



## Parasite exchange and hybridisation at a wild-feral-domestic interface

William J. Smith<sup>a,\*,1</sup>, Michał T. Jezierski<sup>a,1</sup>, Jenny C. Dunn<sup>b,c</sup>, Sonya M. Clegg<sup>a</sup><sup>a</sup> Edward Grey Institute of Field Ornithology, Department of Biology, University of Oxford, UK<sup>b</sup> School of Life and Environmental Sciences, University of Lincoln, UK<sup>c</sup> School of Biology, University of Leeds, UK

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## ABSTRACT

Interactions between wild, feral, and domestic animals are of economic and conservation significance. The pigeon *Columba livia* is a synanthropic species in a feral form, but it also includes the rare Rock Dove. *Columba livia* is an important player at the wild-domestic interface, acting as a carrier of avian diseases, and the feral form threatens Rock Doves with extinction via hybridisation. Despite its abundance, little is known about drivers of disease prevalence in *C. livia*, or how disease and hybridisation represent synergistic threats to Rock Doves. We focused on infection by the parasite *Trichomonas*, first collating prevalence estimates in domestic and free-living populations from relevant studies of *C. livia*. Second, we characterised variation in the diversity and prevalence of *Trichomonas* among three *C. livia* populations in the United Kingdom: a feral, a Rock Dove, and a feral-wild hybrid population. Across multiple continents, free-living pigeons had lower *Trichomonas* infection than captive conspecifics, but the effect was weak. Environmental factors which could impact *Trichomonas* infection status did not explain variation in infection among populations. Among the British populations, strain diversity varied, and there was lower parasite prevalence in Rock Doves than feral pigeons. Individual infection status was not explained by the available covariates, including hybrid score and site. The drivers of *Trichomonas* prevalence are unclear, perhaps due to idiosyncratic local-scale drivers. However, given the population-level variation in both infection prevalence and introgressive hybridisation, the potential combined effects could accelerate the extinction of the Rock Dove. Further study of the synergistic effects of multiple types of biotic interactions at the wild-feral-domestic interface is warranted, especially where vagile, globally distributed and superabundant animals are involved.

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## 1. Introduction

The wild-domestic interface is the sum of interactions between domesticated organisms and the wider environment (Clark et al., 2018). Processes that occur at this interface, including predation, competition, and habitat alteration, can have demographic and health repercussions for both domesticated and wild organisms. These impacts have broad importance, from the economic significance to domesticates such as livestock, through to the conservation implications for endangered wild populations (Gortazar et al., 2015; Rossi et al., 2019). One such interaction that has received significant levels of attention is disease transmission, particularly infection of domestic organisms via contact with wild relatives (Gortazar et al., 2015). For example, migratory waterfowl are

known to transmit avian influenza to domestic poultry (Khan et al., 2018). Interactions at the wild-domestic interface can be mediated by feral (self-sustaining, free-living, naturalised domestic origin) populations which, being synanthropic and tightly associated with anthropogenic environments, could come into contact with both wild and captive domestic populations (Doherty et al., 2017). For example, domestic and feral dogs (*Canis familiaris*) have spread canine distemper to the African wild dog, *Lycaon pictus* (van de Bildt et al., 2002). Another type of interaction at the wild-domestic interface is interbreeding (Smith et al., 2022a). When interbreeding occurs extensively, it can result in genomic homogenisation, rendering a population or species extinct as an evolutionarily distinct lineage; a process known as extinction by hybridisation (Rhymer and Simberloff, 1996). Feral populations pose a particular threat to their wild conspecifics due to relatively recent evolutionary divergence and porous reproductive boundaries (Smith et al., 2022a). For example, contemporary Singaporean Red Junglefowl (*Gallus gallus*) and Scottish wildcats (*Felis silvestris*) each exist as part of mixed wild-feral-domestic hybrid populations

\* Corresponding author at: Edward Grey Institute of Field Ornithology, Department of Biology, University of Oxford, OX1 3SZ, UK.

E-mail address: [william.smith@biology.ox.ac.uk](mailto:william.smith@biology.ox.ac.uk) (W.J. Smith).

<sup>1</sup> Joint first authors.

rather than as entities distinct from their feral and domestic relatives (Senn et al., 2019; Wu et al., 2020).

Knowledge of specific types of wild-domestic interactions is variable, and some are better understood than others. For example, whilst disease transmission receives significant research funding and effort (Gortazar et al., 2015; Clark et al., 2018), the evolution of escaped domesticates is relatively poorly understood (Daniels and Bekoff, 1989). Additionally, there has been little attention paid to the synergistic impacts of different processes which occur upon contact between wild, feral and domestic entities. For example, disease and genetic homogenisation are rarely mentioned in the context of one another. Indeed, studies of extinction by hybridisation rarely explore factors that impact the temporal and spatial dynamics of introgression, and often can only highlight that it is in progress – in itself a challenging endeavour (Randi, 2008). In the well-studied wildcat population of Scotland, multiple papers have been published exploring feral-wild hybrid ancestry (Daniels et al., 1998; Beaumont et al., 2001; Witzemberger and Hochkirch, 2014; Senn et al., 2019; Tiesmeyer et al., 2020). There have also been papers discussing the transmission of diseases from feral cats to wildcats (Daniels et al., 1999; Meredith et al., 2018). Despite this, there has been no published work assessing potential synergistic effects linking disease and hybridisation. If, for example, the severity of a disease is higher in an individual lacking hybrid ancestry (perhaps due to lower genetic diversity in unadmixed individuals), then hybrid individuals may be favoured by natural selection over their wild relatives, potentially accelerating the extinction by hybridisation process.

In birds, trichomoniasis, caused by *Trichomonas gallinae* and possibly related species such as *Trichomonas tenax* and *Trichomonas vaginalis* (Quillfeldt et al., 2018), can have variable effects on both wild and domestic forms. Many infections are apparently asymptomatic (Borji et al., 2011), but in other cases individuals exhibit ruffled feathers, weight loss, and the build-up of lesions and fluid in the crop and mouth, followed by death (Amin et al., 2014). Trichomoniasis can lead to localised population declines, as well as declines at a whole-species level. For example, the Type A strain of *T. gallinae* has been implicated in the decline of the British Greenfinch, *Chloris chloris* (Lawson et al., 2012). In European Turtle Doves (*Streptopelia turtur*) in Britain, all individuals with clinical signs of trichomoniasis also had *T. gallinae* with an internal transcribed spacer (ITS) gene sequence identical to that of the Type A strain (Stockdale et al., 2015). Transmission from wild birds into domestic populations can occur, and *T. gallinae* parasites have been identified in domestic poultry, especially where husbandry standards are low (Willoughby et al., 1995; Williams, 2005). Studies of *Trichomonas* suggest a wide spectrum of transmission capability and virulence, depending on the strain involved and the relative immune response of the host individual and species (Höfle et al., 2004). It is also known that birds can lose as well as gain detectable infections (Bunbury et al., 2008). Strain composition differs geographically and temporally, even within single species (Thomas et al., 2022). In particular, the disease is known to pose a threat to pigeon and dove species, the most affected bird family and the main global hosts (Borji et al., 2011). This includes the declining European Turtle Dove and the endemic Mauritian Pink Pigeon, *Nesoenas mayeri* (Bunbury et al., 2008; Marx et al., 2017). Of the wild pigeons and doves in Europe, *T. gallinae* has been isolated from Woodpigeons, *Columba palambus* (47% infection rate among sampled birds in the UK), Stock Doves, *Columba oenas* (40%), Turtle Doves (86%) and Collared Doves, *Streptopelia decaocto* (86%) (Lennon et al., 2013). More recent work assessing Turtle Doves in the UK reported prevalence of 90% and 100% in 2014 and 2015, respectively (Thomas et al., 2022).

Within the pigeon and dove family, the domestic pigeon *Columba livia* is the main host facilitating the global spread of *T.*

*gallinae* (Chi et al., 2013). This is borne out by studies showing that domestic and feral pigeons host *T. gallinae* (Greguric et al., 1986; Borji et al., 2011; Alkharigiy et al., 2018), often at high prevalence (e.g. 68% prevalence across multiple sites in Spain and Portugal (Santos et al., 2019)). Indeed, the species has been proposed as the ‘ultimate source’ of this particular parasite (Richard et al., 2008) and the feral pigeon acts as an important bi-directional transmission link for disease between domestic poultry or other captive birds, and wild bird species in general (Johnston and Janiga, 1995; Capoccia et al., 2018). Feral pigeons are an example of a superabundant and vagile commensal, whose association with anthropogenic environments allows dispersal across the globe. This category of species, which also includes the Brown Rat (*Rattus norvegicus*) and the House Sparrow (*Passer domesticus*), inevitably plays a disproportionate role at the wild-feral-domestic interface, including in terms of disease transmission. Their study is therefore of particular importance. Feral pigeons differ from both fully captive and free-ranging contemporary domestic pigeons in that they are longstanding unmanaged populations which have undergone adaptation to anthropogenic environments (Johnston and Janiga, 1995). They are now genetically, morphologically, behaviourally and physiologically distinct from their domestic relatives. *Columba livia* also exists in a wild, undomesticated form known as the Rock Dove, that persists in certain locations but is threatened with extinction by hybridisation via introgression with the feral pigeon (Smith et al., 2022b). This wild form is far less studied than its domestic, model species cousin (Shapiro and Domyan, 2013), including with respect to its epidemiology (Smith, 2023). Contact between the different types of pigeon at the wild-feral-domestic interface occurs in various different circumstances (Johnston and Janiga, 1995). For example, domestic pigeons can encounter feral pigeons or Rock Doves through different routes. Shared feeding locations are an obvious site of contact. Feral pigeons or Rock Doves often make use of food provided for domestic pigeons, and domestic pigeons can mix with Rock Doves or feral pigeons on agricultural land. Lost domestic pigeons can also join feral pigeon or Rock Dove colonies. Similarly, Rock Doves and feral pigeons can interact with one another through shared feeding sites on the edges of urban areas, or following expansion of the feral pigeon distribution into areas with relict Rock Dove populations (e.g. sharing roosting and breeding sites within ruined buildings). Disease transmission between these three genetically, morphologically, physiologically and behaviourally different forms of *C. livia* is therefore multi-directional. The only published epidemiological research that has been carried out to date on undomesticated ‘truly wild’ Rock Doves assessed a putative population near Sinj in Croatia (although no genomic assessment was carried out to confirm Rock Dove status), and compared them with feral pigeons in urban Zagreb (Greguric et al., 1986). Whilst *Trichomonas* was detected in 146 of the 268 feral pigeons sampled (54.47%), only two of Sinj’s 17 Rock Doves (11.76%) were found to be infected. The extent to which *Trichomonas* infection varies among *C. livia* populations with different genomic backgrounds is unknown, yet in combination with the hybridisation threat, parasitic disease is likely to be an important consideration for Rock Dove conservation (Smith, 2023).

Here we examine the drivers of *Trichomonas* infection in *C. livia*; its variation among feral, hybrid and Rock Dove populations; its variation among individuals; and, for the Rock Dove in particular, how the joint threats of hybridisation and disease could interact. We collated information from published studies of *Trichomonas* infection in *C. livia* to ask i) if infection prevalence could be explained by captive domestic versus free-living status, and a suite of environmental variables. Next, we sampled from three populations of *C. livia* in the U.K. that had previously been genomically confirmed to represent feral pigeons, Rock Doves and birds from a feral-wild hybrid population where all birds have mixed

ancestry, although most individuals will be backcrosses rather than first generation hybrids (Smith et al., 2022b) to ask ii) if *Trichomonas* prevalence and diversity varied across these populations; and iii) if individual infection status was related to a morphological hybrid score or condition score. Combining these global, population, and individual-level perspectives, we build on our understanding of a globally important actor at the wild-domestic interface, and discuss potential synergies linking disease transmission with the process of extinction by hybridisation in the Rock Dove.

## 2. Materials and methods

### 2.1. Global analysis of prevalence

To assess factors important in explaining variation in the prevalence of *Trichomonas* in *C. livia*, we collected data from all available literature on the prevalence of this infection in the species. We searched the Web of Science using: TS = (feral pigeon OR Rock Dove OR pigeon OR domestic pigeon OR dove OR *Columba livia*) AND TS = trichom\*. We included papers that conducted population screening of *C. livia*, excluding veterinary papers that worked specifically on clinical cases of *Trichomonas*, and studies targeting individuals of known infection status or poor condition. This was done to ensure the dataset did not include works that could artificially increase prevalence. We collated data on the numbers of populations studied, type of pigeon population (domestic pigeons, feral pigeons, wild Rock Doves), identified species of *Trichomonas*, and its prevalence and strains present. We extracted the geographic coordinates of sites either directly from papers, or using GeoHack (<https://geohack.toolforge.org/>).

In recording prevalence, ideally we would have used a binomial term of number infected versus number uninfected. However some studies did not report total numbers, therefore we were constrained in expressing prevalence as a proportion of infected birds in this analysis to maximise the number of observations in the dataset. We used the recommended approach of beta regression when the response variable is bound between 0 and 1 (Douma and Weedon, 2019), within the *glmmTMB* package in R v4.2.1 (Brooks et al., 2017). In addition to the explanatory variable of pigeon type, we included additional variables that have been shown to influence disease prevalence in other systems: urban or rural environment (Evans et al., 2009), temperature (Holand et al., 2019), and precipitation (Ferenczi et al., 2016). Using the geographic co-ordinates from each study, we categorised each site as urban or rural based on the land type designation from NaturalEarthData (<https://www.naturalearthdata.com/>), and extracted average annual temperature and average annual precipitation from WorldClim at 10 m resolution (Fick and Hijmans, 2017). Climate variables were centred (by subtracting column means) and scaled (by dividing the centred columns by their standard deviation) using the command *scale* from *stats* in R. We used the *dredge* function of *MuMIn* scored using Bayesian Information Criterion (BIC) to discriminate among models.

### 2.2. Field data collection

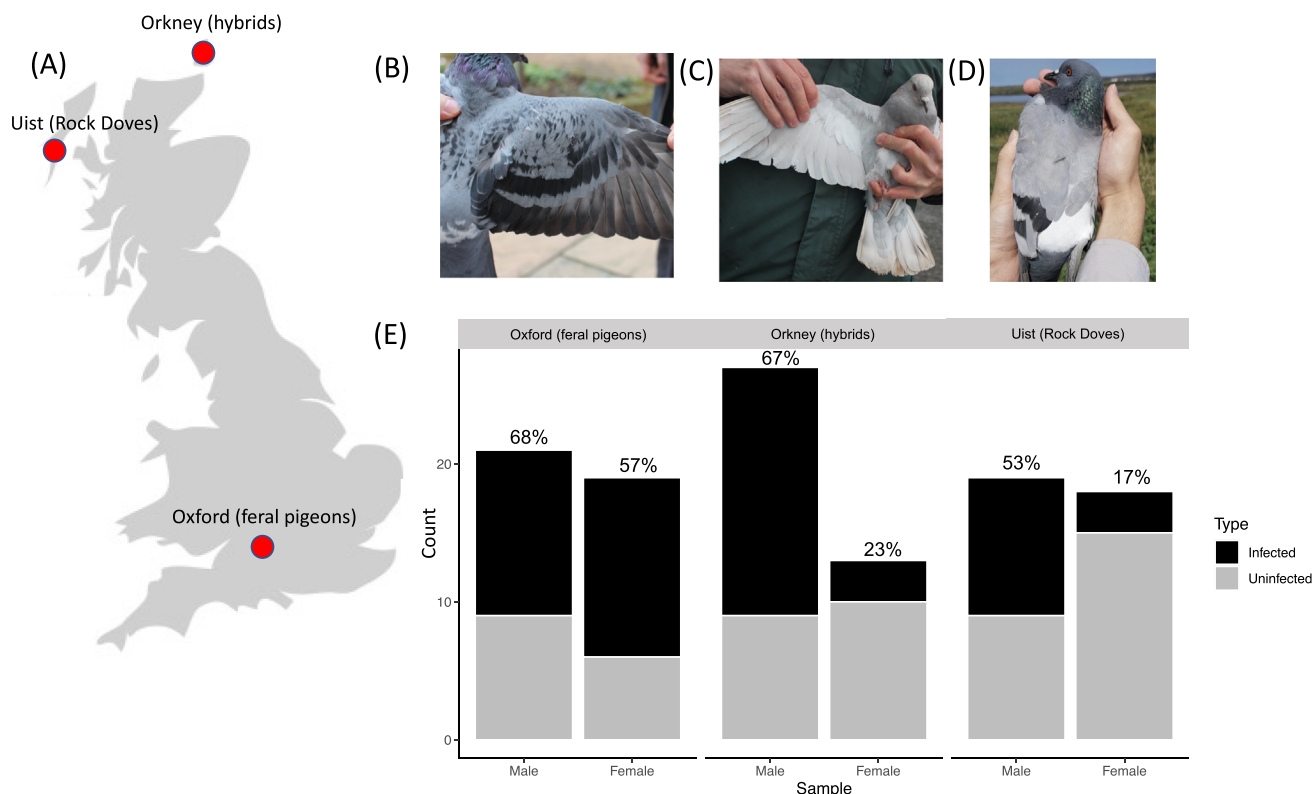
Adult birds ( $n = 120$ ) were captured at three locations across the UK between the 21st of March and the 16th of May 2021 (Fig. 1A) from populations previously identified as one of three types (Smith et al., 2022b): feral domestic pigeons ( $n = 40$ , Fig. 1B) from Oxford in southern England (51°45'00.0"N 1°15'36.0"W); a hybrid swarm population ( $n = 40$ , Fig. 1C) from Orkney, Northern Isles, Scotland (59°03'00.0"N 3°01'12.0"W); and undomesticated Rock Doves ( $n = 40$ , Fig. 1D) from Uist, Outer Hebrides, Scotland

(57°12'00.0"N 7°24'00.0"W). Feral pigeons were caught by hand during the daytime, baited using seed and bread, and ringed with individually coded plastic Darvic blue colour rings to prevent resampling of individuals. Rock Doves and hybrids were caught at night whilst roosting in caves, ruins or farm buildings, and ringed with individually numbered metal leg rings provided by the British Trust for Ornithology, UK. Age was established based on moult progression and plumage traits, and juvenile birds (plus those of unknown age category) were not sampled to avoid age adding confounding morphological variation to our analyses (Baker, 2016). We recorded: mass using a digital Pesola scale ( $\pm 1$  g); maximum wing chord and tarsus length to claw using a butted metal ruler ( $\pm 1$  mm); and cere width, tarsus width, and bill length (to feather edge) using dial callipers ( $\pm 0.1$  mm). For genetic sexing, we took a blood sample from each bird by pricking the brachial vein with a 26 G ( $0.45 \times 25$  mm) hypodermic needle, collecting the resulting blood droplet with a capillary tube, and transferring to a Whatman® FTA card™ (Whatman, Maidstone, United Kingdom) for storage at ambient temperature while in the field. We swabbed each bird's crop and buccal cavity with a sterile viscose swab. The swab was used to inoculate an individual InPouch® TV culture kit (Biomed Diagnostics, Oregon, USA). The kits were incubated in the field at 37 °C for 4–7 days with an Octagon Egg Incubator (Brinsea, Weston-super-Mare, UK). Following incubation, the culture medium of each individual bird was stored in 100% ethanol at 1:1 volume of ethanol:culture medium (Stockdale et al., 2015). On return to the laboratory, the cultured crop swab samples were stored at –20 °C and the blood samples on FTA cards stored at 4 °C.

### 2.3. DNA extraction and PCR

Cultured crop/buccal cavity swab samples were transferred to one or two microcentrifuge tubes per individual. Samples were centrifuged for 5 min at 3200 rpm (572 g), and the ethanol poured off the resulting pellet. Pellets were then washed in 1 ml of PBS using the same centrifugation process, and the PBS poured off. We then added 200 µl of PBS to each microcentrifuge tube and mixed by vortexing the tubes. Samples from the same individual were pooled for DNA extraction. Half of the volume of each pooled sample (the exact volume varied among individual birds) was used, with a 2 h digestion phase (with 4 µl of Proteinase K and 250 µl of DIGSOL extraction buffer) and a standard phenol–chloroform extraction technique (described in Smith et al., 2022b). DNA from blood samples (a punch-out of 1x1 cm of the area of the FTA card®) was extracted using the same phenol–chloroform extraction method but preceded by a 6 h digestion phase. Genomic DNA extracts from both crop/buccal samples and blood samples were visualised via gel electrophoresis on a 1% agarose gel, to confirm the presence of high molecular weight DNA.

We carried out a PCR protocol using a Qiagen AllTaq™ Master-Mix Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations. We included a negative control in each PCR as a check for potential contamination. The ITS1/5.8S/ITS2 ribosomal region of rDNA (referred to as the ITS region) was used to determine positive infections and to identify *Trichomonas* strains ('ribotypes') (Grabensteiner et al., 2010; Alrefaei et al., 2019a). This region has a low mutation rate, meaning that sequences not identical to known strains are assumed to be novel strains. We repeated ITS amplification twice to minimise false negatives. The PCR used the primers TFR1 (5'-TGCTTCAGTTACGCGGTCTTCC-3') and TFR2 (5'-CGGTAGGTGAACCTGCCGTTG G-3'), which give an expected product length of 400 bp (Robinson et al., 2010). PCRs with ITS primers were carried out with thermal cycling of: 2 min denaturation at 94 °C, then 40 cycles of 30 s at 94 °C, 30 s at 67 °C, and 2 min at 72 °C, followed by final elongation for 15 min at 72 °C (Robinson et al., 2010). We then carried out PCR



**Fig. 1.** The species *Columba livia* in Britain varies both in terms of its genetic background and its infection status. (A) Sampling locations in England and Scotland. (B) In Oxford, most feral pigeons display black chequering on the wings (above the black wing bar), a trait known to have been introduced during the domestication process. (C) Hybrid pigeons from Orkney mostly have the 'Rock Dove' plumage seen in Uist, but many display feral pigeon plumage traits including the 'red-white' colouration displayed by this individual. (D) Rock Doves caught in Uist all showed the distinctive plumage of the undomesticated form, including a white rump patch. (E) Sex differences in infection prevalence at each site.

amplification for the hydrogenosomal iron hydrogenase (*Fe-hyd*) gene region (expected product length 900 bp) to identify subtypes of *T. gallinae* (McBurney et al., 2015; Alrefaei et al., 2019a) on all individuals. The PCR was repeated for any individuals that were positive for infection at the ITS region but failed to amplify in the first *Fe-hyd* PCR. We used the primers TrichhydFOR (5'-GTTTGGG ATGGCCTCAGAAT-3') and TrichhydREV (5'-AGCCGAAGATGTTGTC GAAT-3') which had an expected product length of 900 bp (McBurney et al., 2015). Thermal cycling was conducted as follows: 15 min at 94 °C, followed by 35 cycles of 94 °C for 1 min, 66 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The *Trichomonas* PCR products were electrophoresed on a 1% agarose gel to confirm the presence or absence of amplified DNA. For 50 randomly selected positive ITS and 12 *Fe-hyd* samples, the PCR products were purified at Eurofins Genomics (Eurofins, Luxembourg, Luxembourg), and the forward and reverse reactions sequenced using the TubeSeq service. For genetic sexing, we used the CHD1 pigeon sexing primers, which have the following sequences: forward: 5'-TTCTGAGGATGGAAATGAGT-3' and reverse: 5'-AGCAATGGTTACAACACTTC-3', and thermal cycling of: 94 °C for 5 min, then 38 cycles of 94 °C for 30 s, 53.5 °C for 30 s and 72 °C for 30 s, followed by a final extension of 72 °C for 5 min (Liang et al., 2019). A single band (of 474 bp) on a 1% agarose gel signified a male, and two bands (of 319 bp and 474 bp) a female.

#### 2.4. Phylogenetic analysis and strain diversity

Using MEGA11 (Tamura et al., 2021), we trimmed and manually aligned the forward sequence with the reverse sequence for each PCR product. We then assessed them for sequencing errors to gen-

erate a consensus sequence, by identifying unassigned ('wobble') bases on the forward strand that had been successfully determined to a specific base on the corresponding reverse strand. We identified the closest matching known sequence to each consensus sequence using the NCBI BLAST algorithm (Altschul et al., 1997). To construct phylogenetic trees for the ITS and *Fe-hyd* regions, we first generated an alignment using the MUSCLE algorithm (Edgar, 2004) including one example of each consensus sequence isolated in this study, together with single sequences of all known strains reported in a recent study (Thomas et al., 2022). These MEGA alignments were trimmed to 216 bp for the ITS region and 578 bp for the *Fe-hyd* gene region. For the tree generated for the ITS region, we used identical publicly available GenBank sequences to those used in the phylogeny reported in Thomas et al. (2022), except for ITS strain A, where we used sequence A/N KX459445 rather than A/N MN587093 (which is only 214 bp long). We also included representative ITS region sequences of the related species *T. vaginalis* (A/N AY871048), *T. tenax* (A/N KX459486), and *Tetratrichomonas gallinarum* (A/N AY871048), with the latter used as an outgroup. In the *Fe-hyd* alignment, we added the GenBank sequence of novel strain A3 (A/N MZ128143), and used a representative sequence of *T. vaginalis* (A/N XM001310179) as an outgroup (*T. tenax* and *T. gallinarum* did not have any *Fe-hyd* gene sequences published in GenBank). Of the nucleotide substitution models available in the BEAUti graphical user interface (Drummond et al., 2012), we selected the optimal model for each alignment using ModelFinder (via the Bayesian Information Criterion (BIC) (Kalyaanamoorthy et al., 2017)). This was Hasewaga-Kishino-Yano plus gamma with empirical base frequencies for both the ITS and *Fe-hyd* alignments. Using this model, the alignments (of 27 sequences for the ITS region, and 36 sequences for the *Fe-hyd* gene

region) were used as inputs in BEAUti v1.10.4 and BEAST v1.10.4 using a strict clock and the Yule speciation process, and then Markov Chain Monte Carlo simulations with 100,000,000 generations sampled every 1000 generations after a 10% burn-in (Drummond et al., 2012; Suchard et al., 2018). The maximum clade credibility trees were created using TreeAnnotator v1.10.4 (part of the BEAST suite) and visualised in FigTree v1.4.4 (<https://tree.bio.ed.ac.uk/software/figtree/>).

To test for non-random associations between locations and the numbers of birds identified as being infected with each ITS strain, we carried out Fisher's exact test with the Freeman-Halton extension. We then carried out Fisher's exact tests to identify whether there were non-random associations between sex and the number of birds identified as being infected with each ITS strain at either Oxford, Orkney, or Uist. Insufficient *Fe-hyd* sequences were obtained to repeat these tests for the *Fe-hyd* subtypes.

## 2.5. Prevalence and correlates of *Trichomonas* infection and condition

Prevalence was determined as the number of infected versus uninfected individuals in each population, determined by ITS region PCR. Using a generalised linear model (GLM) with a binomial error structure, we determined if variation in infection at the individual level (dependent variable; 0 if negative for infection, 1 if positive for infection) was explained by sample location, sex, and hybrid score. Because we did not have whole genome sequences available from the particular individuals used in this study, we calculated a proxy for individual introgression level using individual principal component 1 scores from a principal component analysis (PCA) using the logged values of wing, bill and tarsus length, cere width and mass. Morphology has been shown in prior work to represent a suitable proxy for the extent of genomic admixture (Smith et al., 2022b). The PCA included individuals screened here for *Trichomonas* plus other Rock Dove and domestic/feral individuals measured in Smith et al. (2022b). Using all morphological data available improves the reliability of hybrid status estimates for the samples from the three focal sites used here. Distance from the mean PC1 value was used to calculate a hybrid score for each individual (the absolute value of the difference from the mean PC1 value).

We next tested whether the body condition of individual pigeons is influenced by *Trichomonas* infection. The condition of an individual was estimated as the residual value of a linear regression of logged mass versus logged wing length (Santos et al., 2019). Using condition as the response variable, we applied a GLM with gaussian error structure, and *Trichomonas* infection status, site, sex, hybrid score and the interaction of hybrid score and infection status, as explanatory variables. Sex was included as males and females differ in their body shape, which influences the condition metric (Johnston, 1990). Models were run within R 4.2.1 (R Core Team, 2022) using the base *stats* package. Within R, data organisation and visualisation were achieved using *tidyverse* (Wickham et al., 2019). For model selection purposes we used the package *MuMIn* (Bartoń, K., 2022. *MuMIn*: multi-model inference – R package ver. 1.47.1. CRAN: The Comprehensive R Archive Network). We used the *dredge* function within *MuMIn* to test all possible models, using BIC as our information criterion (Schwarz, 1978), and using  $\Delta\text{BIC} > 2$  to indicate significant difference between models (Raftery, 1995). Where relevant, we used the traditional threshold of  $P < 0.05$  to represent a significant result.

## 2.6. Data availability

Parasite sequence data is available in GenBank under the accession numbers reported throughout this manuscript. Alignments,

data, and R code required to repeat analyses are publicly available at <https://data.mendeley.com/10.17632/nzmpnf9r5m.1>.

## 3. Results

### 3.1. *Trichomonas* prevalence in pigeons varies widely among published studies

We identified a total of 27 published studies that produced data fitting our selection criteria, covering 47 geographically different populations of *Columba livia* including those assessed as part of this study. Due to low sample size (two publications, three populations) undomesticated wild Rock Doves were included with feral pigeons as a category of 'free-living' *C. livia* for statistical analyses (Fig. 2 shows the Rock Dove and feral pigeon components of the 'free-living' category separately). *Trichomonas* prevalence varied from 1.6% to 78% across studies. Only seven previously published studies reported the *Trichomonas* strain: in 5/7 strain A was reported; in 6/7 strain B was reported. Two studies reported novel strains (Mohammed and Abdulwahed, 2019; Santos et al., 2019). Most studies come from Europe or Asia (39/47 populations) with sparse representation on other continents.

In Table 1, the best performing model suggested that variation in prevalence was primarily explained by pigeon type (free-living or not), with free-living pigeons having decreased prevalence of *Trichomonas* infection (estimate =  $-0.567 \pm 0.26$ ,  $P$ -value = 0.029). However two other well-performing models (with  $\Delta\text{BIC} < 2$  from the best model) were identified. These were the null model and the model that included temperature and type of pigeon. Temperature negatively correlated with prevalence, but not significantly (estimate =  $-0.199 \pm 0.13$ ,  $P$ -value = 0.136), while free-living pigeons again had lower infection prevalence (estimate =  $-0.6105 \pm 0.26$ ,  $P$ -value = 0.0175).

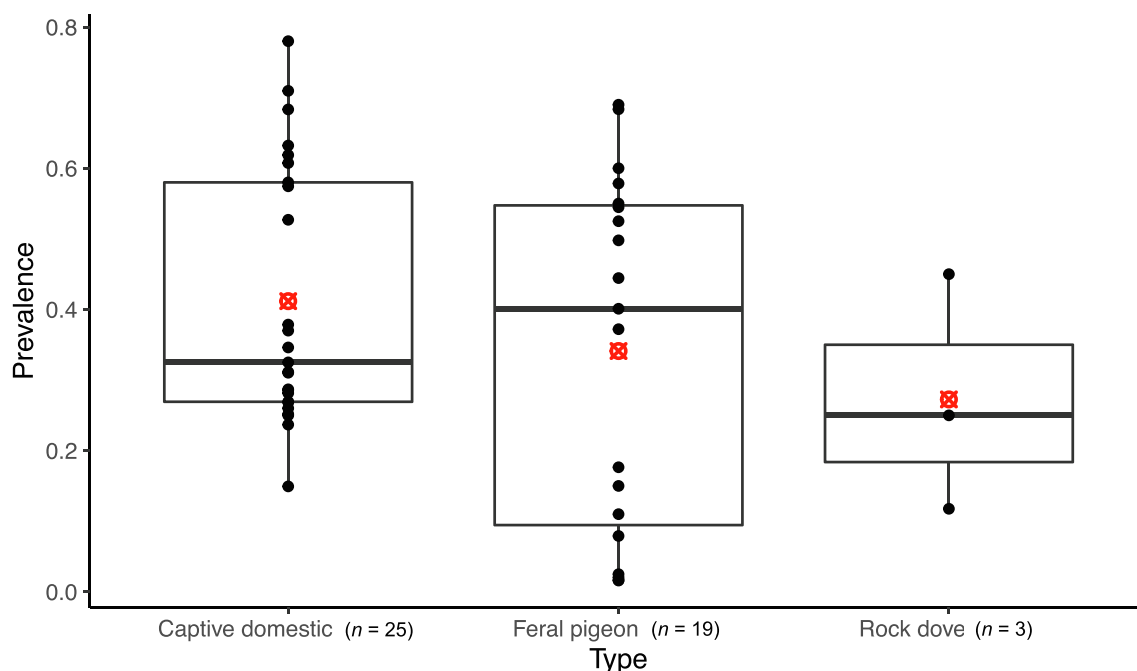
### 3.2. Correlates of infection status and impact of disease on condition are unclear

These analyses included only adult individuals with complete data on all morphological traits measured (113/120 individuals across all three sites). The two top models for infection status were identified (with  $\Delta\text{BIC} < 2$ ). The top model had sex as the explanatory variable where males had higher odds of being infected relative to females (estimate =  $0.9357 \pm 0.394$ ,  $P$ -value = 0.0174). However this model was not clearly better than the null model (Table 2).

Bird condition varied by site and sex (Table 3). Specifically, males had better condition than females; and relative to Uist birds, Oxford birds had significantly worse condition, with Orkney birds trending in the same direction but not significantly so (Table 4). Body condition was not explained by *Trichomonas* infection, hybrid score, or their interaction, however birds with infection do consistently show lower average condition across all three populations in the data (Supplementary Fig. S1).

### 3.3. Parasite prevalence and diversity differ between pigeon types

From the 120 birds caught in the UK, we obtained 59 positive PCRs for the *Trichomonas* ITS gene, giving an overall prevalence of 49.2%. Fifty of these (randomly selected) were sequenced, with 45 producing good quality, useable DNA sequences. We also gained good quality *Fe-hyd* gene sequences from all of the 12 individuals that were successfully amplified. Prevalence of *Trichomonas* infection (based on the proportion of samples with successful ITS gene amplification) was significantly lower in the Uist Rock Dove population (32.5%,  $n = 13/40$ ) than in the Oxford feral pigeons (62.5%,



**Fig. 2.** Prevalence values for *Trichomonas gallinae* reported for individual studies of captive domestic pigeon, feral pigeon, and Rock Dove populations. Mean prevalences are indicated with crosses. The highest 'Rock Dove' point represents the Orkney feral-wild hybrid population.

**Table 1**

Model selection table showing the models best explaining observed variation in disease prevalence among pigeon populations included in the global study.

Model	BIC	$\Delta$ BIC	Akaike's weight	Log-likelihood	df
<i>prevalence ~ pigeon type</i>	−11.116	0.000	0.268	11.333	3
<i>prevalence ~ 1 (null model)</i>	−10.365	0.751	0.184	9.033	2
<i>prevalence ~ temperature + pigeon type</i>	−9.479	1.637	0.118	12.440	4
<i>prevalence ~ urban location</i>	−8.726	2.390	0.081	10.138	3
<i>prevalence ~ pigeon type + urban location</i>	−8.206	2.911	0.063	11.803	4
<i>prevalence ~ temperature</i>	−7.908	3.208	0.054	9.729	3
<i>prevalence ~ precipitation</i>	−7.884	3.233	0.053	9.717	3
<i>prevalence ~ precipitation + pigeon type</i>	−7.689	3.428	0.048	11.545	4

BIC, Bayesian Information Criterion;  $\Delta$ BIC, the difference from the best model (top); df, degrees of freedom. Only the top seven models and the null model are shown for brevity.

**Table 2**

Model selection table showing models best explaining observed variation in infection status among individual pigeons from the three British populations.

Model	BIC	$\Delta$ BIC	Akaike's weight	Log-likelihood	df
<i>Infection status ~ sex</i>	160.269	0.000	0.470	−75.407	2
<i>infection status ~ 1 (null model)</i>	161.370	1.101	0.271	−78.321	1
<i>infection status ~ sex + site</i>	163.873	3.604	0.078	−72.482	4
<i>infection status ~ site</i>	164.445	4.176	0.058	−75.131	3
<i>Infection status ~ hybrid score + sex</i>	164.537	4.268	0.056	−75.177	4
<i>Infection status ~ hybrid score</i>	165.283	5.014	0.038	−77.914	3
<i>infection status ~ hybrid score + sex + site</i>	167.157	6.888	0.015	−71.760	5
<i>infection status ~ hybrid score + site</i>	167.213	6.944	0.015	−74.152	4

BIC, Bayesian Information Criterion;  $\Delta$ BIC, the difference from the best model (top); df, degrees of freedom.

**Table 3**

Model selection table showing five models best explaining observed variation in condition among individual pigeons from the three British populations.

Model	BIC	$\Delta$ BIC	Akaike's weight	Log-likelihood	df
<i>condition ~ sex + site</i>	−238.317	0.000	0.754	130.977	5
<i>condition ~ sex + site + infection status</i>	−234.519	3.798	0.113	131.442	6
<i>condition ~ hybrid score + sex + site</i>	−233.611	4.706	0.072	130.988	6
<i>condition ~ sex</i>	−230.602	7.715	0.016	122.392	3
<i>condition ~ site</i>	−230.193	8.124	0.013	124.551	4

BIC, Bayesian Information Criterion;  $\Delta$ BIC, the difference from the best model (top); df, degrees of freedom.

**Table 4**

Results as presented by summary() function of R of the best linear model explaining bird condition; condition ~ sex + site. R<sup>2</sup> denotes marginal value as applied in MuMIn.

Predictors	Estimates	Standard error	P-value
Intercept - Uist	0.002	0.015	0.892
Site - Orkney	-0.025	0.018	0.168
Site - Oxford	-0.075	0.018	<0.001
Sex - Male	0.054	0.015	<0.001
Sample size	113		
R <sup>2</sup>	0.218		

$n = 25/40$ ,  $P = 0.007$ ,  $\chi^2 = 6.065$ , degrees of freedom (df) = 1) but not in the Orkney hybrid population (52.5%,  $n = 21/40$ ,  $P = 0.0567$ ,  $\chi^2 = 2.506$ , df = 1). The Orkney and Oxford populations did not differ from one another in terms of the proportion of positive samples ( $P = 0.498$ ,  $\chi^2 = 0.4604$ , df = 1). All birds except three from Uist were molecularly sexed successfully. There was lower prevalence of *Trichomonas* infection in females compared with males in the Uist (3/18 females versus 10/19 males) and Orkney (3/13 versus 18/27) populations ( $P = 0.0004$ ,  $\chi^2 = 11.315$ , df = 1) but not in Oxford (13/19 versus 12/21,  $P = 0.6586$ ,  $\chi^2 = 0.167$ , df = 1) (Fig. 1E). Only one bird (a male from Orkney) showed clinical signs of infection (lesions around the oral area). We identified three distinct ITS sequences, two of which were identical to previously known strains (Table 5). These were Type C, found in Oxford feral pigeons (in 15 individuals), and Type A, found in all three populations in varying proportions (69% of infections for which a DNA sequence was generated in Orkney, 25% in Oxford and 44% in Uist). A novel ITS strain, named Ttx-RD (A/N OQ925392), was found in 31% (5/16) of the sequences from infected Orkney hybrids, 56% (5/9) of Uist Rock Dove sequences, and none of the Oxford feral pigeons. This novel strain had 99.71% identity to the 'GEO' *T. tenax*-like strain previously isolated from German Woodpigeons (A/N KX459459), and Turtle Doves from various locations in Europe (A/N MN587090). Phylogenetic analysis (Fig. 3A) showed that, in the alignment of 216 bp, the novel ITS strain Ttx-RD was most similar to the known strain GEO-TD, falling within the 'tenax-like' clade of strains that is composed of the widespread GEO sequence as well as the apparently less common GEO-TD and Ttl-TD sequences. Strain diversity differed by location (Uist, Orkney, Oxford) ( $P < 0.001$ ), but not by sex at any of the three locations (Supplementary Table S1), although it is important to note that sample sizes were small (see Supplementary Fig. S2).

We identified five *Fe-hyd* gene variants (Table 6), three of which were previously known. The strain A1.2 was isolated in one Uist and three Orkney birds. The strains C4 and A3 were isolated in four and two of the Oxford feral pigeons, respectively. The two new *Fe-hyd* region strains isolated were related to the known strain C1, and are hereafter known as C1-FP.1 (identified in one Oxford feral pigeon – 99.39% similar to *Fe-hyd* strain C1, A/N OQ927984) and C1-FP.2 (identified in one Oxford feral pigeon – 99.38% similar to *Fe-hyd* strain C1, A/N OQ911502). The phylogeny constructed for the *Fe-hyd* region (578 bp alignment) showed that the novel strains C1-FP.1 and C1-FP.2 were most closely related to C10/C11 and C8, respectively, falling within the clade containing Type A and Type C variants, and more specifically in the weakly supported Type C clade (Fig. 3B).

#### 4. Discussion

*Trichomonas* appears to be less prevalent in free-living pigeons compared with captive animals, but nevertheless represents a genetically diverse parasite that occurs at variable frequency, even at relatively small regional scales. Lineage diversity is currently underappreciated given the number of new lineages found here.

Surprisingly, among the three populations that we sampled, we did not find significant predictors of individual infection status, and found that condition was not significantly affected by infection status. We believe we have performed the first characterisation of *Trichomonas* prevalence and strain diversity in Rock Doves that have been genomically confirmed to be undomesticated.

*Trichomonas* infection varies substantially in populations of *C. livia* spread across Europe, North America, Asia and Africa. While no clear best model explaining this variation was identified, captive versus free-living (both feral and Rock Dove forms) emerged as an important variable, with higher prevalence in captive birds. This is perhaps not surprising given that domestic pigeons are often kept at very high densities (Johnston and Janiga, 1995). Urbanisation, temperature and precipitation did not explain infection prevalence, unlike in other avian host-parasite systems where these factors play a role (Evans et al., 2009; Holand et al., 2019). Many examples come from studies of avian malaria, a disease which requires an invertebrate vector for its transmission. Because abiotic variables strongly impact vector biology, e.g. the larval stage of mosquitos proliferate where rainwater accumulates (Trewin et al., 2019), and temperature-related delays in sporogonic development of malaria parasites can negatively affect their transmission (Platonova and Palinauskas, 2021), such relationships are expected. In contrast, *Trichomonas* has direct transmission among individuals, and therefore variables that mediate proximity among individuals at a local level, e.g. suitable feeding and drinking sites, may be more important in explaining variation.

Variation in parasite prevalence among feral and wild, undomesticated pigeons is supported by our regional analysis of three populations within the UK. The prevalence of *Trichomonas* infection in Oxford and Orkney falls within the expected range found in prior studies of domestic and feral pigeons, whereas prevalence in the Uist population of wild Rock Doves is lower than is usual in feral or domestic conspecifics (Alkharigiy et al., 2018; Santos et al., 2019). The only other study of *Trichomonas* infection in wild (but not genomically verified) Rock Doves was that of Greguric et al. (1986) in the Mediterranean that reported a prevalence half that of Uist's Rock Doves. Several decades separate that study from ours, and it relied on microscopy alone, a method that has reduced parasite detectability compared with the genetic methods available today (Nabwemyambo et al., 2017). Nevertheless, the low prevalence in both Mediterranean and Scottish Rock Dove populations provides support for undomesticated Rock Doves having lower *Trichomonas* prevalence than feral/domestic pigeons. Three non-mutually exclusive factors could explain lower prevalence in the Uist population, and potentially Rock Doves in general: geographical isolation, behavioural variation reducing opportunities for individuals to mingle, and genetically-based resistance to infection. Geographical isolation reduces opportunities for the spread of parasites and disease. Whilst both are insular, Uist is further away from large feral pigeon colonies than Orkney, and prevailing winds make feral pigeon colonisation from mainland Scotland more difficult. Reduced parasite infection on islands for vector-mediated parasites has been identified in some avian systems (Loiseau et al., 2017), but not others (Ishtiaq et al., 2010). With respect to directly transmitted parasites, there has been less research attention, but it has been shown that ectoparasite prevalence and diversity for Azorean Blackbirds (*Turdus merula*) is higher than on the mainland (Tomás et al., 2021).

Ecological and behavioural characteristics of Uist Rock Doves may contribute to their lower prevalence of *Trichomonas* infection. *Trichomonas* is transmitted through drinking water or at shared feeding sites, or through courtship behaviour or the feeding of offspring by adults (Marx et al., 2017). In urban and village contexts favoured by feral pigeons and hybrids, deliberate or inadvertent provision of food and water by humans acts to concentrate those

**Table 5**

Internal transcribed spacer (ITS) region strains identified from Sanger sequencing of 400 bp fragment. Ttx-RD is a novel strain, and strains A and C are previously known. Pigeons were sampled in three locations in the United Kingdom: Oxford, Orkney, and Uist.

Strain	Oxford	Orkney	Uist	TOTAL
Type C	15	0	0	15
Type A	5	11	4	20
Ttx-RD (novel strain)	0	5	5	10
TOTAL	20	16	9	45

essential sources, resulting in more opportunities for close proximity among individuals (Lawson et al., 2018). Feral pigeons form dense feeding flocks in streets, town squares, and on pavements (Johnston and Janiga, 1995). Rock Doves, at least in Britain, have lower direct dependence on humans for food and water, and form comparatively dispersed groups feeding on open land (Murton and Westwood, 1966). Opportunities for direct transfer of *Trichomonas* are therefore likely reduced in Rock Doves in a foraging context. Roosting behaviour also results in differences in local densities. Some individuals within the hybrid population we sampled in Orkney make use of an ancient 'doocot' (dovecote) together with modern farm buildings, which have limited airflow and can house up to 100 individuals in close proximity to one another. Such an environment would provide increased opportunity for disease transmission compared with the relatively well-ventilated caves and ruined buildings favoured by Rock Doves in Uist. Support for the role of transmission at concentrated roost sites also comes from the higher rates of *Trichomonas* infection seen in free-living domestic pigeons which roost in enclosed buildings, and captive pigeons sampled in pigeon lofts, compared with those roosting in lower density situations (Sansano-Maestre et al., 2009).

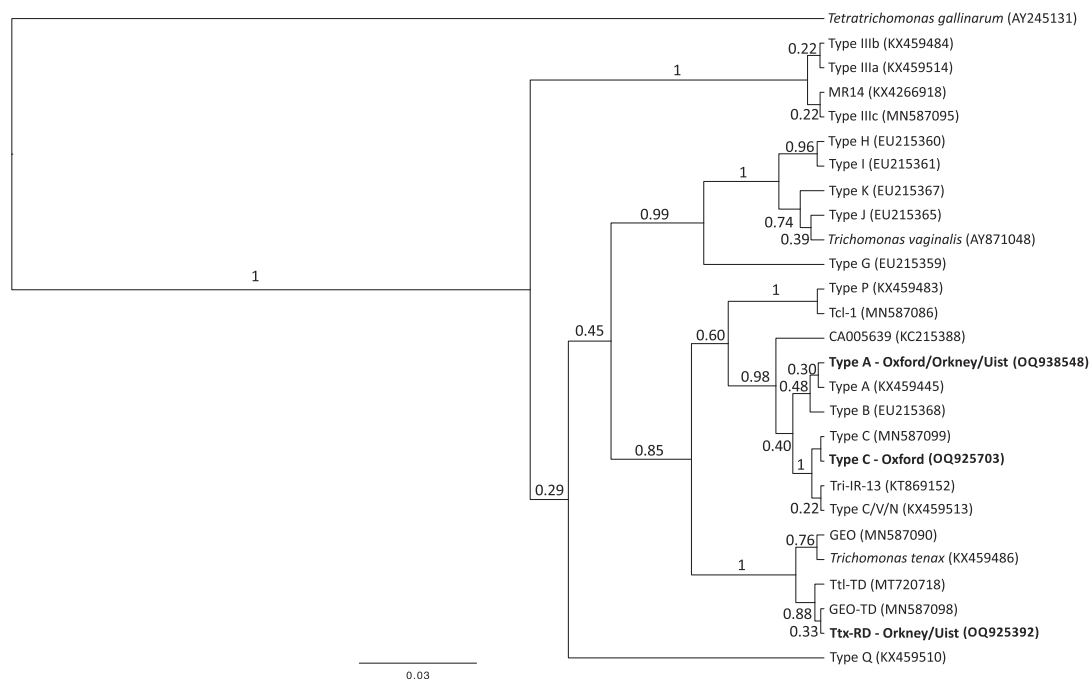
Rock Doves may be more resistant to infection due to genetic factors, and such resistance has been much discussed in relation to various wild relatives of different domestic animal species (Taberlet et al., 2008; Redford and Dudley, 2018; Smith et al., 2022a). For such an argument to hold true, this resistance would need to have been lost in the hybrid swarm in Orkney, which has prevalence similar to that of the Oxford population of feral pigeons. Further research is needed to identify the mechanism responsible. Disease resistance in birds can be due to functional changes in gene expression (Bonneau et al., 2011), which could happen during domestication or feralisation, and therefore explain different immunity or disease tolerance among the types of *C. livia*. One notable result is that, in the Uist and Orkney populations, females had significantly lower prevalence of infection than males. This is not the case in the Oxford population of feral pigeons and has not been seen in comparable studies of feral or domestic pigeons (Santos et al., 2019). The biology of the wild Rock Dove is poorly understood (Johnston et al., 1988) so it is unknown if ecological or immuno-genetic factors could underlie differences seen, or indeed the impact of domestication on sexual selection, and subsequent variation in resource allocation between males and females. More broadly, there is mixed evidence for both male- and female-biased parasite infection among birds (van Oers et al., 2010). Further work across multiple populations, including genomic analysis of immune genes in each sex, could help to explain the observed patterns in our study populations. It is worth noting that, because sampling at each site was not possible simultaneously, sampling date may have impacted parasite prevalence and diversity. Although all of our sampling occurred within a short period, studies of blood parasites have shown that drastic changes in infection status can occur throughout the breeding season (Szöllösi et al., 2016). In *C. livia*, where the breeding season is especially prolonged and where there is extensive individual variation in both the time of breeding and the number of broods hatched per year, it is likely that the birds we sampled were at various stages of breeding,

reducing the impact of breeding status on our results. Nevertheless, it would be interesting to characterise temporal variation in parasite diversity and prevalence in the future, especially within individual birds.

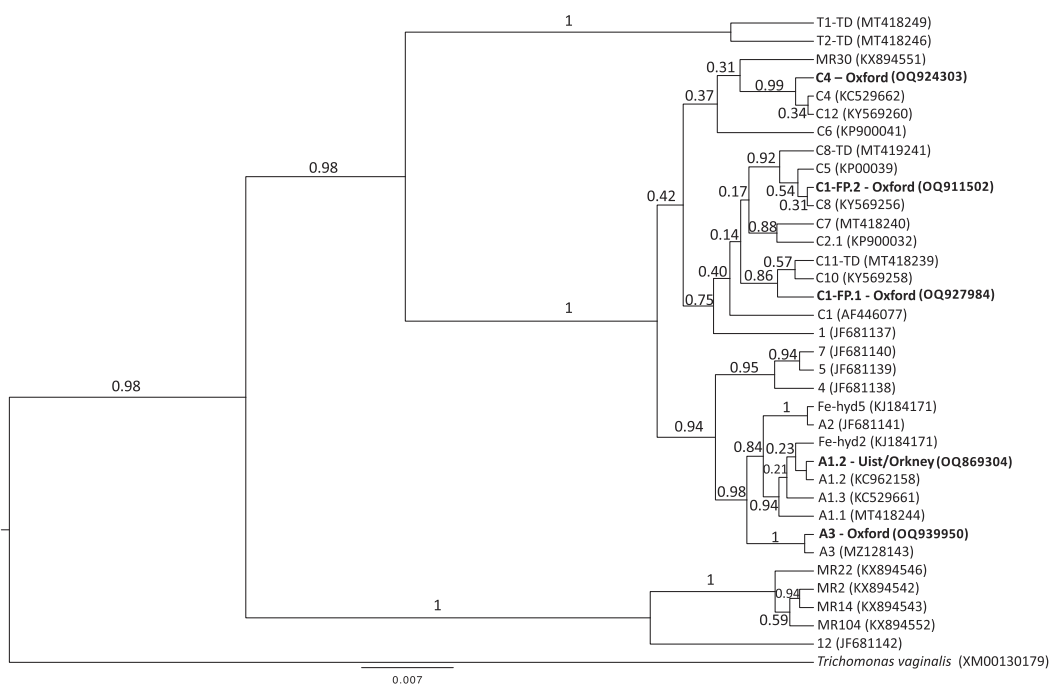
Unlike Santos et al. (2019), we did not find a significant decrease in condition in *Trichomonas*-infected birds, although our data shows they tended to have lower mean conditions. This could be due to sample size limitations. The apparent lack of impact of infection on condition might also be due to a lack of nestling or recently fledged juvenile birds in the sample; summer or autumn sampling would be required to assess this possibility. It is known that adult birds are often less impacted by disease than younger individuals, such as Turkeys (*Meleagris gallopavo*) infected with *Mycoplasma* (Efsa Panel on Animal Health et al., 2017). Very strong impacts of *Trichomonas* infection on body condition were observed in a prior study of free-living domestic pigeons in Spain and Portugal (Santos et al., 2019) but not in our results in either feral, wild or hybrid populations. This might be because the pigeons in the previous study might be relatively immunologically naïve as they were sourced from captive domestic stock that could have been kept almost parasite-free for multiple generations. Whilst the strain we identified may differ from the epidemic strain A at other regions, the identical ITS region is worrying and further research is needed to assess its risk, especially with respect to chicks of the endangered wild Rock Dove. The remaining Rock Dove populations are likely to come into increasing levels of contact with the wider global population of feral pigeons in the coming decades (Johnston et al., 1988). The relative susceptibility of wild Rock Doves to diseases introduced by their feral domestic conspecifics might contribute to their prospects of extinction via hybridisation in the future (Johnston and Janiga, 1995).

Despite similar prevalence of *Trichomonas* between Orkney and Oxford compared with the Uist population, strain diversity at both sequence targets was much more similar between Orkney and Uist compared with Oxford and there was a significant non-random association between site and numbers of birds identified as being infected with different ITS strains. ITS strain Type C, common in Oxford pigeons but not recorded in the other two populations, is present across Europe and is found in various pigeons, doves and birds of prey (Thomas et al., 2022). It has also been isolated from the domestic Budgerigar (*Melopsittacus undulatus*) in Iran (A/N KT869151). Type A, found in all three populations but especially prevalent in the hybridising population in Orkney, has been implicated as a causative factor in the declines of British Greenfinches (Alrefaei et al., 2019b). ITS strains that are more similar to *T. tenax* than *T. gallinae*, such as the novel ITS strain Ttx-RD, have been identified in Europe, Asia and Africa previously (in Woodpigeons, Turtle Doves and Stock Doves), but apparently not in *C. livia* until now (Marx et al., 2017; Thomas et al., 2022). The *Fe-hyd* strains that we identified follow a similar pattern to the ITS strains; of those samples successfully sequenced, the Orkney and Uist birds had the same sequence (A1.2). This strain is commonly isolated in avian epidemiological studies and has been found in birds of prey, pigeons and doves, and passerines (Thomas et al., 2022). Of the strains identified in Oxford pigeons, C4 is commonly found in raptors and pigeons (Thomas et al., 2022) and strain A3 has been

A



B



**Fig. 3.** Phylogenetic trees showing the relationships between different *Trichomonas* strains. Maximum clade credibility tree of the (A) internal transcribed spacer (ITS) and (B) *Fe-hyd* gene regions of known sequences/strains for *Trichomonas gallinae*, as inferred by BEAST. Sequences isolated from pigeons during our study are presented in bold. All sequences are given an example GenBank accession number. Branch support is indicated by Bayesian posterior probabilities.

previously identified in a Brahminy Kite (*Haliastur indus*) and domestic Canary (*Serinus canaria*) sampled in Japan (Chou et al., 2022). The other two Oxford individuals each had a novel *Fe-hyd* strain which was closely related to the known C1 strain, which has been isolated from *C. livia* in the USA. It is clear that much of the diversity of these parasites remains to be characterised. As we only sequenced the ITS and *Fe-hyd* gene regions, there may

have been differences at other functional genes. Such differences may underlie the differences in prevalence between Uist and Orkney, despite the two populations apparently having similar *Trichomonas* strains. Of course, the difference could also be explained by behavioural or environmental differences discussed previously, and by geographical/latitudinal differences in the presence of the particular strains involved. Whilst sex differences in infection by

**Table 6**

*Fe-hyd* gene region strains identified from Sanger sequencing of 900 bp fragment. C1-FP.1 and C1-FP.2 are novel strains. A1.2, A3 and C4 are previously known. Pigeons were sampled in three locations in the United Kingdom: Oxford, Orkney, and Uist.

Strain	Oxford	Orkney	Uist	TOTAL
A1.2	0	3	1	4
A3	2	0	0	2
C4	4	0	0	4
C1-FP.1 (novel strain)	1	0	0	1
C1-FP.2 (novel strain)	1	0	0	1
TOTAL	8	3	1	12

parasite lineages have been detected in certain case studies (Reimchen and Nosil, 2001; Krasnov et al., 2005), we did not detect such differences within the subset of our birds for which an ITS sequence could be generated. This may be due to the small sample size (see Supplementary Fig. S2), and more work on this topic would be valuable.

The UK Rock Dove – feral pigeon case study offers an excellent opportunity to explore the dynamics of hybridisation and disease transfer, with clear variation in *Trichomonas* infection prevalence and diversity among the different types of *Columba livia*. To further develop the system, sampling from multiple populations across the range of the feral pigeon and Rock Dove, including their hybrid zones in e.g. Caithness, Orkney and Shetland, would allow us to tease apart the different factors of geography, environmental variables, and type of pigeon. This will be vital in characterising the interaction between hybrid status and parasite infection. It would also be beneficial to sample the full range of age categories of the birds. Sampling juveniles, which may be more impacted by *Trichomonas* infection than older birds, might also allow us to better quantify the impacts of infection on individuals. Coupled with this, it will be possible to assess individual variation in reproductive success, behaviour, longevity and gain/loss of infections through mark-recapture studies and GPS tracking. This will facilitate a thorough characterisation of fine-scale variation in the role *Trichomonas* plays in populations of differing wild-feral ancestry proportions.

This study is, to our knowledge, the first to genetically characterise *Trichomonas* infection in the wild form of *Columba livia*. The wild Rock Dove and wild-feral hybrid populations that we sampled exhibit different *Trichomonas* strain diversity from their more abundant feral conspecifics. The Rock Doves also have lower infection prevalence. Increasing contact between feral and wild pigeons will increase the transmission of parasitic diseases among populations, likely contributing to the decline of wild Rock Doves. *Columba livia* has immense economic and ecological importance through disease transmission to poultry and other captive and wild birds – and is perhaps the most capable wild avian species in terms of facilitating disease transmission between wild and captive populations. Much of the work studying *Trichomonas* infection that happens in birds today focusses on declining native species (Greenfinches, Turtle Doves, Pink Pigeons). Improved focus on the most globally important transmitter of the parasite would contribute to understanding its history and potential future impacts on both declining native species and captive birds. More broadly, the study of globally superabundant synanthropic commensal species (e.g. Brown Rats (*R. norvegicus*), feral cats (*F. catus*), and feral dogs (*C. familiaris*)) and their diseases is becoming more and more important in an increasingly homogenised global ecosystem.

# Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2023.06.005>.

# References

- Alkharig, F.A., El Naas, A.S., El Maghrbi, A.A., 2018. Survey of parasites in domestic pigeons (*Columba livia*) in Tripoli, Libya. *Open Vet J* 8, 360–366. <https://doi.org/10.4314/ovj.v8i4.2>.
- Alrefaei, A.F., Gerhold, R.W., Nader, J.L., Bell, D.J., Tyler, K.M., 2019a. Improved subtyping affords better discrimination of *Trichomonas gallinae* strains and suggests hybrid lineages. *Infect. Genet. Evol.* 73, 234–241. <https://doi.org/10.1016/j.meegid.2019.05.007>.
- Alrefaei, A.F., Low, R., Hall, N., Jardim, R., Dávila, A., Gerhold, R., John, S., Steinbiss, S., Cunningham, A.A., Lawson, B., Bell, D., Tyler, K., 2019b. Multilocus Analysis Resolves the European Finch Epidemic Strain of *Trichomonas gallinae* and Suggests Introgression from Divergent Trichomonads. *Genome Biol. Evol.* 11, 2391–2402. <https://doi.org/10.1093/gbe/evz164>.
- Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402. <https://doi.org/10.1093/nar/25.17.3389>.
- Amin, A., Bilic, I., Liebhart, D., Hess, M., 2014. Trichomonads in birds – a review. *Parasitology* 141, 733–747. <https://doi.org/10.1017/S0031182013002096>.
- Baker, K., 2016. Identification of European Non-passerines. British Trust for Ornithology, Thetford.
- Beaumont, M., Barratt, E.M., Gottelli, D., Kitchener, A.C., Daniels, M.J., Pritchard, J.K., Bruford, M.W., 2001. Genetic diversity and introgression in the Scottish wildcat. *Mol. Ecol.* 10, 319–336. <https://doi.org/10.1046/j.1365-294x.2001.01196.x>.
- Bonneaud, C., Balenger, S.L., Russell, A.F., Zhang, J., Hill, G.E., Edwards, S.V., 2011. Rapid evolution of disease resistance is accompanied by functional changes in gene expression in a wild bird. *PNAS* 108, 7866–7871. <https://doi.org/10.1073/pnas.1018580108>.
- Borji, H., Razmi, G.H., Movassaghi, A.H., Moghaddas, E., Azad, M., 2011. Prevalence and pathological lesion of *Trichomonas gallinae* in pigeons of Iran. *J. Parasit. Dis.* 35, 186–189. <https://doi.org/10.1007/s12639-011-0047-2>.
- Brooks, M.E., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Machler, M., Bolker, B.M., 2017. glmmTMB balances speed and

- flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* 9, 378–400.
- Bunbury, N., Jones, C.G., Greenwood, A.G., Bell, D.J., 2008. Epidemiology and conservation implications of *Trichomonas gallinae* infection in the endangered Mauritian pink pigeon. *Biol. Conserv.* 141, 153–161. <https://doi.org/10.1016/j.biocon.2007.09.008>.
- Capoccia, S., Boyle, C., Darnell, T., 2018. Loved or loathed, feral pigeons as subjects in ecological and social research. *J. Urban Ecol.* 4, 1, juy024. <https://doi.org/10.1093/jue/juy024>.
- Chi, J.F., Lawson, B., Durrant, C., Beckmann, K., John, S., Alrefaei, A.F., Kirkbride, K., Bell, D.J., Cunningham, A.A., Tyler, K.M., 2013. The finch epidemic strain of *Trichomonas gallinae* is predominant in British non-passerines. *Parasitology* 140, 1234–1245. <https://doi.org/10.1017/S0031182013000930>.
- Chou, S., Hadano, S., Kojima, A., Yorisaki, M., Yasuda, M., Ike, K., Tokiwa, T., 2022. Genetic characterization of *Trichomonas gallinae* (Rivolta, 1878) in companion birds in Japan and the genotypical relationship in the Asia region. *J. Microbiol. Immunol. Infect.* 55, 527–534. <https://doi.org/10.1016/j.jmii.2021.05.010>.
- Clark, N.J., Seddon, J.M., Šlapeta, J., Wells, K., 2018. Parasite spread at the domestic animal – wildlife interface: anthropogenic habitat use, phylogeny and body mass drive risk of cat and dog flea (*Ctenocephalides* spp.) infestation in wild mammals. *Parasit. Vectors* 11, 8. <https://doi.org/10.1186/s13071-017-2564-z>.
- Daniels, M.J., Balharry, D., Hirst, D., Kitchener, A.C., Aspinall, R.J., 1998. Morphological and pelage characteristics of wild living cats in Scotland: implications for defining the ‘wildcat’. *J. Zool.* 244, 231–247.
- Daniels, T.J., Bekoff, M., 1989. Feralization: The making of wild domestic animals. *Behav. Proc.* 19, 79–94. [https://doi.org/10.1016/0376-6357\(89\)90032-6](https://doi.org/10.1016/0376-6357(89)90032-6).
- Daniels, M.J., Golder, M.C., Jarrett, O., MacDonald, D.W., 1999. Feline viruses in wildcats from Scotland. *J. Wildl. Dis.* 35, 121–124. <https://doi.org/10.7589/0090-3558-35.1.121>.
- Doherty, T.S., Dickman, C.R., Glen, A.S., Newsome, T.M., Nimmo, D.G., Ritchie, E.G., Vanak, A.T., Wirsing, A.J., 2017. The global impacts of domestic dogs on threatened vertebrates. *Biol. Conserv.* 210, 56–59. <https://doi.org/10.1016/j.biocon.2017.04.007>.
- Douma, J.C., Weedon, J.T., 2019. Analysing continuous proportions in ecology and evolution: A practical introduction to beta and Dirichlet regression. *Methods Ecol. Evol.* 10, 1412–1430. <https://doi.org/10.1111/2041-210X.13234>.
- Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973. <https://doi.org/10.1093/molbev/mss075>.
- Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinform.* 5, 113. <https://doi.org/10.1186/1471-2105-5-113>.
- Efsa Panel on Animal Health, Welfare, More, S., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., Edwards, S., Garin-Bastuji, B., Good, M., Gortázar Schmidt, C., Michel, V., Miranda, M.A., Nielsen, S.S., Raj, M., Sihvonen, L., Spoolder, H., Stegeman, J.A., Thulke, H.-H., Velarde, A., Willeberg, P., Winckler, C., Baldinelli, F., Broglia, A., Dhollander, S., Beltrán-Beck, B., Kohnle, L., Bicoût, D., 2017. Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): avian mycoplasmosis (*Mycoplasma gallisepticum*, *M. meleagridis*). *EFSA Journal* 15, e04953. doi: <https://doi.org/10.2903/j.efsa.2017.4953>.
- Evans, K.L., Gaston, K.J., Sharp, S.P., McGowan, A., Simeoni, M., Hatchwell, B.J., 2009. Effects of Urbanisation on Disease Prevalence and Age Structure in Blackbird *Turdus merula* Populations. *Oikos* 118, 774–782.
- Ferenczi, M., Beckmann, C., Warner, S., Loyn, R., O’Riley, K., Wang, X., Klaassen, M., 2016. Avian influenza infection dynamics under variable climatic conditions, viral prevalence is rainfall driven in waterfowl from temperate, south-east Australia. *Vet. Res.* 47, 23. <https://doi.org/10.1186/s13567-016-0308-2>.
- Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* 37, 4302–4315. <https://doi.org/10.1002/joc.5086>.
- Gortázar, C., Diez-Delgado, I., Barasona, J.A., Vicente, J., De La Fuente, J., Boadella, M., 2015. The Wild Side of Disease Control at the Wildlife-Livestock-Human Interface: A Review. *Front. Vet. Sci.* 1, 27. <https://doi.org/10.3389/fvets.2014.00027>.
- 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. <https://doi.org/10.1016/j.vetpar.2010.04.015>.
- Greguric, J., Jercic, J., Muzinic, J., Szeleszczuk, P., Pecaric, A., 1986. The influence of the ecological environment on the incidence of *Trichomonas gallinae* in pigeons. *Vet. Glas.* 40, 657–660.
- Höfle, U., Gortázar, C., Ortiz, J.A., Knispel, B., Kaleta, E.F., 2004. Outbreak of trichomoniasis in a woodpigeon (*Columba palumbus*) wintering roost. *European J. Wildlife Res.* 50, 73–77. <https://doi.org/10.1007/s10344-004-0043-2>.
- Holand, H., Jensen, H., Kvalnes, T., Tufto, J., Pärn, H., Sæther, B.E., Ringsby, T.H., 2019. Parasite prevalence increases with temperature in an avian metapopulation in northern Norway. *Parasitology* 146, 1030–1035. <https://doi.org/10.1017/S0031182019000337>.
- Ishtiaq, F., Clegg, S.M., Phillimore, A.B., Black, R.A., Owens, I.P.F., Sheldon, B.C., 2010. Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands. *J. Biogeography* 37, 120–132. <https://doi.org/10.1111/j.1365-2699.2009.02189.x>.
- Johnston, R.F., 1990. Variation in Size and Shape in Pigeons, *Columba livia*. *Wilson Bull.* 102, 213–225.
- Johnston, R., Causey, D., Johnson, S., 1988. European Populations of the Rock Dove *Columba livia* and Genotypic Extinction. *Am. Midl. Nat.* 120, 1–10. <https://doi.org/10.2307/2425881>.
- Johnston, R.F., Janiga, M., 1995. *Feral pigeons*. Oxford University Press, New York, Oxford.
- Kalyanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermini, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589. <https://doi.org/10.1038/nmeth.4285>.
- Khan, S.U., Gurley, E.S., Gerloff, N., Rahman, M.Z., Simpson, N., Rahman, M., Haider, N., Chowdhury, S., Balish, A., Zaman, R.U., Nasreen, S., Chandra Das, B., Azziz-Baumgartner, E., Sturm-Ramirez, K., Davis, C.T., Donis, R.O., Luby, S.P., 2018. Avian influenza surveillance in domestic waterfowl and environment of live bird markets in Bangladesh, 2007–2012. *Sci. Rep.* 8, 9396. <https://doi.org/10.1038/s41598-018-27515-w>.
- Krasnov, B.R., Morand, S., Hawlena, H., Khokhlova, I.S., Shenbrot, G.I., 2005. Sex-biased parasitism, seasonality and sexual size dimorphism in desert rodents. *Oecologia* 146, 209–217. <https://doi.org/10.1007/s00442-005-0189-y>.
- Lawson, B., Robinson, R.A., Colville, K.M., Peck, K.M., Chantrey, J., Pennycott, T.W., Simpson, V.R., Toms, M.P., Cunningham, A.A., 2012. The emergence and spread of finch trichomonosis in the British Isles. *Philos. Trans. R. Soc. B* 367, 2852–2863. <https://doi.org/10.1098/rstb.2012.0130>.
- Lawson, B., Robinson, R.A., Toms, M.P., Risely, K., MacDonald, S., Cunningham, A.A., 2018. Health hazards to wild birds and risk factors associated with anthropogenic food provisioning. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 373. <https://doi.org/10.1098/rstb.2017.0091>.
- Lennon, R.J., Dunn, J.C., Stockdale, J.E., Goodman, S.J., Morris, A.J., Hamer, K.C., 2013. Trichomonad parasite infection in four species of Columbidae in the UK. *Parasitology* 140, 1368–1376. <https://doi.org/10.1017/S0031182013000887>.
- Liang, S.-J., Chen, M.-X., Gao, C.-Q., Yan, H.-C., Zhang, G.-L., Wang, X.-Q., 2019. Sex identification of pigeons using polymerase chain reaction analysis with simple DNA extraction. *Avian Biol. Res.* 12, 45–48. <https://doi.org/10.1177/1758155919832141>.
- Loiseau, C., Melo, M., Lobato, E., Beadell, J.S., Fleischer, R.C., Reis, S., Doutrelant, C., Covas, R., 2017. Insularity effects on the assemblage of the blood parasite community of the birds from the Gulf of Guinea. *J. Biogeogr.* 44, 2607–2617. <https://doi.org/10.1111/jbi.13060>.
- Marx, M., Reiner, G., Willems, H., Rocha, G., Hillerich, K., Masello, J.F., Mayr, S.L., Moussa, S., Dunn, J.C., Thomas, R.C., Goodman, S.J., Hamer, K.C., Metzger, B., Cecere, J.G., Spina, F., Koschkar, S., Calderón, L., Romeike, T., Quillfeldt, P., 2017. High prevalence of *Trichomonas gallinae* in wild columbids across western and southern Europe. *Parasit. Vectors* 10, 242–242. doi: 10.1186/s13071-017-2170-0.
- McBurney, S., Kelly-Clark, W.K., Forzán, M.J., Lawson, B., Tyler, K.M., Greenwood, S.J., 2015. Molecular characterization of *Trichomonas gallinae* isolates recovered from the Canadian Maritime provinces’ wild avifauna reveals the presence of the genotype responsible for the European finch trichomonosis epidemic and additional strains. *Parasitology* 142, 1053–1062. <https://doi.org/10.1017/S0031182015000281>.
- Meredith, A., Bacon, A., Allan, B., Kitchener, A., Senn, H., Brooks, S., Kortland, K., Hetherington, D., Davies, S., 2018. Domestic cat neutering to preserve the Scottish wildcat. *Vet. Rec.* 183, 27–28. <https://doi.org/10.1136/vr.k2905>.
- Mohammed, F.A., Abdulwahed, F.A., 2019. Isolation and Characterization of Novel *Trichomonas gallinae* Ribotypes Infecting Domestic and Wild Birds in Riyadh, Saudi Arabia. *Avian Dis.* 64, 130–134. <https://doi.org/10.1637/0005-2086-64.2.130>.
- Murton, R., Westwood, N., 1966. The foods of the rock dove and feral pigeon. *Bird Study* 13, 130–146.
- Nabwewayambo, S., Kakaïre, O., Sowinski, S., Okeng, A., Ojiambo, H., Kimeze, J., Najjingo, I., Bwanga, F., 2017. Very low sensitivity of wet mount microscopy compared to PCR against culture in the diagnosis of vaginal trichomoniasis in Uganda: a cross sectional study. *BMC. Res. Notes* 10, 259. <https://doi.org/10.1186/s13104-017-2581-1>.
- Platonova, E., Palinauskas, V., 2021. The Impact of Temperature on the Sporogonic Development of the Tropical Avian Malaria Parasite *Plasmodium relictum* (Genetic Lineage pGRW4) in *Culex pipiens* Form *molestus* Mosquitoes. *Microorganisms* 9, 2240.
- Quillfeldt, P., Schumm, Y.R., Marek, C., Mader, V., Fischer, D., Marx, M., 2018. Prevalence and genotyping of *Trichomonas* infections in wild birds in central Germany. *PLoS One* 13, e0200798.
- R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Raftery, A.E., 1995. Bayesian Model Selection in Social Research. *Sociolog. Methodol.* 25, 111–163. <https://doi.org/10.2307/271063>.
- Randi, E., 2008. Detecting hybridization between wild species and their domesticated relatives. *Mol. Ecol.* 17, 285–293. <https://doi.org/10.1111/j.1365-294X.2007.03417.x>.
- Redford, K., Dudley, N., 2018. Why should we save the wild relatives of domesticated animals? *Oryx* 52, 397–398. <https://doi.org/10.1017/S0030605318000601>.
- Reimchen, T.E., Nosil, P., 2001. Ecological causes of sex-biased parasitism in threespine stickleback. *Biological J. Linnean Soc.* 73, 51–63. <https://doi.org/10.1111/j.1095-8312.2001.tb01346.x>.
- Rhymer, J.M., Simberloff, D., 1996. Extinction by Hybridization and Introgression. *Ann. Rev. Ecol. Systemat.* 27, 83–109. <https://doi.org/10.1146/annurev.ecolsys.27.1.83>.

- Richard, W.G., Michael, J.Y., Autumn, J.S., Elissa, O., William, M., Jeff, D.C., John, R.F., 2008. Molecular Characterization of the *Trichomonas gallinae* Morphologic Complex in the United States. *J. Parasitol.* 94, 1335–1341. <https://doi.org/10.1645/GE-1585.1>.
- Robinson, R.A., Lawson, B., Toms, M.P., Peck, K.M., Kirkwood, J.K., Chantrey, J., Clatworthy, I.R., Evans, A.D., Hughes, L.A., Hutchinson, O.C., John, S.K., Pennycott, T.W., Perkins, M.W., Rowley, P.S., Simpson, V.R., Tyler, K.M., Cunningham, A.A., 2010. Emerging Infectious Disease Leads to Rapid Population Declines of Common British Birds. *PLoS One* 5, e12215.
- Rossi, L., Tizzani, P., Rambozzi, L., Moroni, B., Meneguz, P.G., 2019. Sanitary Emergencies at the Wild/Domestic Caprines Interface in Europe. *Animals (Basel)* 9, 922. <https://doi.org/10.3390/ani9110922>.
- Sansano-Maestre, J., Garijo-Toledo, M.M., Gómez-Muñoz, M.T., 2009. Prevalence and genotyping of *Trichomonas gallinae* in pigeons and birds of prey. *Avian Pathol.* 38, 201–207. <https://doi.org/10.1080/03079450902912135>.
- Santos, N., Jambas, J., Monteiro, A., Amaral, J., Martins, N., Garcia, J., Fernández, A.M., Tyler, K.M., Almeida, T., Abrantes, J., Esteves, P.J., 2019. *Trichomonas* Infection in a Community of Free-Ranging Domestic and Wild Columbiformes and Bonelli's Eagle (*Aquila fasciata*). *Front Vet Sci* 6, 148. <https://doi.org/10.3389/fvets.2019.00148>.
- Schwarz, G., 1978. Estimating the Dimension of a Model. *Annals Statistics* 6 (461–464), 464.
- Senn, H.V., Ghazali, M., Kaden, J., Barclay, D., Harrower, B., Campbell, R.D., Macdonald, D.W., Kitchener, A.C., 2019. Distinguishing the victim from the threat: SNP-based methods reveal the extent of introgressive hybridization between wildcats and domestic cats in Scotland and inform future in situ and ex situ management options for species restoration. *Evol App* 12, 399–414. <https://doi.org/10.1111/eva.12720>.
- Shapiro, M.D., Domyan, E.T., 2013. Domestic pigeons. *Curr. Biol.* 23, 302–303. <https://doi.org/10.1016/j.cub.2013.01.063>.
- Smith, W.J., 2023. The undomesticated Rock Dove in Britain and the Isle of Man. *British Birds* 116, 70–76.
- Smith, W.J., Quilodrán, C.S., Jezierski, M.T., Sendell-Price, A.T., Clegg, S.M., 2022a. The wild ancestors of domestic animals as a neglected and threatened component of biodiversity. *Conserv. Biol.* 36, e13867.
- Smith, W.J., Sendell-Price, A.T., Fayet, A.L., Schweizer, T.M., Jezierski, M.T., van de Kerkhof, C., Sheldon, B.C., Ruegg, K.C., Kelly, S., Turnbull, L.A., Clegg, S.M., 2022b. Limited domestic introgression in a final refuge of the wild pigeon. *iScience* 25, <https://doi.org/10.1016/j.isci.2022.104620> 104620.
- Stockdale, J.E., Dunn, J.C., Goodman, S.J., Morris, A.J., Sheehan, D.K., Grice, P.V., Hamer, K.C., 2015. The protozoan parasite *Trichomonas gallinae* causes adult and nestling mortality in a declining population of European Turtle Doves, *Streptopelia turtur*. *Parasitology* 142, 490–498. <https://doi.org/10.1017/S0031182014001474>.
- Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A., 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol.* 4, vey016. <https://doi.org/10.1093/ve/vey016>.
- Szöllösi, E., Garamszegi, L.Z., Hegyi, G., Laczi, M., Rosivall, B., Török, J., 2016. *Haemoproteus* infection status of collared flycatcher males changes within a breeding season. *Parasitol. Res.* 115, 4663–4672. <https://doi.org/10.1007/s00436-016-5258-0>.
- Taberlet, P., Valentini, A., Rezaei, H.R., Naderi, S., Pompanon, F., Negrini, R., Ajmone-Marsan, P., 2008. Are cattle, sheep, and goats endangered species? *Mol. Ecol.* 17, 275–284. <https://doi.org/10.1111/j.1365-294X.2007.03475.x>.
- Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* 38, 3022–3027. <https://doi.org/10.1093/molbev/msab120>.
- Thomas, R.C., Dunn, J.C., Dawson, D.A., Hipperson, H., Horsburgh, G.J., Morris, A.J., Orsman, C., Mallord, J., Grice, P.V., Hamer, K.C., Eraud, C., Hervé, L., Goodman, S. J., 2022. Assessing rates of parasite coinfection and spatiotemporal strain variation via metabarcoding: Insights for the conservation of European turtle doves *Streptopelia turtur*. *Mol. Ecol.* 31, 2730–2751. <https://doi.org/10.1111/mec.16421>.
- Tiesmeyer, A., Ramos, L., Manuel Lucas, J., Steyer, K., Alves, P.C., Astaras, C., Brix, M., Cragnolini, M., Domokos, C., Hegyi, Z., Janssen, R., Kitchener, A.C., Lambinet, C., Mestdagh, X., Migli, D., Monterroso, P., Mulder, J.L., Schockert, V., Youlatos, D., Pfenninger, M., Nowak, C., 2020. Range-wide patterns of human-mediated hybridisation in European wildcats. *Conserv. Genet.* 21, 247–260. <https://doi.org/10.1007/s10592-019-01247-4>.
- Tomás, A., Pereira da Fonseca, I., Valkenburg, T., Rebelo, M.T., 2021. Louse flies in Azorean and mainland populations of four Passeriformes species: A new perspective to parasite Island syndromes. *Int J Parasitol: Parasites Wildlife* 14, 33–40. <https://doi.org/10.1016/j.ijppaw.2020.12.004>.
- Trewin, B.J., Darbro, J.M., Zalucki, M.P., Jansen, C.C., Schellhorn, N.A., Devine, G.J., 2019. Life on the margin: Rainwater tanks facilitate overwintering of the dengue vector, *Aedes aegypti*, in a sub-tropical climate. *PLoS One* 14, e0211167.
- van de Bildt, M.W.G., Kuiken, T., Visee, A.M., Lema, S., Fitzjohn, T.R., Osterhaus, A.D. M.E., 2002. Distemper outbreak and its effect on African wild dog conservation. *Emerg. Infect. Dis.* 8, 211–213. <https://doi.org/10.3201/eid0802.010314>.
- van Oers, K., Richardson, D.S., Sæther, S.A., Komdeur, J., 2010. Reduced blood parasite prevalence with age in the Seychelles Warbler: selective mortality or suppression of infection? *J. Ornithol.* 151, 69–77. <https://doi.org/10.1007/s10336-009-0427-x>.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the Tidyverse. *J Open Source Software* 4, 1686. <https://doi.org/10.21105/joss.01686>.
- Williams, R.B., 2005. Avian malaria: clinical and chemical pathology of *Plasmodium gallinaceum* in the domesticated fowl *Gallus gallus*. *Avian Pathol.* 34, 29–47. <https://doi.org/10.1080/030794504000025430>.
- Willoughby, D.H., Bickford, A.A., Charlton, B.R., Cooper, G.L., 1995. Esophageal Trichomoniasis in Chickens. *Avian Dis.* 39, 919–924. <https://doi.org/10.2307/1592434>.
- Witzenberger, K.A., Hochkirch, A., 2014. The Genetic Integrity of the Ex Situ Population of the European Wildcat (*Felis silvestris silvestris*) Is Seriously Threatened by Introgression from Domestic Cats (*Felis silvestris catus*). *PLoS One* 9, e106083.
- Wu, M.Y., Low, G.W., Forcina, G., van Grouw, H., Lee, B.P.Y.H., Oh, R.R.Y., Rheindt, F. E., 2020. Historic and modern genomes unveil a domestic introgression gradient in a wild red junglefowl population. *Evol App* 13, 2300–2315. <https://doi.org/10.1111/eva.13023>.