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Original Article

# Trichomonosis due to *Trichomonas gallinae* infection in barn owls (*Tyto alba*) and barred owls (*Strix varia*) from the eastern United States

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ABSTRACT

Trichomonosis is an important cause of mortality in multiple avian species; however, there have been relatively few reports of this disease in owls. Two barn owls (*Tyto alba*) and four barred owls (*Strix varia*) submitted for diagnostic examination had lesions consistent with trichomonosis including caseous necrosis and inflammation in the oropharynx. Microscopically, these lesions were often associated with trichomonads and molecular testing, if obtainable, confirmed the presence of *Trichomonas gallinae*, the species most commonly associated with trichomonosis in birds. The *T. gallinae* genotype in one barn owl and two barred owls was identified as ITS-OBT-Tg-1 by sequence analysis. Columbids are the primary hosts for *T. gallinae,* and columbid remains found within the nest box of the barn owls were the likely source of infection. This study is the first to formally describe the strains and genetic variation of *T. gallinae* samples from clinical cases of trichomonosis in barn and barred owls in the eastern USA.

## 1. Introduction

Trichomonosis is a potentially severe disease of multiple avian species, particularly doves and pigeons as well as predatory birds that may feed on infected prey. The causative agents are flagellated protozoa within the genus *Trichomonas* (Family Trichomonadidae), most commonly *Trichomonas gallinae* (Rivolta, 1878) (Forrester and Foster, 2009). The disease is characterized by severe inflammation and necrosis of multiple tissues, primarily the upper gastrointestinal tract (oropharynx and crop) (Amin et al., 2014). Mortality in affected birds are typically the result of starvation from reduced ability to forage, respiratory failure from lesions obstructing the trachea, sepsis due to secondary infections, or visceral organ failure following disseminated infection (Kocan and Herman, 1971; Narcisi et al., 1991). The parasite can cause significant effects at the population level, particularly when introduced into naïve populations. For example, the introduction of *T. gallinae* to Mauritius is considered a major threat to the endangered pink pigeon (*Columba mayeri*) (Bunbury et al., 2008). Additionally, *T.*

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**Table 1**
Case details from six owls with trichomonosis including location of origin, gross and microscopic lesions, and *Trichomonas* genotype detected.

| Case | Species | Age | Year | Location | Nutritional condition | Main gross lesions | Main microscopic lesions | ITS sequence: genbank |
|---|---|---|---|---|---|---|---|---|
| 1 | Barn owl | Juvenile | 2013 | Clarke County, GA | Poor | Multiple friable plaques on caudal palate and sublingual mucosa | Heterophilic stomatitis with bacteria and trichomonads | ITS-OBT-Tg-1: JN007005 |
| 2 | Barn owl | Juvenile | 2013 | Clarke County, GA | Poor | Friable plaque on dorsal palate, nasal sinuses contained opaque fluid | Heterophilic stomatitis with bacteria and trichomonads | ITS-OBT-Tg-1: JN007005 |
| 3 | Barred owl | Juvenile | 2015 | Lyon County, KS | Poor | Oropharyngeal mass extending into maxilla, opaque air sacs | Heterophilic stomatitis with trichomonads | ITS-OBT-Tg-1: KC215387 |
| 4 | Barred owl | Adult | 2012 | Baldwin County, GA | Good | Caseous plaque surrounding glottis and choanae, gray fluid in nasal sinuses | Fibrinous stomatitis and perivascular lymphoplasmacytic infiltrates | ITS-OBT-Tg-1: KC215387 |
| 5 | Barred owl | Adult | 2003 | Glynn County, GA | Moderate | Oropharyx filled with yellow-white debris obstructing pharynx | Heterophilic stomatitis with trichomonads | N/A |
| 6 | Barred owl | Adult | 1989 | Hendry County, FL | Moderate | Tan mass on the palate extending to esophagus | Granulomatous stomatitis with bacteria | N/A |

*gallinae*-associated disease was implicated as a potential cause of declining greenfinch (*Carduelis chloris*) and chaffinch (*Fringilla coelebs*) populations in the British Isles (Lawson et al., 2012). It is speculated that the most common mechanisms of transmission are via contact with infected food material, water, or saliva, which is supported by the ability of trichomonads to persist in these environments (Amin et al., 2014; Purple et al., 2015; McBurney et al., 2017).

Birds in the Order Columbiformes are the primary hosts, and many species in this order are considered to have widespread subclinical infections (Amin et al., 2014). Other hosts, including species in the Orders Passeriformes and Falconiformes, may subclinically harbor the pathogen, but sporadic cases of overt disease in these hosts can occur (Cooper and Petty, 1988; Boal et al., 1998; Anderson et al., 2009; Dudek et al., 2018). Numerous cases of *Trichomonas* infections have been reported in raptors, but are relatively uncommon in owls. However, the disease is less-studied in owls compared to other raptor species (Forrester and Foster, 2009). Infection has been reported in at least nine species of owls within the genera *Bubo, Tyto, Otus, Megascops, Strix,* and *Athene* (Forrester and Foster, 2009). Clinical disease has been documented in barred owls (*Strix varia*), great horned owls (*Bubo virginianus*), spotted owls (*Strix occidentalis*), and barn owls (*Tyto alba*) in the USA, and trichomonosis is considered a major cause of mortality in barn owls in the state of Hawai'i (Jessup, 1980; Pokras et al., 1993; Work and Hale, 1996; Rogers et al., 2016). Molecular characterization of parasites from owls from the USA is limited to one study on the relatedness of trichomonads from spotted owls and band-tailed pigeons (*Patagioenas fasciata monilis*) in California (Rogers et al., 2016).

*Trichomonas gallinae* is cosmopolitan, largely due to the wide geographical range of its primary host, the rock dove (*Columba livia*) (Stabler, 1947). However, multiple genetically distinct strains and subtypes of *T. gallinae* circulate within many avian species across the globe (Gerhold et al., 2008; McBurney et al., 2015; Martínez-Herrero et al., 2017). Within the last ten years, numerous studies have examined the genetic variation in *Trichomonas* strains from a wide variety of hosts, which has led to the detection of many strains or lineages of *T. gallinae* as well as several novel *Trichomonas* species, some of which have been formally described and can cause clinical disease (e.g., *Trichomonas stableri* and *Trichomonas gypaetinii*) (Anderson et al., 2009; Girard et al., 2014; Martínez-Díaz et al., 2015). Avian *Trichomonas* species and strains can occur subclinically or in association with disease, and individual animals can be co-infected with multiple strains (Grabensteiner et al., 2010; Kelly-Clark et al., 2013; Girard et al., 2014).

Due to the increasingly complex genetic variability in both pathogenic and commensal trichomonads in avian species, characterizing the genetic variability of trichomonads in uncommon avian hosts is important to improve our understanding of the epizootiology of this pathogen (Martínez-Herrero et al., 2017). We conducted a review of trichomonosis cases diagnosed in owls submitted to the Southeastern Cooperative Wildlife Disease Study (SCWDS, University of Georgia, Athens, GA, USA). We also present the case details and the first genetic characterization of *T. gallinae* samples from barn owls (*Tyto alba*) and barred owls (*Strix varia*) from the southeastern and midwestern United States.

## 2. Methods

Clinical case records of owls from 1 January 1975, through 31 December 2016, were reviewed for diagnoses of trichomonosis. The cases were defined as lesions in any tissue in association with trichomonad organisms detected histologically or via molecular testing. Data for each case were recorded including history, descriptions of lesions, and any additional testing.

Samples for molecular characterization were available from swabs and plaques from oral lesions from two barn owls and two barred owls. DNA was extracted from samples using a Qiagen DNEasy extraction kit (Germantown, MD) according to the manufacturer's protocol.

Polymerase chain reaction (PCR) was conducted using primers that target the ITS1/5.8S/ITS2 region of *Trichomonas* spp. (Felleisen, 1997; Gerhold et al., 2008). Bidirectional sequencing was conducted at the Georgia Genomics Facility (Athens, GA).

## 3. Results

From 1975 to 2016, a total of 89 owls were submitted for diagnostic evaluation to the Southeastern Cooperative Wildlife Disease Study (SCWDS, Athens, GA, USA). Thirty great horned owls (*Bubo virginianus*), ten Eastern screech owls (*Megascops asio*), and one Puerto Rican screech owl (*Megascops nudipes*) had variable diagnoses including trauma, emaciation, and anticoagulant rodenticide toxicosis without lesions consistent with trichomonosis. Trichomonosis was diagnosed in two species of owls: two of 26 barn owls (8%) and three of 22 barred owls (14%). An additional case in a barred owl was suspected based on gross and histopathologic lesions consistent with trichomonosis, although trichomonads were not observed histologically nor was infection confirmed via molecular methods. A summary of the cases is provided in Table 1.

## 4. Cases: barn owls

### 4.1. Cases 1 and 2

On 23 July 2013, a juvenile, recently fledged, female barn owl was found dead beneath a nest box at the Athens-Clarke County Landfill in Georgia, USA (Case1). The second case (Case 2), a sibling male, was found under the same nest box on 25 July 2013. The second bird was lethargic, moribund, and recumbent and was submitted to the University of Georgia for rehabilitation. Despite supportive care, the bird was found dead the next morning. Both birds were submitted to SCWDS for diagnostic evaluation.

The female was in poor nutritional condition with a prominent keel and little to no subcutaneous adipose tissue. Approximately 90% of the mucosa covering the caudal palate was effaced by yellow-tan, friable, caseous material, and an approximately 4 mm plaque was present on the left sublingual oropharyngeal mucosa (Fig. 1A). The bird also had a small, focal, tan, sub-meningeal plaque on the left cerebral hemisphere. Histologically, degenerating heterophils, fibrin, and mixed populations of bacteria and protozoa consistent with *Trichomonas* spp. were identified within the mucosa and submucosa of the oropharynx (Fig. 1B and C). No histologic lesions were observed in the brain, despite examination of multiple sections of the grossly visible lesion. Aerobic and anaerobic bacterial culture of the brain only showed light growth of bacterial species interpreted as contaminants.

The male was also in poor nutritional condition. The ocular conjunctiva and nares were diffusely red. The oropharyngeal mucosa on the dorsal palate was effaced by a yellow-tan friable plaque. An additional plaque was present on the left sublingual mucosa. The nasal sinuses were filled with a moderate amount of clear to tan, opaque fluid. Histologically, the mucosa and submucosa were diffusely expanded and replaced by sheets of degenerating heterophils, necrotic debris, fibrin, and colonies of mixed-population bacteria and intralesional protozoal organisms.

During the course of the diagnostic evaluation, several likely subclinical bacterial infections were detected, including *Salmonella* and *Mycoplasma*. A swab of the nasal cavity of the male owl chick consisted of a heavy growth of multiple aerobic bacteria and *Salmonella* species. Because these were considered secondary invaders, the aerobic bacteria were not identified, but the *Salmonella* species was determined to be *S. enterica* subspecies *enterica* Serotype Newport by the National Veterinary Services Laboratories (Ames, IA). The female owl chick was PCR positive for *Mycoplasma* based on universal primers as described, and the sequence (GenBank KT003195; 429 bp) was 90% similar to *Mycoplasma lipofaciens* (GenBank AY755604) (Lauerman, 1998).

## 5. Cases: barred owls

### 5.1. Case 3

On 11 June 2015, a juvenile, female barred owl was found in the backyard of a private residence in Lyon County, Kansas, USA. The owl was lethargic, and several blowflies were noted around the neck of bird. The owl was easily captured with a net, euthanized, and submitted to SCWDS for diagnostic evaluation.

At necropsy, the owl was emaciated with no visceral or subcutaneous fat stores and had marked pectoral muscle atrophy. Fly larvae were present around the oropharynx at the commissures of the mouth and the medial commissures of both eyes. The feathers on the

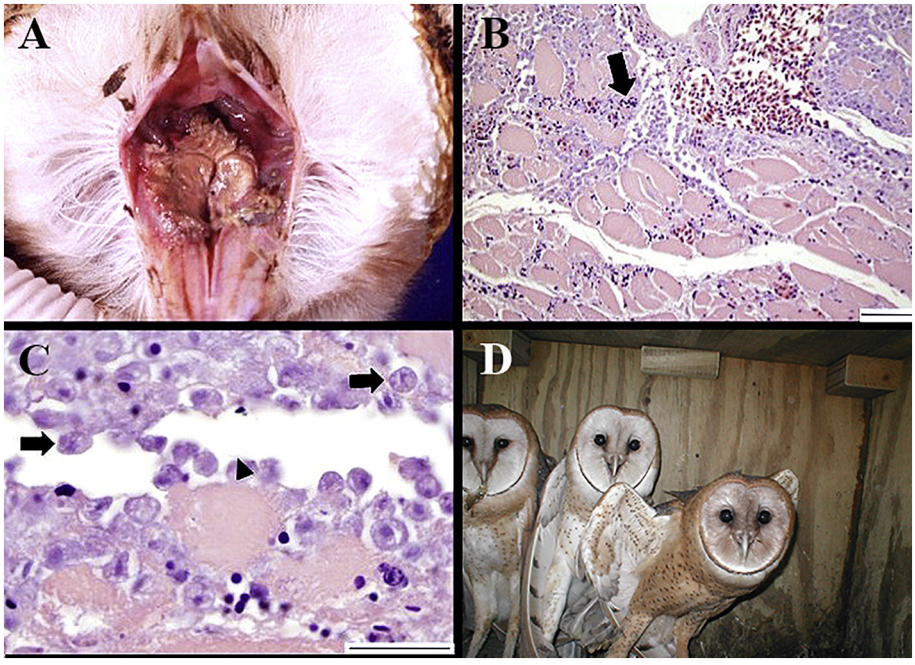


**Fig. 1.** A. Yellow-tan, friable plaque in the oral cavity of a female barn owl. B. Photomicrograph of a histological section of oropharynx from a female barn owl showing degenerated heterophils, fibrin, and mixed populations of bacteria along with numerous trichomonads (arrow) (bar = 50 µm). C. Photomicrograph of the oral lesion from a female barn owl showing trichomonads (arrows), some with visible flagella (arrowhead) (bar = 25 µm). (A-C: Case 1). D. Barn owl chicks observed in the nest box prior to mortality (Cases 1 and 2).

ventral neck were matted and slightly damp, and mucus was present within the oropharynx. A round, tan, firm, multilobulated, raised mass that measured approximately 3.5 × 3 cm was present on the right side of the oropharynx. The mass extended into the right maxilla and right nasal cavity and also into the lumen of the oropharynx where it narrowed the cavity. The air sacs were diffusely opaque and thickened.

Histologically, the oropharyngeal mass was composed of primarily fibrin and cellular debris admixed with degenerating heterophils and macrophages. The inflammatory exudates effaced normal architecture and extended deep within the soft tissue surrounding the oropharynx with associated skeletal muscle damage and infiltration of the surrounding skin and subcutaneous tissue. The fibrinonecrotizing lesions extended down the crop. Rare pyriform-shaped protozoal organisms approximately 12 µm in diameter that stained with Giemsa staining were found among the necrotic debris. The inflammatory exudates effaced the nasal sinuses in some sections and invaded surrounding bone. The surrounding bone marrow was hypercellular with multiple heterophils present.

Additional findings, thought to be incidental, included a small number of adult nematodes approximately 100 µm in diameter with a thin cuticle within the small intestinal lumen. Routine testing of pooled oropharyngeal and cloacal swabs for avian influenza using PCR targeting the matrix gene was negative.

### 5.2. Case 4

On 28 November 2012, an adult, female barred owl was found in the backyard of a private residence in Baldwin County, Georgia, USA. The owl attempted to fly but was unable to sustain flight. The bird was captured and treated by a local veterinarian who provided 0.25 ml of 3 mg dexamethasone and noted blood on the beak. The owl did not improve and was found dead on 30 November 2012. The carcass was frozen and submitted to SCWDS for diagnostic evaluation.

The owl was in good nutritional condition. A 3.5cm-diameter, tan, firm, caseous lesion was centered at the glottis and extended rostrally around the choanae, laterally to the tympanic cavity, and caudally to include the esophagus. The tissues immediately adjacent to the choanae were gray-green and friable. There was gray-green fluid mixed with gray, gritty material in the nasal passage and internal acoustic meatus. The crop, proventriculus, and ventriculus were empty, and there was scant dark gray to green ingesta throughout the intestines.

Histologically, the oropharyngeal mucosa was greatly expanded by sheets of eosinophilic, mononuclear cells that were unidentifiable (presumably heterophils and macrophages) and by brightly eosinophilic fibrillar material. Underlying muscles were largely replaced by sheets of heterophils and brightly eosinophilic cells and material described above. Aggregates of lymphocytes and plasma cells were associated with vessels. A section of dermis was thickened by dense connective tissue. No other significant findings were noted other than a single focus of perivascular hemorrhage in the lung and mild granulocytic and lymphoplasmacytic infiltrates in the liver.

### 5.3. Case 5

On 16 July 2003, an adult, female barred owl from Glynn County, Georgia, USA was observed in the same tree for twelve hours and when the owl tried to fly, it fell to the ground. The bird was euthanized and submitted for diagnostic evaluation.

The owl was slightly thin. The oropharynx was filled with firm yellow-white material, which was invading the soft tissues and obstructing the pharynx. No other gross lesions were noted. Within sections from the oropharynx, the muscle was invaded and replaced by large numbers of macrophages and heterophils with fewer numbers of lymphocytes and plasma cells. Occasional multinucleated giant cells were also noted. Rare protozoal organisms morphologically consistent with *Trichomonas* were noted within these areas of inflammation.

### 5.4. Case 6

In December 1989 an adult, female barred owl from Hendry County, Florida, USA was found dead in the same area as two previous recent owl mortalities. The owl was frozen and submitted to SCWDS.

The bird had atrophied breast musculature, but adequate fat stores. There was a 3.5 cm-diameter, firm, cream-colored mass on the left roof of the mouth that partially protruded into the right side. The mass extended caudally to the opening of the esophagus and dorsally to the ventral surface of the palatine and parasphenoid bones, but no invasion was observed. The owl was extremely autolyzed, so histological evaluation was of limited value. Macrophages, fibroblastic cells, a few multinucleated giant cells, and gram positive cocci were noted within the mass. Because no trichomonads were noted on histology, this case is considered a suspect case, but the gross lesions are highly suggestive of trichomonosis.

## 6. Results: molecular analyses

The sequences from the barn owls (Cases 1 and 2) were 342 bp and were identical to a *T. gallinae* sequence from a captive budgerigar from Austria (GenBank JN007005). The sequences from two of the barred owls (Cases 3 and 4) were 370 bp long and were identical to a *T. gallinae* (KC215387) sequence from a Pacific Coast band-tailed pigeon from California as well as from sparrowhawk (*Accipiter nisus*), rock dove, blackbird (*Turdus merula*), and greenfinch (*Chloris chloris*) samples from Europe (Girard et al., 2014; Quillfeldt et al., 2018). Both *T. gallinae* sequences are included in the ITS-OBT-Tg-1 genotype per terminology suggested by Martínez-Herrero et al. (2014).

## 7. Discussion

Trichomonosis was a relatively uncommon diagnosis in six out of 89 owls (6.7%) submitted to SCWDS between 1975 and 2016. Three out of the six owls were in poor nutritional condition suggesting trichomonosis likely contributed to morbidity and mortality in these animals. Three out of the six owls, including both barn owls, were also juveniles, who are potentially more predisposed to clinical disease and mortality compared to adults, and immunosuppression, particularly in juveniles, may have played a role in the severity of disease (Rogers et al., 2018).

Trichomonosis has been reported in numerous raptor species, but in general, reports in Strigiformes are infrequent compared with Falconiformes. However, there are exceptions. Quillfeldt et al. (2018) found a higher prevalence in strigiformes (58%) and columbiformes (50%) compared to other bird orders. Prevalences in barn owls have varied greatly; prevalences were low in two studies (40 of 1638 (2%) from California (USA) and < 1% of 278 from England were positive), but considerably higher in two other studies (20 of 81 (25%) from Hawai'i (USA) and 10 of 20 (50%) in Italy) (Hardy et al., 1981; Schulz 1986; Work and Hale, 1996; Delogu et al., 1997). The reason(s) for differences in prevalence among these studies is unknown. These studies were predominately based on retrospective causes of mortality in owls examined at wildlife hospitals or diagnostic laboratories, so there is low likelihood of biases between studies regarding differences in demographics (e.g., age, sex). Infections and clinical disease in individual barn owls that were not part of larger surveillance studies have been reported from California and Louisiana (USA), as well as in Spain (Pokras et al., 1993; Martínez-Herrero et al., 2014). The generally low prevalence in barn owls could be due to their diet that consists primarily of small, terrestrial mammals (Colvin and McLean, 1986). No large-scale prevalence studies have been conducted regarding barred owls, and previous reports are individual cases with clinical disease in Louisiana and Massachusetts, or reports of subclinical infections in Florida (USA) (Pokras et al., 1993; Forrester and Spalding, 2003).

A history of barn owl nestling morbidity and mortality due to unknown causes had been noted at the nest site of the two juvenile barn

owl cases and with this particular nesting pair (Muise, unpublished data). Also, columbid (likely Eurasian collared dove (*Streptopelia decaocto*), rock doves, or both), chicken, and occasional American kestrel (*Falco sparverius*) remains were detected inside the nest box. This is particularly interesting because Eurasian collared-doves were first recorded in Georgia in 1988 and were reported in the Piedmont region of Georgia in 1994 (Beaton et al., 2003). Because of their size, these doves could be prey for barn owls. These doves inhabit open spaces where barn owls are more likely to hunt. A single study of *Trichomonas* in Eurasian collared-doves suggests they are commonly infected and can develop disease (Stimmelmayr et al., 2012). Although collared doves share habitat with barn owls, it is unknown if Eurasian collared doves, rock doves, or another columbid were the source of infection in the barn owls in this study. Regardless, future studies are needed to determine the prevalence of *Trichomonas* in Eurasian collared-doves as well as the potential threats to barn owls or other ornithophagous birds that may feed on them, especially as Eurasian collared doves expand throughout North America. Additionally, studies exploring possible shifts in dietary preferences for owls are warranted, particularly in areas where landscape changes may result in a higher proportion of avian prey similar to that described by Dudek et al. in golden eagles (*Aquila chrysaetos*) (Dudek et al., 2018).

This study also highlights additional co-morbidities in owls with clinical trichomonosis. A previous study on barn owls in New Jersey showed that 9% of nestlings from 20% of nest sites were positive for *S. enterica* subsp. *enterica* subtypes Typhimurium, Thompson, and Tuindorp (Kirkpatrick and Colvin, 1986). In adult wild birds, *Salmonella* infections are often asymptomatic, but large epornitics have been reported (Hernandez et al., 2012). However, the impact of *Salmonella* on wild bird health is poorly understood and may be a significant cause of mortality among juvenile wild birds (Phalen et al., 2010). *Mycoplasma lipofaciens* is a potentially zoonotic avian mycoplasma first described in domestic chickens; it has since been reported in domestic turkeys, domestic ducks, and a Goshawk (*Accipiter gentilis*) (Bradbury et al., 1983; Lierz et al., 2007, 2008). Although the *Mycoplasma* detected in this study was most similar to *M. lipofaciens*, it likely represents a novel *Mycoplasma* sp. of unknown pathogenicity. To our knowledge, this is the first reported case of *Mycoplasma* sp. infection in barn owls in North America.

This study provides the first sequences and genetic characterization of *Trichomonas* from barn owls and barred owls from the USA. The genotype (ITS-OBT-Tg-1) we detected has also been found in a single barn owl from Spain with typical trichomonosis lesions (Martínez-Herrero et al., 2014). The ITS-OBT-Tg-1 genotype has been found in a wide range of bird species across a broad geographic range, but importantly, in a study conducted in Spain, this genotype was associated with an increased risk of birds presenting with lesions, often associated with mortality (Martínez-Herrero et al., 2014). Birds that were not strictly ornithophagous (as with barn owls) were more likely to develop lesions due to *Trichomonas* infection compared to bird species that were strictly ornithophagous. In Spain, a different genotype (ITS-OBT-Tg-2) predominated in columbids and in an ornithophagous raptor (Goshawk) that had a low prevalence of lesions (Sansano-Maestre et al., 2016).

The sequences from the barred owls were identical to one from a band-tailed pigeon in California, as well as multiple avian species in Europe, but none from owls (KC215387) (Girard et al., 2014; Quillfeldt et al., 2018). Currently, there are several manners by which to label the *Trichomonas* lineages. Other studies have labelled these lineages as "A/B lineage" or "A1 subtype," which are considered common and highly pathogenic (Robinson et al., 2010; Chi et al., 2013; Quillfeldt et al., 2018). The Fe-hydrogenase locus in spotted owls in California revealed the presence of FeH subtype A2 in all owls examined, and although not used in this study, sequences from the FeH locus may have provided additional data to compare between lineages (Rogers et al., 2016). Similar to a study by Martínez-Herrero et al. (2014), a comprehensive examination of *Trichomonas* from various raptor species and possible avian prey using consistent gene targets is needed in North America to better understand risk and transmission dynamics. This is particularly relevant because of the invasive populations of Eurasian collared-doves which may be a new prey item for raptors in the US.

In conclusion, this study indicates that multiple genetic strains of *T. gallinae* genotype ITS-OBT-Tg-1 can cause clinical disease in both young and adult owls of at least two species in the eastern USA. Few studies have formally described the strains affecting owls in this region, and further studies into the epidemiology of *Trichomonas* species in owls and their prey are warranted.

## Declaration of conflicting interests

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from kevindn@uga.edu.

### *Ethical statement*

No animals were specifically harmed for the purpose of this study but were used opportunistically and abided by all of the authors' respective institutional IACUCs.

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