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
40 ITS WWDO	43 ITS INDO	5
	49 ITS ECDO	28
	70 ITS WWDO	7
	71 ITS WWDO	4
	T. gallinae (A)	21
	T. gallinae (B)	27
	T. gallinae (C)	27
	T. gallinae (D)	24
	T. gallinae (E)	19
	T. gallinae (F)	17
	T. gallinae (G)	28
	T. gallinae (H)	2
	T. gallinae (I)	1
	T. gallinae (J)	7
	T. stableri (K)	5
	T. gallinae (L)	4
	T. gallinae (O)	31
	T. gallinae (P)	22
	T. gallinae (Q)	36
	T. gallinae (II)	19
	T. gallinae (V)	22
	T. gallinae (VI)	9
	T. vaginalis	5
	T. tenax	25
	Tetratrichomonas gallinarum	65
	Tritrichomonas foetus	94

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
43 ITS WWDO	49 ITS ECDO	25
	70 ITS WWDO	4
	71 ITS WWDO	2
	T. gallinae (A)	23
	T. gallinae (B)	28
	T. gallinae (C)	24
	T. gallinae (D)	22
	T. gallinae (E)	17
	T. gallinae (F)	18
	T. gallinae (G)	27
	T. gallinae (H)	5
	T. gallinae (I)	4
	T. gallinae (J)	4
	T. stableri (K)	5
	T. gallinae (L)	1
	T. gallinae (O)	30
	T. gallinae (P)	23
	T. gallinae (Q)	36
	T. gallinae (II)	20
	T. gallinae (V)	19
	T. gallinae (VI)	6
	T. vaginalis	2
	T. tenax	26
	Tetratrichomonas gallinarum	64
	Tritrichomonas foetus	93

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
49 ITS WWDO	70 ITS WWDO	25
	71 ITS WWDO	25
	T. gallinae (A)	4
	T. gallinae (B)	6
	T. gallinae (C)	3
	T. gallinae (D)	0
	T. gallinae (E)	0
	T. gallinae (F)	18
	T. gallinae (G)	29
	T. gallinae (H)	29
	T. gallinae (I)	27
	T. gallinae (J)	22
	T. stableri (K)	23
	T. gallinae (L)	24
	T. gallinae (O)	11
	T. gallinae (P)	13
	T. gallinae (Q)	33
	T. gallinae (II)	16
	T. gallinae (V)	0
	T. gallinae (VI)	19
	T. vaginalis	25
	T. tenax	20
	Tetratrichomonas gallinarum	61
	Tritrichomonas foetus	99

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
70 ITS WWDO	71 ITS WWDO	4
	T. gallinae (A)	25
	T. gallinae (B)	27
	T. gallinae (C)	26
	T. gallinae (D)	23
	T. gallinae (E)	17
	T. gallinae (F)	18
	T. gallinae (G)	29
	T. gallinae (H)	7
	T. gallinae (I)	6
	T. gallinae (J)	6
	T. stableri (K)	7
	T. gallinae (L)	3
	T. gallinae (O)	32
	T. gallinae (P)	25
	T. gallinae (Q)	38
	T. gallinae (II)	20
	T. gallinae (V)	19
	T. gallinae (VI)	6
	T. vaginalis	4
	T. tenax	28
	Tetratrichomonas gallinarum	65
	Tritrichomonas foetus	93

APPENDIX V (CONTINUED)

Sample/Group	Comparison	No. of Differences
71 ITS WWDO	*T. gallinae* (A)	23
	T. gallinae (B)	28
	T. gallinae (C)	24
	T. gallinae (D)	22
	T. gallinae (E)	18
	T. gallinae (F)	18
	T. gallinae (G)	27
	T. gallinae (H)	5
	T. gallinae (I)	4
	T. gallinae (J)	4
	T. stableri (K)	5
	T. gallinae (L)	1
	T. gallinae (O)	30
	T. gallinae (P)	23
	T. gallinae (Q)	35
	T. gallinae (II)	21
	T. gallinae (V)	20
	T. gallinae (VI)	7
	T. vaginalis	2
	T. tenax	26
	Tetratrichomonas gallinarum	62
	Tritrichomonas foetus	93

APPENDIX VI

Pairwise distances of Fe-hydrogenase sequences from this study and known reference sequences from the literature. Pairwise distances were calculated using the number of differences method and nucleotide substitutions. Substitutions included transitions and transversions. Sample/Group: sample from current study; Comparison: sequence compared to sample; No. of differences: number of differences between the two sequences being compared.

Sample	Comparison	No. of Differences
19 FeHyd INDO	49 FeHyd ECDO	15
	55 FeHyd ECDO	19
	59 FeHyd ECDO	18
	T. gallinae subtype A1	0
	T. gallinae subtype A2	5
	T. gallinae subtype C1	18
	T. gallinae subtype C2	18
	T. gallinae subtype C4	15
	T. gallinae subtype C5	12
	T. gallinae subtype C6	15
	T. gallinae subtype C7	15
	T. stableri	157
	T. vaginalis	101
49 FeHyd ECDO	55 FeHyd ECDO	6
	59 FeHyd ECDO	5
	T. gallinae subtype A1	15
	T. gallinae subtype A2	16
	T. gallinae subtype C1	9
	T. gallinae subtype C2	5
	T. gallinae subtype C4	6
	T. gallinae subtype C5	5
	T. gallinae subtype C6	8
	T. gallinae subtype C7	6
	T. stableri	157
	T. vaginalis	95
55 FeHyd ECDO	59 FeHyd ECDO	1
	T. gallinae subtype A1	19
	T. gallinae subtype A2	20
	T. gallinae subtype C1	13
	T. gallinae subtype C2	1
	T. gallinae subtype C4	10
	T. gallinae subtype C5	9

APPENDIX VI (CONTINUED)

Sample	Comparison	No. of Differences
55 FeHyd ECDO	*T. gallinae* subtype C6	12
	T. gallinae subtype C7	8
	T. stableri	154
	T. vaginalis	98
59 FeHyd ECDO	*T. gallinae* subtype A1	18
	T. gallinae subtype A2	19
	T. gallinae subtype C1	12
	T. gallinae subtype C2	0
	T. gallinae subtype C4	9
	T. gallinae subtype C5	8
	T. gallinae subtype C6	11
	T. gallinae subtype C7	7
	T. stableri	153
	T. vaginalis	97

APPENDIX VII

IACUC approval letter for this study.

ANGELO STATE UNIVERSITY
College of Graduate Studies & Research
Institutional Animal Care & Use Committee

October 12, 2017

Dr. Ben Skipper, Assistant Professor
Dept. of Biology
Angelo State University
San Angelo, TX 76909

Your proposed project titled, "*Prevalence of Avian Trichomoniasis in 3 species of dove in the city of San Angelo, Texas*" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective October 12, 2017 and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on August 15 of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 17-01. Please include this number in the subject line of in all future communications with the IACUC regarding the protocol.

Sincerely,

Chase Runyan, Ph.D.
Co-Chair, Institutional Animal Care and Use Committee