scientific reports

Check for updates

OPEN

Legacy parasite collections reveal species-specific population genetic patterns among three species of zoonotic schistosomes

Erika T. Ebbs[1]✉, D'Eldra Malone[2], Sean A. Locke[3], Norm E. Davis[4], Vasyl Tkatch[5] & Sara V. Brant[2]

Studies estimating genetic diversity and population structure in multi-host parasites are often constrained by temporally and spatially limited sampling. This study addresses these limitations by analyzing globally distributed samples of three congeneric avian schistosomes (Trematoda: Schistosomatidae: *Trichobilharzia*), including collections spanning 20 years archived at The Museum of Southwestern Biology, Parasites Division. The three species exhibited significant differences in population genetic parameters across one nuclear and two mitochondrial loci. *Trichobilharzia querquedulae* (TQ) maintained a well-connected, globally diverse metapopulation, with an effective population size approximately three times larger than that of the other two species, *T. physellae* (TP) and *Trichobilharzia* sp. A (TA). TP and TA had lower overall genetic diversity and greater population structure. These differences are likely shaped by the ecologies of the duck definitive hosts that disperse these parasites. This study highlights the value of natural history collections, particularly since *Trichobilharzia* is a key agent of zoonotic cercarial dermatitis, a disease whose etiology and epidemiology remain poorly understood. Within a comparative congeneric framework, population genetic data can provide insights into host-parasite natural history and its influence on microevolutionary patterns, including contributions to zoonotic disease.

Identifying the factors that shape microevolutionary forces in natural populations is a fundamental goal of evolutionary biology[1]. In multi-host helminth systems, the determinants of gene flow, genetic drift and effective population size (*Ne*) remain poorly understood[2–4]. These parameters influence the evolution of drug resistance[5,6], local adaption[7,8], the probability of host-switching[9], and, ultimately, the speciation of parasite lineages[10,11]. Given the ubiquity of parasitism[12], this lack of understanding represents a significant gap in our understanding of microevolutionary processes within natural populations.

The physiology, immunology, genetics and natural history of hosts influence the microevolution of their parasites[13–17]. For example, host traits such as geographic range, migratory behavior, feeding preferences, and vagility, have been shown to shape the population genetic structure of associated parasite populations[11,14,18,19], ultimately influencing microevolutionary forces. Comparative population genetic studies provide an ideal framework for investigating parasite population structure and microevolution. Previous research has primarily considered broad life history differences among distantly related parasite species (as reviewed by Blasco-Costa & Poulin[19]); autogenic versus allogenic life cycles[14] or migratory versus non-migratory hosts[18]. Comparing congeneric parasite species offers a more precise means of assessing the relative influence of host natural history on parasite populations by minimizing the confounding effects of parasite evolutionary history[20,21]. However, in most host-helminth systems—particularly those not of direct medical importance—taxon sampling and systematics remain insufficiently resolved to support this approach. However, the requisite taxon sampling and systematics of few host-helminth systems, specifically those not of direct medical importance, are sufficiently resolved for this approach. This limitation stems from inherent challenges associated with multi-host helminth systems, including the difficulty of identifying infected hosts, low parasite prevalence, obtaining adequate parasite tissue, and the presence of cryptic variation. Consequently, population genetic studies of multi-host helminth

[1]Department of Biology, Purchase College, The State University of New York, Purchase, NY, USA. [2]Department of Biology, Museum of Southwestern Biology Parasite Division, Center for Evolutionary and Theoretical Immunology, University of New Mexico, Albuquerque, NM, USA. [3]Department of Biology, University of Puerto Rico at Mayagüez, Box 9000, Mayaguez 00681-9000, Puerto Rico. [4]University of Otago, Dunedin, New Zealand. [5]Grand Forks Department of Biology, University of North Dakota, Grand Forks, USA. ✉email: erika.ebbs@purchase.edu

systems remain relatively scarce. Using collections housed at the Museum of Southwestern Biology Parasites Division and targeted field collections, this study overcame some of these challenges. This study estimated the genetic diversity and population genetic structure of three congeneric species within the avian schistosome genus *Trichobilharzia* (Trematoda: Schistosomatidae), based on three loci (*cox*1, *nad*4, *ITS*1).

Prior studies have sampled species of *Trichobilharzia* across their global range, leading to an updated and robust understanding of the systematics and host associations of these parasites[22,23]. As adults, *Trichobilharzia* develop and reproduce sexually in a duck definitive host (Anatidae) and usually release parasite eggs in host feces. A free-swimming larval stage (miracidium) hatches from the egg to find the freshwater snail (Pulmonata) intermediate host where asexual amplification occurs. Subsequently, the snail releases free-swimming cercariae, which infect ducks to complete the life cycle. *Trichobilharzia* is a globally distributed, species-rich genus that infects a broad ecological and taxonomic range of anatid hosts (ducks, geese and swans[22,24,25]. Notably, *Trichobilharzia* species have significant public health implications, as they are the causative agents of human cercarial dermatitis (HCD), a globally prevalent zoonotic disease[26]. The etiology and transmission dynamics of HCD remain complex and poorly understood[27].

This study focuses on *Trichobilharzia* species within the species complex known as Clade Q (*sensu* Brant & Loker[22]). The primary objective was to characterize population genetic diversity and structure among three congeneric Clade Q species to better understand species-specific and host-mediated differences in parasite microevolution. To investigate host-parasite associations, we surveyed 1,358 bird hosts for *Trichobilharzia* infections. Here, we compare the phylogeographic, population genetic and demographic patterns of the three most prevalent *Trichobilharzia* species recovered from North America[22], this study): *T. physellae* (TP), *T. querquedulae* (TQ), and *Trichobilharzia* sp. A (TA). Patterns of definitive host-use for these three species will be described in detail below. Regarding their molluscan intermediate hosts, TP and TQ are transmitted by *Physa* spp. (Physidae) snails, whereas TA is transmitted by *Stagnicola* spp. (Lymnaeidae) snails[22,28]. Recent studies of *Trichobilharzia*[22,23] along with the data presented herein, suggests that *Trichobilharzia* species may exhibit a stronger specificity for particular duck host species than previously recognized[29,30]. Consequently, these parasites may possess distinct species-specific population genetic parameters and structures, mediated by duck host use.

Results

Trichobilharzia survey data

A total of 1,358 birds were examined in this study (Additional File 1 & 2). *Trichobilharzia* species were recovered exclusively from hosts within the family Anatidae. Of the 73 anatid species examined, 29 were found to be infected with at least one *Trichobilharzia* species. Among these 29 species, 464 individual hosts (n_{total} = 1,271; 36.5%) were infected.

The three most prevalent *Trichobilharzia* species identified in our North American surveys were *T. querquedulae* (TQ), *T. physellae* (TP), and *Trichobilharzia* sp. A (TA). The three species exhibited substantial variation in host species range; however, their infection patterns were predominantly (>90%) associated with specific host ecological groups (DEG, Table 1; Fig. 1). These ecological groups—dabbling versus diving ducks—are distinguished by traits such as feeding behaviors, habitat selection, and migratory ecology rather than by strict phylogenetic relatedness[31–33]. TP was detected in eight species across five genera, with highest prevalence in *Aythya affinis* (27%), *A. collaris* (26%) and *A. valisineria* (20%). These three species can be considered core hosts, as collectively, *Aythya* spp. accounted for 73% of all TP infections[34]. Lower TP prevalence was recorded in *Anas platyrhynchos* (12.5%), *Clangula hyemalis* (10%), *Mergus merganser* (10%), *Bucephala albeola* (7.7%), and *Mareca strepera* (2.8%). Consistent with previous studies[22,23], TQ was found almost exclusively (98.3%) in the dabbling duck genus *Spatula*[35], with the highest prevalence in *Spatula discors* (90%). All *Spatula* species examined had an infection rate of at least 74%. TA was recovered exclusively (100%) from the American wigeon (*Mareca americana*), a dabbling duck, making it the most host-specific of the three species, with a prevalence of 27%. Notably, *M. americana* exhibited the highest *Trichobilharzia* species diversity among all examined hosts, harboring four distinct *Trichobilharzia* lineages (Additional File 2). In contrast, the average number of *Trichobilharzia* species per host species was 1.5 in North America and 1.8 globally (Additional File 2).

Trichobilharzia species delimitation

A General Mixed Yule Coalescent (GMYC[37]) model was used to assign lineages and delimit species within the *Trichobilharzia* Clade Q species complex[22,23,25], thereby identifying in-groups for subsequent population genetic analyses. Bayesian phylogenetic inference[38] was used to reconstruct ultrametric trees of Clade Q + *Trichobilharzia regenti* (outgroup, *n* = 118, 743 bp of *cox*1). GMYC analysis suggested the presence of 10

Species	Geographic range	Host range	# of hosts examined	# of infected hosts	DEG	DEG specificity
T. querquedulae	Global	5	129	102	Dabbling	1
T. physellae	North America*	8	168	27	Diving	0.93
Trichobilharzia sp. A	Western USA	1	37	10	Dabbling	1

Table 1. Results of a global genetic survey of three *Trichobilharzia* species. DEG Specificity is the proportion of infections recovered from a single DEG (duck ecological group). Host range is the number of different host species infected. Hosts examined and hosts infected refer only to the subset of hosts examined within the known host range. All infections were identified genetically. An asterisk denotes a single report of TP in Austria (Helmer et al.[36]).

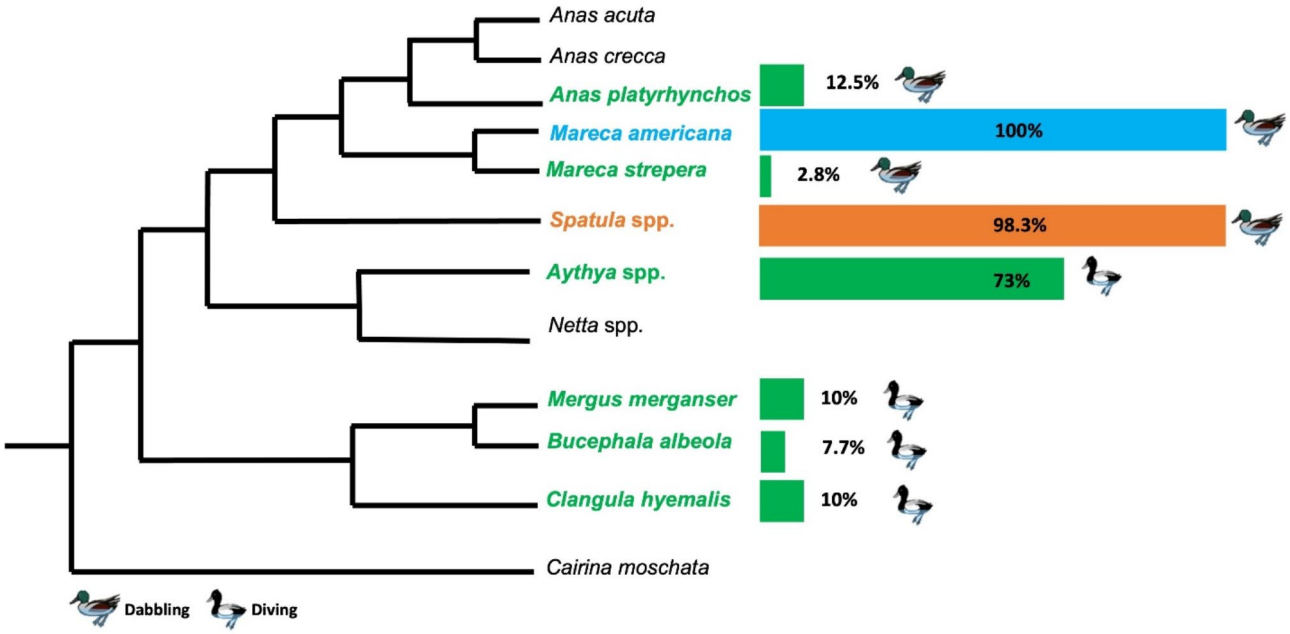

Fig. 1. Proportion of *Trichobilharzia querquedulae* (TQ), *T. physellae* (TP) and *Trichobilharzia* sp. A (TA) infections by duck host; TQ = orange, TP = green and TA = blue. Cladogram is based on Sun et al.[32].

distinct taxonomic units (confidence interval: 8–26) within the dataset (Fig. 2), as supported by a likelihood ratio test (LRT = 25.9199, $p < 0.0001$). The three species of study (TQ, TP & TA) were supported as monophyletic species. Singleton sequences such as W205, W701, W345_2, W750.2 and W345_3, represented distinct lineages, and were removed from all in-group analyses. Pairwise genetic distances within and between Clade Q lineages are provided in Additional File 3.

Species-wide nucleotide diversity (π, Fig. 2B) was estimated for the three species of interest, as well as for *T. franki* (n = *7*) and *T. regenti* (n = *8*). TA was found to have the highest estimate of π followed by TQ and TP. Species wide Θ (Fig. 2C) varied substantially among the three species of interest and was found to be the lowest for TA; TQ = 169.215 (upper = 345, lower = 87), TP = 91.676 (upper = 266, lower = 34), TA = 25.563 (upper = 90, lower = 7). Species wide Θ was not estimated for *T. franki* and *T. regenti* due to small sample sizes.

Phylogeographic patterns among congeners

Trichobilharzia querquedulae

Phylogenetic analysis of TQ supports its global co-distribution with hosts belonging to the genus *Spatula*[23] (Fig. 3). There is little statistical support for resolution of intraspecific clades within TQ (Fig. 3, Additional File 4 A-C). Singleton sequences, associated with TQ (W345.3, W750.2 and W771) which were excluded based on GMYC results and have a pairwise distance (*cox*1) > 4.7%. It is notable that specimens collected from the same collection event, or even the same infrapopulation (within individual host[34]), do not necessarily group together (Additional File 1, determined by shared sample IDs), supporting a well-mixed component population[34]. Minimum spanning networks (Fig. 3) of the total *cox1* dataset revealed high haplotype diversity within TQ, signifying genetically diverse and well-connected populations. Infrapopulation haplotype diversity was similar to component population (infections of a given life stage across host population) haplotype diversity, suggesting infrapopulation recruitment occurs randomly over time and space[2,39]. TQ haplotypes did not partition according to flyway, and Southern Hemisphere haplotypes did not group together and were not found to be more or less divergent than Northern Hemisphere haplotypes (also see[23]).

Trichobilharzia physellae

Overall intraspecific phylogenetic structure was minimal across most phylogenetic analyses. ML and BI analysis of Clade Q based on *cox*1 (Additional File 4A) two subclades (TP1, TP2) that may correlate with eastern and western collection sites. Haplotypes within TP1 were recovered from 3 of the 4 flyways, while 75% (12 of 16) of TP2 individuals were recovered from the Central flyway. However, in a BI in-group phylogeny (Fig. 4A) only TP1 was recovered. Similarly, TP1 and TP2 were not recovered from analyses based on other genetic markers sampled. Minimum spanning networks were coded by host migratory flyway (Fig. 4B), and host species (Additional File 5), which suggested no further sub-structuring. While infrapopulation sampling was limited relative to TQ, it is notable that when multiple individuals from the same TP infrapopulation or the same collection event were sampled (Additional File 1, 4A-C), they generally grouped together, further supporting more prominent population structuring relative to TQ.

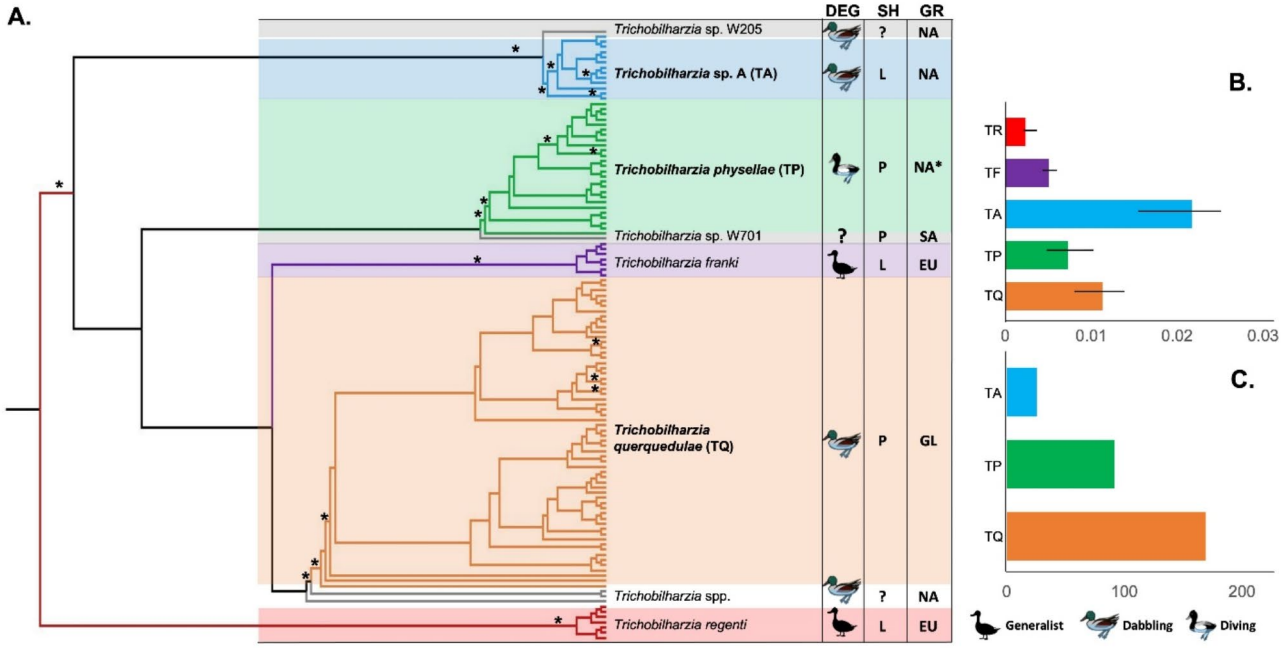

Fig. 2. 'Clade Q' phylogeny. (A) Clade Q + *Trichobilharzia regenti* (outgroup) phylogeny generated in BEAST2[38] under a constant coalescent prior, based on *cox*1 dataset. Branches are colored according to GMYC results, performed using the R package 'Splits'[37]. Nodes with a posterior probability of or greater than 0.98 are noted with an asterisk (*). *Trichobilharzia* spp. includes three monotypic lineages determined by GMYC analysis. *Trichobilharzia* sp. W205 = *Trichobilharzia* sp. B *sensu* Brant and Loker[22]. DEG = Duck Ecological Group. SH = Snail Intermediate Host, where 'P' refers to the snail family Physidae and 'L' to the snail family Lymnaeidae. GR = Geographic Range, where 'EU' denotes Eurasia, 'NA' denotes North America, 'SA' denotes South America and 'GL' denotes global. GR of TP is denoted with an (*) due to the fact that there has been a singular report of TP outside of North America (Helmer et al.[36]). (B) Species-wide nucleotide diversity for the three species of interest plus *Trichobilharzia franki* (TF, $n = 7$) and *Trichobilharzia regenti* (TR, $n = 8$). (C) Species wide theta estimated from average polymorphisms (K) for the three species of interest; estimates for TF and TR not calculated due to low sample size.

Trichobilharzia sp. A

Mitochondrial datasets support the monophyly of TA and its association with *T. franki* and *Trichobilharzia* sp. B W205 (Fig. 2, Additional File 4 A-C). However, *ITS*1 analysis (Additional File 4 C) suggests that TA is paraphyletic with TP, *T. franki*, and *Trichobilharzia* sp. B W205. This result could suggest mito-nuclear discordance within the TA dataset that was not found within either TP or TQ. There is greater sub-structuring within TA (Fig. 5, Additional File 4 A-C) which may suggest phylogeographic structure, however, geographic sampling was too limited to draw general phylogeographic inferences.

Comparative population genetic structure

Trichobilharzia querquedulae

Overall Pairwise Φ_{ST} (Table 2) and multiple hierarchical AMOVA analyses (Table 3) suggest limited genetic structuring within the TQ dataset, specifically in relation to migratory flyway. To determine if Southern Hemisphere populations were obscuring flyway structure within North American samples, hierarchical AMOVA was repeated excluding non-North American samples, but similar results were obtained, though Φ_{sc} was within one standard deviation of significance.

Instead of flyways, TQ populations were partitioned by latitudinal group (high vs. low) as a proxy for genetic structure associated with breeding vs. wintering range. Analyses including and excluding Southern Hemisphere populations do not suggest that latitude (as proxied here) structures TQ populations. No significant structure by host species was found (Table 3).

Pairwise Φ_{ST} values among flyways are summarized in Table 2. When all populations within a flyway were pooled no significant differentiation between flyways was found within TQ, apart from between Pacific Flyway and Southern Hemisphere samples. A Spearman rank test was performed and correlation between geographic distance and both pairwise Φ_{ST} and uncorrected *p*-distance was found to be weak ($\rho = -0.11$ and $\rho = 0.002$, respectively) suggesting no relationship between geographic distance and genetic diversity/structure.

Trichobilharzia physellae

In the TP dataset, which is more limited in terms of sample size, significant differentiation was found between several flyways (Tables 2 and 3). Pairwise Φ_{ST} between localities within flyways and by host species were also

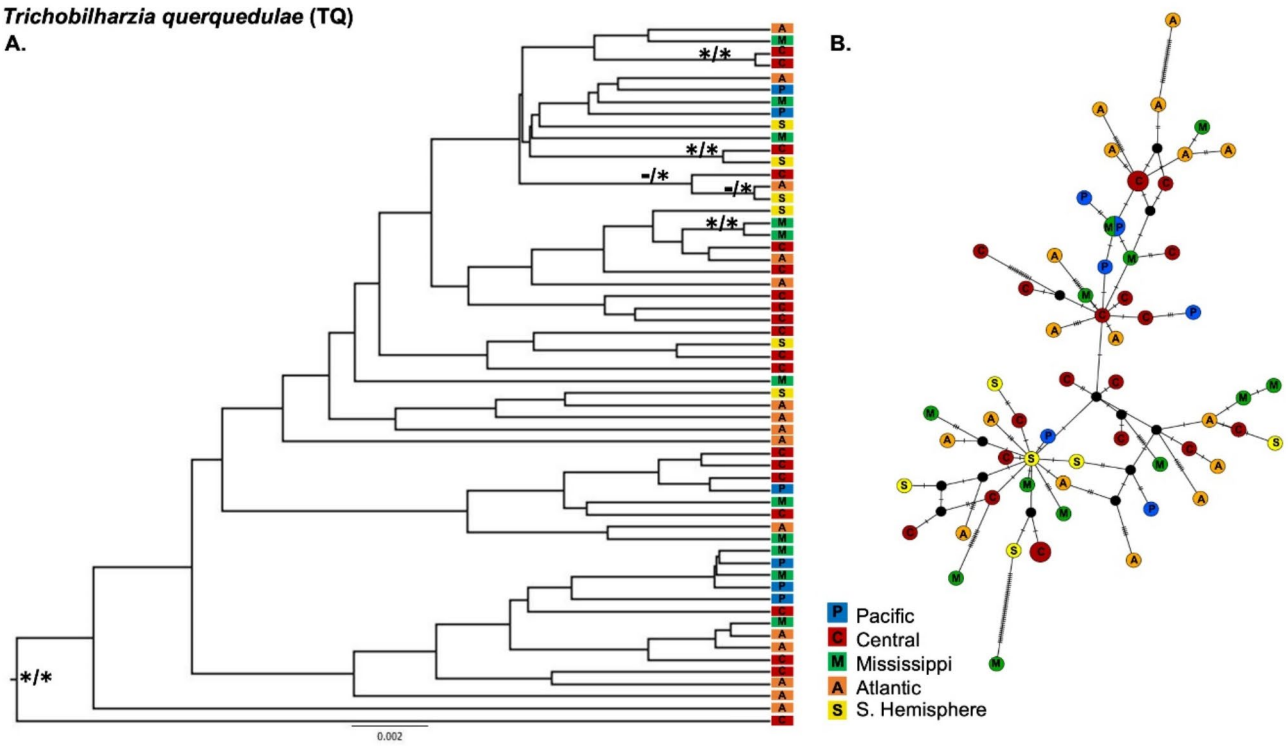

Fig. 3. Intraspecific phylogeny and minimum spanning network of TQ. Based on partial *cox*1. (A) Ultrametric tree generated in BEAST2[38] under a constant coalescent prior. Individual worms are labeled by the migratory flyway they were collected from, denoted by a colored box. (B) Minimum spanning network, haplotypes are colored according to migratory flyway.

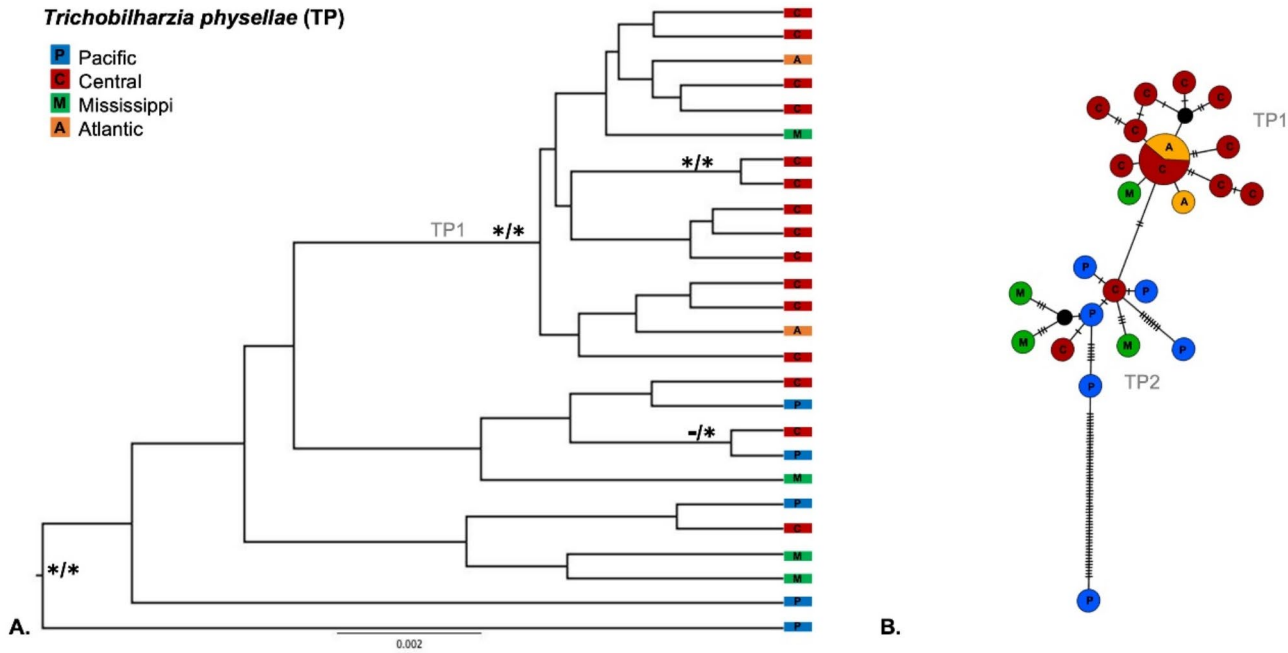

Fig. 4. Intraspecific phylogeny and minimum spanning network of TP. Based on partial *cox*1. (A) Ultrametric tree generated in BEAST2[38] under a constant coalescent prior. Individual worms are labeled by the migratory flyway they were collected from, denoted by a colored box. Subclade TP1 is labelled; TP2 recovered in other analyses was not supported here. (B) Minimum spanning network, haplotypes are colored according to migratory flyway. Haplogroups TP1 and TP2 are labelled.

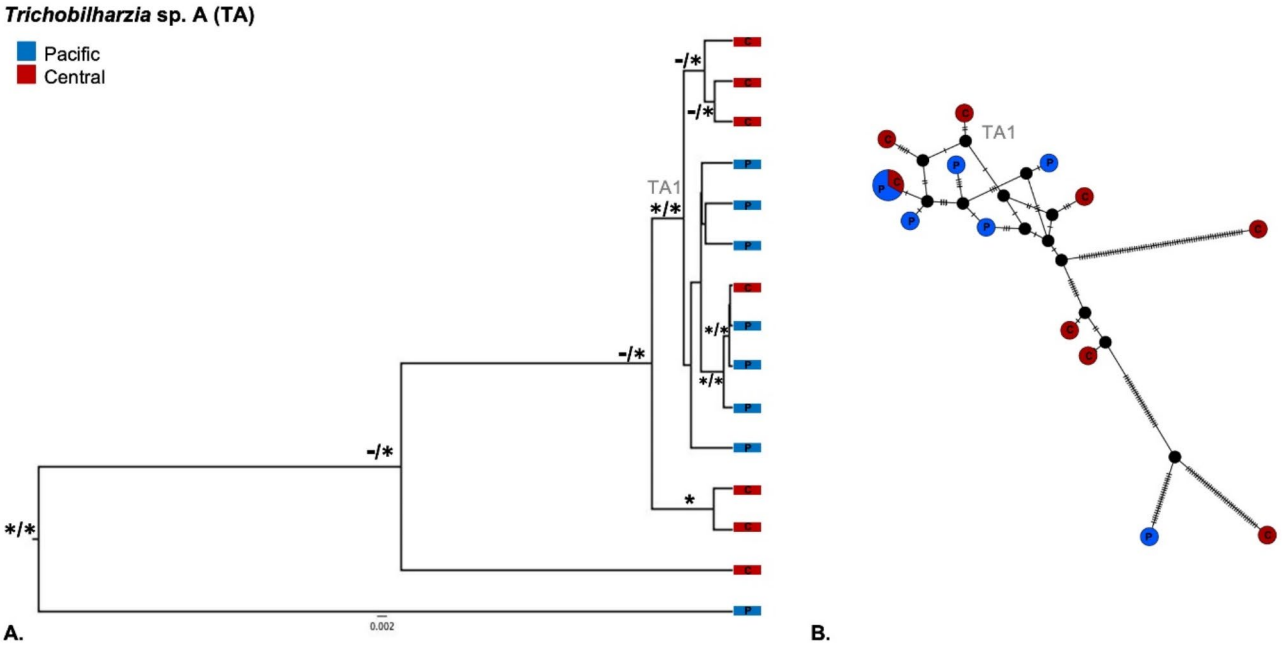

Fig. 5. Intraspecific phylogeny and minimum spanning network of TA. Based on partial *cox*1. (A) Ultrametric tree generated in BEAST2[38] under a constant coalescent prior. Individual worms are labeled by the migratory flyway they were collected from, denoted by a colored box. (B) Minimum spanning network, haplotypes are colored according to migratory flyway.

	TQ				
	Pacific	**Central**	**Mississippi**	**Atlantic**	**SH**
Pacific	**0.0049**	-0.0192	-0.0192	-0.0172	**0.109***
Central	0.0063	**0.0078**	-0.0089	0.0063	0.029
Mississippi	0.0068	0.0082	**0.0088**	-0.0027	0.037
Atlantic	0.0103	0.0114	0.012	**0.015**	0.0012
SH	0.008	0.0086	0.0093	0.0124	**0.0091**
	TP				
	Pacific	**Central**	**Mississippi**	**Atlantic**	
Pacific	**0.0123**	**0.2808****	0.0582	**0.1539**	
Central	0.0116	**0.0095**	**0.228***	-0.0714	
Mississippi	0.0103	0.0086	**0**	**0.1295**	
Atlantic	0.0088	0.0104	0.0097	**0.01023**	
	TA				
	Pacific	**Central**			
Pacific	**0.0458**	**0.0775***			
Central	0.0354	**0.0195**			

Table 2. Pairwise differences and Φ_{ST} values by flyways. The *bottom* diagonal represents average uncorrected genetic distance and the *upper* diagonal represents Φ_{ST} values, based on 701 bp of *cox*1. The diagonal cells, shaded and bolded, are *within* group average uncorrected genetic distance values. Significant pairwise Φ_{ST} values are bolded, a (*) indicates a p-value < 0.05 and (**) indicates a p-value < 0.01. TQ: *Trichobilharzia querquedulae*; TP: *Trichobilharzia physellae*; TA: *Trichobilharzia* sp. A; SH: Southern hemisphere.

measured (results not shown) which identified that TP recovered from New Mexico were distinct from both Alaskan ($\Phi_{ST} = 0.2952$, $p < 0.001$) and Michigan ($\Phi_{ST} = 0.24159$, $p < 0.001$) populations. AMOVA by flyway indicated significant differentiation among flyways ($\Phi_{CT} = 0.52517$, $p = 0.0401$, Table 3), providing some support for the hypothesis that flyways structure TP populations. However, ~90% of the population variation could be attributed to within population differences. When populations were grouped according to latitudinal group (high v. low), no significant population genetic structure was observed. Host genus was associated with significant Φ_{ST}

Source of Variation	d.f.	% Variation	Fixation indices	*p* value
Trichobilharzia querquedulae Flyway				
Among Flyways	4	-5.85	ΦCT= -0.05853	0.6676
Among Populations within Flyways	8	9.97	ΦSC = 0.09420	0.0694
Within Populations	44	95.88	ΦST = 0.04118	0.1603
Flyway ~ NA				
Among Flyways	3	-11.36	ΦCT= -0.1136	0.908
Among Populations within Flyways	6	16.46	ΦSC = 0.1477	**0.0518**±
Within Populations	41	94.91	ΦST = 0.0509	0.1271
Latitude				
Among LG	1	-1.74	ΦCT= -0.01744	0.519
Among Populations within LG	11	5.21	ΦSC = 0.05117	0.0596
Within Populations	44	96.54	ΦST = 0.0346	0.1847
Latitude ~ NA				
Among LG	1	-2.20	ΦCT= -0.0272	0.1026
Among Populations within LG	8	7.06	ΦSC = 0.06905	**0.0205***
Within Populations	41	95.15	ΦST = 0.04855	0.159
Host species				
Among HS	6	-0.47	ΦCT= -0.00472	0.4594
Among Populations within HS	13	8.34	ΦSC = 0.08303	0.0675
Within Populations	35	92.13	ΦST = 0.0787	0.0577
Trichobilharzia physellae Flyway				
Among Flyways	3	52.52	ΦCT = 0.52517	**0.0401***
Among Populations within Flyways	3	-42.96	ΦSC = -0.90482	0.9511
Within Populations	18	90.45	ΦST = 0.0955	0.2933
Latitude				
Among LG	1	2.71	ΦCT = 0.02714	0.50733
Among Populations within LG	4	7.12	ΦSC = 0.07319	0.52884
Within Populations	20	90.17	ΦST = 0.09835	0.15934
Host species				
Among HS	4	20.13	ΦCT = 0.20128	0.12219
Among Populations within HS	3	10.46	ΦSC = 0.13098	0.26588
Within Populations	17	69.41	ΦST = 0.3059	**0.00293****

Table 3. Hierarchical AMOVA of *Trichobilharzia querquedulae* and *T. physellae* cytochrome *c* oxidase 1 (*cox1*) population structure. Alternative hypotheses of population structure were tested and determined significant when $p \leq 0.05$, significant values are bolded. Values denoted with an (±) indicate a *p*-value one standard deviation (±) from significance. NA refers to North America and the exclusion of southern hemisphere samples. LG is latitudinal group; HS is host species.

($p = 0.00293$), however, neither the Φ_{SC} nor Φ_{CT} were significant, suggesting no real influence of host species. There was no evidence that host species influenced population genetic structure.

The Central flyway, predominantly composed of New Mexican specimens, was found to be significantly distinct from the Pacific and Mississippi flyways (Table 2). The Mississippi and Atlantic flyways harbored the highest within flyway divergence values (1.2% and 0.95% respectively) in comparison to the Central (0.01%) and Pacific (0.048%) flyways. Correlation between geographic distance and uncorrected *p*-distance was moderate ($\rho = 0.473$) indicating a relationship between genetic diversity and spatial separation. Geographic distances were not correlated with pairwise Φ_{ST} values ($\rho = -0.025$).

Trichobilharzia sp. A

Since TA was only recovered from two flyways a hierarchical AMOVA was not performed. Pairwise Φ_{ST} between the two flyways sampled (Pacific and Central) was significant ($\Phi_{ST} = 0.0775$, $p < 0.001$). Genetic divergence within the Central flyway was higher (1.95%) than in the Pacific (0.458%) with an average *p*-distance of 3.54% between the two flyways (Table 2). Due to limited geographic sampling a Spearman rank test was not performed.

Genetic diversity and demographic analyses

While haplotype diversity was similar across TP, TQ and TA, nucleotide diversity (π) and Θ were substantially different across the three species (Table 4; Fig. 2B & C).

Species	Locality	MFW	*N*	H	Hd	S	K	π	Θ
TQ									
	California	P	4	4	1	10	5.17	0.00737	0.00778
	Alaska	P	2	2	1	4	4.00	0.00287	0.0057
		Total	6	6	1	9	*3.40*	*0.00487*	*0.00565*
	New Mexico	C	17	16	0.993	40	6.82	0.00974	0.01733
	Nebraska	C	1	-	-	-	-	-	-
	North Dakota	C	2	2	1	5	5.00	0.00713	0.00713
		Total	20	19	0.995	44	*6.75*	*0.00964*	0.01812
	Manitoba	M	1	-	-	-	-	-	-
	Minnesota	M	3	3	1	10	6.67	0.00952	0.00952
	Louisiana	M	6	6	1	16	6.93	0.00989	0.0100
		Total	10	10	1	28	*7.91*	*0.01130*	0.01515
	Florida	A	14	14	1	45	9.33	0.01598	0.02291
	Puerto Rico	A	1	-	-	-	-	-	-
		Total	15	15	1	58	*11.97*	*0.01718*	*0.02647*
	Argentina	SH	2	2	1	6	6.00	0.00857	0.00857
	New Zealand	SH	3	3	1	15	10.00	0.01429	0.01429
	South Africa	SH	1	-	-	-	-	-	-
		Total	6	6	1	21	*7.60*	*0.0108*	*0.01314*
	Species Total		**58**	**50**	**0.986**	***99***	***6.622***	***0.00956***	***0.03242***
TP									
	Alaska	P	4	4	1	18	9.1667	0.01310	0.01403
	California	P	1	-	-	-	-	-	-
		Total	5	5	1	18	*7.60*	0.01086	*0.01234*
	New Mexico	C	13	12	0.987	16	3.538	0.00506	0.00738
	Alberta	C	1	-	-	-	-	-	-
		Total	14	12	0.967	16	*3.307*	*0.00473*	*0.00720*
	Michigan	M	4	4	1	13	6.6667	0.00954	0.01013
		Total	4	4	1	13	*6.6667*	0.00954	0.01013
	Pennsylvania	A	1	-	-	-	-	-	-
	Florida	A	1	-	-	-	-	-	-
		Total	*2*	*2*	*1*	*2*	*2.00*	*0.00285*	*0.00285*
	Species Total		**26**	**23**	**0.982**	***39***	5.105	0.00730	0.01462
TA									
	California	P	2	2	1	37	38	0.0079	0.00843
	Alaska	P	6	5	0.933	15	6.133	0.00909	0.01038
		Total	8	7	0.964	*26*	*25.107*	*0.0079*	0.008
	New Mexico	C	6	6	1	82	32.8667	0.04689	0.05435
	North Dakota	C	*1*	-	-	-	-	-	-
		Total	*7*	7	1	82	*13.667*	0.0195	*0.05373*
	Species Total		**15**	**13**	**0.971**	***56***	**15.199**	**0.0217**	**0.01619**

Table 4. Population genetic summary statistics for the three species of *Trichobilharzia*. Genetic diversity summary statistics for *Trichobilharzia* infrapopulations[34] sampled for the three species of interest: *Trichobilharzia querquedulae* (TQ), *Trichobilharzia physellae* (TP) and *Trichobilharzia* sp. A (TA). MFW = Migratory flyway that the duck host was collected from; P = Pacific, C = Central, M = Mississippi, A = Atlantic, SH = Southern Hemisphere. Summary statistics are abbreviated as follows; N = the number of individual *Trichobilharzia* sequences included; H = the number of haplotypes recovered; Hd = haplotype diversity; S = number of segregating sites; K = average pairwise differences among individuals sampled; π = nucleotide diversity; Θ = Watterson's estimator of theta per site. Italics are used to denote when multiple sample groups are combined.

Figure 6 shows estimates of *Ne* over time (kya) for TP, TQ, and TA based on a 4% change/million years[40,41], a lower rate of 2% was also tested (Additional File 6). Population growth trends and mean *Ne* from Bayesian skyline analysis supports recent population expansion of TQ, and to a lesser extent TP. However, it should be noted that when the highest posterior density (HPD) is considered across analyses there is overlap among the two species (Additional File 6).

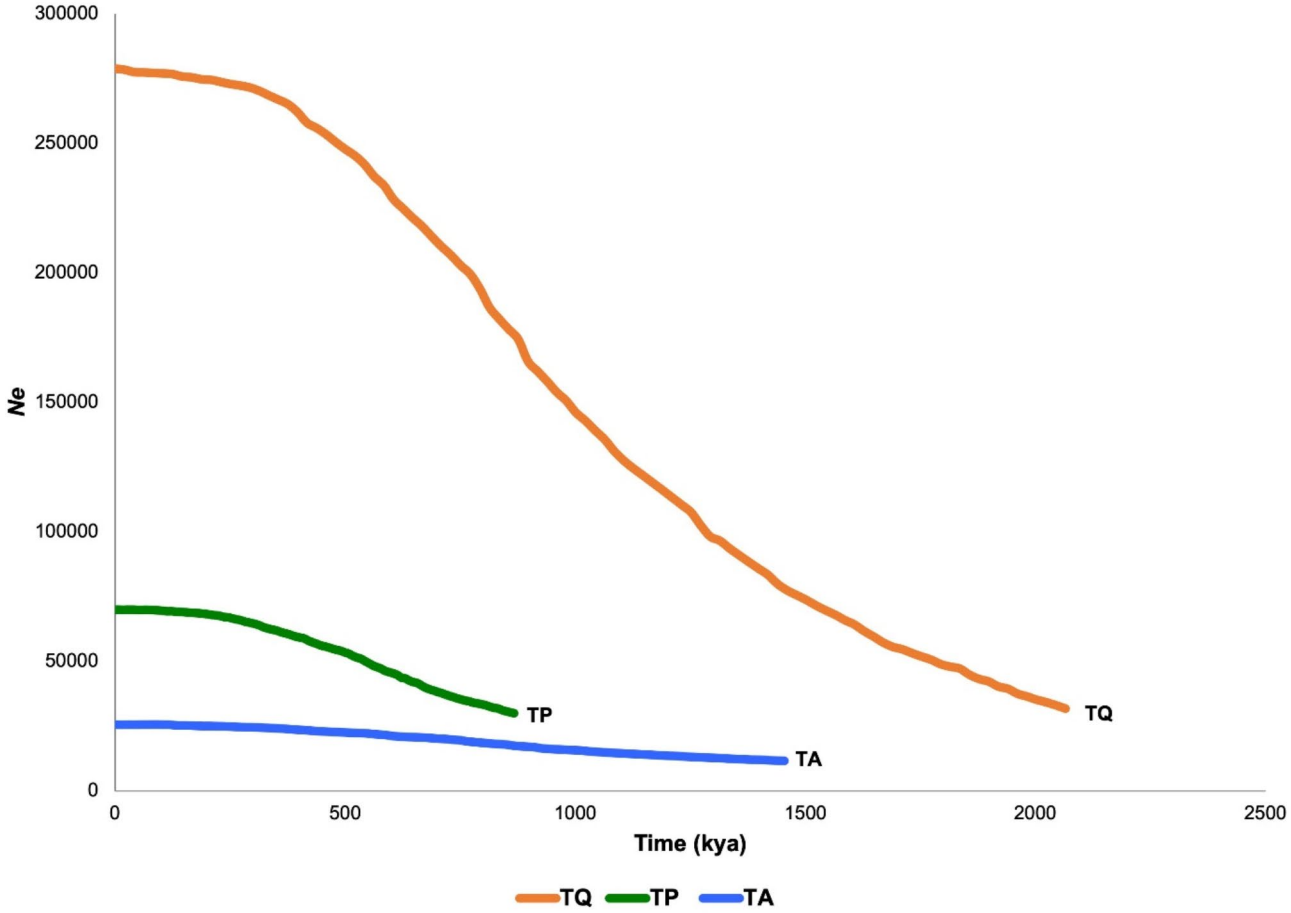

Fig. 6. Bayesian skyline plot of partial cytochrome *c* oxidase 1 (*cox*1) datasets. Species wide estimates of *Ne* (y-axis) over time (kya, x-axis), based on a 4% mutation rate per million years (4E-6). Lines are colored by species; *Trichobilharzia querquedulae* (TQ) = orange, *T. physellae*(TP) = green and *Trichobilharzia* sp. A (TA) = blue. Additional skyline plots testing alternative mutation rates and highest posterior density (HPD) intervals can be found in the supplementary files.

Discussion

This study compared population genetic and phylogeographic patterns among three *Trichobilharzia* Clade Q species, uncovering species-specific microevolutionary dynamics within a complex and widespread host-helminth system. Differences in genetic structure among congeneric parasites correlate with differences in duck host use and ecology (e.g. migratory range, feeding behavior). We observed substantial differences in estimates of *Ne*, π and Θ among the three species, particularly between TQ and TP, despite both utilizing the same intermediate host taxon (*Physa* spp.). In multi-host parasite systems, the determinants of *Ne*, π, and Θ remain poorly understood[42], yet these parameters are fundamental to disease ecology and parasite management. The parameter Θ (Θ = 4*Ne*μ) can serve as an indicator of a species' genetic flexibility or capacity to respond to environmental and demographic shifts[8,14,43], making it a valuable measure of adaptive potential[42]. Given its higher Θ estimates, TQ may exhibit greater adaptive potential than TP. From this perspective, TQ populations may be more resilient to environmental changes, such as host extinction or climate shifts, compared to TP. Here, we examine key aspects of host-*Trichobilharzia* interactions that may shape these genetic parameters and discuss their broader implications in microevolution and disease ecology, as they specifically relate to the epidemiology of human cercarial dermatitis (HCD).

Definitive host migration and ecology

Comparative population genetic analyses of TQ, TP and TA revealed marked differences in their population structure and demographic histories (summarized in Tables 2, 3, 4 and 5). The observed population genetic patterns, particularly the limited structure in TQ, are supported by geographically extensive sampling of parasite species. Spatially restricted sampling can obscure genetic structure in parasites, a challenge that is particularly pronounced in widely distributed hosts such as waterfowl[44]. In these hosts, genetic structuring is typically detected only at intercontinental scales using markers like *cox1*[45].

TQ, a common and genetically diverse schistosomatid infecting *Spatula* spp. across four continents[23]; this study), exhibited weak population co-structure in relation to duck migratory ranges, with latitude serving as

a proxy for breeding and non-breeding distribution. Despite its global presence, including in non-migratory Southern Hemisphere *Spatula* spp., TQ showed no discernible phylogeographic structure. Historical effective population size (*Ne*) estimates for TQ were an order of magnitude higher than those of TP and TA. Furthermore, contemporary regional measures of π and Θ remained high across TQ's range, including in Southern Hemisphere populations, with regional values closely aligning with species-wide and infra-population estimates—suggesting extensive gene flow throughout its distribution. In contrast, TP exhibited lower species-wide π and Θ, indicating comparatively reduced genetic diversity and gene flow.

Overall, TP exhibits lower genetic diversity and greater population structure compared to TQ, with potential geographic structuring between the eastern and western portions of its host's range. The phylogeographic structure of North American *Aythya* spp. remains uncharacterized, making it unclear whether the observed genetic structure in TP reflects that of its host populations. In Eurasia, *Aythya* spp. display moderate to high female philopatry, minimal genetic differentiation between Europe and Asia, and significant admixture within wintering grounds[46,47].

Hierarchical AMOVA (Table 3) and pairwise ΦST values (Table 2) suggest that TP exhibits geographic structuring, potentially influenced by migratory flyways, aligning with an east-west division. Differences in genetic structure between TP and TQ may reflect ecological behaviors specific to their duck hosts, such as distribution, philopatry, microhabitat preferences, and habitat selection. Our sampling only detected TP in North American ducks, indicating a more restricted distribution than TQ. However, Helmer et al.[36] reported TP infections in invasive *Physa acuta* snails in Austria. The cercariae recovered were nearly identical genetically to North American TP[36], suggesting either high gene flow between North American and Eurasian populations or a recent introduction. Given the low rate of TP recovery in Eurasian hosts, the latter seems more likely.

In susceptible avian hosts, TQ was five times more prevalent than TP overall and three times more prevalent when comparing the most commonly infected hosts—*Spatula discors* (TQ) and *Aythya affinis* (TP). This suggests a stark difference in the probability of definitive host infection. Both TP and TQ utilize *Physa* spp. (Physidae) as their snail intermediate hosts[22]; *Physa* spp. are abundant across North and South America[48,49], with *P. acuta* being globally invasive, occurring on six continents[48]. These snails thrive in diverse freshwater habitats and can reach high densities[50].

The higher prevalence of TQ in *Spatula* spp. may be linked to its feeding ecology—dabbling in shallow waters and marshes—compared to the diving habits of *Aythya* spp., which were less frequently infected with TP. While experimental infections are needed to rule out inherent differences in host susceptibility, the stark contrast in TQ and TP prevalence suggests that certain host-microhabitat combinations may enhance parasite transmission, leading to larger effective population sizes and higher genetic diversity. These differences may have important implications for the epidemiology of HCD, a globally re-emerging disease most commonly caused by *Trichobilharzia* spp[26]. The factors influencing the emergence and persistence of HCD outbreaks in waterbodies remain poorly understood[27]. Our findings suggest that due to higher transmission, ecological predictability, and greater genetic diversity, TQ—and *Trichobilharzia* species specific to dabbling ducks—may play a disproportionate role in HCD epidemiology. Notably, a study on Avian Influenza Virus (AIV) found that dabbling ducks in North America exhibited both higher prevalence and greater subtype diversity of AIV compared to diving ducks[51], indicating a pattern of increased pathogen transmission in this group.

Infrapopulation diversity and within-host recruitment

Infrapopulation diversity of TQ was equal to overall component population diversity and the number of unique haplotypes within infrapopulations increased linearly with the number of individuals sampled. This pattern suggests that infrapopulation recruitment (i.e., transmission) occurs randomly across time and space within a well-mixed component population. In other words, ducks do not recruit *Trichobilharzia* in packets of cercarial clones emerging from an individual snail but steadily acquire parasites with unique haplotypes over time. Similar findings have been reported in *Diplostomum pseudospathaceum*, where microsatellite analyses revealed a lack of clonal infections within individual fish hosts[52].

In this study, the majority of recovered worms were males, as female *Trichobilharzia* are more challenging to locate within duck hosts—a common limitation in previous surveys[28,29,53,54]. Female worms migrate to and remain in the intestinal serosa to lay eggs, making them harder to detect and significantly increasing screening time per bird. Consequently, our genetic analyses primarily reflect a diverse pool of male worms. Notably, sex-specific genetic structure has been reported in the related human schistosome, *Schistosoma mansoni*[55], where male genotypes exhibited more random distribution patterns compared to females. Thus, sex-related differences in genetic structure cannot be ruled out as a potential confounding factor in this study.

In contrast to TQ, TP infrapopulations appear to be less heterogeneous (Additional File 4 A-C), possibly indicating that recruitment is structured geographically. This pattern may be linked to the overall greater population subdivision observed within TP compared to TQ (Table 2). However, deeper sampling of TP infrapopulations across different regions is needed to fully elucidate the extent and causes of this structure[56].

Additionally, multiple infrapopulations of TA were sequenced from American wigeon (*Mareca americana*), a host species that harbors the highest diversity of *Trichobilharzia* lineages. Our data suggest that *M. americana* maintains at least four distinct, currently undescribed *Trichobilharzia* genetic lineages[22,23,25]. As a generalist dabbling duck[57], *M. americana* occupies a wide range of seasonal habitats, which may increase its likelihood of encountering diverse snail species and their associated parasites, thereby facilitating greater parasite diversity within its population.

Definitive-host habitat predictability

In North America, the primary hosts of TQ (*Spatula clypeata*, *S. discors*, and *S. cyanoptera*) have broad migratory ranges and utilize diverse local habitats[57], with a preference for shallow marshes[58]. Site selection among *Spatula*

spp. is highly variable[59], driven primarily by habitat quality. As a result, these ducks frequently move locally in search of optimal habitats, increasing their probability of encountering infected snails as they sample different environments[57,60]. This behavior leads *Spatula* spp. to spend a significant amount of time in *Physa*-rich habitats, thereby enhancing their likelihood of acquiring *Trichobilharzia* infections.

In contrast, North American *Aythya* spp. tend to occupy larger, more stable water bodies and spend less time in shallow areas, particularly during winter. *Aythya* spp. are highly philopatric, meaning habitat quality plays a lesser role in site selection, and their local movement is more restricted compared to *Spatula* spp[57,60]. These behaviors reduce their exposure to *Trichobilharzia*, aligning with the lower prevalence of TP observed in this study. Additionally, philopatric host species are more likely to facilitate population genetic structure among their parasites[55], a pattern consistent with the greater genetic structuring observed in TP compared to TQ.

Additionally, the phenology of migration between North American *Spatula* spp. and *Aythya* spp. is markedly different[61,62], potentially conferring temporal separation of ducks hosts and available *Trichobilharzia* spp. within shared water bodies. Although experimental research is necessary to fully understand the life history of these parasites, it is possible that adult TP has a shorter lifespan than TQ, leading to a smaller standing crop and lower prevalence, as observed in our samples. Our survey data suggest that TP prevalence remains stable across seasons and sampling years, though our study design does not allow us to formally test this hypothesis.

The host range of a parasite (i.e. host specificity) is determined by host-parasite *encounter* and host-parasite *compatibility*, which have been conceptualized as successive filters to establishing infections[63]. Studies of the compatibility filter in schistosome-bird interactions are scant. Older studies suggest that species of *Trichobilharzia* are compatible with anatids and some non-anatids. For example, McMullen and Beaver[53] demonstrated that TP could develop to patency in non-anatids (e.g. canaries and pigeons). Similarly, while it is largely accepted that *Trichobilharzia* is anatid specific[25], rare natural infections are also known within non-anatids[54]. On balance, the encounter filter appears to dominate in schistosome-bird interactions. Extensive molecular surveys [22,23, this study] suggest that many, if not most, *Trichobilharzia* species (Additional File 2) are predictably recovered from a specific duck species or ecological group, and are present, but rare, in other compatible host species. Here, we suggest the *encounter* filter is narrowed by the ecology of the host species/ecological group. From our survey of 1,358 birds, 6 of the 9 (~67%) *Trichobilharzia* lineages recovered with adequate sampling were found to have >90% of reported infections occur within a single duck species or ecological group (Table 1, Additional File 2).

Heterogeneous duck-host population dynamics might also contribute to the observed differences among *Trichobilharzia* spp. The population dynamics of some diving duck species, such as *A. affinis*, are more sensitive to anthropogenic disturbance than some dabbling duck species, in both North America and Europe[64–66]. For example, lesser scaup (*Aythya affinis*) populations, a primary host for TP, have been in decline since the 1980's[67,68], for reasons not fully understood, but undoubtedly exacerbated by mass die offs from infection of invasive trematodes, *Cyathocotyle bushiensis* and *Sphaeridiotrema globulus*[69,70]. Since the late 1970s, the sex ratio of lesser scaup has been skewed towards males[67], with an extreme ratio of 2.37 males to 1 female. We were unable to test for host sex bias in *Trichobilharzia* infections because host sex was not always recorded. We may hypothesize that since breeding females spend more time in transmission-rich sites while rearing offspring, they are exposed to more *Trichobilharzia* than males.

Conclusions

Using long-term museum collections in combination with targeted field collections, we investigated the population genetics of three *Trichobilharzia* species and found that estimates of π, θ, *Ne* and population structure varied greatly among species. These species-specific patterns can, in part, be attributed to differences in host ecological traits, which likely influence parasite gene flow. This study highlights the utility—and, in many multi-host parasite systems, the necessity—of museum collections[71,72]. Archived specimens were crucial for expanding the geographic scope of sampling, a scale rarely achieved in non-human parasite population genetics studies.

Trichobilharzia is re-emerging as a globally significant zoonotic parasite, with increasing outbreaks of human cercarial dermatitis (HCD)[26,27]. Our findings suggest that specific aspects of duck natural history and population dynamics influence *Trichobilharzia* transmission, population structure, and microevolution. These factors likely have important implications for understanding and managing HCD outbreaks.

Materials and methods

Host–parasite survey

This study surveyed 1,358 avian hosts across 94 species (73 anatids) and 7 countries (Additional File 1 & 2), for *Trichobilharzia* infection. Host and parasite samples are housed in the Museum of Southwestern Biology (MSB), Parasite Division, at the University of New Mexico, Albuquerque; MSB Parasites has a 20 + year collection of Schistosomatidae from avian and snail hosts, as part of a long-standing goal to understand schistosomatid diversity. This work utilized *Trichobilharzia* collected from past MSB efforts and was supplemented by targeted collections of ducks known to host the *Trichobilharzia* species of interest to improve sample sizes. The methods described below apply to both historical museum samples and specimens collected specifically for this study. All hosts examined were either donated by hunters or obtained from the MSB Bird Division. All records are maintained within the Arctos database (https://arctos.database.museum/) and genetic data was obtained through subsampling. All work with vertebrate hosts was conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at the University of New Mexico, USA (IACUC # 11- 100553-MCC, Animal Welfare Assurance # A4023-01). Most species of *Trichobilharzia* are parasites of the venous system and primarily reside in the mesenteric and hepatic portal veins, with some exceptions, notably *T. regenti*, which occupies the nasal mucosa. Mesenteric veins were inspected for adult worms with the aid of a dissection microscope and were removed using microscissors. Adult worms were also recovered by perfusing the hepatic portal vein with

saline and then crushing the liver. Worms were then isolated in a series of decantation steps. *Trichobilharzia* samples were preserved in 95% ethanol for genetic and 80% ethanol for morphological assessment. In addition to the schistosome species recovered from the anatid hosts, co-occurring parasites (liver, kidney, heart and gastrointestinal tract) were collected and vouchered at the MSB.

For infected hosts we calculated *prevalence*, the proportion of infected hosts[34], and *observed host range*, calculated as the number of host species infected by a parasite species. A *Trichobilharzia* species was determined to have *ecological specificity* if > 90% of infections were recovered from a single ecological group (e.g., dabbling or diving ducks as defined by Sibley[31]). Intensity and consequently abundance are important parameters to consider when estimating host associations[73]. However, the difficulties of detecting all *Trichobilharzia* prevented quantification of parasite *infrapopulation*s, which are the number of individuals of a given parasite species within an individual host[34]. Some parameters were calculated at the scale of the component population, which is the number of individuals of a given parasite species within a host population[34].

DNA extraction, PCR amplification and sequencing

Trichobilharzia DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA), or the QIAmp DNA Micro Kit (Qiagen, Valencia, California, USA) which was found to have higher DNA yield. We amplified two mitochondrial and one nuclear gene region using primarily the Takara *Ex Taq* kit (Takara Biomedicals, Otsu, Japan). For samples that were difficult to amplify, we used either the Go*Taq* Flexi (Promega) or the Platinum *Taq* kit (Invitrogen) using 0.4 ul of *Taq* and 4ul of 2.5 mM $MgCl_2$ per reaction. We sequenced a 743 bp (5' end) region of the cytochrome oxidase I gene (*cox*1), a 409 bp (5' end) region of the NADH 4 gene (*nad*4) and 733 bp of the internal transcribed spacer region 1 (ITS1, including the 3' end of 18 S rRNA and the 5' end of 5.8 S). PCR protocols and primers used were identical to those used by Brant and Loker[22] (*cox*1, *ITS*1) and Ebbs et al.[23] (*nad*4). Our dataset comprised samples collected (and sequenced) over a 20-year period, inclusion of historical samples often meant that tissue/DNA samples were exhausted before all three loci could be successfully obtained, therefore for some samples there is not equal sampling among the three loci (Additional File 1). Sequencing reactions were performed using the BigDye ver. 3.1 sequencing kit (Applied Biosystems, Foster City, California, USA). Sequences were edited using Sequencher 5.3 (Gene Codes Corporation, Ann Arbor, MI, USA) and aligned using Clustal Omega integrated in Geneious Prime 2023.0.4. Sequences generated from this study are accessioned in the NCBI GenBank database under the accession numbers PQ057157-PQ057179; PQ057776- PQ057786.

Phylogenetic analyses and species delimitation

To evaluate intraspecific relationships, phylogenetic analyses of Clade Q (*sensu* Brant and Loker[22]) were carried out. Phylogenetic datasets were analyzed by individual gene sequences (*cox*1, *nad*4, *ITS1*) or concatenated sequences (*cox*1 + *nad*4 + *ITS1*), using *Trichobilharzia regenti* as an outgroup[22,23]. Mitochondrial genes (*cox*1 and *nad*4) are well suited for intraspecific studies as matrilineal inheritance results in a smaller effective size and higher sorting rate[74,75]. Part of the nuclear rRNA complex, *ITS*1 was chosen to evaluate potential mito-nuclear discordance. Appropriate models of nucleotide substitution were selected using JModelTest2[76] based on the Akaike information criterion (AIC). Maximum Likelihood (ML) and Bayesian Inference (BI) were performed on each phylogenetic dataset. ML analyses were performed in RaxML v.8[77], with 1,000 bootstrap replicates to assess topologies. Bayesian Inference (BI) was performed using the program MrBayes v. 3.2.6[78], consisting of two replicated runs for each locus with four Markov chain Monte Carlo (MCMC) chains, one cold and three heated chains. Each analysis ran for 10,000,000 generations and was sampled every 1000 generations, and it was verified that the standard deviation of the split frequencies was or fell below 0.01, indicating search chain convergence. Likelihood parameters and convergence between runs were assessed using the program Tracer v.1.6[79], based on ESS values greater than 200. Maximum clade credibility trees (10% burn in) were generated, then visualized and manipulated using Fig Tree v. 1.4.4[80].

Ingroups for subsequent intra-specific analyses were distinguished using a Generalized Mixed Yule Coalescent (GMYC), a robust approach for single-locus datasets[37]. GMYC analyses were performed in BEAST 2.5[38], both Yule and Constant Coalescent priors were tested. A constant clock and HKY + G model, where the number of gamma categories were set to 4 was used for both, each analysis ran for 10,000,000 generations. The posterior was evaluated and ESS values checked in Tracer v.1.6[79], a summary ultrametric tree was produced (10% burn in). Ultrametric trees were generated using *cox1* alignments, using 'Clade Q' ($n = 118$) to determine interspecific thresholds, TQ ($n = 61$), TP ($n = 26$), and TA ($n = 15$). Ultrametric trees were uploaded into R, and the package 'splits' (Species Limits by Threshold Statistics[37]) was used to perform and visualize GMYC analysis. Additionally, pairwise uncorrected *p*-distances were calculated in MEGA X[81] within and between the three species of *Trichobilharzia* to better understand intraspecific genetic distance[22] (Additional File 3). In-group datasets for *nad*4 were smaller, as not all specimens had sufficient material for additional sequencing ($TQn_{nad4}=41$, $TPn_{nad4}=14$, $TAn_{nad4}=9$).

Intraspecific datasets (TP, TQ and TA) were collapsed into haplotypes, determined by DNAsp v5[82] and haplotype relationships were assessed using Minimum Spanning Network analysis in POPart[83]. Haplotypes were coded by migratory flyway (Figs. 3, 4 and 5) and host species (Additional File 5). Migratory flyways are a common way of partitioning bird populations[84,85] and their symbionts[86,87]. Each of the four North American flyways (Pacific, Central, Mississippi and Atlantic) was sampled for adult *Trichobilharzia* for this study. All samples collected from the Southern Hemisphere were pooled because of the relatively small sample size.

Genetic diversity and population structure

Genetic diversity and population genetic structure were measured across multiple levels of infrapopulation organization: state or province the host was collected from, migratory flyway from which the host was collected,

and host species. For all in-group analyses the complete dataset was used. Indices of genetic diversity were estimated in DNAsp v5[82] for both *cox*1 and *nad*4: number of polymorphic sites (S), average number of nucleotide differences (K), and nucleotide diversity (π). Uncorrected *p*-distances were calculated between flyways, regions and host species in Arlequin 3.5[88].

In the two best-sampled species (TQ and TP), separate hierarchical AMOVAs were performed on *cox*1 datasets using Arlequin v. 3.5[88], to estimate overall population genetic structure. Factors tested included flyway in the Northern Hemisphere, host species, and high versus low latitude. For the TQ dataset AMOVAs were run both with the inclusion and exclusion of Southern Hemisphere populations (Table 3). Estimates of Φ_{ST} variation were generated from all samples across all localities sampled, among flyways, host species, latitudinal group and among localities within the flyways, host species, and latitudinal groups. We also estimated pairwise Φ_{ST}[89] by locality, flyway and host species (Table 3). Significance ($p < 0.05$) was determined by permutation tests of 10,000 random permutations.

To test for isolation by distance patterns we constructed a geographic distance matrix by converting geographic coordinates to Euclidean distances between localities as implemented in Primer 7[90]. Correlation between geographic distances and both uncorrected *p*-distances and pairwise Φ_{ST} matrices were calculated using Spearman's rank correlation also in Primer 7.

Demographic analyses

To assess contemporary and historical demographic patterns, Watterson's estimator (Θ) was calculated (*cox1*) and assessed statistically using coalescent simulations with a 95% confidence interval and 10,000 permutations in DNAsp[82].

Changes in *Ne* over time were estimated by fitting a Bayesian Skyline demographic model[91,92] for each of TQ, TP, and TA based on *cox1* using BEAST v 2.5[38]. The HKY substitution model was used for two simultaneous MCMC runs for 10,000,000 iterations sampling every 1,000 steps. Mutation rates of 2% and 4% change per million years were used to estimate mitochondrial genome evolution, as has been used for *Schistosoma mansoni*[40,41]. As substantial divergence within TA collected between the Pacific and Central flyways was estimated, these populations were analyzed separately. Convergence was checked (effective sample size of 200 or greater) and results visualized using Tracer v1.6[79], all data was exported, aggregated and visualized in Microsoft Excel.

Data availability

All sequence data generated is uploaded to GenBank and accessible through accession numbers SUB14612933, SUB14612849, SUB14612794. All host and parasite records are publicly searchable through the Arctos Database and are curated by the Museum of Southwestern Biology, Parasites Division in Albuquerque New Mexico.

Received: 17 July 2024; Accepted: 11 March 2025
Published online: 19 March 2025

References

1. Slatkin, M. Gene flow in natural populations. *Ann. Rev. Ecol. Sys.* **16**, 393–430 (1985).
2. Nadler, S. A. Microevolution and the genetic structure of parasite populations. *J. Parasit.* **81**, 395–403 (1995).
3. Criscione, C. & Blouin, M. Effective sizes of macroparasite populations: A conceptual model. *TRENDS Parasit.* **21**, 212–217 (2005).
4. Rey, O. et al. Population genetics of African schistosoma species. *Infect. Genet. Evo.* **89**, 104727 (2021).
5. Blouin, M. S., Yowell, C. A., Courtney, C. H. & Dame, J. B. Host movement and the genetic structure of populations of parasitic nematodes. *Genetics* **141**, 1007–1014 (1995).
6. Chevalier, F. D. et al. Oxamniquine resistance alleles are widespread in old world *Schistosoma mansoni* and predate drug deployment. *PLoS Pathog.* **15**, e1007881. https://doi.org/10.1371/journal.ppat.1007881 (2019).
7. Dybdahl, M. F. & Lively, C. M. The geography of Coevolution: comparative population structures for a snail and its trematode parasite. *Evolution* **50**, 2264–2275 (1996).
8. Gandon, S. & Nuismer, S. L. Interactions between genetic drift, gene flow, and selection mosaics drive parasite local adaptation. *Am. Nat.* **173**, 212–224 (2009).
9. Matthee, C. A., Engelbrecht, A. & Matthee, S. Comparative phylogeography of parasitic *Laelaps* mites contribute new insights into the specialist-generalist variation hypothesis (SGVH). *BMC Evol. Biol.* **18**, 1–11. https://doi.org/10.1186/s12862-018-1245-7 (2018).
10. Huyse, T., Poulin, R. & Théron, A. Speciation in parasites: A population genetics approach. *TRENDS Parasit.* **21**, 469–475. https://doi.org/10.1016/j.pt.2005.08.009 (2005).
11. García, B. S., Melin, A. D., Aureli, F. & de León, G. P. P. Unveiling patterns of genetic variation in parasite-host associations: an example with pinworms and Neotropical primates. *Parasit* **146**, 356–362 (2019).
12. Weinstein, S. B. & Kuris, A. M. Independent origins of parasitism in animalia. *Biol. Lett.* **12**, 20160324 (2016).
13. Barrett, L. G., Thrall, P. H., Burdon, J. J. & Linde, C. Pathogen life-history and the evolutionary potential of populations. *TRENDS Ecol. Evol.* **23**, 678–685 (2008).
14. Criscione, C. & Blouin, M. Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. *Evol* **58**, 198–202 (2004).
15. Criscione, C. D., Poulin, R. & Blouin, M. S. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. *Mol. Ecol.* **14**, 2247–2257 (2005).
16. Nieberding, C., Morand, S., Libois, R. & Michaux, J. R. Parasites and the Island syndrome: the colonization of the Western mediterranean Islands by *Heligmosomoides polygyrus* (Dujardin, 1845). *J. Biogeo.* **33**, 1212–1222 (2006).
17. Whitman, N. K., Kimball, R. T. & Parker, P. G. Co-phylogeography and comparative population genetics of the threatened Galápagos Hawk and three ectoparasite species: ecology shapes population histories within parasite communities. *Mol. Ecol.* **16**, 4759–4773 (2007).
18. Blasco-Costa, I., Waters, J. M. & Poulin, R. Swimming against the current: genetic structure, host mobility and the drift paradox in trematode parasites. *Mol. Ecol.* **21**, 207–217 (2012).
19. Blasco-Costa, I. & Poulin, R. Host traits explain the genetic structure of parasites: A meta-analysis. *Parasit* **140**, 1316–1322 (2013).
20. Benton, M. J. The red queen and the court jester: species diversity and the role of biotic and abiotic factors through time. *Science* **323**, 728–732 (2009).

21. Dawson, M. N., Louie, K. D., Barlow, M., Jacobs, D. K. & Swift, C. C. Comparative phylogeography of sympatric sister species, *Clevelandia Ios* and *Eucyclogobius newberryi* (Teleostei, Gobiidae), across the California Transition Zone. *Mol. Ecol.* **11**, 1065–1075 (2002).
22. Brant, S. V. & Loker, E. S. Molecular systematics of the avian schistosome genus *Trichobilharzia* (Trematoda: Schistosomatidae) in North America. *J. Parasit.* **95**, 941–963 (2009).
23. Ebbs, E. T. et al. Schistosomes with wings: how host phylogeny and ecology shape the global distribution of *Trichobilharzia querquedulae* (Schistosomatidae). *Int. J. Parasit.* **46**, 669–677 (2016).
24. Brant, S. V. & Loker, E. S. Discovery-based studies of schistosome diversity stimulate new hypotheses about parasite biology. *TRENDS Parasit.* **29**, 1–11. https://doi.org/10.1016/j.pt.2013.06.004 (2013).
25. Ebbs, E. T. et al. Phylogenomics and diversification of the schistosomatidae based on targeted sequence capture of ultra-conserved elements. *Pathogens* **11**, 769. https://doi.org/10.3390/pathogens11070769 (2022).
26. Horák, P. et al. Avian schistosomes and outbreaks of cercarial dermatitis. *Clin. Micro Rev.* **28**, 165–190 (2015).
27. Loker, E. S., DeJong, R. J. & Brant, S. V. Scratching the itch: updated perspectives on the schistosomes responsible for swimmer's itch around the world. *Pathogens* **11**, 587. https://doi.org/10.3390/pathogens11050587 (2022).
28. McLeod, J. A. Two new schistosomid trematodes from water-birds. *J. Parasit.* **23**, 456–466 (1937).
29. Blair, D. & Islam, K. S. The life-cycle and morphology of *Trichobilharzia australis* N. sp.(Digenea: Schistosomatidae) from the N.sal blood vessels of the black Duck (*Anas superciliosa*) in Australia, with a review of the genus *Trichobilharzia*. *Sys Parasit.* **5**, 89–117 (1983).
30. Horák, P., Kolářová, L. & Adema, C. M. Biology of the schistosome genus *Trichobilharzia*. *Adv. Parasit.* **52**, 155–233 (2002).
31. Sibley, D. A. *Sibley Guide To Birds of North America. The United States of America* (Alfred A. Knopf, 2000).
32. Sun, Z. et al. Rapid and recent diversification patterns in anseriformes birds: inferred from molecular phylogeny and diversification analyses. *PloS ONE.* **12**, e0184529. https://doi.org/10.1371/journal.pone.0184529 (2017).
33. Meehan, T. D. et al. Half-century winter Duck abundance and temperature trends in the Mississippi and Atlantic flyways. *J. Wildl. Manage.* **85**, 713–722 (2021).
34. Bush, A. O., Lafferty, K. D., Lotz, J. M. & Shostak, A. W. Parasitology Meets ecology in its own terms: Margolis et. Al. revisited. *J. Parasit.* **84**, 575–583 (1997).
35. Gonzalez, J., Düttmann, H. & Wink, M. Phylogenetic relationships based on two mitochondrial genes and hybridization patterns in Anatidae. *J. Zool.* **279**, 310–318. https://doi.org/10.1111/j.1469-7998.2009.00622.x (2009).
36. Helmer, N. et al. First record of *Trichobilharzia physellae* (Talbot, 1936) in Europe, a possible causative agent of cercarial dermatitis. *Pathogens*. 10, 1473 (2021).
37. Fujisawa, T. & Barraclough, T. G. Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. *Sys Biol.* **62**, 707–724 (2013).
38. Bouckaert, R. et al. BEAST 2.5: an advanced software platform for bayesian evolutionary analysis. *PLoS Comput. Biol.* **15**, e1006650. https://doi.org/10.1371/journal.pcbi.1006650 (2019).
39. Sire, C., Durand, P., Pointier, J. P. & Theron, A. Genetic diversity of *Schistosoma mansoni* within and among individual hosts (*Rattus rattus*): infrapopulation differentiation at micro Spatial scale. *Int. J. Parasit.* **31**, 1609–1616 (2001).
40. Morgan, J. A. et al. Origin and diversification of the human parasite *Schistosoma mansoni*. *Mol. Ecol.* **14**, 3889–3902 (2005).
41. Jones, B. P. et al. Divergence across mitochondrial genomes of sympatric members of the schistosoma indicum group and clues into the evolution of schistosoma Spindale. *Sci. Rep.* **10**, 1–14. https://doi.org/10.1038/s41598-020-57736-x (2020).
42. Gorton, M. J., Kasl, E. L., Detwiler, J. T. & Criscione, C. D. Testing local-scale panmixia provides insights into the cryptic ecology, evolution, and epidemiology of metazoan animal parasites. *Parasit* **139**, 981–997. https://doi.org/10.1017/s0031182012000455 (2012).
43. Archie, E. A. & Ezenwa, V. O. Population genetic structure and history of a generalist parasite infecting multiple sympatric host species. *Int. J. Parasit.* **41**, 89–98 (2011).
44. Louhi, K. R., Karvonen, A., Rellstab, C. & Jokela, J. Is the population genetic structure of complex life cycle parasites determined by the geographic range of the most motile host? *Infect. Genet. Evol.* **10**, 1271–1277 (2010).
45. Locke, S. A. et al. Intercontinental distributions, phylogenetic position and life cycles of species of *Apharyngostrigea* (Digenea, Diplostomoidea) illuminated with morphological, experimental, molecular and genomic data. *Int. J. Parasit.* **51**, 667–683 (2021).
46. Liu, Y., Keller, I. & Heckel, G. Range-wide genetic population structure of common Pochard (*Aythya ferina*): a potentially important vector of highly pathogenic avian influenza viruses. *Ecol. Evol.* **1**, 529–545 (2011).
47. Liu, Y., Keller, I. & Heckel, G. Breeding site fidelity and winter admixture in a long-distance migrant, the tufted Duck (*Aythya fuligula*). *Heredity* **109**, 108–116 (2012).
48. Ebbs, E. T., Loker, E. S. & Brant, S. V. Phylogeography and genetics of the globally invasive snail physa acuta Draparnaud 1805, and its potential to serve as an intermediate host to larval digenetic trematodes. *BMC Evol. Biol.* **18**, 1–17. https://doi.org/10.1186/s12862-018-1208-z (2018).
49. Wethington, A. R. & Lydeard, C. A molecular phylogeny of Physidae (Gastropoda: Basommatophora) based on mitochondrial DNA sequences. *J. Molluscan Stud.* **73**, 241–257 (2007).
50. Vinarski, M. V. The history of an invasion: phases of the explosive spread of the physid snail physella acuta through Europe, transcaucasia and central Asia. *Biol. Invasions*. **19**, 1273–1288 (2017).
51. Hill, N. J. et al. Waterfowl ecology and avian influenza in California: do host traits inform Us about viral occurrence? *Avian Dis.* **54**, 426–432 (2010).
52. Rauch, G., Kalbe, M. & Reusch, T. B. H. How a complex life cycle can improve a parasite's sex life. *J. Evol. Biol.* **18**, 1069–1075 (2005).
53. McMullen, D. B. & Beaver, P. C. Studies on schistosome dermatitis. *Am. J. Epi.* **42**, 128–154 (1945).
54. Rudolfová, J., Sitko, J. & Horák, P. Unusual finding of *Trichobilharzia* Sp. in *Motacilla Alba* in the Czech Republic. *J. Helminthol.* **80**, 83–85 (2006).
55. Prugnolle, F. Population genetics of complex life-cycle parasites: an illustration with trematodes. *Int. J. Parasit.* **35**, 255–263 (2005).
56. Lively, C. M. Habitat heterogeneity, host population structure, and parasite local adaptation. *J. Heredity*. **109**, 29–37 (2017).
57. Johnson, D. H. & Grier, J. W. Determinants of breeding distributions of ducks. *Wildl. Monogr.* **100**, 1–37 (1988).
58. Osborn, J. M., Hagy, H. M., Mcclanahan, M. D., Davis, J. B. & Gray, M. J. Habitat selection and activities of dabbling ducks during non-breeding periods. *J. Wildl. Manage.* **81**, 1482–1493 (2017).
59. Humphreys, J. M., Murrow, J. L., Sullivan, J. D. & Prosser, D. J. Seasonal occurrence and abundance of dabbling ducks across the continental united States: joint spatio-temporal modeling for the genus *Anas*. *Divers. Distrib.* **25**, 1497–1508. https://doi.org/10.1111/ddi.12960 (2019).
60. Austin, J. E., O'Neil, S. T. & Warren, J. M. Habitat selection by postbreeding female diving ducks: influence of habitat attributes and conspecifics. *J. Avian Biol.* **48**, 295–308 (2016).
61. Anteau, M. J. & Afton, A. D. Nutrient reserves of lesser Scaup (*Aythya affinis*) during spring migration in the Mississippi flyway: a test of the spring condition hypothesis. *Auk* **121**, 917–929 (2004).
62. Arzel, C., Elmberg, J. & Guillemain, M. Ecology of spring-migrating Anatidae: a review. *J. Ornith.* **147**, 167–184 (2006).
63. Combes, C. *Parasitism: the Ecology and Evolution of Intimate Interactions* (University of Chicago Press, 2001).
64. Koons, D. N., Gunnarsson, G., Schmutz, J. A. & Rotella *J. J. Drivers Waterfowl Popul. Dynamics: Teal Swans. Wildfowl* **4**, 169–191 (2014).

65. Pöysä, H. et al. Environmental variability and population dynamics: do European and North American ducks play by the same rules? *Ecol. Evol.* **6**, 7004–7014 (2016).
66. Zhao, Q., Silverman, E., Fleming, K. & Boomer, G. S. Forecasting waterfowl population dynamics under climate change—Does the Spatial variation of density dependence and environmental effects matter? *Biol. Conserv.* **194**, 80–88 (2016).
67. Afton, A. D. & Anderson, M. G. Declining Scaup populations: a retrospective analysis of long-term population and harvest survey data. *J. Wildl. Manage.* **65**, 781–796 (2001).
68. Koons, D. et al. Lesser Scaup population dynamics: what can be learned from available data? *Avian Conserv. Ecol.* **1**, 0–5751. ace-00105-010306 (2006).
69. Herrmann, K. K. & Sorensen, R. E. Differences in natural infections of two mortality-related trematodes in lesser Scaup and American Coot. *J. Parasit.* **97**, 555–558 (2011).
70. England, J. C. et al. Associations of intestinal helminth infections with health parameters of spring-migrating female lesser Scaup (*Aythya affinis*) in the upper Midwest, USA. *Parasit. Res.* **117**, 1877–1890 (2018).
71. Harmon, A., Littlewood, D. T. J. & Wood, C. L. Parasites lost: using natural history collections to track disease change across deep time. *Front. Ecol. Enviro*. **17**, 157–166 (2019).
72. Thompson, C. W. et al. Preserve a voucher specimen! The critical need for integrating natural history collections in infectious disease studies. Mbio. 12, e02698-20; 10.1128/mbio.02698-20 (2021).
73. Poulin, R. & Mouillot, D. Combining phylogenetic and ecological information into a new index of host specificity. *J. Parasit.* **91**, 511–514 (2005).
74. Avise, J. Intraspecific phylogeography: the mitochondrial-DNA Bridge between population genetics and systematics. *Annual Rev. Ecol. Sys*. **18**, 489–522 (1987).
75. Vilas, R., Criscione, C. D. & Blouin, M. S. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. *Parasit* **13**, 839–846 (2005).
76. Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods*. **9**, 772–772 (2012).
77. Stamatakis, A. RAxML V. 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313 (2014).
78. Ronquist, F. et al. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Sys Biol.* **61**, 539–542 (2012).
79. Rambaut, A. TRACER v1. 5. (2009). http://tree.bio.ed.ac.uk/software/tracer/.
80. Rambaut, A. & Drummond, A. FigTree v1. 3.1. (2009). http://tree.bio.ed.ac.uk/software/figtree/
81. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evo*. **35**, 1547 (2018).
82. Librado, P. & Rozas, J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452 (2009).
83. Leigh, J. W., Bryant, D. & Popart Full-feature software for haplotype network construction. *Methods Ecol. Evo*. **6**, 1110–1116 (2015).
84. Belkhiria, J., Alkhamis, M. A. & Martínez-López, B. Application of species distribution modeling for avian influenza surveillance in the united States considering the North America migratory flyways. *Sci. Rep.* **6**, 33161. https://doi.org/10.1038/srep33161 (2016).
85. Swanson, G. A., Hawkins, A. S., Hanson, R. D., Nelson, H. K. & Reeves, H. M. Flyways: pioneering waterfowl management in North America. *J. Wildl. Manag*. **49**, 832 (1985).
86. Lam, T. T. Y. et al. Migratory flyway and geographical distance are barriers to the gene flow of influenza virus among North American birds. *Ecol. Lett.* **15**, 24–33 (2012).
87. Pearce, J. M. et al. Avian influenza at both ends of a migratory flyway: characterizing viral genomic diversity to optimize surveillance plans for North America. *Evol. App*. **2**, 457–468. https://doi.org/10.1111/j.1752-4571.2009.00071.x (2009).
88. Excoffier, L. & Lischer, H. E. L. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and windows. *Mol. Ecol. Resour.* **10**, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x (2010).
89. Reynolds, J., Weir, B. S. & Cockerham, C. C. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics* **105**, 767–779 (1983).
90. Clarke, K. R. & Gorley, R. N. PRIMER v7 user manualp. 296, (2015).
91. Drummond, A. J., Rambaut, A., Shapiro, B. & Pybus, O. G. Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol. Biol. Evol.* **22**, 1185–1192 (2005).
92. Ho, S. Y. & Shapiro, B. Skyline-plot methods for estimating demographic history from nucleotide sequences. *Mol. Ecol. Resour.* **11**, 423–434 (2011).

Acknowledgements

Thank you to the following for invaluable help collecting birds—Museum of Southwestern Biology Bird Division, Andy Johnson, Ernest Valdez, Robert W. Dickerman and Chris Witt; National Museum Bloemfontein: Rick Nuttall and Dawie H. de Swardt.: Illinois Natural History Survey, Heath Hagy and Josh Osborne; Veronica Flores for providing specimens used in this study. Individual duck hunters: Brad Bothner, Josh Butler, Tony Gilyard, James Mckeehen, Bob Mckeehen, Matthew Snyder. Individuals who assisted with host necropsies: Keith Keller and Emily Sarvis. The authors would like to thank Drs. Eric (Sam) Loker, Charles Criscione and Thomas Turner for helpful comments on the early drafts of this manuscript. We acknowledge technical support from the University of New Mexico Molecular Biology Facility, which is supported by National Institutes of Health, USA grant P30GM110907 from the Institute Development Award program of the National Center for Research Resources, USA. This work was primarily funded through a National Science Foundation, (NSF) USA grant to S.V.B. (DEB 1021427). E.T.E was supported by NSF grant DBI 2218190.S.A.L. was supported by NSF grant DEB 1845021.

Author contributions

ETE: Contributed to all aspects of the study; Study conceptualization conceived, designed and carried out the research, analyzed and interpreted data, wrote the manuscript.DM: Contributed to sequence data collectionSAL: Contributed critical specimens, contributed to the final manuscriptND: Contributed critical specimens VVT: Contributed critical specimensSVB: Contributed data and specimens and to the study design, performed research, provided funding, discussed all aspects and contributed to the final manuscript.All authors reviewed the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-93985-4.

Correspondence and requests for materials should be addressed to E.T.E.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.