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Schistosomes with wings: how host phylogeny and ecology shape the global distribution of *Trichobilharzia querquedulae* (Schistosomatidae)☆

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Abstract

Migratory waterfowl play an important role in the maintenance and spread of zoonotic diseases worldwide. An example is cercarial dermatitis, caused when larval stages of schistosomes that normally develop in birds penetrate human skin. Members of the genus *Trichobilharzia* (Schistosomatidae), transmitted mainly by ducks, are considered to be major etiological agents of cercarial dermatitis globally. To better understand the diversity and distribution of *Trichobilharzia* spp., we surveyed ducks from the United States, eastern Canada, Argentina, South Africa and New Zealand. To aid in species identification of the *Trichobilharzia* worms recovered, regions of the *Cox*1 , ND4 and ITS1 were sequenced. Furthermore, we provide molecular phylogenetic evidence for the cosmopolitan distribution and trans-hemispheric gene flow for one species, *Trichobilharzia querquedulae,* previously thought to be restricted to North America. These new samples from endemic non-migratory duck species indicate that *T. querquedulae* transmission occurs within each of the regions we sampled and that it is specific to the blue-winged + silver teal duck clade. Prevalence within this host group is >95% across the known range of *T. querquedulae,* indicating that transmission is common. Genetic divergence is evenly distributed among continents, and no phylogenetic structure associated with geography was observed. The results provide strong support for the global distribution and transmission of *T. querquedulae* and represent, to our knowledge, the first report of a cosmopolitan schistosome confirmed by genetic data. These data are the first known to support trans-hemispheric genetic exchange in a species responsible for causing cercarial dermatitis, indicating that the epidemiology of this group of poorly known zoonotic parasites is more complex than previously expected.

Keywords: *Trichobilharzia,* Schistosome, Cercarial dermatitis, *Anas*, Zoonosis, Emerging disease

1. Introduction

Maintenance of complex life cycles over time and space is influenced by a myriad of both evolutionary and ecological forces. Determinants of host associations are essential in understanding the dynamics of any host-parasite system and for efforts to model the emergence of zoonotic parasites (Ostfeld and Keesing, 2000; Taylor et al., 2001; McKenzie, 2007). For example, the degree to which a parasite has phylogenetic and/or ecological constraints with its host range will largely determine the predictability of transmission (Thompson, 2000; Hoberg and Brooks, 2008). Within this context, we aimed to understand host associations and factors shaping the diversity and distribution of a common group of trematodes in waterfowl which are known for causing the globally re-emerging zoonotic disease human cercarial dermatitis (HCD) (de Gentile et al., 1996; Kolářová et al., 2010; Horak et al., 2015). This disease enters the human population when avian schistosome (Schistosomatidae) cercariae penetrate human skin, resulting in a severe, short-term allergic reaction (Horak et al., 2002; Kourilova et al., 2004; Kolářová et al., 2013). Although the larvae are unable to establish patent infections within a human host, there are intense immune reactions that can be long lasting and painful (Kourilova et al., 2004). While HCD is neither communicable nor fatal, it is still not without economic and epidemiologic importance and is considered a substantial public health problem (Horak and Kolarova, 2011; Soldánová et al., 2013; Horak et al., 2015). Relatively little is known about the epidemiology of HCD due to the diversity of schistosome species, the lack of data on the preferred hosts and the timing of transmission, and the large geographical scale over which outbreaks occur.

An increase in the incidence of HCD outbreaks (de Gentile et al., 1996; Larsen et al., 2004) has been linked to altered or managed habitats that support high densities of migratory and resident anseriform birds and permissive snail host species (Larsen et al., 2004; Horak and Kolarova, 2011) as well as to other forms of environmental degradation such as eutrophication of water bodies (Valdovinos and Balboa, 2008; Soldánová et al., 2013). These conditions have created an urgent need to better understand avian schistosome species diversity, distribution, host use, associated host ecology and life cycle maintenance, within a global framework. The avian schistosome genus *Trichobilharzia* (Skrjabin and Zakharov, 1920) is most frequently implicated in HCD outbreaks (Kolářová et al., 2010, 2013; Soldánová et al., 2013; Horak et al., 2015) and is also the most speciose genus within the family (Blair and Islam, 1983; Brant and Loker, 2009). Adults of *Trichobilharzia* spp. primarily infect ducks and as larvae develop within freshwater pulmonate snails in the families Physidae and Lymnaeidae (Blair and Islam, 1983; Horak et al., 2002; Brant and Loker, 2009). Species diversity and host use of *Trichobilharzia* spp. are best known in Europe and North America (Aldhoun et al., 2009; Brant and Loker, 2009; Jouet et al., 2009, 2010; Rizevsky et al., 2011; Christiansen et al., 2014; Lawton et al., 2014). But even in these well-studied areas, the presence and dynamics of particular *Trichobilharzia* spp. in other bird hosts and in other countries that share those bird hosts remain unknown. For example, are *Trichobilharzia* spp. shared across continents or does each continent have its own species, or both?

Brant and Loker (2009) investigated host-parasite associations among *Trichobilharzia* spp. within North America and found that one species, *Trichobilharzia querquedulae* (McLeod, 1937), has a clear association with a globally distributed clade of dabbling ducks (the ‘blue-wing’ group) that includes the holarctically distributed northern shovelers (*Anas clypeata*); the cinnamon (*Anas cyanoptera*) and blue-wing teal (*Anas discors*) as well as several Southern Hemisphere endemic species (Johnson and Sorenson, 1999). Species in the ‘blue-wing’ group share specific ecological preferences for shallow freshwater bodies across both their summer and winter grounds (Baldassarre et al., 1996; Kear, 2005). Additionally, *T. querquedulae* infects *Physa* spp. snails, which are ubiquitous in North America and one common species, *Physa acuta* (Draparnaud, 1805), is globally invasive (Bousset et al., 2014). Until recently, *T. querquedulae* had been reported from only the North American ‘blue-wing’ species (*A. clypeata, A. cyanoptera* and *A. discors*). Brant and Loker (2009) sampled a phylogenetically diverse range of ducks (n = 299 individuals) within North America and found that *T. querquedulae* occurred with a prevalence greater than 95%, exclusively within ‘blue-wing’ ducks. Endemic, ecologically similar species of the ‘blue-wing’ group also occur in the Southern Hemisphere (Kear, 2005), but heretofore, records for *T. querquedulae* or other schistosome species are largely lacking for these species. Is the association between *T. querquedulae* and members of the ‘blue-wing’ group consistent across hemispheres, and if so, what factors contribute to the global maintenance of this association?

2. Materials and methods

2.1. Parasite collection

Ducks were collected from several locations in both the Northern and Southern Hemispheres (Table 1); North America (United States and Canada), South America (Argentina, 30° 5' 56.4" S, 59° 29' 56.4" W), Africa (South Africa, 29° 12' 28.8" S, 27° 11' 20.4" E) and New Zealand (South Island, 44° 21' 3.6" S, 170° 12' 32.4" E). For the purposes of this paper, reasonable sampling of hosts and localities from Europe is represented in GenBank. The mesenteric and hepatic portal veins of ducks and geese were examined for species of *Trichobilharzia*. Mesenteric veins were inspected visually for adult worms, which were removed using Vanna's micro scissors. Adult worms were removed from the hepatic portal vein and liver by either crushing and washing the liver in a series of decantation steps to isolate the worms or using a syringe to push saline through the hepatic portal vein to collect any worms that washed out. Schistosome samples were preserved in 95% ethanol for genetic, and 80% ethanol for morphological analyses. Parasites were deposited in the Museum of Southwestern Biology, Division of Parasites, University of New Mexico, USA (Table 1). 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).

2.2. Genetic analysis

DNA from the samples was extracted using a DNeasy Tissue Kit (Qiagen, Valenicia, California, USA) following the manufacturer's protocol or by HotShot Lysis (Truett et al., 2000). We targeted two mitochondrial gene regions and one nuclear region for PCR amplification (Takara Ex Taq kit, Takara Biomedicals, Otsu, Japan), following protocols used by Brant and Loker (2009). The mitochondrial cytochrome oxidase subunit I (*Cox*1) gene (743 bp region, 5' end) (primers in Brant and Loker, 2009) and the NADH Dehydrogenase subunit 4 (ND4) gene (409 bp in the central portion of the gene), using the ND4 primers TSND4_F4 5' - AGTCCTTATCCGGAGCGTTA-3', TSND4_R4 5'-AACCAGCAACACACAAAAACA-3'. The ND4 gene was targeted due to high intraspecific variation relative to *Cox*1 and internal transcribed spacer 1 (ITS1) (Blouin et al., 1995; Webster et al., 2007), with a goal of assessing intraspecific relationships across hemispheres and host species. The nuclear gene region of

ITS1 (733 bp, including the 3' portion of 18S rRNA and the 5' portion of 5.8S) was amplified and sequenced using primers from Dvorak et al. (2002). Sequencing reactions were performed using the BigDye sequencing kit 3.1 (Applied Biosystems, Foster City, California, USA). Sequences were edited using Sequencher 5.3 (Gene Codes Corporation, Ann Arbor, MI, USA), aligned using ClustalW (Larkin et al., 2007) and then modified manually. Sequences generated during this study were submitted to GenBank (Table 1).

2.3. Reconstructing evolutionary relationships

Phylogenetic analyses included the samples collected herein as well as the available *T. querquedulae* samples and appropriate outgroups for Clade Q (sensu Brant and Loker, 2009; Table 1). Each gene was treated as independent and the best models of nucleotide substitution was chosen based on the Akaike information criterion (AIC; Akaike, 1974) using JModelTest (Darriba et al., 2012) as follows: TN93+G for *Cox*1 (Tamura and Nei, 1993), K2+G for ITS1 (Kimura, 1980), HKY + I for ND4 (Hasegawa, Kishino and Yano, 1985). These specific models were incorporated into Maximum Likelihood (ML) analyses.

Gene trees were analyzed independently using ML methods in the program PAUP* 4.0 (Swofford, D. L. 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA). Five hundred bootstrap replicates were preformed within each gene tree analysis to statistically assess the resulting topologies.

Bayesian Inference (BI) was performed using the program MrBayes 3.2.1 (Ronquist and Huelsenbeck, 2003), 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 2,000,000 generations and was sampled every 1000 generations. The analysis was terminated when the S.D. of the split frequencies was or fell below 0.01, supporting convergence. Likelihood parameters and convergence between runs were assessed using the program Tracer v.1.5 (Rambaut et al., 2014 Tracer v1.6. http://beast.bio.ed.ac.uk/Tracer). The first 25% of trees from each analysis was discarded as burnin. Resulting phylogenetic trees were visualized and manipulated using Fig Tree v. 1.4 (Rambaut and Drummond, 2009, FigTree v1. 3.1. http://tree.bio.ed.ac.uk/software/figtree) and MEGA 6 (Tamura et al., 2013).

Genetic distances were estimated, based on uncorrected *p*-distances between all sequences for each locus, and then averaged within lineages for comparison (Table 2), using the program MEGA 6 (Tamura et al., 2013).

3. Results

3.1. Parasite infection and molecular identification

From our collections of endemic ducks in the three countries in the Southern Hemisphere, *T. querquedulae* was found to be globally distributed in ducks from the 'blue-wing' clade as well as in the sister clade of silver teal ducks. In all three countries sampled, endemic 'blue-wing' and allied species were infected with a prevalence of 95% or greater, similar to what has been reported from the United States (Table 2).

3.2. Monophyly of T. querquedulae

Molecular phylogenetic analyses (ML, BI) show strong statistical support for the monophyly of *T. querquedulae* populations, regardless of the hemisphere sampled (Figs. 1 - 3). The resultant phylogenies show similar gene trees among nuclear and mitochondrial genes, suggesting that Southern Hemisphere samples belong to *T. querquedulae* and likely do not have a hybrid origin.

This study provides sequence data for the mitochondrial ND4 gene region of *T. querquedulae*, which had more genetic variation (Fig. 3, Table 3) relative to *Cox*1 and ITS1. Gene tree analysis (ML, BI) shows a well supported monophyly of T. *querquedulae* and a lack of phylogenetic resolution for internal nodes, concordant with the *Cox*1 and ITS1 gene trees (Figs. 1, 2).

The low nodal support within the *T. querquedulae* clade, consistent with what has been reported previously (Brant and Loker, 2009), is suggestive of a lack of geographic structure and is consistent with gene flow across hemispheres (Bouzid et al., 2008; Goulding and Cohen, 2014). Genetic distances within and between continents and hemispheres support low intraspecific divergence (Table 3). On average, for all genes analyzed, genetic divergence within North America (approximately 2%) is comparable with genetic divergence between Northern and Southern Hemisphere populations (approximately 2.7%).

4. Discussion

The survey results reported here provide the first known genetic evidence for an avian schistosome, *T. querquedulae*, to have a global distribution spanning three continents and New Zealand. Our sampling indicates that the duck host range of *T. querquedulae* extends in the Southern Hemisphere both to endemic members of the 'blue-wing' group (Cape and New Zealand shovelers), and to members of a sister clade containing the silver teal (see Johnson and Sorenson, 1999). Southern Hemisphere worms were recovered from endemic non-migratory species (Kear, 2005), thus we can infer that the infections were acquired locally. For all loci analyzed, genetic divergence within and between Northern and Southern Hemisphere individuals was low, suggesting their populations are connected by trans-hemispheric (specifically, between Northern and Southern hemispheres) migration of particular duck species. The holarctically distributed northern shoveler (*A. clypeata*), for which annual migrations between the Northern and Southern Hemispheres is common (Peters et al., 2014), is likely the primary host species facilitating gene flow across the range of *T. querquedulae*. It is also likely that populations of blue-wing and cinnamon teal (*A. cyanoptera* and *A. discors*) that breed in North America and winter in the Southern Hemisphere facilitate *T. querquedulae* transmission, as they are seasonally sympatric with the endemic 'blue-winged' ducks and their allies (silver teal) (Wilson et al., 2011). Estimated genetic distances (Table 3) indicate that across its range *T. querquedulae* populations are connected and genetically homogenized, although increased population sampling is necessary to fully elucidate phylogeographic patterns. More variable genetic markers, increased sampling and population genetic analyses are required to determine how much gene flow occurs across the hemispheres and the relative importance of different duck species in maintaining the global transmission of *T. querquedulae.*

Through targeted collections of Southern Hemisphere endemic host species, we find evidence to support the phylogenetic association between *T. querquedulae* and the 'blue-wing' group uncovered by Brant and Loker (2009) (host associations are summarized in Fig. 4). Our data suggest that the duck host range of *T. querquedulae* includes, in addition to the 'blue-wing' group, the sister clade that includes the silver teal. It is clear that the definitive hosts of *T. querquedulae* are not a random sample of *Anas* duck species and that definitive host phylogeny is important in life cycle maintenance. Below we discuss several aspects of the host and parasites biology which could maintain the observed phylogenetic association among *T. querquedulae* and their specific duck hosts.

It is possible that these patterns are maintained as a consequence of specialization that has occurred over an evolutionary time frame based on host physiology or immune competence. It has been generally thought that definitive host specificity is low within species of *Trichobilharzia* (Blair and Islam, 1983; Horak et al., 2002), however with increased taxonomic sampling of hosts and the inclusion of molecular data, patterns of duck host specificity are becoming increasingly apparent in some *Trichobilharzia* spp. (Brant and Loker, 2009, 2013a; Jouet et al., 2015). Similar phylogenetic affinities are known to occur within other avian schistosome genera. For example, *Allobilharzia* (Kolářová et al., 2006) in swans and *Anserobilhariza* (Brant et al., 2013b) in geese*,* are just two examples where avian schistosome transmission shows some phylogenetic constraints at the definitive host level. The physiological, genetic and/or immunological barriers mediating *Trichobilharzia* infections within duck hosts is currently unknown (Horak et al., 2002) and, as suggested by the present results, in need of experimental study. Experimentally, non-'blue wing' ducks such as mallards (McLeod and Little, 1942) can support egg-laying infections of *T. querquedulae*. However, natural infections of non-'blue wing' hosts appear to be rare, and our surveys over the last 10 years have recovered a single infection of an immature worm fragment within an American wigeon (*Anas americana*) in North America (S.V. Brant, unpublished data).

It is likely that host ecological preferences, which are also influenced by the hosts' evolutionary history, play a key role in the persistence of the association of *T. querquedulae* in one clade of ducks versus another. 'Blue-wing' ducks are ecological specialists (Baldassarre et al., 1996), with a preference for shallow marshy habitats. It can be hypothesized that shared habitat preferences specifically relating to nesting site selection and natal habitats can act as an encounter filter (Combes, 2001) among 'blue-wing' and allied species to restrain *T. querquedulae* host use. Nesting sites, where the majority of *Trichobilharzia* transmission is likely occurring (Rau et al., 1975; Brant and Loker, 2009), are non-randomly chosen (Clark and Shutler, 1999). Blums et al. (2002) showed that over a 23 year period female northern shovelers (*A. clypeata*) exhibited greater than 88% nest site fidelity, significantly more so than other sympatric duck species measured. Further, northern shovelers and other ‘blue-wing’ species are known to select fragmented small water bodies similar to wetland islands for nesting (Blums et al., 2002), where the pair will live and breed. Males are known to guard these ‘islands’ and exhibit territorial behavior (Poston, 1974), so much so that availability of nesting sites limits population sizes (Vickery and Nudds, 1984). Generally, single to a few pairs occur at individual nesting sites, in contrast to other dabbling and diving duck species (Vickery and Nudds, 1984), which may have more concentrated nesting sites and may be sympatric with several different species of birds. Site and habitat fidelity exhibited by the duck host likely results in stable transmission dynamics for *T. querquedulae.* This also assumes that the intermediate host is present and that at least peak cercarial shedding of *T. querquedulae* occurs during this time as well, when the shovelers and teal are isolated for nesting and raising of ducklings.

It could also be the case that the potential for temporal isolation is a mechanism for the maintenance of this global host-parasite association. Members of the ‘blue-wing’ group are the first duck species to migrate in both the spring and the winter (Arzel et al., 2006), thus it would follow that their ducklings may be the first to encounter schistosome cercariae in the spring. This hypothesis may hinge on the importance of overwintering infected snails in sustaining a majority of the transmission (Crews and Esch, 1986; Goater et al., 1989). This idea is plausible for a snail host of *T. querquedulae, Physa gyrina,* as it is bivoltine, with overwintering snails producing an early spring generation (Brown, 1979), and it is likely an abundant permissive snail host within the duck host natal habitats (Pip, 1987). Data from this study support the hypothesis that migrating birds transfer infections among continents, in particular northern shovelers, suggesting that transmission may not be strictly confined to natal habitats; however, habitat requirements by this particular clade of ducks would be the same. Nonetheless, these stimulating ideas require empirical studies documenting the dynamics of transmission at relevant spatial and temporal scales.

It is clear that for *T. querquedulae* to persist in the Southern Hemisphere, suitable snail intermediate hosts must be present. Although the identity of the intermediate host(s) for *T. querquedulae* in the Southern Hemisphere remains unknown, several possibilities exist, some of which can help account for the broad geographic range and even definitive host associations observed. In North America, *Physa* spp. are confirmed hosts (McLeod and Little, 1942; Brant and

Loker, 2009; Brant et al., 2011). Physid diversity is highest in North America (Wethingtion, 2007), but several species are native to, or have invaded, the Southern Hemisphere. South America harbors several endemic physids, which could sustain *T. querquedulae* transmission, for example, *Physa marmorata* (Guilding, 1828), which has now invaded parts of South Africa (Appleton, 2003). *Physa acuta* (Draparnaud, 1805), is a well-documented global invader that has established in much of the Southern Hemisphere (Bousset et al., 2014) and may well serve as a host for *T. querquedulae*. Physid snails commonly colonize the shallow freshwater habitats favored by 'blue wing' duck species.

Generally, *Trichobilharzia* spp. are specific to a single snail species or a group of closely-related congeners (Kock, 2001; Rudolfova et al., 2005; Brant and Loker, 2009; Jouet et al., 2009), and experimental and genetic confirmation that a species of *Trichobilharzia* can infect multiple genera of snails within a family has yet to be achieved. There is a report of an avian schistosome *Dendritobilhariza pulverulenta* (Cheatum, 1941) found in both *Gyraulus* (North America, New Zealand) and *Anisus* (Europe) snails but the genetic identity of the worms from *Anisus* have not been confirmed as *D. pulverulenta,* since there exists more than one species (Khalifa, 1976; Vusse, 1980; Brant et al., 2011) and *Dendritobilhariza loossi* has been found experimentally to use *Anisus vortex* in Europe (Akramova et al., 2011). Snail surveys carried out in Europe (Rudolfova et al., 2005; Jouet et al., 2009b) and Africa (Appleton and Brackenbury, 1998; Laamrani et al., 2005), have failed to recover any *Trichobilharzia* spp. from native or invasive physids. Two studies report schistosome cercariae from other physids in Europe (*Aplexa hypnorum,* Gerard, 2004; *Physa fontinalis,* Rudolfova et al., 2005), but neither schistosome belongs to the genus *Trichobilharzia.* It should be noted, however, that even within North America the prevalence of *T. querquedulae* in *Physa* spp. is very low and snail surveys for trematodes frequently fail to recover *T. querquedulae* and other *Trichobilharzia* spp. (Loy and Haas, 2001) from their presumptive snail hosts. However, survey collections of physids at times of the year when these duck host have ducklings are lacking, and that might be peak time for cercarial shedding.

It is even less common to find a genetically confirmed schistosome conspecific infecting two different families of snails (Blair et al., 2001). However one species, *Trichobilharzia jequitibaensis* (Leite et al., 1978), has been reported to infect both Physidae and Lymnaeidae. Miracidia obtained from naturally infected domestic Muscovy ducks (*Cairina moschata domestica*) successfully infected both *P. marmorata* and *Lymnaea columella* under experimental conditions. If such a situation were confirmed and shown to be true for *T. querquedulae*, then its broad geographic range would be more explicable since lymnaeid species diversity is much greater in the Eastern Hemisphere than physid species diversity. It will be interesting to pursue and verify these intriguing results with further experimentation, field collections and sequence analyses. These details of both definitive and intermediate host associations across a parasite's range are necessary to understand transmission dynamics as well as to model zoonotic cycles.

The example of *T. querquedulae* globally distributed within a rather narrowly defined clade of ducks is instructive for those interested in the epidemiology of HCD, and more broadly the transmission of waterfowl zoonoses. The trans-hemispheric occurrence of *T. querquedulae* is certainly aided both by the migratory behavior (trans-hemispheric migration of the northern shoveler) and habitat preferences of definitive hosts as well as habitat preferences of the snail hosts. Perhaps the shared host ecology of duck hosts (habitat preference, nest site fidelity and timing of migration) across both the Northern and Southern Hemispheres sustain transmission and perpetuate the clear phylogenetic association between *T. querquedulae* and the 'blue-wing' group. Further research is necessary to partition the relative roles of host ecology and phylogeny in shaping the transmission of *T. querquedulae* over evolutionary time. For example, experimental infections evaluating permissiveness of non 'blue-wing' duck species and other snail host species would be a logical next step providing data vital to model transmission dynamics. The greater role duck host ecology plays, the more dynamic one would expect host associations and transmission to be in response to ecological change over evolutionary time, as opposed to a strict specialist model. Human-mediated habitat alteration and climate change have been implicated in affecting the transmission of other waterfowl zoonoses (Reed et al., 2003; Fuller et al., 2012; Dijk et al., 2014) and are likely to alter *Trichobilharzia* host associations over time as well. Together with the fact that avian schistosomes of other genera infect different bird and snail lineages, we must remember that HCD is caused by a number of schistosome species, with markedly different patterns of host use, posing a challenge for those interested in understanding the epidemiology of HCD. Thus future studies on the specific ecological relationships among hosts and worms, and on how permissive other species of ducks and snails are for *T. querquedulae* will be an exciting path forward to both understanding parasite-host biology as well as the ability to more specifically model zoonotic disease emergence.

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Legends to Figures

Fig. 1. Maximum likelihood (ML) tree based on mitochondrial cytochrome oxidase subunit I (*Cox*1) sequence data. Branch support values less than 50% were not shown. Values are reported as bootstrap support from ML analysis/ posterior probabilities from Bayesian analysis. The shaded box includes all *Trichobilharzia querquedulae* individuals analyzed, bolded taxa indicate samples collected from the Southern Hemisphere. Accession numbers of other analyzed *Trichobilharzia* samples can be found in Table 1.

Fig. 2. Maximum likelihood (ML) tree based on internal transcribed spacer region 1 (ITS1) sequence data. Branch support values less than 50% are not shown; values are reported as bootstrap support from ML analysis/posterior probabilities from Bayesian analysis. The shaded box includes all *T*richobilharzia *querquedulae* individuals analyzed, bolded taxa indicate samples collected from the Southern Hemisphere. Accession numbers of other analyzed *Trichobilharzia* samples can be found in Table 1.

Fig. 3. Maximum likelihood (ML) tree based on NADH Dehydrogenase subunit 4 (ND4) sequence data. Branch support values less than 50% were not shown; values are reported as bootstrap support from ML analysis/posterior probabilities from Bayesian analysis. The shaded box includes all *Trichobilharzia querquedulae* individuals analyzed, bolded taxa indicate samples collected from the Southern Hemisphere. Accession numbers of other analyzed *Trichobilharzia* samples analyzed can be found in Table 1.

Fig. 4. Simplified *Anas* phylogeny reconstructed from Johnson and Sorensen (1999). (A) Tree depicts phylogenetic relationships within *Anas.* Clades are collapsed to include: pintails (*A. acuta, A. georgica, A. bahamensis, A. erythrorhyncha, A. capensis*), green-winged teals (*A. flavirostris, A. carolinensis, A. crecca*), Mallards (*A. laysanensis, A. luzonica, A. platyrhynchos, A. poecilorhyncha, A. zonorhyncha, A. diazi, A. rubripes, A. fulvigula, A. superciliosa, A. melleri, A. undulata, A. sparsa*), gray teals (*A. gibberifrons, A. castanea, A. bernieri*), Brown teals (*A. aucklandica, A. chlorotis*), Wigeons (*A. americana, A. sibilatrix, A. penelope, A. strepera, A. falcata*), silver teals (*A. versicolor, A. puna, A. hottentota, A. querquedula*), blue-winged teals (*A. smithii, A. rhynchotis, A. clypeata, A. cyanoptera, A. discors, A. platalea*), South American ducks (*Speculanas specularis, Amazonetta brasiliensis, Tachyeres pteneres, Lophonetta specularioides*), outgroup (*Marmaronetta, Pteronetta, Cyanochen, Aythya, Asarcornis, Chenonetta, Callonetta, Tadorna,Cairina, Aix, Sarkidiorni*). (B) Tree depicts interspecific relationships within the ‘blue-winged’ teal clade (shaded in A). Host taxa are labeled by infection status with *Trichobilharzia querquedulae* (★· experimental host (McLeod and Little, 1942); □, host not examined; ◆, host examined/positive; ■, host examined/negative). All survey data summarized was preformed by the authors with the exception of *A. querquedulae* (Kolarova et al.,1997).

Figure 1

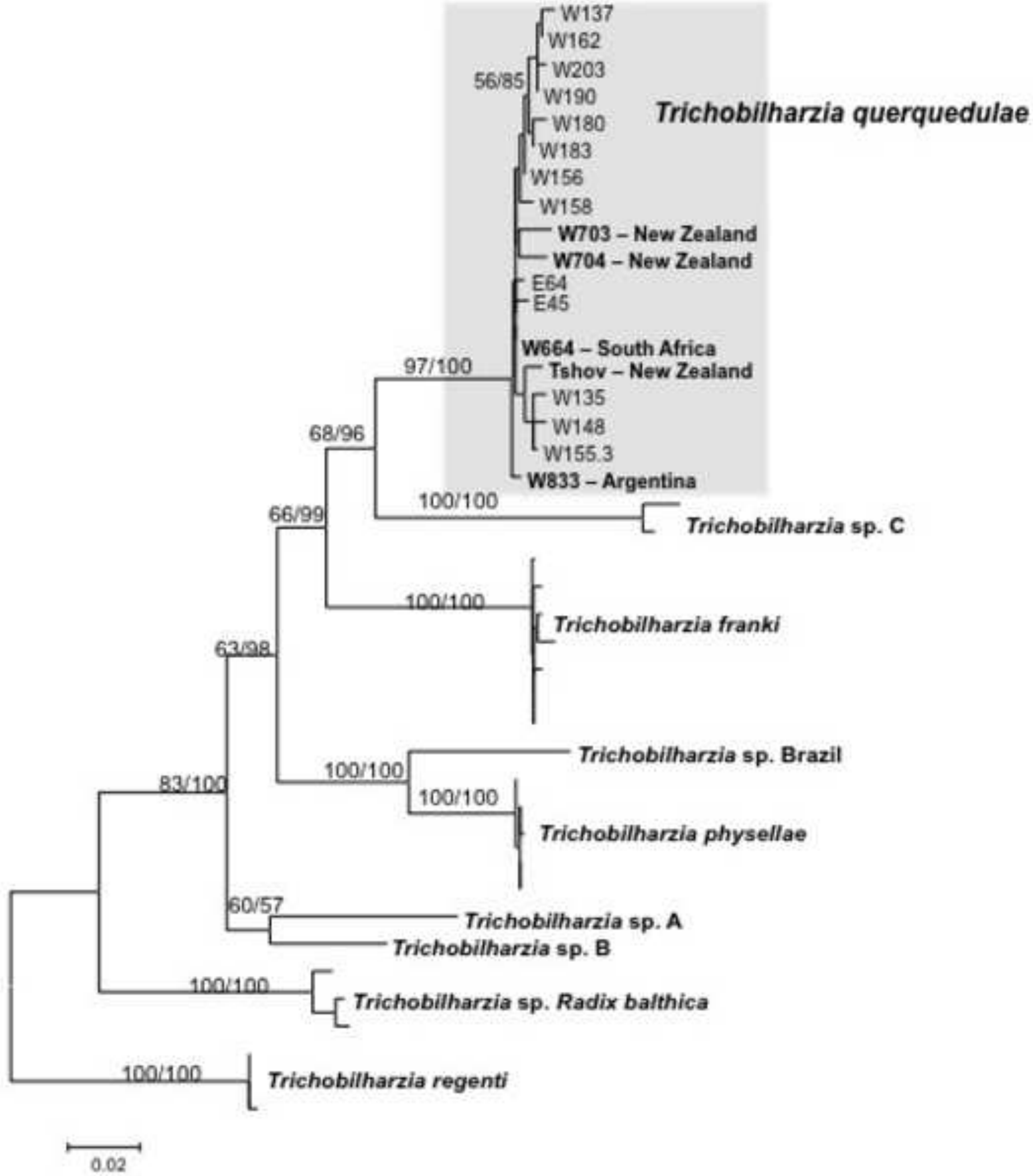

W137
W162
W203
W190
56/85
W180
W183
W156
W158
Trichobilharzia querquedulae
W703 – New Zealand
W704 – New Zealand
E64
E45
W664 – South Africa
97/100
Tshov – New Zealand
W135
W148
W155.3
68/96
W833 – Argentina
100/100
Trichobilharzia sp. C
66/99
100/100
Trichobilharzia franki
63/98
Trichobilharzia sp. Brazil
100/100
83/100
100/100
Trichobilharzia physellae
60/57
Trichobilharzia sp. A
Trichobilharzia sp. B
100/100
Trichobilharzia sp. Radix balthica
100/100
Trichobilharzia regenti
0.02

Figure 2

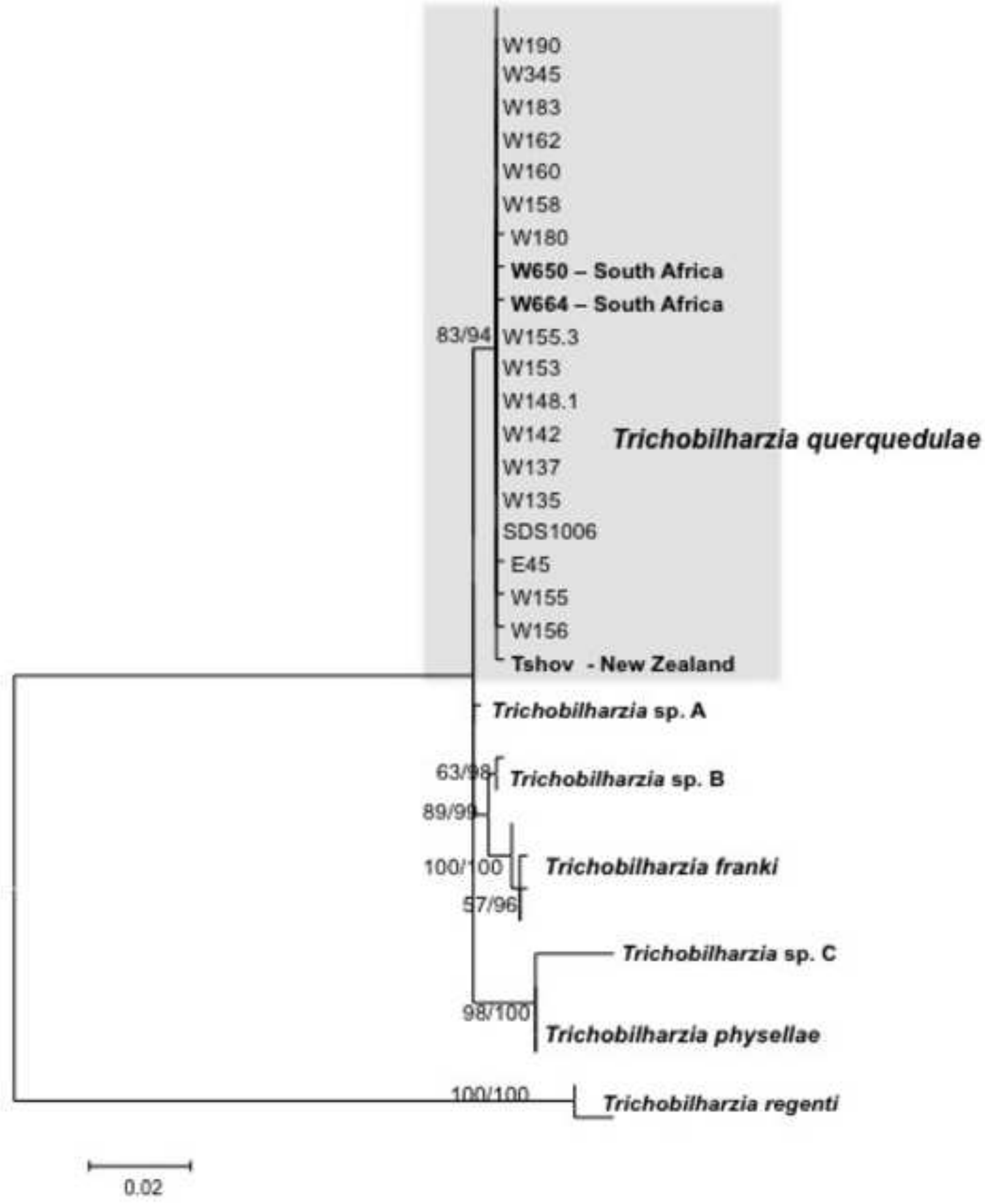

W190
W345
W183
W162
W160
W158
W180
W650 – South Africa
W664 – South Africa
83/94
W155.3
W153
W148.1
W142
W137
W135
SDS1006
E45
W155
W156
Tshov - New Zealand
Trichobilharzia querquedulae
Trichobilharzia sp. A
63/98
Trichobilharzia sp. B
89/99
100/100
Trichobilharzia franki
57/96
Trichobilharzia sp. C
98/100
Trichobilharzia physellae
100/100
Trichobilharzia regenti
0.02

Figure 3

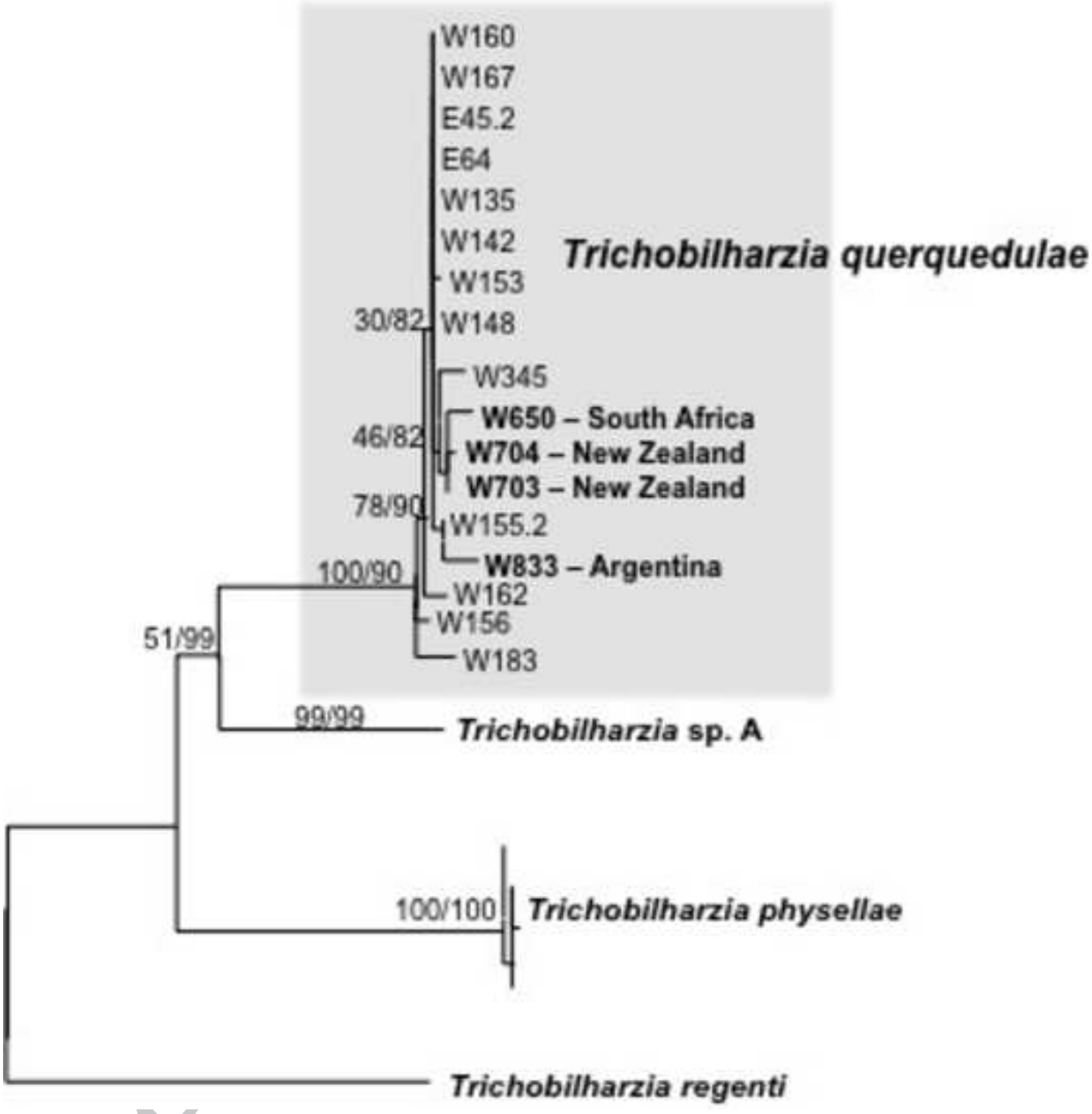

W160
W167
E45.2
E64
W135
W142
W153
30/82
W148
Trichobilharzia querquedulae
W345
46/82
W650 – South Africa
W704 – New Zealand
W703 – New Zealand
78/90
W155.2
W833 – Argentina
100/90
W162
W156
51/99
W183
99/99
Trichobilharzia sp. A
100/100
Trichobilharzia physellae
Trichobilharzia regenti

Figure 4

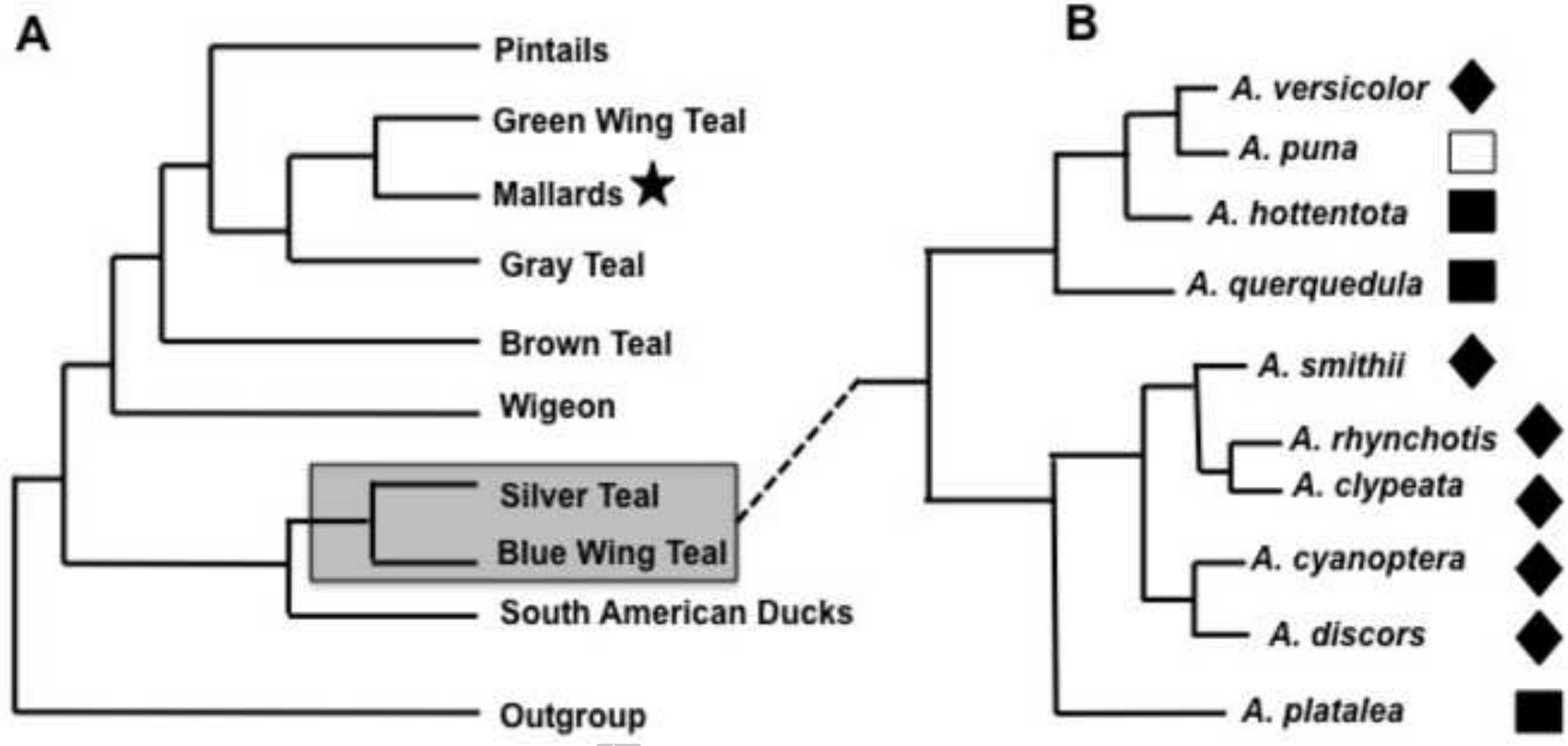

A
Pintails
Green Wing Teal
Mallards ★
Gray Teal
Brown Teal
Wigeon
Silver Teal
Blue Wing Teal
South American Ducks
Outgroup
B
A. versicolor ◆
A. puna □
A. hottentota ■
A. querquedula ■
A. smithii ◆
A. rhynchotis ◆
A. clypeata ◆
A. cyanoptera ◆
A. discors ◆
A. platalea ■

Table 1. Host association and locality origin of the specimens used in this study.

				Gene Accession Number				
	Species	**Host**	**Locality**	***Cox*1**	**ND4**	**ITS**	**MSB Catalog #[a]**	**Reference**
W135	*Trichobilharzia querquedulae*	*Anas clypeata*	LA	FJ174497	KU057197	FJ174557	183	Brant and Loker, 2009
W137	*T. querquedulae*	*Anas discors*	LA	FJ174498	-	FJ174558	19497	Brant and Loker, 2009
W142	*T. querquedulae*	*A. discors*	LA	-	KU057196	KP788761	19502	Brant and Loker, 2009
W148.1	*T. querquedulae*	*Anas cyanoptera*	NM	FJ174499	KU057194	FJ174559	18584	Brant and Loker, 2009
W148.2	*T. querquedulae*	*A. cyanoptera*	NM	FJ174500	-	-	18584	Brant and Loker, 2009
W153	*T. querquedulae*	*A. clypeata*	NM	-	KU057192	KP788763	18587	Brant and Loker, 2009
W155	*T. querquedulae*	*A. cyanoptera*	NM	-	-	FJ174553	18589	Brant and Loker, 2009
W155.2	*T. querquedulae*	*A. cyanoptera*	NM	-	KU057200	-	18589	Brant and Loker, 2009
W155.3	*T. querquedulae*	*A. cyanoptera*	NM	FJ174501	-	KP788764	18589	Brant and Loker, 2009
W156	*T. querquedulae*	*A. discors*	NM	FJ174502	KU057191	FJ174554	18590	Brant and Loker, 2009

W158	*T. querquedulae*	*A. clypeata*	NM	FJ174503	-	FJ174549	18592	Brant and Loker, 2009
W160	*T. querquedulae*	*A. discors*	NM	-	KU057201	KP788766	18594	Brant and Loker, 2009
W162	*T. querquedulae*	*A. clypeata*	NM	FJ174504	KU057190	FJ174551	18602	Brant and Loker, 2009
W167	*T. querquedulae*	*A. discors*	NM	-	KU057202	-	-	Brant and Loker, 2009
W180	*T. querquedulae*	*A. cyanoptera*	CA	FJ174505	-	FJ174556	18573	Brant and Loker, 2009
W183	*T. querquedulae*	*A. clypeata*	CA	FJ174506	KU057189	FJ174560	18575	Brant and Loker, 2009
W190	*T. querquedulae*	*A. discors*	CA	FJ174507	-	FJ174550	18582	Brant and Loker, 2009
W203	*T. querquedulae*	*A. clypeata*	AK	FJ174508	-	FJ174552	18636	Brant and Loker, 2009
W345	*T. querquedulae*	*A. clypeata*	MB	FJ174509	KU057185	FJ174547	18626	Brant and Loker, 2009
W650	*T. querquedulae*	*Anas smithii*	ZA	-	KU057205	KP788765	18990	This study
W664	*T. querquedulae*	*A. smithii*	ZA	KU057180	-	KP788762	19000	This study
W703	*T. querquedulae*	*Anas rhynchotis*	NZ	KU057181	KU057204	-	20792	This study
W704	*T. querquedulae*	*A. rhynchotis*	NZ	KU057182	KU057203	-	20793	This study
Tshov	*T. querquedu*	*A. rhynchoti*	NZ	KU057183	-	KP788760	2079	This study

	lae	*s*					4	
W833	*T. querquedulae*	*Anas versicolor*	AR	KU057184	KU057206	-	-	This study
E45	*T. querquedulae*	*A. discors*	FL	FJ174510	-	FJ174555	-	Brant and Loker, 2009
E45.2	*T. querquedulae*	*A. discors*	FL	-	KU057199	-	-	Brant and Loker, 2009
E64	*T. querquedulae*	*A. discors*	FL	FJ174511	KU057198	-	-	Brant and Loker, 2009
SDS1006	*T. querquedulae*	*A. clypeata*	NE	-	-	FJ174548	-	Brant and Loker, 2009
W206.2	*Trichobilharzia sp. A*	*Anas americana*	AK	-	-	KP788771	-	Brant and Loker, 2009
W210	*Trichobilharzia sp. A*	*A. americana*	AK	-	-	KP788772	-	Brant and Loker, 2009
W213	*Trichobilharzia sp. A*	*A. americana*	AK	FJ174527	-	FJ174570	18636	Brant and Loker, 2009
W149	*Trichobilharzia sp. A*	*A. americana*	NM	-	KU057193	-	18585	Brant and Loker, 2009
W205	*Trichobilharzia sp. B*	*A. americana*	AK	FJ174528	-	KP788770	18638	Brant and Loker, 2009
W173	*Trichobilharzia sp. C*	*Lophodytes cucullatus*	PA	FJ174529	-	FJ174576	-	Brant and Loker, 2009
W175	*Trichobilharzia physellae*	*Anas platyrhynchos*	PA	-	-	KP788768	-	Brant and Loker,

								2009
W196	*Trichobilharzia physellae*	*Aythya affinis*	NM	-	-	KP788767	-	Brant and Loker, 2009
W263	*T. physellae*	*Physa gyrina*	NM	FJ174523	*	FJ174562	178	Brant and Loker, 2009
RSFO1	*Trichobilharzia franki*	*Radix auricularia*	FR	-	-	AY795572	-	Ferte et al., 2005
RSFO3	*T. franki*	*R. auricularia*	FR	HM131198	-	AY795573	-	Ferte et al. 2005
HamRa6	*T. franki*	*R. auricularia*	GB	-	-	KJ775868	-	Lawton et al. 2014
HamRa7	*T. franki*	*R. auricularia*	GB	-	-	KJ775869	-	Lawton et al, 2014
CYA18	*Trichobilharzia regenti*	*Cygnus olor*	FR	HM439500	-	HM439497	-	Jouet et al. 2010
DQ859919	*Trichobilharzia regenti*	*Radix peregra*		-	GeneID: 5333425	-	-	Webster et al. 2007

[a] All samples collected for this study have been deposited in the Museum of Southwestern Biology (MSB) Division of Parasites at the University of New Mexico, USA; parasite and hosts records (including GPS coordinates) can be accessed by the associated catalog number via the Arctos database (http://arctos.database.museum/).

LA, Louisiana USA; NM, New Mexico USA; CA, California USA; AK, Alaska USA; FL, Florida USA, NE, Nebraska USA; PA, Pennsylvania USA; FR, France; GB, England United Kingdom; MB, Manitoba Canada; ZA, Freestate South Africa; NZ, South Island New Zealand; AR, Corrientes Argentina; CO1, cytochrome oxidase subunit I; ND4, NADH Dehydrogenase subunit 4; ITS1, internal transcribed spacer 1.

Table 2. Prevalence of *Trichobilharzia querquedulae* within members of the 'blue-wing' clade of dabbling ducks and their allies (Johnson and Sorenson, 1999).

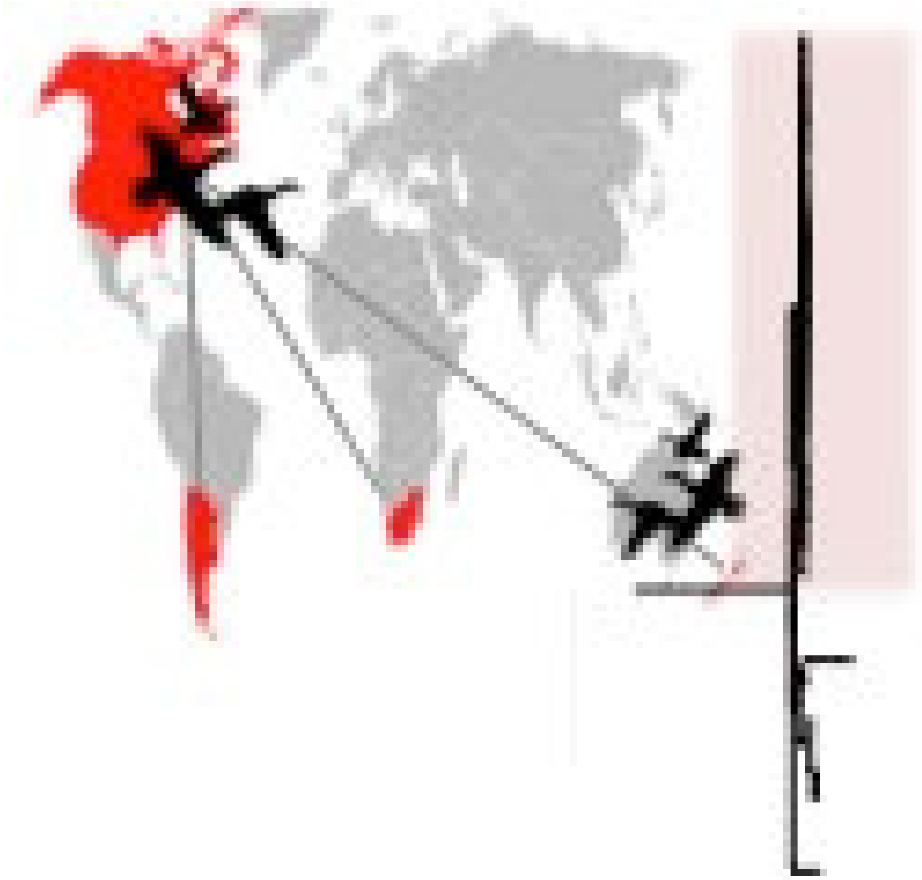
Species	**Location**	**Examined (*n*)**	**Infected (*n*)**	**Reference**
Anas clypeata	North America	22	20	Brant and Loker, 2009
Anas discors	North America	20	20	Brant and Loker, 2009
Anas discors	North America	184	175	Garvon et al., 2011
Anas cyanoptera	North America	12	12	Brant and Loker, 2009
Anas smithii	South Africa	2	2	This study
Anas hottentota	Kenya	2	0	This study
Anas rhynchotis	New Zealand	3	3	This study
Anas versicolor	Argentina	3	3	This study
Anas platalea	Argentina	1	0	This study

Table 3. Genetic distances comparing internal transcribed spacer 1 (ITS1), cytochrome oxidase subunit I (*Cox*1) and NADH Dehydrogenase subunit 4 (ND4) gene regions within and between global samples of *Trichobilharzia querquedulae* and closely related *Trichobilharzia* spp.

	Gene		
Species	***Cox*1**	**ND4**	**ITS1**
T. querquedulae within NA	0.010	0.012	0.001
T. querquedulae within SH	0.012	0.017	0.004
T. querquedulae between NA and SH	0.012	0.015	0.003
T. querquedulae all sequences	0.011	0.012	0.002
T. querquedulae vs. *Trichobilharzia franki*	0.077	-	0.002
T. querquedulae vs. *Trichobilharzia* sp. A	0.082	0.11	0.002
T. querquedulae vs. *Trichobilharzia physellae*	0.082	0.13	0.002

NA, North America; SH, Southern Hemisphere.

Graphical abstract



Highlights

- Endemic Southern Hemisphere duck species were sampled for *Trichobilharzia* adults
- *Trichobilharzia querquedulae* were recovered from a clade of non-migratory Southern Hemisphere ducks
- A globally maintained phylogenetic association of *T. querquedulae* with a duck host clade was revealed
- We report the first known cosmopolitan schistosome species confirmed by molecular data