# An Exploration of Gastropod-Borne Parasites with a Focus on Intestinal Schistosomiasis in *Biomphalaria* Snails Inhabiting the Great African Lakes

By

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for a Doctor of Philosophy Degree (PhD).

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## Declaration:

I declare that this thesis has been composed by myself and that the work has not been submitted for any other degree or professional qualification. I confirm that the work submitted is my own.

Materials and data were supplied as follows: Chapters 3, 4 and 5 use *Biomphalaria* samples originally collected by Candia Rowel and Besigye Fred as a part of the Schistosomiasis in Mothers and Infants (SIMI) project. The SIMI project was funded by the Wellcome Trust foundation and headed by Professor J. Russell Stothard at the Liverpool School of Tropical Medicine from 2008-2012. Additionally, Chapter 5 uses some of the *Biomphalaria* samples and abiotic datasets and some of the genetic diversity and sequencing data published by Claire J. Standley in her 2011 PhD thesis. All *Biomphalaria* samples were provided to me by Professor J. Russell Stothard.

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I dedicate this PhD thesis to my entire family for their support during my studies. I would like to extend my deepest gratitude to my grandmother, Susan, whose support has made this thesis a reality and I am forever grateful to her. Additionally, I would like to express my appreciation to my mother, Jennifer, who has been my constant source of love, support, and care throughout my life. Her sacrifices and unwavering support have been the backbone that has kept me going through the toughest times. Her dedication and love has been an inspiration to me, and I am eternally grateful for the sacrifices she has made for me, despite winding her up every chance I get. Lastly, I would like to express my unwavering love to my fiancée, Yitong, who I met during the first year of my PhD. Her encouragement throughout my PhD has been a source of constant motivation, and her support has been invaluable in these last four years.

*"What happens on Earth stays on Earth"* - Kendrick Lamar, 2017

Abstract:

Gastropod-borne parasitic diseases are a significant concern for public health all over the world, but particularly in developing countries. Many species of terrestrial and freshwater gastropods serve as the intermediate host for various parasites with medical and/or veterinary significance. One such example is the intravascular trematode species, *Schistosoma mansoni* (Digenea: Schistosomatidae), which is the leading cause of intestinal schistosomiasis globally. The obligatory intermediate host of *S. mansoni* is the freshwater snail genus, *Biomphalaria* (Gastropoda: Planorbidae). The vast majority of intestinal schistosomiasis cases occur in sub-Saharan regions of Africa, with countries in East Africa suffering from a high prevalence of *S. mansoni* in and around the Great African Lakes. The hyper-endemic nature of intestinal schistosomiasis at Lake Albert and Lake Victoria is a significant public health concern.

In addition to trematodes, other gastropod-borne parasites such as lungworms (Strongylida) are common causes of disease in humans and companion animals. Unlike digenetic trematodes, lungworms do not specialise in their choice of obligatory intermediate host and can use numerous species of both terrestrial and freshwater gastropods to infect their definitive host, typically through consumption. The parasitic nematode genus, *Angiostrongylus* (Chromadorea: Angiostrongylidae) for example, contains species that cause angiostrongyliasis in humans (*An. cantonensis*) and dogs (*An. vasorum*). Despite being endemic to tropical regions, an *An. cantonensis* infection was recently reported in France as the first ever autochthonous human case of angiostrongyliasis in mainland Europe. Conversely, *An. vasorum* is found globally. In the UK, it is most prevalent in Southern England and Southern Wales, though there have been reports of the parasite as far north as Scotland.

Main Findings:

Chapter 3 "Comparing shell size and shape with canonical variate analysis of sympatric *Biomphalaria* species within Lake Albert and Lake Victoria" utilises landmark-based geometric morphometric techniques to differentiate the conchological characteristics of four *Biomphalaria* species inhabiting the Great African Lakes of Uganda. The study found that it was possible to accurately discriminate and identify all *Biomphalaria* species present at the Great African Lakes in Uganda (*B. choanomphala*, *B. pfeifferi, B. stanleyi and B. sudanica*) using a canonical variate analysis (CVA) of the apical and apertural angles of the shell.

Chapter 4 "*Schistosoma mansoni* infection in *Biomphalaria* snails at the Ugandan shorelines of Lake Albert and Lake Victoria" uses PCR-based molecular infection detection methods to quantify the prevalence of *S. mansoni* infection among the *Biomphalaria* species present at the Ugandan shorelines of Lake Albert (*B. pfeifferi*, *B. stanleyi* and *B. sudanica*) and Lake Victoria (*B. choanomphala*). It also measures prevalence of infection for each of the wet and dry seasons over a two year period for both lakes. The study found that the mean prevalence of *S. mansoni* infection was higher at Lake Albert (12.5%) than Lake Victoria (5%), with *B. stanleyi* (15%) having the highest mean infection prevalence of the four species tested. In addition, the wet seasons at both lakes had a higher mean prevalence of infection than the dry seasons, though this difference was not statistically significant.

Chapter 5 "*Schistosoma mansoni* infection and population genetic structure of *Biomphalaria choanomphala* snails in Lake Victoria" uses PCR-based molecular infection detection methods to quantify the prevalence of *S. mansoni* infection across the Kenyan, Tanzanian and Ugandan shorelines of Lake Victoria. Additionally, several abiotic (temperature, pH, physiochemical etc.) and biotic (snail genetic diversity) factors were measured to investigate which factors are involved in schistosomiasis transmission. The study found the mean prevalence of *S. mansoni* infection among *B. choanomphala* snails across Lake Victoria was 9.3%, with the Tanzanian shoreline having the highest prevalence, followed by the Ugandan and Kenyan shorelines. There was a significant positive relationship with infection prevalence and *B. choanomphala* abundance, calcium, and magnesium concentrations. Conversely, there was a significant negative correlation between infection prevalence and increasing water alkalinity. Lastly, populations of *B. choanomphala* where *S. mansoni* infection was present had a higher mean haplotype diversity score and less private haplotypes than populations without infection present.

Chapter 6 "Nematodes and trematodes associated with terrestrial gastropods in Nottingham, England" implements traditional parasitological and molecular identification techniques to survey the terrestrial gastropod populations in and around the city of Nottingham, with the intention of finding medical (or veterinary) important parasites. The study found the mean infection prevalence of terrestrial gastropods was 28.3%, with slugs and snails having similar prevalence of infection. Of the gastropod-borne parasites extracted, seven nematode species and four trematode species were identified. No medical or veterinary important parasites were discovered in or around the city of Nottingham.

## Preface:

This thesis is comprised of seven chapters:

Chapter 1 entitled "General Introduction" details an introductory background on the relationship between gastropods and helminthic parasites (nematodes and trematodes), with the main focus being on intestinal schistosomiasis.

Chapter 2 entitled "General Material and Methods" details a description of the overall general techniques used in each of my result chapters.

Chapter 3 entitled "Comparing shell size and shape with canonical variate analysis of sympatric *Biomphalaria* species within Lake Albert and Lake Victoria" is the first results chapter and details the conchological differences between the *Biomphalaria* species found at the Ugandan Great African Lakes using landmark-based geometric morphometrics. This chapter was published in the Zoological Journal of the Linnean Society, 2023.

Chapter 4 entitled "*Schistosoma mansoni* infection in *Biomphalaria* snails at the Ugandan shorelines of Lake Albert and Lake Victoria" is the second results chapter and details the prevalence of *S. mansoni* infection among the *Biomphalaria* species found at the Great African Lakes using molecular based infection detection techniques.

Chapter 5 entitled "*Schistosoma mansoni* infection and population genetic structure of *Biomphalaria choanomphala* snails in Lake Victoria" is the third results chapter and details the effects abiotic factors and snail host genetic diversity have on the prevalency of *S. mansoni* infection among *B. choanomphala* populations across Lake Victoria using molecular based infection detection techniques.

Chapter 6 entitled "Nematodes and trematodes associated with terrestrial gastropods in Nottingham, England" is the last results chapter and details the common nematode and trematode parasites found inside terrestrial gastropods in the city of Nottingham. This chapter was published in the Journal of Helminthology, 2022.

Chapter 7 entitled "General Discussion" details a general discussion of the main findings of my study.

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# Chapter 1 General Introduction:

## 1.1 Gastropod-Borne Parasitic Disease:

Zoonotic diseases are responsible for as much as 75% of emerging infectious disease worldwide (Taylor et al. 2001). These diseases can be difficult to control as they often have life cycles involving multiple hosts and can be transmitted from a variety of animal reservoirs. However, it is important to understand the transmission pathways and other factors that contribute to the spread of zoonotic diseases in order to develop effective strategies for disease management and prevention. The phylum Mollusca is the second-largest phylum in the animal kingdom. It has approximately 85,000 extant species, the majority of which belong to the class, Gastropoda (~62,000; Chapman, 2009; Barker, 2001). Gastropods are well-known to serve as hosts for various helminthic parasites (such as nematodes and trematodes) due to their ability to colonise and thrive in a variety of terrestrial, freshwater and marine ecosystems (Adema et al., 2012). Gastropod-borne parasitic diseases continue to be a significant concern for global public health, especially in developing countries. Lu et al. (2018) lists over 140 species from 20 gastropod families that act as vectors and/or intermediate hosts for well-known nematode (*Angiostrongylus*) and trematode (*Clonorchis, Fasciolopsis, Fasciola, Opisthorchis*, *Paragonimus* and *Schistosoma*) species which affect hundreds of millions of people in around 90 countries. In addition to causing human disease, gastropod-borne parasitic diseases cause a significant impact on the health of livestock and companion animals (Taubert et al., 2009; Knubben-Schweizer & Torgerson, 2015). Moreover, its estimated over 18,000 digenean trematode species and around 50 metastrongyloid nematode species use gastropods as their intermediate hosts and are of medical (or veterinary) concern (Giannelli et al., 2016).

### 1.1.1 Gastropod-Borne Parasitic Diseases Caused by Nematodes:

Nematodes evolved various types of symbiotic relationships with terrestrial and aquatic gastropods, with some species having phoretic (*Caenorhabditis elegans*), parasitic (*Agfa flexilis*), paratenic or pathogenic (*Phasmarhabditis hermaphrodita*) relationships with their gastropod host (Grewal et al., 2003). Based on current phylogenetic relationships of the different nematode families, it appears that relationships with gastropods have evolved independently multiple times (Grewal et al., 2003). However, these instances are primarily limited to two groups of nematodes: those who use gastropods as their intermediate host (e.g. metastrongyloids) and those who use gastropods as their definitive host (e.g. rhabditids). Among the 61 nematode species that use gastropods as their intermediate host, 49 of them belong to the order Strongylida. Similarly, of the 47 nematode species that use gastropods as their definitive host, 33 belong to the Order Rhabditida. A prominent example of a metastrongyloid species is the rat lungworm, *Angiostrongylus* (*An.*) *cantonensis*, a zoonotic parasite that infects rats and humans (Figure 1.1). In addition to *An. cantonensis*, there are multiple lungworm species that have veterinary importance as they infect important livestock (*Dictyocaulus filaria* and *Protostrongylus rufescens*) and companion animals (*Angiostrongylus vasorum* and *Crenosoma vulpis*; Figure 1.1).

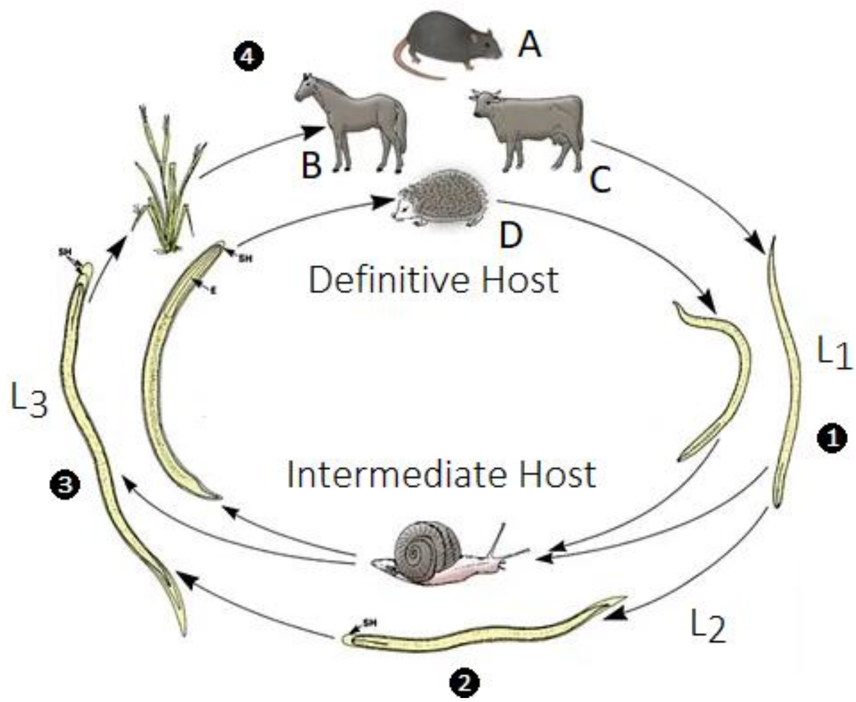


Figure 1.1. The generic life cycle of several different lungworms species. (A) *Angiostrongylus spp.*, (B) *Dictyocaulus spp.*, (C) *Protostrongylus spp.* and (D) *Crenosoma spp.* The first stage (Larva stage 1) is the rhabditiform stage of the lifecycle and is directly after the excreted eggs hatch. The second stage (L2) occurs when the rhabditiform larva moults and matures into a filariform larva. The third stage (L3) is known as the infective stage. At this stage, the larva is mature enough to infect its definitive host when it comes into contact with it. The fourth stage is the final stage, where the larva is fully developed into an adult and is ready to reproduce within its definitive host. Adapted from Mehlhorn, (2008).

### 1.1.2 Gastropod-Borne Parasitic Diseases Caused by Trematodes:

The Digenea are a subclass of the Trematoda that are obligatory internal parasites. Digenetic trematode species have a more complex lifecycle than nematodes, which can involve one to four hosts and various morphologically distinct forms (Cribb et al., 2003; Figure 1.2). The Digenea are comprised of around 80 families and 6,000 described species, with only a dozen species being known to actively infect humans (Olson et al., 2003; Table 1.1). The *Schistosoma* genus is the most important among these species, with an estimated 240 million people being afflicted with schistosomiasis worldwide (WHO, 2022a). The other non-schistosome species that infect humans have a prevalence ranging from the tens of millions (clonorchiasis, fascioliasis, fasciolopsiasis, heterophyiasis, opisthorchiasis and paragonimiasis) to hundreds of thousands (metagonimiasis) to just only a few recorded cases (brachylaimiasis and gastrodiscoidiasis; Cribb et al., 2003; Olson et al., 2003; Table 1.1).

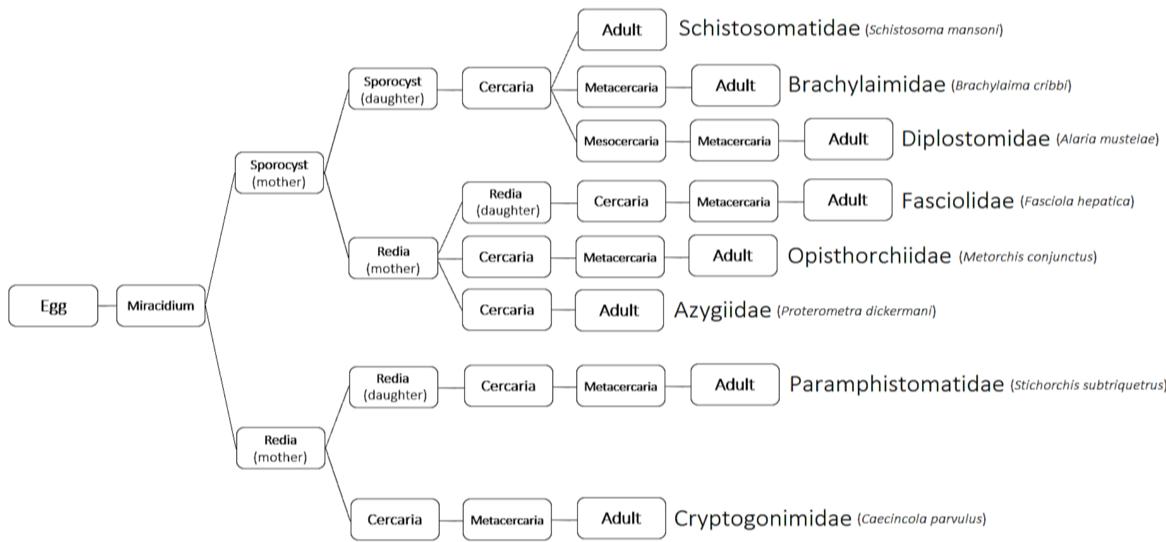


Figure 1.2. Life cycles stages of different digenean trematode families. Adapted from Schell, (1970).

Table 1.1 Examples of human pathogenic digenic trematode species. Information taken from Cribb et al. (2003) and Olson et al. (2003).

| Family | Species | Gastropod Host | Mode of Human Infection |
|---|---|---|---|
| Brachylaimidae | *Brachylaima cribbi* | *Cernuella spp.* | Snails |
| Fasciolidae | *Fasciolopsis buski*<br>*Fasciola hepatica* | *Segmentina spp.*<br>*Galba spp.* | Plants<br>Plants |
| Heterophyidae | *Heterophyes heterophyes*<br>*Metagonimus yokogawaii* | *Pirinella spp.*<br>*Semisulcospira spp.* | Fish<br>Fish |
| Opisthorchiidae | *Clonorchis sinensis*<br>*Opisthorchis viverrini* | *Bulinus spp.*<br>*Bithynia spp.* | Fish<br>Fish |
| Paragonimidae | *Paragonimus westermani* | *Oncomelania spp.* | Crustacea |
| Paramphistomidae | *Gastrodiscoides hominis* | *Helicorbis spp.* | Plants |
| Schistosomatidae | *Schistosoma mansoni*<br>*Schistosoma haematobium*<br>*Schistosoma japonicum* | *Biomphalaria spp.*<br>*Bulinus spp.*<br>*Oncomelania spp.* | Snails<br>Snails<br>Snails |

## 1.2 An Introduction to Schistosomiasis:

In 1851, Theodor Bilharz discovered schistosomiasis (initially naming it Bilharzia), with Pirajá da Silva subsequently detailing the entire transmission cycle of the disease in 1908 (Mahdy et al., 2017). Human schistosomiasis is a tropical disease caused by six intravascular trematode species in the genus *Schistosoma* (Trematoda: Schistosomatidae). Of the 23 recognised species of *Schistosoma*, *S. haematobium* and *S. mansoni* cause the vast majority (>99%) of schistosomiasis infections globally (Jamison et al., 2006; Merrifield et al., 2016). Depending on the species of *Schistosoma*, the disease can take the form of either intestinal or urogenital schistosomiasis (Table 1.2). Other uncommon *Schistosoma* species such as *S.*

*guineensis*, *S. intercalatum, S. japonicum* and *S. mekongi* also cause intestinal schistosomiasis in humans, but are restricted to specific regions of Central Africa, East Asia and Southeast Asia, respectively (Crompton, 1999; Sturrock, 2001). Each species of *Schistosoma* uses a specific snail genus as an intermediate host to spread the disease through freshwater sources contaminated by infected human (or animal) waste (Gryseels et al., 2006; Table 1.2). The World Health Organisation (WHO) considers schistosomiasis as the third worst tropical disease after tuberculosis and malaria, while the Center for Disease Control and Prevention (CDC) says it is the second most devastating parasitic disease after malaria. Today, an estimated 240 million people are currently infected worldwide, with approximately 90% of cases concentrated in Africa (~85% in sub-Saharan Africa; Boko et al., 2016; WHO, 2022a). The sub-Saharan African countries Nigeria (29 million cases of schistosomiasis), the United Republic of Tanzania (19 million), Mozambique (19 million), Ghana (15 million) and the Democratic Republic of Congo (15 million) have the highest prevalence of the disease and make up more than a third of global schistosomiasis cases (Boko et al., 2016; Onasanya et al., 2021). Furthermore, schistosomiasis is found in 78 countries and is endemic in 54 countries worldwide, with a potential 700-800 million people at risk of infection from daily work-related (farming, fishing etc.) and recreational (swimming, bathing etc.) activities (WHO, 2013; Figure 1.3; Table 1.3).

Table 1.2 The prevalence of human infection for the six major *Schistosoma* species that account for all human infection and their most common intermediate hosts. Information collected from Crompton (1999); Van Der Werf et al. (2003); Colley et al. (2014); Zhu et al. (2017) and Khieu et al. (2019).

| | Form | Infected and at Risk | Intermediate Host |
|---|---|---|---|
| *Schistosoma haematobium* | Urogenital | ~113.9 million infected (~436 million at risk) | *Bulinus* (O. F. Müller, 1781) |
| *Schistosoma mansoni* | Intestinal | ~83.3 million infected (~393 million at risk) | *Biomphalaria* (Preston, 1910) |
| *Schistosoma intercalatum* | Intestinal | ~1.73 million infected | *Bulinus* (O. F. Müller, 1781) |
| *Schistosoma guineensis* | | | |
| *Schistosoma japonicum* | Intestinal | ~1.55 million infected (~65 million at risk) | *Oncomelania* (Gredler, 1881) |
| *Schistosoma mekongi* | Intestinal | ~0.91 million infected (~0.15 at risk) | *Neotricula* (Temcharoen, 1971) |

Note: Illustrations from Pilsbry (1915) and Oberholzer & Van Eeden (1967).

Table 1.3 Global statics of schistosomiasis infection. Estimations supplied by Utroska et al. (1990); Zhou (2007); Danso-Appiah et al. (2013); WHO (2013); PAHO (2014); CDC (2017) and WHO (2019).

| | People Infected (Million) | People at Risk (Million) | No. of Countries |
|---|---|---|---|
| Worldwide | ~240 | ~700-800 | 78/195 |
| Africa | ~216-232 | ~595-680 | 53/54 |
| Asia | ~1-12 | ~65-100 | 16/48 |
| The Americas | ~16 | ~25 | 8/35 |
| Europe | ~0.05 | ~0.5 | 1/44 |

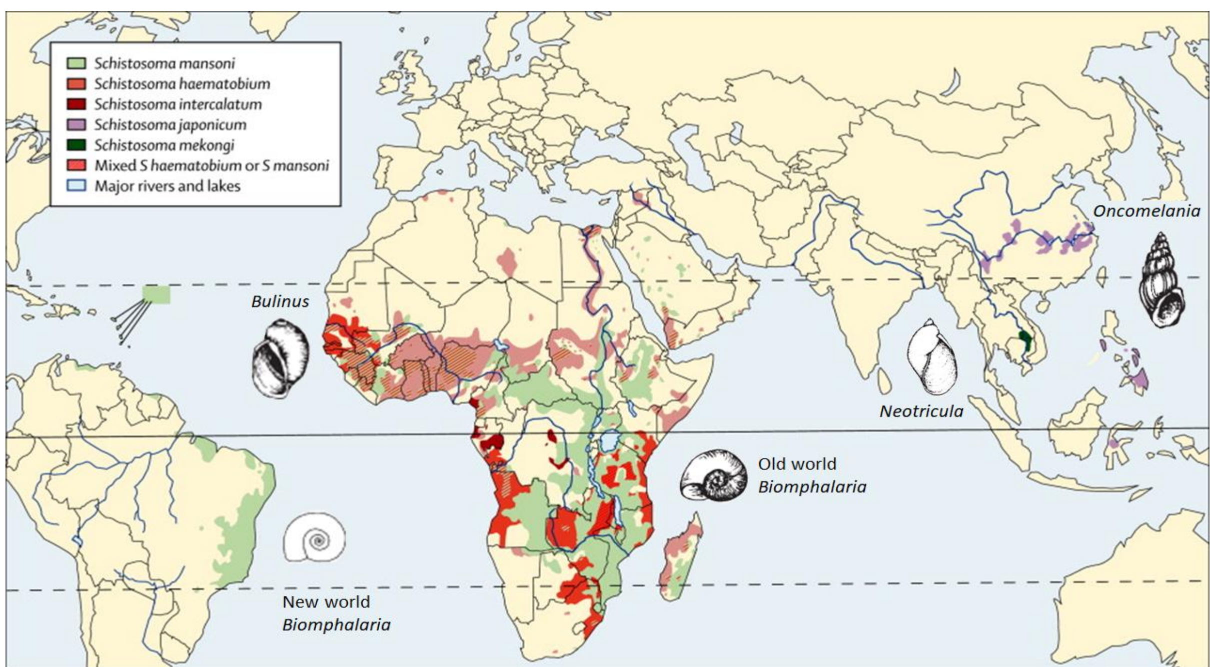


Figure 1.3. Global distribution of schistosomiasis infection. Adapted from Gryseels et al. (2006).

Of the estimated 240 million people infected with schistosomiasis, an estimated 60% of patients are symptomatic, 30% are asymptomatic and 10% suffer from severe symptoms causing morbidity (Chitsulo et al., 2000). However, the idea of whether schistosomiasis can be asymptomatic is still debated (King, 2015). It is estimated schistosomiasis claims between 24,000-200,000 lives globally every year (WHO, 2019). However, these values need to be re-evaluated, as they are outdated and have likely decreased from large-scale preventative chemotherapy campaigns over the past several decades. Mortality is not as large as an issue as the morbidity caused by the disease. Disability-adjusted life years (DALYs) are a time based measurement that summarises the health of a population caused by a medical condition into a single indicator (Murray et al., 2002).

$$DALY = YLD + YLL$$
$$YLL = Number\ of\ deaths * \ Standard\ life\ expectancy\ at\ age\ of\ death$$
$$YLD = (\text{Number of incident cases} * Disability\ weight) * Mean\ disease\ duration$$

DALYs were developed by the World Bank in 1990 and adopted by the WHO as a way of measuring the amount of mortality (Years of Life Lost) and morbidity (Years Lived with Disease) caused by a disease (Anand & Hanson, 1997). A single DALY is equivalent to the loss of one healthy year of life, with Hotez et al. (2014) estimating schistosomiasis accounted for 3.31 million DALYs globally in 2010. However, there is still an ongoing debate about how much of a sequela can be attributed to schistosomiasis, with King (2010) estimating the global DALYs lost to schistosomiasis being as high as 56 million. It is agreed that the majority (>90%) of the DALYs caused by schistosomiasis are due to the Years of Life Lived with Disease (YLD), instead of Years of Life Lost (YLL; King and Dangerfield, 2008).

The majority of those infected with schistosomiasis have mild symptoms, with both anaemia and malnutrition being common. There are many symptoms caused by schistosomiasis that fluctuate in seriousness, with the initial infection causing dermatitis from the cercariae penetrating the skin (Inobaya et al., 2014). As the disease develops, another primary condition called Katayama fever (acute schistosomiasis) can cause fever, lethargy, a severe itching (urticarial) rash, enlargement of the liver (hepatomegaly) and/or spleen (splenomegaly) and coughing (bronchospasms; Mogawer et al., 2019). However, the more severe morbidity is caused by the reproductive activities of the adult schistosomes. The majority of eggs released by the adult worms become lodged in different tissues (typically the intestinal/bladder walls), but it is possible for them to reach the liver, spleen, lungs, heart and/or brain (de Oliveira, 2013). The eggs cause chronically active schistosomiasis within several organ systems, which can lead to high blood pressure and fluid build-up throughout the gastrointestinal (or genitourinary) system causing debilitating and potentially life-threatening symptoms (Colley & Secor, 2014).

### 1.2.1 The Pathology and Lifecycle of Schistosomiasis:

The *Schistosoma* lifecycle consists of two stages; the sporocyst (asexual stage) that lives inside the intermediate gastropod host and the worm (sexual stage) that lives inside the definitive mammalian host (Figure 1.4). Whilst inside the definitive host, a single pair of schistosomes can produce several hundred (*S. mansoni* & *S. haematobium*) or even thousands (*S. japonicum*) of eggs daily during their lifetime (3-10 years). Only 20-55% of these eggs successfully pass through the host and reach the water, while the rest are trapped in the intestinal (*S. mansoni* and *S. japonicum*) or urinary (*S. haematobium*) tissues (Costain et al., 2018). Depending on the species, the currently migrating or trapped eggs inside the host can cause either gastrointestinal disease (*S. mansoni* and *S. japonicum*) or genitourinary disease (*S. haematobium*). The former condition can lead to the narrowing of the colon or rectum, as well as fibrosis of the liver in long-term infected hosts, while the latter condition can lead to fibrosis of the urinary tract, genital lesions (female and male genital schistosomiasis), hydronephrosis, kidney failure and even bladder cancer (Gray et al., 2011). Furthermore, a more serious third condition, central nervous system disease, can

develop if eggs migrate and get stuck in either the brain (*S. japonicum*) or spinal cord (*S. mansoni* and *S. haematobium*; Ross et al., 2002).

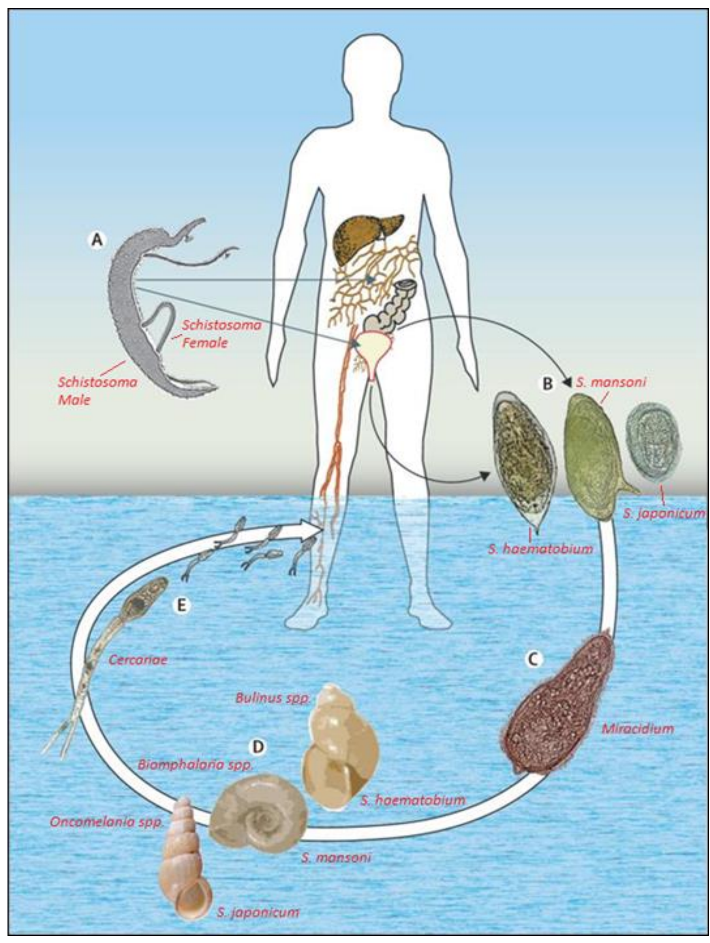


Figure 1.4. Simplified life cycle of *Schistosoma*. (A) the adult schistosome male carries the female and sexually reproduce in their definitive mammalian host; (B) Eggs are released from the definitive mammalian host through either defecation (*S. mansoni* and *S. japonicum*) or urination (*S. haematobium*); (C) the eggs hatch into a larval stage called a miracidium, and seek out and penetrate a suitable intermediate snail host; (D) successful miracidium develop into asexual sporocysts within their intermediate snail host; (E) a second free-swimming larval stage called a cercaria is released from the intermediate snail host, and seeks out a suitable definitive mammalian host to penetrate. Adapted from Colley et al. (2014).

If eggs are passed through the host into freshwater and optimal conditions are met, then the eggs will hatch into miracidia in less than an hour. Miracidia have a lifespan of less than 72 hours and have to successfully find and penetrate into the appropriate snail host (Figure 1.4). One week after successfully penetrating the correct snail host, the miracidium transforms into a mother sporocyst and remains inside the head-foot tissue of the snail for the following 2-3 weeks (Hanington et al., 2010; Humphries, 2011). Four weeks after exposure, the mother sporocyst produces daughter sporocysts, which migrate to the snail's digestive gland-gonad complex. By the 6th week, these daughter sporocysts eventually rupture and cercariae are released into the water. Once released, these infective cercariae have 4-6 days to seek out an appropriate mammalian host (Figure 1.4). Once found, the

cercariae penetrates into the skin, losing their tail, and travel through the blood vessels as schistosomula. Many perish from the immune system before reaching the liver, but successful schistosomula mature into adult worms that coat themselves with host antigens to avoid future detection. Sexual maturation happens 4-6 weeks after initial infection, in which individual worms seek out the opposite sex and mate.

### 1.2.2 The Treatment of Schistosomiasis:

In the 21st century, schistosomiasis is considered an easy disease to cure, as the average cost for each person treated is on average £1.13 (Salari et al., 2020). Yet it remains widespread throughout the developing world due to the ease of infection and reinfection among patients. Biltricide, or its more common name praziquantel, is a safe and highly effective anthelmintic drug, which is taken orally and is used to treat a large range of helminthic infections (Chai, 2013). Praziquantel (PZQ) is the main method for controlling infection in high-risk groups (e.g. school children, pregnant women, etc.) and is extremely effective at curing schistosomiasis (cure defined as the percentage of reduction in the number of patients who either cease to excrete eggs or the reduction in the mean number of eggs excreted). However, praziquantel is less effective when dealing with heavily infected individuals (> 400 eggs per gram of faeces) or individuals co-infected with more than one *Schistosoma* species at one time, and it does not prevent reinfection (Midzi et al., 2008; Olliaro et al., 2011; Lovis, et al., 2012).

The intensity and rate of reinfection after treatment varies depending on what species are present, the transmission dynamics and the endemicity level of an area (Tchuenté, et al., 2013). Furthermore, praziquantel is only effective on adult worms, miracidia and cercariae, but ineffective against eggs, sporocysts and schistosomula (Wu et al., 2011). Despite its faults, praziquantel is the most preferred antischistosomal drugs over others like metrifonate (severer side effects) or oxamniquine (only effective against *S. mansoni*). However, due to the heavy overreliance on praziquantel, inevitable immunity of the drug will occur within *Schistosoma* populations. Alongside praziquantel-resistance, many other geographical, environmental, socio-economic, ecological and epidemiological factors all contribute to the rate of reinfection (Ernould et al., 2004; Cundill et al., 2011). Reinfection is so common due to both halves of the lifecycle, helping to sustain the other half. Control programs heavily focus only on the human side of the infection cycle, which aims at and succeeds in treating the afflicted local people. This helps to reduce future cases of infection by introducing sanitation services and curing current human hosts with chemotherapeutic drugs, which stops any new eggs reaching freshwater sources and infecting new snail hosts. However, only focusing on one half (the definitive) of the infection cycle leaves the other half (the intermediate) rampant and uncontrolled. The intermediate stage relies on sporocysts to asexually create new cercariae. This means even if all new eggs are prevented from reaching the water, it will only stop new snails from becoming infected, but will not stop already infected snails from producing new cercariae for the rest of their lives. Furthermore, human *Schistosoma* species can exist in other mammals (bovine, canines, primates, ruminants, rodents, swine etc.), indicating animal reservoirs will allow infection to continue independently of human involvement. Therefore, breaking the cycle of infection

requires a more sophisticated solution than just controlling and curing the infection found in afflicted people.

### 1.2.3 Current Progress on Schistosomiasis Control:

Prior to the discovery of anthelmintic drugs, schistosomiasis control used to be heavily reliant on policies involving behaviour and sanitation. However with the introduction of anthelmintic drugs, the focus of subsequent schistosomiasis control strategies changed to chemotherapy and the elimination of the intermediate snail host (Inobaya et al., 2014). The current roadmap for the implementation of neglected tropical diseases control was developed by the WHO's World Assembly in 2020, setting new global targets for the prevention, control or eradication of 20 diseases (including schistosomiasis) by 2030 (WHO, 2020). Schistosomiasis is one of 17 recognised neglected tropical diseases (NTDs) which currently affect developing countries (WHO, 2022a). The WHO considers schistosomiasis to be one of six NTDs which can be controlled and eliminated through the use of safe water, sanitation, and hygiene (WASH) practices, environmental intervention of vectors/hosts (molluscicides and water engineering) and providing access to preventative chemotherapy (praziquantel; WHO, 2022b). Furthermore, if the total eradication of schistosomiasis were ever achieved, the control measures put in place would go on to help control other diseases caused by unsanitary and unhygienic practices such as soil-transmitted helminthiasis. In addition, eliminating schistosomiasis would help towards; (I) the eradication of extreme poverty and hunger; (II) achieving universal primary school education; (III) promoting gender equality and empowering women; (IV) reducing child mortality rates and improving maternal health and (V) combat the transmission of HIV/AIDs (WHO, 2005).

As previously mentioned in section 1.2.2, praziquantel-based mass drug administration (MDA) programs are currently the main method for controlling schistosomiasis and lead the way as the main method of control. Mass drug administration programs have had considerable success in reducing both the prevalence and intensity of schistosomiasis infection in hyper-endemic areas (Webster et al., 2014). The popularity of PZQ-MDA is likely to continue due to the large-scale reach it has within the global community and their primary focus on helping school-age children (and occasionally adolescents) who suffer from the highest prevalence and intensity of schistosomiasis. However, despite the advantages prioritising only one demographic has (preventing infection in the next generation and protects the most vulnerable group), it also has a lot of disadvantages such as missing out the majority of infected individuals (out of school children, older children and adults) who continue to suffer and contribute to the continuous lifecycle of the disease. Furthermore, it also excludes adults whose occupation (sand harvesters, anglers, farmers etc.) regularly exposes them to schistosomes infested waters (Onkanga et al., 2016). For example, the most up to date estimates show that fewer than half of the 240 million people who require preventative treatment, received it (WHO, 2021a).

Current PZQ-MDA programs have many direct and indirect benefits such as the decline of schistosomiasis-associated paediatric malnutrition and mitigating the cognitive deficits in children during development (Ezeamama et al., 2018). However, for all the good it has done, praziquantel is seen as a unidimensional approach that is insufficient at controlling and

ultimately eliminating schistosomiasis in the resource-poor regions of Africa, South America and the Middle East. The problems lie within its current delivery model, which cannot reliably reach areas with high transmission rates and high post-treatment reinfection rates for frequent deliveries of repeated doses. Moreover, there is little evidence showing PZQ-MDA alone can sufficiently and effectively interrupt the transmission cycle in said areas, even with several years of constant praziquantel coverage (Hotez et al. 2019).

### 1.2.4 Diagnosing Schistosomiasis Infection in Human and Snails:

The primary diagnostic method for schistosomiasis in humans is to isolate and identify whether schistosome eggs are present in the urine (urogenital) or stool (intestinal) of a patient. Microscopic egg detection is considered the diagnostic “gold standard” of detecting schistosomiasis in people as false-positive results are not possible. However it does have limitations, particularly when it comes to detecting infection in low-intensity infection cases or prepatent patients. The WHO recommends several different techniques for diagnosing both intestinal (e.g. Kato-Katz smear method) and urogenital schistosomiasis (e.g. urine filtration method; Ross et al., 2002; Gryseels et al., 2006). However, the need for more sensitive, accurate and time-efficient diagnostic methods is still sought after, especially in areas where PZQ-MDA campaigns are being currently implemented. As a result of this need, various molecular techniques have been developed in recent years which can detect schistosome infection in a patient’s urine (Sandoval et al., 2006), stool (ten Hove et al., 2008) or even blood (Wichmann et al., 2013).

In addition to human infection prevalence, assessing infection prevalence within the intermediate snail host is becoming just as important. The traditional diagnostic method for detecting schistosome infection within an intermediate snail host is to identify whether cercariae are being produced (Webbe, 1965). However, this traditional cercarial shedding method has limitations, as its very time-consuming taking anywhere from 35 to 49 days to perform and any snails which die during this period can skew infection results. To overcome these limitations, various novel techniques have been developed to address the need for a more direct and rapid diagnostic method for detecting schistosome infection within snails. Such molecular methods like loop-mediated isothermal amplification (LAMP) or polymerase chain reaction (PCR) can be used to rapidly detect *Schistosoma* DNA from an extracted snail sample (Notomi et al., 2000; Nagamine et al., 2002; Sandoval et al., 2006; Abbasi et al., 2010; Lu et al., 2016). Moreover, these techniques can even detect infection in early prepatent snails giving more accurate, rapid, and species-specific results than the traditional cercarial shedding method (Joof et al., 2020). The first PCR-based detection method for *S. mansoni* infection in *Biomphalaria* snails were the $Sm^{1-7}$ primers designed by Hamburger et al. (1998). However, the reliability of these primers were recently questioned since they were unable to consistently detect *S. mansoni* DNA in laboratory infected *B. glabrata* snails (Joof et al., 2020). Conversely, alternative primer sets such as $Sm^{F/R}$ (Sandoval et al., 2006) and ND5 (Lu et al., 2016) have demonstrated greater accuracy and reliability in detecting *S. mansoni* infection in various species of *Biomphalaria* snails (Joof et al., 2020).

### 1.2.5 The Future of Schistosomiasis Control:

There is a growing concern for other alternative methods of controlling schistosomiasis, instead of depending solely on mass chemotherapeutic programs like PZQ-MDA. Any new alternative control methods for schistosomiasis are highly sought after and there are many potential areas from which it can come. For example, immunisation is a common prophylactic method used to control the prevalence of many diseases. The invention of vaccines has successfully led to the near eradication (e.g. poliomyelitis) or complete eradication (e.g. smallpox) of many diseases which once devastated humanity. However, due to schistosomiasis being caused by a metazoan parasite, the development process is much more complicated than that of a conventional vaccine made for a disease caused by a virus or unicellular organism. Currently, more than 100 potential antigens have been identified and could be used to create a vaccine for schistosomiasis. However, only a few of these antigens have advanced to human clinical trials, with the Sm14/GLA-SE, Sm28-GST, Sh28-GST and Sm/TSP-2 vaccines still in development (Table 1.4; Merrifield et al., 2016; Tendler et al., 2018).

Table 1.4 Information about vaccines against *Schistosoma mansoni* (sm) and *S. haematobium* (sh) infection. Information supplied by Tsuji (2020).

| | Targets | Progress |
|---|---|---|
| Sm28-GST | Glutathione S-transferase | Undergoing Phase 3 |
| Sh28-GST | Glutathione S-transferase | Undergoing Phase 3 |
| Sm14/GLA-SE | Fatty Acid Binding Protein | Undergoing Phase 2 |
| Sm/TSP-2 | Tetraspanin Integral Membrane Protein | Undergoing Phase 1 |
| Sm-p80 | Calpain Neutral Cysteine Peptidase | Preparing for Phase 1 |

Creating a vaccine for schistosomiasis is both difficult and expensive as it requires a lot of research and resources in order to ensure it is safe and effective. Schistosomiasis (and other NTDs) mostly affect people living in poor countries, which gives multinational pharmaceutical companies no commercial incentive to create a vaccine, as if one were ever made it would not be very profitable (Sabin, 2020). Additionally, the governments of developing countries prioritise other diseases such as HIV/AIDs, malaria and tuberculosis as these diseases have greater immediate impact on population health and economic development than NTDs. Therefore, most pharmaceutical companies and governments do not prioritise the eradication of NTDs. Consequently, this lack of support makes it hard for researchers to find the funding to continue their work, which makes the development of a vaccine often reliant on funding from charitable groups, but this funding is often limited.

Other than Immunisation, other control methods like integrated sanitation, access to safe water and educational programs can be promoted in endemic areas to help reduce prevalence of NTDs like schistosomiasis. Furthermore, acknowledging the fundamental role freshwater snails play in the transmission of the disease and focusing on stopping them can also prevent infection (Ross et al., 2017a). There are three genera responsible for vectoring

the majority of infections, *Bulinus* (*S. haematobium*), *Biomphalaria* (*S. mansoni*) and *Oncomelania* (*S. japonicum*; Table 1). *Biomphalaria* is especially problematic due to its invasive nature, as it is found in Africa, East Asia, the Middle East and the Americas (Colley et al., 2014; Habib et al., 2021). Currently, *Biomphalaria* is single-handedly responsible for all *S. mansoni* infections, with a global estimate of 83 million people currently infected and a further 393 million at risk (DeJong et al., 2001; Van der Werf et al., 2003).

## 1.3 An Introduction to *Schistosoma mansoni* and *Biomphalaria*:

### 1.3.1 The Origins of *Schistosoma mansoni*:

There are approximately 23 nominal species of *Schistosoma*, with each schistosome species being classified into five clades, the *S. haematobium* clade, the *S. hippopotami* clade, the *S. indicum* clade, the *S. japonicum* clade and the *S. mansoni* clade (Figure 1.5; Lawton et al., 2011). Lawton et al. (2011) proposes *Schistosoma* originated in Asia approximately 60-70 million years as parasites of murid rodents. The invasion of primordial Asian *Schistosoma* species to Africa happened at least two separate times, with the first invasion giving rise to the *S. hippopotami* clade and latter invasion (or invasions) giving rise to a *S. mansoni*-like ancestor of the *S. haematobium*, *S. indicum* and *S. mansoni* clades (Figure 1.5; Lawton et al., 2011). Subsequent genomic research suggests that *S. mansoni* emerged in East Africa as recently as 126,500 years ago and remained isolated in the east for 119,000 years before spreading outwards to the rest of Africa (Rey et al., 2021).

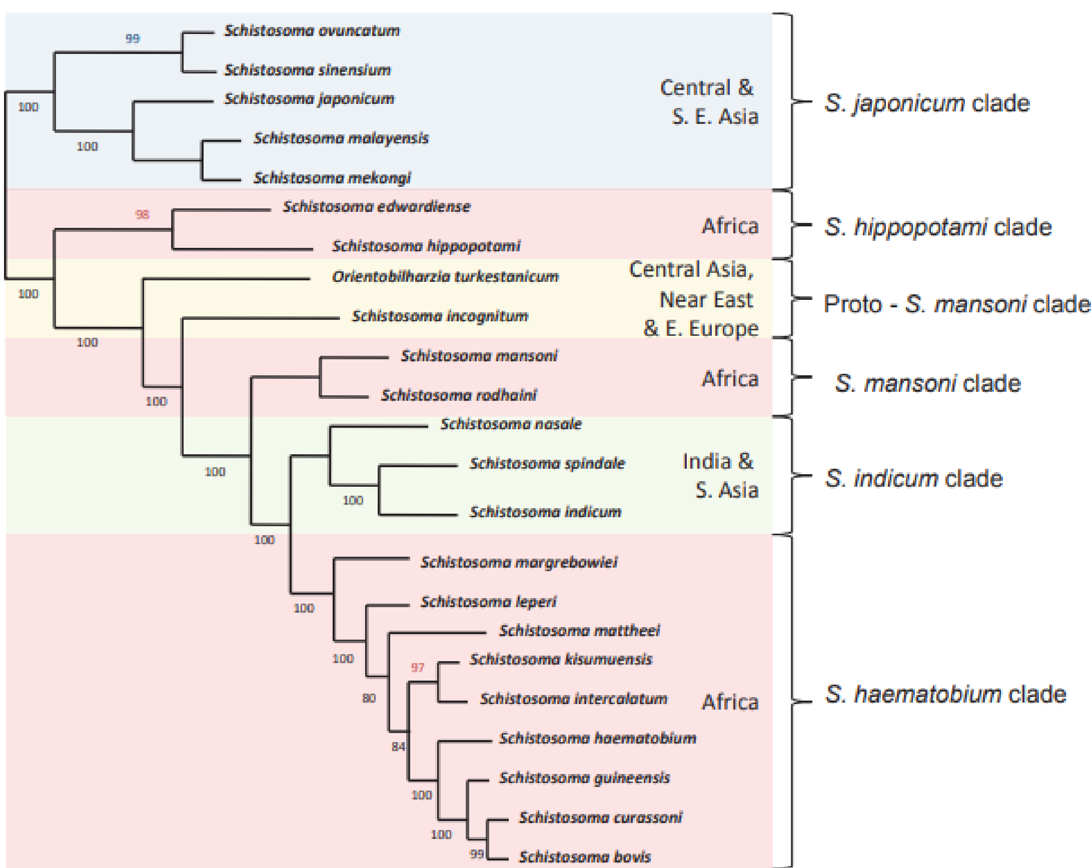

Figure 1.5. Phylogenetic summary of the *Schistosoma* genus using Bayesian analysis of the complete small ribosomal subunit (SSU) , partial large ribosomal subunit (LSU) and the partial Cytochrome Oxidase 1 Subunit (COI). Adapted from Lawton et al. (2011).

It is estimated that the parasitic relationship between *S. mansoni* and African *Biomphalaria* species developed approximately 2-5 million years ago in Africa, with the introduction of proto-*B. glabrata* snails from South America (Campbell et al., 2000; Morgan et al. 2001). Subsequently, *S. mansoni* was then introduced into the New World via the colonisation of the Americas and the Trans-Atlantic slave trade, using the convenient South American *Biomphalaria* species as its intermediate host (Platt et al. 2022).

1.3.2 An Introduction to *Biomphalaria*:

*Biomphalaria* (Gastropoda: Planorbidae) are the intermediate host for *Schistosoma mansoni*, the primary cause of intestinal schistosomiasis. In addition to *S. mansoni*, *Biomphalaria* acts as an intermediate host to approximately 50 other helminthic parasitic species with medical or veterinary importance (Habib et al., 2021; Table 1.5).

Table 1.5 Examples of nematode and trematode species that use *Biomphalaria* as their intermediate host. Information supplied by Habib et al. (2021).

| | Parasite species | Definitive host |
|---|---|---|
| Nematoda | *Angiostrongylus cantonensis*<br>*Angiostrongylus costaricensis*<br>*Angiostrongylus siamensis*<br>*Angiostrongylus vasorum* | Mammals |
| Trematoda | *Echinoparyphium spp.*<br>*Echinostoma barbosai*<br>*Echinostoma caproni*<br>*Echinostoma friedi*<br>*Echinostoma liei*<br>*Echinostoma lindoense*<br>*Echinostoma luisreyi*<br>*Echinostoma noacrorchi*<br>*Echinostoma paraensei*<br>*Echinostoma revolutum*<br>*Echinostoma rodriguesi*<br>*Echinostoma togoensis*<br>*Echinostoma trivolvis*<br>*Schistosoma mansoni*<br>*Paryphostomum segregatum*<br>*Zygocotyle lunata* | Birds and Mammals |
| | *Austrodiplostomum compactum*<br>*Ribeiroia spp.* | Fish and Amphibians |

*Biomphalaria* are described as sinistrally coiled, with a flat discoidal shell. They inhabit a multitude of natural and human-made freshwater environments (lakes, rivers, ponds,

marshes, irrigation channels etc.), which are rich in algae or organic detritus (faeces). However, despite living in freshwater, *Biomphalaria* breathe air via a lung-like pulmonary cavity. Like most snail species, they are hermaphroditic meaning they possess both male and female gametes. This allows *Biomphalaria* to reproduce both sexually (cross-fertilisation) and asexually (self-fertilisation). A single snail can lay over 10,000 eggs during its 2-3 year lifespan, with a clutch of eggs hatching within 6-8 days and reaching sexual maturity in less than two months (depending on environmental conditions) (Eveland & Haseeb, 2011). Certain abiotic factors such as temperature, illuminance and the physiochemical parameters of the water (pH, salinity, conductivity, major anions and cations levels) can affect the growth rate and abundance of a *Biomphalaria* population (McCreesh, and Booth, 2014).

### 1.3.3 The Origins and Current Distribution of *Biomphalaria*:

The current theorised origins of *Biomphalaria* are within South America after the separation of the supercontinent Gondwana (~95-106 million years ago), with the genus first appearing in the fossil record in the late Cretaceous period (~65-100 million years ago; Abou-El-Naga, 2013; Cabrera et al., 2018). Early allozyme phylogenetic studies by Bandoni et al. (1995) and more informative phylogenetic studies by DeJong et al. (2001) and Jørgensen et al. (2007) confirm this hypothesis as *B. glabrata* is the ancestral sister group to all African *Biomphalaria* species. This led to current theory that *Biomphalaria* were introduced into Africa via aquatic birds or rafts made of vegetation carrying primordial *B. glabrata*-like snails from South America approximately 2-5 million years ago (Campbell et al., 2000; Jørgensen et al., 2007). Currently, *Biomphalaria* species are native to the tropical (and sub-tropical) regions of Africa and South America (Taylor, 1988). However, *Biomphalaria* are notoriously invasive, as despite only making up 15% of Planorbid species, they constitute approximately half of the reported invasive Planorbidae species globally (Pointer et al., 2005). Habib et al. (2021) reviewed the current global distribution of *Biomphalaria* species and found South American species have invaded China, Romania, Mexico and the Caribbean (Figure 1.6).

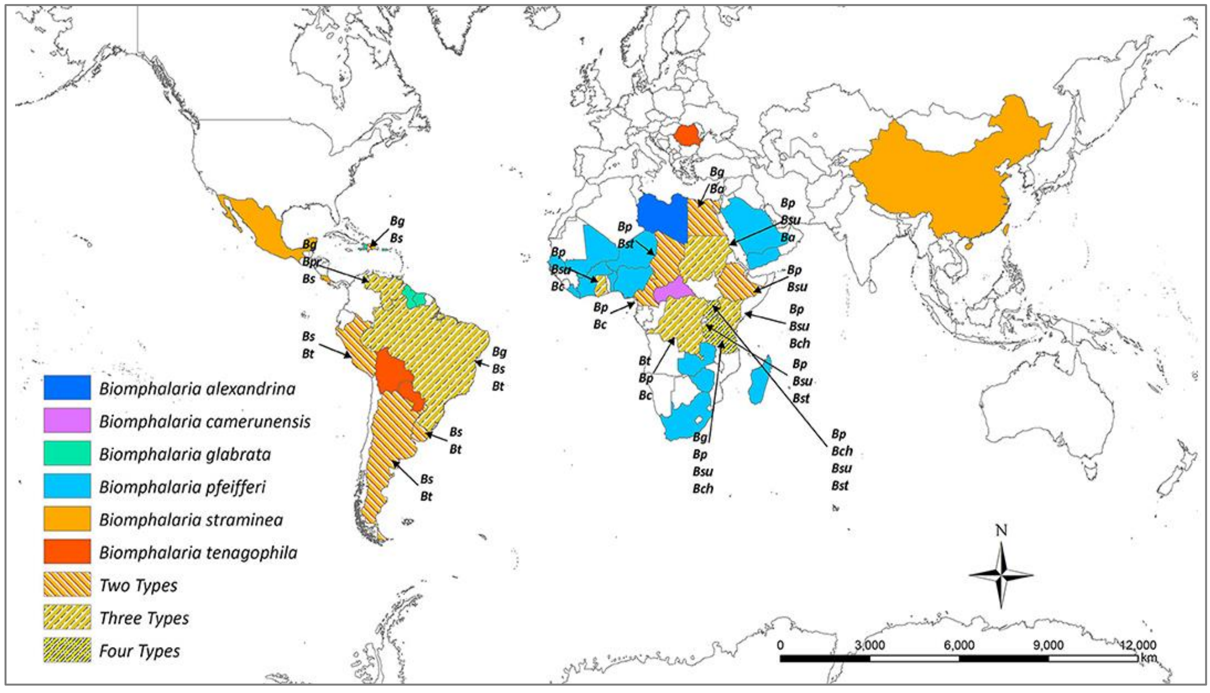

Figure 1.6. Global distribution of *Biomphalaria* species. Adapted from Habib et al. (2021).

Mandahl-Barth (1957) was the first to classify the African *Biomphalaria* species using a combination of different morphological characteristics. He categorised 12 species into four groups comprised of the *B. alexandrina* group; the *B. choanomphala* group; the *B. pfeifferi* group and the *B. sudanica* group (Mandahl-Barth, 1957). However, through the use of more sophisticated molecular techniques, Dejong et al. (2001) found that *B. alexandrina*, *B. choanomphala* and *B. sudanica* were all closely related. This disproves the previously proposed four group system, showing that morphology-based identification methods have many inconsistencies. Moreover, certain species previously defined exclusively by morphological characteristics such as *B. arabica*, *B. barthi, B. rhodesiensis, B. ruppellii, B. salinarum* and *B. tchadiensis* were invalidated by molecular methods (Brown, 1994; Jørgensen et al., 2007). In total, there are approximately 20 new world species and 8 African species of *Biomphalaria* (Dejong et al., 2001; Jørgensen et al., 2007). Unlike the new world species, all of the African *Biomphalaria* species are fully capable of harbouring *S. mansoni* infection. However, the current phylogenetic structure of the African *Biomphalaria* species is problematic, as the only clearly defined species are *B. camerunensis* and *B. pfeifferi*, while the remaining species (*B. alexandrina*, *B. angulosa*, *B. choanomphala*, *B. smithi*, *B. stanleyi* and *B. sudanica)* form a poorly defined clade named the Nilotic species complex (Figure 1.7). The Nilotic species complex is named so as all of the species inhabit regions connected to the river Nile (Abou-El-Naga, 2013).

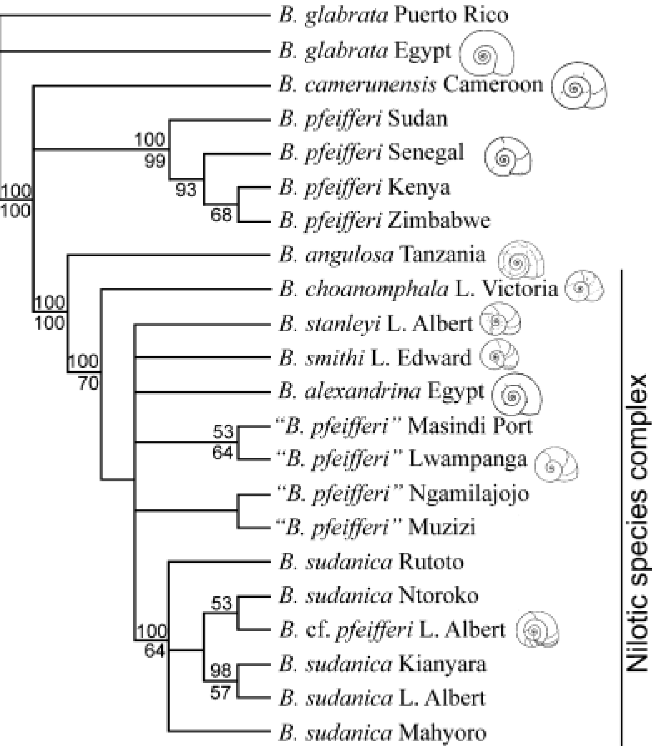


Figure 1.7. Phylogenetic analysis of the African *Biomphalaria* species, rooted on the common ancestor, *B. glabrata*. Adapted from Jørgensen et al. (2007).

## 1.4 Research Aims:

This thesis explores the relationship between gastropod-borne parasites and their gastropod hosts. It focusses primarily on the digenetic trematode species, *Schistosoma mansoni* and its relationship with *Biomphalaria* snails inhabiting the Great African Lakes.

Chapter 3 aimed to use landmark-based geometric morphometric techniques to identify what morphological characteristics of *Biomphalaria* shells are important when differentiating specimens at the species-level, with the hope of improving conchological identification methods used in the field.

Chapter 4 focuses on investigating the prevalence of *Schistosoma mansoni* infection and the level of genetic diversity in the *Biomphalaria* species inhabiting the Great African Lakes. Additionally, *S. mansoni* prevalency were measured for the wet and dry seasons over a two year period with the intension of understanding transmission patterns throughout the year.

Chapter 5 focused on *Biomphalaria choanomphala* snails in Lake Victoria with the aim of identifying the major factors driving the prevalence of *S. mansoni* infection in *B. choanomphala* populations across Lake Victoria. This chapter investigates the influence of biotic and abiotic factors in the lake on *S. mansoni* prevalence.

Chapter 6 is distinct from the three previous chapters, with the focus being on whether medical and veterinary important gastropod-borne parasites are present in and around the city of Nottingham. A parasitological survey of local terrestrial gastropod populations was undertaken with the gastropods collected and screened for potentially pathogenic nematode and trematode species.

## Chapter 2 General Material and Methods:

### 2.1 Samples Analysed and their Collection Localities:

Malacological collection surveys were undertaken by Candia Rowel and Besigye Fred (Rowel et al. 2015) from January 2009 to May 2011 as a part of the Schistosomiasis in Mothers and Infants (SIMI) project at the Ugandan shorelines of both Lake Albert (Buliisa District) and Lake Victoria (Mayuge District). They collected *Biomphalaria* snails once a month for 28 consecutive months from three sites at Lake Albert (Bugoigo: 1.908°N, 31.409°E; Piida: 1.819°N, 31.328°E and Walukuba: 1.842°N, 31.378°E) and three sites at Lake Victoria (Bugoto: 0.319°N, 33.628°E; Bukoba: 0.312°N, 33.492°E and Lwanika: 0.351°N, 33.446°E; Figure 2.1). These whole snail samples were originally collected and analysed by the Rowel et al. (2015) study, with approximately half of the snails collected (collections from January 2009 to December 2010) being held as a reference archival collection at the Liverpool School of Tropical Medicine, UK. In total, 2,645 snails preserved from the original 6,183 snails collected at Lake Albert, and 6,382 snails preserved from the original 13,172 snails collected at Lake Victoria were provided by Professor J. Russell Stothard from the LSTM.

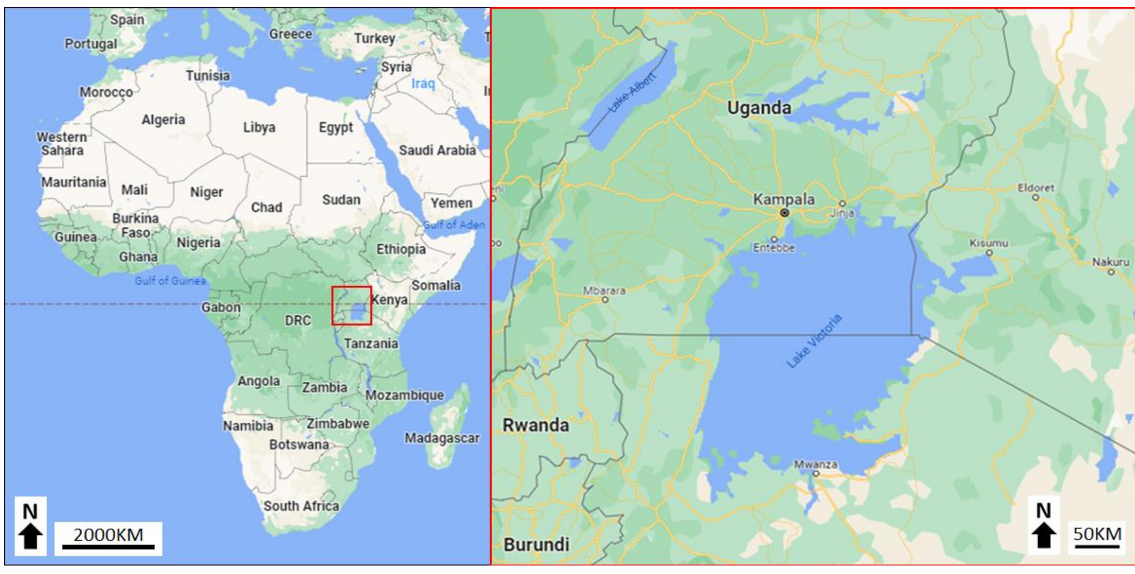


Figure 2.1. Map of the Great African Lakes, Lake Albert and Lake Victoria.

In addition to the SIMI samples, malacological samples from the Kenyan, Tanzanian and Ugandan shorelines of Lake Victoria were collected by Claire J. Standley from February 2008 to February 2010 (Standley et al., 2011; Standley et al., 2012; Standley et al., 2014). Genomic DNA (gDNA) samples of her *Biomphalaria choanomphala* collections were provided by Professor J. Russell Stothard from the LSTM, with accompanying quantitative and qualitative data relating to the abiotic factors at each site. This included: the date, time, current weather, GPS coordinates, the number of snails present, the physiochemical parameters of the water, temperature, water conductivity, total dissolved solids, salinity, pH, habitat type, substrate type, water depth and wave action. The samples and datasets provided to us by Professor J. Russell Stothard had been previously analysed and subsequently published in Standley et al. (2011), Standley et al. (2012) and Standley et al. (2014).

### 2.1.1 Morphological Identification of *Biomphalaria* and Counting Snails:

The preserved *Biomphalaria* snails provided by the LSTM were contained in 15ml falcon tubes containing 70% ethanol, with each tube being labelled with when and where the collection occurred. The first step of analysis was to identify what species were present at each site and quantify the number of each species collected per site every month. This involved individually emptying each of the 15ml falcon tubes one at a time into a 9cm Petri dish and separating the snails out based on shell morphology (Figure 2.2). Once counted, each of the specimens were identified at the species-level using conchological identification methods described by the 'Freshwater Snails of Africa and their Medical Importance' monograph by Brown (1994). In addition to quantifying each of the species present, the number of morphotype-A and morphotype-B shell per collection were counted as well (Figure 2.2). After being counted, the specimens were placed back into the 15ml falcon tube, and the tube was topped up with fresh 70% ethanol and resealed. After counting all of the specimens, 20 individuals of each species from each of the six sites were selected for DNA extraction and molecular analysis. In this case, the August collections of 2010 were chosen as they had the highest number of preserved snails for both Lake Albert and Lake Victoria. The information of each *Biomphalaria* collection was documented into an Excel spreadsheet, which listed the number of snails collected each month, which species were present for each monthly collection and how many of each morphotype were present.

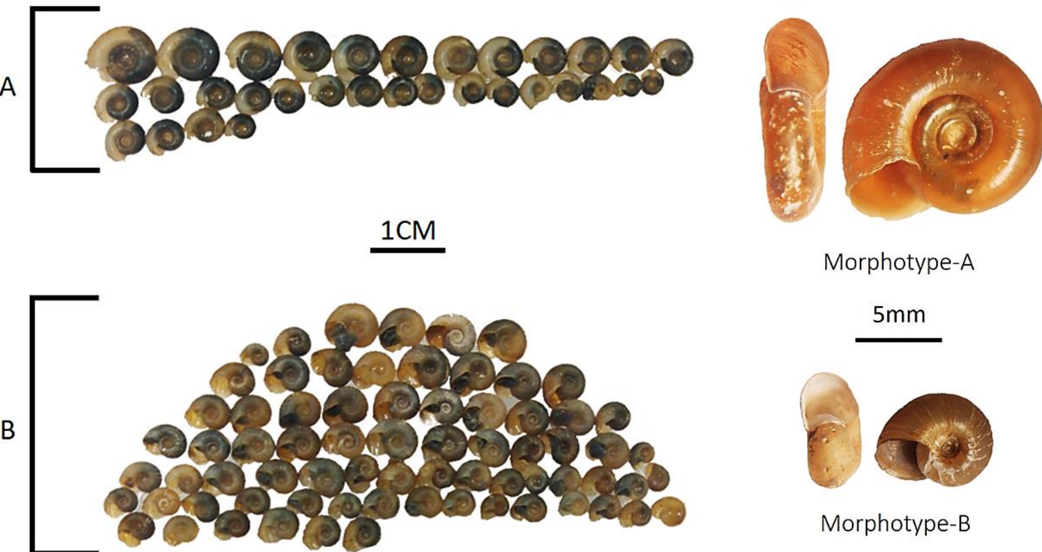


Figure 2.2. Example of counting and categorising the *Biomphalaria* snails by morphotype (left). Examples of what morphotype-A and morphotype-B shells look like up-close (right). Samples originally collected as a part of the SIMI project and were provided by the LSTM.

### 2.2 Malacological Survey of the City of Nottingham:

Malacological collections of terrestrial gastropods were performed in and around the city of Nottingham from June to November 2020 and June to November 2021. All slugs and snails were collected by hand and placed into separate plastic tubs based on species. At each site, the goal was to collect exactly 50 specimens, with a maximum of 10 individuals per species. In total, 16 sites were surveyed in and around the city of Nottingham (Figure 2.3). The sites selected were all popular dog walking locations such as recreational grounds, country parks,

public gardens and nature reserves. In addition, to collecting gastropods at each site, the time, date, and GPS coordinates (provided by Google Maps) of each collection was also recorded.

Figure 2.3. Map of the City of Nottingham, United Kingdom.

### 2.2.1 Morphological Identification of Terrestrial Gastropods:

All terrestrial gastropods collected from Nottingham were identified using the 'Terrestrial Mollusc Key' (https://idtools.org/id/mollusc/key.php) by White-McLean (2011) and the illustrated guide 'Slugs of Britain and Ireland' by Rowson et al. (2014). Identifications were performed during collections and again before gastropods were processed. The identification process involved looking at the shell characteristics (hairs, dents, striae, lirae, ribs or wrinkles), body characteristics (breathing pore location, keel or no keel), body markings (spots, blotches, stripes or bands) and sole/mucus colour (white, yellow, orange or clear) of the collected gastropods.

### 2.2.2 Helminthic Parasite Extraction from Terrestrial Gastropods:

After being identified, specimens were cryo-anaesthetised at -20°C for a short period and washed in 70% ethanol (to remove external organisms such as mites and phoretic nematodes) within 12 hours of collection. Gastropods were sliced into four equal pieces (snails were crushed prior to slicing) and artificially digested in a 50ml falcon tube containing Ash's digesting solution (0.7% pepsin in 0.5% HCl) for four to eight hours depending on the body size (Figure 2.4).

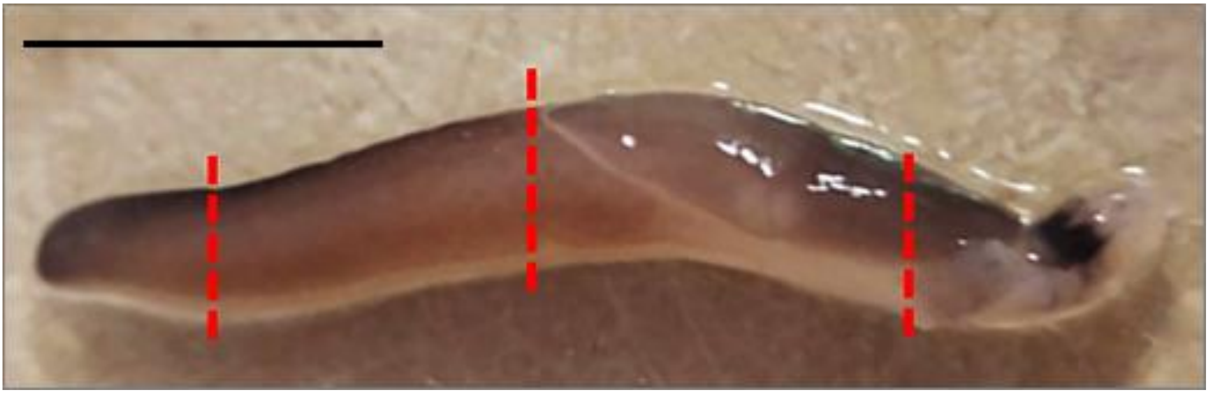

Figure 2.4. Example of how washed gastropod were cut into four pieces. Scalebar: 0.5cm.

After artificial digestion, the solution was diluted using water and poured into a 9cm Petri dish. A dissection microscope was used to check for nematodes or metacercariae for ten minutes (Figure 2.5). Nematodes were classified as either juveniles or adults depending on their body size and morphology. If found, nematodes and metacercariae were counted, picked using a worm pick and placed into a 0.2ml tube containing 70% ethanol (juvenile worms, adult worms and metacercariae were placed in separate tubes) and preserved at -20°C. The information of each gastropod digested was documented into an Excel spreadsheet, which included where the gastropod was collected from (time/date/location), the sample number, the species name, the body size, the number of juvenile and adult nematodes counted, the number of metacercariae counted and the number of parasites (juvenile/adult nematodes and/or metacercariae) picked and preserved in ethanol.

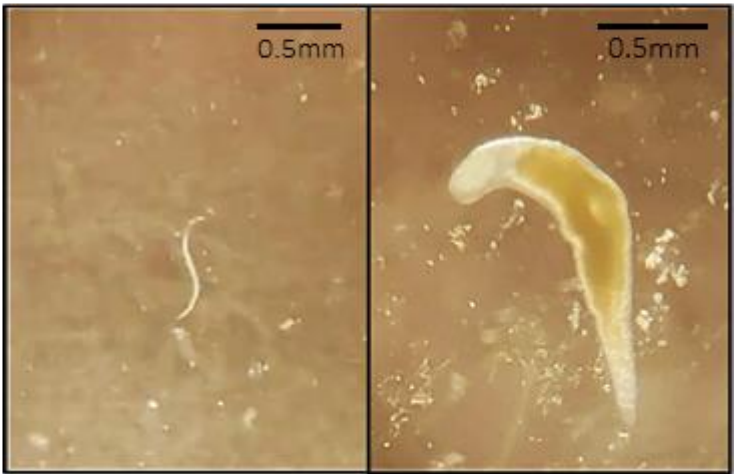


Figure 2.5. Juvenile nematode (left) and metacercaria (right) found in Ash's digestive fluid.

### 2.3 Molecular Processing of Snails and Parasites:

#### 2.3.1 DNA extraction:

DNA was extracted using a modified CTAB (hexadecyltrimethylammonium bromide) method. In the case of *Biomphalaria*, the first step was removing the snail from the 70% ethanol. Next, the whole body was carefully removed from the shell using needle-nose tweezers. Shells of extracted individuals were labelled and stored in a separate 1.5ml

Eppendorf for later use in morphometric analysis (Chapter 2.5). Next, an approximate 2x2mm slice of head-tissue (Figure 2.6) was taken and pressed between paper towels to remove any excess ethanol. The flattened tissue was then placed into a 1.5ml Eppendorf containing 500µl of CTAB solution (100mM Tris base, 20mM EDTA, 1.4M NaCl, 2% CTAB) and a spatula full of 100µm glass beads. A plastic pestle was used to grind the tissue for 5-10 minutes, until disintegrated. Next, 10µl of Proteinase-K (10mg/ml) and 1µl of β-mercaptoethanol was added and the solution was incubated at 56°C for 2 hours with frequent vortexing every 30 mins.

Figure 2.6. Where tissue was taken from shell-extracted *Biomphalaria*. Scalebar: 1cm.

In the case of the helminthic parasites (from Chapter 2.2.2), a single nematode (or metacercaria) were removed from the 70% ethanol and carefully placed into a 1.5ml Eppendorf containing 500µl of CTAB solution, 10µl of Proteinase-K and 1µl of β-mercaptoethanol. No glass beads or plastic pestle were used, as the available tissue was small and easily digested when incubated at 56°C for 2 hours with frequent vortexing every 30 mins.

After the tissue was fully digested, 500µl of Chloroform-Isoamyl alcohol (24:1) was added. Samples were then gently inverted for 5-10 mins to mix the contents. They were then centrifuged at 13,000 rpm for 10 mins. The aqueous layer was then transferred off the top of the chloroform layer into a new 1.5ml Eppendorf and the previous step was repeated. After the second Chloroform-Isoamyl alcohol wash, the aqueous layer was transferred into a new 1.5ml Eppendorf. Next, 1ml of 95% ice-cold ethanol was added, along with 4µl of sodium acetate (3M). The 1.5ml Eppendorf was then placed into a -70°C freezer for 12-24 hours to allow the DNA to precipitate. Next, each sample was centrifuged at 13,000 rpm for 10 mins to form a DNA pellet at the bottom of the 1.5ml Eppendorf. Next, all ethanol was extracted, leaving a pellet at the bottom. The samples were then placed onto a heat block at 45°C for 5-10 mins to evaporate any remaining ethanol. Finally, the pellet was resuspended in 50-200µl of either TE buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) or Tris-HCl buffer (10mM TRIS-HCl, pH 8.0 buffer) depending on whether the pellet was *Biomphalaria* or helminthic DNA, respectively. All DNA samples were stored at -20°C for later use in PCR amplification.

2.3.2 PCR Amplification for Identifying *Biomphalaria* Snails and Helminthic Parasites:

All PCR reactions used a 25µl reaction mixture containing 24µl of 1X PCR buffer and 1µl of DNA template. In the case of *Biomphalaria* DNA, all samples were amplified using both the ribosomal RNA 16S (rRNA 16S) and Cytochrome Oxidase subunit 1 (COI) primers (Table 2.1). The 1X PCR buffer used was Promega GoTaq® G2 Master Mix and contained: 1 Unit of Taq polymerase, 0.2µM of forward/reverse primers, 200µM of each deoxynucleotide triphosphate (dNTP) and 3mM of magnesium chloride ($MgCl_2$). The reaction protocol for both the 16S and COI primers was 96°C for 1 min, then 34 cycles of 94°C for 1 min, then 50°C for 1 min, then 72°C for 1 min and a final extension stage at 70°C for 10 mins.

Table 2.1 Summary information of primer sets used and their intended target organism.

| | Primer Sequence | Gene Complex | Target Organism |
|---|---|---|---|
| 16Sarm/brm Palumbi et al. (1991) | 16Sarm-F: 5′-CTT CTC GAC TGT TTA TCA AAA ACA-3′ 16Sbrm-R: 5′-GCC GGT CTG AAC TCA GAT CAT-3′ | ribosomal RNA 16S (Mitochondrial) 450bp | *Biomphalaria* |
| LCO/HCO Folmer et al. (1994) | LCO1490-F: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' HCO2198-R: 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' | Cytochrome oxidase subunit 1 (Mitochondrial) 650bp | *Biomphalaria* |
| N93/N94 Nadler et al. (2000) | N93-F: 5′-TTG AAC CGG GTA AAA GTC G-3′ N94-R: 5′-TTA GTT TCT TTT CCT CCG CT-3′ | ITS1/ITS2 (Nuclear) 850bp | Nematodes |
| $Sm^{F/R}$ Sandoval et al. (2006) | Sm-F: 5′- GAG ATC AAG TGT GAC AGT TTT GC-3′ Sm-R: 5′- ACA GTG CGC GCG TCG TAA GC-3′ | 28S (Nuclear) 350bp | *S. mansoni* |
| ND5 (Lu et al., 2016) | ND5-F: 5′-ATT AGA GGC AAT GCG TGC TC-3′ ND5-R: 5′-ATT GAA CCA ACC CCA AAT CA-3′ | ND5 (Mitochondrial) 302bp length | *S. mansoni* |
| LSU1iii/3iii Fontanilla et al. (2017) | LSU-1iii-F: 5′-TGC GAG AAT TAA TGT GAA TTG C-3′ LSU-3iii-R: 5′-ACG GTA CTT GTC CGC TAT CG-3′ | 5.8S-ITS2-28S (Nuclear) 1000bp | Snails |
| LPF/R Kim et al. (2019) | LP-F: 5′-AGG GAA TGG GTG GAT TTA TT-3′ LP-R: 5′-AGA CAC GAC TGA AAG GTT GC-3′ | ribosomal RNA 18S (Nuclear) 550bp | Trematodes |

In the case of the helminthic parasites, samples were amplified using either the N93/N94 primer set (for nematode DNA) or the LPF/LPR primer set (for trematode DNA; Table 2.1). The 1X PCR buffer used was Promega GoTaq® G2 Master Mix (1U TAQ, 0.2µM primers, 200µM dNTP, 1.5mM $MgCl_2$). The PCR protocol used for both the N93/N94 and LPF/LPR primer sets was an initial denaturation at 95°C for 2 mins, followed by 35 cycles of 95°C for 30 seconds, 50°C for 30 secs, 72°C for 2 mins and a final extension of 72°C for 10 mins.

After amplification was completed, 5µl of PCR product was visualised on an ethidium bromide infused 2% agarose gel and observed under UV light. Successful PCR products were then purified and sequenced using Macrogen's EZ-Seq or Eco-Seq service (https://order.macrogen-europe.com).

### 2.3.3 Detecting *Schistosoma mansoni* Infection in *Biomphalaria* Snails using Polymerase Chain Reaction (PCR):

All samples were amplified using a 25µl reaction volume consisting of 24µl of 1X Promega GoTaq® G2 Master Mix (1U TAQ, 0.2µM primers, 200µM dNTP, 3mM $MgCl_2$) and 1µl of DNA template (diluted to 50ng/µl). All extracted *Biomphalaria* DNA samples were tested for *S. mansoni* infection using two different infection detection primer sets. Samples were first tested for infection using the $Sm^{F/R}$ primer set (Table 2.1). The PCR cycling conditions used was an initial denaturation at 96°C for 1 min, followed by 35 cycles of 94°C for 20 secs, 65°C for 20 secs and 72°C for 30 secs. If found $Sm^{F/R}$ positive, samples were then tested using the ND5 primer set in order to confirm whether the positive samples were indeed infected with *S. mansoni*. The ND5 primer set was chosen as a preventative measure to reduce the chance of false positive results as it can determine whether an infected *Biomphalaria* snail is infected with the human schistosome species, *S. mansoni* (~302bp) or the rat schistosome species, *S. rodhaini* (~800bp) based on the length of the diagnostic band (Lu et al., 2016). The PCR cycling conditions of the ND5 primer set was an initial denaturation at 95°C for 5 mins, followed by 30 cycles of 95°C for 1min, 58°C for 1min, 72°C for 30sec and a final extension step at 72°C for 10 mins. Both the PCR reaction mixtures and cycling conditions for the $Sm^{F/R}$ and ND5 primer sets were followed precisely as described by Sandoval et al. (2006) and Lu et al. (2016), respectively.

In addition to the two infection detection primers, all of the extracted *Biomphalaria* samples were tested using the LSU-1iii/3iii primer set (Table 2.1). This was used as a quality control measure to ensure that the nuclear DNA of an extracted sample was of high quality; this was used as a preventative measure to reduce the chance of false negative results. The PCR reaction mixture for the LSU-1iii/3iii primer set, was the same as the PCR mixture used for the $Sm^{F/R}$ and ND5 primer sets. The LSU-1iii/3iii PCRs were performed on the same day as the infection detection PCRs. The PCR cycling conditions of the LSU-1iii/3iii primer set was an initial denaturation at 96°C for 2 mins, followed by 35 cycles of 94°C for 30 secs, 45°C for 1 min, 72°C for 2 mins and a final extension step at 72°C for 5 mins.

Alongside the *Biomphalaria* samples, two negative controls (water and uninfected *B. glabrata* DNA) and two positive controls (pure *S. mansoni* DNA and infected *B. glabrata* DNA) were also included. These controls were provided by Professor Mike Doenhoff, School of Biology, University of Nottingham. After amplification was completed, 10µl of PCR

product was electrophoresed on a 2% agarose gel containing ethidium bromide and observed under UV light. The information of each infection detection PCR was documented into an Excel spreadsheet and detailed the species name, the location, whether the DNA was good-quality (LSU-1iii/3iii positive) or not and whether the sample was infected with *S. mansoni* ($Sm^{F/R}$ and ND5 positive) or not.

### 2.4 Sequence Analysis and Bioinformatics:

Sequence data was used to perform several different phylogenetic and genealogical analyses. This was a multistep process involving several different programs. The step by step process was: (I) sequence processing; (II) phylogenetics and (III) population genetics. Step one used FinchTV (created by the Geospiza research team) and SeaView (created by Manolo Gouy and colleagues). Step two used PhyML (created by Stephane Guindon and Olivier Gascuel) and FigTree (created by Andrew Rambaut). The third step used DNASP (created by Julio Rozas and colleagues) and Network (created by Fluxus Technology Ltd).

All programs listed were used on a Windows 10 operating system:

- FinchTV v1.4: a program for viewing, editing, and analysing chromatograms. It also can be used to export sequence data in a variety of formats for further analysis. https://digitalworldbiology.com/FinchTV

- SeaView v5.0.4: a program for aligning multiple sequences using a graphical user interface (GUI) that provides a number of useful tools for aligning and analysing sequence data. SeaView has additional built-in software like Muscle for sequence alignment and Gblocks for alignment selection (Gouy et al., 2021). https://doua.prabi.fr/software/seaview

- PhyML v3.1: a program for inferring phylogenetic relationships using the maximum likelihood-based method (Guindon et al., 2010). http://www.atgc-montpellier.fr/phyml

- FigTree v1.4.3: a Java-based program that allows users to view and edit phylogenetic trees produced by PhyML (Rambaut, 2010). http://tree.bio.ed.ac.uk/software/figtree

- DNASP v6.12.3: a program used for population genetic analyses on multiple aligned sequences (Rozas et al., 2017). http://www.ub.edu/dnasp/index_v5.html

- Network v10.2: a program used to generate genealogical networks from multiple aligned sequences. https://www.fluxus-engineering.com/sharenet.htm

#### 2.4.1 Sequence Processing:

All sequencing was performed by Macrogen (https://order.macrogen-europe.com), who sent the sequence data as applied biosystem DNA electropherogram files (.ab1). Sequence data was processed using FinchTV, which was used to view and edit DNA chromatograms. The first step was to remove the forward and reverse primers from the start and end of

each sequence. Next, the sequence was checked from begin to end for sequencing errors and if ambiguous base pairs were found, they were replaced with the appropriate IUPAC code. Additionally, FinchTV was used to confirm the identity of a sequence by using the Basic Local Alignment Search Tool (BLASTn) to compare it to similar sequences. This was done by selecting Edit > BLAST sequence > Nucleotide, BLASTn. Once processed, sequences were imported into SeaView and aligned using MUSCLE (Multiple Sequence Comparison by Log-Expectation). This was done by selecting Align > Alignment options > Muscle > Align all. Afterwards, alignments were checked by hand to fix any inconsistencies and to ensure accuracy. In the case of the rRNA sequences, Gblocks was used to remove poorly aligned regions. This was done by selecting Site > Create set > Gblocks. Finished alignments were saved as .fasta file and exported as a .phylip file. All of the alignments generated and used can be viewed in the appendix.

### 2.4.2 Phylogenetics:

Alignments were imported into PhyML, and the following setting were used to build a tree:

Data type: DNA
Input sequences: Interleaved
Non-parametric bootstrap analysis: Yes (1000 replicates)
Approximate likelihood ratio test: Yes
Model of nucleotide substitution: GTR
Base frequency estimates: ML
Ts/Tv ratio: Estimated
Proportion of invariable sites: Estimated
One category of substitution rate: No
Number of substitution rate categories: 4
Gamma distributed rates across sites: Yes
Gamma distribution parameter: Estimated
Optimise tree topology: Yes
Input tree (BIONJ/user tree): BioNJ
Optimise tree topology: Yes
Tree topology search operations: Best of NNI and SPR
Add random starting trees: Yes
Number of random starting trees: 5

After the phylogenetic tree was generated, trees were imported, viewed and edited in FigTree.

### 2.4.3 Population genetics:

Alignments were imported into DNASP, and Haplotype (gene) diversity (Hd) was estimated by selecting Generate > Haplotype Diversity File > Site with gaps/missing: Considered > Invariable Sites: Included. This creates a .nexus file and produced a descriptive output of the sequences including the number of haplotypes and the Hd score of population. DNASP was also used to estimate the nucleotide diversity ($\pi$) of a population, this was done by selecting Overview > Multi-Domain Analysis. Lastly, DNASP was used to determine the structure of a population ($F_{st}$). This was done by selecting Data > Define Sequence Sets in order to name

and define each of the populations within the aligned sequences. Next, the $F_{st}$ values were obtained by selecting Analysis > Gene Flow and Genetic Differentiation > Sites with Alignment Gaps are: Considered (as a fifth state) > Perform the Permutation Test: 10,000 Replicates.

In addition to DNASP, Network was used to generate a median-joining haplotype network using the .nexus file created by DNASP. This was performed by selecting Calculate Network > Network Calculations > Median-Joining Network > File > Open > Calculate Network. This created a Network output (.out) file, which can then be view by selecting Draw Network > File > Open.

### 2.5 Landmark-based Geometric Morphometric Analysis:

Morphometric analysis was performed to compare the conchological morphologies of each *Biomphalaria* species identified at Lake Albert and Lake Victoria. This was a multistep process involving several different programs. The step by step process of the analysis was: (I) sample preparation and photography; (II) data preparation and landmark placement; (III) importing data and morphometric analysis. Step two used the TPS (thin-plate spline) suit of software created by F. James Rohlf, while step three used MorphoJ created by Peter Klingenberg and by F. James Rohlf.

All programs listed were used on a Windows 10 operating system:

- tpsUtil v1.74: a program that has multiple utilities for thin-plate spline (tps) analysis, including tools for formatting photographs and creating .tps files (Rohlf, 2015). https://www.sbmorphometrics.org/soft-dataacq.html
- tpsDig2 v2.31: a program that allows the user to digitise landmarks and outlines onto photographs of specimens (Rohlf, 2015). https://www.sbmorphometrics.org/soft-dataacq.html
- MorphoJ v1.07a: is a Java plug-in program for the image analysis software ImageJ. It provides a wide range of tools for morphometric analysis (Klingenberg, 2011). https://morphometrics.uk/MorphoJ_page.html

#### 2.5.1 Sample Preparation and Photography:

The first step was to photograph the apertural and apical angles of ten shells (with no or minimal damage) for each species present at each site. This was accomplished by placing the shells under a dissection microscope with a 64MegaPixel smartphone camera attached to the eyepiece (Figure 2.7). Each digital picture had a 5mm and 10mm scalebar present, with the positioning of the shell and magnification setting of the microscope being standardised. Digital pictures were labelled and saved in a .jpeg format and transferred over to a Windows 10 based computer for landmark placement.

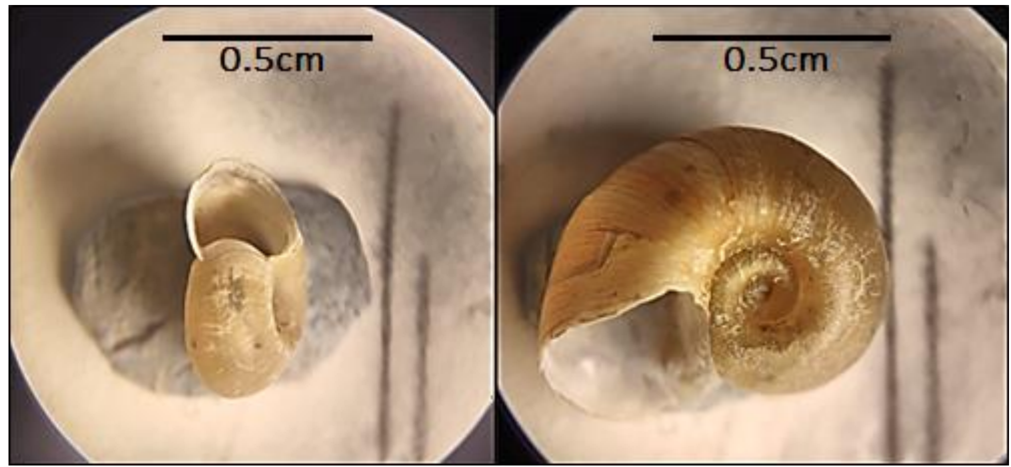


Figure 2.7. Examples of apertural (left) and apical (right) pictures of a *Biomphalaria* shell using smartphone microscopic photography.

### 2.5.2 Data Preparation and Landmark Placement:

After transferring, pictures were organised into folders. The structural information of the folders and pictures was then converted into a .tps file using tpsUtil. This was done by selecting Operation > Build tps file from images > Input directory > Output file > Setup > Create. Next, the newly created .tps file was imported into tpsDig2 for landmark placement. This was done by selecting File > Input source > File. The number and location of all landmarks placed were the same for every shell. The scale of each shell was quantified using the scalebars present in each photograph. This was done by selecting Option > Image tools > Measure > Set scale. Once all landmarks were placed and scale data was set for all specimens, the .tps file was ready for analysis.

### 2.5.3 Importing Data and Morphometric Analysis:

After landmark placement, the .tps file was imported into MorphoJ by selecting File > Create New Project > File Type: TPS. Next, the data was classified into groups based on species ID and location, this was performed by selecting Preliminaries > Extract New Classifier from ID String. After being placed into groups, a Procrustes Fit was performed on the data by selecting Preliminaries > New Procrustes Fit > Align by principal axes. Next the landmark placement data was assessed to ensure each specimen had a consistent number and placement of each landmark. This was done by selecting Preliminaries > Find Outliers. Next a covariance matrix was produced using the Procrustes fit data and the groups created earlier. This was performed by selecting Preliminaries > Generate Covariance Matrix. Lastly, a canonical variate analysis was performed by selecting Comparison > Canonical Variate Analysis.

## 2.6 Statistical Analysis:

All datasets were imported into SPPS from Excel. All non-parametric tests were performed using IBM SPSS Statistics version 26 (IBM, Armonk, USA). A Pearson's chi-squared ($X^2$) test was performed by selecting Analyse > Non-parametric Tests > One Sample. Similarly, a Mann-Whitney U or Kruskal-Wallis H test was performed by selecting Analyse > Non-

parametric Tests > Independent Samples. A Spearman's rank correlation test was performed by selecting Analyse > Correlate > Bivariate.

# Chapter 3 Comparing Shell Size and Shape with Canonical Variate Analysis of Sympatric *Biomphalaria* Species within Lake Albert and Lake Victoria[1]

[1]This chapter was published as "*Andrus, P. S., Stothard, J. R., Kabatereine, N. B. & Wade, C. M. (2023). Comparing shell size and shape with canonical variate analysis of sympatric Biomphalaria species within Lake Albert and Lake Victoria. Zoological Journal of the Linnean Society, 198(2), 1-10.*"


## Abstract:

The Great African Lakes in Uganda (Lake Albert and Lake Victoria) are known habitats to several sympatric species of *Biomphalaria*, intermediate snail hosts of the human parasite *Schistosoma mansoni*. Accurate identification of snails by morphology alone, however, can be problematic highlighting a need for robust, on-site identification methods, since only certain species have important roles in parasite transmission. This study investigates the conchological variation within *Biomphalaria* species collected from these two Great East African Lakes. We compared the shell morphologies of *Biomphalaria* species using landmark-based morphometric techniques and were able to distinguish *Biomphalaria* species through canonical variate analysis (CVA) of the apical and apertural shell angles. After identification with molecular methods, three *Biomphalaria* species (*B. pfeifferi*, *B. stanleyi* and *B. sudanica*), with heterogenous occurrences along the shoreline, were identified at Lake Albert that could be differentiated from one another using CVA of apical and apertural datasets; by contrast, a single *Biomphalaria* species was identified at Lake Victoria (*B. choanomphala*). When snails from both lakes were compared together, CVA was able to differentiate all four species using the apical dataset but not the apertural dataset. Of the *Biomphalaria* species identified, ecological phenotypic variation was only found in *B. choanomphala*, which exhibited two distinct ecological morphotypes. Furthermore, these two *B. choanomphala* morphotypes from Lake Victoria, overlapped upon analysis of the apical dataset yet were clearly separated upon analysis of the apertural dataset. Our study demonstrates that landmark-based morphometrics could play a future role in distinguishing sympatric *Biomphalaria* species in Uganda.


## 3.1 Introduction:

Freshwater snails of the genus *Biomphalaria* (Gastropoda: Planorbidae) are found in South and Central America, Africa, the Middle East and Madagascar (Brown, 1994; Dejong et al., 2001; Rollinson, 2011). They act as the obligatory intermediate hosts of *Schistosoma mansoni* (Trematoda: Schistosomatidae), a globally important trematode responsible for intestinal schistosomiasis (Brown, 1994; Colley et al., 2014). In Africa, a total of 15 species of *Biomphalaria* are recognised, Mandahl-Barth (1957) being the first to categorise them into four main taxonomic groups based on a combination of several morphological characters (shell, genital organs and radula).

The four groups of *Biomphalaria* comprise the *B. alexandrina*-group (*B. alexandrina, B. angulosa, B. salinarum* and *B. tchadiensis*), the *B. choanomphala*-group (*B. barthi, B. choanomphala, B. smithi* and *B. stanleyi*), the *B. pfeifferi*-group (*B. pfeifferi and B. rhodesiensis*) and the *B. sudanica*-group (*B. camerunensis* and *B. sudanica*). Of these

morphological identification methods, genital morphology is the most dependable as complementary reproductive organs are essential for intraspecies mating (Gómez, 2001). However, identifying *Biomphalaria* using genital morphology requires both time and expertise, as the genitals need to be cautiously dissected from relaxed snails, then carefully fixed and mounted for viewing. Subsequently, fine detail measurements are collected under a suitable light microscope. This precludes rapid identification of snails at the sight of collection and makes identification of snails by shell morphology more preferrable. Although conchological identification has its drawbacks, its rapid and inexpensive when compared to other morphological, or molecular identification methods.

The introduction of molecular studies has partially clarified the taxonomy and phylogeography of African *Biomphalaria*. Both Dejong et al. (2001) and Jørgensen et al. (2007) found that the only clearly defined African species were *B. camerunensis* and *B. pfeifferi*, while the six other *Biomphalaria* species (*B. alexandrina*, *B. angulosa*, *B. choanomphala*, *B. smithi*, *B. stanleyi* and *B. sudanica*) formed a poorly defined clade named the 'Nilotic species complex'. Dejong et al. (2001) confirmed the topology was consistent with the proposed Neotropical origins of the genus, with the oldest *Biomphalaria* fossils being dated from approximately 60 million years ago (Jarne et al., 2011). However, all of the African *Biomphalaria* species have a low level of genetic diversification (Morgan et al., 2002; Van Damme & Van Bocxlaer, 2009), which is likely the result of their relatively recent evolutionary history. Campbell et al. (2000) places the introduction of proto-*B. glabrata* taxon to the African continent from South America and the evolution of all African *Biomphalaria* at approximately 1.8 to 3.6 Mya, while Morgan et al. (2001) estimated a longer time frame of 2 to 5 Mya based on the current fossil record. Furthermore, the remaining nominal African *Biomphalaria* species (*B. arabica*, *B. barthi, B. rhodesiensis, B. ruppellii, B. salinarum* and *B. tchadiensis*) previously defined exclusively by morphological characteristics are becoming increasingly invalidated by modern molecular methods, with further investigation needed to confirm whether these species are valid taxa (Jørgensen et al., 2007). The large number of invalid taxa within the *Biomphalaria* literature is likely the result of several (if not all) species of *Biomphalaria* being subject to various sources of intraspecific variation such as ecophenotypic variation and indeterminate shell growth (Jarne et al., 2011). Collectively, this can make two individuals within a single nominal species appear taxonomically distinct entities (Jarne et al., 2011). Standley et al. (2011) and Zhang et al. (2018) both found that *B. choanomphala* snails present at Lake Victoria exhibited contrastingly different conchological morphologies, likely due to presence or absence of wave action, but were very genetically similar.

Similarly to *Biomphalaria*, the closely related Planorbidae genus *Helisoma* also exhibits a striking amount of morphological variation (Hoverman et al., 2005). Dillon (2019) discusses the conchological variation found in two genetically identical populations of *H. trivolvis*, which had two contrasting shell morphologies dependent on whether the snails lived in lentic (still) or lotic (flowing) water (Figure 3.1). Dillon (2019) hypothesised that these two

contrasting shell morphologies were ecological phenotypes (or ecophenotypes) that helped the snails adapt to their micro-environments. The lentic morphotype (morphotype-A, Figure 3.1) is large, narrow, flat and has an arithmetic spiral. This morphology allows for the trapping of air, which the snail uses to regulate its buoyancy in still water to reach and graze on floating vegetation. Conversely, the lotic morphotype (morphotype-B, Figure 3.1) is small, broad, round and has a logarithmic spiral. This morphology cannot trap air and allows for less drag in flowing water, with their wide aperture/foot being used for better grip while grazing onto rocks in flowing water.

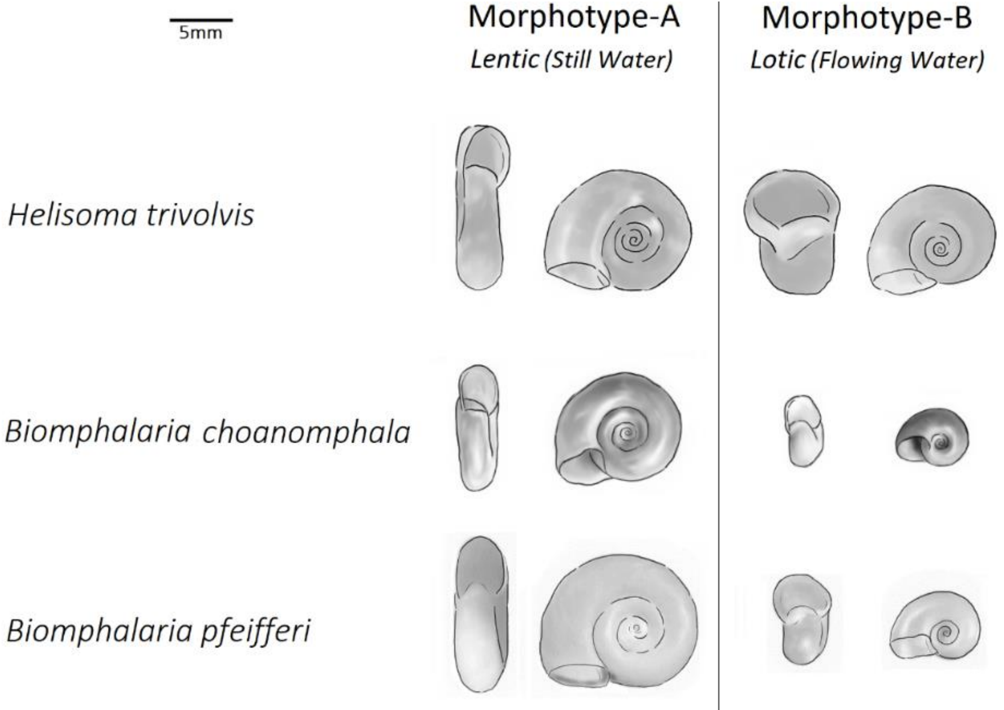


Figure 3.1. Morphological examples of ecological phenotypic plasticity in Planorbidae snails. Morphotype-A is the form found in lentic (still) water, while morphotype-B is the form found in lotic (flowing) water. Morphotype-A shells are larger, slow-whorling and have narrow apertures compared to morphotype-B shells. The *Biomphalaria pfeifferi* and *Helisoma trivolvis* shells were adapted from Plam et al. (2008) and Dillon (2019), respectively. The shells are viewed from the apertural (left) and apical (right) shell angles.

In light of the morphological comparison within *Helisoma* described by Dillon (2019), African *Biomphalaria* species frequently resemble shell morphologies that are similar to the lentic (e.g. *B. alexandrina*, *B. angulosa*, *B. camerunensis*, *B. pfeifferi* and *B. sudanica*) and lotic morphotypes (e.g. *B. choanomphala*, *B. smithi* and *B. stanleyi*) (Brown, 1994; DeJong et al., 2001; Kazibwe et al., 2006; Jørgensen et al., 2007; Plam et al., 2008; Kazibwe et al., 2010; Standley et al., 2011; Zhang et al., 2018). Furthermore, studies that use both conchological and molecular identification methods have shown that *B. choanomphala* and *B. pfeifferi* snails can exhibit these contrasting ecomorphotypes depending on their habitat (Figure 3.1; Plam et al., 2008; Standley et al., 2011; Standley et al., 2014; Zhang et al., 2018). It is plausible that a parallel adaptation occurs in *Biomphalaria* similar to *Helisoma*.

A potential solution to the issues conchological identification methods have when trying to differentiate *Biomphalaria* species, is to incorporate geometric morphometric techniques. Landmark-based geometric morphometrics is a powerful tool used to quantify and analyse the size and shape variation between organisms (Webster & Sheets, 2010) and has been widely used in differentiating medically important invertebrates, insects in particular (Goncalves et al., 2016; de Souza et al., 2020; Jiménez-Martín et al., 2020). Although landmark-based geometric morphometric techniques have been applied previously to medically important snail genera (Vasallo et al., 2013; Parra & Liria 2017; Hammoud et al., 2022), they are yet to be fully explored and applied for differentiating species within *Biomphalaria*. To this end, we utilise molecular identification methods and landmark-based morphometric techniques to undertake a conchological investigation of *Biomphalaria* snails collected from the Ugandan shorelines of Lake Albert and Lake Victoria.

## 3.2 Materials and Methods:

### 3.2.1 Sample Sites:

The *Biomphalaria* used in this study were previously collected by the Rowel et al. (2015) team, further information about these collections can be found in Chapter 2.1. Snails were routinely collected from three disease surveillance sites along the Ugandan shorelines of Lake Albert and three sites along the Ugandan shoreline of Lake Victoria between 2009 and 2010 (Figure 3.2; Table 3.1). The snails were collected from both the lake edge and within the lake (to a depth of ~1m).

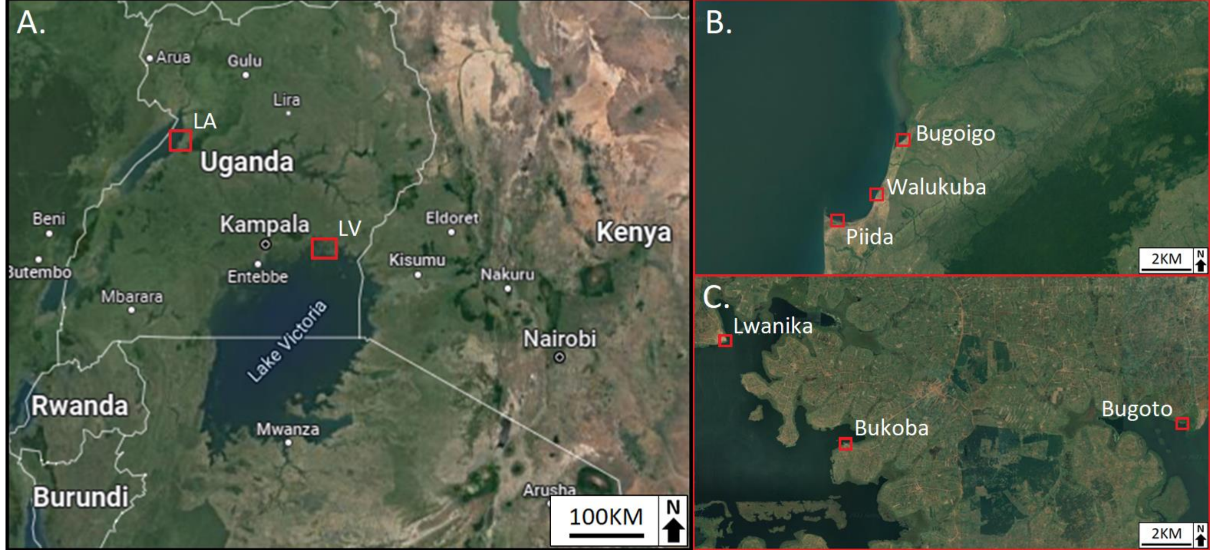


Figure 3.2. (A) Map showing the location of Lake Albert (LA) and Lake Victoria (LV). (B) Lake Albert collection sites (Bugoigo, Piida and Walukuba). (C) Lake Victoria collection sites (Bugoto, Bukoba and Lwanika). Satellite imaging was provided by Google Maps (Google, 2022).

Table 3.1. *Biomphalaria* collection information.

| | Site | Preserved specimens | | | Latitude, Longitude | Elevation |
|---|---|---|---|---|---|---|
| | | No. | A | B | | |
| Lake Albert | Bugoigo | 977 | 84% | 16% | 1.908, 31.409 | 615m |
| | Piida | 521 | 100% | 0% | 1.819, 31.328 | 618m |
| | Walukuba | 1147 | 13% | 87% | 1.842, 31.378 | 617m |
| Lake Victoria | Bugoto | 4005 | 13% | 87% | 0.319, 33.628 | 1153m |
| | Bukoba | 1264 | 44% | 56% | 0.312, 33.492 | 1133m |
| | Lwanika | 1113 | 32% | 68% | 0.351, 33.446 | 1128m |

Note: A & B indicate what percentage of preserved *Biomphalaria* snails were morphotype-A or -B.

3.2.2 Sample Selection, Shell Categorisation and Species Identification:

All of the preserved *Biomphalaria* snails from Lake Albert and Lake Victoria were first categorised into whether they exhibited a morphotype-A or morphotype-B shell morphology (Figure 1). Once shells were categorised as either morphotype A or morphotype B, they were then placed into species groups based on conchological homogeneity (following the identification guide of Brown, 1994). This was conducted based upon how similar the shells looked to one another using specific shell characteristics such as whorl number, shell diameter, shell height and aperture shape. Once all of the shells were categorised, 20 individuals from each species group were randomly selected from each of the six sites.

For each snail, DNA was extracted using a modified CTAB extraction method as described in Chapter 2.3.1 with the extracted samples resuspended in 100-200µl TE, pH 8.0 (10mM Tris-HCl, 0.1mM EDTA) buffer. Species identifications were confirmed by molecular methods using both 16S rRNA (16S) and cytochrome c oxidase subunit I (COI) genotyping. PCR amplifications were performed using a modified version of the 16S primers designed by Palumbi et al. (1991) and the universal COI primers designed by Folmer et al. (1994) (Table 2.1). All PCR reactions were performed using Promega GoTaq® G2 Master Mix buffer, with 1µl of DNA template added to 24µl of 1X Master Mix buffer (1U TAQ, 0.2µM primers, 200µM dNTP, 3mM $MgCl_2$). The PCR cycling conditions used for both the 16S and COI primer sets were identical, with an initial denaturation at 96°C for 1minute, followed by 34 cycles of 94°C for 1min, 50°C for 1min, 72°C for 1min and a final extension at 72°C for 10mins. PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide and were observed under UV light. All 16S and COI PCR products were purified and sequenced using Macrogen's EZ-Seq service.

Both the 16S and COI sequences had their primer sequences removed and were cut down to match the base pair length of chosen GenBank references. Sequences were aligned using the Muscle algorithm in the program Seaview v5 (Gouy et al., 2021), with misaligned sections of the 16S and COI being fixed manually. Conserved sites were selected using the Gblocks program (Castresana et al., 2000). Samples were identified to the species-level

using a concatenated 16S and COI phylogenetic tree incorporating GenBank reference sequences from studies that utilised both conchological and molecular identification methods (Jørgensen et al., 2007; Plam et al., 2008; Standley et al., 2014; Zhang et al., 2018, Supplementary Table 1). Phylogenetic trees were constructed using the Maximum Likelihood method, using a General Time Reversible model incorporating gamma rate correction (GTR+Γ) in the program PhyML v3.1 (Guindon et al., 2010), with bootstrap analysis undertaken using 1000 replicates.

### 3.2.3 Morphometric analysis:

In order to reduce any error associated with *Biomphalaria* shells due to indeterminate shell growth, several preventive steps were implemented into our morphometric analysis: (I) multiple individuals from different populations were used to average the plastic variation within the dataset; (II) only adult specimens were selected (shell diameter greater than ~4mm), to minimize the morphological variation between adult and juvenile shells; (III) a Procrustes fit analysis was used to remove the unwanted effects of translation, rotation and scaling of the dataset during landmark placement and (IV) outlier detection was used to excluding individuals that exhibit extreme morphological differences from the final analysis.

After identifying the *Biomphalaria* species found at the great lakes using the molecular identification methods, ten shells from each species with no (or minimal) damage were selected from each site for photography. In addition to the African *Biomphalaria* samples, five laboratory-bred *B. glabrata* were also included as a comparative control, they were provided by Professor Mike Doenhoff, School of Biology, University of Nottingham. All shells were photographed using a dissection microscope with a 64MegaPixel mobile phone camera attached. All shells were positioned and photographed from the apical and apertural shell angles with a 1mm, 5mm and 10mm scalebar present. Shell diameter and shell height were measured using a dial caliper before each photo. Photographs were imported into the tpsDig2 v2.31 program (Rohlf, 2015), with each image being digitised using 14 landmarks for the apical view (4 fixed and 10 semi-landmark) and 15 landmarks for the apertural view (2 fixed and 13 semi-landmark) of the shell (Figure 3).

The landmark placement of the apical shell photos was guided by the landmark placement of Parra & Liria (2017). The coordinate data for the apical and apertural photos were stored in separate TPS files, and each sample was scaled and had a unique ID (e.g., ID=BS-1). The TPS files were then imported into the MorphoJ v1.07 program (Klingenberg, 2011). The apical shell data was treated as non-symmetrical, while the apertural shell data was treated as symmetrical due to the bilateral symmetry of the 15 landmarks. The data was grouped based on species (as defined by the molecular data) and a full Procrustes fit was performed to help standardise the data and minimise any differences in object orientation or size.

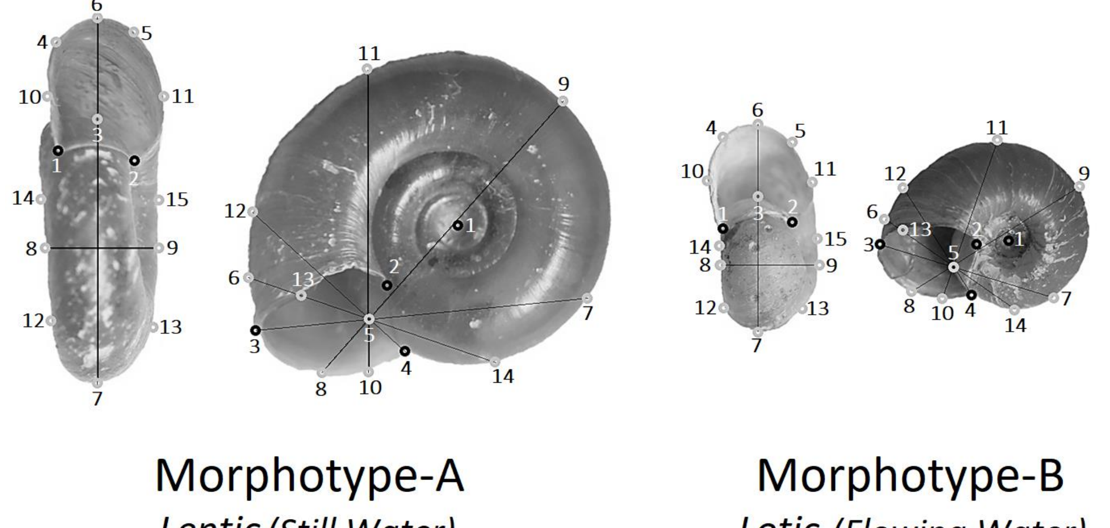


Figure 3.3. Fixed landmarks (black) and semi-landmarks (grey) on the morphotype-A (lentic) and morphotype-B (lotic) forms of *Biomphalaria* from the apertural (left) and apical (right) shell angles.

A Canonical Variate Analysis (CVA) (also known as Canonical Correlation Analysis or Linear Discrimination Analysis) was used across all landmarks using 10,000 permutations. A CVA is defined as a statistical technique used to analyse the relationship between two sets of variables. In this case, our landmark coordinate data is the independent variable, while the dependent variables are our species groups as defined by 16S and COI genotyping. CVA is a multivariate analysis that is used to extract the most important information (called canonical variables) from a large and complex dataset. It is particularly useful when the goal is to identify patterns in the data that are not immediately obvious from the raw data itself. These newly created canonical variables are linear combinations of the original variables and are chosen based on how well they explain the variation between the original two datasets, with the first canonical variable (CV1) explaining the most variation, followed by the second (CV2) explaining the second most, and so on. CVA was chosen over other multivariate statistical techniques (like Principal Component Analysis) due to CVA being optimised for the classification and discrimination of groups within large datasets.

### 3.2.4 GenBank Accessions:

GenBank accession numbers for the *Biomphalaria* 16S and COI sequences used from Jørgensen et al. (2007), Plam et al., 2008, Standley et al. (2014) and Zhang et al. (2018) can be found in Supplementary Table 3.1. The DNA sequences generated in this study are available in GenBank accession numbers OQ924749-OQ924929 for the 16S gene and OQ849817-OQ849997 for the COI gene (Supplementary Table 3.1).

3.3 Results:

3.3.1 Species Found and Shell Morphologies:

At Lake Albert, three *Biomphalaria* species (*B. pfeifferi*, *B. stanleyi* and *B. sudanica*) were identified using conchological and molecular methods (Figure 3.4). Walukuba had all three species present, Bugoigo had two species (*B. pfeifferi* and *B. sudanica*) and Piida had one (*B. sudanica*). At Lake Victoria, only *B. choanomphala* was present and was found at all three sites (Figure 3.4).

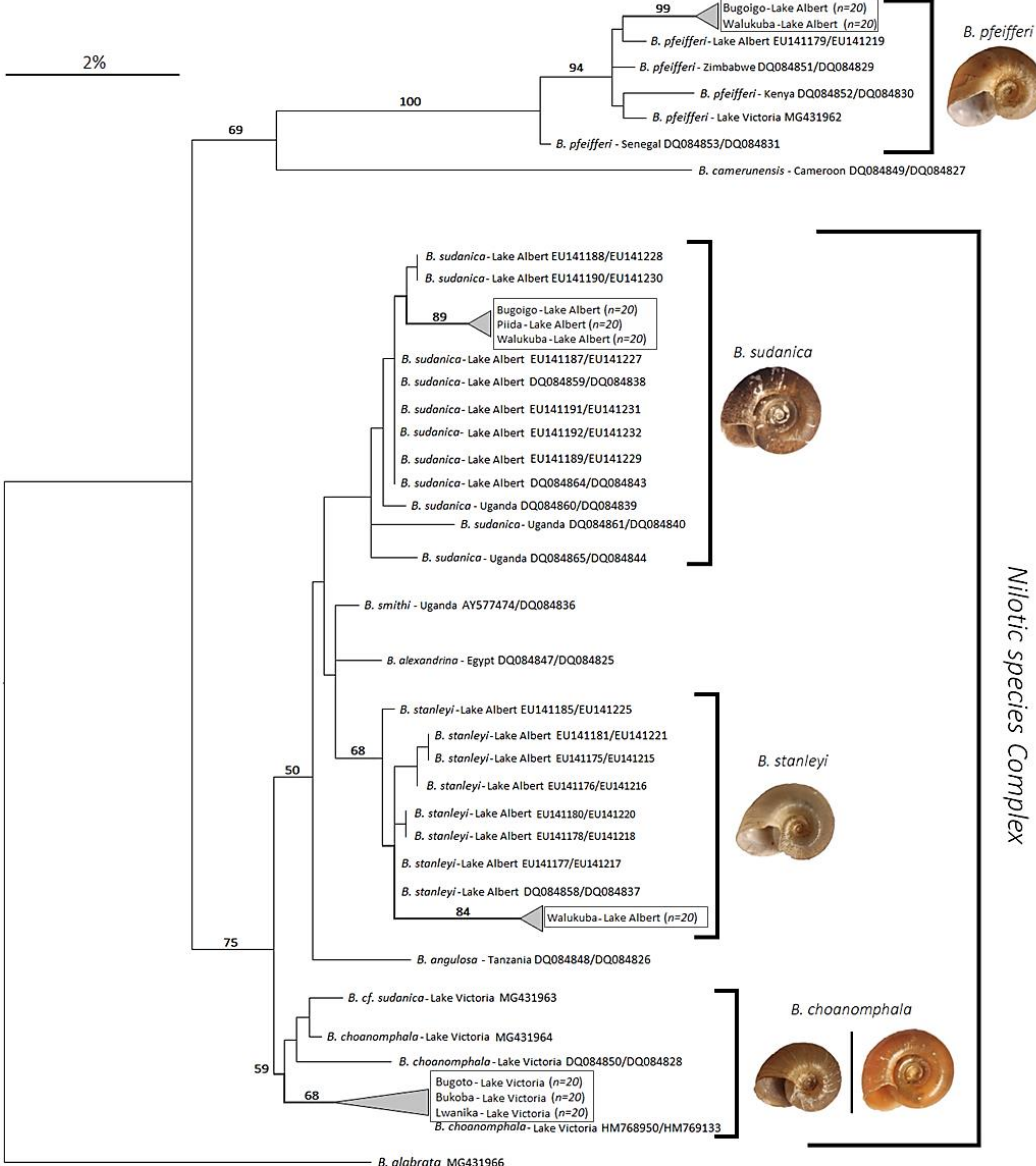


Figure 3.4. Maximum likelihood tree of the combined 16S rRNA (330bp) and cytochrome c oxidase subunit I (500bp) gene fragments. This tree was generated using PhyML v3.1 using a GTR+Γ model and is rooted on *Biomphalaria glabrata.* Numbers on branches indicate the bootstrap percentages for 1000 replicates (bootstrap values under 50% were not shown). The scale bar represents 2% sequence divergence. Samples labelled '*cf.'* had shell morphologies' that looked like a specific species but were identified as another species using

molecular methods (from Jørgensen et al., 2007; Plam et al., 2008; Standley et al., 2014; Zhang et al., 2018).

At Lake Albert, there was no ecophenotypic variation within species. All 60 of the *B. sudanica* identified had morphotype-A shells, all 40 *B. pfeifferi* had morphotype-B shells and all 20 *B. stanleyi* had morphotype-B shells (Table 3.2). Of the 2,645 preserved *Biomphalaria* snails from Lake Albert, approximately 54% of the shells were morphotype-A and were morphologically homogenous to *B. sudanica*. The remaining shells were morphotype-B, with approximately 43% of them being morphologically homogenous to *B. pfeifferi* and 3% being morphologically homogenous to *B. stanleyi*. Conversely, at Lake Victoria there was ecophenotypic variation within *B. choanomphala* with 45 of the 60 *B. choanomphala* identified having morphotype-B shells and the remaining 15 having morphotype-A shells. Of the 6,382 preserved *Biomphalaria* snails from Lake Victoria, approximately 75% of the shells were morphologically homogenous to morphotype-B *B. choanomphala*. The remaining 25% were morphologically homogenous to morphotype-A *B. choanomphala*.

The largest species of *Biomphalaria* found at the great lakes was *B. sudanica*, with a mean whorl number of 5.62, a mean shell diameter of 11mm and a mean shell height of 3.3mm (Table 3.2). The second largest species was the morphotype-A form of *B. choanomphala* with a mean whorl number of 5.86, a mean shell diameter of 9.9mm and a mean shell height of 3.6mm (Table 3.2). The third largest species was *B. pfeifferi* with a mean whorl number of 3.18, a mean shell diameter of 7.7mm and a mean shell height of 3.8mm (Table 3.2). The fourth largest species was the Morphotype-B form of *B. choanomphala* with a mean whorl number of 4.22, a mean shell diameter of 6.7mm and a mean shell height of 3.1mm. The smallest species found was *B. stanleyi* with a mean whorl number of 3.35, a mean shell diameter of 5.3mm and a mean shell height of 2.4mm (Table 3.2). In addition to the four *Biomphalaria* species found, an invasive Asian *Gyraulus* species was identified at both Lake Albert and Lake Victoria (Supplementary Figure 3.1). It had an appearance similar to juvenile *B. sudanica* but was significantly thinner with a mean shell height of 0.9mm, a mean whorl number of 4.55 and a mean shell diameter of 3.7mm.

Table 3.2. Mean shell diameter and height of photographed *Biomphalaria* shells.

| | Sites | Species | Morphotype | Mean Shell Dimensions (mm) | |
|---|---|---|---|---|---|
| | | | | Diameter (+SD) | Height (+SD) |
| - | Control | *B. glabrata (n=10)* | A | 15.5 (+4.1) | 5.2 (+0.9) |
| Lake Albert | Bugoigo | *B. sudanica* (*n=10*) | A | 11.7 (+1.6) | 3.3 (+0.4) |
| | | *B. pfeifferi* (*n=10*) | B | 7.6 (+1.8) | 3.7 (+0.8) |
| | Piida | *B. sudanica* (*n=10*) | A | 11.6 (+2.9) | 3.4 (+0.3) |
| | Walukuba | *B. sudanica* (*n=10*) | A | 9.5 (+2.1) | 3.1 (+0.4) |
| | | *B. pfeifferi* (*n=10*) | B | 7.8 (+1.6) | 3.9 (+0.8) |
| | | *B. stanleyi* (*n=10*) | B | 5.3 (+0.5) | 2.4 (+0.1) |
| Lake Victoria | Bugoto | *B. choanomphala* (*n=10*) | B | 6.6 (+0.5) | 3.1 (+0.2) |
| | Bukoba | *B. choanomphala* (*n=10*) | B | 6.2 (+0.6) | 3.3 (+0.1) |
| | Lwanika | *B. choanomphala* (*n=10*) | B | 7.2 (+0.5) | 3 (+0.4) |
| | All Sites | *B. choanomphala* (*n=10*) | A | 9.9 (+1.3) | 3.6 (+0.3) |

Note: 'S.D' stands for Standard Deviation.

3.3.2 Morphometrics:

When a canonical variate analysis (CVA) was performed on the *Biomphalaria* samples found at Lake Albert, we found that the three species of *Biomphalaria* present at Lake Albert were clearly separated from one other when using both the apical (CV1: 95.6% and CV2: 4.4%) and apertural (CV1: 98.1% and CV2: 1.9%) datasets (Figure 3.5a). For Lake Victoria, the two morphotypes of *B. choanomphala* overlapped with one another in CVA analysis using the apical dataset (CV1: 85.6% and CV2: 14.4%) (Figure 3.5b) but were separated when using the apertural dataset (CV1: 95% and CV2: 5%) (Figure 3.5b).

When a CVA was performed on all *Biomphalaria* samples obtained from both lakes combined, we found that all four species were clearly separated from one another using the apical dataset (CV1: 79.3% and CV2: 17.3%) (Figure 3.5c). For the apertural dataset, *B. pfeifferi* showed overlap (albeit minimal) with *B. stanleyi*, and *B. sudanica* showed large amounts of overlap with the morphotype-A form of *B. choanomphala* (CV1: 76.9% and CV2: 16.9%) (Figure 3.5c). The two morphotypes of *B. choanomphala* (morphotypes A and B) overlapped for the apical dataset but when using the apertural dataset the morphotype A and B forms of *B. choanomphala* were separate (Figure 3.5c).

Our CVA plots found the apical dataset more informative at differentiating the Lake Albert species from one another than the apertural dataset (Figure 3.5a). The apical dataset was also capable of differentiating the Lake Albert species from *B. choanomphala* (Figure 3.5c). While the apertural dataset was able to differentiate the Lake Albert species and both of the *B. choanomphala* morphotypes when tested separately, it was only able to differentiate the

morphotype-B form of *B. choanomphala* when testing all samples together. Additional information relating to the morphological differences between species that had overlapping morphological characteristics can be found in Supplementary Figure 3.2.

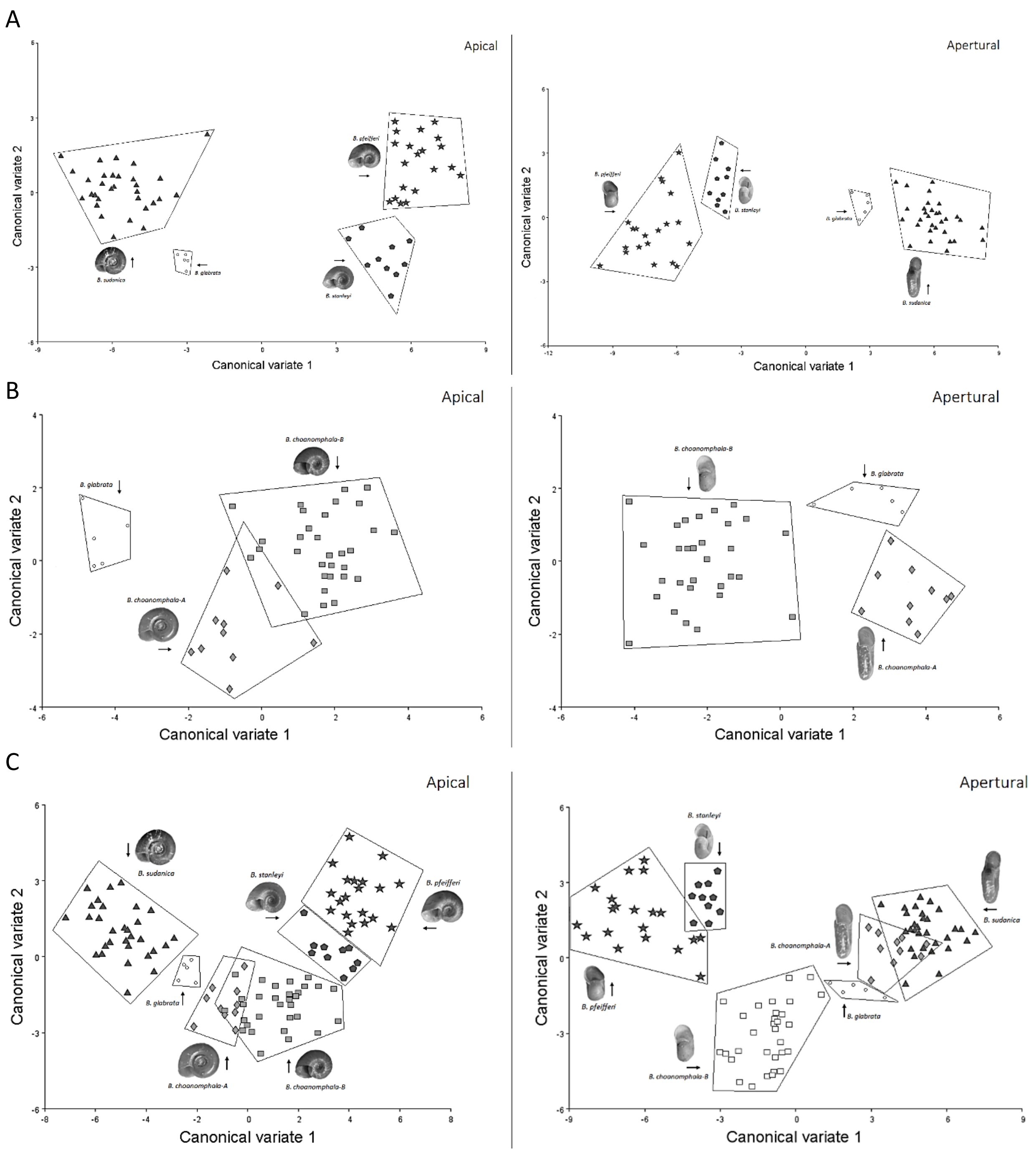


Figure 3.5. Canonical variate analysis plots of the apical and apertural shell landmark datasets. (A) CVA plot of the *Biomphalaria* species present at Lake Albert (*B. pfeifferi* (*n=20*), *B. stanleyi* (*n=10*) and *B. sudanica* (*n=30*)). (B) CVA plot of *Biomphalaria* species present at Lake Victoria (*B. choanomphala* morphotype-A (*n=10*) and *B. choanomphala* morphotype-B (*n=30*)). (C) CVA plot of *Biomphalaria* species present at both lakes (*B. choanomphala* morphotype-A (*n=10*), *B. choanomphala* morphotype-B (*n=30*), *B. pfeifferi* (*n=20*), *B. stanleyi* (*n=10*) and *B. sudanica* (*n=30*)). All CVA plots contain *B. glabrata* (*n=5*) as a control. All

samples from Lake Albert are coloured black, samples from Lake Victoria are coloured grey and the *B. glabrata* samples are coloured white. *B. choanomphala-A* = ◊, *B. choanomphala-B* = □, *B. glabrata* = ○, *B. stanleyi* = ⬠, *B. sudanica* = △ and *B. pfeifferi* = ☆.

3.4 Discussion:

The morphological identification of intermediate snail hosts is the first step in the 'active surveillance and monitoring system' proposed by Abe et al. (2018), as morphological identification can be done in the field and can be used to determine where transmission and potential risk of *Schistosoma* infection can occur. Whilst identification conducted in the field using morphology is useful due to its simplicity and low cost, certain species (such as sympatric *Biomphalaria*) cannot be easily distinguished by morphology alone. Alternatively, more precise methods are required when trying to identify similar species that co-inhabit the same environment (Webster, & Sheets, 2010; Palasio et al., 2017; Vaux et al., 2018). Our study is the first contribution that utilises landmark-based geometric morphometric techniques to differentiate sympatric *Biomphalaria* species. Previous conchological morphology studies of *Biomphalaria* have categorised species based on whether they exhibited a "lacustrine" morphology (found within a lake) or a "non-lacustrine" morphology (found elsewhere) (DeJong et al., 2001; Kazibwe et al., 2006; Plam et al., 2008; Kazibwe et al., 2010). However, these categories are contradictory to the terminology proposed by Dillon (2019) as the lacustrine morphology is equivalent to the lotic (morphotype-B) morphotype, but lakes are described as lentic ecosystems. Moreover, we found both "lacustrine" (morphotype-B) and "non-lacustrine" (morphotype-A) shells at both Lake Albert and Lake Victoria, making the differentiation arbitrary.

The ratio of morphotype-A and morphotype-B shells present at each site varied (Table 3.1). This could indicate that some of the sites were more preferable for one of the morphotype than another. For example, at Lake Albert, morphotype-A shells were predominantly found at Bugoigo and Piida, while morphotype-B shells were predominantly found at Walukuba. It is worth noting that the shoreline of Walukuba is much more open to wave action than that at Bugoigo and Piida, which are each nested behind large spits that protrude into the lake. Bugoigo and Piida both have lentic ecosystems in the form of sheltered marshlands, conversely to Walukuba which is a more lotic ecosystem (Figure 3.2; Supplementary figure 3.3). Likewise, Bugoto had the lowest number of morphotype-A shells and was an unprotected shoreline, while Bukoba (and Lwanika) had the highest number of morphotype-A shells and had a lentic ecosystem in the form of sheltered vegetation protected by coves (Figure 3.2; Supplementary Figure 3.3). Alternatively, shell morphology could also be influenced by other factors besides the flowrate of the ecosystem such as parasitism, predation and temperature (Haas, 2003; Hoverman et al. 2005; Holomuzki & Biggs, 2006; Hoverman & Relyea, 2007; Lagrue et al., 2007; Vasallo et al., 2013; Parra & Liria, 2017; Tamburi et al., 2018). Of the four *Biomphalaria* species we identified at the great lakes, only *B. choanomphala* was found to exhibit more than one ecophenotype. Our findings are consistent with both Standley et al. (2011) and Zhang et al. (2018) who found that *B.*

*sudanica*-like snails at Lake Victoria were more genetically similar to *B. choanomphala* than to *B. sudanica* from other African countries and should in fact be classified as *B. choanomphala* and not *B. sudanica*. Similarly, Standley et al. (2012) found the morphotype-A and morphotype-B forms of *B. choanomphala* were present across the entire shoreline of Lake Victoria. Moreover, their Bayesian analysis found each ecophenotype inhabited separate ecological niches from one another, with specific abiotic variables (e.g. chloride, nitrate, sulphate, pH and water depth) being significant predictors of which morphotype would be present in a given ecosystem.

When measuring each of the *Biomphalaria* species found at the great lakes, *B. sudanica* had the largest mean shell diameter (11mm) followed by the morphotype-A form of *B. choanomphala* (9.9mm), then *B. pfeifferi* (7.7mm), then the morphotype-B form of *B. choanomphala* (6.7mm) and finally *B. stanleyi* (5.3mm). Conversely, the *Biomphalaria* species with the largest mean shell height was the morphotype-A form of *B. choanomphala* (3.6mm) followed by *B. pfeifferi* (3.8mm), then *B. sudanica* (3.3mm), then the morphotype-B form of *B. choanomphala* (3.1mm) and finally *B. stanleyi* (2.4mm). However, these shell characteristics alone were not dependable enough to distinguish *Biomphalaria* species. Further examination using a CVA found the apical shell angle was more informative at distinguishing *Biomphalaria* species from one another than using the apertural shell angle (Figure 3.5). The *Biomphalaria* species found at Lake Albert were morphologically distinct from each other when using both the apical and (to a lesser extent) the apertural dataset (Figure 3.5a). When a CVA was performed on the two *B. choanomphala* morphotypes found at Lake Victoria, the apical dataset showed an overlap between the two, while the apertural dataset did not (Figure 3.5b). This showed despite the apparent difference in shell diameter and height, the apical dataset was able to find homogenous characteristics between the two *B. choanomphala* morphotypes.

Previous studies of the great lakes consistently report *B. pfeifferi*, *B. sudanica* and *B. stanleyi* at Lake Albert and *B. choanomphala* at Lake Victoria (Brown, 1994; Jørgensen et al., 2007; Plam et al., 2008; Adriko et al., 2013; Zhang et al., 2018; Rowel et al., 2015; Mutuku et al., 2019). However, despite the long-established history of *B. choanomphala* being endemic to Lake Victoria, Plam et al. (2008) found *B. choanomphala* at Lake Albert, though this might have been an ephemeral presence. Similarly, Zhang et al. (2018) found *B. pfeifferi* in streams leading into Lake Victoria. These cases of atypical *Biomphalaria* species being found at each of the great lakes is likely due to the invasive nature of *Biomphalaria* (Pointer et al., 2005). Therefore, the possibility of *Biomphalaria* species from one lake being introduced to another is very likely. Newly introduced *Biomphalaria* species could affect the transmission rates of intestinal schistosomiasis at the great lakes as some *Biomphalaria* species are more compatible with *S. mansoni* than others (Brown, 1994; Morgan et al., 2001; Campbell et al., 2010; Stensgaard et al., 2013; Lu et al., 2016).

When CVA was used to compare the *Biomphalaria* species at both Lake Albert and Lake Victoria using the apical dataset, the Lake Albert species (*B. pfeifferi*, *B. stanleyi* and *B.*

*sudanica*) were distinguishable from each other and from *B. choanomphala*. However, the two *B. choanomphala* morphotypes overlapped with each other (Figure 3.5c). The apertural dataset was not as effective as the apical dataset at distinguishing the species, with only the morphotype-B form of *B. choanomphala* being distinct. The morphotype-A form of *B. choanomphala* overlaps with *B. sudanica.* Likewise, *B. pfeifferi* overlaps with *B. stanleyi* (Figure 3.5c).

Our novel use of CVA has been proven able to differentiate the shell morphologies of four *Biomphalaria* species. CVA in future has the potential for improving conchological identification methods used in the field. Further research is needed encompassing a larger variety of specimens, populations, species, and locations to confirm the true effectiveness and integrity of this technique.

## 3.5 Supplementary Material:

Supplementary Table 3.1. GenBank accession numbers and corresponding references for the 16S/COI phylogenetic tree.

| GenBank | | | | |
|---|---|---|---|---|
| Code | Accession no. | | Species | Reference |
| | 16S rRNA | COI | | |
| BsmRwe1 | AY577474 | DQ084836 | *smithi* | Jørgensen et al. (2007) |
| BalDBL1 | DQ084847 | DQ084825 | *alexandrina* | Jørgensen et al. (2007) |
| BanRua1 | DQ084848 | DQ084826 | *angulosa* | Jørgensen et al. (2007) |
| BcaBak1 | DQ084849 | DQ084827 | *camerunensis* | Jørgensen et al. (2007) |
| BchVic1 | DQ084850 | DQ084828 | *choanomphala* | Jørgensen et al. (2007) |
| BpfChi1 | DQ084851 | DQ084829 | *pfeifferi* | Jørgensen et al. (2007) |
| BpfKib1 | DQ084852 | DQ084830 | *pfeifferi* | Jørgensen et al. (2007) |
| BpfDeG1 | DQ084853 | DQ084831 | *pfeifferi* | Jørgensen et al. (2007) |
| BstBut1 | DQ084858 | DQ084837 | *stanleyi* | Jørgensen et al. (2007) |
| BsuBut1 | DQ084859 | DQ084838 | *sudanica* | Jørgensen et al. (2007) |
| BsuKin1 | DQ084860 | DQ084839 | *sudanica* | Jørgensen et al. (2007) |
| BsuMah1 | DQ084861 | DQ084840 | *sudanica* | Jørgensen et al. (2007) |
| BsuNto1 | DQ084864 | DQ084843 | *sudanica* | Jørgensen et al. (2007) |
| BsuRut1 | DQ084865 | DQ084844 | *sudanica* | Jørgensen et al. (2007) |
| FL1 | EU141175 | EU141215 | *stanleyi* | Plam et al. (2008) |
| FL2 | EU141176 | EU141216 | *stanleyi* | Plam et al. (2008) |
| FL3 | EU141177 | EU141217 | *stanleyi* | Plam et al. (2008) |
| FL4 | EU141178 | EU141218 | *stanleyi* | Plam et al. (2008) |
| FL5 | EU141179 | EU141219 | *pfeifferi* | Plam et al. (2008) |
| FL6 | EU141180 | EU141220 | *Stanleyi* | Plam et al. (2008) |
| FN1 | EU141181 | EU141221 | *Stanleyi* | Plam et al. (2008) |
| FN5 | EU141185 | EU141225 | *stanleyi* | Plam et al. (2008) |
| SN1 | EU141187 | EU141227 | *sudanica* | Plam et al. (2008) |
| SN2 | EU141188 | EU141228 | *sudanica* | Plam et al. (2008) |
| SN3 | EU141189 | EU141229 | *sudanica* | Plam et al. (2008) |
| SN4 | EU141190 | EU141230 | *sudanica* | Plam et al. (2008) |

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|---|---|---|---|---|
| SN5 | EU141191 | EU141231 | *sudanica* | Plam et al. (2008) |
| SN6 | EU141192 | EU141232 | *sudanica* | Plam et al. (2008) |
| - | MG431962 | MG431962 | *pfeifferi* | Zhang et al. (2018) |
| - | MG431963 | MG431963 | *cf. sudanica* | Zhang et al. (2018) |
| - | MG431964 | MG431964 | *choanomphala* | Zhang et al. (2018) |
| - | MG431966 | MG431966 | *glabrata* | Zhang et al. (2018) |
| CJS-2010 | HM768950 | HM769133 | *choanomphala* | Standley et al. (2014) |

Our sequences

| Code | Accession no. | | Species | Site/Lake |
|---|---|---|---|---|
| | 16S | COI | | |
| BA1R1 | OQ924829 | OQ849897 | *sudanica* | Bugoigo/LA |
| BA102 | OQ924830 | OQ849898 | *sudanica* | Bugoigo/LA |
| BA103 | OQ924831 | OQ849899 | *sudanica* | Bugoigo/LA |
| BA104 | OQ924832 | OQ849900 | *sudanica* | Bugoigo/LA |
| BA105 | OQ924833 | OQ849901 | *sudanica* | Bugoigo/LA |
| BA106 | OQ924834 | OQ849902 | *sudanica* | Bugoigo/LA |
| BA107 | OQ924835 | OQ849903 | *sudanica* | Bugoigo/LA |
| BA108 | OQ924836 | OQ849904 | *sudanica* | Bugoigo/LA |
| BA109 | OQ924837 | OQ849905 | *sudanica* | Bugoigo/LA |
| BA111 | OQ924838 | OQ849906 | *sudanica* | Bugoigo/LA |
| BA112 | OQ924839 | OQ849907 | *sudanica* | Bugoigo/LA |
| BA113 | OQ924840 | OQ849908 | *sudanica* | Bugoigo/LA |
| BA114 | OQ924841 | OQ849909 | *sudanica* | Bugoigo/LA |
| BA115 | OQ924842 | OQ849910 | *sudanica* | Bugoigo/LA |
| BA116 | OQ924843 | OQ849911 | *sudanica* | Bugoigo/LA |
| BB103 | OQ924844 | OQ849912 | *sudanica* | Bugoigo/LA |
| BB110 | OQ924845 | OQ849913 | *sudanica* | Bugoigo/LA |
| BB111 | OQ924846 | OQ849914 | *sudanica* | Bugoigo/LA |
| BB113 | OQ924847 | OQ849915 | *sudanica* | Bugoigo/LA |
| B2G04 | OQ924848 | OQ849916 | *sudanica* | Bugoigo/LA |
| B1B15 | OQ924789 | OQ849857 | *pfeifferi* | Bugoigo/LA |
| B1B17 | OQ924790 | OQ849858 | *pfeifferi* | Bugoigo/LA |
| B1B19 | OQ924791 | OQ849859 | *pfeifferi* | Bugoigo/LA |
| B1B20 | OQ924792 | OQ849860 | *pfeifferi* | Bugoigo/LA |
| B2B01 | OQ924793 | OQ849861 | *pfeifferi* | Bugoigo/LA |
| B2B03 | OQ924794 | OQ849862 | *pfeifferi* | Bugoigo/LA |
| B2B04 | OQ924795 | OQ849863 | *pfeifferi* | Bugoigo/LA |
| B2B07 | OQ924796 | OQ849864 | *pfeifferi* | Bugoigo/LA |
| B2B08 | OQ924797 | OQ849865 | *pfeifferi* | Bugoigo/LA |
| B7A10 | OQ924798 | OQ849866 | *pfeifferi* | Bugoigo/LA |
| B7B10 | OQ924799 | OQ849867 | *pfeifferi* | Bugoigo/LA |
| B7C10 | OQ924800 | OQ849868 | *pfeifferi* | Bugoigo/LA |
| B7D08 | OQ924801 | OQ849869 | *pfeifferi* | Bugoigo/LA |
| B7D09 | OQ924802 | OQ849870 | *pfeifferi* | Bugoigo/LA |
| B7E09 | OQ924803 | OQ849871 | *pfeifferi* | Bugoigo/LA |
| B7E10 | OQ924804 | OQ849872 | *pfeifferi* | Bugoigo/LA |
| B7F07 | OQ924805 | OQ849873 | *pfeifferi* | Bugoigo/LA |
| B7F09 | OQ924806 | OQ849874 | *pfeifferi* | Bugoigo/LA |
| B7H07 | OQ924807 | OQ849875 | *pfeifferi* | Bugoigo/LA |

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|---|---|---|---|---|
| B7H09 | OQ924808 | OQ849876 | *pfeifferi* | Bugoigo/LA |
| PA101 | OQ924849 | OQ849917 | *sudanica* | Piida/LA |
| PA102 | OQ924850 | OQ849918 | *sudanica* | Piida/LA |
| PA103 | OQ924851 | OQ849919 | *sudanica* | Piida/LA |
| PA107 | OQ924852 | OQ849920 | *sudanica* | Piida/LA |
| PA108 | OQ924853 | OQ849921 | *sudanica* | Piida/LA |
| PA109 | OQ924854 | OQ849922 | *sudanica* | Piida/LA |
| PA110 | OQ924855 | OQ849923 | *sudanica* | Piida/LA |
| PA112 | OQ924856 | OQ849924 | *sudanica* | Piida/LA |
| PA117 | OQ924857 | OQ849925 | *sudanica* | Piida/LA |
| PA118 | OQ924858 | OQ849926 | *sudanica* | Piida/LA |
| PA119 | OQ924859 | OQ849927 | *sudanica* | Piida/LA |
| PA201 | OQ924860 | OQ849928 | *sudanica* | Piida/LA |
| PA203 | OQ924861 | OQ849929 | *sudanica* | Piida/LA |
| PA207 | OQ924862 | OQ849930 | *sudanica* | Piida/LA |
| PA211 | OQ924863 | OQ849931 | *sudanica* | Piida/LA |
| PA212 | OQ924864 | OQ849932 | *sudanica* | Piida/LA |
| PA214 | OQ924865 | OQ849933 | *sudanica* | Piida/LA |
| PA215 | OQ924866 | OQ849934 | *sudanica* | Piida/LA |
| PA217 | OQ924867 | OQ849935 | *sudanica* | Piida/LA |
| PA219 | OQ924868 | OQ849936 | *sudanica* | Piida/LA |
| WA109 | OQ924809 | OQ849877 | *sudanica* | Walukuba/LA |
| WA111 | OQ924810 | OQ849878 | *sudanica* | Walukuba/LA |
| W2B09 | OQ924811 | OQ849879 | *sudanica* | Walukuba/LA |
| W2B10 | OQ924812 | OQ849880 | *sudanica* | Walukuba/LA |
| W2E9 | OQ924813 | OQ849881 | *sudanica* | Walukuba/LA |
| W2E10 | OQ924814 | OQ849882 | *sudanica* | Walukuba/LA |
| W2F01 | OQ924815 | OQ849883 | *sudanica* | Walukuba/LA |
| W2F02 | OQ924816 | OQ849884 | *sudanica* | Walukuba/LA |
| W2F03 | OQ924817 | OQ849885 | *sudanica* | Walukuba/LA |
| W2F04 | OQ924818 | OQ849886 | *sudanica* | Walukuba/LA |
| W2F05 | OQ924819 | OQ849887 | *sudanica* | Walukuba/LA |
| W2F06 | OQ924820 | OQ849888 | *sudanica* | Walukuba/LA |
| W2F07 | OQ924821 | OQ849889 | *sudanica* | Walukuba/LA |
| W2F08 | OQ924822 | OQ849890 | *sudanica* | Walukuba/LA |
| W2F09 | OQ924823 | OQ849891 | *sudanica* | Walukuba/LA |
| W2G01 | OQ924824 | OQ849892 | *sudanica* | Walukuba/LA |
| W2G03 | OQ924825 | OQ849893 | *sudanica* | Walukuba/LA |
| W2G06 | OQ924826 | OQ849894 | *sudanica* | Walukuba/LA |
| W2G07 | OQ924827 | OQ849895 | *sudanica* | Walukuba/LA |
| W2G08 | OQ924828 | OQ849896 | *sudanica* | Walukuba/LA |
| WA103 | OQ924769 | OQ849837 | *pfeifferi* | Walukuba/LA |
| WA104 | OQ924770 | OQ849838 | *pfeifferi* | Walukuba/LA |
| WA107 | OQ924771 | OQ849839 | *pfeifferi* | Walukuba/LA |
| WA108 | OQ924772 | OQ849840 | *pfeifferi* | Walukuba/LA |
| WA112 | OQ924773 | OQ849841 | *pfeifferi* | Walukuba/LA |
| WB101 | OQ924774 | OQ849842 | *pfeifferi* | Walukuba/LA |
| WB102 | OQ924775 | OQ849843 | *pfeifferi* | Walukuba/LA |
| WB103 | OQ924776 | OQ849844 | *pfeifferi* | Walukuba/LA |

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|---|---|---|---|---|
| WB104 | OQ924777 | OQ849845 | *pfeifferi* | Walukuba/LA |
| WB105 | OQ924778 | OQ849846 | *pfeifferi* | Walukuba/LA |
| WB106 | OQ924779 | OQ849847 | *pfeifferi* | Walukuba/LA |
| WB107 | OQ924780 | OQ849848 | *pfeifferi* | Walukuba/LA |
| WB108 | OQ924781 | OQ849849 | *pfeifferi* | Walukuba/LA |
| WB109 | OQ924782 | OQ849850 | *pfeifferi* | Walukuba/LA |
| WB110 | OQ924783 | OQ849851 | *pfeifferi* | Walukuba/LA |
| WB111 | OQ924784 | OQ849852 | *pfeifferi* | Walukuba/LA |
| WB112 | OQ924785 | OQ849853 | *pfeifferi* | Walukuba/LA |
| WB114 | OQ924786 | OQ849854 | *pfeifferi* | Walukuba/LA |
| WB116 | OQ924787 | OQ849855 | *pfeifferi* | Walukuba/LA |
| WB118 | OQ924788 | OQ849856 | *pfeifferi* | Walukuba/LA |
| WA101 | OQ849817 | OQ924749 | *stanleyi* | Walukuba/LA |
| WA102 | OQ849818 | OQ924750 | *stanleyi* | Walukuba/LA |
| WA105 | OQ849819 | OQ924751 | *stanleyi* | Walukuba/LA |
| W6A03 | OQ849820 | OQ924752 | *stanleyi* | Walukuba/LA |
| W6A04 | OQ849821 | OQ924753 | *stanleyi* | Walukuba/LA |
| W6A05 | OQ849822 | OQ924754 | *stanleyi* | Walukuba/LA |
| W7A07 | OQ849823 | OQ924755 | *stanleyi* | Walukuba/LA |
| W7C09 | OQ849824 | OQ924756 | *stanleyi* | Walukuba/LA |
| W7D10 | OQ849825 | OQ924757 | *stanleyi* | Walukuba/LA |
| W7F10 | OQ849826 | OQ924758 | *stanleyi* | Walukuba/LA |
| W9A01 | OQ849827 | OQ924759 | *stanleyi* | Walukuba/LA |
| W9B01 | OQ849828 | OQ924760 | *stanleyi* | Walukuba/LA |
| W9C01 | OQ849829 | OQ924761 | *stanleyi* | Walukuba/LA |
| W9D01 | OQ849830 | OQ924762 | *stanleyi* | Walukuba/LA |
| W9D02 | OQ849831 | OQ924763 | *stanleyi* | Walukuba/LA |
| W9E01 | OQ849832 | OQ924764 | *stanleyi* | Walukuba/LA |
| W9F01 | OQ849833 | OQ924765 | *stanleyi* | Walukuba/LA |
| W9F03 | OQ849834 | OQ924766 | *stanleyi* | Walukuba/LA |
| W9G01 | OQ849835 | OQ924767 | *stanleyi* | Walukuba/LA |
| W9H01 | OQ849836 | OQ924768 | *stanleyi* | Walukuba/LA |
| BG2A08 | OQ924869 | OQ849937 | *choanomphala* | Bugoto/LV |
| BG2A10 | OQ924870 | OQ849938 | *choanomphala* | Bugoto/LV |
| BG2E04 | OQ924871 | OQ849939 | *choanomphala* | Bugoto/LV |
| BG2G09 | OQ924872 | OQ849940 | *choanomphala* | Bugoto/LV |
| BG6B05 | OQ924873 | OQ849941 | *choanomphala* | Bugoto/LV |
| BG6C05 | OQ924874 | OQ849942 | *choanomphala* | Bugoto/LV |
| BG6C06 | OQ924875 | OQ849943 | *choanomphala* | Bugoto/LV |
| BG6C07 | OQ924876 | OQ849944 | *choanomphala* | Bugoto/LV |
| BG6D05 | OQ924877 | OQ849945 | *choanomphala* | Bugoto/LV |
| BG6D08 | OQ924878 | OQ849946 | *choanomphala* | Bugoto/LV |
| BG6D09 | OQ924879 | OQ849947 | *choanomphala* | Bugoto/LV |
| BG6E05 | OQ924880 | OQ849948 | *choanomphala* | Bugoto/LV |
| BG6E06 | OQ924881 | OQ849949 | *choanomphala* | Bugoto/LV |
| BG6E07 | OQ924882 | OQ849950 | *choanomphala* | Bugoto/LV |
| BG6E09 | OQ924883 | OQ849951 | *choanomphala* | Bugoto/LV |
| BG6F08 | OQ924884 | OQ849952 | *choanomphala* | Bugoto/LV |
| BG6F09 | OQ924885 | OQ849953 | *choanomphala* | Bugoto/LV |

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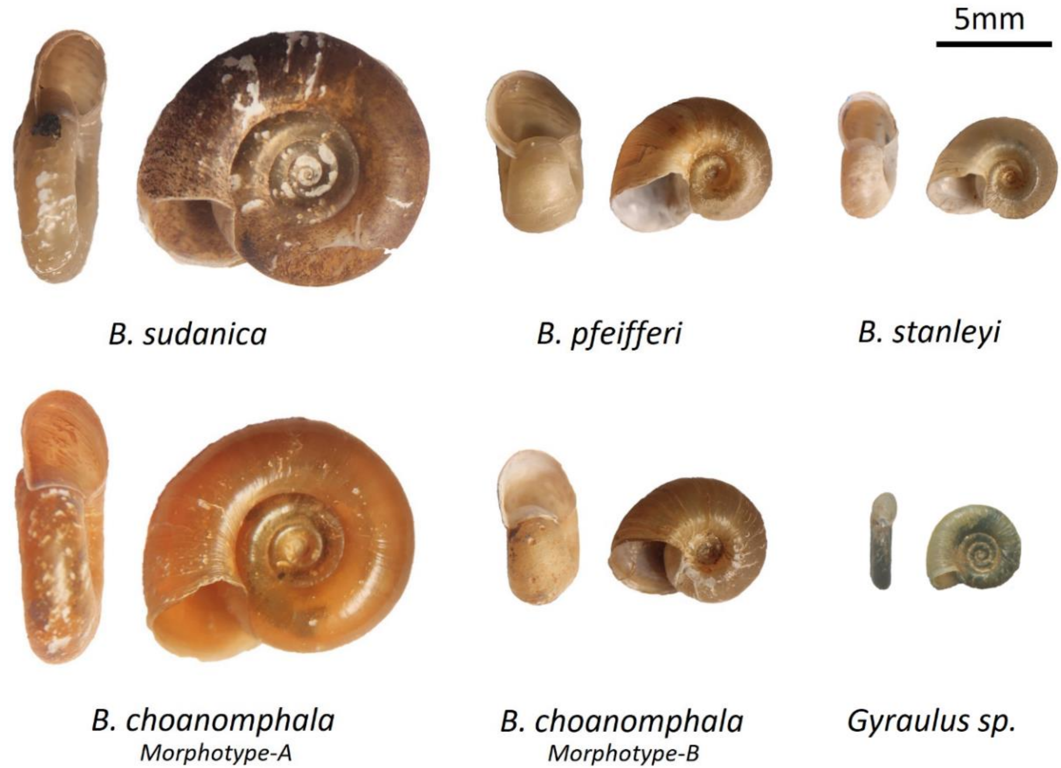
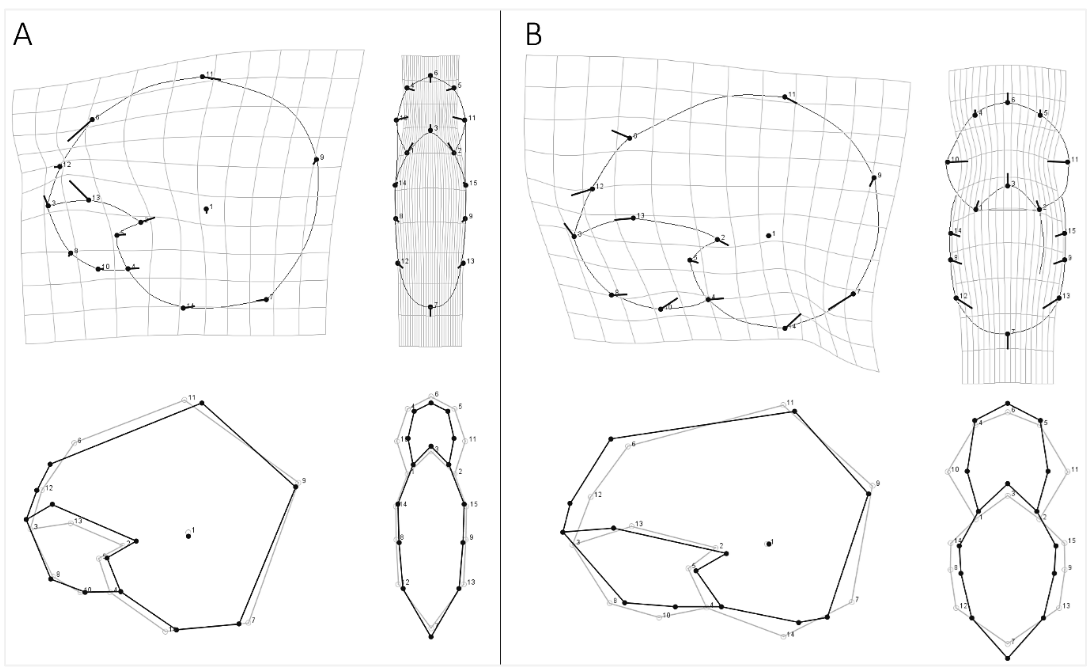
|---|---|---|---|---|
| BG6G06 | OQ924886 | OQ849954 | *choanomphala* | Bugoto/LV |
| BG6G07 | OQ924887 | OQ849955 | *choanomphala* | Bugoto/LV |
| BG6G09 | OQ924888 | OQ849956 | *choanomphala* | Bugoto/LV |
| BKLV01 | OQ924889 | OQ849957 | *choanomphala* | Bukoba/LV |
| BKLV08 | OQ924890 | OQ849958 | *choanomphala* | Bukoba/LV |
| BKLV10 | OQ924891 | OQ849959 | *choanomphala* | Bukoba/LV |
| BK2A04 | OQ924892 | OQ849960 | *choanomphala* | Bukoba/LV |
| BK2A07 | OQ924893 | OQ849961 | *choanomphala* | Bukoba/LV |
| BK2C06 | OQ924894 | OQ849962 | *choanomphala* | Bukoba/LV |
| BK2E01 | OQ924895 | OQ849963 | *choanomphala* | Bukoba/LV |
| BK2E03 | OQ924896 | OQ849964 | *choanomphala* | Bukoba/LV |
| BK2E05 | OQ924897 | OQ849965 | *choanomphala* | Bukoba/LV |
| BK2E06 | OQ924898 | OQ849966 | *choanomphala* | Bukoba/LV |
| BK2E07 | OQ924899 | OQ849967 | *choanomphala* | Bukoba/LV |
| BK2E08 | OQ924900 | OQ849968 | *choanomphala* | Bukoba/LV |
| BK6G08 | OQ924901 | OQ849969 | *choanomphala* | Bukoba/LV |
| BK7D11 | OQ924902 | OQ849970 | *choanomphala* | Bukoba/LV |
| BK7E11 | OQ924903 | OQ849971 | *choanomphala* | Bukoba/LV |
| BK7E12 | OQ924904 | OQ849972 | *choanomphala* | Bukoba/LV |
| BK7H12 | OQ924905 | OQ849973 | *choanomphala* | Bukoba/LV |
| BK9B03 | OQ924906 | OQ849974 | *choanomphala* | Bukoba/LV |
| BK9C03 | OQ924907 | OQ849975 | *choanomphala* | Bukoba/LV |
| BK9F04 | OQ924908 | OQ849976 | *choanomphala* | Bukoba/LV |
| LW2D02 | OQ924909 | OQ849977 | *choanomphala* | Lwanika/LV |
| LW2D03 | OQ924910 | OQ849978 | *choanomphala* | Lwanika/LV |
| LW2D08 | OQ924911 | OQ849979 | *choanomphala* | Lwanika/LV |
| LW2H07 | OQ924912 | OQ849980 | *choanomphala* | Lwanika/LV |
| LW2H09 | OQ924913 | OQ849981 | *choanomphala* | Lwanika/LV |
| LW6B02 | OQ924914 | OQ849982 | *choanomphala* | Lwanika/LV |
| LW6C02 | OQ924915 | OQ849983 | *choanomphala* | Lwanika/LV |
| LW6D03 | OQ924916 | OQ849984 | *choanomphala* | Lwanika/LV |
| LW6D10 | OQ924917 | OQ849985 | *choanomphala* | Lwanika/LV |
| LW6E10 | OQ924918 | OQ849986 | *choanomphala* | Lwanika/LV |
| LW6F02 | OQ924919 | OQ849987 | *choanomphala* | Lwanika/LV |
| LW6F04 | OQ924920 | OQ849988 | *choanomphala* | Lwanika/LV |
| LW6F10 | OQ924921 | OQ849989 | *choanomphala* | Lwanika/LV |
| LW6G01 | OQ924922 | OQ849990 | *choanomphala* | Lwanika/LV |
| LW6G02 | OQ924923 | OQ849991 | *choanomphala* | Lwanika/LV |
| LW7B11 | OQ924924 | OQ849992 | *choanomphala* | Lwanika/LV |
| LW7C11 | OQ924925 | OQ849993 | *choanomphala* | Lwanika/LV |
| LW7D12 | OQ924926 | OQ849994 | *choanomphala* | Lwanika/LV |
| LW7F12 | OQ924927 | OQ849995 | *choanomphala* | Lwanika/LV |
| LW9E04 | OQ924928 | OQ849996 | *choanomphala* | Lwanika/LV |
| Gsp | OQ924929 | OQ849997 | *Gyraulus sp.* | - |

Note: '*cf.*' indicates the shell morphology looked like a specific species but was identified as a different species using molecular methods.




Supplementary Figure 3.1. Shell morphologies of Planorbidae snails found at the Ugandan shorelines of Lake Albert and Lake Victoria. *Biomphalaria pfeifferi, B. stanleyi, B. sudanica* and the unknown *Gyraulus sp.* were present at Lake Albert. *Biomphalaria choanomphala* and the unknown *Gyraulus sp.* were present at Lake Victoria. The shells are viewed from the apertural (left) and apical (right) shell angles.




Supplementary Figure 3.2. Morphometric comparison between (A) *B. choanomphala*-A **and** *B. sudanica* and (B) *B. pfeifferi* **and** *B. stanleyi* using a lollipop graph (top) and wireframe graph (bottom) of the apical and apertural shell angles.

Supplementary Figure 3.3. Aerial views of the Lake Albert (A: Bugoigo; B: Piida; C: Walukuba) and Lake Victoria (D: Bugoto; E: Bukoba; F: Lwanika) collection sites.

# Chapter 4 *Schistosoma mansoni* Infection in *Biomphalaria* Snails at the Ugandan Shorelines of Lake Albert and Lake Victoria

Abstract:

Intestinal schistosomiasis is hyperendemic in many sub-Saharan African countries. In Uganda, it is endemic at both Lake Albert and Lake Victoria due to the presence of *S. mansoni* and its obligatory freshwater snail host, *Biomphalaria*. We utilised a molecular method to detect *S. mansoni* infection in *Biomphalaria* species found at the Ugandan shorelines of Lake Albert and Lake Victoria. Overall, Lake Albert had a higher mean prevalence of *S. mansoni* infection (12.5%) than Lake Victoria (5%), with Walukuba (13.3%) having the highest prevalence at Lake Albert, while Lwanika (10%) had the highest prevalence at Lake Victoria. Overall, three species of *Biomphalaria*, *B. pfeifferi*, *B. stanleyi* and *B. sudanica*, were identified at our Lake Albert collection sites, while only one species, *B. choanomphala*, was identified at our Lake Victoria collection sites. *Biomphalaria stanleyi* (15%) had the highest *S. mansoni* prevalence, followed by *B. sudanica* (13.3%), *B. pfeifferi* (10%) and *B. choanomphala* (5%). Of the *Biomphalaria* species identified, *B. choanomphala* had the highest genetic diversity, followed by *B. stanleyi*, *B. sudanica* and *B. pfeifferi*; sites with a higher mean prevalence of *S. mansoni* infection had higher intra-species haplotype diversity scores than sites with a lower mean prevalence. The wet seasons had a consistently higher mean prevalence of *S. mansoni* infection than the dry seasons for all species and all sites tested at both Lake Albert and Lake Victoria, though the difference was not statistically significant.

## 4.1 Introduction:

Schistosomiasis is a parasitic disease caused by the digenetic trematode genus, Schistosoma. It is estimated that 133 million children and 108 million adults are infected with schistosomiasis worldwide, with over 700 million people being at risk of infection (WHO, 2021b). Schistosomiasis is most prevalent in sub-Saharan African countries, with approximately 93% of infections and up to 90% of individuals at risk of infection living within sub-Saharan African countries (Boko et al., 2016; Onasanya et al., 2021). The disease can be expressed as either intestinal schistosomiasis (caused by *Schistosoma mansoni*, *S. intercalatum*, *S. japonicum* or *S. mekongi*) or urogenital schistosomiasis (caused by *S. haematobium*; Colley & Secor, 2014). *Schistosoma mansoni* is the leading global cause of intestinal schistosomiasis in humans and accounts for 33% of all schistosomiasis cases (WHO, 2021a).

Intestinal schistosomiasis is particularly prevalent in East Africa, with the national prevalence in Tanzania being an estimated 31 million people infected, followed by Uganda with 11 million and Kenya with 6 million (Mazigo et al., 2012; Musuva et al., 2014; Exum et al., 2019). The distribution of schistosomiasis is dependent on the ecological requirements of the intermediate snail host, with the availability of freshwater habitats limiting the spread of schistosomiasis (Sturrock, 2001; Steinmann et al., 2006). East Africa has a high prevalence of schistosomiasis due to the abundance of diverse freshwater environments (lakes, ponds, streams, dams and irrigation canals) that intermediate snail hosts inhabit (Kazibwe et al.,

2006). Combined with poor water hygiene and sanitation, this provides an optimal environment for the transmission of schistosomiasis (Kazibwe et al., 2006).

The freshwater snail genus *Biomphalaria* acts as the intermediate host for *S. mansoni* (Crompton, 1999; Sturrock, 2001; Jamison et al., 2006), with the African Great Lakes, Lake Albert and Lake Victoria providing a favourable habitat for multiple species of *Biomphalaria* (Steinmann et al., 2006). All African *Biomphalaria* species are capable of transmitting *S. mansoni* infection (Brown, 1994), though some species (e.g. *B. pfeifferi*) are considered more important than others (Morgan et al., 2001). Previous studies of Lake Albert and Lake Victoria have shown that multiple species of *Biomphalaria* are present (Brown, 1994; Rowel et al., 2015). Using conchological identification methods, three species, *B. pfeifferi*, *B. stanleyi* and *B. sudanica*, were found at Lake Albert (Brown, 1994; Kazibwe et al., 2006; Kazibwe et al., 2010; Adriko et al., 2013; Levitz et al., 2013; Rowel et al., 2015), and three species, *B. choanomphala*, *B. pfeifferi* and *B. sudanica*, were found at Lake Victoria (Brown, 1994; Adriko et al., 2013; Rowel et al., 2015; Mutuku et al., 2019). However, the lack of non-plastic shell characteristics makes the conchological identification of *Biomphalaria* species difficult, with many morphological features overlapping between species (Plam et al., 2008) and with shell morphology influenced by other factors besides genetics (Haase, 2003; Holomuzki & Biggs, 2006; Lagrue et al., 2007; Vasallo et al., 2013; Parra & Liria, 2017; Tamburi et al., 2018). Molecular identification methods have improved the reliability of *Biomphalaria* species identification but are still not perfect; the only definitive African species are *B. camerunensis*, and *B. pfeifferi* (Dejong et al., 2001) with the remaining African *Biomphalaria* species (*B. alexandrina*, *B. angulosa*, *B. choanomphala*, *B. smithi*, *B. stanleyi* and *B. sudanica*) forming a poorly differentiated clade termed the ‘Nilotic species complex’ (Jørgensen et al., 2007).

The prevalence of schistosome infection within a *Biomphalaria* population has traditionally been measured by observing how many snails shed cercariae over a 35-42 day period (Webbe, 1965). Previous studies using this traditional cercarial shedding method have shown that snails at Lake Albert consistently have a higher infection prevalence than snails at Lake Victoria (Adriko et al., 2013; Rowel et al., 2015). Of the *Biomphalaria* species found at the African Great Lakes, *B. stanleyi* is reported as consistently having a high prevalence of *S. mansoni* infection (Kazibwe et al., 2006; Kazibwe et al., 2010; Adriko et al., 2013; Rowel et al., 2015), while *B. choanomphala* is reported as consistently having a low infection prevalence (Odongo-Aginya et al,. 2008; Adriko et al., 2013; Rowel et al., 2015). Molecular methods for detection of schistosome infection (Jannotti-Passos et al., 1997; Hamburger et al., 1998; Notomi et al., 2000; Sandoval et al., 2006) have several advantages over traditional cercarial shedding methods as they can specifically detect *S. mansoni*, can detect infection in both prepatent and shedding snails, do not require live snail specimens and are considerably less time consuming (Abbasi et al., 2010; Hamburger et al., 2013; Lu et al., 2016; Caldeira et al., 2017). However, while the use of molecular methods for detecting schistosome infection in humans (via urine and faeces) and in the environment (via sediment and water) is widespread, these methods are underutilised for the detection of schistosome infection in intermediate snail hosts collected from the field (ten Hove et al. 2008; Sengupta et al., 2019; Nwoko et al. 2021).

The prevalence of *S. mansoni* infection is affected by multiple factors. Past studies have associated snail populations with low levels of genetic variability with a higher prevalence of *S. mansoni* infection (Jarne & Théron, 2001; Campbell et al., 2010). Additionally, environmental factors such as altitude, water conductivity, water depth, water pH, temperature, droughts and floods have been shown to affect the prevalence of schistosome infection (Shiff et al., 1975; Sturrock et al., 2001; Kabatereine et al., 2004; Rubaihayo et al., 2008; Perez-Saez et al., 2016; Tabo et al., 2022). East Africa has a bimodal climate with two wet seasons (from March to May and from September to November) and two dry seasons (from December to February and from June to August) that take place each year. Adoka et al. (2014) reported that people living at the shoreline of Lake Victoria believed that intestinal schistosomiasis was more prevalent in the wet seasons. Rowel et al. (2015) found evidence in support of this, with their results showing that the number of *Biomphalaria* shedding cercariae was higher during the wet seasons than the dry seasons.

Here we use a PCR-based, molecular infection detection method to investigate the prevalence of *S. mansoni* infection in the *Biomphalaria* species found at the Ugandan shorelines of Lake Albert and Lake Victoria. We measure the extent of genetic diversity in *Biomphalaria* species to determine whether there is any correlation between infection prevalence and snail diversity. We also investigate the effect seasonality has on the prevalence of *S. mansoni* infection within *Biomphalaria* snails by comparing the infection prevalence infection during the wet and dry seasons.

## 4.2 Materials and Methods:

### 4.2.1 Sample Sites and Sample Selection:

*Biomphalaria* snails were previously collected at the Ugandan shorelines of Lake Albert and Lake Victoria by the Rowel et al. (2015) team, for further details about the collections please see Chapters 2.1 and 3.2.1.

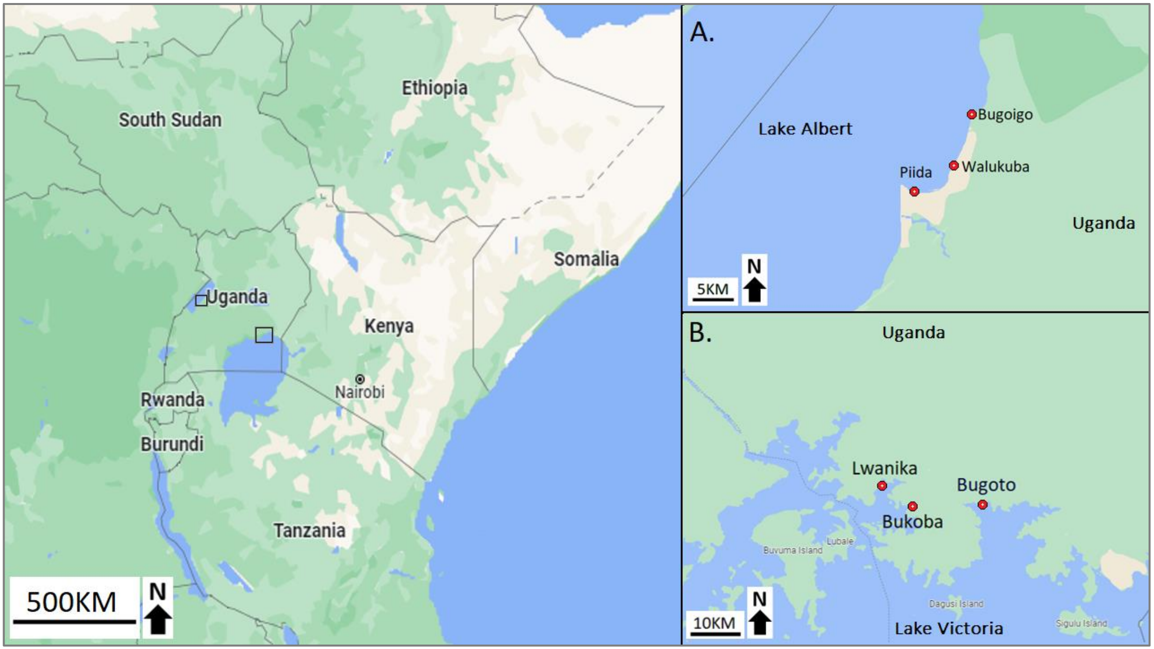


Figure 4.1. Map showing the collection site locations at Lake Albert and Lake Victoria in Uganda. (A) The three collection sites of Lake Albert (Bugoigo, Piida and Walukuba) and (B) the three collection sites of Lake Victoria (Bugoto, Bukoba and Lwanika) (Google, 2022).

### 4.2.2 Snail Identification and Genetic Diversity:

All of the preserved *Biomphalaria* species collected over the two year period were initially identified to the species level using conchological identification methods (Brown, 1994). Next, 20 individuals of each species identified at each of the Lake Albert and Lake Victoria sites were selected for further molecular analysis. In order for consistency, these selected individuals all came from the August 2010 collection, as this period had the highest number of viable specimens available. For each snail, DNA was extracted using a modified CTAB extraction method as described in Joof et al. (2020), with extracted samples being resuspended in 100µl of TE, pH 8.0 (10mM Tris-HCl, 0.1mM EDTA) buffer. After extraction, DNA yields were measured using a NanoPhotometer N50 (Implen, München, Germany) and each DNA extract were tested using the LSU-1iii/LSU-3iii primers (Fontanilla et al. 2017; Table 2.1) in order to ensure that the DNA was not degraded and was amplifiable. All PCR reactions were performed using Promega GoTaq® G2 Master Mix buffer, with 1µl of DNA template added to 24µl of 1X Master Mix buffer (1U TAQ, 0.2µM primers, 200µM dNTPs, 3mM $MgCl_2$). The PCR cycling conditions for the LSU-1iii/LSU-3iii primers was an initial denaturation at 96°C for 2min, followed by 35 cycles of 94°C for 30sec, 45°C for 1 min, 72°C for 2min and a final extension step at 72°C for 5 min.

The identification of each specimen was confirmed using 16S and COI genotyping. For the 16S gene, we used a modified version of the 16Sar/16Sbr primers designed by Palumbi et al. (1991; Table 2.1). For COI, we used the universal COI primers designed by Folmer et al. (1994; Table 2.1). The PCR cycling conditions used for both the 16S and COI primer sets were identical, with an initial denaturation at 96°C for 1minute, followed by 34 cycles of 94°C for 1min, 50°C for 1min, 72°C for 1min and a final extension at 72°C for 10mins. All PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide and were observed under UV light. All 16S and COI PCR products were purified and sequenced using Macrogen's EZ-Seq service.

Sequenced samples were aligned using the Muscle algorithm in the program Seaview v5 (Gouy et al., 2021), with misaligned sections of the 16S and the COI being fixed by hand and sites for tree building were selected using the Gblocks program (Castresana, 2000). Samples were identified to the species-level using a concatenated 16S and COI phylogenetic tree incorporating GenBank references from Jørgensen et al. (2007), Plam et al. (2008), Standley et al. (2014) and Zhang et al. (2018). Phylogenetic trees were constructed using the Maximum Likelihood method, using a General Time Reversible model incorporating gamma correction (GTR+Γ) in the program PhyML v3.1 (Guindon et al., 2010), with bootstrap analysis undertaken using 1000 replicates. After confirming what species were present at the African Great Lakes, we measured genetic variability using DNASP v6 (Rozas et al., 2017) to calculate Haplotype (Gene) Diversity (Hd) scores and Nucleotide diversity (π) values (Nei, 1987). MEGA-X (Kumar et al. 2018) was used to calculate pairwise distances using the

Maximum Composite Likelihood (MCL) method. Genealogical relationships of the 16S and COI haplotypes were constructed using Median-Joining (MJ) networks (Bandelt et al., 1999) using the software NETWORK v5 (Fluxus Technology Ltd. www.Fluxus-engineering.com).

### 4.2.3 Infection Detection:

The prevalence of *S. mansoni* infection for each site was measured using snails from a single time-point (August 2010). Twenty individuals of each species present at each site were tested for *S. mansoni* infection. In total, 120 snails (60 *B. sudanica*, 40 *B. pfeifferi* and 20 *B. stanleyi*) were selected from Lake Albert and 60 *B. choanomphala* snails from Lake Victoria (Supplementary Table 4.3). Next, to examine seasonal prevalence of infection we tested the prevalence of infection of *Biomphalaria* species found at Lake Albert and Lake Victoria for each of the four wet and four dry seasons that occurred within the two year collection period (January 2009 to December 2010). Due to the limited number of samples available at Piida and Bukoba and the limited number of samples available for *B. stanleyi* these analyses were restricted to Bugoigo, Walukuba, Bugoto and Lwanika and to *B. choanomphala*, *B. pfeifferi* and *B. sudanica*. For *B. sudanica*, we tested 20 individuals for each of the wet (March to May and September to November) and dry (December to February and June to August) seasons that occurred over the two year collection period for both Bugoigo and Walukuba. For *B. pfeifferi*, we tested 20 individuals for each of the wet and dry seasons, but only for Walukuba. For *B. choanomphala*, we tested 20 individuals for each of the wet and dry seasons for both Bugoto and Lwanika. In total, 480 snails (320 *B. sudanica* and 160 *B. pfeifferi*) from Lake Albert and 320 *B. choanomphala* snails from Lake Victoria were tested for infection (Supplementary Table 4.3).

All samples were tested for *S. mansoni* infection using two different primer sets, firstly $Sm^{F/R}$ (designed by Sandoval et al. 2006) and then ND5 (designed by Lu et al. 2016; Table 2). Only the samples that tested positive with the $Sm^{F/R}$ primer set were subjected to further testing using the ND5 primer set. This additional testing was carried out because the ND5 primer set possesses the ability to differentiate between human and non-human schistosome species based on the length of the diagnostic band (Lu et al., 2016). All PCR reactions were performed using Promega GoTaq® G2 Master Mix buffer, with 1µl of DNA template diluted to 50ng/µl. Alongside the *Biomphalaria* samples, two negative controls (water and uninfected *B. glabrata* DNA) and two positive controls (pure *S. mansoni* DNA and infected *B. glabrata* DNA) were also included. These controls were provided by Professor Mike Doenhoff, School of Biology, University of Nottingham. The PCR reaction mixture and cycling conditions for the $Sm^{F/R}$ and ND5 primer sets were followed precisely as described by Sandoval et al. (2006) and Lu et al. (2016), respectively (as described in Chapter 2.3.3). *Schistosoma mansoni* infection was confirmed by running the PCR products on a 2% agarose gel containing ethidium bromide and observing whether a diagnostic band was present under UV light. A Pearson's chi-squared ($X^2$) test with Yates' correction was used to compare the prevalence of infection using SPSS v26 (IBM, Armonk, USA).

### 4.2.4 GenBank Accessions:

GenBank accession numbers for the *Biomphalaria* 16S and COI sequences used from Jørgensen et al. (2007), Plam et al., 2008, Standley et al. (2014) and Zhang et al. (2018) can

be found in Supplementary Table 3.1. The DNA sequences generated in this study are available in GenBank accession numbers OQ924749-OQ924929 for the 16S gene and OQ849817-OQ849997 for the COI gene (further information can be found in Supplementary Tables 3.1, 4.1 and 4.2).

## 4.3 Results:

### 4.3.1 Prevalence of Infection at the African Great Lakes:

Lake Albert had the highest prevalence of *S. mansoni* infection, with an overall infection prevalence of 12.5% (15 PCR positive snails out of 120). Lake Victoria had a lower prevalence of 5% (3/60). When partitioned by site, the Lake Albert sites had a higher mean prevalence of infection than the Lake Victoria sites (Table 4.1). Walukuba had the highest infection prevalence of the Lake Albert sites with 13.3% (8/60), followed by Bugoigo with 12.5% (5/40) and Piida with 10% (2/20) (Table 4.1). Of the Lake Victoria sites, Lwanika had the highest prevalence of infection with 10% (2/20), followed by Bugoto and Bukoba with 5% (1/20) for both sites (Table 4.1). All of our $Sm^{F/R}$ positive *Biomphalaria* samples were confirmed to be infected with *S. mansoni* as every sample gave a diagnostic band length of ~302bp when tested with the ND5 primer set.

Table 4.1. Mean prevalence of *S. mansoni* infection and the number of unique 16S/COI haplotypes (No.), haplotype diversity scores (Hd) and nucleotide diversity values (π) of each *Biomphalaria* species genotyped at the Lake Albert and Lake Victoria collection sites.

| Lake Albert | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Species | No. Infected (*n*=20) | Site Infection | 16S | | | COI | | |
| | | | | No. | Hd | π | No. | Hd | π |
| Bugoigo | *B. sudanica* | 3 | 12.5% | 7 | 0.784 | 0.000 | 3 | 0.532 | 0.002 |
| | *B. pfeifferi* | 2 | | 2 | 0.337 | 0.000 | 4 | 0.489 | 0.001 |
| Piida | *B. sudanica* | 2 | 10% | 6 | 0.716 | 0.000 | 2 | 0.521 | 0.002 |
| Walukuba | *B. stanleyi* | 3 | 13.3% | 10 | 0.884 | 0.002 | 10 | 0.815 | 0.003 |
| | *B. sudanica* | 3 | | 10 | 0.884 | 0.001 | 4 | 0.553 | 0.002 |
| | *B. pfeifferi* | 2 | | 3 | 0.468 | 0.001 | 6 | 0.832 | 0.002 |
| Lake Victoria | | | | | | | | | |
| | Species | No. Infected (*n*=20) | Site Infection | 16S | | | COI | | |
| | | | | No. | Hd | π | No. | Hd | π |
| Bugoto | *B. choanomphala* | 1 | 5% | 11 | 0.884 | 0.008 | 5 | 0.774 | 0.004 |
| Bukoba | *B. choanomphala* | 1 | 5% | 15 | 0.958 | 0.007 | 10 | 0.89 | 0.005 |
| Lwanika | *B. choanomphala* | 2 | 10% | 16 | 0.963 | 0.008 | 9 | 0.826 | 0.005 |

Note: *Schistosoma mansoni* infection was determined based on whether snails had a diagnostic band for both $Sm^{F/R}$ (~350bp) and ND5 (~302bp).

We found three species, *B. pfeifferi*, *B. stanleyi* and *B. sudanica*, at Lake Albert and one species, *B. choanomphala*, at Lake Victoria (Supplementary Figure 3.1; Figure 3.4). Of the

four species identified, *B. stanleyi* had the highest prevalence of *S. mansoni* infection with 15% (3/20), followed by *B. sudanica* with 13.3% (8/60), *B. pfeifferi* with 10% (4/40), and *B. choanomphala* with 5% (3/60) (Table 4.1). In addition to the four *Biomphalaria* species, we identified an Asian *Gyraulus* species at both Lake Albert and Lake Victoria (Supplementary Figure 3.1). There have been no published reports of *Schistosoma* infection in *Gyraulus,* and we detected no cases of *S. mansoni* infection in the Asian *Gyraulus* species found at Lake Albert (0/10) or Lake Victoria (0/10).

### 4.3.2 Genetic Diversity of the *Biomphalaria* species at the African Great Lakes:

Of the *Biomphalaria* species found at the African Great Lakes, *B. choanomphala* (*n*=60) had the most haplotypes for the 16S gene fragment with 31, followed by *B. sudanica* (*n*=60) with 14, *B. stanleyi* (*n*=20) with 10 and *B. pfeifferi* (*n*=40) with four (Table 4.1; Figure 4.2a). For the COI gene fragment, *B. choanomphala* had the most haplotypes with 14, followed by *B. stanleyi* with 10, *B. pfeifferi* with six and *B. sudanica* with four (Table 4.1; Figure 4.2b). The haplotype diversity (Hd) scores for the 16S were highest for *B. choanomphala* with 0.945, followed by *B. sudanica* with 0.833, *B. stanleyi* with 0.884 and *B. pfeifferi* with 0.422. For the COI, haplotype diversity (Hd) scores were highest for *B. choanomphala* with 0.842, followed by *B. stanleyi* with 0.815, *B. pfeifferi* with 0.618 and *B. sudanica* with 0.553. Overall, the haplotypes were not highly divergent for both the 16S and COI. The nucleotide diversity values were highest for the *B. choanomphala* populations at Lake Victoria for both the 16S (0.007-0.008) and COI (0.004-0.005), while all of the *Biomphalaria* species at Lake Albert had very low nucleotide diversity values for both the 16S (0.000-0.002) and COI (0.001-0.003; Table 4.1). The intra-species pairwise distances of the 16S was the highest for *B. choanomphala* (0.0-1.8%), followed by *B. stanleyi* (0.0-0.8%), *B. sudanica* (0.0-0.8%) and *B. pfeifferi* (0.0-0.1%). Conversely, the intra-species pairwise distances of the COI was the highest for *B. pfeifferi* (0.0-1.4%), followed by *B. stanleyi* (0.0-1.3%), *B. choanomphala* (0.0-1.2%) and *B. sudanica* (0.0-0.4%).

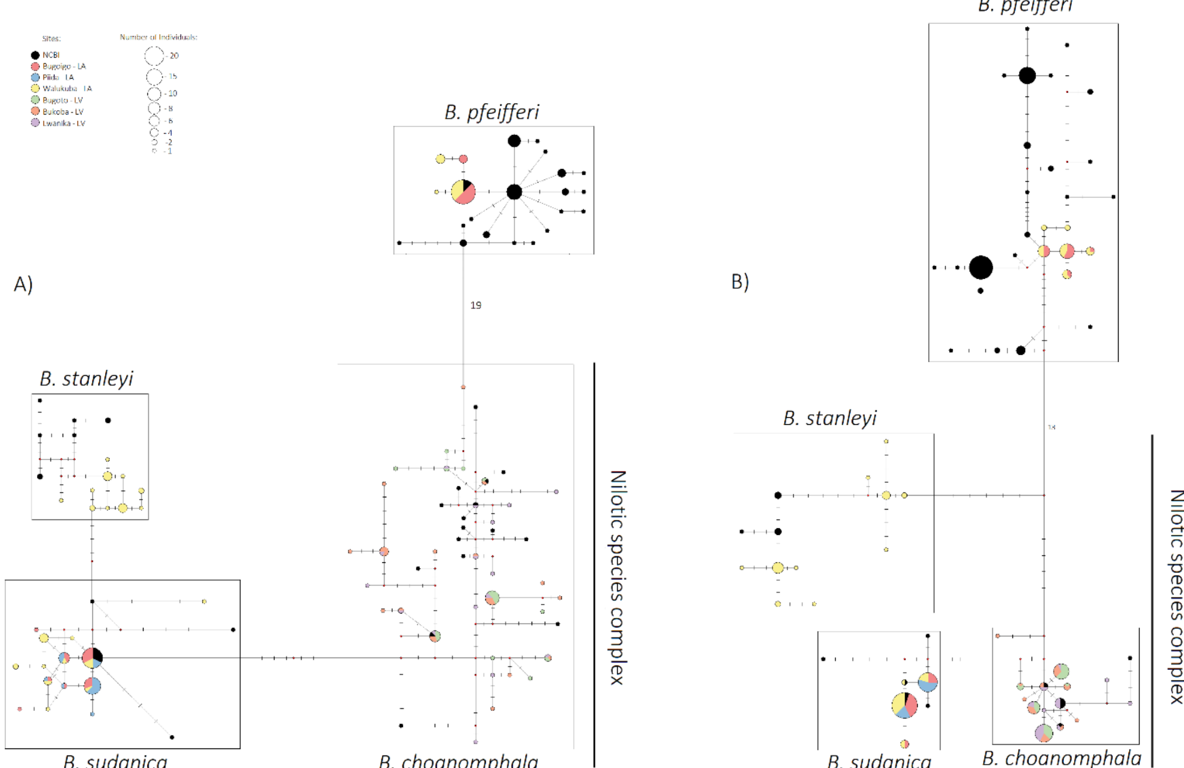



Figure 4.2. Median-Joining haplotype network of the *Biomphalaria* species found at Lake Albert (*B. pfeifferi n*=40; *B. stanleyi n*=20; *B. sudanica n*=60) and Lake Victoria (*B. choanomphala n*=60) using (A) 16S rRNA gene fragment (395bp) and (B) Cytochrome Oxidase Subunit I gene fragment (520bp). This network was generated using the software NETWORK v5. Circles represent each haplotype and circle size represents the numbers of individuals sharing a haplotype. Diamonds represent intermediate haplotypes, while hatch marks between points represent the number of nucleotide substitutions (substitutions more than five are indicated by numbers). Gaps were included in the 16S and COI alignments. Reference sequence information for the 16S and COI networks can be found in Supplementary Tables 4.1 and 4.2, respectively.

### 4.3.3 Seasonal Prevalence of Infection:

At Lake Albert we examined the seasonal changes in prevalence of infection at two sites (Bugoigo and Walukuba). One species (*B. sudanica*) was tested at Bugoigo, while two species (*B. pfeifferi* and *B. sudanica*) were tested at Walukuba. Piida and *B. stanleyi* were not tested due to a lack of samples. At Bugoigo, the wet seasons had a mean infection prevalence of 12.5% (10/80), while the dry seasons had a mean infection prevalence of 10% (8/80) (Table 4.2). At Walukuba, the wet seasons had a mean infection prevalence of 13.8% (22/160), while the dry seasons had a mean infection prevalence of 9.4% (15/160) (Table 4.2).

At Lake Victoria, we examined the seasonal changes in prevalence of infection among *Biomphalaria* populations (*B. choanomphala*) at two sites (Bugoto and Lwanika). Bukoba was not tested due to a lack of samples. At Lwanika, the wet seasons had a mean infection prevalence of 8.8% (7/80), while the dry seasons had a mean infection prevalence of 6.3%

(5/80) (Table 4.2). Bugoto had a mean infection prevalence of 8.8% (7/80) for the wet seasons and 3.8% (3/80) for the dry seasons (Table 4.2).

Table 4.2. Mean **prevalence of** infection of the wet and dry seasons at Lake Albert and Lake Victoria between 2009-2010.

| Lake Albert | | | | | | |
|---|---|---|---|---|---|---|
| Site | Species | First Dry (*n*=40) | First Wet (*n*=40) | Second Dry (*n*=40) | Second Wet (*n*=40) | Overall Infection (*n*=160) |
| Walukuba | *B. pfeifferi* | 4 (10%) | 6 (15%) | 4 (10%) | 6 (15%) | 20 (12.5%) |
| | *B. sudanica* | 3 (7.5%) | 5 (12.5%) | 4 (10%) | 5 (12.5%) | 17 (10.6%) |
| Bugoigo | *B. sudanica* | 4 (10%) | 5 (12.5%) | 4 (10%) | 5 (12.5%) | 18 (11.3%) |
| Lake Victoria | | | | | | |
| Site | Species | First Dry (*n*=40) | First Wet (*n*=40) | Second Dry (*n*=40) | Second Wet (*n*=40) | Overall Infection (*n*=160) |
| Lwanika | *B. choanomphala* | 2 (5%) | 3 (7.5%) | 3 (7.5%) | 4 (10%) | 12 (7.5%) |
| Bugoto | *B. choanomphala* | 1 (2.5%) | 3 (7.5%) | 2 (5%) | 4 (10%) | 10 (6.3%) |

Note: First Dry: Dec-Feb; First Wet: Mar-May; Second Dry: Jun-Aug; Second Wet: Sep-Nov.

Overall, the prevalence of *S. mansoni* infection was consistently higher in the wet seasons than the dry seasons for both Lake Albert and Lake Victoria (Table 4.2; Supplementary Figure 4.1). The overall mean prevalence of infection at Lake Albert for the four wet seasons was 13.3% (32/240), while the four dry seasons was 9.5% (23/240) (Table 4.2). Similarly, the overall mean prevalence of infection at Lake Victoria was 8.7% (14/160) for the wet seasons and 5% (8/160) for the dry seasons (Table 4.2). Nevertheless, a chi-square ($X^2$) analysis found there was no significant difference in the prevalence of infection between the wet and dry seasons ($p$= 0.252 for Lake Albert and $p$= 0.269 for Lake Victoria). When comparing the prevalence of infection for the first and second wet season we found no difference for the Lake Albert sites. Likewise, there was no difference in infection prevalence for the first and second dry season. For Lake Victoria, we found that the first wet season had a lower mean prevalence of infection than the second wet season. Similarly, the first dry season also had a lower prevalence of infection than the second dry season (Table 4.2).

In order to test consistency in our infection prevalence estimates, we compared the prevalence of infection measured in our seasonality dataset against our single time point (August 2010) dataset. The single time point dataset found a mean infection prevalence of 12.5% (15/120) for Lake Albert, while the seasonality dataset found a mean infection prevalence of 11.5% (55/480). Lake Victoria had an infection prevalence of 5% (3/60) for the single time point dataset, while the seasonality dataset had an infection prevalence of 7.2% (23/320). Of the species tested, *B. sudanica* had an infection prevalence of 13.3% for the single time point dataset and an infection prevalence of 10.9% for the seasonality dataset. The mean infection prevalence of the *B. pfeifferi* snails was 10% for the single time point dataset and 12.5% for the seasonality dataset. Lastly, the *B. choanomphala* snails had a mean infection prevalence of 5% for the single time point dataset and 6.9% for the

seasonality dataset. A chi-square ($X^2$) analysis found there was no significant ($P > 0.05$) difference in the prevalence of *S. mansoni* infection in *Biomphalaria* snails between the two datasets. The overall averages for both datasets can be found in Supplementary Table 4.3.

### 4.4 Discussion:

At Lake Albert, we identified three *Biomphalaria* species, *B. pfeifferi*, *B. stanleyi* and *B. sudanica*. This is consistent with the findings of the original Rowel et al. (2015) study, which is to be expected as both studies used the same dataset. Other studies of Lake Albert have similarly also reported these same three *Biomphalaria* species (Brown, 1994; Kazibwe et al., 2006; Jørgensen et al., 2007; Kazibwe et al., 2010; Adriko et al., 2013; Levitz et al., 2013). At Lake Victoria, we identified only one *Biomphalaria* species, *B. choanomphala*. This differs from the findings of the original Rowel et al. (2015) study which reported three species, *B. choanomphala*, *B. pfeifferi* and *B. sudanica*. This discrepancy is almost certainly due to the use of only conchological identification methods when differentiating the *Biomphalaria* snails in the original Rowel et al. (2015) study; our study used both conchological and molecular identification methods to determine species identifications. The lack of non-plastic shell characteristics in *Biomphalaria,* with many morphological features overlapping between species, makes the conchological identification of *Biomphalaria* species unreliable (Plam et al., 2008). Standley et al. (2011) showed that *B. sudanica*-like snails found at Lake Victoria were genetically more similar to *B. choanomphala* snails than to other *B. sudanica* snails found elsewhere in Africa. They hypothesised that these *B. sudanica*-like snails were ecological phenotypic variants (ecophenotypes) of *B. choanomphala*. This finding was further supported by Zhang et al. (2018) who showed that the complete mitochondrial genomes of the *B. choanomphala* and *B. sudanica*-like snails from Lake Victoria were genetically very similar. The *B. sudanica*-like snails reported at Lake Victoria by Rowel et al. (2015) are thus most likely ecophenotypes of *B. choanomphala*. As for the reported *B. pfeifferi* snails at Lake Victoria, Plam et al. (2008) found that *B. pfeifferi* had similar shell morphologies to *B. choanomphala*, demonstrating that conchological methods of identification can be misled by plastic shell characteristics. We are confident that our approach of using conchological identification methods in conjunction with molecular methods is superior and more accurate than the solely conchological approach used in the Rowel et al. (2015) study.

#### 4.4.1 Infection Prevalence of the *Biomphalaria* Species found at the African Great Lakes:

We found Lake Albert (12.5%) had a higher mean prevalence of *S. mansoni* infection than Lake Victoria (5%). Similarly, Rowel et al. (2015) also reported a higher rate of cercarial shedding in *Biomphalaria* snails at Lake Albert (8.9%) than Lake Victoria (2.1%). Of the sites tested, we found Walukuba (13.8%) had the highest prevalence of infection at Lake Albert, while Lwanika (10%) had the highest prevalence of infection at Lake Victoria. Similarly, Rowel et al. (2015) found Walukuba (12.3%) had the highest rates of cercarial shedding at Lake Albert and Lwanika (3.8%) had the highest rates of shedding at Lake Victoria.

Our study observed a higher mean prevalence of *S. mansoni* infection compared to the Rowel et al (2015) study. Molecular detection methods (as used here) typically show higher levels of infection when compared to the cercarial shedding method (Born-Torrijos et al.,

2014; Lu et al., 2016; Joof et al., 2020). Infected *Biomphalaria* snails do not always produce cercariae during the usual 35-49 day incubation period. Cold temperatures can lead to delays in sporocyst development and shedding (Shiff et al., 1975). Similarly, delays to sporocyst development and shedding can arise due to an immune response to *S. mansoni* infection; the snail's immunological response to infection does not guarantee the complete eradication of all sporocysts and some sporocysts can release cercariae up to ten months post infection (Borges et al., 1998; Lemos & Andrade, 2001). Ultimately these prepatent snails will be undetectable by the cercarial shedding method but are still detectable by molecular methods (Lu et al., 2016; Joof et al., 2020). However, molecular methods can also overestimate the number of snails that present a risk. Lu et al. (2016) found that not all PCR positive *Biomphalaria* snails went on to shed cercariae; some snails were able to successfully encapsulate and degrade the sporocysts during the prepatent period, which resulted in the infection failing. The chance of this happening was shown to be dependent on the species, with the majority of PCR positive *B. pfeifferi* snails (60%) going on to shed cercariae, while only a minority of PCR positive *B. sudanica* snails (10%) went on to shed cercariae.

It seems whether an infection is successful or not is dependent on schistosome-snail compatibility, with compatible schistosomes being able to successfully evade the host's immune defences (Théron et al., 1997; Mitta et al., 2012; Théron et al., 2014). This means that a snail that is PCR positive for infection may not necessarily be capable of spreading that infection on to humans. Rowel et al. (2015) reported that of the snails shedding cercariae, only 15.8% at Lake Albert and 13.9% at Lake Victoria were shedding *S. mansoni* cercariae (identified using general anatomical appearance; Frandsen & Christensen, 1984) as opposed to shedding cercariae of trematode species with no medical importance. When snails are co-infected with both *S. mansoni* and non-*S. mansoni* sporocysts simultaneously (Born-Torrijos et al., 2014; Outa et al., 2020) it is more difficult to reliably identify the presence of *S. mansoni* cercariae, since these *S. mansoni* cercariae can be obscured by other non-medically important cercariae and therefore missed. Moreover, *S. mansoni* (human) infections in snails cannot be distinguished from *S. rodhaini* (rat) infections in snails using general anatomical identification methods. Molecular detection methods are able to detect whether or not *S. mansoni* is present, while ignoring non-*S. mansoni* sporocysts, though some detection methods are more specific than others. For example, $Sm^{F/R}$ is able to distinguish *S. mansoni* from other schistosome species with the exception of *S. rodhaini*, while ND5 is capable of distinguishing between *S. mansoni* and *S. rodhaini.*

### 4.4.2 Infection Prevalence and Host-Snail Genetic Diversity:

We found that the *Biomphalaria* species found at Lake Victoria (*B. choanomphala*) had a higher intra-species genetic diversity than the *Biomphalaria* species (*B. pfeifferi*, *B. stanleyi* and *B. sudanica*) found at Lake Albert. Furthermore, Lake Victoria had a lower prevalence of infection than Lake Albert. This is consistent with previous studies that have reported higher levels of intra-species genetic variation in host snails being linked to a lower prevalence of infection (Jarne & Théron, 2001; Campbell et al., 2010). However, when we examined each of the sites individually, we found that the sites with the highest prevalence of infection also had *Biomphalaria* species with the highest intra-specific genetic diversity (Table 4.1). For

example, when comparing the haplotype diversity scores of the 16S and COI genes for the *B. pfeifferi* snails found at Walukuba with the *B. pfeifferi* snails found at Bugoigo, we find Walukuba had both a higher amount of genetic diversity (16S Hd: 0.468; COI Hd: 0.832) and a higher prevalence of infection (13.3%) than Bugoigo (16S Hd: 0.337; COI Hd: 0.489; prevalence: 12.5%) (Table 4.1). We also find this trend for *B. sudanica* and *B. choanomphala* (Table 4.1). For *B. sudanica*, Walukuba had both a higher amount of genetic diversity (16S Hd: 0.884; COI Hd: 0.553) and a higher prevalence of infection (13.3%) than Bugoigo (16S Hd: 0.784; COI Hd: 0.532; prevalence: 12.5%) and Piida (16S Hd: 0.716; COI Hd: 0.521; prevalence: 10%) (Table 4.1). Similarly, the *B. choanomphala* snails at Lwanika had a higher genetic diversity (16S Hd: 0.963; COI Hd: 0.826) and infection prevalence (10%) than the *B. choanomphala* snails at Bugoto (16S Hd: 0.884; COI Hd: 0.774; prevalence: 5%) (Table 4.1).

The prevalence of *S. mansoni* infection within a snail population is associated with multiple factors, not just snail host genetic diversity. Sandland et al. (2009) found that there was no significant difference in susceptibility to *S. mansoni* infection of *Biomphalaria glabrata* snails that came from either inbred or outcrossed lineages. This is contradictory to previous studies which associate low amounts of genetic variability within an intermediate host population with a higher prevalence of *S. mansoni* infection (Jarne & Théron, 2001; Campbell et al., 2010). Our results suggested that populations with lower amounts of *S. mansoni* infection also had lower amounts of genetic diversity. A possible explanation for this could be attributed to the 'coevolution selective sweep' phenomenon, which is when a beneficial gene (e.g. resistance to *S. mansoni*) quickly becomes widespread throughout a population. This in turn reduces the genetic diversity within a population as individuals that do not possess this gene are less successful and die off. As for the sites with higher amounts of *S. mansoni* infection and higher amounts of genetic diversity, this beneficial gene could not be present, and the selective sweep has not occurred.

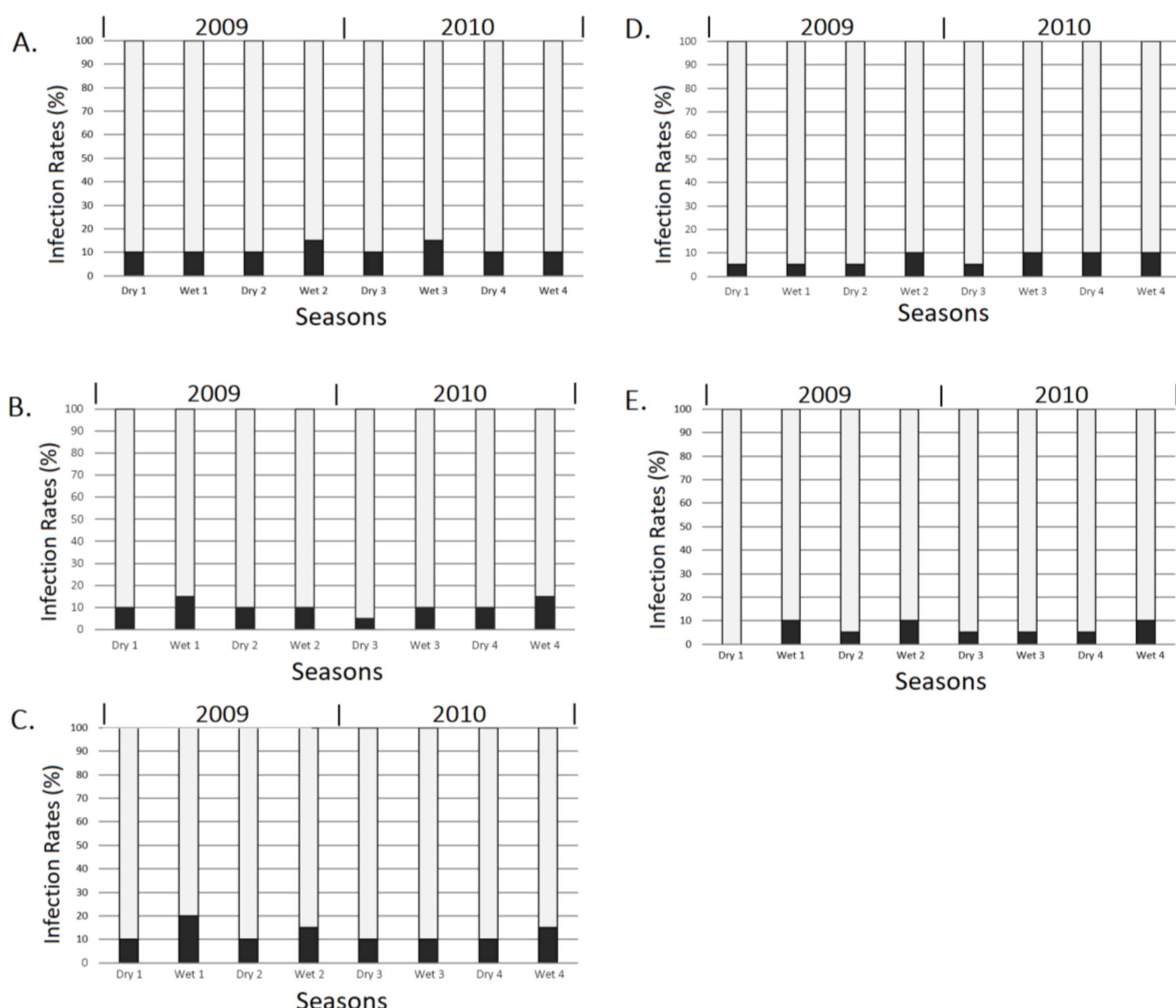
### 4.4.3 Infection Prevalence and Seasonality:

At Lake Albert, we found that the wet seasons (March to May and September to November) had a higher mean prevalence of *S. mansoni* infection (13.3%) than the dry seasons (December to February and June to August) (9.6%). Similarly, the wet seasons at Lake Victoria had a higher mean prevalence of infection (8.7%) than the dry seasons (5%). Rowel et al. (2015) also observed a higher number of shedding *Biomphalaria* snails during the wet seasons at both Lake Albert and Lake Victoria. Kazibwe et al. (2006) also found the highest rates of cercarial shedding in *B. stanleyi* and *B. sudanica* snails at Lake Albert was during the wet seasons. Similarly, in South Africa, Wolmarans et al. (2002) found *B. pfeifferi* collected during the wet season (January to April) had a higher cercarial shedding rate than *B. pfeifferi* collected during either the cold (May to August) or the warm (September to December) dry season.

Ouma et al. (2016) found that the physicochemical composition of the lake water was significantly different between the wet and dry seasons. This change is due to the increase in rainfall, which effects the water pH, water conductivity, oxygen saturation and phosphate, nitrate, chloride and ammonia levels in the water. In the original Rowel et al. (2015) study, *S. mansoni* infection was found to be positively correlated with *Biomphalaria*

abundance and temperature, while it was negatively correlated with higher water pH and conductivity. Similarly, *Biomphalaria* abundance itself positively correlates with higher temperature and oxygen saturation, but negatively correlates with higher water pH and water conductivity (Kazibwe et al., 2006; Rowel et al., 2015; Bakhoum et al. 2021). During the wet seasons, the levels of dissolved oxygen in the lake water is significantly higher than the dry seasons, while water conductivity levels are significantly lower (Ouma et al., 2016). This significant difference in water conductivity and oxygen saturation could explain why the wet seasons consistently have a higher prevalence of *S. mansoni* infection than the dry seasons. Conversely, the dry seasons have a significantly higher temperatures than the wet seasons, while pH levels are significantly lower (Ouma et al., 2016). Alternatively, the increased rainfall from the wet seasons also allows for a higher risk of flooding which can transport *Biomphalaria* snails to new areas; create and sustain new snail habitats; increase the amount of human/animal waste found in freshwater by the flooding of latrines and sewerage systems. However, our chi-square ($X^2$) analysis found that the prevalence of infection during the wet seasons was not significantly higher than the prevalence of infection during the dry seasons for both Lake Albert and Lake Victoria. Moreover, depending on where the parasitological survey is undertaken can lead to contradictory results as studies undertaken in Ethiopia (Hailegebriel et al., 2022), Nigeria (Okeke & Ubachukwu, 2017), Tanzania (Nzalawahe, 2021) and Sudan (Ismail et al., 2022) have found the opposite trend, with the dry seasons having a higher prevalence of *S. mansoni* infection in snails than the wet seasons.

4.5 Supplementary Material:




Supplementary Figure 4.1. Prevalence of *Schistosoma mansoni* infection at Lake Albert (A-C) and Lake Victoria (D-E) over the course of two years (2009-2010). *Biomphalaria sudanica* (*n*=320) was tested at two sites in Lake Albert (A: Bugoigo & B: Walukuba), while *B. pfeifferi* (*n*=160) was tested at one site (C: Walukuba). *Biomphalaria choanomphala* (*n*=320) was tested at two sites at Lake Victoria (D: Bugoto & E: Lwanika). Black bars indicate the percentage of infected individuals (*n*=20). (Dry 1: January-February 2009; Wet 1: March-May 2009; Dry 2: June-August 2009; Wet 2: September-November 2009; Dry 3: December 2009-February 2010; Wet 3: March-May 2010; Dry 4: June-August 2010; Wet 4: September-November 2010).

Supplementary Table 4.1. GenBank accession numbers for the 16S haplotype network.

| | Accession no. | Species |
|---|---|---|
| FN2 | EU141182 | *cf. choanomphala* |
| - | AY030202 | *choanomphala* |
| H44 | HM768993 | *choanomphala* |

| | | |
|---|---|---|
| H60 | HM769009 | *choanomphala* |
| H68 | HM769017 | *choanomphala* |
| H80 | HM769029 | *choanomphala* |
| H84 | HM769033 | *choanomphala* |
| H85 | HM769034 | *choanomphala* |
| H86 | HM769035 | *choanomphala* |
| H101 | HM769050 | *choanomphala* |
| H137 | HM769086 | *choanomphala* |
| H145 | HM769094 | *choanomphala* |
| H147 | HM769096 | *choanomphala* |
| H150 | HM769099 | *choanomphala* |
| H160 | HM769109 | *choanomphala* |
| - | MG431964 | *choanomphala* |
| AJ-2005 | DQ084854 | *cf. pfeifferi* |
| FL5 | EU141179 | *cf. pfeifferi* |
| LS-131 | AY030193 | *pfeifferi* |
| LS-135 | AY030194 | *pfeifferi* |
| LS-82 | AY030195 | *pfeifferi* |
| LS-336 | AY030196 | *pfeifferi* |
| - | AY126599 | *pfeifferi* |
| - | AY126600 | *pfeifferi* |
| - | AY126601 | *pfeifferi* |
| - | AY126602 | *pfeifferi* |
| - | AY126603 | *pfeifferi* |
| - | AY126604 | *pfeifferi* |
| - | AY126605 | *pfeifferi* |
| - | AY126606 | *pfeifferi* |
| 1126 | AY198048 | *pfeifferi* |
| 1132 | AY198049 | *pfeifferi* |
| 1133 | AY198050 | *pfeifferi* |

| | | |
|---|---|---|
| 1136 | AY198051 | *pfeifferi* |
| 115 | AY198052 | *pfeifferi* |
| 116 | AY198053 | *pfeifferi* |
| 117 | AY198054 | *pfeifferi* |
| 131 | AY198055 | *pfeifferi* |
| 133 | AY198056 | *pfeifferi* |
| 135 | AY198057 | *pfeifferi* |
| 137 | AY198058 | *pfeifferi* |
| 2004 | AY198059 | *pfeifferi* |
| 2005a | AY198060 | *pfeifferi* |
| 2098 | AY198061 | *pfeifferi* |
| 2100 | AY198062 | *pfeifferi* |
| 2005b | AY198063 | *pfeifferi* |
| 228a | AY198064 | *pfeifferi* |
| 262 | AY198065 | *pfeifferi* |
| 271 | AY198066 | *pfeifferi* |
| 274 | AY198067 | *pfeifferi* |
| 296 | AY198068 | *pfeifferi* |
| 311 | AY198069 | *pfeifferi* |
| 336 | AY198070 | *pfeifferi* |
| 337 | AY198071 | *pfeifferi* |
| 343 | AY198072 | *pfeifferi* |
| 350 | AY198073 | *pfeifferi* |
| 351 | AY198074 | *pfeifferi* |
| 82 | AY198075 | *pfeifferi* |
| 83 | AY198076 | *pfeifferi* |
| 92 | AY198077 | *pfeifferi* |
| - | AY577475 | *pfeifferi* |
| BpfChi1 | DQ084851 | *pfeifferi* |
| BpfKib1 | DQ084852 | *pfeifferi* |

| BpfDeG1 | DQ084853 | *pfeifferi* |
|---|---|---|
| BpfAbu1 | DQ084857 | *pfeifferi* |
| - | MG431962 | *pfeifferi* |
| - | AY030197 | *cf. stanleyi* |
| BstBut1 | DQ084858 | *stanleyi* |
| FL1 | EU141175 | *stanleyi* |
| FL2 | EU141176 | *stanleyi* |
| FL3 | EU141177 | *stanleyi* |
| FL4 | EU141178 | *stanleyi* |
| FL6 | EU141180 | *stanleyi* |
| FN1 | EU141181 | *stanleyi* |
| FN5 | EU141185 | *stanleyi* |
| - | AY126608 | *cf. sudanica* |
| - | MG431963 | *cf. sudanica* |
| BsuBut1 | DQ084859 | *sudanica* |
| BsuKin1 | DQ084860 | *sudanica* |
| BsuMah1 | DQ084861 | *sudanica* |
| BsuNto1 | DQ084864 | *sudanica* |
| BsuRut1 | DQ084865 | *sudanica* |
| SN1 | EU141187 | *sudanica* |
| SN2 | EU141188 | *sudanica* |
| SN3 | EU141189 | *sudanica* |
| SN4 | EU141190 | *sudanica* |
| SN5 | EU141191 | *sudanica* |
| SN6 | EU141192 | *sudanica* |

Note: ‘cf.’ indicates the shell morphology looked like a specific species but was identified as a different species by the original authors using molecular methods.

Supplementary Table 4.2. GenBank accession numbers for the COI haplotype network.

| | Accession no. | Species |
|---|---|---|
| - | MG431964 | *choanomphala* |
| BchVic1 | DQ084828 | *choanomphala* |
| - | OL423116 | *pfeifferi* |
| - | MG431962 | *pfeifferi* |
| 1792 | AF199097 | *pfeifferi* |
| 1869 | AF199102 | *pfeifferi* |
| 1903 | AF199100 | *pfeifferi* |
| 1907 | AF199101 | *pfeifferi* |
| 1914 | AF199104 | *pfeifferi* |
| 1915 | AF199099 | *pfeifferi* |
| BpfChi1 | DQ084829 | *pfeifferi* |
| BpfDeG1 | DQ084831 | *pfeifferi* |
| BpfKib1 | DQ084830 | *pfeifferi* |
| MP05Bi1 | OM535896 | *pfeifferi* |
| MP05Bi2 | OM535897 | *pfeifferi* |
| SUDAN 0 | MG780151 | *pfeifferi* |
| SUDAN 22 | MG780154 | *pfeifferi* |
| SUDAN 23 | MG780155 | *pfeifferi* |
| SUDAN 24 | MG780156 | *pfeifferi* |
| SUDAN 26 | MG780157 | *pfeifferi* |
| SUDAN 27 | MG780158 | *pfeifferi* |
| SUDAN 28 | MG780160 | *pfeifferi* |
| SUDAN 29 | MG780161 | *pfeifferi* |
| SUDAN 3 | MG780150 | *pfeifferi* |
| SUDAN 31 | MG780159 | *pfeifferi* |
| SUDAN 32 | MG780162 | *pfeifferi* |
| SUDAN 33 | MG780163 | *pfeifferi* |
| SUDAN 34 | MG780164 | *pfeifferi* |

| SUDAN 35 | MG780165 | *pfeifferi* |
|---|---|---|
| SUDAN 36 | MG780166 | *pfeifferi* |
| SUDAN 37 | MG780167 | *pfeifferi* |
| SUDAN 38 | MG780168 | *pfeifferi* |
| SUDAN 40 | MG780174 | *pfeifferi* |
| SUDAN 41 | MG780175 | *pfeifferi* |
| SUDAN 42 | MG780176 | *pfeifferi* |
| SUDAN 43 | MG780177 | *pfeifferi* |
| SUDAN 44 | MG780178 | *pfeifferi* |
| SUDAN 45 | MG780179 | *pfeifferi* |
| SUDAN 7 | MG780152 | *pfeifferi* |
| SUDAN 8 | MG780153 | *pfeifferi* |
| SUDAN S13 | MG780170 | *pfeifferi* |
| SUDAN S15 | MG780169 | *pfeifferi* |
| SUDAN S20 | MG780171 | *pfeifferi* |
| SUDAN S21 | MG780172 | *pfeifferi* |
| SUDAN S25 | MG780173 | *pfeifferi* |
| ZWE 2 | MG780180 | *pfeifferi* |
| ZWE 232 | MG780207 | *pfeifferi* |
| ZWE 236 | MG780208 | *pfeifferi* |
| ZWE 254 | MG780197 | *pfeifferi* |
| ZWE 258 | MG780196 | *pfeifferi* |
| ZWE 260 | MG780195 | *pfeifferi* |
| ZWE 261 | MG780194 | *pfeifferi* |
| ZWE 262 | MG780193 | *pfeifferi* |
| ZWE 263 | MG780192 | *pfeifferi* |
| ZWE 264 | MG780191 | *pfeifferi* |
| ZWE 266 | MG780190 | *pfeifferi* |
| ZWE 267 | MG780189 | *pfeifferi* |
| ZWE 268 | MG780188 | *pfeifferi* |

| ZWE 269 | MG780187 | *pfeifferi* |
|---|---|---|
| ZWE 27 | MG780198 | *pfeifferi* |
| ZWE 270 | MG780186 | *pfeifferi* |
| ZWE 271 | MG780185 | *pfeifferi* |
| ZWE 276 | MG780184 | *pfeifferi* |
| ZWE 277 | MG780183 | *pfeifferi* |
| ZWE 278 | MG780182 | *pfeifferi* |
| ZWE 280 | MG780181 | *pfeifferi* |
| ZWE 30 | MG780202 | *pfeifferi* |
| ZWE 32 | MG780203 | *pfeifferi* |
| ZWE 33 | MG780199 | *pfeifferi* |
| ZWE 33 | MG780204 | *pfeifferi* |
| ZWE 37 | MG780200 | *pfeifferi* |
| ZWE 37 | MG780201 | *pfeifferi* |
| ZWE 38 | MG780205 | *pfeifferi* |
| ZWE 41 | MG780206 | *pfeifferi* |
| BstBut1 | DQ084837 | *stanleyi* |
| - | OL423117 | *cf. sudanica* |
| - | MG431963 | *cf. sudanica* |
| 1986 | AF199106 | *cf. sudanica* |
| 1987 | AF199107 | *cf. sudanica* |
| 1091 | AF199088 | *sudanica* |
| 1908 | AF199087 | *sudanica* |
| 1925 | AF199108 | *sudanica* |
| BsuBut1 | DQ084838 | *sudanica* |
| BsuKin1 | DQ084839 | *sudanica* |
| BsuMah1 | DQ084840 | *sudanica* |
| BsuNto1 | DQ084843 | *sudanica* |
| BsuRut1 | DQ084844 | *sudanica* |

Note: 'cf.' indicates the shell morphology looked like a specific species but was identified as a different species by the original authors using molecular methods.

Supplementary Table 4.3. Mean infection prevalence of both the single time point dataset and the seasonality dataset for the Lake Albert and Lake Victoria collection sites.

| Lake Albert | | | | | | | |
|---|---|---|---|---|---|---|---|
| Site | Samples Preserved | Species | Samples Tested | | Number Infected | | Total |
| | | | D1 | D2 | | | |
| Bugoigo | 977 | *B. pfeifferi* | *n*=20 | - | 10% | (2/20) | 11.7% (70/600) |
| | | *B. sudanica* | *n*=20 | *n*=160 | 11.7% | (21/180) | |
| Piida | 521 | *B. sudanica* | *n*=20 | - | 10% | (2/20) | |
| Walukuba | 1147 | *B. pfeifferi* | *n*=20 | *n*=160 | 12.2% | (22/180) | |
| | | *B. stanleyi* | *n*=20 | - | 15% | (3/20) | |
| | | *B. sudanica* | *n*=20 | *n*=160 | 11.1% | (20/180) | |
| Lake Victoria | | | | | | | |
| Site | Samples Preserved | Species | Samples Tested | | Number Infected | | Total |
| | | | D1 | D2 | | | |
| Bugoto | 4005 | *B. choanomphala* | *n*=20 | *n*=160 | 6.1% | (11/180) | 6.8% (26/380) |
| Bukoba | 1264 | *B. choanomphala* | *n*=20 | - | 5% | (1/20) | |
| Lwanika | 1113 | *B. choanomphala* | *n*=20 | *n*=160 | 7.8% | (14/180) | |

Note: D = Dataset. D1 was the single time point dataset and D2 was the seasonality dataset.

# Chapter 5 *Schistosoma mansoni* Infection and Population Genetic Structure of *Biomphalaria choanomphala* Snails in Lake Victoria

Abstract:

Lake Victoria is a well-known hot spot for intestinal schistosomiasis, with the *Biomphalaria* snail species *B. choanomphala* acting as the predominant intermediate host for *Schistosoma mansoni* transmission. Prevalence of *S. mansoni* infection within snail populations is influenced by abiotic/physicochemical factors of the water, incidence of infection in human populations (and reservoir hosts) and the level of genetic compatibility between the parasite and the host. We measured the prevalence of *S. mansoni* infection within *B. choanomphala* populations along the Kenyan, Tanzanian and Ugandan shorelines of Lake Victoria and related this to abiotic/physicochemical characteristics of the lake, *B. choanomphala* abundance and genetic diversity of host snail populations. The overall mean prevalence of *S. mansoni* infection at Lake Victoria was 9.3%, with the highest prevalence of infection occurring on the Tanzanian shoreline (13.1%), followed by the Ugandan (8.2%) and Kenyan (4.7%) shorelines. There was a significant difference in median water temperature, conductivity, salinity, total dissolved solids and major anions/cations concentrations between the Kenyan, Tanzanian and Ugandan shorelines of Lake Victoria. Spearman's rank analysis found there was a significant negative correlation between prevalence of *S. mansoni* infection and increasing water alkalinity. Conversely, *S. mansoni* infection had a significant positive relationship with *B. choanomphala* abundance, calcium and magnesium concentration. We observed that sites with *S. mansoni* infection correlated with *B. choanomphala* populations with a higher mean haplotype diversity score compared to sites found without infection. However, there was no significant relationship between the prevalence of infection and the haplotype diversity scores of the *B. choanomphala* populations.

## 5.1 Introduction:

Schistosomiasis is a parasitic disease caused by the intravascular parasite genus, *Schistosoma*. Schistosomiasis is a neglected tropical disease (NTD) that affects over 240 million people globally, with over 700 million people being at risk of infection (WHO, 2022b). The disease is endemic in 78 countries worldwide and seriously impacts developing countries, especially sub-Saharan Africa (WHO, 2022a). It is estimated that 3.3 million Disability-Adjusted Life Years (DALYs) were lost in 2010 alone, from urogenital or intestinal schistosomiasis (Hotez et al., 2014). The majority of intestinal schistosomiasis cases are caused by *Schistosoma mansoni* and its intermediate freshwater snail host, *Biomphalaria* (Gryseels et al., 2006; Colley & Secor, 2014). East Africa is a well-known regional hotspot for schistosomiasis with the disease being as prevalent as 18% in Kenya, 86% in Tanzania and 88% in Uganda (Ngowi, 2020). The high prevalence of *S. mansoni* infection in East Africa is due to the large number of freshwater environments that *Biomphalaria* snails can inhabit, with the largest source of freshwater being Lake Victoria (Kazibwe et al., 2006; Steinmann et al., 2006). These favourable habitats combined with poor water hygiene and sanitation standards make the shoreline of Lake Victoria a hot spot for intestinal schistosomiasis

(Kazibwe et al., 2006). *Biomphalaria* are notoriously invasive and are capable of rapidly expanding their territory due to their high fecundity and ability to self-fertilise (Kengne-Fokam et al., 2016). This rapid expansion can lead to outbreaks of schistosomiasis as self-fertilisation and inbreeding leads to genetically homogenous populations at the expense of schistosome resistance (Jarne & Théron, 2001; Campbell et al., 2010). However, the distribution of *S. mansoni* is dependent on the ecological requirements of its intermediate host, with the availability of suitable freshwater habitats limiting the potential geographical reach of the parasite (Sturrock, 2001; Steinmann et al., 2006). *Biomphalaria* populations are known to be sensitive to a variety of abiotic factors in their habitat, which limits what pristine environments they can inhabit (Woolhouse, 1992; Brown, 1994).

At Lake Victoria, two *Biomphalaria* species, *B. choanomphala* and *B. sudanica* have been reported to inhabit the lake shore (Standley et al. 2012; Adriko et al., 2013; Rowel et al., 2015; Mutuku et al. 2021). However, Standley et al. (2011) and Zhang et al. (2018) found that the *B. sudanica*-like snails in Lake Victoria were genetically more similar to *B. choanomphala* than to *B. sudanica* populations found in the rest of Africa. They suggested that the *B. sudanica*-like snails from Lake Victoria were ecological phenotypes of *B. choanomphala*. Other studies have described these morphotypes of *B. choanomphala* as being either "lacustrine" (*B. choanomphala*) or "non-lacustrine" (*B. sudanica*-like) due to the former morphotype being commonly found in the lake and the latter morphotype being commonly found in swamps adjacent to the shoreline (Supplementary Figure 5.1; Brown, 1994; DeJong et al., 2001; Kazibwe et al., 2006; Plam et al., 2008; Kazibwe et al., 2010).

Before the advent of molecular identification and diagnostic methods, Webb (1962) and Prentice et al. (1970) were the first to document that *B. choanomphala* snails at Lake Victoria were capable of transmitting *Schistosoma mansoni* using traditional morphological identification methods and artificial mouse infection experiments. Subsequent parasitological surveys have consistently found that *B. choanomphala* has the lowest prevalence of *S. mansoni* infection when compared to *B. pfeifferi*, *B. stanleyi* and *B. sudanica* (Odongo-Aginya et al., 2008; Adriko et al., 2013; Rowel et al., 2015; Gouvras et al., 2017; Mutuku et al., 2021; Trienekens et al. 2022). *Schistosoma mansoni* infection can be determined using both traditional cercarial shedding methods (Webbe, 1965) and molecular infection detection methods (Sandoval et al., 2006). Molecular detection methods provide significant advantages over the traditional cercarial shedding method, as they are more time-efficient, can be used with preserved snail tissue, are species specific and are capable of detecting prepatent infection (Kane et al., 2014; Joof et al., 2020). However despite these benefits, molecular detection methods are underutilised in detecting schistosome infection in intermediate snail hosts collected from the field.

In this study we investigate the prevalence of *S. mansoni* infection in *B. choanomphala* snails collected from the Kenyan, Tanzanian and Ugandan shorelines of Lake Victoria. We examine genetic diversity in host snail populations around the lake shore and investigate the physicochemical characteristics of our collection sites in order to determine the effect of snail host genetic diversity and abiotic factors on infection prevalence.

## 5.2 Materials and Methods:

### 5.2.1 Collection Sites:

Collections were undertaken from 2008 to 2011 and included 170 sites from the Kenyan (*n*=35), Tanzanian (*n*=82) and Ugandan (*n*=53) shorelines of Lake Victoria (Figure 5.1). At each site, five qualitative measurements (*B. choanomphala* abundance, which morphotype was present, habitat type, water depth and water turbulence) were recorded *in situ*. *Biomphalaria choanomphala* abundance was measured as either being absent (0 snails), low (< 10 snails), medium (10-30 snails) or high (> 30 snails). When present, *B. choanomphala* snails were collected using scoops and placed into jars filled with lake water for later processing. Collected snail populations were analysed via shell morphometrics to see whether they exhibited non-lacustrine (morphotype-A) or lacustrine shell (morphotype-B) morphologies as described by Standley et al. (2011) and Chapter 3 (Supplementary Figure 5.1). Habitat type was categorised as being marshlands (a), lake edge (b) or other (c). Water depth was assessed as being shallow (< 10cm), moderately-shallow (10cm-30cm), moderate (30cm-50cm), moderately-deep (50cm-70cm) and deep (> 70cm). Water turbulence was classified as being low, medium or high. Alongside the qualitative measurements, the temperature (°C), conductivity (µS), total dissolved solids (g/L), salinity (g/L) and pH of the water at each site was measured using a portable water meter (Hanna Instruments, Inc., Woonsocket, USA or 430 Enterprise, Jenway Ltd, Stone, UK). At each site, a 15ml sample of lake water was collected and frozen prior to compositional analysis. Anions (fluoride, chloride, nitrate, phosphate and sulphate) concentrations were determined using Reagent-Free Ion Chromatography (RFIC-EG), while cation (calcium, potassium, magnesium and sodium) concentrations were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Collections were undertaken by Standley et al. (2012) and Rowel et al. (2015). Further information on the collection protocols can be found in Standley et al. (2012), Rowel et al. (2015) and is summarised in Chapter 2.1.

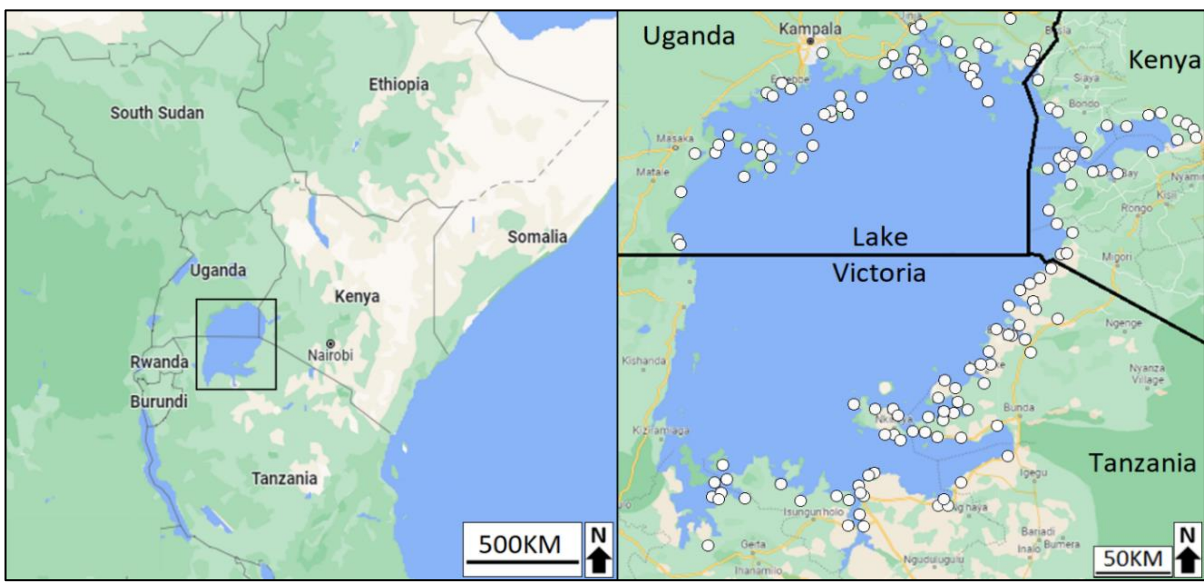



Figure 5.1. Map of the collection sites at Lake Victoria, East Africa (Google, 2022).

### 5.2.2 *Biomphalaria choanomphala* Collection, Identification and DNA Extraction:

When found, *B. choanomphala* snails were identified using conchological identification methods as described by Mandahl-Barth (1962) and Brown (1994). Snails were collected from all sites where *B. choanomphala* was found and their DNA was extracted using a modified CTAB extraction method (as described in Chapter 2.3.1). Where numbers permitted, 12 snails were extracted per site, while for sites with fewer than 12 individuals all snails were extracted. After extraction, samples were resuspended in 100-200µl of TE, pH 8.0 (10mM Tris-HCl, 0.1mM EDTA) buffer and DNA yields were measured using a NanoPhotometer N50 (Implen, München, Germany).

### 5.2.3 PCR Amplification and Population Genetics of *Biomphalaria choanomphala*:

Genomic DNA samples from 27 sites were selected to measure the genetic diversity and population structure of the *B. choanomphala* snails found across Lake Victoria. Chosen sites had a minimum of ten individuals and were evenly distributed along the lakeshore. Population genetic analysis was done using 16S and COI genotyping, which used a modified version of the 16Sar/16Sbr primers designed by Palumbi et al. (1991) and the universal COI primers designed by Folmer et al. (1994; Table 2.1). All PCR reactions were performed using a 25µl reaction volume containing 24µl of PCR master mix (1U TAQ, 0.2µM primers, 200µM dNTP, 1.5mM $MgCl_2$) and 1µl of DNA template. The PCR cycling conditions used for both the 16S and COI primer sets were identical, with an initial denaturation at 96°C for 1minute, followed by 34 cycles of 94°C for 1min, 50°C for 1min, 72°C for 1min and a final extension at 72°C for 10mins. PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide and observed under UV light, with PCR products purified and sequenced by either the Natural History Museum or using Macrogen's EZ Seq service.

### 5.2.4 Detecting *S. mansoni* Infection in *Biomphalaria choanomphala*:

All of the extracted *B. choanomphala* samples were tested for *S. mansoni* infection, first using the $Sm^{F/R}$ primer set designed by Sandoval et al. (2006) and then if found positive, with the ND5 primer set designed by Lu et al. (2016; Table 2.1). The second round of ND5 PCR was used to determine whether the infection present in the snail was caused by *S. mansoni* or its closely-related sister species, *S. rodhaini*. The PCR reaction mixture and cycling conditions for the $Sm^{F/R}$ and the ND5 primers were followed precisely as described by Sandoval et al. (2006) and Lu et al. 2016, respectively (further information can be found in Chapter 2.3.3). Alongside the *B. choanomphala* samples, two negative controls (water and uninfected *B. glabrata* DNA) and two positive controls (pure *S. mansoni* DNA and infected *B. glabrata* DNA) were also included. These controls were provided by Professor Mike Doenhoff, School of Biology, University of Nottingham. Additionally, all samples were tested using the LSU-1iii/LSU-3iii primers (Fontanilla et al. 2017; Table 2.1) to ensure that the DNA was not degraded and was amplifiable. The PCR cycling conditions for these primers was an initial denaturation at 96°C for 2min, followed by 35 cycles of 94°C for 30sec, 45°C for 1 min, 72°C for 2min and a final extension step at 72°C for 5 min. All PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide and amplicons were observed under UV light. *Schistosoma mansoni* infection was confirmed based on whether a diagnostic band was present for both the $Sm^{F/R}$ (~350bp) and ND5 (~302bp) primer sets.

### 5.2.5 Bioinformatics and Statistical Analysis:

*Biomphalaria choanomphala* sequences were aligned using the Muscle algorithm in the program Seaview v5 (Gouy et al., 2021), with misaligned sections of the 16S and the COI fixed by hand and sites for tree building sites selected using the Gblocks program (Castresana, 2000). Phylogenetic trees were constructed using the Maximum Likelihood method, using a General Time Reversible model incorporating gamma correction (GTR+Γ) in the program PhyML v3.1 (Guindon et al., 2010), with bootstrap analysis undertaken using 1000 replicates. DNASP v6 (Rozas et al., 2017) was used to determine haplotype (gene) diversity scores (Hd), nucleotide diversity (π) and to examine population structure among populations between countries using Wright's F-statistics ($F_{st}$). Pairwise distances were calculated using MEGA-X using the Maximum Composite Likelihood method (Kumar et al. 2018).

Using SPSS v26 (IBM, Armonk, USA), correlations were performed to determine the relationships between the prevalence of *S. mansoni* infection, snail host haplotype diversity, *B. choanomphala* abundance and the physicochemical/abiotic factors of Lake Victoria. This was done using a two-tailed bivariate Spearman’s rank correlation analysis. Similarly, SPSS v26 was used to perform a Kruskal-Wallis H test with pairwise comparisons, Mann-Whitney U test and Pearson's chi-squared ($X^2$) test with Yates’ correction in order to compare the abundance of *B. choanomphala*, haplotype diversity, prevalence of infection and physicochemical factors between the Kenyan, Tanzanian and Ugandan shorelines of Lake Victoria.

### 5.2.6 GenBank Accessions:

Both previously published datasets and our datasets used in this study can be accessed on GenBank. The accession numbers for the Standley et al. (2014) *B. choanomphala* 16S gene sequences are HM768950-HM769131, and HM769132-HM769258 for the COI gene. Likewise, our *B. choanomphala* 16S gene sequences are OQ924869-OQ924928 and OQ849937-OQ849996 for the COI gene.

## 5.3 Results:

### 5.3.1 *Biomphalaria choanomphala* Abundance at Lake Victoria:

*Biomphalaria choanomphala* was present at 107 of the 170 sites surveyed at Lake Victoria (Supplementary Table 5.1). Of these 107 sites, 44 had a low abundance (< 10) of *B. choanomphala*, 25 had a medium abundance (10-30), and 38 had a high abundance (> 30); Table 5.1). The Ugandan sites had the highest abundance of *B. choanomphala*, followed by the Tanzanian sites and the Kenyan sites (Table 5.1). When categorised by morphotype, we found 64 sites had morphotype-A and 57 sites had morphotype-B (Table 5.1). Only 14 sites had both morphotypes present, with the majority of these sites being lake-marsh hybrid ecosystems on the Ugandan and the Tanzanian shorelines (Table 5.1). When partitioned by country, we found morphotype-A was more prevalent than morphotype-B at the Kenyan and Tanzanian sites, while morphotype-B more prevalent at the Ugandan sites (Table 5.1). The distribution of *B. choanomphala* along the shoreline of Lake Victoria is shown in Figure 5.2.

Table 5.1. Summary of the abiotic factors collected across the Kenyan (*n*=35), Tanzanian (*n*=82) and Ugandan (*n*=53) sites of Lake Victoria.

| | Category | Number of sites | | |
|---|---|---|---|---|
| | | Kenya | Tanzania | Uganda |
| *Biomphalaria* Abundance | No Snails | 14 | 37 | 12 |
| | Low (< 10) | 9 | 24 | 11 |
| | Medium (11 - 30) | 9 | 4 | 12 |
| | High (> 30) | 3 | 17 | 18 |
| Ecophenotypes | No Snails | 14 | 37 | 12 |
| | Only Morphotype-A | 19 | 32 | 13 |
| | Only Morphotype-B | 2 | 16 | 39 |
| | Both Morphotypes | 0 | 3 | 11 |
| Habitat | Marsh (a) | 12 | 30 | 9 |
| | Lake (b) | 14 | 35 | 36 |
| | Other (c)* | 9 | 17 | 8 |
| Water Depth | Shallow (< 10cm) | 15 | 48 | 22 |
| | Mod-Shallow (10-30cm) | 3 | 4 | 8 |
| | Moderate (30-50cm) | 7 | 12 | 9 |
| | Mod-Deep (50-70cm) | 2 | 2 | 7 |
| | Deep (> 70cm) | 7 | 8 | 5 |
| | Missing (N/A) | 1 | 8 | 2 |
| Water Turbulence | Low | 28 | 47 | 12 |
| | Medium | 2 | 7 | 9 |
| | High | 4 | 9 | 9 |
| | Missing (N/A) | 1 | 19 | 23 |

Note: * Breakdown of the other (c) habitats as follows: Rice paddy: 3; Pond: 8; Ditch/Canal: 1; Lake/Marsh: 16 and other combination: 6.

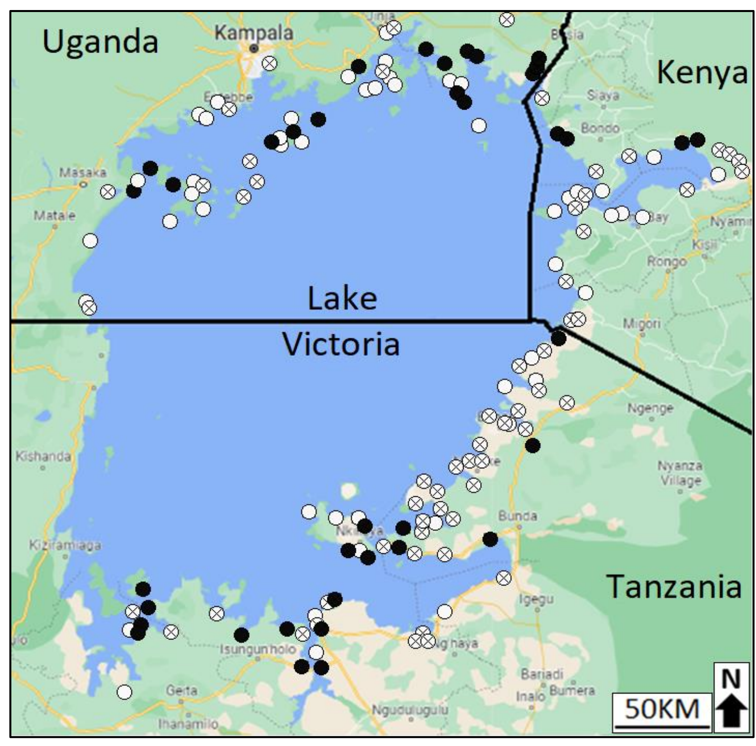



Figure 5.2. Map of collection sites at Lake Victoria showing where *B. choanomphala* snails were found and the incidence of *S. mansoni* infection in these snail populations. Collection sites with no *B. choanomphala* are shown with a cross. White circles denote sites where *B. choanomphala* snails were found but were uninfected with *S. mansoni*. Black circles denote sites where *S. mansoni* infected *B. choanomphala* snails were found.

5.3.2 Prevalence of *S. mansoni* Infection at Lake Victoria:

Of the 107 sites with *B. choanomphala* present, we found *S. mansoni* infection at 35.5% of sites (38 of 107 sites; Figure 5.2). All of our $Sm^{F/R}$ positive *Biomphalaria* samples were confirmed to be infected with *S. mansoni* as every sample gave a diagnostic band length of ~302bp when tested with the ND5 primer set. When partitioned by country, the Tanzanian shoreline had the highest number of infected sites, with 40% of the sites with *B. choanomphala* snails present (18/45) infected with *S. mansoni*. This was followed closely by the Ugandan shoreline with 39% (16/41) of sites with *B. choanomphala* infected and the Kenyan shoreline with only 19% (4/21) of sites with *B. choanomphala* infected (Figure 5.2, Supplementary Table 5.1). The sites with the highest number of infected *B. choanomphala* snails were in the Sengerema district of Tanzania, with T027b (7 of 10 snails) and T033a (4 of 10 snails) having the highest prevalence of infection at Lake Victoria. The remaining Lake Victorian sites had a maximum of two (or less) infected snails (Supplementary Table 5.1). Of the 107 sites with *B. choanomphala* present, 40 were marshlands (a), 50 were from the lake edge (b) and the remaining 17 were from a mixture of ecosystems (c) such as canals, paddies and ponds bordering the lake and hybrid environments. Of the 40 marshland sites (a), 14 had infection present (35%), while 16 of the 50 lake edge sites (b) had infection present (32%) and the mixed sites (c) had infection present at 8 of the 17 sites (47%). A chi-square ($X^2$) analysis found there was no significant difference in the amount of infection found at each of the three ecosystems ($p$= 0.53).

The overall prevalence of *S. mansoni* infection for Lake Victoria was 9.3%, with 59 of the 635 *B. choanomphala* snails testing positive for *S. mansoni* infection. When partitioned by country, we found the Tanzanian shoreline of Lake Victoria had the highest mean prevalence of infection with 13.1% (31/237) of snails infected, followed by the Ugandan shoreline with 8.2% (22/269) and the Kenyan shoreline with 4.7% (6/129). A chi-square ($X^2$) analysis found there was a significantly higher number of infected *B. choanomphala* snails at the Tanzanian and Ugandan shorelines when compared to the Kenyan shoreline, $X^2$ (2, *n*= 635) = 7.73, *p*= 0.02. However, there was no significant difference between the number of infected *B. choanomphala* snails between the Tanzanian and Ugandan shorelines (*p*= 0.09). When categorised by morphotype, the morphotype-A variants of *B. choanomphala* had an infection prevalence of 7.8% (27/347), while the morphotype-B form had an infection prevalence of 10.8% (31/288). However, there was no significant difference in the prevalence of *S. mansoni* infection between the two morphotypes of *B. choanomphala*, $X^2$ (1, *n*= 635) = 1.35, *p*= 0.246.

### 5.3.3 Host Snail Genetic Diversity and its Effect on Infection Prevalence

Of the 27 sites selected for host snail population genetic analysis, 168 unique 16S haplotypes (*n*=315) and 113 unique COI haplotypes (*n*=306) were found (Supplementary Table 5.2; Supplementary Figure 5.2). The mean haplotype diversity (Hd) scores of the 27 sites were 0.845 (+ 0.16) for 16S, and 0.787 (+ 0.17) for COI. The mean nucleotide diversity (π) value for all of the Lake Victorian sites were 0.015 (+ 0.009) for 16S, and 0.008 (+ 0.005) for COI. The overall range of pairwise distances of the 27 sites genotyped at Lake Victoria was 0.0-3.5% for the 16S and 0.0-4% for the COI.

When proportioned by shoreline, we found the Ugandan shoreline had the highest number of haplotypes with 94 16S haplotypes (*n*=150) and 60 COI haplotypes (*n*=156). Of the 12 Ugandan sites sampled, the mean Hd score was 0.883 (+ 0.19) for 16S and 0.747 (+ 0.19) for COI. The mean nucleotide diversity value for Ugandan sites was 0.018 (+ 0.01) for 16S, and 0.007 (+ 0.005) for COI. Pairwise distances for the Ugandan sites were 0.0-3.1% for 16S and 0.0-3.9% for COI. The Tanzanian shoreline had the second highest number of haplotypes with 50 16S haplotypes (*n*=93) and 39 COI haplotypes (*n*=93). Of the 9 Tanzanian sites sampled, the 16S and COI had a mean Hd score of 0.863 (+ 0.09) and 0.802 (+ 0.16), respectively. The mean nucleotide diversity value for Tanzanian sites was 0.016 (+ 0.008) for 16S, and 0.008 (+ 0.005) for COI. Pairwise distances for the Tanzanian sites were 0.0-3.4% for 16S and 0.0-3.8% for COI. Lastly, the Kenyan shoreline had the lowest number of haplotypes with 24 16S haplotypes (*n*=62) and 23 COI haplotypes (*n*=64). Of the six Kenyan sites sampled, the mean Hd score was 0.748 (+ 0.16) for 16S and 0.844 (+ 0.07) for COI. The mean nucleotide diversity value for Kenyan sites was 0.007 (+ 0.003) for 16S, and 0.009 (+ 0.006) for COI. Pairwise distances for the Kenyan sites were 0.0-1.9% for 16S and 0.0-1.2% for COI.

Next, when comparing the amount of haplotype diversity (Hd) at the 13 sites found with infection against the 14 sites found without infection, we found sites with infection had a higher mean Hd score than sites without infection (Table 5.2). The mean Hd score of the 13 *B. choanomphala* collection sites with infection was 0.881 (± 0.1) for 16S and 0.841 (± 0.11) for COI, while the mean Hd score of the 14 collection sites with no infection was 0.814 (± 0.2) for 16S and 0.737 (± 0.19) for COI (Table 5.2). However, a Mann-Whitney U test found this difference in mean Hd score was not significant for either the 16S (U = 93.5, *p*= 0.903) or the COI (U = 118.5, *p*= 0.182). When partitioned by country, we find not all of the countries share this trend of sites with infection having a higher mean Hd score than sites without infection. For example, the mean Hd score of the 16S was higher for sites found without infection (0.933) than sites found with infection (0.828) on the Tanzanian shoreline (Table 5.2). Likewise, the mean Hd score of the COI was higher for sites found without infection (0.879) than sites found with infection (0.774) on the Kenyan shorelines (Table 5.2). A Spearman's rank correlation test found that there was positive correlation between haplotype diversity scores and the prevalence of *S. mansoni* infection for both the 16S ($R_s$=0.003) and COI ($R_s$=0.229). However, the correlations between haplotype diversity scores and the prevalence of *S. mansoni* infection were not statistically significant for both the 16S (*p*= 0.989) and COI (*p*= 0.251).

Table 5.2. Comparing the mean haplotype diversity (Hd) scores of Lake Victorian sites found with and without *S. mansoni* infection.

| | Mean Haplotype Diversity Scores (± SD) | | | |
|---|---|---|---|---|
| | 16S | | COI | |
| | Uninfected | Infected | Uninfected | Infected |
| Overall mean (*n=27*) | 0.814 (+ 0.2) | 0.881 (± 0.1) | 0.737 (+ 0.2) | 0.841 (± 0.1) |
| Kenya (*n*=6) | 0.691 (± 0.1) | 0.862 (± 0.1) | 0.879 (± 0.0) | 0.774 (± 0.1) |
| Tanzania (*n*=9) | 0.933 (± 0.0) | 0.828 (± 0.1) | 0.715 (± 0.2) | 0.846 (± 0.1) |
| Uganda (*n*=9) | 0.833 (± 0.0) | 0.953 (± 0.0) | 0.666 (± 0.2) | 0.862 (± 0.1) |

Next, when measuring the population structure ($F_{st}$) between *B. choanomphala* populations using the 16S, we found the population structure was highest among the Kenyan and Ugandan populations (0.305), followed by the Tanzanian and Ugandan populations (0.242), while the Kenyan and Tanzanian populations had the lowest amount of structure (0.098; Table 5.3). Likewise for the COI, we found the population structure was highest among the Kenyan and Ugandan populations (0.195). However, the second highest $F_{st}$ value was between the Kenyan and Tanzanian populations (0.118), followed by the Tanzanian and Ugandan populations (0.067; Table 5.3). Lastly, we mapped the distribution of private and shared 16S and COI haplotypes of *B. choanomphala* throughout Lake Victoria (Figure 5.3). The mean percentage of private haplotypes found within each *B. choanomphala* population was 46.7% for 16S haplotypes and 29.5% for COI haplotypes, while the mean percentage of shared haplotypes was higher for both the 16S (53.3%) and COI (70.5%). When partitioned

by country, we found the Kenyan sites had the highest mean percentage of shared haplotypes with 71% for the 16S and 71.9% for the COI. Next, was the Tanzanian sites had the second highest mean percentage of shared haplotypes with 58% for the 16S and 70.5% for the COI. The Ugandan sites had the lowest mean percentage of shared haplotypes with 43% for the 16S and 69.9% for the COI. When comparing the number of shared haplotypes between *B. choanomphala* populations found with and without *S. mansoni* infection, we found sites with infection had more shared haplotypes for both the 16S (58.6%) and COI (78.9%) than sites found without infection (16S: 47.2%; COI: 61.1%; Figure 5.3). However, when partitioned by country, we found on average the uninfected Kenyan sites had more shared haplotypes for both the 16S (75.6%) and COI (70%) than infected sites (16S: 61.9%; COI: 72.7%). Conversely, on average the infected Tanzanian sites had more shared haplotypes for both the 16S (61.9%) and COI (78.2%) than uninfected sites (16S: 50%; COI: 54.8%). Likewise, on average the infected Ugandan sites had more shared haplotypes for both the 16S (55%) and COI (81.7%) than uninfected sites (16S: 29.6%; COI: 56.8%).

Table 5.3. Comparing the $F_{st}$ values of *B. choanomphala* populations across Lake Victoria.

| Population 1 | Population 2 | $F_{st}$ Value | |
|---|---|---|---|
| | | 16S | COI |
| Kenya | Tanzania | 0.098 | 0.118 |
| Kenya | Uganda | 0.305 | 0.195 |
| Tanzania | Uganda | 0.242 | 0.067 |

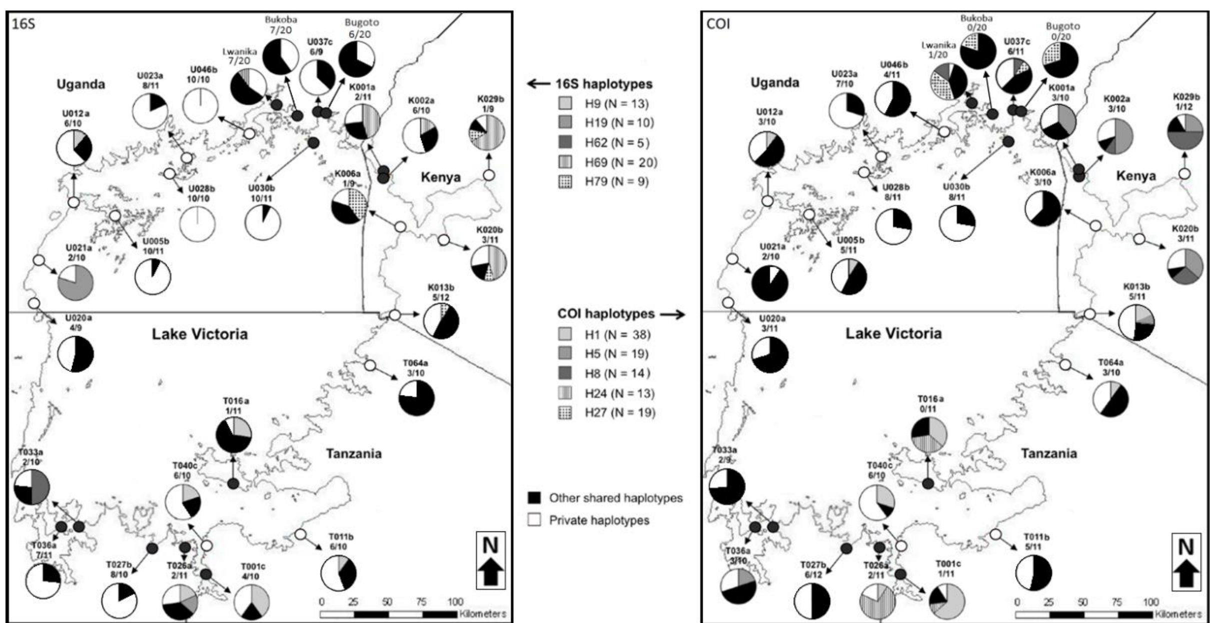



Figure 5.3. Distribution of 16S and COI haplotypes of *B. choanomphala* populations (*n*=27) found in Lake Victoria. Private haplotypes are coloured white in the pie chart, while shared haplotypes are shaded. Sites found without *S. mansoni* infection are shown with a white dot (*n*=14), while sites found with infection are shown with a black dot (*n*=13).

5.3.4 Abiotic Factors, *B. choanomphala* Abundance and ***S. mansoni*** Infection:

When comparing the physicochemical factors between the Kenyan and Tanzanian sites, a Kruskal-Wallis test with post hoc pairwise comparisons (Bonferroni adjusted) found the median temperature, fluoride, nitrate and sulphate levels were significantly higher at the Kenyan sites than at the Tanzanian sites (Table 5.4; Supplementary Table 5.3). For the Kenyan and Ugandan sites, the median conductivity, TDS, salinity, fluoride, nitrate, phosphate, sulphate and sodium levels were significantly higher at the Kenyan sites than at the Ugandan sites (Table 5.4). Finally, for the Tanzanian and Ugandan sites, the median conductivity, TDS, salinity, fluoride, sulphate and sodium levels were significantly higher at the Tanzanian sites than at the Ugandan sites (Table 5.4). Conversely, the median temperature and potassium levels were significantly higher at the Ugandan sites than at the Tanzanian sites (Table 5.4).

Table 5.4. Pairwise comparison of the Kruskal-Wallis analysis comparing the prevalence of *S. mansoni* infection, *B. choanomphala* abundance and abiotic factors of the Kenyan (*n*=35), Tanzanian (*n*=82) and Ugandan (*n*=53) collection sites.

| | Kenya - Tanzania | | Kenya - Uganda | | Tanzania - Uganda | |
|---|---|---|---|---|---|---|
| | *H*(2) | *p* value | *H*(2) | *p* value | *H*(2) | *p* value |
| Infection | - | *Not sig.* | - | *Not sig.* | - | *Not sig.* |
| *B. choan* Abundance | - | *Not sig.* | - 24.96 | 0.045 | - 26.89 | 0.004 |
| Morphotype-A Abund. | - | *Not sig.* | 23.54 | 0.033 | - | *Not sig.* |
| Morphotype-B Abund. | - | *Not sig.* | - 58.51 | 0.000 | - 48.59 | 0.000 |
| Temperature (°C) | 27.38 | 0.004 | - | *Not sig.* | 36.48 | 0.001 |
| Conductivity (µS) | - | *Not sig.* | 62.71 | 0.000 | 44.82 | 0.000 |
| pH | - | *Not sig.* | - | *Not sig.* | - | *Not sig.* |
| Total Dissolved Solids (g/L) | - | *Not sig.* | 47.22 | 0.000 | 59.66 | 0.000 |
| Salinity (g/L) | - | *Not sig.* | 32.79 | 0.000 | 280.02 | 0.000 |
| Fluoride ($F^-$) | 22.58 | 0.027 | 69.77 | 0.000 | 47.19 | 0.000 |
| Chloride ($Cl^-$) | - | *Not sig.* | - | *Not sig.* | - | *Not sig.* |
| Nitrate ($NO_3^-$) | 19.64 | 0.031 | 25.30 | 0.013 | - | *Not sig.* |
| Phosphate ($PO_4^{3-}$) | - | *Not sig.* | 25.16 | 0.021 | - | *Not sig.* |
| Sulphate ($SO_4^{3-}$) | 290.01 | 0.002 | 59.86 | 0.000 | 30.83 | 0.000 |
| Sodium ($Na^+$) | - | *Not sig.* | 45.43 | 0.000 | 270.02 | 0.003 |
| Magnesium ($Mg_2^+$) | - | *Not sig.* | - | *Not sig.* | - | *Not sig.* |
| Calcium ($Ca_2^+$) | - | *Not sig.* | - | *Not sig.* | - | *Not sig.* |
| Potassium ($K^+$) | - | *Not sig.* | - | *Not sig.* | - 20.71 | 0.030 |

Note: Non-significant differences were greyed out; *H*(df) value is the result from the Kruskal-Wallis H test. The *p* values displayed are adjusted using Bonferroni corrections.

When comparing prevalence of infection between countries, a Kruskal-Wallis test showed there was no significant difference in the number of sites found with *S. mansoni* infection between the Kenyan ($n$=35), Tanzanian ($n$=82) and Ugandan ($n$=53) shorelines, $H(2) = 4.05$, $p = 0.132$. However, a Spearman's rank correlation analysis found there were several significant relationships between infection prevalence and *B. choanomphala* abundance (0.445), morphotype-B abundance (0.306), morphotype-A abundance (0.271), pH (- 0.199), calcium (0.184) and magnesium (0.175; Table 5.5).

When comparing the abundance of *B. choanomphala* between countries, a Kruskal-Wallis test found Ugandan sites had a significantly higher abundance of *B. choanomphala* compared to the Kenyan and Tanzanian sites (Table 5.4). When categorised by morphotype, we found the abundance of morphotype-A snails was significantly higher only at Kenyan sites compared to the Ugandan, while Tanzanian and Ugandan sites had similar amounts (Table 5.4). However, the abundance of morphotype-B snails was significantly higher at Ugandan sites compared to both Kenyan and Tanzanian sites (Table 5.4). A Spearman's rank correlation analysis found *B. choanomphala* abundance had several significant relationships with chloride (0.354), magnesium (0.322), phosphate (0.319), potassium (0.316), pH (- 0.311), calcium (0.238), nitrate (0.215) and water turbulence (- 0.214; Table 5.5). Likewise, morphotype-A abundance had a significant negative relationship with morphotype-B abundance (- 0.177) and *vice versa*. (Table 5.5). This relationship indicates that each morphotype prefers inverse environmental factors to one another. For example, morphotype-A abundance had a significant positive relationship with sulphate (0.508), water conductivity (0.421), nitrate (0.404), sodium (0.402), calcium (0.398), phosphate (0.394), chloride (0.379), TDS (0.336), magnesium (0.307), salinity (0.252) and potassium (0.241). Whereas morphotype-B abundance has a significant negative relationship with sulphate (- 0.359), water conductivity (- 0.363), nitrate (- 0.181), sodium (- 0.316), TDS (- 0.379), salinity (- 0.256) and fluoride (- 0.391; Table 5.5). Moreover, morphotype-A abundance had a significant negative relationship with water turbulence (- 0.447), pH (- 0.386) and water depth (- 0.170), while morphotype-B abundance had a significant positive relationship with water turbulence (0.269) and water depth (0.161; Table 5.5).

Table 5.5. Spearman's rank correlation coefficients relating **prevalence** of *S. mansoni* infection, *B. choanomphala* abundance and abiotic factors of the Kenyan (*n*=35), Tanzanian (*n*=82) and Ugandan (*n*=53) collection sites.

| | Sites (*n=*) | Correlation Coefficient | | | |
|---|---|---|---|---|---|
| | | Infection | Abundance | Morphotype-A | Morphotype-B |
| Infection | 170 | - | - | - | - |
| *B. choan* Abund. | 170 | 0.445 ** | - | - | - |
| Morphotype-A Abund. | 170 | 0.271 ** | 0.619 ** | - | - |
| Morphotype-B Abund. | 170 | 0.306 ** | 0.563 ** | - 0.177 * | - |
| Conductivity | 166 | 0.064 | 0.125 | 0.421 ** | - 0.363 ** |
| pH | 165 | - 0.199 * | - 0.311 ** | - 0.386 ** | - 0.032 |
| Temperature | 165 | 0.075 | 0.067 | 0.063 | 0.041 |
| TDS | 164 | - 0.023 | 0.050 | 0.336 ** | - 0.379 ** |
| Water Depth | 159 | 0.009 | - 0.058 | - 0.170 * | 0.161 * |
| Salinity | 154 | 0.060 | 0.064 | 0.252 ** | - 0.256 ** |
| Fluoride ($F^-$) | 141 | - 0.092 | - 0.144 | 0.154 | - 0.391 ** |
| Chloride ($Cl^-$) | 141 | 0.154 | 0.354 ** | 0.379 ** | 0.013 |
| Phosphate ($PO_4^{3-}$) | 141 | 0.113 | 0.319 ** | 0.394 ** | - 0.064 |
| Sulphate ($SO_4^{3-}$) | 141 | - 0.004 | 0.157 | 0.508 ** | - 0.359 ** |
| Sodium ($Na^+$) | 140 | 0.131 | 0.139 | 0.402 ** | - 0.316 ** |
| Magnesium ($Mg_2^+$) | 140 | 0.175 * | 0.322 ** | 0.307 ** | 0.009 |
| Calcium ($Ca_2^+$) | 140 | 0.184 * | 0.238 ** | 0.398 ** | - 0.155 |
| Nitrate ($NO_3^-$) | 134 | - 0.041 | 0.215 * | 0.404 ** | - 0.181 * |
| Potassium ($K^+$) | 139 | 0.159 | 0.316 ** | 0.241 ** | 0.140 |
| Water Turbulence | 127 | - 0.105 | - 0.214 * | - 0.447 ** | 0.268 ** |

Note: Non-significant correlations were greyed out; * indicates a significance of $p < 0.05$ and ** indicates a significance of $p < 0.001$.

5.1 Discussion:

Our study investigated the prevalence of *S. mansoni* infection in *B. choanomphala* snails in Lake Victoria and whether certain biotic (snail host abundance and genetic diversity) and abiotic (temperature, pH, physiochemical parameters etc.) had an effect on infection prevalence. Standley et al. (2014) reported that *B. choanomphala* snails found at Lake Victoria had high amounts of genetic diversity, high levels of both inter- and intra-population diversity, low levels of gene flow between populations and low levels of inbreeding. They theorised that this high level of genetic diversity could be caused by several factors relating to the environment (homogenous habitats), human activity (mass treatment and snail control programs) and *S. mansoni* infection. However, Standley et al. (2014) were unable to examine whether *S. mansoni* infection prevalence was influenced by *B. choanomphala* population structure due to the lack of data on whether a snail was

infected or not. Our study provides this missing infection data and incorporates it with host genetic diversity, host abundance and abiotic/physiochemical datasets.

### 5.4.1 Prevalence of *S. mansoni* infection in *B. choanomphala* snails in Lake Victoria

Our study found a mean prevalence of *S. mansoni* infection of 9.3% in *B. choanomphala* snails at Lake Victoria, with the highest prevalence of infection observed on the Tanzanian shoreline with 13.1%, followed by the Ugandan shoreline with 8.2% and the Kenyan shoreline with 4.7%. Our study found a higher mean prevalence of *S. mansoni* infection when compared to previous parasitological studies. Previously, Gouvras et al. (2017) reported 1.2% of snails on the Tanzanian shoreline were shedding cercariae, while 1.8-2.1% were shedding on the Ugandan shoreline (Odongo-Aginya et al., 2008; Rowel et al., 2015) and 0.7-1.5% were shedding on the Kenyan shoreline (Mutuku et al., 2019; Odero et al., 2019). The reason for this increase in infection prevalence is most likely attributed to the use of molecular methods to detect infection in the present study rather than the traditional cercarial shedding method. Molecular detection methods tend to show a higher number of infected snails as they are able to detect infection in both prepatent and actively shedding snails and are thus less likely to give false negative results (Hamburger et al., 2013; Lu et al., 2016; Joof et al., 2020).

When categorised by morphotype, we found the morphotype-B form of *B. choanomphala* had a higher mean infection prevalence (10.8%) than the morphotype-A form (7.8%). Similarly, a Spearman's rank test found morphotype-B variants of *B. choanomphala* (0.306) had a stronger relationship with infection than the morphotype-A variant (0.271). However, a chi squared analysis found this difference in infection prevalence was not statistically significant. Consistent with our findings, Mutuku et al. (2021) reported that *S. mansoni* infection and cercarial production was significantly higher in the morphotype-B forms of *B. choanomphala* than the morphotype-A form, regardless of miracidium dosage or whether the eggs came from allopatric or sympatric sources. However, Rowel et al. (2015) and Gouvras et al. (2017) found the opposite trend, with the morphotype-A form of *B. choanomphala* having a higher *S. mansoni* infection prevalence than the morphotype-B form.

### 5.4.2 The Effect Abiotic Factors Have on *B. choanomphala* Abundance and Shell Morphology:

A Spearman's rank correlation test found *B. choanomphala* abundance had a significant positive relationship with calcium, chloride, magnesium, nitrate, phosphate and potassium levels in the water. Conversely, *B. choanomphala* abundance had a significant negative relationship with high water turbulence and pH levels (*B. choanomphala* abundance decreased with increasing alkalinity). When comparing *B. choanomphala* abundance between the Kenyan, Tanzanian and Ugandan sites, we found the Ugandan sites had a significantly higher amount of *B. choanomphala* snails compared to the Kenyan and Tanzanian sites. When categorised by morphotype, the majority of the *B. choanomphala* snails collected from the Ugandan shoreline were morphotype-B, while the majority of the *B. choanomphala* snails collected from the Kenyan and Tanzanian shorelines were morphotype-A. This difference in morphology could be explained by the difference in abiotic factors between the Kenyan, Tanzanian and Ugandan sites, as a Kruskal-Wallis test found

the Kenyan and Tanzanian sites had significantly higher levels of nitrate, potassium, salinity, sodium, sulphate, TDS and water conductivity than the Ugandan sites. Likewise, a Spearman's rank analysis found morphotype-A abundance had a positive relationship with higher levels of nitrate, potassium, salinity, sodium, sulphate, TDS and water conductivity, while morphotype-B abundance had a negative relationship with higher levels of nitrate, salinity, sodium, sulphate, TDS and water conductivity.

A Spearman's rank analysis found morphotype-A abundance had a negative relationship with water depth and water turbulence, while morphotype-B abundance had a positive relationship with water depth and water turbulence. The morphotype-A form of *B. choanomphala* was predominately found in shallow and lentic (still) environments, while the morphotype-A form was predominately found in deep lotic (flowing) environments. Dillon (2019) found the American Planorbidae species, *Helisoma trivolvis* also exhibit different ecological phenotypes depending on whether they inhabited shallow, lentic waters, or deep, lotic waters. Dillon (2019) hypothesised that these two contrasting shell morphologies helped the snails adapt to their environment, as the morphotype found in the shallow, lentic waters use their shell to trap air, in order to regulate their buoyancy and reach floating vegetation. Conversely, the morphotype found in deep, lotic waters use their wide aperture/foot to grip onto rocks while grazing in flowing water. This functionality could be analogous to the *B. choanomphala* ecophenotypes found in Lake Victoria.

### 5.4.3 The Factors Affecting Infection Prevalence:

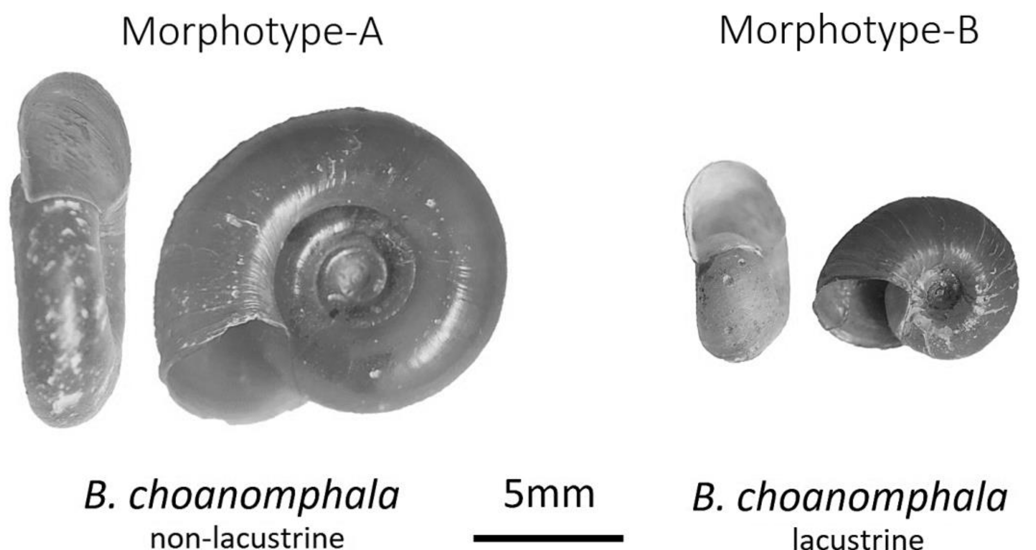
A Spearman's rank correlation test found *S. mansoni* infection in *B. choanomphala* snails had a significant positive relationship with *B. choanomphala* abundance, calcium levels and magnesium levels. Conversely, infection prevalence had a significant negative correlation with pH levels of the lake water (*S. mansoni* infection decreased with increasing alkalinity). Rowel et al. (2015) also observed this trend, with *S. mansoni* infection having a significant positive relationship with *Biomphalaria* abundance and a significant negative relationship with alkaline pH levels. However, *B. choanomphala* abundance itself also has a significant positive relationship with calcium and magnesium levels, as well as a significant negative relationship with alkaline pH levels. Therefore, it is likely that *B. choanomphala* abundance is the only direct factor influencing infection prevalence as the other factors indirectly affect infection via *Biomphalaria* abundance. However, we found the Ugandan shoreline had a significantly higher abundance of *B. choanomphala* snails than the Tanzanian shoreline, yet the Tanzanian shoreline had a higher number of infected *B. choanomphala* snails than the Ugandan shoreline. This is odd as we found no significant difference in other factors affecting infection (calcium, magnesium and pH levels) between the Tanzanian and Ugandan shorelines which could explain this increase. Likewise, Ngowi, (2020) found reports from 1941 to 2019 that showed that Tanzania (86%) and Uganda (88%) have a similar prevalence of schistosomiasis within their respective populations. Therefore, other socioeconomic, behavioural or ecological factors not measured in this study could be increasing the frequency and spread of *S. mansoni* infected *B. choanomphala* within Tanzania. It is important to acknowledge that our analysis lacks any human-based data for each of the sites investigated.

### 5.4.4 The Effects of Snail Host Genetic Diversity on Infection:

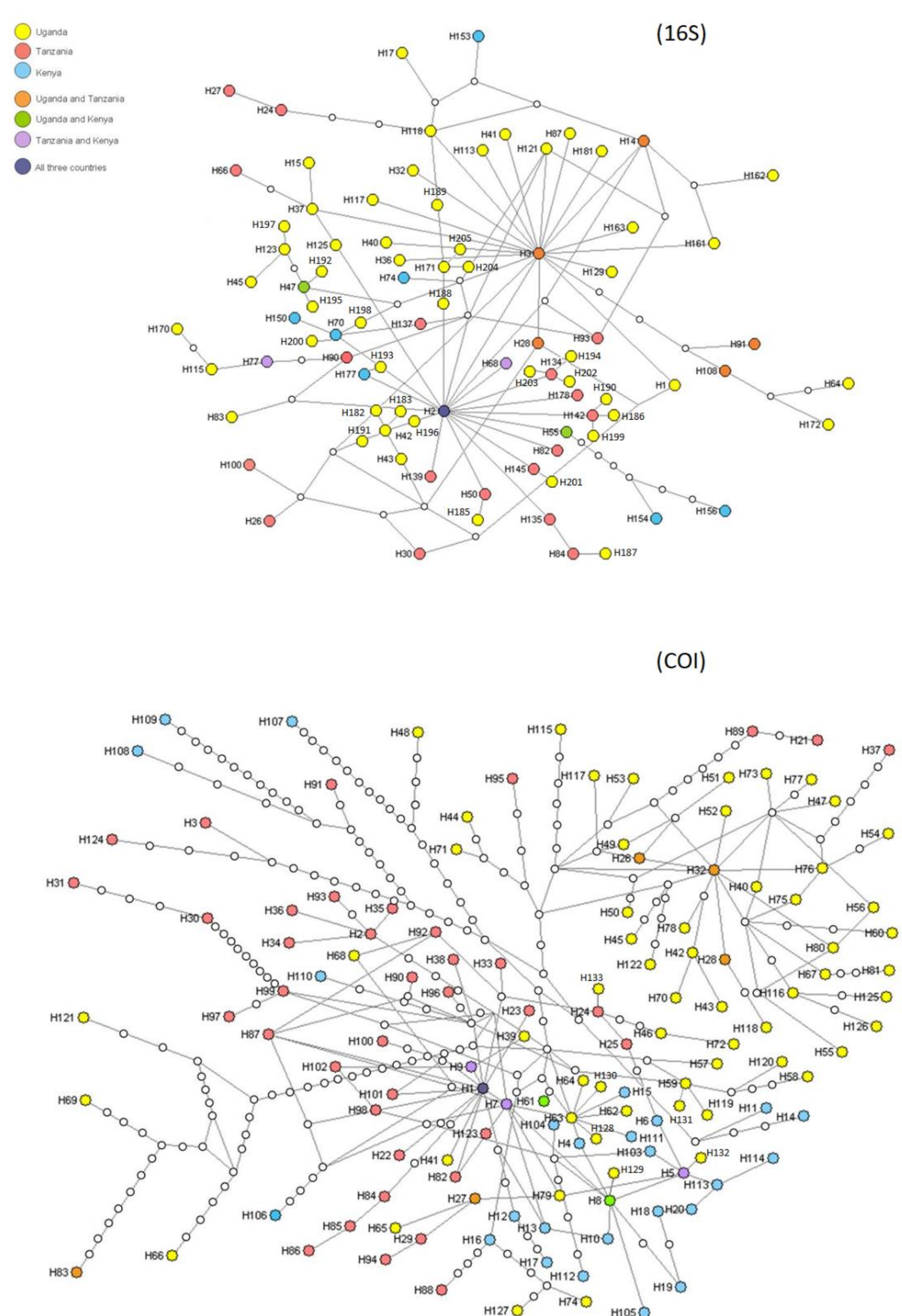
We found the *B. choanomphala* populations found on the Ugandan shoreline had the highest genetic diversity, followed by the Tanzanian and Kenyan populations. When we compared the level of genetic diversity at sites with and without *S. mansoni* infection, we found sites with infection had a higher mean haplotype diversity score (16S: 0.881; COI: 0.841) than sites without infection (16S: 0.814; COI: 0.737). A Spearman's rank correlation test found both the 16S and COI Hd scores correlated positively with infection prevalence, but this relationship between haplotype diversity and infection was not statistically significant. When we mapped the distribution of the 16S and COI haplotypes across Lake Victoria, we found infected *B. choanomphala* populations on average had fewer private and more shared 16S and COI haplotypes than *B. choanomphala* populations without infection (Figure 5.3), indicating there is greater amounts of gene flow occurring among infected sites than among uninfected sites. Our findings are contradictory to previous studies that found a link between lower genetic diversity within a host population and increased susceptibility to parasite infection (Coltman et al., 1999; Jarne & Théron, 2001; Campbell et al., 2010). One possible explanation for this contradiction could be due to the higher amounts of gene flow previously mentioned. This is because the migration of *B. choanomphala* snails between sites helps to maintain a high amount of genetic diversity (via gene flow) and could explain why sites with infection have higher amounts of genetic diversity than sites without infection.

An alternatively explanation for this contradiction could be explained by the 'coevolution selective sweep' phenomenon, where a host-parasite relationship results in selective sweeps of host resistance adaptations and parasite counter-adaptations (Kawecki et al., 2012; Auld & Tinsley, 2015). This causes a reduction in genetic diversity as individuals without this adaption (e.g. *S. mansoni* resistance) are less successful than those who have it. Populations with high genetic diversity and high *S. mansoni* infection prevalence may not have undergone this selective sweep, while populations with low genetic diversity and low infection prevalence could have. Another explanation could be due to whether non-random mating behaviour is being exhibited or not. This is because non-random mating behaviour is involved in maintaining resistance to *S. mansoni*, and ultimately reduces the genetic diversity of a population (Webster & Gower, 2006). Populations with high genetic diversity and high infection levels may not exhibit non-random mating behaviour, favouring random mating as it promotes genetic diversity over *S. mansoni* resistance, resulting in longer life span, higher fecundity, and more successful offspring (Sandland et al., 2007). Conversely, populations with low genetic diversity and low infection levels may exhibit this non-random mating behaviour, favouring resistance over genetic diversity. Overall, the relationship between *S. mansoni* and *Biomphalaria* snails is complex and can depend on many factors such as the genetic constitution of the snails, the environment in which they live, and the prevalence and virulence of *S. mansoni* within an area.

5.5 Supplementary Material:




Supplementary Figure 5.1. Morphological examples of non-lacustrine (morphotype-A) and lacustrine (morphotype-B) forms of *B. choanomphala* found at Lake Victoria. The shells are viewed from the apertural (left) and apical (right) angle.




Supplementary Figure 5.2. Median-joining network of the gapless 16S (top) and the gapless COI (bottom) haplotypes observed at Lake Victoria. Adapted from Standley et al. 2014 and includes the 23 new 16S haplotypes (H183-205) and the six new COI haplotypes (H128-133) from the Rowel et al. 2015 dataset. Haplotypes are coloured based on location.

Supplementary Table 5.1. Site information of Lake Victoria collections performed by Standley et al. (2012) and Rowel et al. (2015).

| | Extracted Individuals | Infected | Latitude | Longitude |
|---|---|---|---|---|
| K001a | 11 | 2 | - 0.073 | 34.058 |
| K001b | 0 | 0 | - 0.073 | 34.058 |
| K002a | 11 | 1 | - 0.110 | 34.065 |
| K003a | 4 | 0 | - 0.187 | 34.387 |
| K003b | 0 | 0 | - 0.187 | 34.387 |
| K004b | 4 | 2 | - 0.141 | 34.594 |
| K005c | 5 | 0 | - 0.420 | 34.207 |
| K006a | 10 | 0 | - 0.434 | 34.171 |
| K007a | 4 | 0 | - 0.431 | 34.129 |
| K007b | 0 | 0 | - 0.431 | 34.129 |
| K008a | 8 | 0 | - 0.398 | 34.160 |
| K010b | 0 | 0 | - 0.381 | 34.213 |
| K011c | 4 | 0 | - 0.453 | 34.323 |
| K012b | 2 | 0 | - 0.474 | 34.288 |
| K013a | 0 | 0 | - 1.010 | 34.130 |
| K013b | 12 | 0 | - 1.010 | 34.130 |
| K014b | 0 | 0 | - 1.001 | 34.099 |
| K015a | 5 | 0 | - 0.856 | 34.187 |
| K016b | 0 | 0 | - 0.817 | 34.119 |
| K017a | 4 | 0 | - 0.726 | 34.058 |
| K018c | 0 | 0 | - 0.538 | 34.164 |
| K019a | 6 | 0 | - 0.437 | 34.015 |
| K020b | 11 | 0 | - 0.523 | 34.455 |
| K021a | 4 | 0 | - 0.354 | 34.663 |
| K021b | 0 | 0 | - 0.354 | 34.663 |
| K022c | 2 | 0 | - 0.312 | 34.848 |
| K023c | 0 | 0 | - 0.226 | 34.967 |
| K024c | 0 | 0 | - 0.175 | 34.936 |
| K025c | 0 | 0 | - 0.171 | 34.907 |
| K026c | 0 | 0 | - 0.156 | 34.850 |
| K028b | 2 | 1 | - 0.105 | 34.718 |
| K029b | 12 | 0 | - 0.096 | 34.749 |
| K030b | 3 | 0 | - 0.328 | 34.267 |
| K032c | 0 | 0 | - 0.181 | 34.263 |
| K033a | 5 | 0 | 0.099 | 33.968 |
| T001c | 11 | 1 | - 2.713 | 32.894 |
| T002a | 6 | 0 | - 2.726 | 32.870 |
| T003c | 5 | 0 | - 2.643 | 32.960 |
| T004c | 4 | 1 | - 2.531 | 32.901 |
| T005c | 4 | 0 | - 2.414 | 32.941 |
| T006a | 8 | 1 | - 2.405 | 32.950 |
| T006b | 0 | 0 | - 2.405 | 32.950 |
| T007b | 0 | 0 | - 2.502 | 32.880 |
| T008c | 0 | 0 | - 2.585 | 33.390 |
| T009a | 3 | 0 | - 2.525 | 33.395 |
| T009b | 0 | 0 | - 2.526 | 33.395 |

| | | | | |
|---|---|---|---|---|
| T010c | 0 | 0 | - 2.588 | 33.415 |
| T011b | 11 | 0 | - 2.452 | 33.517 |
| T012a | 0 | 0 | - 2.262 | 33.809 |
| T013b | 0 | 0 | - 2.168 | 33.351 |
| T014a | 0 | 0 | - 2.112 | 33.210 |
| T014b | 5 | 2 | - 2.112 | 33.210 |
| T015a | 4 | 1 | - 2.128 | 33.048 |
| T016a | 11 | 0 | - 2.117 | 33.071 |
| T017b | 4 | 0 | - 2.124 | 33.065 |
| T018a | 4 | 1 | - 2.018 | 33.103 |
| T019c | 3 | 0 | - 1.996 | 33.111 |
| T020a | 4 | 0 | - 1.984 | 33.018 |
| T021a | 4 | 0 | - 1.943 | 32.861 |
| T022a | 3 | 0 | - 2.048 | 33.312 |
| T023a | 4 | 1 | - 2.131 | 33.328 |
| T024a | 0 | 0 | - 2.157 | 33.477 |
| T025c | 3 | 1 | - 2.080 | 33.742 |
| T026a | 11 | 2 | - 2.535 | 32.755 |
| T027b | 10 | 7 | - 2.546 | 32.542 |
| T028c | 0 | 0 | - 2.538 | 32.233 |
| T029a | 1 | 1 | - 2.507 | 32.015 |
| T030b | 5 | 0 | - 2.494 | 31.986 |
| T031b | 5 | 0 | - 2.461 | 31.984 |
| T032b | 1 | 0 | - 2.440 | 32.010 |
| T033a | 10 | 4 | - 2.405 | 32.059 |
| T034a | 4 | 1 | - 2.348 | 32.040 |
| T035b | 0 | 0 | - 2.383 | 31.967 |
| T036a | 11 | 2 | - 2.407 | 31.945 |
| T037b | 4 | 2 | - 2.415 | 31.923 |
| T038b | 0 | 0 | - 2.436 | 32.411 |
| T038b | 3 | 0 | - 2.436 | 32.411 |
| T039c | 0 | 0 | - 2.539 | 32.840 |
| T040c | 10 | 0 | - 2.528 | 32.895 |
| T041a | 0 | 0 | - 1.962 | 33.530 |
| T042a | 4 | 0 | - 1.956 | 33.467 |
| T042b | 0 | 0 | - 1.956 | 33.467 |
| T043a | 2 | 1 | - 2.047 | 33.381 |
| T043b | 0 | 0 | - 2.047 | 33.381 |
| T043c | 0 | 0 | - 2.047 | 33.381 |
| T044b | 2 | 0 | - 2.015 | 33.386 |
| T044c | 0 | 0 | - 2.015 | 33.386 |
| T045b | 2 | 0 | - 1.984 | 33.433 |
| T047a | 4 | 0 | - 1.809 | 33.412 |
| T047b | 0 | 0 | - 1.809 | 33.412 |
| T048a | 3 | 0 | - 1.908 | 33.397 |
| T048b | 0 | 0 | - 1.908 | 33.397 |
| T049b | 0 | 0 | - 1.847 | 33.466 |
| T050a | 0 | 0 | - 1.782 | 33.622 |
| T050b | 0 | 0 | - 1.782 | 33.622 |
| T051b | 0 | 0 | - 1.680 | 33.541 |

| | | | | |
|---|---|---|---|---|
| T052b | 0 | 0 | - 1.677 | 33.618 |
| T053a | 0 | 0 | - 1.683 | 33.687 |
| T053b | 0 | 0 | - 1.683 | 33.687 |
| T054b | 0 | 0 | - 1.605 | 33.695 |
| T056b | 0 | 0 | - 1.496 | 33.739 |
| T057b | 4 | 0 | - 1.330 | 33.813 |
| T059b | 0 | 0 | - 1.454 | 33.856 |
| T060a | 0 | 0 | - 1.498 | 33.895 |
| T060b | 0 | 0 | - 1.498 | 33.895 |
| T061c | 4 | 1 | - 1.599 | 33.913 |
| T062c | 0 | 0 | - 1.516 | 33.821 |
| T063c | 4 | 0 | - 1.526 | 33.832 |
| T064a | 10 | 0 | - 1.347 | 33.970 |
| T064b | 0 | 0 | - 1.347 | 33.970 |
| T065c | 0 | 0 | - 1.401 | 34.134 |
| T066b | 5 | 0 | - 1.306 | 33.955 |
| T067a | 4 | 0 | - 1.125 | 33.999 |
| T068b | 9 | 1 | - 1.038 | 34.085 |
| T069b | 0 | 0 | - 1.193 | 33.943 |
| T070a | 4 | 0 | - 1.255 | 33.868 |
| T070b | 0 | 0 | - 1.255 | 33.868 |
| U001b | 3 | 0 | 0.078 | 32.448 |
| U002b | 0 | 0 | - 0.234 | 32.575 |
| U003b | 0 | 0 | - 0.352 | 32.572 |
| U004c | 0 | 0 | - 0.320 | 32.576 |
| U005b | 11 | 0 | - 0.364 | 32.295 |
| U006a | 2 | 0 | - 0.334 | 32.332 |
| U007c | 0 | 0 | - 0.325 | 32.309 |
| U008b | 4 | 0 | - 0.511 | 32.158 |
| U009b | 1 | 0 | - 0.310 | 32.292 |
| U010b | 1 | 1 | - 0.324 | 32.194 |
| U011b | 2 | 1 | - 0.248 | 32.068 |
| U012a | 10 | 0 | - 0.273 | 32.027 |
| U013b | 1 | 1 | - 0.301 | 32.035 |
| U014b | 2 | 0 | 0.004 | 32.432 |
| U015b | 2 | 0 | 0.015 | 32.388 |
| U016b | 4 | 0 | 0.016 | 32.381 |
| U017b | 0 | 0 | - 0.009 | 32.432 |
| U019b | 0 | 0 | - 0.915 | 31.767 |
| U020a | 11 | 0 | - 0.939 | 31.763 |
| U021a | 10 | 0 | - 0.655 | 31.797 |
| U022b | 0 | 0 | - 0.348 | 31.880 |
| U023a | 11 | 0 | 0.015 | 32.767 |
| U024b | 10 | 1 | - 0.042 | 32.764 |
| U025b | 7 | 1 | 0.002 | 32.901 |
| U026b | 1 | 0 | - 0.110 | 32.764 |
| U027a | 3 | 2 | - 0.100 | 32.653 |
| U028b | 11 | 0 | - 0.086 | 32.652 |
| U029b | 8 | 0 | 0.141 | 33.602 |
| U030b | 11 | 2 | 0.112 | 33.602 |

| U034b | 2 | 0 | 0.003 | 33.659 |
|---|---|---|---|---|
| U035b | 9 | 1 | 0.156 | 33.566 |
| U036b | 1 | 0 | 0.173 | 33.562 |
| U037c | 11 | 1 | 0.318 | 33.627 |
| U038a | 0 | 0 | 0.263 | 33.985 |
| U038b | 3 | 1 | 0.263 | 33.985 |
| U039a | 4 | 2 | 0.253 | 33.989 |
| U040b | 4 | 2 | 0.241 | 33.992 |
| U041a | 0 | 0 | 0.535 | 33.891 |
| U043c | 0 | 0 | 0.476 | 33.281 |
| U044b | 4 | 0 | 0.438 | 33.241 |
| U045b | 0 | 0 | 0.290 | 32.655 |
| U046b | 11 | 0 | 0.173 | 33.184 |
| U047b | 4 | 0 | 0.186 | 33.215 |
| U048b | 4 | 0 | 0.198 | 33.265 |
| U049b | 4 | 0 | 0.234 | 33.243 |
| U050c | 4 | 0 | 0.247 | 33.219 |
| U051b | 0 | 0 | 0.270 | 33.206 |
| U052b | 3 | 0 | 0.271 | 33.153 |
| U053b | 8 | 2 | 0.240 | 33.137 |
| U055b | 7 | 0 | - 0.092 | 32.684 |
| Bugoto c | 20 | 1 | 0.319 | 33.628 |
| Bukoba c | 20 | 1 | 0.312 | 33.492 |
| Lwanika c | 20 | 2 | 0.351 | 33.446 |

Note: The (a), (b) and (c) indicates whether the collection site was either (a) marshlands, (b) the lake edge or from (c) another ecosystem such as a canal, paddy, pond or a hybrid environment (e.g. lake shore/marshland). Sites that had samples genotyped using the 16S and COI gene fragments are highlighted.

Supplementary Table 5.2. The 16S and COI haplotype frequencies of the sequenced *B. choanomphala* populations.

| Site | 16S | | COI | |
|---|---|---|---|---|
| | Haplotype | Frequency | Haplotype | Frequency |
| K001a | 69 | 5 | 4 | 4 |
| | 70 | 3 | 5 | 4 |
| | 71 | 2 | 6 | 1 |
| | 147 | 1 | 104 | 1 |
| K002a | 68 | 2 | 5 | 5 |
| | 70 | 1 | 7 | 1 |
| | 72 | 1 | 8 | 1 |
| | 73 | 1 | 10 | 1 |
| | 75 | 1 | 13 | 1 |
| | 85 | 1 | 105 | 1 |
| | 148 | 1 | | |
| | 149 | 1 | | |
| | 150 | 1 | | |
| K006a | 79 | 6 | 9 | 3 |
| | 80 | 3 | 20 | 2 |
| | | | 113 | 4 |

| | | | | |
|---|---|---|---|---|
| | | | 114 | 1 |
| K013b | 51 | 2 | 1 | 2 |
| | 78 | 4 | 5 | 1 |
| | 79 | 1 | 12 | 2 |
| | 157 | 2 | 15 | 2 |
| | 175 | 1 | 17 | 1 |
| | 176 | 1 | 110 | 2 |
| | 177 | 1 | 111 | 1 |
| K020b | 69 | 5 | 5 | 4 |
| | 70 | 1 | 8 | 3 |
| | 79 | 1 | 18 | 1 |
| | 158 | 1 | 19 | 1 |
| | 159 | 2 | 61 | 1 |
| | 160 | 1 | 112 | 1 |
| K029b | 69 | 6 | 5 | 3 |
| | 79 | 1 | 7 | 2 |
| | 85 | 1 | 8 | 6 |
| | 146 | 1 | 103 | 1 |
| T001c | 8 | 1 | 1 | 7 |
| | 9 | 4 | 7 | 1 |
| | 67 | 1 | 24 | 1 |
| | 89 | 1 | 82 | 1 |
| | 90 | 1 | 83 | 1 |
| | 91 | 1 | | |
| | 92 | 1 | | |
| T011b | 9 | 1 | 21 | 2 |
| | 29 | 1 | 22 | 1 |
| | 30 | 1 | 23 | 2 |
| | 51 | 1 | 84 | 4 |
| | 93 | 3 | 85 | 1 |
| | 94 | 1 | 86 | 1 |
| | 95 | 1 | | |
| | 96 | 1 | | |
| T016a | 9 | 3 | 1 | 4 |
| | 10 | 4 | 23 | 3 |
| | 11 | 4 | 24 | 4 |
| T026a | 9 | 2 | 1 | 1 |
| | 11 | 3 | 24 | 8 |
| | 12 | 1 | 25 | 1 |
| | 19 | 2 | 87 | 1 |
| | 65 | 1 | | |
| | 97 | 2 | | |
| T027b | 24 | 1 | 30 | 2 |
| | 26 | 1 | 31 | 1 |
| | 27 | 1 | 88 | 3 |
| | 61 | 1 | 89 | 2 |
| | 99 | 1 | 90 | 1 |
| | 100 | 2 | 91 | 1 |
| | 101 | 1 | | |
| | 102 | 2 | | |

| | | | | |
|---|---|---|---|---|
| T033a | 25 | 1 | 32 | 5 |
| | 28 | 1 | 33 | 3 |
| | 62 | 5 | 92 | 1 |
| | 84 | 3 | | |
| T036a | 31 | 5 | 5 | 2 |
| | 134 | 1 | 29 | 5 |
| | 135 | 1 | 94 | 1 |
| | 136 | 1 | 95 | 1 |
| | 137 | 1 | 96 | 1 |
| | 138 | 1 | | |
| | 139 | 1 | | |
| T040c | 9 | 2 | 1 | 3 |
| | 23 | 1 | 38 | 1 |
| | 57 | 1 | 97 | 1 |
| | 140 | 1 | 98 | 1 |
| | 141 | 1 | 99 | 1 |
| | 142 | 1 | 100 | 1 |
| | 143 | 1 | 101 | 1 |
| | 144 | 1 | 123 | 1 |
| | 174 | 1 | | |
| T064a | 51 | 1 | 1 | 1 |
| | 65 | 2 | 3 | 2 |
| | 66 | 3 | 9 | 4 |
| | 67 | 2 | 98 | 1 |
| | 68 | 1 | 102 | 1 |
| | 145 | 1 | 124 | 1 |
| U005b | 4 | 2 | 1 | 1 |
| | 5 | 1 | 32 | 1 |
| | 33 | 1 | 39 | 1 |
| | 103 | 1 | 40 | 5 |
| | 104 | 1 | 41 | 1 |
| | 105 | 1 | 68 | 1 |
| | 106 | 1 | 69 | 1 |
| | 107 | 1 | | |
| | 108 | 1 | | |
| | 109 | 1 | | |
| U012a | 6 | 1 | 1 | 1 |
| | 7 | 1 | 42 | 6 |
| | 9 | 1 | 43 | 1 |
| | 34 | 1 | 70 | 2 |
| | 35 | 1 | | |
| | 110 | 2 | | |
| | 111 | 3 | | |
| U020a | 21 | 1 | 44 | 1 |
| | 36 | 1 | 45 | 9 |
| | 112 | 6 | 71 | 1 |
| | 113 | 1 | | |
| U021a | 19 | 8 | 46 | 9 |
| | 20 | 1 | 72 | 1 |
| | 83 | 1 | | |

| U023a | 17 | 2 | 47 | 1 |
|---|---|---|---|---|
| | 18 | 2 | 48 | 2 |
| | 37 | 1 | 49 | 1 |
| | 38 | 1 | 50 | 1 |
| | 161 | 1 | 52 | 1 |
| | 162 | 1 | 115 | 1 |
| | 163 | 1 | 116 | 1 |
| | 164 | 1 | 125 | 1 |
| | 179 | 1 | 126 | 1 |
| U028b | 40 | 1 | 54 | 1 |
| | 41 | 1 | 55 | 1 |
| | 53 | 1 | 56 | 3 |
| | 114 | 1 | 73 | 1 |
| | 115 | 1 | 74 | 1 |
| | 116 | 1 | 75 | 1 |
| | 117 | 1 | 76 | 2 |
| | 118 | 1 | 77 | 1 |
| | 119 | 1 | | |
| | 120 | 1 | | |
| U030b | 42 | 1 | 32 | 1 |
| | 43 | 1 | 57 | 2 |
| | 44 | 1 | 58 | 1 |
| | 46 | 2 | 59 | 2 |
| | 169 | 1 | 119 | 1 |
| | 170 | 1 | 120 | 1 |
| | 171 | 1 | 121 | 1 |
| | 172 | 1 | 122 | 1 |
| | 173 | 1 | 127 | 1 |
| | 180 | 1 | | |
| U037c | 2 | 1 | 8 | 1 |
| | 47 | 1 | 27 | 1 |
| | 48 | 1 | 60 | 1 |
| | 54 | 1 | 61 | 1 |
| | 55 | 1 | 62 | 2 |
| | 56 | 1 | 63 | 1 |
| | 58 | 1 | 64 | 2 |
| | 60 | 2 | 65 | 1 |
| | 121 | 1 | 78 | 1 |
| U046b | 3 | 1 | 32 | 2 |
| | 64 | 1 | 66 | 1 |
| | 86 | 1 | 67 | 5 |
| | 87 | 1 | 80 | 2 |
| | 88 | 1 | 81 | 1 |
| | 124 | 1 | | |
| | 128 | 1 | | |
| | 129 | 1 | | |
| | 130 | 1 | | |
| Bugoto c | 48 | 6 | 27 | 6 |
| | 60 | 4 | 61 | 1 |
| | 126 | 1 | 62 | 7 |

| | | | | |
|---|---|---|---|---|
| | 127 | 2 | 64 | 4 |
| | 160 | 1 | 128 | 2 |
| | 185 | 1 | | |
| | 187 | 1 | | |
| | 202 | 1 | | |
| | 203 | 1 | | |
| | 204 | 1 | | |
| | 205 | 1 | | |
| Bukoba c | 42 | 1 | 27 | 4 |
| | 48 | 4 | 61 | 2 |
| | 60 | 2 | 62 | 5 |
| | 160 | 1 | 63 | 1 |
| | 183 | 1 | 64 | 3 |
| | 184 | 2 | 128 | 1 |
| | 185 | 1 | 129 | 1 |
| | 186 | 1 | 130 | 1 |
| | 188 | 1 | 131 | 1 |
| | 189 | 1 | 132 | 1 |
| | 190 | 1 | | |
| | 191 | 1 | | |
| | 192 | 1 | | |
| | 193 | 1 | | |
| | 194 | 1 | | |
| Lwanika c | 48 | 1 | 8 | 3 |
| | 60 | 1 | 27 | 8 |
| | 65 | 1 | 46 | 1 |
| | 69 | 2 | 62 | 1 |
| | 127 | 1 | 63 | 2 |
| | 183 | 4 | 64 | 2 |
| | 184 | 1 | 79 | 1 |
| | 185 | 1 | 132 | 1 |
| | 186 | 1 | 133 | 1 |
| | 195 | 1 | | |
| | 196 | 1 | | |
| | 197 | 1 | | |
| | 198 | 1 | | |
| | 199 | 1 | | |
| | 200 | 1 | | |
| | 201 | 1 | | |

Note: sites with *S. mansoni* infection present are highlighted.

Supplementary Table 5.3. The median (IQR) values of the abiotic factors recorded across the Kenyan ($n$=35), Tanzanian ($n$=82) and Ugandan ($n$=53) sites of Lake Victoria.

| | Kenya | | Tanzania | | Uganda | |
|---|---|---|---|---|---|---|
| Temperature (°C) | 28.2 | (27.2 - 29.3) | 26.6 | (25 - 28) | 27.7 | (26.1 - 29) |
| Conductivity (µS) | 191 | (129 - 480) | 144 | (110 - 348) | 107 | (93 - 120) |
| pH | 8.2 | (7.9 - 8.7) | 7.9 | (7.4 - 8.7) | 8.3 | (7.4 - 9.2) |
| Total Dissolved Solids | 105 | (79 - 190) | 90 | (67 - 217) | 63 | (58 - 71) |
| Salinity | 0.4 | (0.4 - 0.4) | 0.4 | (0.4 - 0.4) | 0.4 | (0.3 - 0.4) |
| Fluoride ($F^-$) | 0.5 | (0.3 - 0.7) | 0.4 | (0.3 - 0.5) | 0.2 | (0.2 - 0.3) |
| Chloride ($Cl^-$) | 9.4 | (6.7 - 25) | 8.5 | (6 - 16) | 9.3 | (5.6 - 13.9) |
| Nitrate ($NO_3^-$) | 0.1 | (0 - 0.4) | 0 | (0 - 0.2) | 0 | (0 - 0.1) |
| Phosphate ($PO_4^{3-}$) | 0.3 | (0.1 - 0.6) | 0.2 | (0 - 0.7) | 0 | (0 - 0.2) |
| Sulphate ($SO_4^{3-}$) | 3.1 | (1.7 - 6.6) | 1.3 | (0.6 - 4.2) | 0.5 | (0.4 - 0.9) |
| Sodium ($Na^+$) | 16.3 | (12.9 - 30) | 13.3 | (10.4 - 21.3) | 11 | (10.5 - 11.5) |
| Magnesium ($Mg_2^+$) | 2.9 | (2.4 - 6.5) | 2.7 | (2.5 - 5.6) | 2.9 | (2.8 - 3.2) |
| Calcium ($Ca_2^+$) | 8.3 | (6.2 - 22.4) | 10 | (5.6 - 28.7) | 7.2 | (6.8 - 7.8) |
| Potassium ($K^+$) | 7.7 | (5.6 - 15.1) | 6.7 | (4.9 -12.5) | 11.1 | (6.4 - 19.6) |

# Chapter 6 Nematodes and Trematodes Associated with Terrestrial Gastropods in Nottingham, England[1]

[1]*This chapter was published as "Andrus, P. S., Rae, R., & Wade, C. M. (2022). Nematodes and trematodes associated with terrestrial gastropods in Nottingham, England. Journal of Helminthology, 96, e81."*


## Abstract:

A parasitological survey of terrestrial slugs and snails was conducted at popular dog walking locations across the city of Nottingham, with the intensions of finding gastropods infected with parasites of medical (or veterinary) importance such as lungworms (metastrongyloids) and trematodes. A total of 800 gastropods were collected from 16 sites over a 225km$^2$ area. The extracted nematodes and trematodes were identified by molecular barcoding. Of the 800 gastropods collected, 227 were infected (172 had nematode infections, 37 had trematode infections and 18 had both nematode and trematode infections). Of the nematode infected gastropods genotyped, seven species were identified, *Agfa flexilis*, *Angiostoma gandavense*, *Angiostoma margaretae*, *Cosmocerca longicauda*, *Phasmarhabditis hermaphrodita*, *Phasmarhabditis neopapillosa* and an unknown Cosmocercidae species. Of the trematode infected gastropods genotyped, four species were identified, *Brachylaima arcuate*, *Brachylaima fuscata*, *Brachylaima mesostoma* and an unknown Plagiorchioidea species. No lungworm species were found within the city of Nottingham. To our knowledge, this study represents the first survey of gastropod-associated nematodes and trematodes in the East midlands of the United Kingdom.


## 6.1 Introduction:

Slugs and snails (Class: Gastropoda) comprise approximately 35,000 extant species and can host a diverse range of metazoan parasites (and parasitoids) such as cestodes, trematodes, nematodes, insects and acarids (Barker & Efford, 2004; Chapman, 2009). There are approximately 25,000 extant species of nematodes, of which 3500 are parasites of invertebrates (Grewal et al., 2003). Of these, 50 metastrongyloid (lungworms) species are of medical or veterinary importance, with notable genera being *Aelurostrongylus*, *Angiostrongylus*, *Crenosoma*, *Elaphostrongylus*, *Muellerius*, *Neostrongylus*, *Oslerus*, *Prostrongylus* and *Troglostrongylus* (Alicata, 1965; Skorping & Halvorsen, 1980; Campbell and Little, 1988; Diez-Baños et al., 1989; Schjetlein & Skorping, 1995; Majoros et al., 2010; Panayotova-Pencheva, 2011; Kim et al., 2014; Patel et al., 2014; Conboy, 2015; Helm et al., 2015; Aziz et al., 2016; Hadi, 2018; Hicklenton & Betson, 2019; Penagos-Tabares et al., 2020). Nematodes have evolved diverse relationships with gastropods, with some species using them as an intermediate host (e.g. juveniles of lungworm species) while others (Rhabditidae, Mermithidae and Ascarididae) parasitise gastropods and use them as their definitive host; or for other means such as necromeny or transportation (paratenic; Grewal et al., 2003; Ivanova et al., 2019).

Digenetic trematodes comprise approximately 40,000 extant species, with more than 18,000 described species (Cribb et al., 2001; Kostadinova & Pérez-del-Olmo, 2014). Unlike nematodes, digenetic trematodes use invertebrates exclusively as an intermediate host, with a vertebrate (typically a fish, mammal, or bird) being used as their definitive host (Barker & Efford, 2004). Notable genera of medical or veterinary importance are *Clonorchis*,

*Fasciola*, *Fasciolopsis*, *Gastrodiscoides*, *Heterophyes*, *Metagonimus*, *Opisthorchis*, *Paragonimus* and *Schistosoma* (Doughty, 1996; Kostadinova & Pérez-del-Olmo, 2014). Trematode species which infect terrestrial gastropods use them in order to infect bird, mammal, or reptile definitive hosts which prey on gastropods (Morley & Lewis, 2008). Most species specialise in infecting one type of definitive host, but some species can infect multiple hosts (Butcher & Grove, 2005). The lifecycle of these trematodes first involves a gastropod host being infected through the ingestion of eggs (excreted by an infected definitive host). After ingestion, it takes one to three months for asexual sporocysts to produce cercariae within the first intermediate gastropod host (Butcher & Grove, 2003). Gastropods can act as both the first and second intermediate host, as infected snails (first intermediate) shed cercariae in their mucus which can infect other gastropods through bodily contact (or themselves making them a first and second intermediate host simultaneously; Butcher & Grove, 2005). The successful cercariae develop into mature metacercariae after four months and can survive up to another four months within the gastropod host. The transmission cycle is completed when a bird, mammal, or reptile (definitive host) ingests the secondary intermediate gastropod host (Morley & Lewis, 2008).

The current understanding of nematodes and trematodes associated with terrestrial gastropods in Europe is based on parasitological surveys conducted in Austria (Penagos-Tabares et al., 2020), Belgium (Singh et al., 2020), Bulgaria (and Crimea; Ivanova et al., 2013), the Czech Republic (Heneberg et al., 2016), Denmark (Taubert et al., 2009), England (Morley & Lewis, 2008; Patel et al., 2014; Hicklenton & Betson, 2019), France (Nguyen et al., 2017), Germany (Ross et al., 2016; Lange et al., 2018; Gérard et al., 2020), Hungary (Majoros et al., 2010), the Netherlands and Norway (Antzée-Hyllseth et al., 2020), Poland (Filipiak et al., 2020), Italy (Ivanova et al., 2019), Slovenia (Laznik et al., 2010), Scotland (Helm et al., 2015), Spain (Foronda et al., 2010; Jefferies et al., 2010; Paredes-Esquivel et al., 2019; Martín-Carrillo et al., 2021) and Wales (Ross et al., 2010a/b; Aziz et al., 2016). The majority of these studies found no medically important nematode or trematode species, with primarily free-living, gastropod-specific and veterinary important species being reported. Four common lungworm genera (Metastrongyloidea) of medical/veterinary importance were present in Europe (*Angiostrongylus*, *Crenosoma*, *Aelurostrongylus* and *Troglostrongylus*) with *Angiostrongylus* (*An.*) *cantonensis* the only medically important species reported.

*Angiostrongylus cantonensis* is a parasite endemic to Asia, the Caribbean and Pacific Islands. In Europe it has been found infecting black rats (*Rattus rattus*) in the Canary and Balearic Islands and the Algerian hedgehog (*Atelerix algirus*) in mainland Spain (Foronda et al., 2010; Paredes-Esquivel et al., 2019; Martín-Carrillo et al., 2021). Furthermore, Nguyen et al. (2017) reported the first autochthonous human case of *An. cantonensis* infection in France. In addition to the metastrongyloids, seven additional gastropod-related nematode families were reported in Europe, the Agfidae, Alloionematidae, Angiostomatidae, Cosmocercidae, Diplogasteridae, Mermithidae and Rhabditidae. The most common genera of trematodes found were *Brachylaima*, *Eurytrema*, *Michajlovia*, *Urogonimus* and *Urotocus*. Certain species of *Brachylaima* (Brachylaimiasis) and *Eurytrema* (Eurytrematosis) have been found to cause infection within humans in Australia and Brazil, respectively (Schwertz et al., 2015; Gracenea

& Gállego, 2017) though there have as yet been no reports of human infection in Europe. Trematodes associated with terrestrial gastropods in Europe have not been as well studied as nematodes, most probably due to the majority of these species of medical or veterinary importance being associated with aquatic snail species.

Lungworm nematode infections have been extensively studied in Europe (Taubert et al., 2009; Patel et al., 2014; Helm et al., 2015; Taylor, 2015; Aziz et al., 2016; Helm & Morgan, 2017; Lange et al., 2018; Elsheikha et al., 2019; Hicklenton & Betson, 2019; Fuehrer et al., 2020; Penagos-Tabares et al., 2020). Lungworm infections are fatal to companion animals due to the severe respiratory disease and bleeding disorders caused by the parasite (Taubert et al., 2009). *Angiostrongylus* (*An.*) *vasorum* and *Crenosoma vulpis* are widespread across the United Kingdom, with domesticated dogs and red foxes (*Vulpes vulpes*) acting as their definitive hosts (Helm & Morgan, 2017). Geography is one of the main risk factors for *An. vasorum* infections in dogs, with the most endemic areas of the United Kingdom being Southern England and Southern Wales (Patel et al., 2014; Helm & Morgan, 2017; Hicklenton & Betson, 2019) though *An. vasorum* in the United Kingdom is spreading northwards, with the parasite already established in Northern England and Scotland (Helm et al., 2015; Aziz et al., 2016). Reasons for the spread of *An. vasorum* include a warmer climate which favours the parasites' development and the urbanisation of wild red fox populations acting as a reservoir of infection, with an estimated one in five infected (Taylor, 2015; Helm & Morgan, 2017). *Crenosoma vulpis* transmission is similar to *An. vasorum* but is more commonly reported in wild canid species than domesticated dogs (Lange et al., 2018). Similarly, *Aelurostrongylus* (*Ae.*) *abstrusus* is a globally distributed lungworm species that infects wild and domesticated cat species, with a prevalence of 1.7% in United Kingdom house cats (Helm & Morgan, 2017; Elsheikha et al., 2019). Lungworm infections in domesticated cats and dogs are thought to be underreported as some infections can be asymptomatic and milder cases are commonly misdiagnosed as other disorders such as hypersensitivity (Wright, 2009; Penagos-Tabares et al., 2018; Pohly et al., 2022).

The primary aim of this study was to investigate which species of terrestrial gastropods are commonly found at dog walking sites in the city of Nottingham and the county of Nottinghamshire, to determine which nematode and trematode species are associated with these gastropods and to determine prevalence of infection. The secondary aim was to investigate whether lungworm nematode species that cause veterinary disease are found at popular dog walking sites across the city of Nottingham and the county of Nottinghamshire.

6.2 Materials and Methods:

6.2.1 Collection sites and gastropod identification:

Slugs and snails were collected from 16 sites across Nottingham from June to November 2020 and June to November 2021. All sites were popular dog walking locations and included recreational grounds, country parks, public gardens, and nature reserves (Table 6.1; Figure 6.1). A total of 800 gastropods were collected by hand with 50 specimens collected from each site and with a maximum of ten individuals per species being taken. Specimens were identified morphologically using a Terrestrial Mollusc Key (https://idtools.org/id/mollusc/key.php; White-McLean, 2011) and the 'Slugs of Britain and Ireland' as an illustrated guide (Rowson et al., 2014).

Figure 6.1. Map of collection sites (*n*=16) across the city of Nottingham and surrounding areas (Google, 2022).

Table 6.1. Collection sites surveyed across the city of Nottingham and surrounding areas.

| | Collection site | Code | Search area ($Km^2$) | Coordinates |
|---|---|---|---|---|
| 1 | Basford (Nottingham) | BAS | 15,288 | 52.977957, -1.180909 |
| 2 | Bestwood Country Park (Nottinghamshire) | MILL | 116,987 | 53.025337, -1.184712 |
| 3 | Forest Fields (Nottingham) | FOR | 5,132 | 52.96401, -1.159410 |
| 4 | University Park Campus (Nottingham) | UNI | 20,506 | 52.938199, -1.12508 |
| 5 | Beeston (Nottinghamshire) | BEE | 1,583 | 52.922972, -1.214944 |
| 6 | Toton (Nottinghamshire) | TOT | 6,469 | 52.915726, -1.264259 |
| 7 | Attenborough Nature Reserve (Nottinghamshire) | ATEN | 33,371 | 52.909117, -1.221000 |
| 8 | Kimberley (Nottinghamshire) | KIM | 5,095 | 52.997686, -1.268583 |
| 9 | Clifton South (Nottingham) | C-SOU | 11,135 | 52.899179, -1.185660 |
| 10 | Iremongers Pond (Nottingham) | POND | 17,958 | 52.936184, -1.152757 |
| 11 | Woodthorpe Grange Park (Nottingham) | GRAN | 143, 670 | 52.982888, -1.135721 |
| 12 | Arnot Hill Park (Nottingham) | ARNOT | 45,220 | 52.997488, -1.133526 |
| 13 | Edwalton (Nottinghamshire) | EDW | 8,181 | 52.917332, -1.124678 |
| 14 | Gamston (Nottinghamshire) | GAM | 24,538 | 52.928595, -1.108470 |
| 15 | Carlton (Nottinghamshire) | CARL | 37,525 | 52.965511, -1.103516 |
| 16 | Colwick (Nottinghamshire) | COLW | 15,920 | 52.952945, -1.091540 |

### 6.2.2 Gastropod Dissection:

Specimens were dissected into four equal pieces within 24 hours of collection and placed into a 50ml falcon tube containing Ash’s digestion solution (0.7% pepsin in 0.5% HCl) for four to eight hours (Ash, 1970). The solution was then placed into a 9cm Petri dish and examined under a dissection microscope for the presence of nematodes, or the metacercariae stage of trematodes. Nematodes were categorised as either juvenile or adult worms. When found, nematodes and metacercariae were individually picked and placed into 0.2ml tubes containing 70% ethanol (adult worms were separated from juveniles) and stored at -20°C.

### 6.2.3 DNA extraction, PCR amplification and Sequencing:

DNA extractions were done on single nematodes or trematodes using a modified CTAB extraction method (Goodacre & Wade, 2001). Extracted samples were resuspended in 100µl of 10mM TRIS-HCl, pH 8.0) buffer. A list of extracted and genotyped samples for each site can be found in Supplementary Tables 6.1 and 6.2. Promega GoTaq® G2 Master Mix buffer was used for all PCR reactions: 1µl of DNA template was added to 24µl of 1X Master Mix buffer (1U TAQ, 0.2µM primers, 200µM each dNTP, 1.5mM $MgCl_2$). The nematode DNA samples were identified using the region of the ribosomal RNA spanning the 18S-ITS1-5.8S-ITS2, which was amplified using the universal nematode primer set developed by Nadler et al. (2000; Table 2.1). The trematode DNA samples were identified using the 18S rRNA gene, which was amplified using the universal trematode primer set developed by Kim et al. (2019; Table 2.1). The PCR conditions used were an initial 2 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 secs at 50°C and 2 mins at 72°C, and finally 10 mins at 72°C. PCR products were run and visualised on an ethidium bromide infused 1.5% agarose gel. PCR products were purified and sequenced using Macrogen's Eco-Seq service. Problematic sequences were re-amplified and sequenced using a higher annealing temperature of 60°C to try and eliminate fungal contaminates amplifying instead of the parasite DNA.

### 6.2.4 Parasite identification:

Parasite sequences were first grouped together based on similarity, with sequences that were 99% identical being placed together. Next, the NCBI 'MOLE-BLAST Neighbor Search Tool' was used to find the closest matching reference sequences on the GenBank database (Altschul et al., 1990; Benson et al., 2013). This tool creates an alignment and a neighbor-joining tree to show the relationship the query sequence has to the reference sequences in the GenBank non-redundant proteins database. Next, a secondary analysis was performed by placing our sequences within an alignment with all of the relevant closest matching GenBank reference sequences. This allowed us to create a maximum likelihood tree to see relationships between our sequences and the references taken from GenBank. The sequences were aligned in Seaview v5 (Gouy et al., 2021) using the Muscle algorithm, with conserved sites being selected using the Gblocks program (Castresana et al., 2000). The phylogenetic trees were constructed using the Maximum Likelihood method, using a General Time Reversible model incorporating gamma correction (GTR+Γ) in PhyML v3.1 (Guindon et al., 2010), with bootstrap analysis undertaken using 1000 replicates.

### 6.2.5 GenBank Accession Numbers:

The DNA sequences generated in this study are available in GenBank accession numbers OP626191 – OP626254. Both the GenBank references used, and the DNA sequences generated in this study are available in Supplementary Table 6.3.

## 6.3 Results:

### 6.3.1 Infection Prevalence:

Of the 800 gastropods collected, 581 were slugs (Agriolimacidae, Arionidae, Boettgerillidae, Limacidae and Milacidae) and 219 were snails (Discidae, Helicidae, Hygromiidae and Oxychilidae). The most common slug species found were *Deroceras invadens* (15%), *Tandonia budapestensis* (13%), *Deroceras reticulatum* (13%), *Arion hortensis* (10%), *Ambigolimax valentianus* (8%), *Limacus maculatus* (7%), *Arion vulgaris* (7%), *Tandonia sowerbyi* (6%), *Arion ater* (6%), *Arion subfuscus* (4%), *Arion rufus* (3%), *Arion silvaticus* (2%), *Limacus flavus* (2%), *Ambigolimax nyctelius* (1%), *Limax maximus* (1%), *Milax gagates* (<1%) and *Boettgerilla pallens* (<1%). The most common snail species found were *Cepaea nemoralis* (28%), *Cornu aspersum* (25%), *Cepaea hortensis* (20%), *Trochulus striolatus* (10%), *Oxychilus alliarius* (7%), *Monacha cantiana* (5%), *Discus rontundas* (3%), *Trochulus hispidus* (1%) and *Arianta arbustorum* (1%).

Overall, 227 specimens were infected (28%) with nematodes or trematodes (or both). Of those, 163 were slugs (28%) and 64 were snails (29%; Table 6.2; Figure 6.2). The only gastropod species without any recorded infections were *A. arbustorum, B. pallens, D. rotundatus and T. hispidus*. Nematodes were found in all other gastropods, with *T. budapestensis*, *D. invadens*, *C. aspersum*, *D. reticulatum*, *A. ater* and *C. nemoralis* accounting for over half of all infections. A total of 533 nematodes were recorded from 190 infected specimens (145 slugs and 45 snails). Of those, only 12 juvenile nematodes were found in 12 hosts (8 slugs and 4 snails; Table 6.2). Trematodes were rarer than nematodes, with *A. ater*, *A. hortensis*, *A. nyctelius*, *A. rufus*, *A. silvaticus*, *A. subfuscus*, *A. vulgaris*, *L. flavus*, *L. maximus* and *O. alliarius* having no recorded trematode infections. A total of 242 trematodes were recorded from 55 specimens (30 slugs and 25 snails; Table 6.2). Lastly, co-infections of both nematodes and trematodes were even rarer, with only 18 specimens being recorded as co-infected (13 slugs and 5 snails; Table 6.2).

Table 6.2. Gastropods collected and details of number of nematode and trematode (metacercaria) infections.

| Family | Species | No. | Infected | Nematode | Trematode | Both |
|---|---|---|---|---|---|---|
| Agriolimacidae | *Deroceras invadens* | 90 | 25 | 15 | 8 | 2 |
| | *Deroceras reticulatum* | 75 | 19 | 13 | 3 | 3 |
| Arionidae | *Arion ater* | 33 | 13 | 13 | 0 | 0 |
| | *Arion hortensis* | 59 | 11 | 11 | 0 | 0 |
| | *Arion rufus* | 20 | 5 | 5 | 0 | 0 |
| | *Arion silvaticus* | 14 | 2 | 2 | 0 | 0 |
| | *Arion subfuscus* | 25 | 6 | 6 | 0 | 0 |
| | *Arion vulgaris* | 42 | 8 | 8 | 0 | 0 |
| Boettgerillidae | *Boettgerilla pallens* | 2 | 0 | 0 | 0 | 0 |
| Discidae | *Discus rotundatus* | 6 | 0 | 0 | 0 | 0 |
| Helicidae | *Arianta arbustorum* | 2 | 0 | 0 | 0 | 0 |
| | *Cepaea hortensis* | 44 | 7 | 6 | 1 | 0 |
| | *Cepaea nemoralis* | 62 | 14 | 9 | 4 | 1 |
| | *Cornu aspersum* | 54 | 24 | 14 | 7 | 3 |
| Hygromiidae | *Trochulus hispidus* | 3 | 0 | 0 | 0 | 0 |
| | *Trochulus striolatus* | 22 | 7 | 4 | 3 | 0 |
| | *Monacha cantiana* | 10 | 7 | 1 | 5 | 1 |
| Limacidae | *Ambigolimax nyctelius* | 5 | 1 | 1 | 0 | 0 |
| | *Ambigolimax valentianus* | 47 | 18 | 8 | 5 | 5 |
| | *Limacus flavus* | 10 | 3 | 3 | 0 | 0 |
| | *Limacus maculatus* | 42 | 9 | 8 | 0 | 1 |
| | *Limax maximus* | 3 | 2 | 2 | 0 | 0 |
| Milacidae | *Milax gagates* | 2 | 1 | 0 | 0 | 1 |
| | *Tandonia budapestensis* | 78 | 31 | 30 | 1 | 0 |
| | *Tandonia sowerbyi* | 34 | 8 | 7 | 0 | 1 |
| Oxychilidae | *Oxychilus alliarius* | 16 | 6 | 6 | 0 | 0 |
| Total | | 800 | 227 | 172 | 37 | 18 |

Note: Gastropod species with zero infections are greyed out. ‘Both’ means a co-infection of nematodes and trematodes within a single specimen.

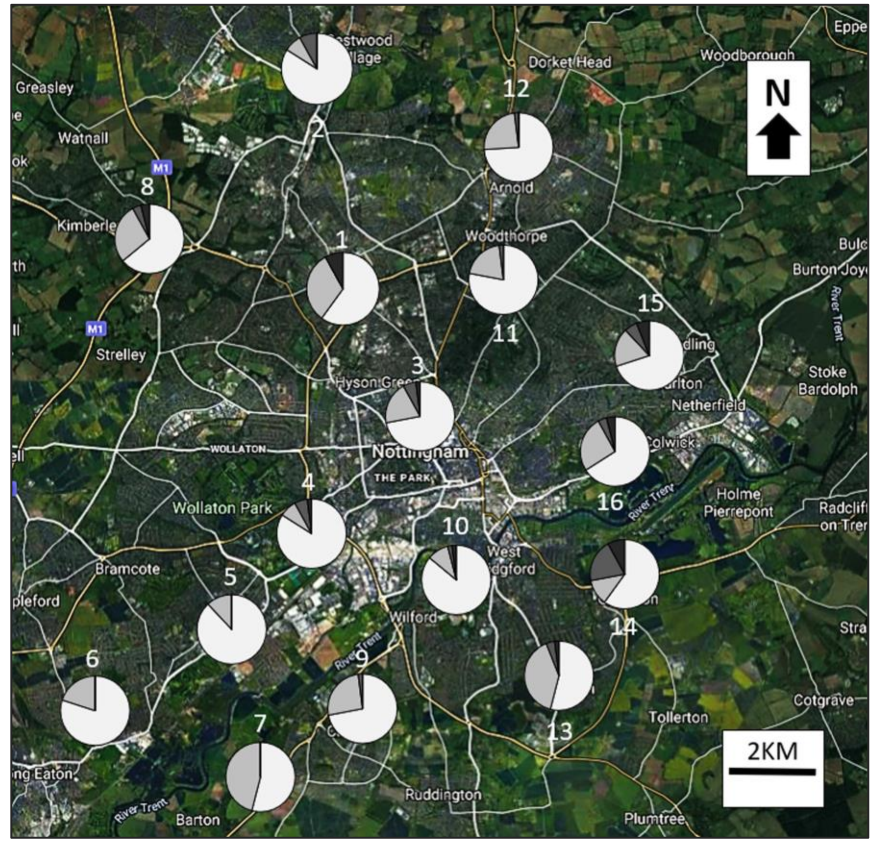



Figure 6.2. Map of collection sites (*n*=16) across the city of Nottingham and the surrounding areas showing infection prevalence at each collection site. White = uninfected, grey = nematode infection, dark grey = trematode infection and black = nematode/trematode co-infection (Google, 2022).

Of the 16 sites surveyed, infection was found at all of them (Table 6.3). The highest recorded prevalence of infection was 46% at site 7 (The Attenborough Nature Reserve) and site 13 (Edwalton). The lowest recorded prevalence of infection was 12% at site 5 (Beeston). Nematode infections were found at all 16 sites, with trematode infections found at 13 of the 16 sites (Figure 6.2). Specimens infected with both nematodes and trematodes were found at 9 of the 16 sites.

Table 6.3. Infection prevalence of collected gastropods (*n*=50) at each site.

| | Collection site | Code | Infection | Nematode | Trematode |
|---|---|---|---|---|---|
| 1 | Basford | BAS | 40% | 40% | 8% |
| 2 | Bestwood Country Park | MILL | 16% | 8% | 8% |
| 3 | Forest Fields | FOR | 28% | 22% | 8% |
| 4 | University Park Campus | UNI | 16% | 10% | 8% |
| 5 | Beeston | BEE | 12% | 12% | 0% |
| 6 | Toton | TOT | 20% | 20% | 0% |
| 7 | Attenborough Nature Reserve | ATEN | 46% | 46% | 0% |
| 8 | Kimberley | KIM | 36% | 32% | 8% |
| 9 | Clifton South | C-SOU | 28% | 26% | 2% |
| 10 | Iremongers Pond | POND | 14% | 12% | 4% |
| 11 | Woodthorpe Grange Park | GRAN | 22% | 20% | 2% |
| 12 | Arnot Hill Park | ARNOT | 26% | 24% | 2% |
| 13 | Edwalton | EDW | 46% | 42% | 6% |
| 14 | Gamston | GAM | 40% | 20% | 28% |
| 15 | Carlton | CARL | 30% | 24% | 12% |
| 16 | Colwick | COLW | 34% | 30% | 8% |

6.3.2 Nematode and trematode identifications:

A total of 35 (23 adults, 12 juveniles) nematodes (Supplementary Table 6.1) and 29 trematodes (Supplementary Table 6.2) were genotyped. All sequences were grouped together based on similarity (>99%) and those groups were then matched with their closest GenBank references using the BLAST and MOLE-BLAST tool (ranked by lowest E-value). The nematode sequences fitted into seven groups, with all groups except group C2 having a GenBank reference match greater than 99% (Table 6.4). The trematode sequences fitted into four groups, with all groups except group F1 having a GenBank reference match greater than 99% (Table 6.4).

Table 6.4. BLAST-MOLE results (ranked by E-value) for grouped nematode (groups A-D) and trematode (groups E-F) sequences with their top five closest references.

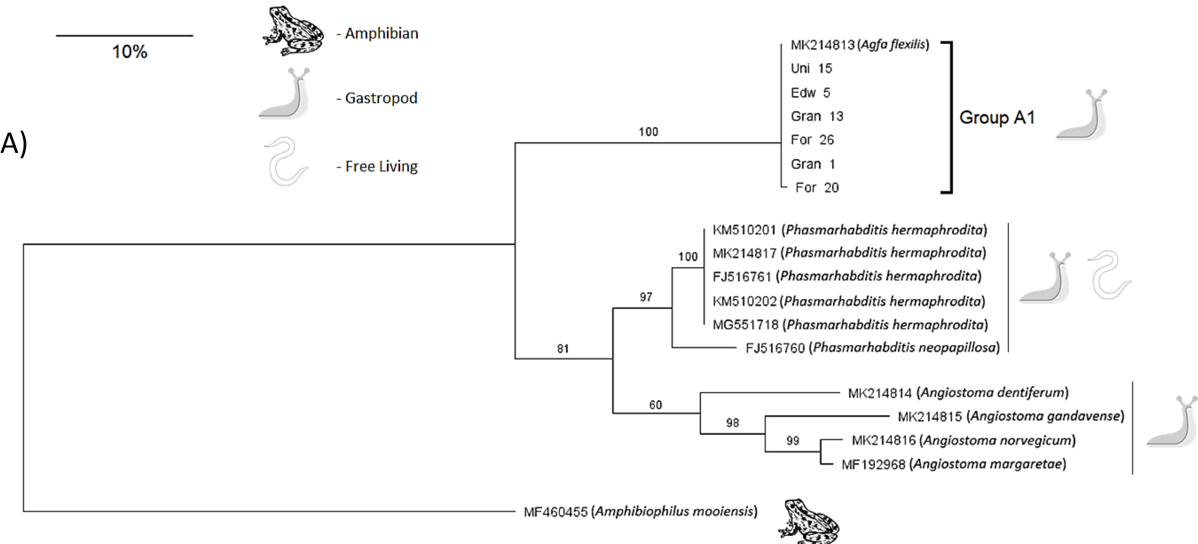
| Nematodes | | | | |
|---|---|---|---|---|
| Group | Samples | Closest references | Reference name | % Match |
| A1 | EDW 5<br>FOR 20<br>FOR 26<br>GRAN 1<br>GRAN 13<br>UNI 15 | MK214813 | *Agfa flexilis* | 99.4 |
| | | FJ516760 | *Phasmarhabditis neopapillosa* | 87 |
| | | MF192968 | *Angiostoma margaretae* | 86 |
| | | FJ516761 | *Phasmarhabditis hermaphrodita* | 85 |
| | | MK214815 | *Angiostoma gandavensis* | 81 |
| B1 | ARNOT 1<br>ARNOT 11<br>ARNOT 35 (J)<br>BAS 45<br>BEE 12<br>BEE 14<br>CARL 18<br>COLW 13 (J)<br>C-SOU 1<br>C-SOU 7<br>C-SOU 9<br>EDW 1 (J)<br>EDW 2<br>FOR 36 (J)<br>GAM 1 | MF192968 | *Angiostoma margaretae* | 99.4 |
| | | MK214816 | *Angiostoma norvegicum* | 92 |
| | | MK214815 | *Angiostoma gandavensis* | 87 |
| | | FJ516761 | *Phasmarhabditis hermaphrodita* | 83 |
| | | FJ516760 | *Phasmarhabditis neopapillosa* | 82 |
| B2 | BEE 16<br>C-SOU 3<br>KIM 1<br>KIM 33 | MK214815 | *Angiostoma gandavensis* | 99.7 |
| | | MF192968 | *Angiostoma margaretae* | 86 |
| | | MK214816 | *Angiostoma norvegicum* | 88 |
| | | FJ516761 | *Phasmarhabditis hermaphrodita* | 84 |
| | | FJ516760 | *Phasmarhabditis neopapillosa* | 85 |
| C1 | POND 14 | OL472311 | *Cosmocerca longicauda* | 99.9 |
| | | LC018444 | *Cosmocercoides pulcher* | 90 |
| | | MH178312 | *Cosmocercoides qingtianensis* | 90 |
| | | AB908161 | *Cosmocercoides tonkinensis* | 90 |
| | | MN839761 | *Cosmocerca simile* | 96 |
| C2 | BAS 1 (J)<br>BEE 1 (J)<br>KIM 40 (J)<br>MILL 19 (J) | OL472311 | *Cosmocerca longicauda* | 90 |
| | | LC018444 | *Cosmocercoides pulcher* | 88 |
| | | MH178312 | *Cosmocercoides qingtianensis* | 88 |
| | | AB908161 | *Cosmocercoides tonkinensis* | 88 |
| | | MN839761 | *Cosmocerca simile* | 96 |

| | | | | |
|---|---|---|---|---|
| D1 | ATEN 12 (J)<br>TOT 24<br>TOT 25 (J) | FJ516761 | *Phasmarhabditis hermaphrodita* | 99.3 |
| | | FJ516760 | *Phasmarhabditis neopapillosa* | 90 |
| | | MK214815 | *Angiostoma gandavensis* | 84 |
| | | MF192968 | *Angiostoma margaretae* | 79 |
| | | MK214813 | *Agfa flexilis* | 85 |
| D2 | C-SOU 10 (J)<br>GAM 16 (J) | FJ516760 | *Phasmarhabditis neopapillosa* | 99.2 |
| | | FJ516761 | *Phasmarhabditis hermaphrodita* | 90 |
| | | MK214815 | *Angiostoma gandavensis* | 82 |
| | | MF192968 | *Angiostoma margaretae* | 78 |
| | | MK214813 | *Agfa flexilis* | 86 |
| Trematodes | | | | |
| Group | Samples | Closest references | Reference name | % Match |
| E1 | BAS 11<br>FOR 23<br>GRAN 8<br>KIM 3<br>KIM 10<br>KIM 37<br>MILL 4a<br>MILL 4b<br>MILL 31<br>MILL 32<br>MILL 35<br>POND 5<br>POND 8<br>UNI 5 | KT074950 | *Brachylaima arcuata* | 99.6% |
| | | KT074955 | *Brachylaima mesostoma* | 98% |
| | | KT074952 | *Brachylaima fuscata* | 97% |
| | | AY222085 | *Brachylaima thompsoni* | 97% |
| | | KP903630 | *Urotocus rossitensis* | 94% |
| E2 | ARNOT 18<br>BAS 26<br>COLW 2<br>EDW 8<br>EDW 25<br>FOR 4<br>GAM 3<br>GAM 15<br>GAM 16<br>GAM 26<br>KIM 40 | KT074952 | *Brachylaima fuscata* | 99.8 |
| | | AY222085 | *Brachylaima thompsoni* | 99.4 |
| | | KT074955 | *Brachylaima mesostoma* | 99.2 |
| | | KT074950 | *Brachylaima arcuata* | 98 |
| | | KP903638 | *Michajlovia migrata* | 96 |
| E3 | CARL 12<br>CARL 13<br>C-SOU 19 | KT074955 | *Brachylaima mesostoma* | 100 |
| | | AY222085 | *Brachylaima thompsoni* | 99.6 |
| | | KT074952 | *Brachylaima fuscata* | 99.2 |
| | | KT074950 | *Brachylaima arcuata* | 99 |
| | | KP903638 | *Michajlovia migrata* | 96 |

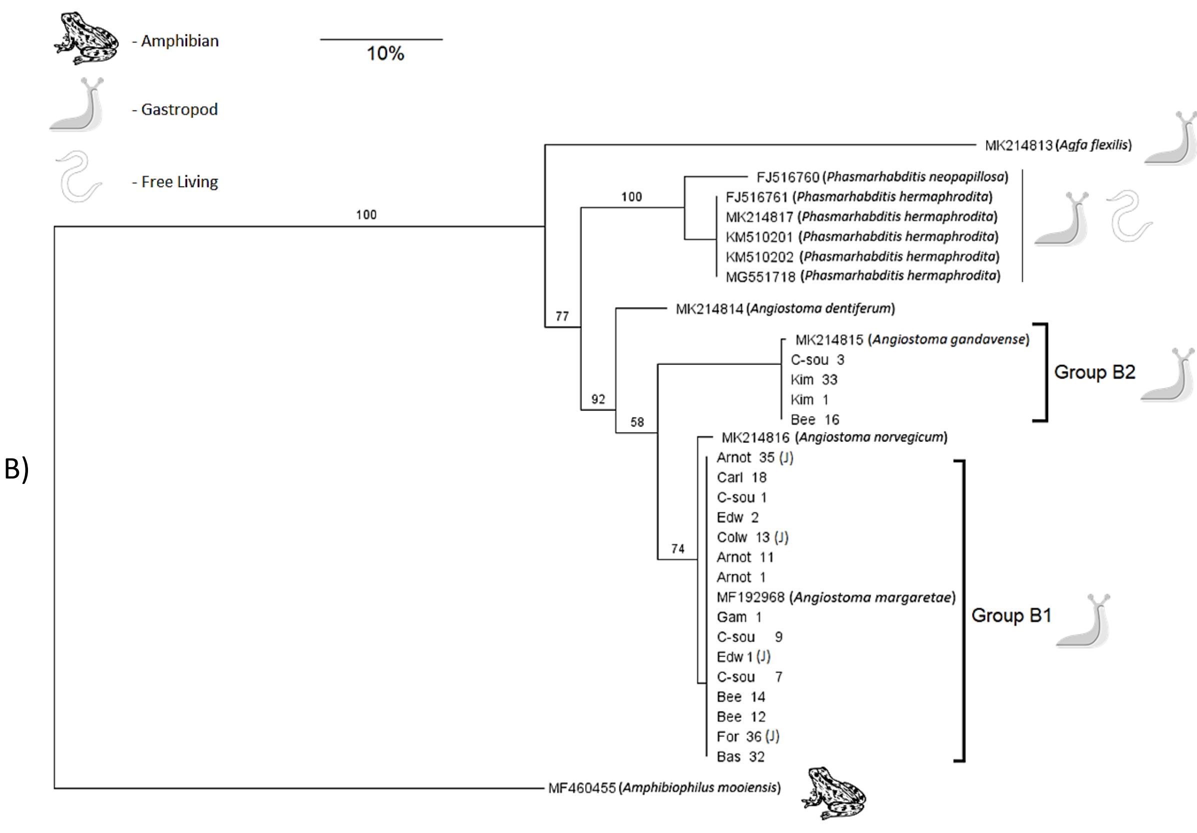
| F1 | UNI 39 | AY222156 | *Telorchis assula* | 97 |
|---|---|---|---|---|
| | | AY222160 | *Brachycoelium salamandrae* | 96 |
| | | AY222159 | *Auridistomum chelydrae* | 96 |
| | | JQ886404 | *Mesocoelium lanfrediae* | 96 |
| | | MZ787582 | *Opisthioglyphe ranae* | 96 |

Note: (J) indicates it was a juvenile nematode. Each of the different designated grouping of ITS (nematode) and 18S (trematode) sequences are less than 1% different. Nematode and trematode groups with less than 99% GenBank reference match are coloured grey.

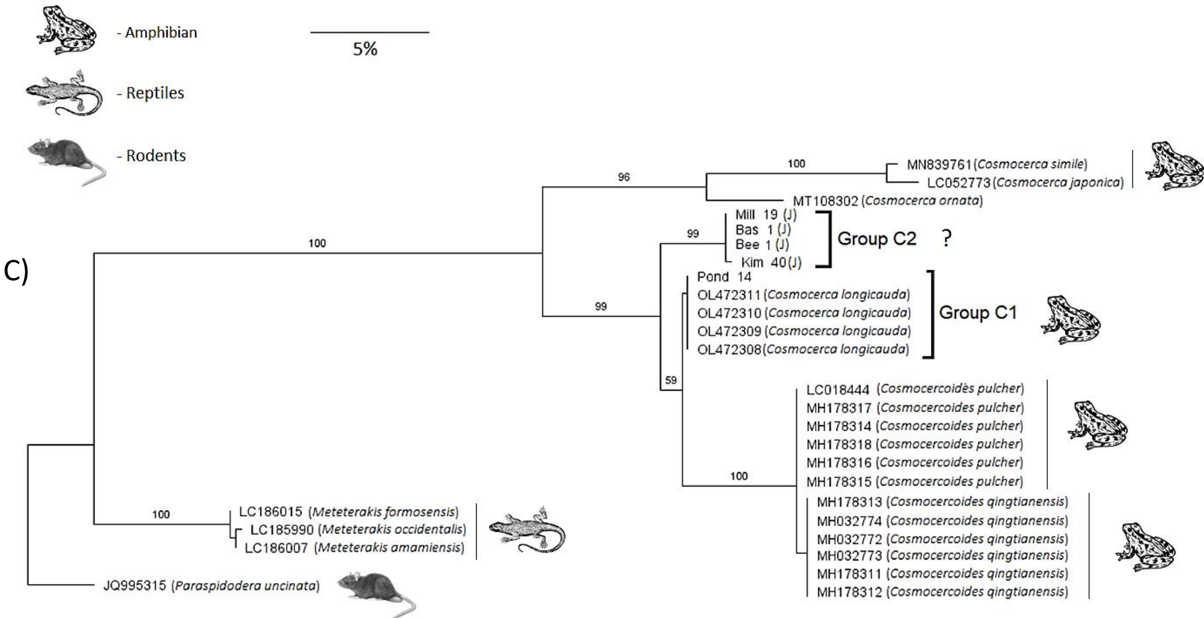
Next, maximum likelihood trees were created for the nematode and trematode sequences by placing each group together with a range of related GenBank references. The majority of the groups were identified at the species level (Figure 6.3). Only groups C2 and F1 were not identifiable at the species level. Group C2 was outside of the *Cosmocerca*/*Cosmocercoides* genera (Figure 6.3C) and group F1 was outside of the *Opisthioglyphe*/ *Macroderoides*/ *Brachycoelium*/ *Mesocoelium*/ *Auridistomum*/ *Telorchis* genera, respectively (Figure 6.3F).

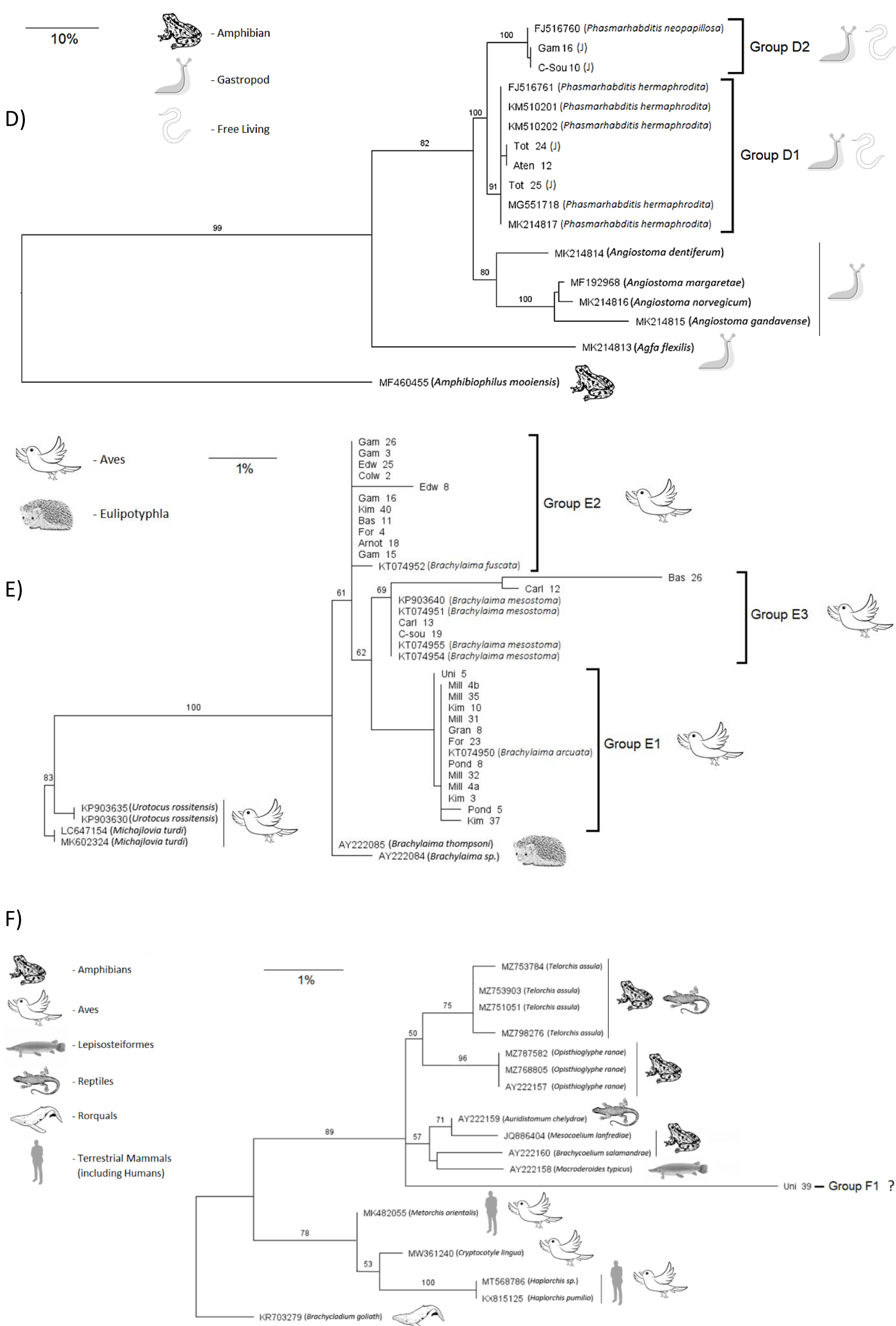


B)




C)





10%
- Amphibian
- Gastropod
- Free Living
D)
FJ516760 (Phasmarhabditis neopapillosa)
Gam 16 (J)
C-Sou 10 (J)
Group D2
FJ516761 (Phasmarhabditis hermaphrodita)
KM510201 (Phasmarhabditis hermaphrodita)
KM510202 (Phasmarhabditis hermaphrodita)
Tot 24 (J)
Aten 12
Tot 25 (J)
MG551718 (Phasmarhabditis hermaphrodita)
MK214817 (Phasmarhabditis hermaphrodita)
Group D1
MK214814 (Angiostoma dentiferum)
MF192968 (Angiostoma margaretae)
MK214816 (Angiostoma norvegicum)
MK214815 (Angiostoma gandavense)
MK214813 (Agfa flexilis)
MF460455 (Amphibiophilus mooiensis)
100
100
82
91
80
100
99
- Aves
- Eulipotyphla
1%
E)
Gam 26
Gam 3
Edw 25
Colw 2
Edw 8
Gam 16
Kim 40
Bas 11
For 4
Arnot 18
Gam 15
KT074952 (Brachylaima fuscata)
Group E2
Bas 26
Carl 12
KP903640 (Brachylaima mesostoma)
KT074951 (Brachylaima mesostoma)
Carl 13
C-sou 19
KT074955 (Brachylaima mesostoma)
KT074954 (Brachylaima mesostoma)
Group E3
Uni 5
Mill 4b
Mill 35
Kim 10
Mill 31
Gran 8
For 23
KT074950 (Brachylaima arcuata)
Pond 8
Mill 32
Mill 4a
Kim 3
Pond 5
Kim 37
Group E1
KP903635 (Urotocus rossitensis)
KP903630 (Urotocus rossitensis)
LC647154 (Michajlovia turdi)
MK602324 (Michajlovia turdi)
AY222085 (Brachylaima thompsoni)
AY222084 (Brachylaima sp.)
61
69
62
100
83
F)
- Amphibians
- Aves
- Lepisosteiformes
- Reptiles
- Rorquals
- Terrestrial Mammals
(including Humans)
1%
MZ753784 (Telorchis assula)
MZ753903 (Telorchis assula)
MZ751051 (Telorchis assula)
MZ798276 (Telorchis assula)
MZ787582 (Opisthioglyphe ranae)
MZ768805 (Opisthioglyphe ranae)
AY222157 (Opisthioglyphe ranae)
AY222159 (Auridistomum chelydrae)
JQ886404 (Mesocoelium lanfrediae)
AY222160 (Brachycoelium salamandrae)
AY222158 (Macroderoides typicus)
Uni 39 — Group F1 ?
MK482055 (Metorchis orientalis)
MW361240 (Cryptocotyle lingua)
MT568786 (Haplorchis sp.)
KX815125 (Haplorchis pumilio)
KR703279 (Brachycladium goliath)
75
50
96
71
57
89
78
53
100

Figure 6.3. Maximum likelihood phylogenetic trees of different nematode (trees A-D) and trematode (trees E-F) species using the ITS and 18S rRNA gene, respectively. The definitive host of each helminth is displayed on each tree. Tree A was created using 325 base pairs (bp) of the ITS and is rooted on *Amphibiophilus mooiensis*. Tree B was created using 306 bp of the ITS and is rooted on *A. mooiensis*. Tree C was created using 402 bp of the ITS and is rooted on *Paraspidodera uncinate*. Tree D was created using 409 bp of the ITS and is rooted on *A. mooiensis*. Tree E was created using 450 bp of the 18S rRNA and is rooted on *Michajlovia turdi*. Tree F was created using 456 bp of the 18S rRNA and is rooted on *Brachycladium goliath*. All trees were generated using PhyML v3.1; the numbers on the branches indicate the bootstrap percentages for 1000 replicates (bootstrap values under 50% are not shown). The scale bar represents percentage sequence divergence. Differing alignment lengths are due to the limited length of GenBank references. Accession numbers for all sequences can be found in Supplementary Table 6.3.

## 6.4 Discussion:

### 6.4.1 Prevalence of infection:

The vast majority of gastropods collected and examined were slugs (73%), of which five families were represented (Agriolimacidae, Arionidae, Boettgerillidae, Limacidae and Milacidae). The remaining gastropods were snails, of which four families were represented (Discidae, Helicidae, Hygromiidae and Oxychilidae). The largest families represented were the Arionidae (24%), Agriolimacidae (20%), Helicidae (20%), Milacidae (16%), Limacidae (13%), Hygromiidae (4%), Oxychilidae (2%), Discidae (<1%) and Boettgerillidae (<1%). The overall prevalence of infections for the gastropods collected was 28%. Both slugs (28%) and snails (29%) had a similar prevalence of infection. No medically or veterinary important lungworm species were found within the city of Nottingham. However, of the 26 gastropod species found, 16 are potential hosts for *Angiostrongylus vasorum*, eight are potential hosts for *Crenosoma vulpis* and five are potential hosts for *Aelurostrongylus abstrusus* (Supplementary Table 6.4).

### 6.4.2 Nematodes:

A total of 533 nematodes were isolated, with only 12 being juveniles. Juvenile nematodes are a useful indication for the possible presence of lungworm (metastrongyloid) species of veterinary importance like *An. vasorum*. Of those 12 juvenile nematodes, no lungworm species were found. Instead, four of them were identified as *Angiostoma margaretae* (Angiostomatidae), a parasite whose definitive host has been reported to be a milacid slug species (Ross et al., 2017b). We also found it inside *D. invadens* (Agriolimacidae) and *A. valentianus* (Limacidae). Four were identified as an unknown Cosmocercidae species, a family of parasitic nematodes whose definitive host are reptiles and amphibians (Baker, 1984). Two were identified as *Phasmarhabditis hermaphrodita* and two were identified as *Phasmarhabditis neopapillosa* (Rhabditidae). *Phasmarhabditis* is a genus of facultative parasitic nematodes that can parasitise a broad range of gastropod species (Andrus & Rae, 2019). Of the adult nematodes identified, all were non-medically (or veterinary) relevant, belonging to four of the seven gastropod-related nematode families (Agfidae, Angiostomatidae, Cosmocercidae and Rhabditidae).

The interactions these nematode families have with terrestrial gastropods are poorly understood (Wilson & Grewal, 2005). The most understood species is *Phasmarhabditis hermaphrodita*, which has been developed into an effective biological alternative molluscicide (Nemaslug®) that reduces agricultural damage done by gastropod pests (Rae et al., 2007). Unlike chemical molluscicides, Nemaslug has no adverse effects on non-target organisms like beneficial organisms (acarids, annelids, carabids, collembolans, dipterans, isopods and nematodes), or gastropod predators (amphibians, birds, mammals and reptiles; Iglesias et al., 2003). However, unlike chemical molluscicides, Nemaslug cannot kill every gastropod pest species. This is due to *P. hermaphrodita* only being able to kill smaller gastropod species (e.g., *Deroceras spp.*, *Arion hortensis*) and the juveniles of some larger species (*Arion ater*, *Cornu aspersum*; Rae, 2017), while larger gastropod species (*Ambigolimax spp.*, *Cepaea hortensis*, *Limacus spp.*, *Limax spp.*, *Lissachatina fulica*) are resistant to the fatal effects of *P. hermaphrodita* (Williams & Rae, 2015; Rae, 2017).

### 6.4.3 Trematodes:

A total of 242 trematodes were isolated. Of these, 29 were genotyped, 14 were identified as *Brachylaima arcuata*, 11 were identified as *B. fuscata* and three were identified as *B. mesostoma*. All these *Brachylaima* species are common gastrointestinal parasites of the bird families Corvidae, Sylviidae and Turdidae (Heneberg et al., 2016). One other trematode sample (belonging to group F1) could not be identified at the species-level. It clustered closely with the genera *Opisthioglyphe*, *Macroderoides*, *Brachycoelium*, *Mesocoelium*, *Auridistomum* and *Telorchis*, placing it within the Plagiorchioidea superfamily. Genera of this Plagiorchioidea superfamily are common parasites of amphibians, fishes and reptiles (Tkach et al., 2001).

*Brachylaima* is a common gastrointestinal parasite of birds, mammals, and reptiles. There are over 60 described species, with *Brachylaima* being found in Africa, the Americas, Asia, Europe, and Oceania (Nasir & Rodriguez, 1966; Wheeler et al., 1989; Richards et al., 1995; Awharitoma et al., 2003; Butcher & Grove, 2005; Richardson & Campo, 2005; Gállego et al., 2014; Gracenea & Gállego, 2017; Nakao et al., 2017; Gérard et al., 2020; Termizi & Him, 2021). *Brachylaima cribbi* is the only documented species capable of infecting humans (Butcher & Grove, 2001) with brachylaimiasis first documented in 1996, with 13 more cases in the subsequent decades after its discovery, all occurring in Australia (Butcher et al., 1996; Gállego et al., 2015). Brachylaimiasis causes diarrhoea, abdominal pain, anorexia, eosinophilia, and weight loss (or decreased weight gain) in infected humans, with a predicted mortality rate of 5-10% in untreated patients (Gállego & Gracenea, 2015). Transmission is typically from either the consumption of undercooked land snails (such as *Cornu aspersum*) infected with metacercariae, or the unintentional consumption of infected gastropod slime/faeces/corpse contaminated fruits and vegetables (Butcher & Grove, 2001).

While the consumption of snails is unpopular in the United Kingdom, on average the world consumes 450,000 tonnes of edible snails every year, of which only 15% come from snail farms (López et al., 2015). Spain, France, Portugal and Belgium are the biggest importers of snails, with approximately 17 million kilograms of snails being imported as a whole from 2020-2021 (United Nations, 2022). Concerns about the prevalence of *Brachylaima* infection

in *Cornu aspersum* at farms and markets has already been raised in France and Spain (Gállego & Gracenea, 2015; Gracenea & Gállego, 2017; Gérard et al., 2020). It is unknown what effect non-*Brachylaima cribbi* species have on public health as there are no studies exploring the possibility of brachylaimiasis caused by European *Brachylaima* species. Furthermore, brachylaimiasis could be frequently misdiagnosed or overlooked in Europe due to either a lack of experience identifying it or due to how small *Brachylaima* eggs are in human faeces (<30μm in length; Gracenea & Gállego, 2017).

## 6.5 Supplementary Material:

Supplementary Table 6.1. Nematode PCR information

| Site (Sample no.) | Sequencing result (N93/N94) | Host | Nematodes found |
|---|---|---|---|
| Arnot Hill (ARNOT) | | | |
| 1 | *Angiostoma margaretae* | *Tandonia budapestensis* | 1 |
| 11 | *Angiostoma margaretae* | *Tandonia budapestensis* | 1 |
| 16 | Fungal contamination | *Tandonia sowerbyi* | 1 |
| 18 | Fungal contamination | *Trochulus striolatus* | 1 |
| 21 | Fungal contamination | *Arion hortensis* | 1 |
| 26 | Fungal contamination | *Deroceras reticulatum* | 1 |
| 30 | Fungal contamination | *D. reticulatum* | 1 |
| 35 (J) | *Angiostoma margaretae* | *Tandonia sowerbyi* | 1 |
| 39 | Not extracted | *T. sowerbyi* | 1 |
| 44 | Fungal contamination | *T. striolatus* | 1 |
| 47 | Not extracted | *T. striolatus* | 1 |
| 50 | Fungal contamination | *Cepaea nemoralis* | 1 |
| Attenborough (ATEN) | | | |
| 1 | Fungal contamination | *Arion Ater* | 3 |
| 2 | Not Extracted | *A. Ater* | 3 |
| 3 | Not Extracted | *A. Ater* | 1 |
| 5 | Fungal contamination | *Arion vulgaris* | 1 |
| 8 | Not Extracted | *A. Ater* | 2 |
| 11 | Not Extracted | *A. Ater* | 1 |
| 12 (J) | *Phasmarhabditis hermaphrodita* | *Arion rufus* | 2 |
| 13 | Not Extracted | *A. rufus* | 1 |
| 15 | Fungal contamination | *A. Ater* | 6 |
| 16 | Not Extracted | *A. Ater* | 5 |
| 20 | Fungal contamination | *C. nemoralis* | 1 |
| 21 | Fungal contamination | *Oxychilus alliarius* | 2 |
| 24 | Fungal contamination | *Cornu aspersum* | 4 |
| 25 | Not Extracted | *A. Ater* | 1 |
| 29 | Not Extracted | *A. Ater* | 3 |
| 31 | Fungal contamination | *A. vulgaris* | 1 |
| 33 | Not Extracted | *A. rufus* | 1 |
| 34 | Not Extracted | *A. rufus* | 1 |
| 37 | Fungal contamination | *C. aspersum* | 1 |
| 40 | Not Extracted | *C. aspersum* | 1 |
| 43 | Not Extracted | *C. aspersum* | 1 |
| 46 | Fungal contamination | *C. nemoralis* | 1 |
| 50 | Not Extracted | *C. nemoralis* | 1 |
| Basford (BAS) | | | |
| 1 (J) | *Cosmocercidae sp.* | *Cornu aspersum* | 27 |
| 6 | Fungal contamination | *D. reticulatum* | 2 |
| 11 | Fungal contamination | *T. sowerbyi* | 3 |
| 12 | Fungal contamination | *Ambigolimax valentianus* | 3 |
| 14 | Not Extracted | *T. budapestensis* | 21 |
| 24 | Fungal contamination | *A. valentianus* | 1 |
| 26 | Not Extracted | *T. budapestensis* | 6 |
| 27 | Not Extracted | *T. budapestensis* | 4 |

| | | | |
|---|---|---|---|
| 30 | Not Extracted | *D. reticulatum* | 1 |
| 31 | Not Extracted | *D. reticulatum* | 1 |
| 38 | Not Extracted | *Milax gagates* | 2 |
| 39 | Not Extracted | *Limacus maculatus* | 17 |
| 40 | Not Extracted | *L. maculatus* | 13 |
| 41 | Not Extracted | *D. reticulatum* | 2 |
| 42 | Not Extracted | *Arion subfuscus* | 4 |
| 43 | Not Extracted | *A. hortensis* | 2 |
| 45 | *Angiostoma margaretae* | *Tandonia budapestensis* | 2 |
| 47 | Not Extracted | *A. hortensis* | 4 |
| 49 | Not Extracted | D. reticulatum | 1 |
| 50 | Not Extracted | D. reticulatum | 2 |
| Beeston (BEE) | | | |
| 1 (J) | *Cosmocercidae sp.* | *Cornu aspersum* | 27 |
| 12 | *Angiostoma margaretae* | *Tandonia budapestensis* | 1 |
| 14 | *Angiostoma margaretae* | *Tandonia budapestensis* | 2 |
| 16 | *Angiostoma gandavense* | *Deroceras invadens* | 1 |
| 25 | Fungal contamination | *D. invadens* | 1 |
| 28 | Fungal contamination | *A. ater* | 2 |
| Carlton (CARL) | | | |
| 2 | Fungal contamination | *C. aspersum* | 2 |
| 3 | Not Extracted | *C. aspersum* | 1 |
| 8 | Fungal contamination | *C. aspersum* | 2 |
| 13 | Fungal contamination | *C. nemoralis* | 2 |
| 18 | *Angiostoma margaretae* | *Deroceras reticulatum* | 3 |
| 26 | Fungal contamination | *T. budapestensis* | 1 |
| 27 | Not Extracted | *T. budapestensis* | 1 |
| 28 | Not Extracted | *T. budapestensis* | 2 |
| 29 | Not Extracted | *T. budapestensis* | 3 |
| 35 | Not Extracted | *T. budapestensis* | 3 |
| 37 | Fungal contamination | *D. invadens* | 1 |
| 41 | Not Extracted | *D. invadens* | 5 |
| Colwick (COLW) | | | |
| 2 | Fungal contamination | *C. aspersum* | 1 |
| 7 | Fungal contamination | *Limacus flavus* | 1 |
| 13 (J) | *Angiostoma margaretae* | *Deroceras invadens* | 4 |
| 18 | Fungal contamination | *Arion silvaticus* | 1 |
| 21 | Fungal contamination | *A. valentianus* | 1 |
| 24 | Not Extracted | *A. valentianus* | 1 |
| 29 | Fungal contamination | *C. hortensis* | 1 |
| 30 | Not Extracted | *C. hortensis* | 1 |
| 31 | Not Extracted | *C. hortensis* | 1 |
| 36 | Not Extracted | *C. hortensis* | 1 |
| 41 | Fungal contamination | *A. hortensis* | 1 |
| 44 | Not Extracted | *A. hortensis* | 1 |
| 46 | Not Extracted | *T. budapestensis* | 1 |
| 47 | Not Extracted | *T. budapestensis* | 1 |
| 49 | Not Extracted | *T. budapestensis* | 2 |
| Clifton south (C-SOU) | | | |
| 1 | *Angiostoma margaretae* | *Deroceras invadens* | 3 |
| 3 | *Angiostoma gandavense* | *Deroceras reticulum* | 3 |

| | | | |
|---|---|---|---|
| 7 | *Angiostoma margaretae* | *Tandonia budapestensis* | 1 |
| 9 | *Angiostoma margaretae* | *Tandonia sowerbyi* | 6 |
| 10 (J) | *Phasmarhabditis neopapillosa* | *Ambigolimax nyctelius* | 4 |
| 23 | Fungal contamination | *A. vulgaris* | 1 |
| 26 | Not Extracted | *D. invadens* | 1 |
| 29 | Fungal contamination | *D. reticulatum* | 1 |
| 30 | Not Extracted | *D. reticulatum* | 1 |
| 32 | Not Extracted | *T. sowerbyi* | 1 |
| 36 | Not Extracted | *T. budapestensis* | 1 |
| 42 | Fungal contamination | *C. nemoralis* | 1 |
| 45 | Fungal contamination | *A. vulgaris* | 1 |
| Edwalton (EDW) | | | |
| 1 (J) | *Angiostoma margaretae* | *Tandonia budapestensis* | 31 |
| 2 | *Angiostoma margaretae* | *Tandonia budapestensis* | 5 |
| 3 | Not Extracted | *T. budapestensis* | 11 |
| 4 | Not Extracted | *T. budapestensis* | 2 |
| 5 | *Agfa flexilis* | *Limacus maculatus* | 4 |
| 6 | Fungal contamination | *A. silvaticus* | 2 |
| 7 | Fungal contamination | *A. hortensis* | 1 |
| 9 | Fungal contamination | *D. invadens* | 1 |
| 23 | Not Extracted | *C. aspersum* | 8 |
| 27 | Not Extracted | *C. aspersum* | 22 |
| 30 | Not Extracted | *T. budapestensis* | 1 |
| 32 | Not Extracted | *T. budapestensis* | 1 |
| 34 | Not Extracted | *C. aspersum* | 1 |
| 38 | Not Extracted | *D. invadens* | 1 |
| 40 | Not Extracted | *D. invadens* | 1 |
| 41 | Not Extracted | *D. invadens* | 1 |
| 44 | Not Extracted | *D. invadens* | 1 |
| 46 | Not Extracted | *T. striolatus* | 1 |
| 47 | Not Extracted | *C. hortensis* | 1 |
| 50 | Not Extracted | *C. hortensis* | 1 |
| Forest field (FOR) | | | |
| 18 | Not Extracted | *L. maculatus* | 1 |
| 20 | *Agfa flexilis* | *Limacus maculatus* | 1 |
| 26 | *Agfa flexilis* | *Limax maximus* | 3 |
| 27 | Not Extracted | *L. maximus* | 4 |
| 30 | Fungal contamination | *C. aspersum* | 5 |
| 33 | Not Extracted | *L. maculatus* | 4 |
| 35 | Not Extracted | *L. maculatus* | 4 |
| 36 (J) | *Angiostoma margaretae* | *Ambigolimax valentianus* | 1 |
| 38 | Fungal contamination | *A. hortensis* | 1 |
| 45 | Fungal contamination | *T. budapestensis* | 1 |
| 46 | Not Extracted | *T. budapestensis* | 1 |
| Gamston (GAM) | | | |
| 1 | *Angiostoma margaretae* | *Deroceras invadens* | 2 |
| 6 | Fungal contamination | *L. maculatus* | 1 |
| 9 | Not Extracted | *L. maculatus* | 1 |
| 13 | Fungal contamination | *A. valentianus* | 1 |
| 14 | Fungal contamination | *A. valentianus* | 1 |
| 15 | Not Extracted | *A. valentianus* | 2 |

| | | | |
|---|---|---|---|
| 16 (J) | *Phasmarhabditis neopapillosa* | *Ambigolimax valentianus* | 1 |
| 17 | Not Extracted | *A. valentianus* | 2 |
| 27 | Fungal contamination | *M. cantiana* | 1 |
| Grange Park (GRAN) | | | |
| 1 | *Agfa flexilis* | *Tandonia Budapestensis* | 1 |
| 13 | *Agfa flexilis* | *Arion vulgaris* | 1 |
| 15 | Fungal contamination | *A. Vulgaris* | 1 |
| 23 | Fungal contamination | *A. subfuscus* | 7 |
| 27 | Not Extracted | *A. subfuscus* | 1 |
| 29 | Fungal contamination | *A. subfuscus* | 2 |
| 34 | Fungal contamination | *T. budapestensis* | 6 |
| 37 | Fungal contamination | *D. invadens* | 2 |
| 42 | Fungal contamination | *D. reticulatum* | 1 |
| 48 | Fungal contamination | *A. Vulgaris* | 1 |
| Kimberley (KIM) | | | |
| 1 | *Angiostoma gandavense* | *Deroceras invadens* | 3 |
| 9 | Not Extracted | *D. invadens* | 1 |
| 12 | Fungal contamination | *O. alliarius* | 1 |
| 14 | Not Extracted | *O. alliarius* | 1 |
| 18 | Not Extracted | *O. alliarius* | 1 |
| 19 | Fungal contamination | *O. alliarius* | 1 |
| 20 | Not Extracted | *O. alliarius* | 4 |
| 21 | Fungal contamination | *A. hortensis* | 1 |
| 24 | Not Extracted | *A. hortensis* | 2 |
| 32 | Not Extracted | *A. hortensis* | 2 |
| 33 | *Angiostoma gandavense* | *Arion hortensis* | 3 |
| 37 | Not Extracted | *D. reticulatum* | 1 |
| 40 (J) | *Cosmocercidae sp.* | *Cornu aspersum* | 32 |
| 42 | Not Extracted | *C. nemoralis* | 1 |
| 45 | Not Extracted | *C. nemoralis* | 1 |
| 46 | Not Extracted | *C. nemoralis* | 1 |
| Bestwood Country Park (MILL) | | | |
| 3 | Fungal contamination | *C. aspersum* | 1 |
| 19 (J) | *Cosmocercidae sp.* | *Cornu aspersum* | 19 |
| 21 | Fungal contamination | *A. vulgaris* | 2 |
| 33 | Fungal contamination | *M. cantiana* | 1 |
| Iremongers pond (POND) | | | |
| 5 | Fungal contamination | *D. reticulatum* | 1 |
| 14 | *Cosmocerca longicauda* | *Limax flavus* | 7 |
| 21 | Fungal contamination | *D. reticulatum* | 1 |
| 25 | Fungal contamination | *D. invadens* | 1 |
| 37 | Fungal contamination | *L. flavus* | 1 |
| 45 | Fungal contamination | *A. rufus* | 1 |
| Toton (TOT) | | | |
| 11 | Fungal contamination | *A. ater* | 2 |
| 13 | Fungal contamination | *T. sowerbyi* | 4 |
| 21 | Fungal contamination | *C. nemoralis* | 1 |
| 24 | *Phasmarhabditis hermaphrodita* | *Cepaea nemoralis* | 1 |
| 25 (J) | *Phasmarhabditis hermaphrodita* | *Arion subfuscus* | 6 |
| 26 | Fungal contamination | *A. ater* | 2 |
| 33 | Fungal contamination | *A. subfuscus* | 1 |

| 39 | Fungal contamination | *A. ater* | 1 |
|---|---|---|---|
| 47 | Fungal contamination | *T. sowerbyi* | 1 |
| University Park (UNI) | | | |
| 2 | Fungal contamination | *A. valentianus* | 1 |
| 3 | Fungal contamination | *A. valentianus* | 1 |
| 15 | *Agfa flexilis* | *Tandonia budapestensis* | 1 |
| 17 | Fungal contamination | *T. budapestensis* | 1 |
| 39 | Fungal contamination | *A. valentianus* | 1 |

Note: (J) indicates it was a juvenile nematode.

Supplementary Table 6.2. Trematode PCR information

| Site (Sample no.) | Sequencing result (LPF/LPR) | Host | Trematodes found |
|---|---|---|---|
| Arnot Hill (ARNOT) | | | |
| 18 | *Brachylaima fuscata* | *Trochulus striolatus* | 1 |
| Basford (BAS) | | | |
| 11 | *Brachylaima arcuata* | *Tandonia sowerbyi* | 1 |
| 26 | *Brachylaima fuscata* | *Cornu aspersum* | 2 |
| 38 | Fungal contamination | *Milax gagates* | 4 |
| 41 | Fungal contamination | *Deroceras reticulatum* | 1 |
| Carlton (CARL) | | | |
| 12 | *Brachylaima mesostoma* | *Cepaea nemoralis* | 4 |
| 13 | *Brachylaima mesostoma* | *Cepaea nemoralis* | 3 |
| 19 | Fungal contamination | *D. reticulatum* | 5 |
| 37 | Not Extracted | *Deroceras invadens* | 4 |
| 41 | Fungal contamination | *D. invadens* | 2 |
| 45 | Not Extracted | *D. invadens* | 3 |
| Colwick (COLW) | | | |
| 2 | *Brachylaima fuscata* | *Cornu aspersum* | 3 |
| 11 | Fungal contamination | *D. invadens* | 2 |
| 21 | Fungal contamination | *Ambigolimax valentianus* | 2 |
| 25 | Not Extracted | *A. valentianus* | 2 |
| Clifton south (C-SOU) | | | |
| 19 | *Brachylaima mesostoma* | *Cepaea nemoralis* | 10 |
| Edwalton (EDW) | | | |
| 8 | *Brachylaima fuscata* | *Deroceras invadens* | 2 |
| 20 | Fungal contamination | *Trochulus striolatus* | 1 |
| 23 | Fungal contamination | *C. aspersum* | 11 |
| 25 | *Brachylaima fuscata* | *Cornu aspersum* | 3 |
| Forest field (FOR) | | | |
| 4 | *Brachylaima fuscata* | *Deroceras invadens* | 1 |
| 18 | Fungal contamination | *Limacus maculatus* | 1 |
| 23 | *Brachylaima arcuata* | *Tandonia budapestensis* | 2 |
| 47 | Fungal contamination | *D. invadens* | 1 |
| Gamston (GAM) | | | |
| 3 | *Brachylaima fuscata* | *Ambigolimax valentianus* | 2 |
| 13 | Not Extracted | *A. valentianus* | 9 |
| 15 | *Brachylaima fuscata* | *Ambigolimax valentianus* | 15 |

| | | | |
|---|---|---|---|
| 16 | *Brachylaima fuscata* | *Ambigolimax valentianus* | 23 |
| 23 | Not Extracted | *T. striolatus* | 11 |
| 26 | *Brachylaima fuscata* | *Cepaea hortensis* | 2 |
| 27 | Not Extracted | *Monacha cantiana* | 4 |
| 28 | Not Extracted | *Cornu aspersum* | 36 |
| 29 | Not Extracted | *C. aspersum* | 2 |
| 30 | Not Extracted | *C. aspersum* | 3 |
| 33 | Not Extracted | *C. aspersum* | 2 |
| 34 | Not Extracted | *C. aspersum* | 5 |
| 37 | Fungal contamination | *D. invadens* | 1 |
| 46 | Not Extracted | *C. nemoralis* | 2 |
| 48 | Not Extracted | *C. nemoralis* | 2 |
| Grange Park (GRAN) | | | |
| 8 | *Brachylaima arcuata* | *Deroceras reticulatum* | 4 |
| Kimberley (KIM) | | | |
| 3 | *Brachylaima arcuata* | *Deroceras invadens* | 3 |
| 10 | *Brachylaima arcuata* | *Deroceras invadens* | 2 |
| 37 | *Brachylaima arcuata* | *Deroceras reticulatum* | 1 |
| 40 | *Brachylaima fuscata* | *Cornu aspersum* | 8 |
| Bestwood Country Park (MILL) | | | |
| 4a | *Brachylaima arcuata* | *Monacha cantiana* | 14 |
| 4b | *Brachylaima arcuata* | *Monacha cantiana* | 14 |
| 31 | *Brachylaima arcuata* | *Monacha cantiana* | 3 |
| 32 | *Brachylaima arcuata* | *Monacha cantiana* | 1 |
| 35 | *Brachylaima arcuata* | *Monacha cantiana* | 1 |
| Iremongers pond (POND) | | | |
| 5 | *Brachylaima arcuata* | *Deroceras reticulatum* | 3 |
| 8 | *Brachylaima arcuata* | *Deroceras reticulatum* | 4 |
| University Park (UNI) | | | |
| 5 | *Brachylaima arcuata* | *Ambigolimax valentianus* | 2 |
| 37 | Fungal contamination | *A. valentianus* | 1 |
| 39 | *Plagiorchioidea sp.* | *Ambigolimax valentianus* | 8 |
| 41 | Fungal contamination | *A. valentianus* | 1 |

Supplementary Table 6.3. GenBank accession numbers for the nematode and trematode ML phylogenetic trees.

| Nematodes | | |
|---|---|---|
| Isolate | Accession no. | Species |
| - | MK214813 | *Agfa flexilis* |
| - | MF460455 | *Amphibiophilus mooiensis* |
| - | MK214814 | *Angiostoma dentiferum* |
| - | MK214815 | *Angiostoma gandavense* |
| - | MF192968 | *Angiostoma margaretae* |
| - | MK214816 | *Angiostoma norvegicum* |
| - | LC052773 | *Cosmocerca japonica* |
| - | OL472308 | *Cosmocerca longicauda* |
| - | OL472309 | *Cosmocerca longicauda* |
| - | OL472310 | *Cosmocerca longicauda* |
| - | OL472311 | *Cosmocerca longicauda* |
| - | MT108302 | *Cosmocerca ornata* |
| - | MN839761 | *Cosmocerca simile* |
| - | LC018444 | *Cosmocercoides pulcher* |
| - | MH178314 | *Cosmocercoides pulcher* |
| - | MH178315 | *Cosmocercoides pulcher* |
| - | MH178316 | *Cosmocercoides pulcher* |
| - | MH178317 | *Cosmocercoides pulcher* |
| - | MH178318 | *Cosmocercoides pulcher* |
| - | MH032772 | *Cosmocercoides qingtianensis* |
| - | MH032773 | *Cosmocercoides qingtianensis* |
| - | MH032774 | *Cosmocercoides qingtianensis* |
| - | MH178311 | *Cosmocercoides qingtianensis* |
| - | MH178312 | *Cosmocercoides qingtianensis* |
| - | MH178313 | *Cosmocercoides qingtianensis* |
| - | LC186007 | *Meteterakis amamiensis* |
| - | LC186015 | *Meteterakis formosensis* |

| - | LC185990 | *Meteterakis occidentalis* |
|---|---|---|
| - | JQ995315 | *Paraspidodera uncinata* |
| - | FJ516761 | *Phasmarhabditis hermaphrodita* |
| - | KM510201 | *Phasmarhabditis hermaphrodita* |
| - | KM510202 | *Phasmarhabditis hermaphrodita* |
| - | MG551718 | *Phasmarhabditis hermaphrodita* |
| - | MK214817 | *Phasmarhabditis hermaphrodita* |
| - | FJ516760 | *Phasmarhabditis neopapillosa* |
| Edwalton 5 | OP626220 | *Agfa flexilis* |
| Forest 20 | OP626221 | *Agfa flexilis* |
| Forest 26 | OP626222 | *Agfa flexilis* |
| Grange 1 | OP626223 | *Agfa flexilis* |
| Grange 13 | OP626224 | *Agfa flexilis* |
| University 15 | OP626225 | *Agfa flexilis* |
| Arnot 1 | OP626226 | *Angiostoma margaretae* |
| Arnot 11 | OP626227 | *Angiostoma margaretae* |
| Arnot 35 | OP626228 | *Angiostoma margaretae* |
| Basford 45 | OP626229 | *Angiostoma margaretae* |
| Beeston 12 | OP626230 | *Angiostoma margaretae* |
| Beeston 14 | OP626231 | *Angiostoma margaretae* |
| Beeston 16 | OP626232 | *Angiostoma gandavensis* |
| Carlton 18 | OP626233 | *Angiostoma margaretae* |
| Colwick 13 | OP626234 | *Angiostoma margaretae* |
| Clifton south 1 | OP626235 | *Angiostoma margaretae* |
| Clifton south 3 | OP626236 | *Angiostoma gandavensis* |
| Clifton south 7 | OP626237 | *Angiostoma margaretae* |
| Clifton south 9 | OP626238 | *Angiostoma margaretae* |
| Edward 1 | OP626239 | *Angiostoma margaretae* |
| Edward 2 | OP626240 | *Angiostoma margaretae* |
| Forest 36 | OP626241 | *Angiostoma margaretae* |

| | | |
|---|---|---|
| Gamston 1 | OP626242 | *Angiostoma margaretae* |
| Kimberley 1 | OP626243 | *Angiostoma gandavensis* |
| Kimberley 33 | OP626244 | *Angiostoma gandavensis* |
| Aten 12 | OP626245 | *Phasmarhabditis hermaphrodita* |
| Clifton South 10 | OP626246 | *Phasmarhabditis neopapillosa* |
| Gamston 16 | OP626247 | *Phasmarhabditis neopapillosa* |
| Toton 24 | OP626248 | *Phasmarhabditis hermaphrodita* |
| Toton 25 | OP626249 | *Phasmarhabditis hermaphrodita* |
| Basford 1 | OP626250 | *Cosmocercidae sp.* |
| Beeston 1 | OP626251 | *Cosmocercidae sp.* |
| Kimberley 40 | OP626252 | *Cosmocercidae sp.* |
| Mill 19 | OP626253 | *Cosmocercidae sp.* |
| Pond 14 | OP626254 | *Cosmocerca longicauda* |
| Trematode | | |
| Isolate | Accession no. | Species |
| - | AY222159 | *Auridistomum chelydrae* |
| - | KR703279 | *Brachycladium goliath* |
| - | AY222160 | *Brachycoelium salamandrae* |
| - | KT074950 | *Brachylaima arcuata* |
| - | KT074952 | *Brachylaima fuscata* |
| - | KP903640 | *Brachylaima mesostoma* |
| - | KT074951 | *Brachylaima mesostoma* |
| - | KT074954 | *Brachylaima mesostoma* |
| - | KT074955 | *Brachylaima mesostoma* |
| - | AY222084 | *Brachylaima sp.* |
| - | AY222085 | *Brachylaima thompsoni* |
| - | MW361240 | *Cryptocotyle lingua* |
| - | KX815125 | *Haplorchis pumilio* |
| - | MT568786 | *Haplorchis sp.* |
| - | AY222158 | *Macroderoides typicus* |

| - | JQ886404 | *Mesocoelium lanfrediae* |
|---|---|---|
| - | MK482055 | *Metorchis orientalis* |
| - | LC647154 | *Michajlovia turdi* |
| - | MK602324 | *Michajlovia turdi* |
| - | AY222157 | *Opisthioglyphe ranae* |
| - | MZ768805 | *Opisthioglyphe ranae* |
| - | MZ787582 | *Opisthioglyphe ranae* |
| - | MZ751051 | *Telorchis assula* |
| - | MZ753784 | *Telorchis assula* |
| - | MZ753903 | *Telorchis assula* |
| - | MZ798276 | *Telorchis assula* |
| - | KP903630 | *Urotocus rossitensis* |
| - | KP903635 | *Urotocus rossitensis* |
| Arnot 18 | OP626191 | *Brachylaima fuscata* |
| Basford 11 | OP626192 | *Brachylaima arcuata* |
| Basford 26 | OP626193 | *Brachylaima fuscata* |
| Carlton 12 | OP626194 | *Brachylaima mesostoma* |
| Carlton 13 | OP626195 | *Brachylaima mesostoma* |
| Colwick 2 | OP626196 | *Brachylaima fuscata* |
| Clifton South 19 | OP626197 | *Brachylaima mesostoma* |
| Edwalton 8 | OP626198 | *Brachylaima fuscata* |
| Edwalton 25 | OP626199 | *Brachylaima fuscata* |
| Forest 4 | OP626200 | *Brachylaima fuscata* |
| Forest 23 | OP626201 | *Brachylaima arcuata* |
| Gamston 3 | OP626202 | *Brachylaima fuscata* |
| Gamston 15 | OP626203 | *Brachylaima fuscata* |
| Gamston 16 | OP626204 | *Brachylaima fuscata* |
| Gamston 26 | OP626205 | *Brachylaima fuscata* |
| Grange 8 | OP626206 | *Brachylaima arcuata* |
| Kimberley 3 | OP626207 | *Brachylaima arcuata* |

| | | |
|---|---|---|
| Kimberley 10 | OP626208 | *Brachylaima arcuata* |
| Kimberley 37 | OP626209 | *Brachylaima arcuata* |
| Kimberley 40 | OP626210 | *Brachylaima fuscata* |
| Mill 4a | OP626211 | *Brachylaima arcuata* |
| Mill 4b | OP626212 | *Brachylaima arcuata* |
| Mill 31 | OP626213 | *Brachylaima arcuata* |
| Mill 32 | OP626214 | *Brachylaima arcuata* |
| Mill 35 | OP626215 | *Brachylaima arcuata* |
| Pond 5 | OP626216 | *Brachylaima arcuata* |
| Pond 8 | OP626217 | *Brachylaima arcuata* |
| University 5 | OP626218 | *Brachylaima arcuata* |
| University 39 | OP626219 | *Plagiorchioidea sp.* |

Supplementary Table 6.4. The terrestrial gastropod species commonly found at popular dog walking sites in and around city of Nottingham and their relevance as intermediate hosts for different lungworm nematode species. Intermediate host status confirmed by Alicata (1965); Skorping & Halvorsen (1980); Campbell and Little (1988); Diez-Baños et al. (1989); Schjetlein & Skorping (1995); Grewal et al. (2003); Majoros et al. (2010); Panayotova-Pencheva (2011); Patel et al. (2014); Helm et al. (2015); Conboy (2015); Aziz et al. (2016); Hadi (2018); Lange et al. (2018); Hicklenton & Betson (2019); Fuehrer et al. (2020) and Penagos-Tabares et al. (2020).

| Family | Species | | Intermediate Host? |
|---|---|---|---|
| Agriolimacidae | *Deroceras invadens* | (Reise, Hutchinson, Schunack & Schlitt, 2011) | Yes [3] |
| | *Deroceras reticulatum* | (Müller, 1774) | Yes [1, 2, 3, 4, 6, 11] |
| Arionidae | *Arion ater* | (Linnaeus, 1758) | Yes [3] |
| | *Arion hortensis* | (Férussac, 1819) | Yes [3, 4, 5, 6, 7] |
| | *Arion rufus* | (Linnaeus, 1758) | Yes [3] |
| | *Arion silvaticus* | (Lohmander, 1937) | Yes [5] |
| | *Arion subfuscus* | (O.F. Müller, 1774) | Yes [3, 5, 6, 7] |
| | *Arion vulgaris* | (Moquin-Tandon, 1855) | Yes [1, 3, 4, 10] |
| Boettgerillidae | *Boettgerilla pallens* | (Simroth, 1912) | No |
| Discidae | *Discus rotundatus* | (Müller, 1774) | Yes [3] |
| Helicidae | *Arianta arbustorum* | (Linnaeus, 1758) | Yes [3, 4, 5, 6, 7] |
| | *Cepaea hortensis* | (O.F. Müller, 1774) | Yes [4, 6, 7] |
| | *Cepaea nemoralis* | (O.F. Müller, 1774) | Yes [3, 4, 6, 7] |
| | *Cornu aspersum* | (O.F. Müller, 1774) | Yes [1, 3, 4, 7, 8, 9] |
| Hygromiidae | *Trochulus hispidus* | (Linnaeus, 1758) | Yes [5] |
| | *Trochulus striolatus* | (Pfeiffer, 1828) | No |
| | *Monacha cantiana* | (Montagu, 1803) | Yes [5, 6, 9] |
| Limacidae | *Ambigolimax nyctelius* | (Bourguignat, 1861) | No |
| | *Ambigolimax valentianus* | (Férussac, 1821) | No |
| | *Limacus flavus* | (Linnaeus, 1758) | Yes [1, 2, 3, 6] |
| | *Limacus maculatus* | (Kaleni-czenko, 1851) | Yes [3] |
| | *Limax maximus* | (Linnaeus, 1758) | Yes [1, 3, 4, 8, 11] |
| Milacidae | *Milax gagates* | (Draparnaud, 1801) | Yes [3] |
| | *Tandonia budapestensis* | (Hazay, 1880) | No |
| | *Tandonia sowerbyi* | (Férussac, 1823) | Yes [3, 6] |
| Oxychilidae | *Oxychilus alliarius* | (Miller, 1822) | Yes [2] |

Note: Gastropods species that are not associated as intermediated hosts of lungworms are greyed out. [1]*Aelurostrongylus abstrusus;* [2]*Angiostrongylus cantonensis;* [3]*Angiostrongylus vasorum;* [4]*Crenosoma vulpis;* [5]*Elaphostrongylus rangiferi;* [6]*Muellerius capillaris*; [7]*Neostrongylus linearis*; [8]*Oslerus rostratus*; [9]*Prostrongylus rufescens*; [10]*Troglostrongylus wilsoni*; [11]*Umingmakstrongylus pallikuukensis*.

# Chapter 7 General Discussion:

The research presented for this thesis aimed to explore the relationship helminthic parasites have with their intermediate gastropod hosts in an effort to better understand the dynamics of transmission for gastropod-borne parasitic disease. This was done by investigating the morphology, distribution, prevalence of infection and genetic diversity of the intermediate snail host. The overall major themes found in each of the chapters are highlighted and discussed below.

## 7.1 Ecological Phenotypic Variation of African *Biomphalaria* Species:

In Chapter 3, we investigated the conchological variation of African *Biomphalaria* species found on the Ugandan shoreline of the Great African Lakes and used novel landmark-based geometric morphometric techniques to differentiate species using only shell morphology. This novel approach was able to distinguish all four species groups using landmark placement data. Our findings are contrary to Plam et al. (2008), who found that sympatric *Biomphalaria* species at Lake Albert could not be distinguished using shell morphology. However, Plam et al. (2008) did not use landmark-based morphometric techniques, but instead a mixture of six distance-based measurements and seven non-distance based shell characteristics.

The use of conchological identification methods has been consistently problematic, as the literature on *Biomphalaria* (and other planorbid snails) have been overloaded with a large number of species, the majority of which are not valid (Jarne et al., 2011). This is likely the consequence of many *Biomphalaria* species (and other planorbid snails) being first identified during the 1800s and their classification being often based on the appearance of their shell morphology alone (Jarne et al., 2011). This problem has been recently addressed with the introduction of 'lacustrine' and 'non-lacustrine' terminology, which addresses that some species of *Biomphalaria* exhibit ecological phenotypic variation in their shell morphology depending on what type of environment they inhabit when developing (Brown, 1996; DeJong et al., 2001; Kazibwe et al., 2006; Plam et al., 2008; Kazibwe et al., 2010). However, the 'lacustrine' and 'non-lacustrine' categories lack detail in explaining what factor (or factors) cause the morphological difference within a species, as lacustrine simply refers to 'relating to lakes' and non-lacustrine refers to 'everything else'.

Another issue is the lack of information in the literature on what specific environmental factors contribute to the contrasting shell morphologies between the two morphotypes. For example, Jarne et al. (2011) describes the lacustrine forms of some African and American species as tending to have smaller shells and larger apertures, as the opening of the shell widens quickly during growth, resulting in a smaller height-to-diameter ratio compared to the non-lacustrine form. However, Jarne et al. (2011) only describes the process of how lacustrine shells develop differently to non-lacustrine shells, not what factor (or factors) is causing the shell to widen during development. Standley et al. (2011) was the first to find that the morphotypes of *B. choanomphala* at Lake Victoria were strongly associated with certain habitat types, with the majority of morphotype-A snails being found in marsh-like habitats, while morphotype B snails were located within the lake. Standley et al. (2012) expanded upon this association further by finding habitat-type, water depth and pH were

significant predictors for morphotype-A abundance. This is a similar finding to the results of Chapter 5, which found morphotype-A variants of *B. choanomphala* had a significant negative relationship with water turbulence, pH and water depth, indicating morphotype-A are commonly found in shallow environments with a neutral pH and low flow rate. Conversely, morphotype-B shells had a significant positive relationship with water turbulence and water depth, being commonly found in deep environments with high flow rates. However, an alternative viewpoint of this relationship between morphotype abundance and habitat preference, is these abiotic factors (water turbulence and depth) are causing this change in morphology.

Dillon (2019) had a similar hypothesis when investigating the ecophenotypic variation in another closely related Planorbidae species, *Helisoma trivolvis*. He found two genetically identical populations of *H. trivolvis*, had two contrasting shell morphologies depending on whether the snails lived in a still, pond (morphotype-A) or the base of a flowing dam (morphotype-B) in Lake Wakendaw, South Carolina. He proposed that the snails in the pond had a different shell morphology than the snails inhabiting the base of the dam in response to the difference in flowrate of the water. This was a rational idea as the large discoidal shape and small apertural opening of the morphotype-A shells allows them to enfold air into their shell cavity and use it to reach floating vegetation. Conversely, the low and broad shape of the morphotype-B shells are better suited at reducing drag from the flowing water and allows the snail to hold onto rocks more effectively with their larger aperture. Furthermore, if these morphotypes were placed into the opposite environment, they would struggle to survive. The morphotype-A snails would be constantly swept away by the water current due to their large surface area, while the morphotype-B snails would not be able to access floating food sources as their large aperture cannot trap air. Additionally, the morphotype-B snails would be vulnerable to predation as their large apertural opening will not stop predators attacking their body. When theorising what abiotic factors influence *Biomphalaria* morphology, it is rational to assume that *Biomphalaria* snails use a similar adaptation to *Helisoma* snails in response to an environment with a high flowrate. Moreover, this could be the missing factor in the Jarne et al. (2011) explanation, as the water pressure entering the shell cavity through the aperture could result in the shell widening during development. It is however possible that other factors, such as water chemistry (pH, salinity, total dissolved solids, conductivity etc.) or the presence of predators could also contribute to the morphological changes seen in *Biomphalaria* (Haas, 2003; Hoverman et al. 2005; Hoverman & Relyea, 2007).

One potential future application of the landmark-based geometric morphometric technique displayed in Chapter 3, is to implement the data generated by the technique into identification software that uses machine learning to identify medically important hosts. For example, the website application programming interface (webAPI) tool, 'snail host detector' uses artificial intelligence to detect the presence of medically important intermediate snail species in real-time, using images uploaded by smart phones (Figure 7.1). It was developed by the Action Towards Reducing Aquatic Snail Borne Parasitic Diseases (ATRAP) research group and is a collaborative project between the Mbarara University of Science and Technology (MUST) and The Royal Museum for Central Africa (RMCA)

(https://snaildetector.africamuseum.be/). The tool is specifically designed to detect the presence of snail shells in a photo and identify whether they are capable of spreading gastropod-borne diseases such as schistosomiasis (*Biomphalaria*) or fascioliasis (*Lymnaea*). It currently utilises the 'You Only Look Once' (YOLOv4) algorithm created by Bochkovskiy et al. (2020), which is a convolutional neural network object detection program that was trained using 2,500 images of snails collected by locals living in the Southern region of Lake Albert, Uganda. The model can currently detect four main groups of snails: *Biomphalaria spp.*, *Lymnaea spp.*, *Gyraulus spp.* (commonly misidentified as *Biomphalaria*, but is not medically relevant), and other non-intermediate host species (Figure 7.1). The researchers behind this tool aim to collect more images to improve its performance and also build an image database to develop region-specific models.

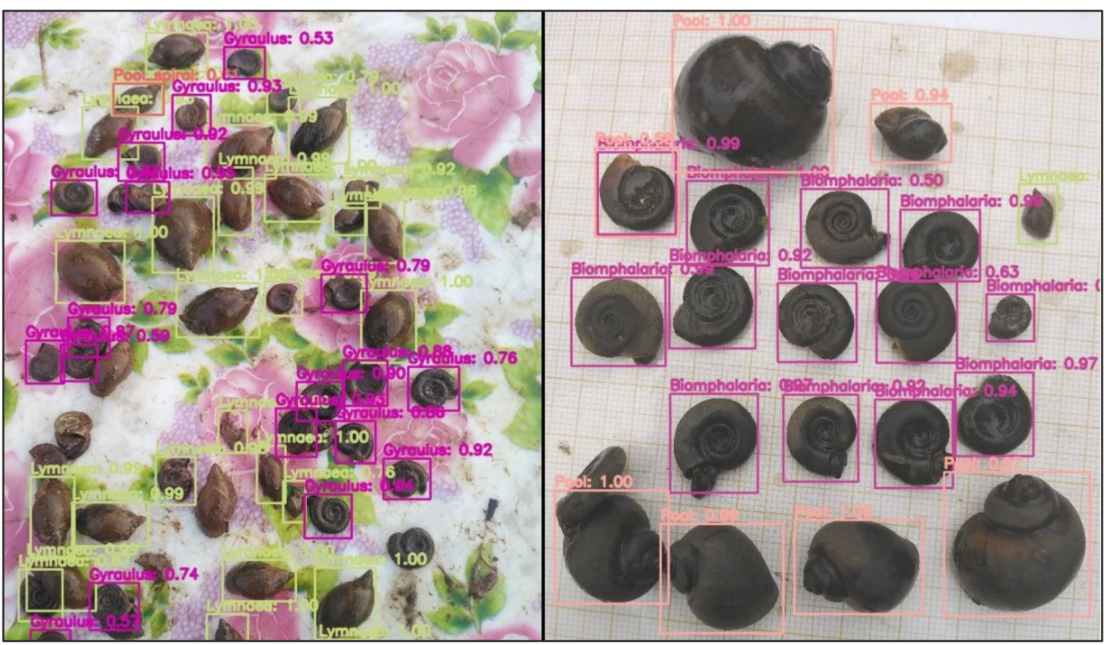



Figure 7.1. Example of the snail host detector tool created by ATRAP discriminating between different freshwater snail genera collected from Lake Albert. Photos adapted from https://snaildetector.africamuseum.be/.

The identification of *Biomphalaria* species is crucial for monitoring and controlling schistosomiasis (Abe et al., 2018). The more we understand about what abiotic factors influence shell morphology, the better we will understand what constitutes a valid species, which will undoubtedly help the future efforts of conchological identification methods and schistosomiasis control.

## 7.2 The Prevalence of *Schistosoma mansoni* at the African Great Lake, and the Abiotic and Biotic Factors Influencing Transmission:

The East African countries of Kenya, Tanzania, and Uganda are frequently surveyed and researched due to their high levels of endemic gastropod-borne parasitic and soil-transmitted helminthic diseases (Brooker et al., 2009). In Chapters 4 and 5, we investigated the prevalence of *S. mansoni* infection within the *Biomphalaria* species found at Lake Albert and Lake Victoria. When looking at just the Ugandan shorelines of each lake, we found the Masindi district of Lake Albert (12.5%) had a higher mean prevalence of *S. mansoni* infection among its *Biomphalaria* populations compared to the Mayuge district of Lake Victoria (5%). This finding was consistent with the original Rowel et al. (2015) study, which also investigated the prevalence of *S. mansoni* infection using traditional parasitological methods. However, when we compare the prevalence of *S. mansoni* infection of Lake Victoria in Chapter 5, we find the mean prevalence of infection for the Ugandan shoreline increases to 8.2%. This is likely due to the inclusion of more *B. choanomphala* populations, with infection being found in the Busia, Kalangala, Masaka and Mukono districts of Lake Victoria, in addition to the previously mentioned Mayuge district. When we look at the whole of Lake Victoria, the mean prevalence of infection increases again to 9.3% following the inclusion of the Kenyan and Tanzanian *B. choanomphala* populations, with infection being found in the Kisumu and Siaya counties of Kenya, as well as the Ilemela, Rorya, Nyamagana, Sengerema and Ukerewe districts of Tanzania.

In addition to measuring infection prevalence, we also investigated what abiotic factors influenced the prevalence of *S. mansoni* infection of the *Biomphalaria* species found at Lake Albert and Lake Victoria. In Chapter 4, we found the mean infection prevalence among *Biomphalaria* populations at the Lake Albert sites (Bugoigo and Walukuba) were consistently higher during the wet seasons than the dry seasons. Similarly, this trend was also found at the Lake Victoria sites (Bugoto and Lwanika), with *B. choanomphala* populations having consistently higher infection prevalence during the wet seasons compared to the dry seasons. We proposed the reason for this increase in infection prevalence during the wet seasons was due to the change in water chemistry of the lake water (higher dissolved oxygen levels and lower water conductivity levels) from the increased rainfall, creating more favourable conditions for snail population growth and infection. Additionally, the increased rainfall and flooding could transport snails to new habitats and increase the amount of human/animal waste washed into freshwater sources. In Chapter 5, we investigated the effects of specific factors on the prevalence of *S. mansoni* infection among *B. choanomphala* populations across Lake Victoria. We found *S. mansoni* infection prevalence had a significant positive relationship with only one biotic factor (*B. choanomphala* abundance) and two abiotic factors (calcium and magnesium). Conversely, *S. mansoni* infection prevalence had a significant negative relationship with the abiotic factor of water pH (increasing water alkalinity). However, we deemed all three of the abiotic factors to not be directly influencing infection prevalence, as we found *B. choanomphala* abundance also had a significant positive relationship with high levels of calcium and magnesium, and a significant negative relationship with increasing water alkalinity. Therefore, *Biomphalaria* abundance was likely to be the only primary factor influencing infection prevalence, while all of the abiotic factors

like calcium/magnesium levels or the alkalinity of the water were indirectly affecting infection prevalence by reducing the number of available hosts. Similarly in Chapter 4, we deemed seasonality as a non-significant factor, as the differences in infection prevalence between the wet and dry seasons were not statistically significant. Furthermore, similar studies in other parts of Africa found contradictory results to us (higher prevalence of infection in the dry season), leading us to conclude that seasonality was not a significant factor of *S. mansoni* infection prevalence.

In addition to *Biomphalaria* abundance, the other biotic factor investigated was snail host genetic diversity. In Chapter 4, we found the *Biomphalaria* species from Lake Victoria (*B. choanomphala*) had a greater level of intra-species genetic diversity than the species from Lake Albert (*B. pfeifferi*, *B. stanleyi*, and *B. sudanica*). This low level of intra-species genetic diversity of the *B. pfeifferi*, *B. stanleyi*, and *B. sudanica* snails at Lake Albert could indicate these populations prefer to self-fertilize or inbreed compared to the *B. choanomphala* populations of Lake Victoria (Campbell et al., 2010). We also found the infection prevalence of the *B. choanomphala* at Lake Victoria were the lowest when compared to the *B. pfeifferi*, *B. stanleyi*, and *B. sudanica* populations of Lake Albert. This higher genetic diversity in the *B. choanomphala* populations could explain why there is a lower infection prevalence than the *Biomphalaria* populations at Lake Albert, as previous parasitological studies (Coltman et al., 1999; Jarne & Théron, 2001; Campbell et al., 2010) found host populations with higher levels of genetic diversity have less prevalence of parasites and disease compared to populations with low levels of genetic diversity. Therefore, we believed our result was intuitive and logical as one of the key reasons why sexual reproduction evolved was to help increase the genetic diversity and adaptability of a population in response to an ever-changing environment such as the introduction of a new parasite (Hamilton et al., 1990). This is due to the likelihood of a parasitic infection being successful or not, can depend on the level of genomic compatibility between an invading parasite and its host. In the case of *S. mansoni*, the level of genomic compatibility between the snail and schistosome, can determine whether the invading miracidium will successfully evade the host's immune defences or not (Mitta et al., 2012; Theron et al., 2014). Moreover, schistosome resistance in snails can be expressed in several different ways, such as preventing the miracidium from entering (via cytotoxic agents in the mucus) (Coyne et al., 2015) or by containing the infection by encapsulating the mother sporocyst (Théron et al., 1997; Lu et al., 2016). However, when we examined the Lake Albert and Lake Victoria sites individually, we discovered the *Biomphalaria* populations at sites with a higher prevalence of infection, also had higher levels of intra-species genetic diversity (Table 4.1; Table 5.2).

In response to this contradictory trend, we proposed in Chapters 4 and 5 that the reason for this high prevalence of infection among *Biomphalaria* populations with high genetic diversity could be from the 'coevolution selective sweep' phenomenon, which occurs when an antagonistic relationship between a coevolving host and parasite results in selective sweeps of host resistance adaptations and consecutive counter-adaptations of the parasite (Kawecki et al., 2012; Auld & Tinsley, 2015). For example, a beneficial host adaptation such as resistance to *S. mansoni*, could become widespread throughout a population of *Biomphalaria*, resulting in less genetic diversity as individuals without the gene will be at a

selective disadvantage and eventually die off. It is therefore possible, that *Biomphalaria* populations with low genetic diversity and low levels of infection have experienced this 'selective sweep', while populations with higher levels of genetic diversity and higher levels of *S. mansoni* infection have not.

Alternatively, in Chapter 5 we discuss this trend could be the result of the migratory habits of *B. choanomphala* snails. This is because the introduction of snails from one site to another could cause higher levels of genetic diversity as it would allow for the exchange of new alleles. Consequently, this migration could also result in the introduction of *S. mansoni* from one population to another and potentially create a positive feedback loop in infection prevalence between the intermediate snail hosts and the definitive mammal hosts. Alternatively, we discussed how Webster & Gower (2006) found that *B. glabrata* snails preferred to mate with individuals that were less infected with *S. mansoni,* and that this selection preference was stronger when the proportion of infected individuals within a population was higher. This suggests that non-random mating behaviour plays a role in the maintenance of resistance to *S. mansoni* and reduces genetic diversity within a population. It is therefore possible, populations within Lake Victoria which have a high genetic diversity and high levels of infection may not exhibit this non-random mating behaviour. Instead, they favour random mating, which promotes higher amounts of genetic diversity instead of *S. mansoni* resistance, as *Biomphalaria* with greater genetic diversity have a longer mean life span, a higher fecundity rate and more successful offspring (faster incubation period and more successful hatch rate) (Sandland et al., 2007). Conversely, populations which have low genetic diversity and low levels of infection could exhibit non-random mating behaviour, favouring *S. mansoni* resistance over higher levels of genetic diversity.

In summary, the relationship between *S. mansoni* infections among *Biomphalaria* snails is complex and multifaceted, with a wide range of factors influencing the likelihood of *Schistosoma* infection becoming hyper endemic within a snail population. Environmental factors such as water temperature, pH levels and the physiochemical composition of the water play a significant role in shaping the transmission dynamics of *S. mansoni* prevalence within *Biomphalaria* snails. Likewise, biological factors such as the presence of alternative hosts or the amount of genetic diversity within a population can influence the transmission dynamics of the parasite as certain snail host species or definitive hosts may exhibit higher levels of compatibility with *S. mansoni*, or support higher parasite loads, respectively. Furthermore, human activities and socio-economic factors can influence the likelihood of hyperendemic schistosome infections within a population, with poor sanitation, inadequate access to clean water and lack of health education contribute to the persistence and spread of infection. Given the complex interplay of abiotic and biotic factors, researching and understanding which abiotic and biotic factors contribute to high levels of schistosome infection is essential when developing sustainable control strategies to reduce the burden of schistosomiasis, such as modifying water sources and combining drug treatments with snail control measures will effectively disrupt the life cycle of the parasite.

### 7.3 Gastropod-Borne Parasitic Disease in Europe:

In Chapter 6, we investigated the prevalence of helminthic parasites found within nine families of terrestrial gastropods found in and around the city of Nottingham. In total 533 nematodes were counted, but not one medically or veterinary important nematode species was identified. However, of the 26 gastropod species identified in and around the city of Nottingham, 16 are potential hosts for *Angiostrongylus vasorum*, eight are potential hosts for *Crenosoma vulpis* and five are potential hosts for *Aelurostrongylus abstrusus* (Supplementary Table 6.4). The presence of these intermediate gastropod species would facilitate the transmission of these veterinary lungworms if they were ever introduced into the city of Nottingham. The introduction of *An. vasorum* in the Midlands is a likely eventuality, as there are documented migration of the *An. vasorum* northwards from Southeast England (Helm et al., 2015; Aziz et al., 2016).

In addition to the veterinary important lungworm species, three of the terrestrial gastropod species commonly found in and around the city of Nottingham are potential intermediate hosts of the medically important lungworm species, *Angiostrongylus cantonensis* (Supplementary Table 6.4). *Angiostrongylus cantonensis* can cause severe gastrointestinal and/or central nervous system disease in humans and is typically transmitted through the consumption of infected gastropods (intermediate host), freshwater crustacea/amphibians (paratenic hosts) or fruits and vegetables contaminated with gastropod slime/faeces/corpses. Despite being endemic to tropical and subtropical regions (such as Southeast Asia and the Pacific islands), 22 cases of human angiostrongyliasis have been reported in Europe from 1988 to 2019 (Federspiel et al., 2020), with the first autochthonous case being reported in mainland France (Nguyen et al., 2017). Alongside human cases, *An*. *cantonensis* has a low host specificity and can infect a large number of mammals, with reports of *An*. *cantonensis* infecting common Eulipotyphla (*Atelerix algirus*) and Rodentia (*Mus musculus* and *Rattus norvegicus*) species in the Canary and Balearic Islands (Paredes-Esquivel et al., 2019; Gonzálvez & Ruiz de Ybanez, 2022). The current distribution and presence of *An*. *cantonensis* is heavily dependent on the availability of intermediate (gastropods) and definitive hosts (typically rats). The current amounts of intercontinental travel, immigration and trade with countries endemic with *An*. *cantonensis*, creates an increased risk of *An*. *cantonensis* being introduced and established in Europe. The low host specificity of *An*. *cantonensis* allows it to infect a wide variety of European gastropod and mammal species, which further increases its chances of successfully invading new territory.

The idea of diseases being introduced from developing countries to developed countries is a concerning issue. This is typically seen as a result of globalisation and ever increasing amounts of intercontinental travel and trading from countries where disease is more prevalent. This is especially concerning, as newly introduced diseases can spread quickly due to the high population density and high levels of mobility found within developed countries. This can lead to potentially serious outbreaks, which can have a significant impact on public health. However, the majority of reports and concern about the introduction of tropical diseases into Europe are commonly vector-borne diseases perpetrated by arthropod species like mosquitoes (Schaffner et al., 2014, Rezza et al., 2007, Gould et al., 2010), with the potential introduction of gastropod-borne parasitic diseases being often overlooked. This is

likely due to the perception that developed countries are ‘protected from’ diseases caused by helminthic parasites as they have access to safe drinking water and adequate hygiene and sanitation practises. However, this assumption is often proven false when gastropod-borne parasitic diseases unexpectedly appear and establish themselves in areas where it was believed they could not occur. One recent and on-going example of this, was the 2013 outbreak of urogenital schistosomiasis in the French, Mediterranean island of Corsica.

The 2013 outbreak in Corsica had reports of over 100 cases of urogenital schistosomiasis, which was a result of the introduction of *Schistosoma haematobium* and a *S. haematobium-S. bovis* hybrid species from Senegal (Boissier et al., 2016). The suspected intermediate snail host was *Bulinus truncatus*, an endemic freshwater snail species present in the Cavu river, a popular location for recreational bathing and swimming by tourists and locals (Oleaga et al., 2019). Boissier et al. (2016) explains that Corsica's popularity as a tourist destination adds an additional risk factor as the intermediate snail, *B. truncatus*, is found in several southern European countries (France, Greece, Italy and Spain) and approximately 3 million visitors travel to Corsica annually. This poses the risk of a ‘worst-case scenario’, where large numbers of visitors and the abundance of suitable intermediate snail hosts across southern Europe could result in the rapid spread of urogenital schistosomiasis, regardless of the protective measures of good hygiene and sanitation practice found in European countries. Moreover, the nature of this newly emerged *S. haematobium-S. bovis* hybrid is still not fully understood, making it even more difficult to evaluate the situation.

Gastropod-borne diseases continue to pose a significant health challenge in the world, particularly in developing nations (Lu et al., 2018). The risk of introducing and spreading gastropod-borne parasitic diseases is ever-increasing due to the always increasing levels of intercontinental travel and trade between endemic and non-endemic countries. Malacological surveys are essential for gaining an in-depth understanding of the epidemiology of gastropod-borne parasitic diseases and formulating effective control strategies to reduce and prevent the spread of gastropod-borne parasitic disease. Through such surveys, relevant data can be collected to identify the distribution and abundance of key intermediate hosts and help identify areas of high disease risk and can assist in the development of effective management strategies.

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# Appendix:

Pages 170-185: Alignment 1 - rRNA 16S *Biomphalaria* - Chapter 3

Pages 186-204: Alignment 2 - COI *Biomphalaria* - Chapter 3

Pages 205-208: Alignment 3 - ITS Nematode Tree A - Chapter 6

Pages 209-213: Alignment 4 - ITS Nematode Tree B - Chapter 6

Pages 214-217: Alignment 5 - ITS Nematode Tree C - Chapter 6

Pages 218-220: Alignment 6 - ITS Nematode Tree D - Chapter 6

Pages 221-225: Alignment 7 - 18S Trematode Tree E - Chapter 6

Pages 226-228: Alignment 8 - 18S Trematode Tree F - Chapter 6

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Bugoigo_P7F07    --------------------------------------------------------------------
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| Lwanika_P6G02 | C T G C C C A A T |
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| Lwanika_P7C11 | C T G C C C A A T |
| Lwanika_P7D12 | C T G C C C A A T |
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| Walukuba_P2G03 | C T G C C C - A T |
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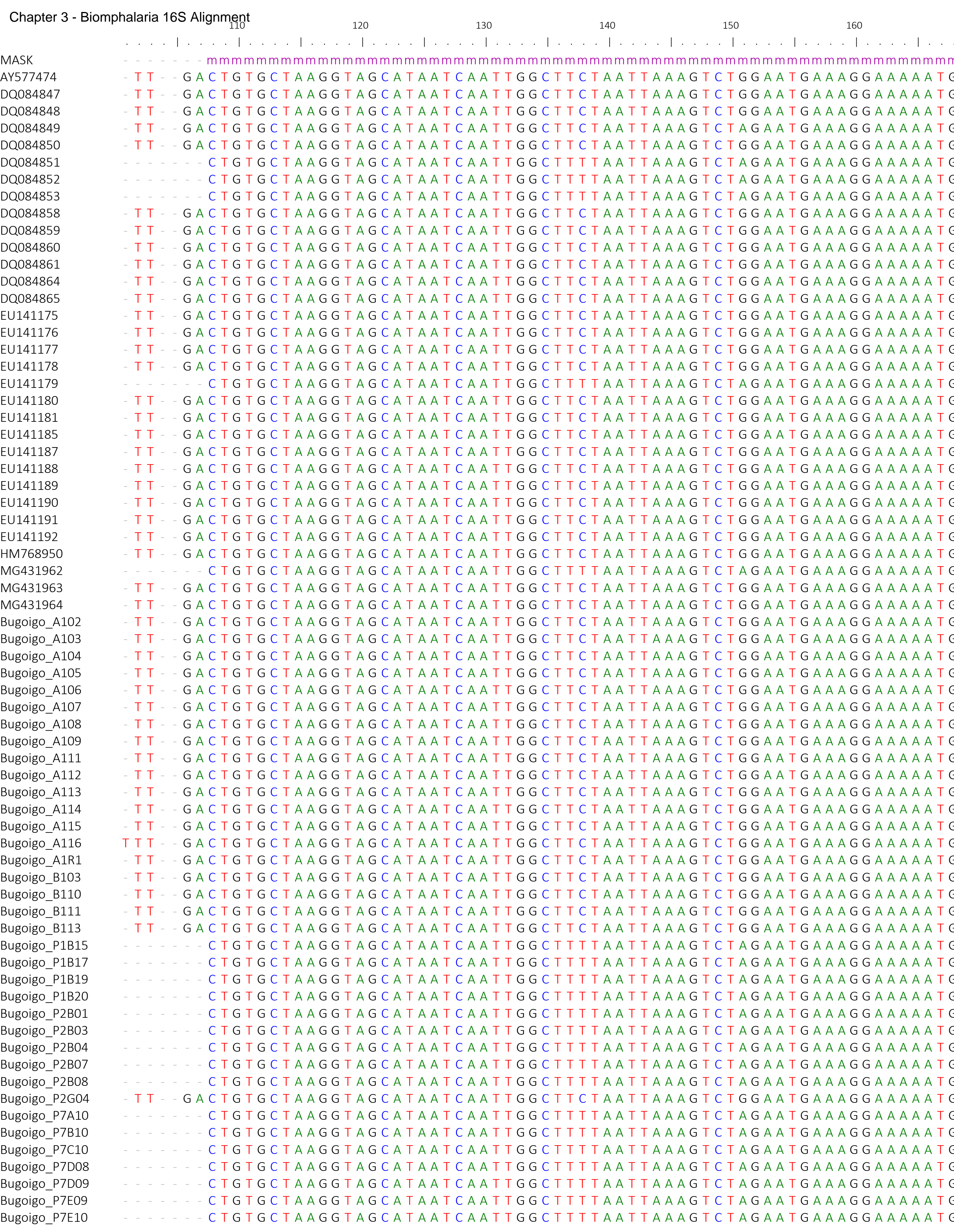
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Bukoba_P2A04    - T T - - G A C K G T G C T A A G G T A G C A W A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
Bukoba_P2A07    - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
Bukoba_P2C06    - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
Bukoba_P2E01    - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
Bukoba_P2E03    - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
Bukoba_P2E05    - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
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Bukoba_P2E08    - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
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Bukoba_P7E11    - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
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Piida A102      - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
Piida A103      - T T T - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
Piida A107      - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
Piida A108      - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
Piida A109      - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
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Piida A112      - T T - - G A C T G T G C T A A G G T A G C A T A A T C A A T T G G C T T C T A A T T A A A G T C T G G A A T G A A A G G A A A A A T G
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| Sample | Sequence |
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| Lwanika_P6E10 | TTTATTTAAATGATGAAAATATCA-TTTTTTAGAAAAAAGACGAGAAGACCCTTAGAGTTTTTATTA- |
| Lwanika_P6F02 | TTTATTTAAATGATGAAAATATCATTTTTTTAGAAAAAAGACGAGAAGACCCTTAGAGTTTTTATTA- |
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| Walukuba_P9E01 | TTTATTTAAATGATGAAAATATCA-TTTTTTAGAAAAAAGACGAGAAGACCCTTAGAGTTTTTATTA- |
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## Chapter 3 - Biomphalaria 16S Alignment

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MG431964        TGAACTTTACTAATTTTTTTTAAATCGATTTTAATAATAAGATTAATTAAACTACCTTAGGGATAACA
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Bugoigo_P7D09   TAAACTTTACTAT--ATTATTTAATCGA-TTTGGTAATAAAAATAATTAAACTACCTTAGGGATAACA
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Bukoba_LV08     TAAACTTTACTAA--TTTTTTTAATCGA-TTTAATAATAAAATTAATTAAACTACCTTAGGGATAACA
Bukoba_LV10     TAAACTTTACAAT--TTTTTTAAATCGA--TTAATAATAAGATTAATTAAACTACCTTAGGGATAACA
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Bukoba_P2A07    TGAACTTTACTAA--TTTTTTAAATCGA--TTAATAATAAGATTAATTAAACTACCTTAGGGATAACA
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Bukoba_P9C03    TGAACTTTACTAA--TTTTTTTAATCGA--TTAATAATAAGATTAATTAAACTACCTTAGGGATAACA
Bukoba_P9F04    TGAACTTTACTAA--TTTTTTAAATCGA--TTAATAATAAGATTAATTAAACTACCTTAGGGATAACA
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Lwanika_P6G02     TAAACTTTACAA---TTTTTTAAATCGA-TTTAATAATAAGATTAATTAAACTACCTTAGGGATAACA
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Lwanika_P9E04     TGAACTTTACTAA--TTTTTTAAATCGA--TTAATAATAAGATTAATTAAACTACCTTAGGGATAACA
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Walukuba_P9G01   TAAACTTTACTAA---TTTTTTAATCGA--TTAATAATAAGATTAATTAAACTACCTTAGGGATAACA
Walukuba_P9H01   TAAACTTTACTAA---TTTTTTAATCGATTTAATAATAAGATTAATTAAACTACCTTAGGGATAACA
MASK             mmmmmmmmmm------mmmmmmmmmmmm--mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
```



```
                 410       420       430       440       450       460
MASK             mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm-------------------------------
AY577474         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTCGAACAATTTATCCTACG
DQ084847         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAA-------------------------------
DQ084848         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTCGAACAATTTT-------
DQ084849         TGTTGGACTAGGAACTTTATAACTAGCCGTTAGAAAAGATTTGTTCTGTTCGAACAATTT--------
DQ084850         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTCGAACAATTTTATCCTAC
DQ084851         TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
DQ084852         TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAACATTTGTTCTGTTC-----------------
DQ084853         TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
DQ084858         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
DQ084859         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
DQ084860         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
DQ084861         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
DQ084864         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
DQ084865         TGTTGGACTAGGAACTTTATGGTTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141175         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141176         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141177         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141178         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141179         TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
EU141180         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141181         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141185         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141187         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141188         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141189         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141190         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141191         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
EU141192         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
HM768950         TGTTGGACTAGGAACTTTATGGCTAGCCGTCTTAAAAGATTTGTTCTGTTCGAACAATTTTATCCTAC
MG431962         TGTTGGACTAGGAACTTTATGGTTAGCCACCATAAAAGATTTGTTCTGTTC-----------------
MG431963         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
MG431964         TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTCGAACAATTTTATCCTAC
Bugoigo_A102     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A103     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A104     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A105     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A106     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A107     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A108     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A109     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A111     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A112     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A113     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A114     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A115     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A116     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_A1R1     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_B103     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_B110     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_B111     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_B113     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P1B15    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P1B17    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P1B19    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P1B20    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P2B01    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P2B03    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P2B04    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P2B07    TGTTGGACTAGGAACTTTATGGTTAGCCATCAKAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P2B08    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P2G04    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P7A10    TGTTGGACTAGGAACTTTATGGTTAGCCRKCGYAAAAGATTTGTTCTGTTY-----------------
Bugoigo_P7B10    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P7C10    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
Bugoigo_P7D08    TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------
```

```
Bugoigo_P7D09   TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC----------------
Bugoigo_P7E09   TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC----------------
Bugoigo_P7E10   TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC----------------
Bugoigo_P7F07   TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC----------------
Bugoigo_P7F09   TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC----------------
Bugoigo_P7H07   TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC----------------
Bugoigo_P7H09   TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P2A08    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P2A10    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P2E04    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P2G09    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6B05    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6C05    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6C06    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6C07    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6D05    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6D08    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6D09    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6E05    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6E06    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6E07    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6E09    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6F08    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6F09    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6G06    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6G07    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bugoto_P6G09    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_LV01     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_LV08     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_LV10     TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P2A04    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAARATTTGTTCTGTTC----------------
Bukoba_P2A07    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P2C06    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P2E01    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P2E03    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P2E05    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
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Bukoba_P2E07    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P2E08    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P6G08    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P7D11    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P7E11    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P7E12    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P7H12    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P9B03    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P9C03    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Bukoba_P9F04    TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A101      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A102      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAARATTTGTTCTGTTC----------------
Piida A103      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAARATTTGTTCTGTTC----------------
Piida A107      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A108      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A109      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A110      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAARATTTGTTCTGTTC----------------
Piida A112      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A117      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A118      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A119      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A201      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A203      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A207      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A211      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A212      TGTTGGACTAGGARCYTTWTGGCTARCCGTCATARRARATTTGTTCTGTTC----------------
Piida A214      TGTTGGACTAGGAACTTTATGGCTAGCCGTCRTAAAARATTTGTTCTGTTC----------------
Piida A215      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A217      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Piida A219      TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Lwanika_P2D02   TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Lwanika_P2D03   TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Lwanika_P2D08   TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Lwanika_P2H07   TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
Lwanika_P2H09   TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC----------------
```

Lwanika_P6B02 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P6C02 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P6D03 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P6D10 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P6E10 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P6F02 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P6F04 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P6F10 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P6G01 TGTTGGACTAGGAACTTTATGGCTAGCCGYCAWAAAAGATTTGTTCTGTTC-----------------

Lwanika_P6G02 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P7B11 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P7C11 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P7D12 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P7F12 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Lwanika_P9E04 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A101 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A102 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A103 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A104 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A105 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A107 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A108 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A109 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A111 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_A112 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B101 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B102 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B103 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B104 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B105 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B106 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B107 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B108 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B109 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B110 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B111 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B112 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B114 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B116 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_B118 TGTTGGACTAGGAACTTTATGGTTAGCCATCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2B09 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2B10 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2E09 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2E10 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2F01 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2F02 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2F03 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2F04 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2F05 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2F06 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2F07 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2F08 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2F09 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2G01 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2G03 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2G06 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2G07 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P2G08 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P6A03 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P6A04 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P6A05 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P7A07 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P7C09 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P7D10 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P7F10 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P9A01 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P9B01 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P9C01 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P9D01 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P9D02 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P9E01 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

Walukuba_P9F01 TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------

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Walukuba_P9F03   TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Walukuba_P9G01   TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
Walukuba_P9H01   TGTTGGACTAGGAACTTTATGGCTAGCCGTCATAAAAGATTTGTTCTGTTC-----------------
MASK             mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm-------------------------------
```

Chapter 3 - Biomphalaria 16S Alignment

```
                 ....|..
MASK             -------
AY577474         -------
DQ084847         -------
DQ084848         -------
DQ084849         -------
DQ084850         -------
DQ084851         -------
DQ084852         -------
DQ084853         -------
DQ084858         -------
DQ084859         -------
DQ084860         -------
DQ084861         -------
DQ084864         -------
DQ084865         -------
EU141175         -------
EU141176         -------
EU141177         -------
EU141178         -------
EU141179         -------
EU141180         -------
EU141181         -------
EU141185         -------
EU141187         -------
EU141188         -------
EU141189         -------
EU141190         -------
EU141191         -------
EU141192         -------
HM768950         -------
MG431962         -------
MG431963         -------
MG431964         CAGTTTC
Bugoigo_A102     -------
Bugoigo_A103     -------
Bugoigo_A104     -------
Bugoigo_A105     -------
Bugoigo_A106     -------
Bugoigo_A107     -------
Bugoigo_A108     -------
Bugoigo_A109     -------
Bugoigo_A111     -------
Bugoigo_A112     -------
Bugoigo_A113     -------
Bugoigo_A114     -------
Bugoigo_A115     -------
Bugoigo_A116     -------
Bugoigo_A1R1     -------
Bugoigo_B103     -------
Bugoigo_B110     -------
Bugoigo_B111     -------
Bugoigo_B113     -------
Bugoigo_P1B15    -------
Bugoigo_P1B17    -------
Bugoigo_P1B19    -------
Bugoigo_P1B20    -------
Bugoigo_P2B01    -------
Bugoigo_P2B03    -------
Bugoigo_P2B04    -------
Bugoigo_P2B07    -------
Bugoigo_P2B08    -------
Bugoigo_P2G04    -------
Bugoigo_P7A10    -------
Bugoigo_P7B10    -------
Bugoigo_P7C10    -------
```

First Three Frames: ATT (Ile)

```
                 10        20        30        40        50        60
MASK             ------------------------------------------------------------mmmmmmmm
DQ084828         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
DQ084829         ------------------------------------------TACTGGACTATCATTATTAATTCGTT
DQ084830         ------------------------------------------TACTGGACTATCATTATTAATTCGTT
DQ084831         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
DQ084837         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
DQ084838         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
DQ084839         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
DQ084840         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
DQ084843         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
DQ084844         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
MG431962         ------------------------------------------TACTGGACTATCATTATTAATTCGTT
MG431963         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
MG431964         ------------------------------------------TACTGGATTATCATTATTAATTCGTT
HM769133         TACTTTATATATAATTTTTGGGATTTGATGTGGTCTGGTTGGTACTGGATTATCATTATTAATTCGTT
MG431966         TACTTTATACATAATTTTTGGGATTTGATGTGGTCTAGTTGGTACTGGGTTATCATTATTGATTCGTT
EU141215         ------------------------------------------------------------AATTCGTG
EU141216         ------------------------------------------------------------AATTCGTG
EU141217         ------------------------------------------------------------AATTCGTG
EU141218         ------------------------------------------------------------AATTCGTG
EU141219         ------------------------------------------------------------AATTCGTG
EU141220         ------------------------------------------------------------AATTCGTG
EU141221         ------------------------------------------------------------AATTCGTG
EU141225         ------------------------------------------------------------AATTCGTG
EU141227         ------------------------------------------------------------AATTCGTG
EU141228         ------------------------------------------------------------AATTCGTG
EU141229         ------------------------------------------------------------AATTCGTG
EU141230         ------------------------------------------------------------AATTCGTG
EU141231         ------------------------------------------------------------AATTCGTG
EU141232         ------------------------------------------------------------AATTCGTG
DQ084836         ----TTATATATAATTTTTGGGATTTGATGTGGTCTGGTTGGTACTGGATTATCATTATTAATTCGTT
DQ084825         ---------TATAATTTTTGGGATTTGATGTGGTCTGGTAGGTACTGGATTATCATTATTAATTCGTT
DQ084826         ----TTATATATAATTTTTGGGATTTGATGTGGTCTAGTTGGTACTGGATTATCATTATTAATTCGTT
DQ084827         --CTTTATATATAATTTTTGGAATTTGATGTGGTCTAGTTGGTACTGGGTTATCATTATTAATTCGTT
Bugoigo A102     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A103     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A104     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A105     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A106     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A107     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A108     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A109     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A111     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A112     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A113     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A114     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A115     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A116     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo A1R1     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo B103     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo B110     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo B111     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo B113     ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo P1B15    ------------------------------------------TACTGGACTATCATTATTAATTCGTT
Bugoigo P1B17    ------------------------------------------TACTGGACTATCATTATTAATTCGTT
Bugoigo P1B19    ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo P1B20    ------------------------------------------TACTGGACTATCATTATTAATTCGTT
Bugoigo P6E09    ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo P6F08    ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo P6F09    ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo P6G06    ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo P6G07    ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo P6G09    ------------------------------------------TACTGGATTATCATTATTAATTCGTT
Bugoigo P7A10    ------------------------------------------TASWGGACTATCATTATKAATTYKTT
Bugoigo P7B10    ------------------------------------------TACTGGACTATCATTATTAATTCGTT
Bugoigo P7C10    ------------------------------------------TACTGGACTATCATTATKAATTCGTT
Bugoigo P7D08    ------------------------------------------TACTGGACTATCATTATTAATTCGTT
Bugoigo P7D09    ------------------------------------------TACWGGASTATCATYATKAWTTYKTK
Bugoigo P7E09    ------------------------------------------TRSWGGWCWATYWTYATGATTTYKTK
Bugoigo P7E10    ------------------------------------------TRSWGGAYWATYWTYRTGAWTTYKTK
```

| Sample | Sequence |
|---|---|
| Bugoigo P7F07 | ----------------------------------------TACTGGACTATCATTATKAATTCGTK |
| Bugoigo P7F09 | ----------------------------------------TRSWGGATTATYWTYATKAWTTYKTK |
| Bugoigo P7H07 | ----------------------------------------TACTGGATTATCATTATTAATTCGTK |
| Bugoigo P7H09 | ----------------------------------------TRSWGKAYTATYWTYATKAWTTYKTK |
| Bugoto P2A08 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P2A10 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P2B01 | ----------------------------------------TACTGGACTATCATTATTAATTCGTT |
| Bugoto P2B03 | ----------------------------------------TACTGGACTATCATTATTAATTCGTT |
| Bugoto P2B04 | ----------------------------------------TACTGGACTATCATTATTAATTCGTT |
| Bugoto P2B07 | ----------------------------------------TACTGGACTATCATTATTAATTCGTT |
| Bugoto P2B08 | ----------------------------------------TACTGGACTATCATTATTAATTCGTT |
| Bugoto P2E04 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P2G04 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P2G09 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P6B05 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P6C05 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P6C06 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P6C07 | ----------------------------------------TACTGGATTATCATTATKAATTCGTT |
| Bugoto P6D05 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P6D08 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P6D09 | ----------------------------------------TACTGGATTATCATTATKAATTCGTT |
| Bugoto P6E05 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P6E06 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bugoto P6E07 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba LV01 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba LV08 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba LV10 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P2A04 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P2A07 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P2C06 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P2E01 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P2E03 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P2E05 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P2E06 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P2E07 | ----------------------------------------TACTGGATTATCATTGTTAATTCGTT |
| Bukoba P2E08 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P6G08 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P7D11 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P7E11 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P7E12 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P7H12 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P9B03 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P9C03 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Bukoba P9F04 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P2D02 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P2D03 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P2D08 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P2H07 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P2H09 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P6B02 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P6C02 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P6D03 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P6D10 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P6E10 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P6F02 | ----------------------------------------AACTGGATTATCATTATTAATTCGTT |
| Lwanika P6F04 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P6F10 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P6G01 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P6G02 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P7B11 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P7C11 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P7D12 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P9D04 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Lwanika P9E04 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Piida A101 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Piida A102 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Piida A103 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Piida A107 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Piida A108 | ----------------------------------------TACTGGATTATCATTATTAATTCKTT |
| Piida A109 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |
| Piida A110 | ----------------------------------------TACTGGATTATCATTATTAATTCGWT |
| Piida A112 | ----------------------------------------TACTGGATTATCATTATTAATTCGTT |

Piida A117 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Piida A118 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Piida A119 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Piida A201 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Piida A203 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Piida A207 --------------------------------------------- TACTGGATTATCATTATTAATTCKTT
Piida A211 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Piida A212 --------------------------------------------- TACTGGATTATCATTATKAWTTCKTK
Piida A214 --------------------------------------------- TACTGGATTATCATTATTWATTCGWT
Piida A215 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Piida A217 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Piida A219 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba A101 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba A102 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba A103 --------------------------------------------- TACWGGACTATCATTATKAATTCKTT
Walukuba A104 --------------------------------------------- TACWGGACTATCATTATKAATTCKTK
Walukuba A105 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba A107 --------------------------------------------- TACTGGACTATCATTATKAWTTCKTT
Walukuba A108 --------------------------------------------- TACWGGACTATYATTATKAWTTYKTK
Walukuba A109 --------------------------------------------- TACTGGATTATCATTATKAATTCKTK
Walukuba A111 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba A112 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B101 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B102 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B103 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba B104 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B105 --------------------------------------------- TACTGGACTATCATTATTAWTTCKTT
Walukuba B106 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B107 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B108 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B109 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B110 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B111 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B112 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba B114 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B116 --------------------------------------------- TACTGGACTATCATTATTAATTCGTT
Walukuba B118 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2B09 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2B10 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2E09 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2E10 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2F01 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2F02 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2F03 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2F04 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2F05 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2F06 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2F07 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2F08 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2F09 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2G01 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2G03 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2G06 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2G07 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P2G08 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P6A03 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P6A04 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P6A05 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P7A07 --------------------------------------------- TACASSAWWATCWTCGTGATTTYTTG
Walukuba P7C09 --------------------------------------------- TACWGGAWTATCATTATKAATTCTTG
Walukuba P7D10 --------------------------------------------- KASAGGAWTATCATYRTGAWTTCKTG
Walukuba P7F10 --------------------------------------------- KACAGGAWTATCATYRTGAWTTCKTG
Walukuba P9A01 --------------------------------------------- KASAGGWTTATCATCRTGAWTTCTTG
Walukuba P9B01 --------------------------------------------- TACWGGAWTATCATCKTGATTTCTTG
Walukuba P9C01 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P9D01 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P9D02 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P9E01 --------------------------------------------- TACAGKWTTATYATCKTGATTTCTTG
Walukuba P9F01 --------------------------------------------- TACAGSAWTATCATCGTGATTTCTTG
Walukuba P9F03 --------------------------------------------- TACTGGATTATCATTATTAATTCGTT
Walukuba P9G01 --------------------------------------------- KRSAGSMWAATYWTCGTGATTTYTTG
Walukuba P9H01 --------------------------------------------- TACTGGATTATCATTATKAATTCGTT

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MASK           -----------------------------------------------------------mmmmmmmmm
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## Chapter 3 - Biomphalaria COI Alignment

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                        110       120       130       140       150       160
               ....|....|....|....|....|....|....|....|....|....|....|....|...
MASK           mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
DQ084828       GAACATTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
DQ084829       GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
DQ084830       GAACATTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
DQ084831       GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
DQ084837       GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
DQ084838       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
DQ084839       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
DQ084840       GAACACTTTTATAACGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
DQ084843       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
DQ084844       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
MG431962       GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
MG431963       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
MG431964       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
HM769133       GAACACTTTTATAATGTCATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
MG431966       GAACATTTTTATAATGTTGTTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
EU141215       GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
EU141216       GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
EU141217       GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
EU141218       GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
EU141219       GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
EU141220       GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
EU141221       GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
EU141225       GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
EU141227       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
EU141228       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
EU141229       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
EU141230       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
EU141231       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
EU141232       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
DQ084836       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
DQ084825       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
DQ084826       GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
DQ084827       GAACATTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A102   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A103   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A104   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A105   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A106   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A107   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A108   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A109   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A111   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A112   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A113   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A114   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A115   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A116   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo A1R1   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo B103   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo B110   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo B111   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo B113   GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P1B15  GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P1B17  GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P1B19  GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P1B20  GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P6E09  GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P6F08  GAACACTTTTTTAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P6F09  GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P6G06  GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P6G07  GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P6G09  GAACACTTTTTTAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7A10  GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7B10  GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7C10  GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7D08  GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7D09  GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
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Bugoigo P7E09 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7E10 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7F07 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7F09 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7H07 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoigo P7H09 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2A08 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2A10 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2B01 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2B03 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2B04 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2B07 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2B08 GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2E04 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2G04 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P2G09 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P6B05 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P6C05 GAACACTTTTTTAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P6C06 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P6C07 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P6D05 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P6D08 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Bugoto P6D09 GAACACTTTTTTAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
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Bukoba LV08 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
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Bukoba P9C03 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
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Lwanika P6C02 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
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Lwanika P9D04 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
Lwanika P9E04 GAACACTTTTTTAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT
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Piida A109 GAACACTTTTATAATGTTATTGTTACGGCTCATGCTTTTATTATAATTTTTTTTATAGTTATACCTAT

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Walukuba B103     GAACACTTTTATAATGTTATTGTTACAGCTCATGCTTTCATTATAATTTTTTTTATAGTTATACCTAT
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Walukuba P7A07    GAACACTTTKATAATGWTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATARTTATACCTAT
Walukuba P7C09    GAACATTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
Walukuba P7D10    GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
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Walukuba P9F01    GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
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Walukuba P9H01   GAACACTTTTATAATGTTATTATTACGGCTCATGCTTTTATTATGATTTTTTTTATAGTTATACCTAT
MASK             mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
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Chapter 3 - Biomphalaria COI Alignment

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                       210       220       230       240       250       260
                 ....|....|....|....|....|....|....|....|....|....|....|....|...
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DQ084829         CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA
DQ084830         CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA
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DQ084838         CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
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MG431962         CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA
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Bugoigo A109     CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
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Bugoigo A112     CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
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Bugoigo B111     CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
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Bugoigo P6E09    CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
Bugoigo P6F08    CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
Bugoigo P6F09    CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
Bugoigo P6G06    CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
Bugoigo P6G07    CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
Bugoigo P6G09    CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA
Bugoigo P7A10    CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA
Bugoigo P7B10    CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA
Bugoigo P7C10    CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA
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| Sample | Sequence |
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| Bugoigo P7D09 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoigo P7E09 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoigo P7E10 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoigo P7F07 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoigo P7F09 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoigo P7H07 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoigo P7H09 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoto P2A08 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P2A10 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P2B01 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoto P2B03 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoto P2B04 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoto P2B07 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoto P2B08 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTGCTTCCA |
| Bugoto P2E04 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P2G04 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P2G09 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6B05 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6C05 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6C06 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6C07 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6D05 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6D08 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6D09 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6E05 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6E06 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bugoto P6E07 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba LV01 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGGATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba LV08 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba LV10 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P2A04 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P2A07 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P2C06 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P2E01 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P2E03 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P2E05 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P2E06 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P2E07 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P2E08 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P6G08 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P7D11 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P7E11 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P7E12 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P7H12 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P9B03 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P9C03 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Bukoba P9F04 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P2D02 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P2D03 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P2D08 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P2H07 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P2H09 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6B02 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6C02 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6D03 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6D10 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6E10 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6F02 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6F04 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6F10 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6G01 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P6G02 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P7B11 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P7C11 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P7D12 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P9D04 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Lwanika P9E04 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Piida A101 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Piida A102 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Piida A103 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |
| Piida A107 | CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCTCGAATAAATAATATATCATTTTGATTACTTCCA |

Piida A108 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A109 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A110 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A112 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A117 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A118 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A119 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A201 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A203 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A207 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A211 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A212 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A214 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A215 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A217 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Piida A219 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba A101 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba A102 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba A103 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba A104 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba A105 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba A107 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba A108 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba A109 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba A111 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba A112 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B101 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B102 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B103 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B104 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B105 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B106 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B107 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B108 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B109 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B110 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B111 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B112 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B114 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B116 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba B118 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G G A T A A A T A A T A T A T C A T T T T G A T T G C T T C C A
Walukuba P2B09 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2B10 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2E09 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2E10 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2F01 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2F02 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2F03 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2F04 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2F05 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2F06 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2F07 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2F08 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2F09 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2G01 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2G03 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2G06 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2G07 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P2G08 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C T C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P6A03 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P6A04 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P6A05 C T T T A T T A A T T G G T G C T C C C G A T A T A A A T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P7A07 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P7C09 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P7D10 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P7F10 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P9A01 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P9B01 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P9C01 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P9D01 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P9D02 C T T T A T T A A T T G G T G C T C C T G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A
Walukuba P9E01 C T T T A T T A A T T G G T G C T C C C G A T A T A A G T T T T C C C C G A A T A A A T A A T A T A T C A T T T T G A T T A C T T C C A

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Walukuba P9G01 CTTTATTAATTGGTGCTCCTGATATAAGTTTTCCCCGAATAAATAATATATCATTTTGATTACTTCCA
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MASK           mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
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| Piida A101 | AGTTGAAGGTGGGGTAGGTACAGGTTGAACTGTATACCCTCCTTTAAGTGGTCCTATTGCTCATGGTG |
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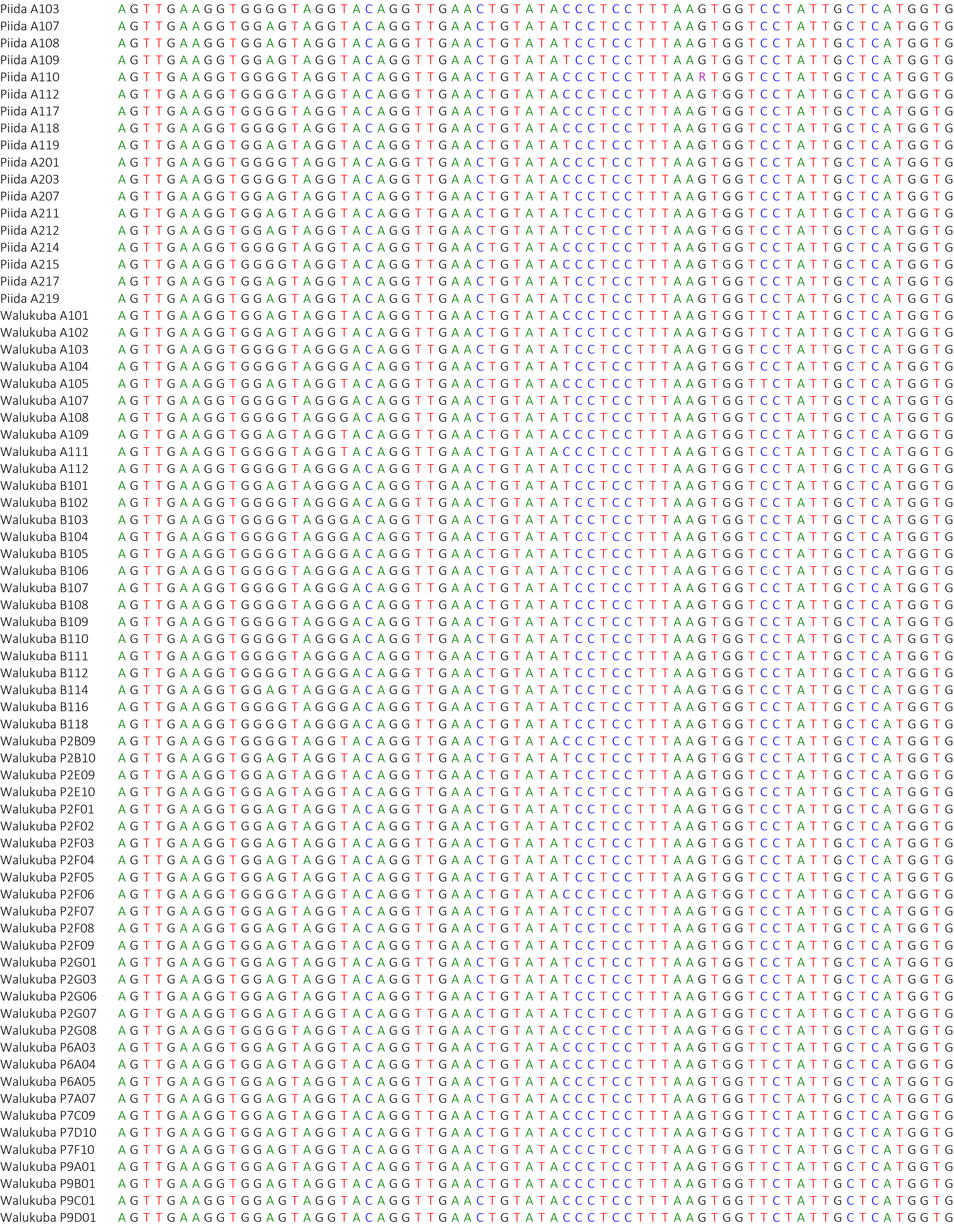

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Piida A107 A G T T G A A G G T G G A G T A G G T A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
Piida A108 A G T T G A A G G T G G A G T A G G T A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
Piida A109 A G T T G A A G G T G G A G T A G G T A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
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Walukuba A105 A G T T G A A G G T G G A G T A G G T A C A G G T T G A A C T G T A T A C C C T C C T T T A A G T G G T T C T A T T G C T C A T G G T G
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Walukuba B104 A G T T G A A G G T G G G G T A G G G A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
Walukuba B105 A G T T G A A G G T G G G G T A G G G A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
Walukuba B106 A G T T G A A G G T G G G G T A G G G A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
Walukuba B107 A G T T G A A G G T G G G G T A G G G A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
Walukuba B108 A G T T G A A G G T G G G G T A G G G A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
Walukuba B109 A G T T G A A G G T G G G G T A G G G A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
Walukuba B110 A G T T G A A G G T G G G G T A G G G A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
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Walukuba P2B10 A G T T G A A G G T G G A G T A G G T A C A G G T T G A A C T G T A T A T C C T C C T T T A A G T G G T C C T A T T G C T C A T G G T G
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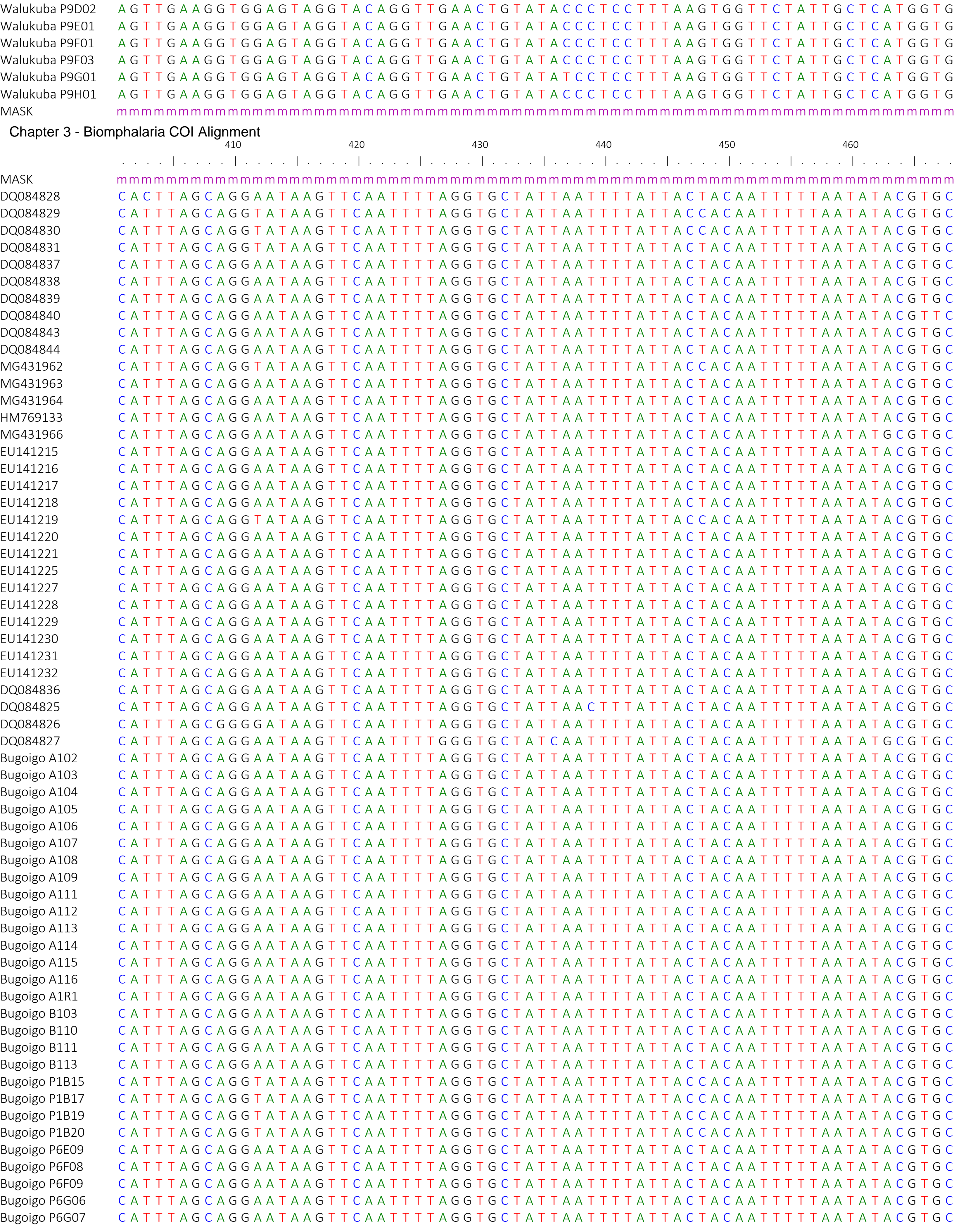

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MASK mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
Chapter 3 - Biomphalaria COI Alignment
410 420 430 440 450 460
MASK mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
DQ084828 CACTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
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| Bugoigo P6G09 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
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| Bugoigo P7B10 | CATTTAGCAGGTATMASTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACRTGC |
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| Bukoba P7H12 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Bukoba P9B03 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Bukoba P9C03 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Bukoba P9F04 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P2D02 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P2D03 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P2D08 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P2H07 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P2H09 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6B02 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6C02 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6D03 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6D10 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6E10 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6F02 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6F04 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6F10 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6G01 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P6G02 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P7B11 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P7C11 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P7D12 | CATTTAGCAGGAATAAGTTCAATTTTAKGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P9D04 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |
| Lwanika P9E04 | CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC |

Piida A101 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A102 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A103 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A107 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A108 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A109 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A110 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGWGC
Piida A112 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
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Piida A118 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
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Piida A201 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A203 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A207 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A211 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
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Piida A214 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A215 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A217 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Piida A219 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba A101 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
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Walukuba A103 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba A104 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba A105 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba A107 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba A108 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba A109 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba A111 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba A112 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B101 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B102 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B103 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B104 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B105 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B106 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B107 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B108 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B109 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba B110 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
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Walukuba B114 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
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Walukuba B118 CATTTAGCAGGTATAAGTTCAATTTTAGGTGCTATTAATTTTATTACCACAATTTTTAATATACGTGC
Walukuba P2B09 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2B10 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
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Walukuba P2E10 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2F01 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
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Walukuba P2F03 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2F04 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
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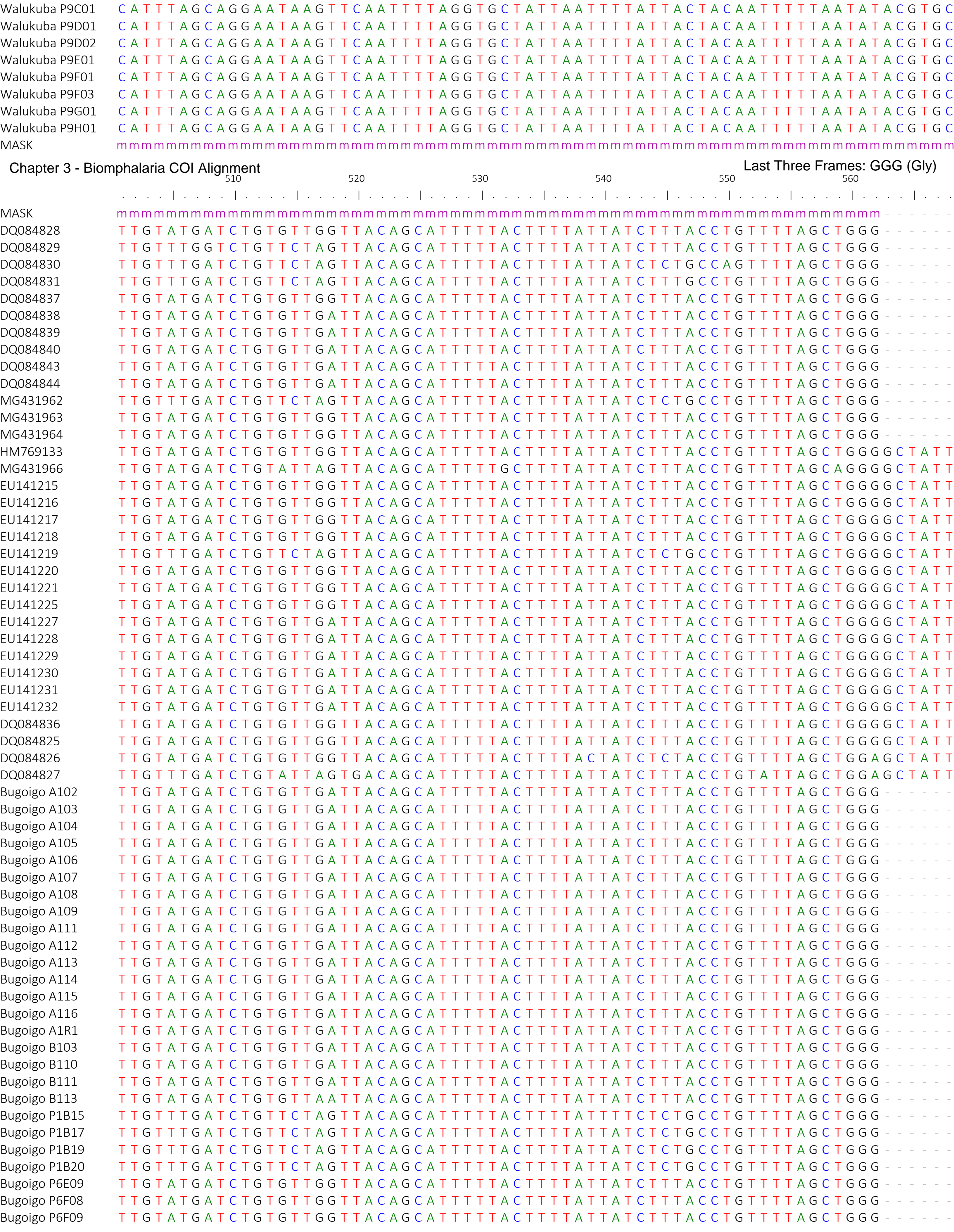
Walukuba P2F07 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2F08 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2F09 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2G01 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2G03 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2G06 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2G07 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P2G08 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P6A03 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P6A04 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P6A05 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P7A07 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P7C09 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P7D10 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P7F10 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P9A01 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC
Walukuba P9B01 CATTTAGCAGGAATAAGTTCAATTTTAGGTGCTATTAATTTTATTACTACAATTTTTAATATACGTGC



Walukuba P9C01
Walukuba P9D01
Walukuba P9D02
Walukuba P9E01
Walukuba P9F01
Walukuba P9F03
Walukuba P9G01
Walukuba P9H01
MASK
Chapter 3 - Biomphalaria COI Alignment
Last Three Frames: GGG (Gly)
510
520
530
540
550
560
MASK
DQ084828
DQ084829
DQ084830
DQ084831
DQ084837
DQ084838
DQ084839
DQ084840
DQ084843
DQ084844
MG431962
MG431963
MG431964
HM769133
MG431966
EU141215
EU141216
EU141217
EU141218
EU141219
EU141220
EU141221
EU141225
EU141227
EU141228
EU141229
EU141230
EU141231
EU141232
DQ084836
DQ084825
DQ084826
DQ084827
Bugoigo A102
Bugoigo A103
Bugoigo A104
Bugoigo A105
Bugoigo A106
Bugoigo A107
Bugoigo A108
Bugoigo A109
Bugoigo A111
Bugoigo A112
Bugoigo A113
Bugoigo A114
Bugoigo A115
Bugoigo A116
Bugoigo A1R1
Bugoigo B103
Bugoigo B110
Bugoigo B111
Bugoigo B113
Bugoigo P1B15
Bugoigo P1B17
Bugoigo P1B19
Bugoigo P1B20
Bugoigo P6E09
Bugoigo P6F08
Bugoigo P6F09

| | |
|---|---|
| Bugoigo P6G06 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoigo P6G07 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoigo P6G09 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoigo P7A10 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoigo P7B10 | TTGTTTGATCTGTTCTAGTTMCASCATTTTTACTTTTATTATCTCTGCCTGTTTTASCTGGG------ |
| Bugoigo P7C10 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoigo P7D08 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoigo P7D09 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoigo P7E09 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoigo P7E10 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoigo P7F07 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoigo P7F09 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoigo P7H07 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoigo P7H09 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoto P2A08 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P2A10 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P2B01 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoto P2B03 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoto P2B04 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoto P2B07 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoto P2B08 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Bugoto P2E04 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P2G04 | TTGTATGATCTGTGTTAATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P2G09 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6B05 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6C05 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6C06 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6C07 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6D05 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6D08 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6D09 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6E05 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6E06 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bugoto P6E07 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba LV01 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba LV08 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba LV10 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P2A04 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P2A07 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P2C06 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P2E01 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P2E03 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P2E05 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P2E06 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P2E07 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P2E08 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P6G08 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P7D11 | TTGTATGATCTGTGTTGGTTRCRGCRTTTTTACTTTTATTATSTTWCCTGTTTTRGCTGGG------ |
| Bukoba P7E11 | TTGTATGATCTGTGTTGGTTACRGCATWTTTACTTTTATTATCTTTACCTGTYYTAGCTGGR------ |
| Bukoba P7E12 | TTGTATGATCTGTGTTGGTTACRGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGKG------ |
| Bukoba P7H12 | TTGTATGATCTGTGTTGGTTACAGMATTTTTACTTTTWTTAWCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P9B03 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P9C03 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Bukoba P9F04 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P2D02 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P2D03 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P2D08 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P2H07 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P2H09 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6B02 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6C02 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6D03 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6D10 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6E10 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6F02 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6F04 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6F10 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6G01 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P6G02 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P7B11 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P7C11 | TTGTATGATCTGTGTTGGTTRCRGCATWTTTACTTTTATTATCTTTACCTGTYTTAGCTGRG------ |
| Lwanika P7D12 | TTGTATGATCTGTGTKGGTTACAGCATWTTTACTTTTATTAWMTTTACCTGTYTTAGMTGRG------ |

| Sample | Sequence |
|---|---|
| Lwanika P9D04 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Lwanika P9E04 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A101 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A102 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A103 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A107 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A108 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A109 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A110 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A112 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A117 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A118 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A119 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A201 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
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| Piida A207 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
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| Piida A217 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Piida A219 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba A101 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba A102 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba A103 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba A104 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba A105 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba A107 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
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| Walukuba A112 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba B101 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
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| Walukuba B105 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba B106 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTACCTGTTTTAGCTGGG------ |
| Walukuba B107 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba B108 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTTTCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba B109 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
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| Walukuba B111 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTTTCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba B112 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba B114 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba B116 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba B118 | TTGTTTGATCTGTTCTAGTTACAGCATTTTTACTTTTATTATCTCTGCCTGTTTTAGCTGGG------ |
| Walukuba P2B09 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P2B10 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P2E09 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P2E10 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P2F01 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P2F02 | TTGTATGATCTGTGTTAATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P2F03 | TTGTATGATCTGTGTTAATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
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| Walukuba P2F07 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P2F08 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P2F09 | TTGTATGATCTGTGTTGATTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
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| Walukuba P6A04 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P6A05 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P7A07 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P7C09 | TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------ |
| Walukuba P7D10 | TTGTATGATCTGTGTTGGTTRCAGCATTTTTACTTTTATTATCTTTWCCTSTTTTAGCTGGG------ |
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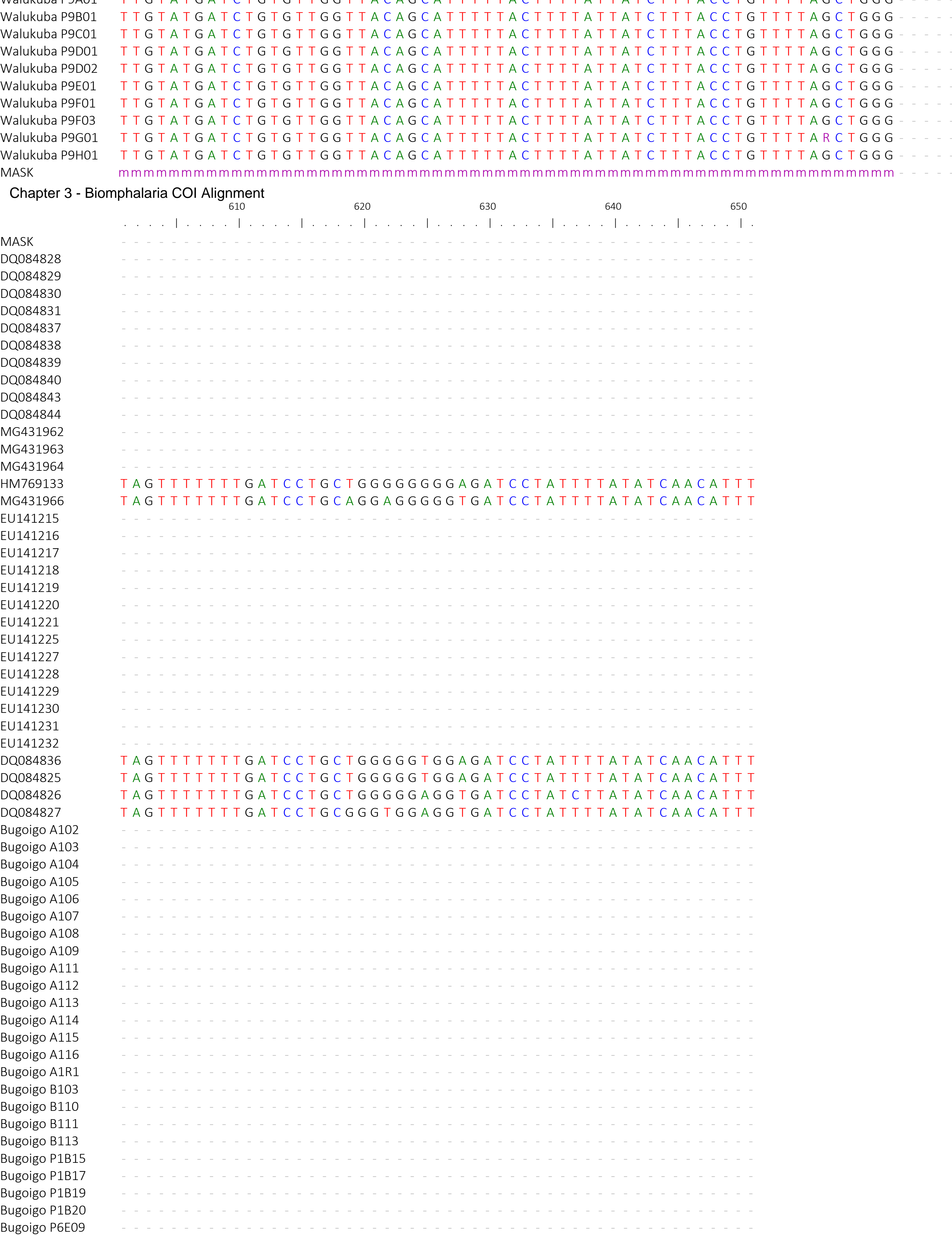

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Walukuba P9C01 TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------
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Walukuba P9F01 TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------
Walukuba P9F03 TTGTATGATCTGTGTTGGTTACAGCATTTTTACTTTTATTATCTTTACCTGTTTTAGCTGGG------
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MASK mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm------
Chapter 3 - Biomphalaria COI Alignment
610 620 630 640 650
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Bugoigo A1R1 ---------------------------------------------------
Bugoigo B103 ---------------------------------------------------
Bugoigo B110 ---------------------------------------------------
Bugoigo B111 ---------------------------------------------------
Bugoigo B113 ---------------------------------------------------
Bugoigo P1B15 ---------------------------------------------------
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Bugoigo P1B20 ---------------------------------------------------
Bugoigo P6E09 ---------------------------------------------------

Chapter 6 Tree A - Nematode Alignment 1

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                              10        20        30        40        50
MF460455_-_Outgroup  CCGAGGCTT-TCAACATGCTAGACTTCTCGA-CTTTGTCGCGAACGTTGGAAGCCT--
For_20               TCGAGGATT-CTGCGTTAAC-TGTTTATCTGGGGCTATAGCC-ACGTTGAGTGCACTT
Uni_15               TCGAGGATT-CTGCGTTAACTTGTTTATCTGGGGCTATAGCC-ACGTTGAGTGCACTT
Edw_5                TCGAGGATT-CTGCGTTAACTTGTTTATCTGGGGCTATAGCC-ACGTTGAGTGCACTT
MK214813             TCGAGGATT-CTGCGTTAAC-TGTTTATCTGGGGCTATAGCC-ACGTTGAGTGCACTT
Gran_13              TCGAGGATT-CTGCGTTAAC-TGTTTATCTGGGGCTATAGCC-ACGTTGAGTGCACTT
For_26               TCGAGGATT-CTGCGTTAAC-TGTTTATCTGGGGCTATAGCC-ACGTTGAGTGCACTT
Gran_1               TCGAGGATT-CTGCGTTAAC-TGTTTATCTGGGGCTATAGCC-ACGTTGAGTGCACTT
FJ516760             AGGAGGACT-CCGCTATA---TTCTTAGCAA-GGCGATAGTTGTTACTAAATGCCGGT
MG551718             CGGAGGACT-CCGCTATA---TTCTTAGCAA-GGCGATAGCTGACACTAGATGCCTGT
KM510202             CGGAGGACTCCCGCTATA---TTCTTAGCAA-GGCGATAGCTGACACTAGATGCCTGT
FJ516761             CGGAGGACT-CCGCTATA---TTCTTAGCAA-GGCGATAGCTGACACTAGATGCCTGT
MK214817             CGGAGGACT-CCGCTATA---TTCTTAGCAA-GGCGATAGCTGACACTAGATGCCTGT
KM510201             ---------------------TTCTTAGCAAGGGCGATAGCTGACACTAGATGCCTGT
MK214815             AAGGGGATT-CCGCTATT---CACTTAGCAA-GGCGATAGTTGGTCTTGGTTGCTTGT
MF192968             AGGAGGATT-CCGCTATT---CACTTAGCAA-GGCGATAGTTGGTTTTGGTTGCCTGA
MK214816             TGGAGGATT-CCGCTATT---CACATAGCAG-GGCGATAGTTGGTYTTGGTTGCCTGA
KX267675             CGGAGGATT-CCGCTATA---CTTTTAGCAA-GGCGATAGCCGGTCTTGGTTGCCTGT
MT819980             CGGAGGATT-CCGCTATA---CTTTTAGCAA-GGCGATAGCCGGTCTTGGTTGCCTGT
MK214814             CGGAGGTTT-CCGCTATA---GTTTTAGCAA-GGCGATAGCTGGTCTTGGTTGCCTGT
MASK                 -------------------------------------------mmmmmmmmmmmmm--

                             110       120       130       140       150
MF460455_-_Outgroup  --------------CTCTACGTGAGGCGTCTATAAACGTGATCA---------TGAGC
For_20               ACTTACTATTTCCCTTCAAATTGAAGC-CCTGCAGAGGTGCTCG-TATCCGACTAGAC
Uni_15               ACTTACTATTTCCCTTCAAATTGAAGC-CCTGCAGAGGTGCTCG-TATCCGACTAGAC
Edw_5                ACTTACTATTTCCCTTCAAATTGAAGC-CCTGCAGAGGTGCTCG-TATCCGACTAGAC
MK214813             ACTTACTATTTCCCTTCAAATTGAAGC-CCTGCAGAGGTGCTCG-TATCCGACTAGAC
Gran_13              ACTTACTATTTCCCTTCAAATTGAAGC-CCTGCAGAGGTGCTCG-TATCCGACTAGAC
For_26               ACTTACTATTTCCCTTCAAATTGAAGC-CCTGCAGAGGTGCTCG-TATCCGACTAGAC
Gran_1               ACTTACTATTTCCCTTCAAATTGAAGC-CCTGCAGAGGTGCTCG-TATCCGACTAGAC
FJ516760             ----------CATCCTCAACCTTAAGC-TCTGCAGAGGTGCTCA-TATTTGACTGAAT
MG551718             ----------CACCCCCAACTTCAAGC-TCTGCAGAGGTGCTCA-TATTTGACTGAAT
KM510202             ----------CACCCCCAACTTTAAGC-TCTGCAGAGGTGCTCA-TATTTGACTGAAT
FJ516761             ----------CACCCCCAACTTTAAGC-TCTGCAGAGGTGCTCA-TATTTGACTGAAT
MK214817             ----------CACCCCCAACTTTAAGC-TCTGCAGAGGTGCTCA-TATTTGACTGAAT
KM510201             ----------CACCCCCAACTTTAAGC-TCTGCAGAGGTGCTCA-TATTTGACTGAAT
MK214815             ----------------------------------------------------------
MF192968             ----------CATCCCCAATTAGACGC-TCCGCAGAGGTGCTCAGTACTTGACTAGAT
MK214816             ----------CACCCTCAATTAGACGC-TCCACAGAGGTGCTTA-TGCCTGGCTAGAC
KX267675             ----------CACCCCCAAAAAGAAGC-TCTGCAGAGGTGTTCTGTGCTTGACTAGAT
MT819980             ----------CACCCCCAAAAAGAAGC-TCTGCAGAGGTGTTCTGTGCTTGACTAGAT
MK214814             ----------CACCCCCAACTTTAAGC-TCTGCAGAGGTACTCAATACTTAATTAGAT
MASK                 ----------------------------------------------------------

                             210       220       230       240       250
MF460455_-_Outgroup  CTTTTCAAGAGCTTTAGACTCAATGAGCAT-------TTGCTAGAAATGCCGCCTCGC
For_20               GTCAGCAGCCAGCAGCAGCCGTTTGAGCGTAAGACAACGGCCTG-GTTATCGCCTCGA
Uni_15               GTCAGCAGCCAGCAGCAGCCGTTTGAGCGTAAGACAACGGCCTG-GTTATCGCCTCGA
Edw_5                GTCAGCAGCCAGCAGCAGCCGTTTGAGCGTAAGACAACGGCCTG-GTTATCGCCTCGA
MK214813             GTCAGCAGCCAGCAGCAGCCGTTTGAGCGTAAGACAACGGCCTG-GTTATCGCCTCGA
Gran_13              GTCAGCAGCCAGCAGCAGCCGTTTGAGCGTAAGACAACGGCCTG-GTTATCGCCTCGA
For_26               GTCAGCAGCCAGCAGCAGCCGTTTGAGCGTAAGACAACGGCCTG-GTTATCGCCTCGA
Gran_1               GTCAGCAGCCAGCAGCAGCCGTTTGAGCGTAAGACAACGGCCTG-GTTATCGCCTCGA
FJ516760             TCTATC-----------ACTGCGTGAGCTCAAAGATATTGCAAG-TATATCGCCTCAC
MG551718             TCTATC-----------ACTGCTTGAGCTCAAAGACATAGCAAG-TATGTCGCCTCAC
KM510202             TCTATC-----------ACTGCTTGAGCTCAAAGACATAGCAAG-TATGTCGCCTCAC
FJ516761             TCTATC-----------ACTGCTTGAGCTCAAAGACATAGCAAG-TATGTCGCCTCAC
MK214817             TCTATC-----------ACTGCTTGAGCTCAAAGACATAGCAAG-TATGTCGCCTCAC
KM510201             TCTATC-----------ACTGCTTGAGCTCAAAGACATAGCAAG-TATGTCGCCTCAC
MK214815             GACACCCCAAA-------------------AAATACATTACTGG-TCTGTGATGTTAA
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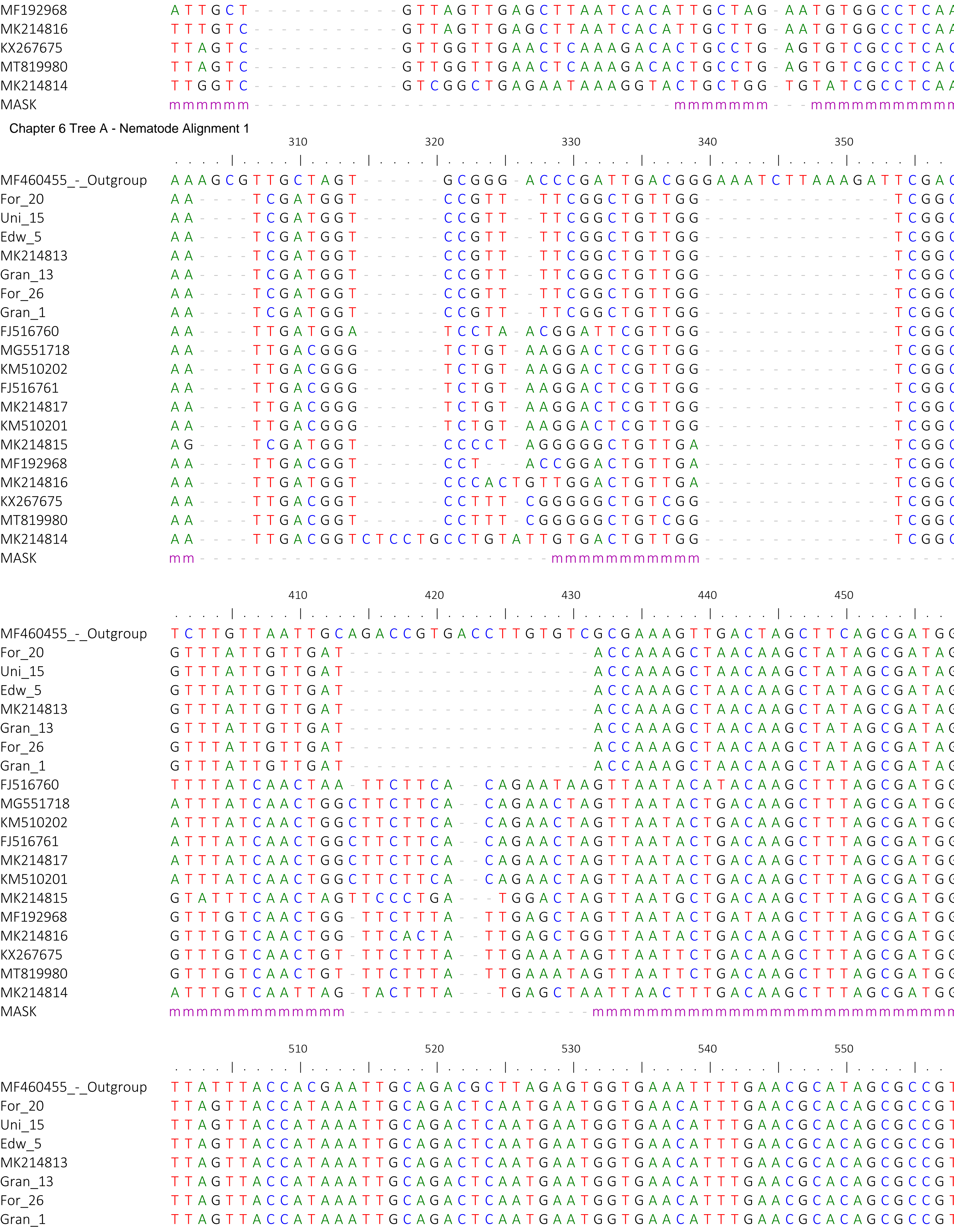

Chapter 6 Tree A - Nematode Alignment 1
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350
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520
530
540
550
MF460455_-_Outgroup
For_20
Uni_15
Edw_5
MK214813
Gran_13
For_26
Gran_1
FJ516760
MG551718
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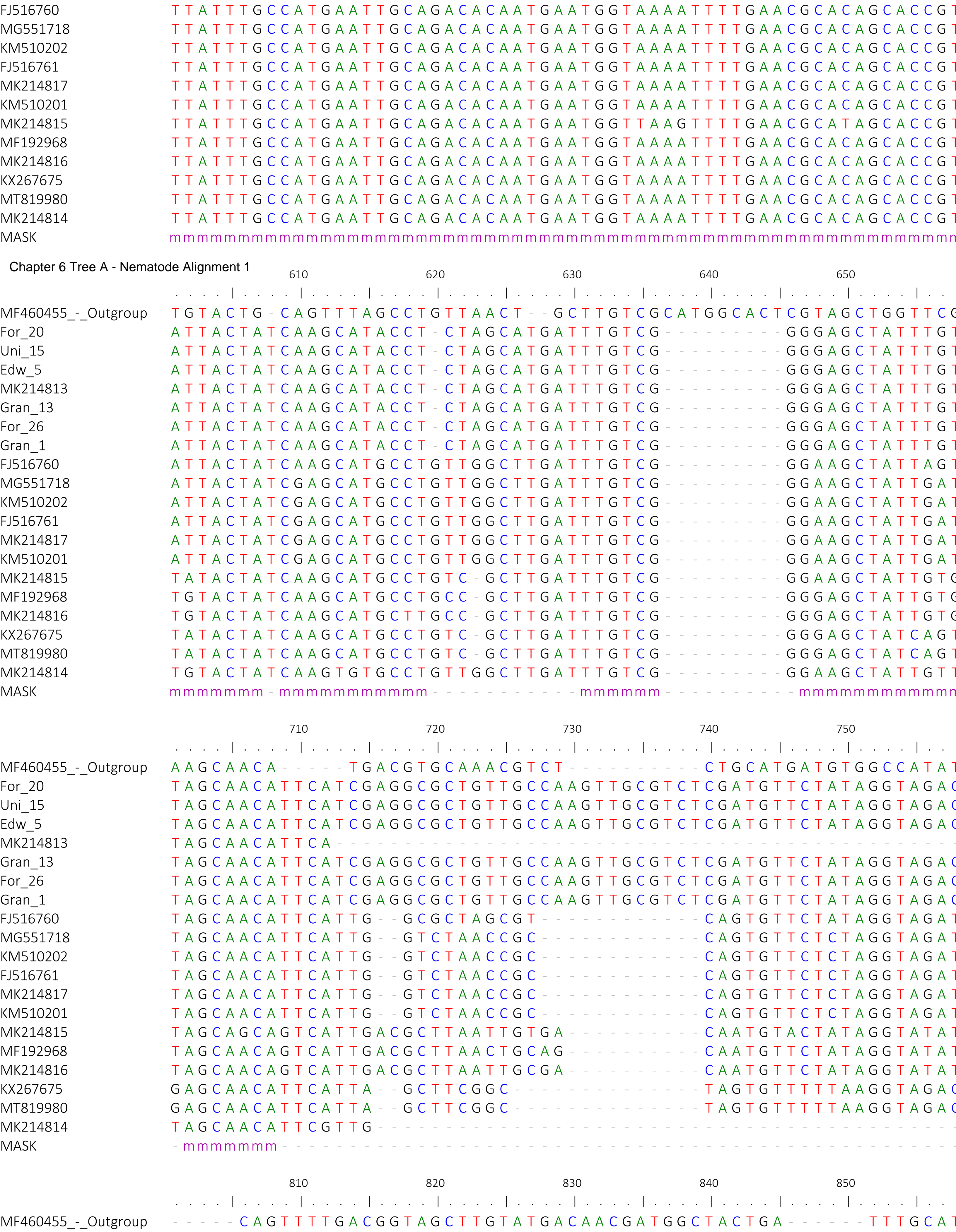
MASK


Chapter 6 Tree A - Nematode Alignment 1
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KM510202
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MK214816
KX267675
MT819980
MK214814
MASK
610
620
630
640
650
MF460455_-_Outgroup
For_20
Uni_15
Edw_5
MK214813
Gran_13
For_26
Gran_1
710
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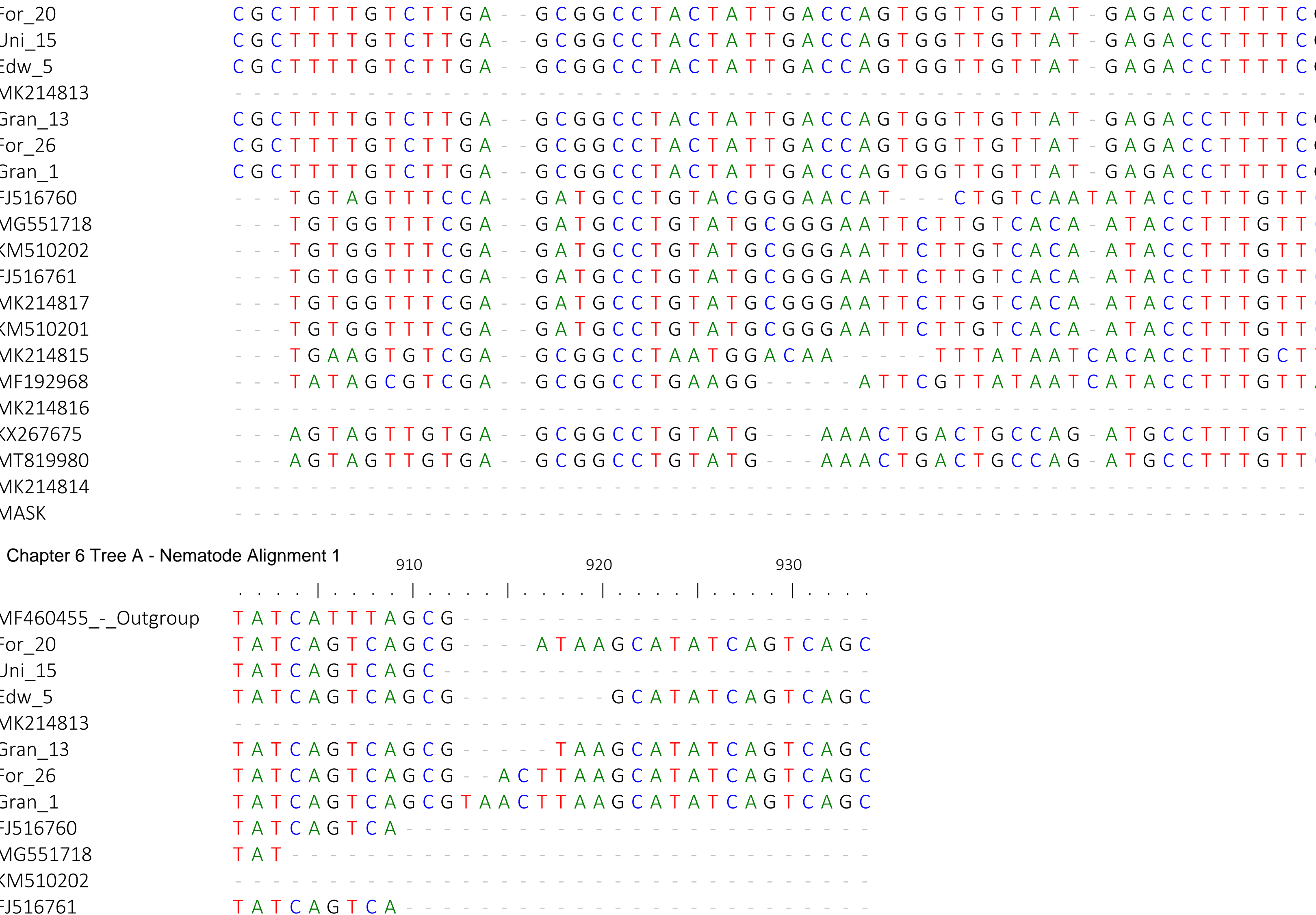

Chapter 6 Tree A - Nematode Alignment 1
910
920
930
MF460455_-_Outgroup TATCATTTAGCG
For_20 TATCAGTCAGCG---ATAAGCATATCAGTCAGC
Uni_15 TATCAGTCAGC
Edw_5 TATCAGTCAGCG-------GCATATCAGTCAGC
MK214813
Gran_13 TATCAGTCAGCG----TAAGCATATCAGTCAGC
For_26 TATCAGTCAGCG--ACTTAAGCATATCAGTCAGC
Gran_1 TATCAGTCAGCGTAACTTAAGCATATCAGTCAGC
FJ516760 TATCAGTCA
MG551718 TAT
KM510202
FJ516761 TATCAGTCA
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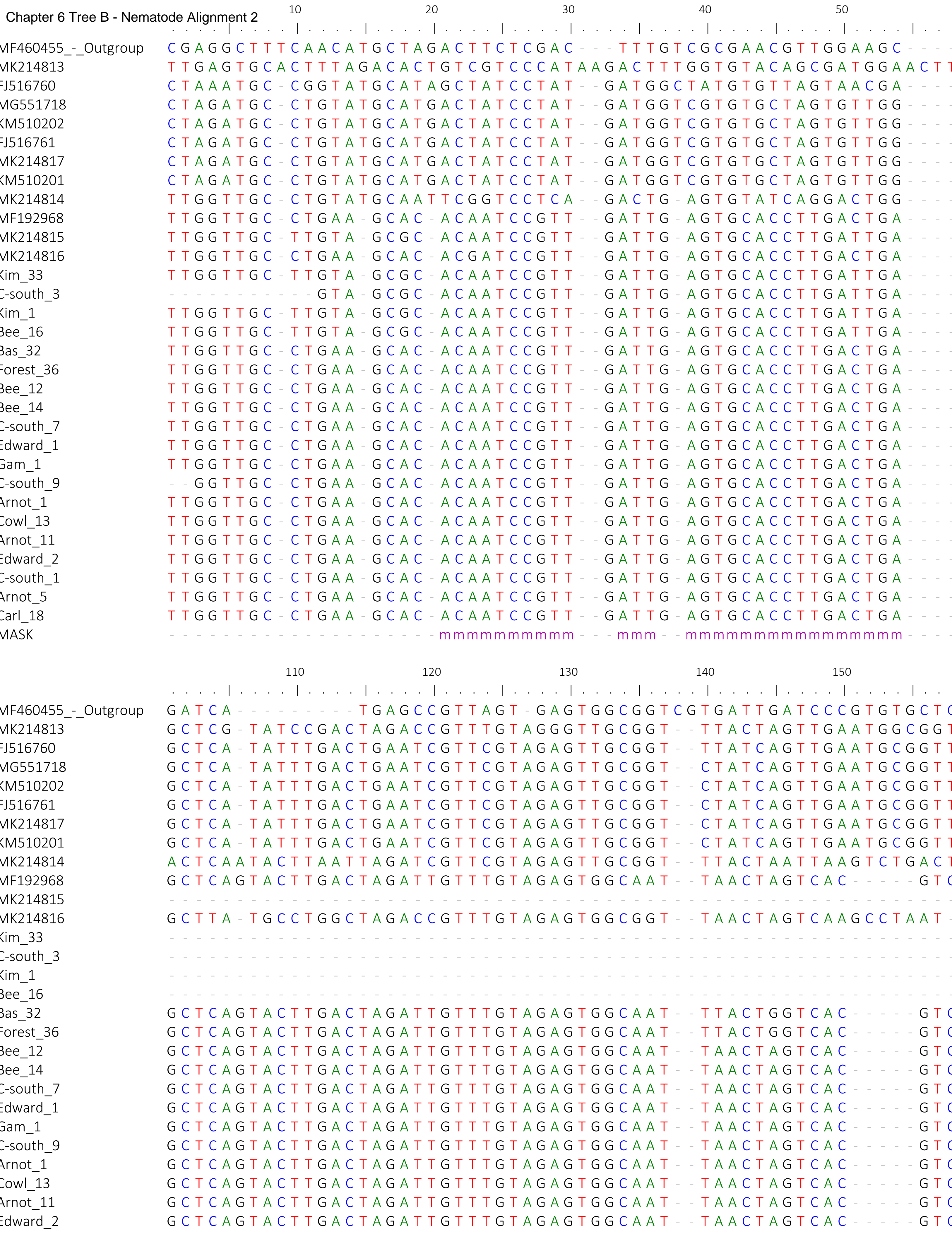
Chapter 6 Tree B - Nematode Alignment 2
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MG551718
KM510202
FJ516761
MK214817
KM510201
MK214814
MF192968
MK214815
MK214816
Kim_33
C-south_3
Kim_1
Bee_16
Bas_32
Forest_36
Bee_12
Bee_14
C-south_7
Edward_1
Gam_1
C-south_9
Arnot_1
Cowl_13
Arnot_11
Edward_2
C-south_1
Arnot_5
Carl_18
MASK
110
120
130
140
150
MF460455_-_Outgroup
MK214813
FJ516760
MG551718
KM510202
FJ516761
MK214817
KM510201
MK214814
MF192968
MK214815
MK214816
Kim_33
C-south_3
Kim_1
Bee_16
Bas_32
Forest_36
Bee_12
Bee_14
C-south_7
Edward_1
Gam_1
C-south_9
Arnot_1
Cowl_13
Arnot_11
Edward_2

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C-south_1             GCTCAGTACTTGACTAGATTGTTTGTAGAGTGGCAAT--TAACTAGTCAC-----GTC
Arnot_5               GCTCAGTACTTGACTAGATTGTTTGTAGAGTGGCAAT--TAACTAGTCAC-----GTC
Carl_18               GCTCAGTACTTGACTAGATTGTTTGTAGAGTGGCAAT--TAACTAGTCAC-----GTC
MASK                  ----------------------------------------------------------
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Chapter 6 Tree B - Nematode Alignment 2

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MF460455_-_Outgroup   AGCATTTGCTAGAAATGCCGCCTCGCTCGTTGGTAGGTATTGGTG-ATTATGCA--GA
MK214813              CAA--CGGCCTG-GTTATCGCCTCGAA-GTTGC-AACTGCTGGTGTA-TTTGCCTGGT
FJ516760              ATA--TTGCAAG-TATATCGCCTCACCCGTTTT-AACTGCTGATGTAATAAGCTACAT
MG551718              ACA--TAGCAAG-TATGTCGCCTCACCCGTTTT-AACTGCTGGTGTAATAAGCTCCAT
KM510202              ACA--TAGCAAG-TATGTCGCCTCACCCGTTTT-AACTGCTGGTGTAATAAGCTCCAT
FJ516761              ACA--TAGCAAG-TATGTCGCCTCACCCGTTTT-AACTGCTGGTGTAATAAGCTCCAT
MK214817              ACA--TAGCAAG-TATGTCGCCTCACCCGTTTT-AACTGCTGGTGTAATAAGCTCCAT
KM510201              ACA--TAGCAAG-TATGTCGCCTCACCCGTTTT-AACTGCTGGTGTAATAAGCTCCAT
MK214814              GTA--CTGCTGG-TGTATCGCCTCAAACGTTTC-AACTGCTGGTGTATCTTGCTTGAT
MF192968              ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCATTCT
MK214815              ACA--TTACTGG-TCTGTGATGTTAAAGGTCTCTCGTTGCTGGTGTAAATTGCTTGAT
MK214816              ACA--TTGCTTG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCTACTT
Kim_33                ACA--TTACTGG-TCTGTGATGTTAAAGGTCTCTCGTTGCTGGTGTAAATTGCTTGAT
C-south_3             ACA--TTACTGG-TCTGTGATGTTAAAGGTCTCTCGTTGCTGGTGTAAACCGCTTGAT
Kim_1                 ACA--TTACTGG-TCTGTGATGTTAAAGGTCTCTCGTTGCTGGTGTAAATTGCTTGAT
Bee_16                ACA--TTACTGG-TCTGTGATGTTAAAGGTCTCTCGTTGCTGGTGTAAATTGCTTGAT
Bas_32                ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCAATCT
Forest_36             ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCAATCT
Bee_12                ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCAATCT
Bee_14                ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCAATCT
C-south_7             ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCATTCT
Edward_1              ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCATTCT
Gam_1                 ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCATTCT
C-south_9             ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCATTCT
Arnot_1               ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCATTCT
Cowl_13               ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCATTCT
Arnot_11              ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCAATCT
Edward_2              ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCAATCT
C-south_1             ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCAATCT
Arnot_5               ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCAATCT
Carl_18               ACA--TTGCTAG-AATGTGGCCTCAAACGTTTCTCGCTGCTGGTGTAACTTGCAATCT
MASK                  -------------mmmmmmmmmmmmmm------mmmmmmmmmmmm----------mm

                               310       320       330       340       350
                      ....|....|....|....|....|....|....|....|....|....|....|..
MF460455_-_Outgroup   GATTGACGGGAAATCTTAAAGATTCGACAGACATGAGGTCGGACGCCAATGCATTACC
MK214813              GTTGGTCGGCAGCTTTGTAA--------------------------CCACATATT---
FJ516760              GTTGGTCGGCAGCTCTAAAA--------------------------CAACCCTTTC-A
MG551718              GTTGGTCGGCAGCTCTGAAA--------------------------CAACCTTTTC-A
KM510202              GTTGGTCGGCAGCTCTGAAA--------------------------CAACCTTTTC-A
FJ516761              GTTGGTCGGCAGCTCTGAAA--------------------------CAACCTTTTC-A
MK214817              GTTGGTCGGCAGCTCTGAAA--------------------------CAACCTTTTC-A
KM510201              GTTGGTCGGCAGCTCTGAAA--------------------------CAACCTTTTC-A
MK214814              GTTGGTCGGCAGCTCTGTAG--------------------------CAACCTTTTC--
MF192968              GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
MK214815              GTTGATCGGCAGC-ATGAGA--------------------------CAA---TCCGA
MK214816              GTTGATCGGCAGC-CTGAGA--------------------------CAATCAATTCGA
Kim_33                GTTGATCGGCAGC-ATGAGA--------------------------CAA---TCCGA
C-south_3             GTTGATCGGCAGC-ATGAGA--------------------------CAA---TCCGA
Kim_1                 GTTGATCGGCAGC-ATGAGA--------------------------CAA---TCCGA
Bee_16                GTTGATCGGCAGC-ATGAGA--------------------------CAA---TCCGA
Bas_32                GTTGATCGGCAGC-TTGAGA--------------------------CAACCAATCCGA
Forest_36             GTTGATCGGCAGC-TTGAGA--------------------------CAACCAATCCGA
Bee_12                GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
Bee_14                GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
C-south_7             GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
Edward_1              GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
Gam_1                 GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
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C-south_9            GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
Arnot_1              GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
Cowl_13              GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
Arnot_11             GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
Edward_2             GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
C-south_1            GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
Arnot_5              GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
Carl_18              GTTGATCGGCAGC-CTGAGA--------------------------CAACCAATCCGA
MASK                 mmmmmmmmmmmmm-mmmmmm--------------------------------------
```

Chapter 6 Tree B - Nematode Alignment 2

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                              410       420       430       440       450
                     ....|....|....|....|....|....|....|....|....|....|....|..
MF460455_-_Outgroup  GTGTCGCGAAAGTT------GACTAGCTTCAGCGATGGATCGGTCGATTCGCGTATCG
MK214813             -----------ACCAAAGCTAACAAGCTATAGCGATAGATCGGTCGATTCCCTTAACG
FJ516760             TCACAGAATAAGTTAATACATACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
MG551718             TCACAGAACTAGTTAATACTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
KM510202             TCACAGAACTAGTTAATACTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
FJ516761             TCACAGAACTAGTTAATACTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
MK214817             TCACAGAACTAGTTAATACTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
KM510201             TCACAGAACTAGTTAATACTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
MK214814             TTAT-GAGCTAATTAACTTTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
MF192968             TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
MK214815             CTGATGGACTAGTTAATGCTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
MK214816             CTATTGAGCTGGTTAATACTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Kim_33               CTGATGGACTAGTTAATGCTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
C-south_3            CTGATGGACTAGTTAATGCTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Kim_1                CTGATGGACTAGTTAATGCTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Bee_16               CTGATGGACTAGTTAATGCTGACAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Bas_32               TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Forest_36            TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Bee_12               TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Bee_14               TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
C-south_7            TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Edward_1             TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Gam_1                TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
C-south_9            TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Arnot_1              TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Cowl_13              TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Arnot_11             TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Edward_2             TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
C-south_1            TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Arnot_5              TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
Carl_18              TTATTGAGCTAGTTAATACTGATAAGCTTTAGCGATGGATCGGTCGATTCCCTTAACG
MASK                 --------------------mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
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                              510       520       530       540       550
                     ....|....|....|....|....|....|....|....|....|....|....|..
MF460455_-_Outgroup  ACGCTTAGAGTGGTGAAATTTTGAACGCATAGCGCCGTTGGGTTTTCCCTTCGGCACG
MK214813             ACTCAATGAATGGTGAACATTTGAACGCACAGCGCCGTTGGGTTTTCCCTTCGGCACG
FJ516760             ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
MG551718             ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
KM510202             ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
FJ516761             ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
MK214817             ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
KM510201             ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
MK214814             ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
MF192968             ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
MK214815             ACACAATGAATGGTTAAGTTTTGAACGCATAGCACCGTTGGGTTTTCCCTTCGGTACG
MK214816             ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Kim_33               ACACAATGAATGGTTAAGTTTTGAACGCATAGCACCGTTGGGTTTTCCCTTCGGTACG
C-south_3            ACACAATGAATGGTTAAGTTTTGAACGCATAGCACCGTTGGGTTTTCCCTTCGGTACG
Kim_1                ACACAATGAATGGTTAAGTTTTGAACGCATAGCACCGTTGGGTTTTCCCTTCGGTACG
Bee_16               ACACAATGAATGGTTAAGTTTTGAACGCATAGCACCGTTGGGTTTTCCCTTCGGTACG
Bas_32               ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Forest_36            ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
```

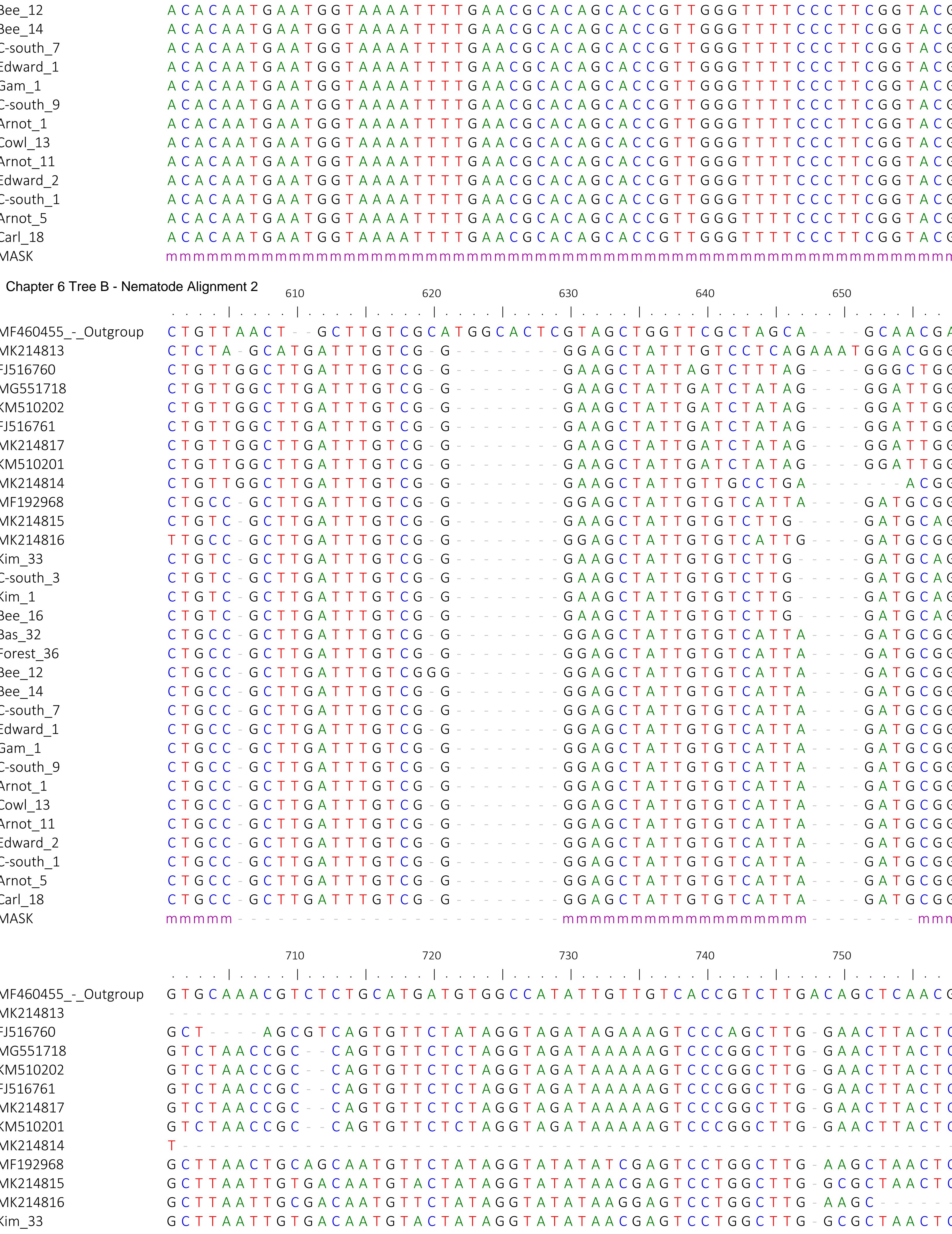

Bee_12 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Bee_14 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
C-south_7 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Edward_1 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Gam_1 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
C-south_9 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Arnot_1 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Cowl_13 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Arnot_11 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Edward_2 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
C-south_1 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Arnot_5 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
Carl_18 ACACAATGAATGGTAAAATTTTGAACGCACAGCACCGTTGGGTTTTCCCTTCGGTACG
MASK mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
Chapter 6 Tree B - Nematode Alignment 2
610 620 630 640 650
MF460455_-_Outgroup CTGTTAACT--GCTTGTCGCATGGCACTCGTAGCTGGTTCGCTAGCA----GCAACGA
MK214813 CTCTA-GCATGATTTGTCG-G--------GGAGCTATTTGTCCTCAGAAATGGACGGG
FJ516760 CTGTTGGCTTGATTTGTCG-G--------GAAGCTATTAGTCTTTAG----GGGCTGG
MG551718 CTGTTGGCTTGATTTGTCG-G--------GAAGCTATTGATCTATAG----GGATTGG
KM510202 CTGTTGGCTTGATTTGTCG-G--------GAAGCTATTGATCTATAG----GGATTGG
FJ516761 CTGTTGGCTTGATTTGTCG-G--------GAAGCTATTGATCTATAG----GGATTGG
MK214817 CTGTTGGCTTGATTTGTCG-G--------GAAGCTATTGATCTATAG----GGATTGG
KM510201 CTGTTGGCTTGATTTGTCG-G--------GAAGCTATTGATCTATAG----GGATTGG
MK214814 CTGTTGGCTTGATTTGTCG-G--------GAAGCTATTGTTGCCTGA-------ACGG
MF192968 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
MK214815 CTGTC-GCTTGATTTGTCG-G--------GAAGCTATTGTGTCTTG-----GATGCAG
MK214816 TTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTG----GATGCGG
Kim_33 CTGTC-GCTTGATTTGTCG-G--------GAAGCTATTGTGTCTTG-----GATGCAG
C-south_3 CTGTC-GCTTGATTTGTCG-G--------GAAGCTATTGTGTCTTG-----GATGCAG
Kim_1 CTGTC-GCTTGATTTGTCG-G--------GAAGCTATTGTGTCTTG-----GATGCAG
Bee_16 CTGTC-GCTTGATTTGTCG-G--------GAAGCTATTGTGTCTTG-----GATGCAG
Bas_32 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Forest_36 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Bee_12 CTGCC-GCTTGATTTGTCGGG--------GGAGCTATTGTGTCATTA----GATGCGG
Bee_14 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
C-south_7 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Edward_1 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Gam_1 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
C-south_9 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Arnot_1 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Cowl_13 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Arnot_11 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Edward_2 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
C-south_1 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Arnot_5 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
Carl_18 CTGCC-GCTTGATTTGTCG-G--------GGAGCTATTGTGTCATTA----GATGCGG
MASK mmmmm------------------------mmmmmmmmmmmmmmmmmm--------mmm
710 720 730 740 750
MF460455_-_Outgroup GTGCAAACGTCTCTGCATGATGTGGCCATATTGTTGTCACCGTCTTGACAGCTCAACG
MK214813 ----------------------------------------------------------
FJ516760 GCT---AGCGTCAGTGTTCTATAGGTAGATAGAAAGTCCCAGCTTG-GAACTTACTC
MG551718 GTCTAACCGC--CAGTGTTCTCTAGGTAGATAAAAAGTCCCGGCTTG-GAACTTACTC
KM510202 GTCTAACCGC--CAGTGTTCTCTAGGTAGATAAAAAGTCCCGGCTTG-GAACTTACTC
FJ516761 GTCTAACCGC--CAGTGTTCTCTAGGTAGATAAAAAGTCCCGGCTTG-GAACTTACTC
MK214817 GTCTAACCGC--CAGTGTTCTCTAGGTAGATAAAAAGTCCCGGCTTG-GAACTTACTC
KM510201 GTCTAACCGC--CAGTGTTCTCTAGGTAGATAAAAAGTCCCGGCTTG-GAACTTACTC
MK214814 T---------------------------------------------------------
MF192968 GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
MK214815 GCTTAATTGTGACAATGTACTATAGGTATATAACGAGTCCTGGCTTG-GCGCTAACTC
MK214816 GCTTAATTGCGACAATGTTCTATAGGTATATAAGGAGTCCTGGCTTG-AAGC------
Kim_33 GCTTAATTGTGACAATGTACTATAGGTATATAACGAGTCCTGGCTTG-GCGCTAACTC

```
C-south_3    GCTTAATTGTGACAATGTACTATAGGTATATAACGAGTCCTGGCTTG-GCGCTAACTC
Kim_1        GCTTAATTGTGACAATGTACTATAGGTATATAACGAGTCCTGGCTTG-GCGCTAACTC
Bee_16       GCTTAATTGTGACAATGTACTATAGGTATATAACGAGTCCTGGCTTG-GCGCTAACTC
Bas_32       GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTGAAAGCTAACTC
Forest_36    GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTGAAAGCTAACTC
Bee_12       GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
Bee_14       GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
C-south_7    GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
Edward_1     GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
Gam_1        GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
C-south_9    GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
Arnot_1      GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
Cowl_13      GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
Arnot_11     GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
Edward_2     GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
C-south_1    GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
Arnot_5      GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
Carl_18      GCTTAACTGCAGCAATGTTCTATAGGTATATATCGAGTCCTGGCTTG-AAGCTAACTC
MASK         ----------------------------------------------------------
```

Chapter 6 Tree B - Nematode Alignment 2

```
                                 810       820       830       840       850
                             ....|....|....|....|....|....|....|....|....|....|..
MF460455_-_Outgroup  AACGATGGCTACTGATTTGCATTGCAACCTGAGCTCAGGCGTGACTACCCGCTGAACT
MK214813
FJ516760             ATCTGTCAATATACCTTTGTTCTACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
MG551718             TCTTGTCACAATACCTTTGTTCTACAACCTGAACTCAGTCGTGATTACCCGCTGAACT
KM510202             TCTTGTCACAATACCTTTGTTCTACA--------------------------------
FJ516761             TCTTGTCACAATACCTTTGTTCTACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
MK214817             TCTTGTCACAATACCTTTGTTCTACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
KM510201             TCTTGTCACAATACCTTTGTTCT-----------------------------------
MK214814             ----------------------------------------------------------
MF192968             CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTG---------------
MK214815             ATTTATAATCACACCTTTGCTTTATAACCTGAACTCAGTCGTGAT-------------
MK214816             ----------------------------------------------------------
Kim_33               ATTTATAATCACACCTTTGCTTTATAACCTGAACTCAGTCGTGATTACCCGCTCAACT
C-south_3            ATTTATAATCACACCTTTGCTTTATAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Kim_1                ATTTATAATCACACCTTTGCTTTATAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Bee_16               ATTTATAATCACACCTTTGCTTTATAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Bas_32               CTTTATAATCATACCTTTGTTTTACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Forest_36            CTTTATAATCATACCTTTGTTTTACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Bee_12               CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Bee_14               CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
C-south_7            CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Edward_1             CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Gam_1                CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
C-south_9            CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Arnot_1              CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Cowl_13              CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Arnot_11             CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Edward_2             CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
C-south_1            CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Arnot_5              CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
Carl_18              CGTTATAATCATACCTTTGTTATACAACCTGAACTCAGTCGTGATTACCCGCTCAACT
MASK                 ----------------------------------------------------------
```

# Chapter 6 Tree C - Nematode Alignment 3

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                             10        20        30        40        50
                    ....|....|....|....|....|....|....|....|....|....|....
JQ995315_-_Outgroup --------------------------CTGTGCATACGCACCATTTGCACACATGTGAG
LC186007            TTATCGAGCTTCTAAAAAGCCTCATACTGTGCATAAGAACCATTTGCACACAATTGAG
LC185990            TTATCGAGCTTCTAAAAAGCCTCATACTGTGCATAAGAACCATTTGCACACAGTTGAG
LC186015            TTATCGAGCTTCTAAAAAGCCTCATACTGTGCATAAGAACCATTTGCACACAATTGAG
MH032773            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTGAATGAG
MH178311            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTGAATGAG
MH178312            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTGAATGAG
MH178317            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTTAATGAG
MH032772            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTGAATGAG
MH032774            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTGAATGAG
MH178313            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTGAATGAG
LC018444            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTTAATGAG
MH178314            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTTAATGAG
MH178315            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTTAATGAG
MH178316            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTTAATGAG
MH178318            TTATCGAGCTTCCAAAAAGCCTCATACCGTGCATAC-ATCCATTTGTGCGTTAATGAG
OL472308            TTATCGAGCTTCC-AAAAGCCTCATACCGTGCATACTTTCCATTTGTGCGATAATGAG
OL472309            TTATCGAGCTTCC-AAAAGCCTCATACCGTGCATACTTTCCATTTGTGCGATAATGAG
OL472310            TTATCGAGCTTCC-AAAAGCCTCATACCGTGCATACTTTCCATTTGTGCGATAATGAG
OL472311            TTATCGAGCTTCC-AAAAGCCTCATACCGTGCATACTTTCCATTTGTGCGATAATGAG
MT108302            TTATCGAGCTTCTAAAAAGCCTCATACCGTACATACCATCCATTTGTACGATTGTGAG
LC052773            TTATCGAGCTTCTAAAAAGCCTCATACCGTACATAGCATTCATTTGTACGATACTGAG
MN839761            TTATCGAGCTTCTAAAAAGCCTCATACCGTACAT-----CCATTTGTACGATACTGAG
Pond_14             TTATCGAGCTTCC-AAAAGCCTCATACCGTGCATACTTTCCATTTGTGCGATAATGAG
Bas_1               TTATCGAGCTTCC-AAAAGCCTCATACCGTGCATACTTTCCATTTGTGCGATGATGAG
Mill_19             TTATCGAGCTTCC-AAAAGCCTCATACCGTGCATACTTTCCATTTGTGCGATGATGAG
Kim_40              TTATCGAGCTTCC--AAAGCCTCA-ACCGTGCATACTTTCCATTTGTGCGATGATGAG
MASK                ---------------------------------------mmmmmmmmmmmmmmmmmmm
```

```
                            110       120       130       140       150
                    ....|....|....|....|....|....|....|....|....|....|....
JQ995315_-_Outgroup GTGTTT----TTGGCTTCCAGTGACTTGCAGCAACGCAC-GTTTTCGCGATGGTGTAT
LC186007            GA---ATTCGTTGGCTTGCAATGGCT---AGCAATGCAC-GTTTTCGCGATTGTGT-T
LC185990            GA---ATTCCTTGGCTTGCAATGGCT---AGCAATGCAC-GTTTTCGCGATTGTGT-T
LC186015            GA---ATTCCTTGGCTTGCAATGGCT---AGCAATGCAC-GTTTTCGCGATTGTGT-T
MH032773            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH178311            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH178312            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH178317            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH032772            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH032774            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH178313            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
LC018444            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH178314            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH178315            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH178316            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
MH178318            GT---G----TTGGCTGACAATGGCT---AGCAAATCGC-GTATGCGCGTTTAGGTTT
OL472308            GT---A----TTGGCTGACAATGGCT---GGCAAATCGC-ATATGCGCGATAAGGTTT
OL472309            GT---A----TTGGCTGACAATGGCT---GGCAAATCGC-ATATGCGCGATAAGGTTT
OL472310            GT---A----TTGGCTGACAATGGCT---GGCAAATCGC-ATATGCGCGATAAGGTTT
OL472311            GT---A----TTGGCTGACAATGGCT---GGCAAATCGC-ATATGCGCGATAAGGTTT
MT108302            GTTCGT----TTGGCTGACAATGACT---AGCGAAACGC-GTTAGCGCGATAGGATAT
LC052773            GTTCGT----TTGGCTGACAATGGCC---CGCGAAACGC-GTTTGCGTGATAGGATGC
MN839761            GTTCGT----TTGGCTGACAATGGCC---CGCGAAACGC-GTTTGCGTGATAGGATGA
Pond_14             GT---A----TTGGCTGACAATGGCT---GGCAAATCGC-ATATGCGCGATAAGGTTT
Bas_1               GT---A----CTGGCTGACAATGGCT---GGCAAATCGCGAAATGTGCGA-AAGGTTT
Mill_19             GT---A----CTGGCTGACAATGGCT---GGCAAATCGCGAAATGTGCGA-AAGGTTT
Kim_40              GT---A----CTGGCTGACAATGGCT---GGCAAATCGCGAAATGTGCGA-AAGGTTT
MASK                mm--------mmmmmmmmmmmmmmmm---Gmmmmmmmmm-mmmmmmmmmm--------
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                            210       220       230       240       250
                    ....|....|....|....|....|....|....|....|....|....|....
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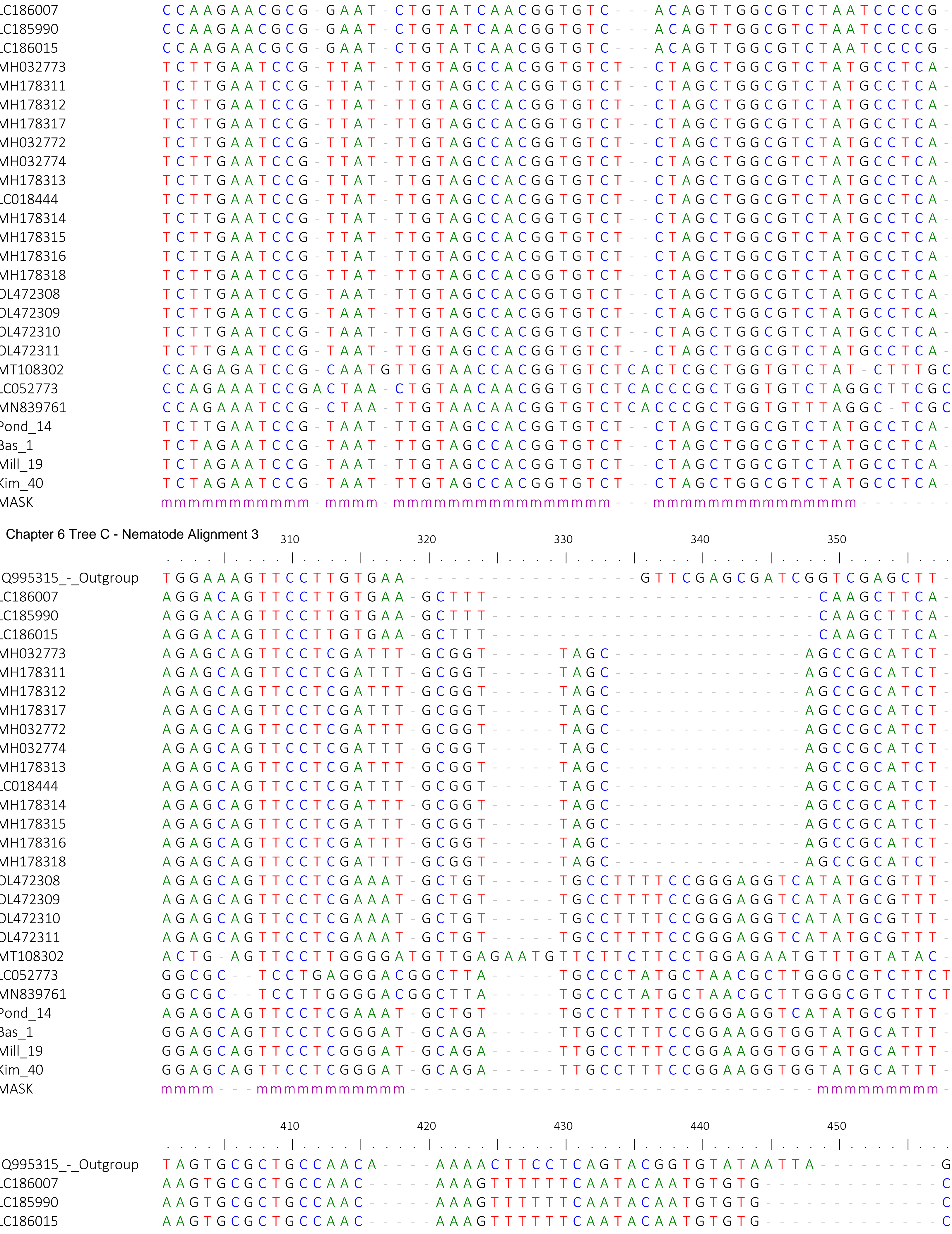
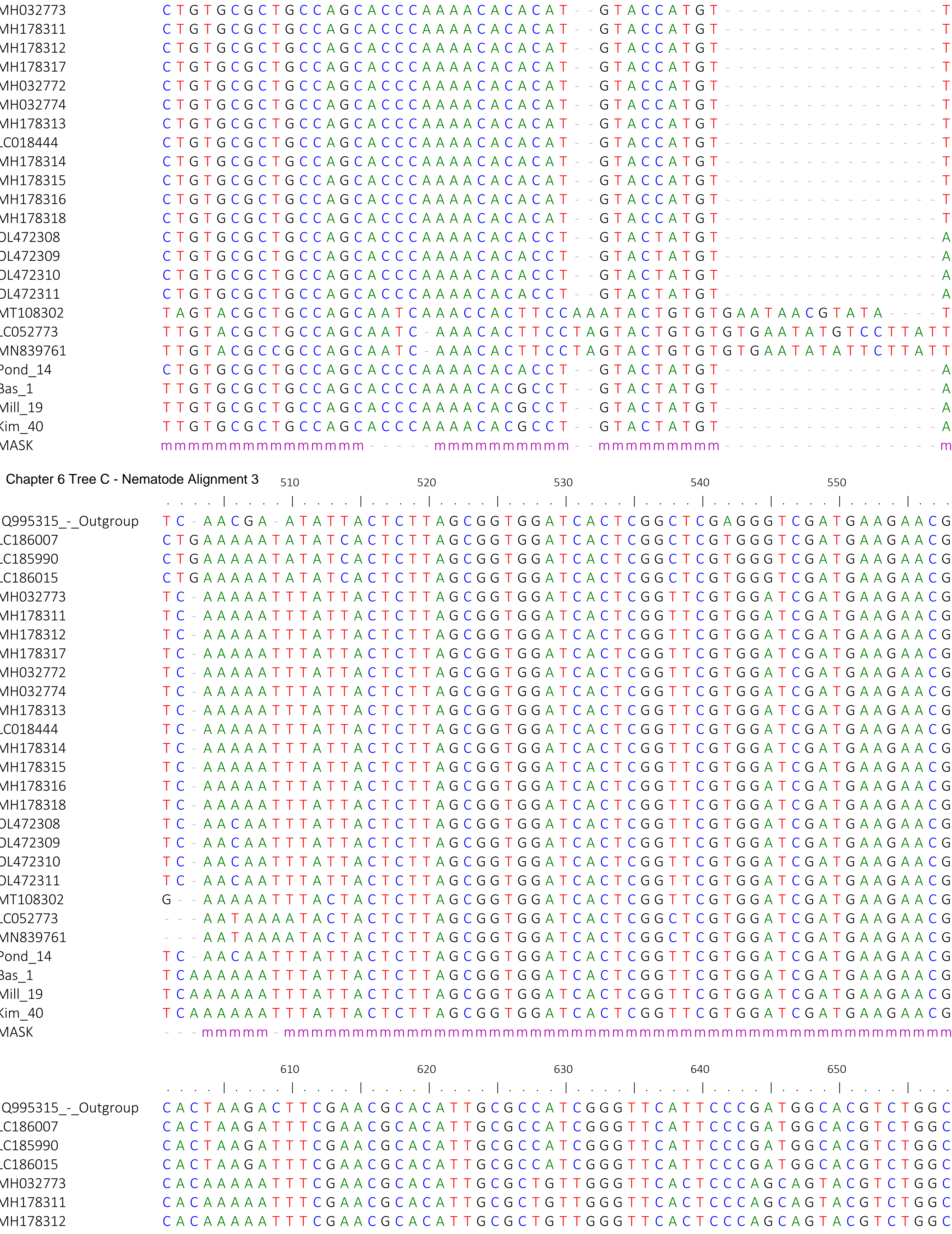


Chapter 6 Tree C - Nematode Alignment 3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620
630
640
650

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OL472308   CACAAAAATTTCGAACGCACATTGCGCCATTGGGTTCACTCCCAATGGTACGTCTGGC
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OL472310   CACAAAAATTTCGAACGCACATTGCGCCATTGGGTTCACTCCCAATGGTACGTCTGGC
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MN839761   CACTAAAATTTCGAACGCACATTGCGCCATTGGGTTCATTCCCATTGGCACGTCTGGC
Pond_14    CACAAAAATTTCGAACGCACATTGCGCCATTGGGTTCACTCCCAATGGTACGTCTGGC
Bas_1      CACAAAAATTTCGAACGCACATTGCGCCATTGGGTTCACTCCCAATGGTACGTCTGGC
Mill_19    CACAAAAATTTCGAACGCACATTGCGCCATTGGGTTCACTCCCAATGGTACGTCTGGC
Kim_40     CACAAAAATTTCGAACGCACATTGCGCCATTGGGTTCACTCCCAATGGTACGTCTGGC
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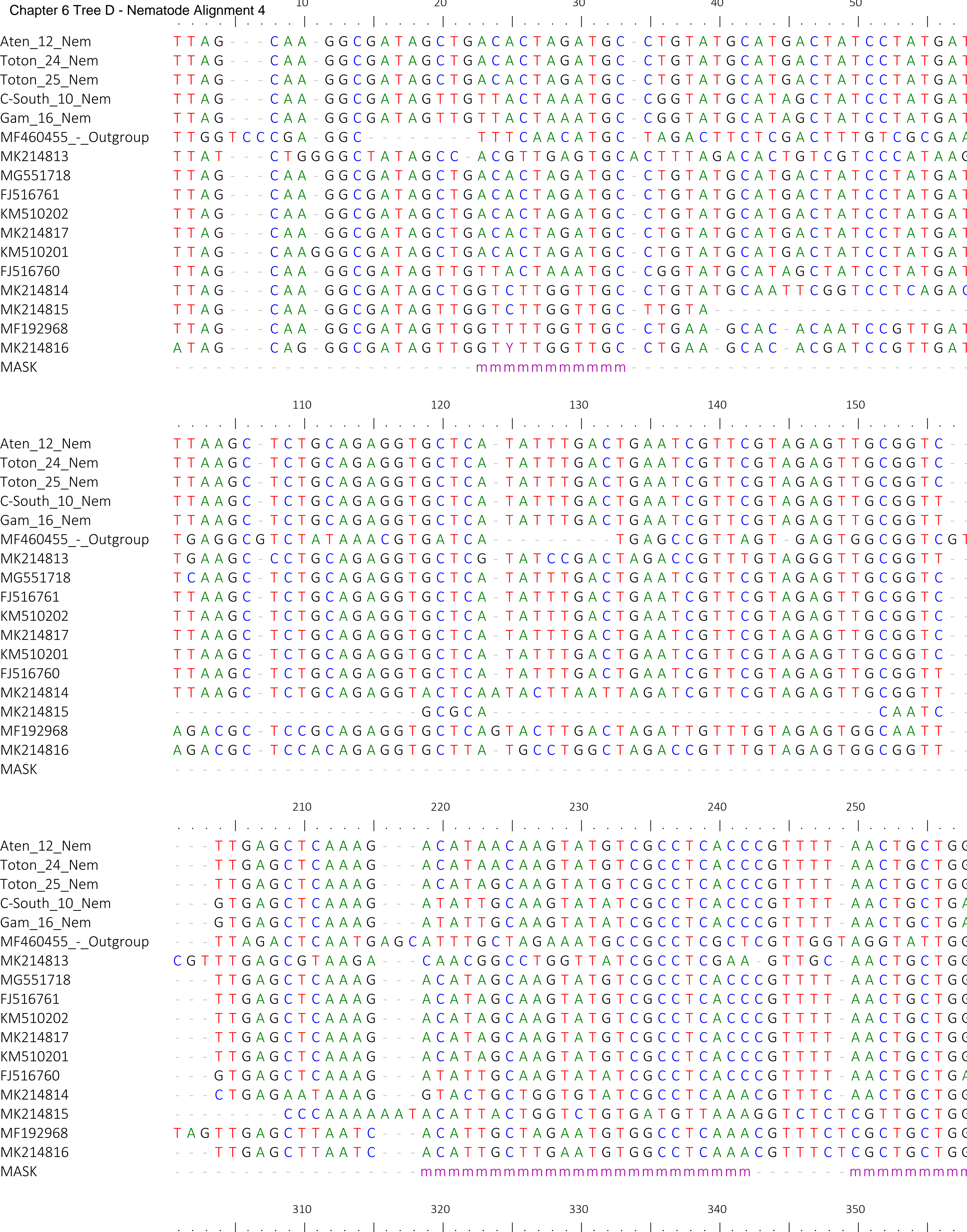
Chapter 6 Tree C - Nematode Alignment 3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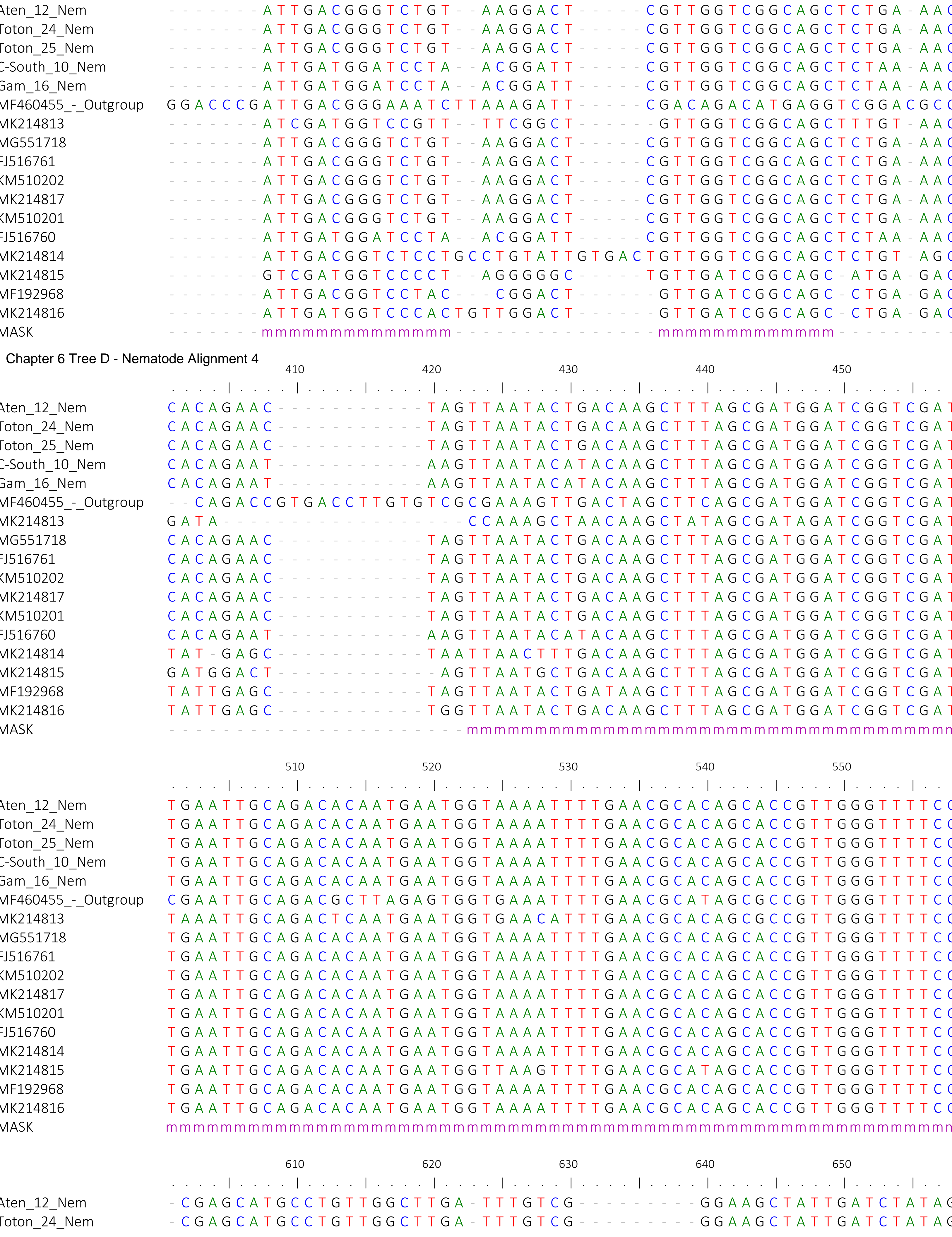
Toton_24_Nem
Toton_25_Nem
C-South_10_Nem
Gam_16_Nem
MF460455_-_Outgroup
MK214813
MG551718
FJ516761
KM510202
MK214817
KM510201
FJ516760
MK214814
MK214815
MF192968
MK214816
MASK



Chapter 6 Tree D - Nematode Alignment 4
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540
550
610
620
630
640
650
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Toton_24_Nem
Toton_25_Nem
C-South_10_Nem
Gam_16_Nem
MF460455_-_Outgroup
MK214813
MG551718
FJ516761
KM510202
MK214817
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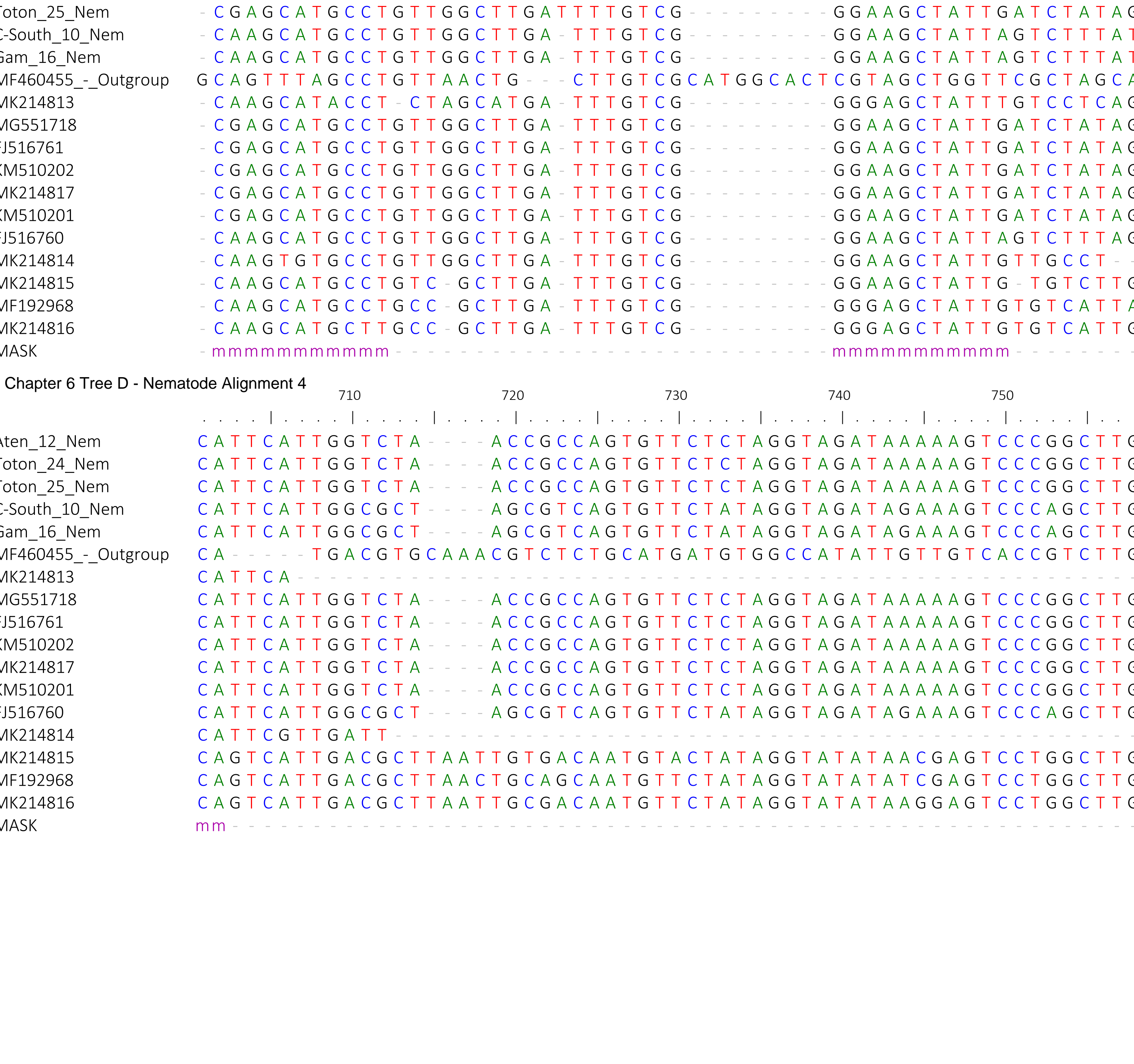
MASK


Chapter 6 Tree D - Nematode Alignment 4
Toton_25_Nem
C-South_10_Nem
Gam_16_Nem
MF460455_-_Outgroup
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MG551718
FJ516761
KM510202
MK214817
KM510201
FJ516760
MK214814
MK214815
MF192968
MK214816
MASK
710
720
730
740
750
Aten_12_Nem
Toton_24_Nem
Toton_25_Nem
C-South_10_Nem
Gam_16_Nem
MF460455_-_Outgroup
MK214813
MG551718
FJ516761
KM510202
MK214817
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FJ516760
MK214814
MK214815
MF192968
MK214816
MASK

Chapter 6 Tree E - Trematode Alignment 1

```
                                   10        20        30        40        50
Carl_12                  ---------CAGCTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Bas_26                   ------CTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Edw_8                    ---GGCTTTCGGTT-TGCCTGTTTGTTACTTCCTGTTGATGACTCCTGGATAACTT--C
Kim_37                   ----GCTTTCGGTT-TGCCTG-TTGT-ACTTCCTG-TGATGACTCCTGGATAACTTA-C
Pond_5                   -----CTTTCGGTT-TGCCTG-TTGT-AC-TCCTG-TGATGACTCCTGGATAACTT--C
Uni_5                    ---TGGCTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Kim_3                    GTTGGCTTTCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACTCCTGGATAACTTT-C
Mill_4a                  ---TGGCTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Mill_32                  -------TTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Pond_8                   ------CTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
For_23                   --GTGGCTTCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACT-CTGGATAACTTA-C
Gran_8                   --GTGGCTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Mill_31                  -TGTGGCTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Kim_10                   -TGTGGCTTCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACT-CTGGATAACTT--C
Mill_35                  -TGTGGCTTCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACT-CTGGATAACTT--C
Mill_4b                  -TGTGGCTTCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACTCCTGGATAACTT--C
Edw_25                   -----CTTTCGGTT-TGCCTG-TTGTTACTTCCTG-TGATGACTCCTGGATAACTTT-C
Gam_16                   ---GGCTTCCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACTCCTGGATAACTTTTC
Arnot_18                 ---------CGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Bas_11                   ----GGCTTCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACT-CTGGATAACTTA-C
For_4                    -------------TATGCCTG-TTGT-AC-TCCTG-TGATGACT-CTGGATAACTTA-C
Kim_40                   ------CTTCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACT-CTGGATAACTTA-C
Gam_3                    ----GGCTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Gam_15                   ----GGCTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
Colw_2                   ----GGCTTCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACT-CTGGATAACTT--C
Gam_26                   -TATGGCTTCGGTTATGCCTG-TTGT-AC-TCCTG-TGATGACT-CTGGATAACTT--C
C-sou_19                 -TATGGCTTCGGCTATGCCTG-TTGT-AC-TCCTG-TGATGACTCCTGGATAACTTTAC
Carl_13                  ------------CTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KP903636                 -------TTCGGCTATCCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KP903638                 -------TTCGGCTATCCCTG-TTGT-AA-T-CTG-TGATGACT-CTGGATAACTT--C
MK602324                 -------TTCGGCTGTCCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
LC647154 - Outgroup      -----------------CCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KP903630                 ------CTTCGGCTATCCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KP903635                 ------CTTCGGCTGTCCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KT074950                 ------CTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
AY222084                 -------TTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KT074952                 ------CTTCGGTTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KT074955                 ------CTTCGGCTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KT074954                 ------CTTCGGCTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KT074951                 ------CTTCGGCTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
KP903640                 ------CTTCGGCTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
AY222085                 ------CTTCGGCTATGCCTG-TTGT-AC-T-CTG-TGATGACT-CTGGATAACTT--C
MASK                     ---------------------------------------------mmmmmmmmmmm--m

                                   110       120       130       140       150
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Bas_26                   CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Edw_8                    CAAATTGTCTGCTCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Kim_37                   CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Pond_5                   CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Uni_5                    CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Kim_3                    CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Mill_4a                  CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Mill_32                  CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Pond_8                   CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
For_23                   CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Gran_8                   CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Mill_31                  CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Kim_10                   CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Mill_35                  CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
Mill_4b                  CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAACG
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Gam_16                 CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
Arnot_18               CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
Bas_11                 CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
For_4                  CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
Kim_40                 CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
Gam_3                  CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
Gam_15                 CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
Colw_2                 CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
Gam_26                 CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
C-sou_19               CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
Carl_13                CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
KP903636               CAAA-TATCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
KP903638               CAAA-TATCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
MK602324               CAAA-TATCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
LC647154 - Outgroup    CAAA-TATCTGCCCTATCAAC--TCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
KP903630               CAAA-TATCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
KP903635               CAAA-TATCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
KT074950               CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
AY222084               CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
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KT074955               CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
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KT074951               CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
KP903640               CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
AY222085               CAAA-TGTCTGCCCTATCAACTGTCGATGGTAGGTGACCTGCCTACCATGGTGATAAC
MASK                   -----mmmmmmmmmmmmmmmm--mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
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Chapter 6 Tree E - Trematode Alignment 1

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                                 210       220       230       240       250
                       ....|....|....|....|....|....|....|....|....|....|...
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Bas_26                 AAAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Edw_8                  AGAAACGGCTACCACTTACTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Kim_37                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Pond_5                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Uni_5                  AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Kim_3                  AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Mill_4a                AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Mill_32                AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Pond_8                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
For_23                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Gran_8                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Mill_31                AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Kim_10                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Mill_35                AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Mill_4b                AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Edw_25                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Gam_16                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Arnot_18               AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Bas_11                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
For_4                  AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Kim_40                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
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Gam_15                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Colw_2                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Gam_26                 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
C-sou_19               AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
Carl_13                AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
KP903636               AGAAACGGCTACCACTT-CCAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
KP903638               AGAAACGGCTACCACTT-CCAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
MK602324               AGAAACGGCTACCACTT-CCAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
LC647154 - Outgroup    AGAAACGGCTACCACTT-CCAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
KP903630               AGAAACGGCTACCACTT-CCAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
KP903635               AGAAACGGCTACCACTT-CCAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
KT074950               AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGC
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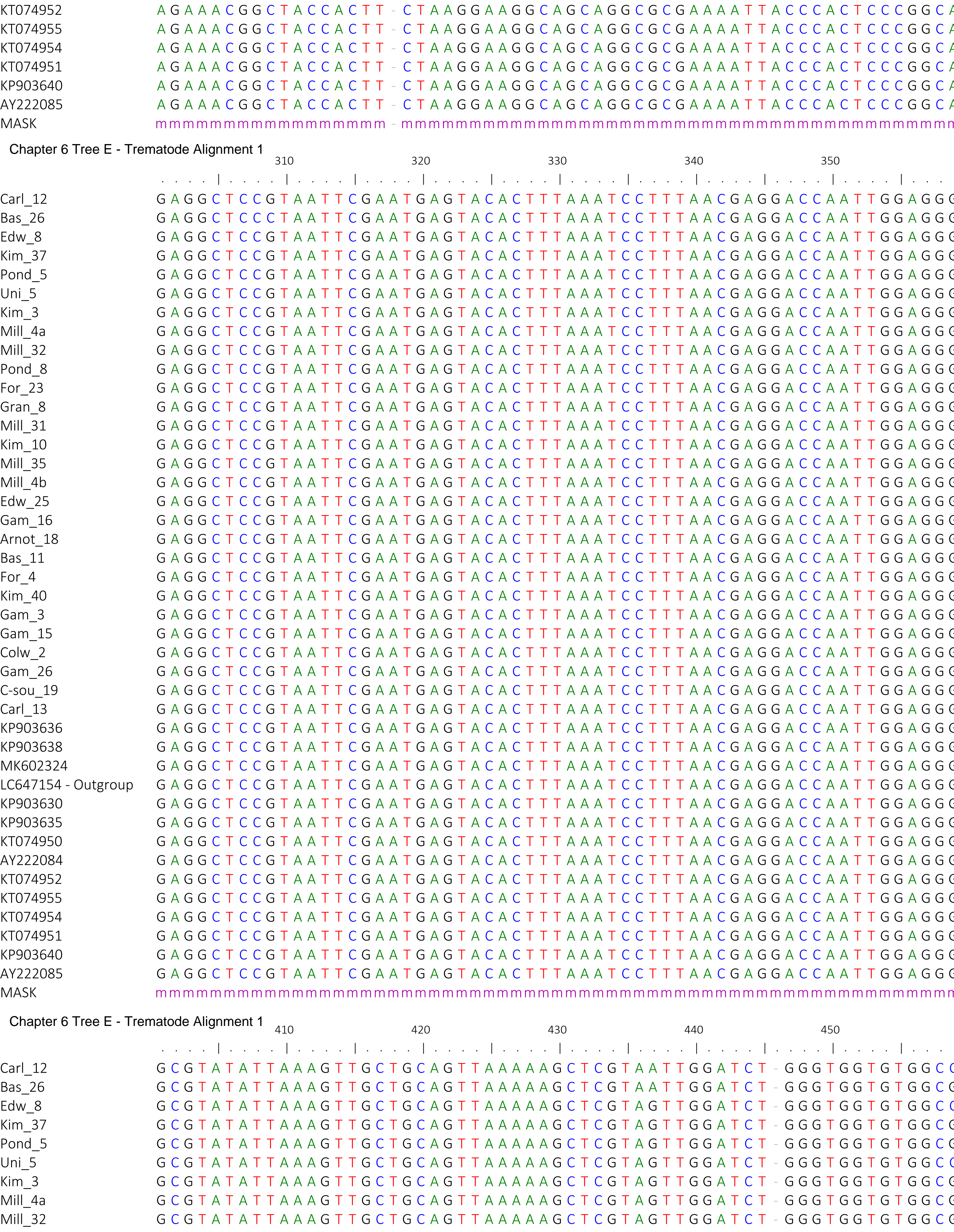
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KP903640 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGCA
AY222085 AGAAACGGCTACCACTT-CTAAGGAAGGCAGCAGGCGCGAAAATTACCCACTCCCGGCA
MASK mmmmmmmmmmmmmmmmm-mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
Chapter 6 Tree E - Trematode Alignment 1
310 320 330 340 350
Carl_12 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Bas_26 GAGGCTCCCTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Edw_8 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Kim_37 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Pond_5 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Uni_5 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Kim_3 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Mill_4a GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Mill_32 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Pond_8 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
For_23 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Gran_8 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Mill_31 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Kim_10 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Mill_35 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Mill_4b GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Edw_25 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Gam_16 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Arnot_18 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Bas_11 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
For_4 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Kim_40 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
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Gam_15 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Colw_2 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Gam_26 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
C-sou_19 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
Carl_13 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
KP903636 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
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MK602324 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
LC647154 - Outgroup GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
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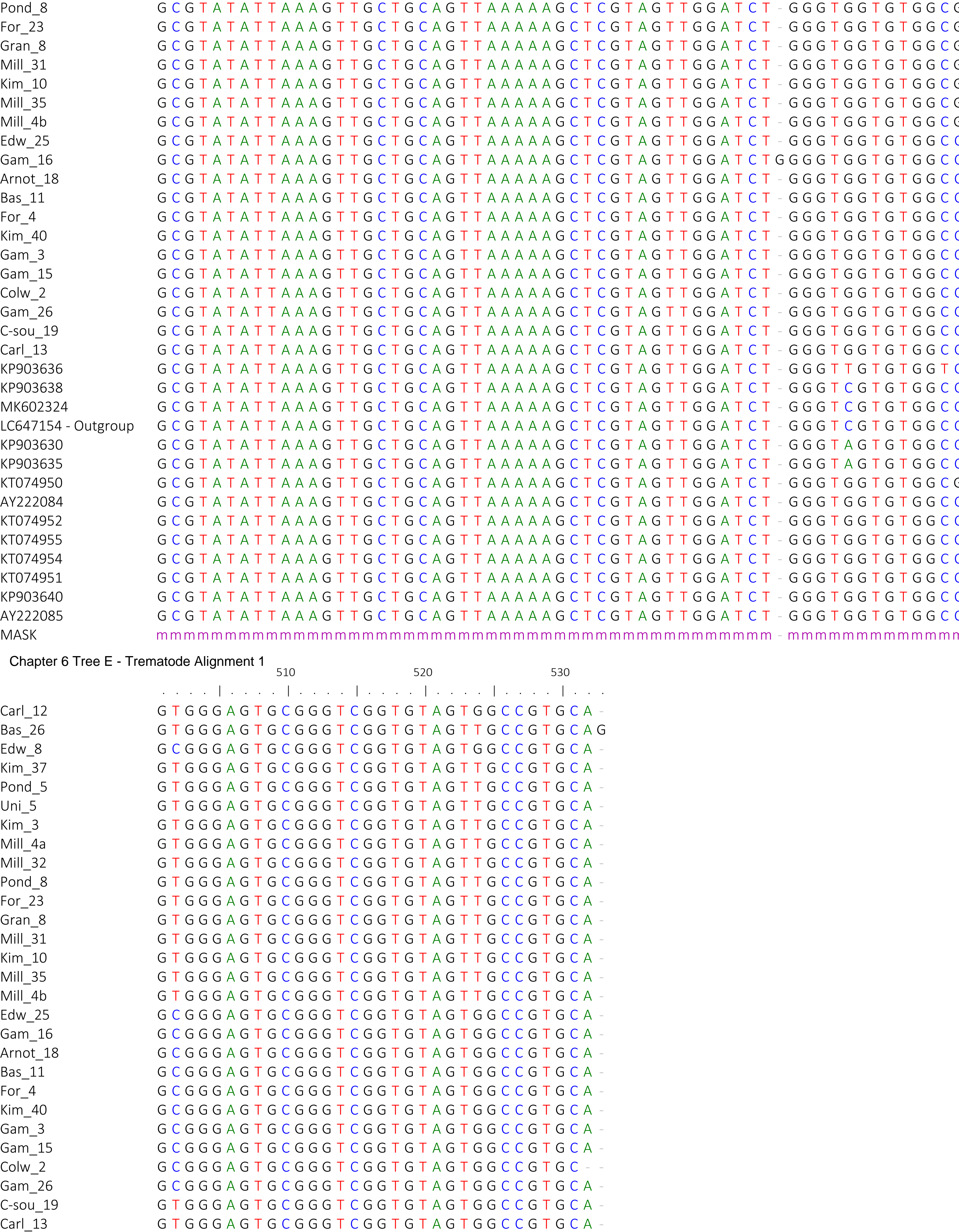
AY222085 GAGGCTCCGTAATTCGAATGAGTACACTTTAAATCCTTTAACGAGGACCAATTGGAGGG
MASK mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
Chapter 6 Tree E - Trematode Alignment 1
410 420 430 440 450
Carl_12 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAATTGGATCT-GGGTGGTGTGGCC
Bas_26 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAATTGGATCT-GGGTGGTGTGGCC
Edw_8 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Kim_37 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Pond_5 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Uni_5 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Kim_3 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Mill_4a GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Mill_32 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC



Pond_8 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCG
For_23 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCG
Gran_8 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCG
Mill_31 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCG
Kim_10 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCG
Mill_35 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCG
Mill_4b GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCG
Edw_25 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Gam_16 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCTGGGGTGGTGTGGCC
Arnot_18 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Bas_11 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
For_4 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Kim_40 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Gam_3 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Gam_15 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Colw_2 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Gam_26 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
C-sou_19 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
Carl_13 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
KP903636 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTTGTGTGGTC
KP903638 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTCGTGTGGCC
MK602324 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTCGTGTGGCC
LC647154 - Outgroup GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTCGTGTGGCC
KP903630 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTAGTGTGGCC
KP903635 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTAGTGTGGCC
KT074950 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCG
AY222084 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
KT074952 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
KT074955 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
KT074954 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
KT074951 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
KP903640 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
AY222085 GCGTATATTAAAGTTGCTGCAGTTAAAAAGCTCGTAGTTGGATCT-GGGTGGTGTGGCC
MASK mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm-mmmmmmmmmmmmm
Chapter 6 Tree E - Trematode Alignment 1
510 520 530
Carl_12 GTGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Bas_26 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCAG
Edw_8 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Kim_37 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Pond_5 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Uni_5 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Kim_3 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Mill_4a GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Mill_32 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Pond_8 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
For_23 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Gran_8 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Mill_31 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Kim_10 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Mill_35 GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Mill_4b GTGGGAGTGCGGGTCGGTGTAGTTGCCGTGCA-
Edw_25 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Gam_16 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Arnot_18 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Bas_11 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
For_4 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Kim_40 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Gam_3 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Gam_15 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Colw_2 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGC--
Gam_26 GCGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
C-sou_19 GTGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-
Carl_13 GTGGGAGTGCGGGTCGGTGTAGTGGCCGTGCA-

| | |
|---|---|
| KP903636 | A C G G G A G T T C G G G T C G G T G T A G T G A C C G T G C A - |
| KP903638 | A C G G G A G T G C G G G T C G G T G T A G T G A C C G T G C A - |
| MK602324 | A C G G G A G T A C G G G T C G G T G T A G T G A C C G T G C A - |
| LC647154 - Outgroup | A C G G G A G T A C G G G T C G G T G T A G T G A C C G T G C A - |
| KP903630 | A C G G G A G T A C G G G T C G G T G T A G T G A C C G T G C A - |
| KP903635 | A C G G G A G T A C G G G T C G G T G T A G T G A C C G T G C A - |
| KT074950 | G T G G G A G T G C G G G T C G G T G T A G T T G C C G T G C A - |
| AY222084 | G C G G T A G T A C G G G T C G G T G T A G T G G C C G T G C A - |
| KT074952 | G C G G G A G T G C G G G T C G G T G T A G T G G C C G T G C A - |
| KT074955 | G T G G G A G T G C G G G T C G G T G T A G T G G C C G T G C A - |
| KT074954 | G T G G G A G T G C G G G T C G G T G T A G T G G C C G T G C A - |
| KT074951 | G T G G G A G T G C G G G T C G G T G T A G T G G C C G T G C A - |
| KP903640 | G T G G G A G T G C G G G T C G G T G T A G T G G C C G T G C A - |
| AY222085 | G C G G G A G T G C G G G T C G G T G T A G T G G C C G T G C A - |
| MASK | mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm - - |

Chapter 6 Tree E - Trematode Alignment 1

Chapter 6 Tree F - Trematode Alignment 2

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                             10        20        30        40        50
                    .........|.........|.........|.........|.........|........
MASK                -----------~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Uni_39              ----~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~TGCATC
KR703279_-_Outgroup CGAGGGGACGGGTGGATTTATTAGAACAGAACCAACCGGTTGTAGCCTCGGCTGCTTC
KX815125            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTGC
MT568786            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTGC
MK482055            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGTTGCTGT
MW361240            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGTTGCTGC
AY222157            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
MZ768805            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
MZ787582            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
MZ410795            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
MZ798276            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
MZ753784            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
MZ751051            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
MZ753903            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
AY222158            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
AY222160            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCATC
JQ886404            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGTGTC
AY222159            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~CGGCTGCTTC
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                             110       120       130       140       150
                    .........|.........|.........|.........|.........|........
MASK                mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
Uni_39              ATCGGCCTTGAGTCGGCAATGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
KR703279_-_Outgroup GTCGGCCTTGTGTCGGCGACGTATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
KX815125            GTCGGCCTTGAGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MT568786            GTCGGCCTTGAGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MK482055            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MW361240            GTCGGCCTTGTGTCGGCGACGGATCCTTCAAATGTCTGCCCTATCAATTTTCGATGGT
AY222157            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MZ768805            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MZ787582            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MZ410795            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MZ798276            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MZ753784            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MZ751051            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
MZ753903            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
AY222158            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
AY222160            GTCGGCCTTGCGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
JQ886404            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
AY222159            GTCGGCCTTGTGTCGGCGACGGATCTTTCAAATGTCTGCCCTATCAATTTTCGATGGT
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Chapter 6 Tree F - Trematode Alignment 2

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                             210       220       230       240       250
                    .........|.........|.........|.........|.........|........
MASK                mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
Uni_39              AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
KR703279_-_Outgroup AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
KX815125            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MT568786            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MK482055            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MW361240            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
AY222157            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MZ768805            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MZ787582            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MZ410795            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MZ798276            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MZ753784            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MZ751051            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
MZ753903            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
AY222158            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
AY222160            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
JQ886404            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
AY222159            AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACTTCCAAGGAAGGCAGC
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                           310       320       330       340       350
                     ....|....|....|....|....|....|....|....|....|....|....|...
MASK                 mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
Uni_39               GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
KR703279_-_Outgroup  GAAAAATACGGATACGGGACTCATCAGAGGCTCCGTAATTCGAATGAGTACAATTCAA
KX815125             GAAAAATACGGATACGGGACTCGTTAGAGGCTCCGTAATTCGAATGAGTACACTTTAA
MT568786             GAAAAATACGGATACGGGACTCGTTAGAGGCTCCGTAATTCGAATGAGTACACTTTAA
MK482055             GAAAAATACGGATACGGGACTCATTAGAGGCTCCGTAATTCGAATGAGTACAATTTAA
MW361240             GAAAAATACGGATACGGGACTCATTAGAGGCTCCGTAATTCGAATGAGTACAATTTAA
AY222157             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
MZ768805             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
MZ787582             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
MZ410795             GAAAAATACGGATACGGGACTCGAATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
MZ798276             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
MZ753784             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
MZ751051             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
MZ753903             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
AY222158             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
AY222160             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA
JQ886404             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACACTTTAA
AY222159             GAAAAATACGGATACGGGACTCGTATGAGGCTCCGTAATTCGAATGAGTACAATTTAA

                           410       420       430       440       450
                     ....|....|....|....|....|....|....|....|....|....|....|...
MASK                 mmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmmm
Uni_39               CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATAAAAGTTGTTGCAGTTAAAAAGCT
KR703279_-_Outgroup  CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
KX815125             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MT568786             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MK482055             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MW361240             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
AY222157             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MZ768805             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MZ787582             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MZ410795             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MZ798276             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MZ753784             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MZ751051             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
MZ753903             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
AY222158             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
AY222160             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
JQ886404             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
AY222159             CAGCCGCGGTAACTCCAGCTCCAGAAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCT
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Chapter 6 Tree F - Trematode Alignment 2

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                           510       520       530       540       550
                     ....|....|....|....|....|....|....|....|....|....|....|...
MASK                 mmmmmmmmmmmmmmmm
Uni_39               ATTCTTGGCTTGGTTA
KR703279_-_Outgroup  ATTCCTGGCTTGGTTACGACTGGGTCGGGTTTGCGAGTCGGTGTCGTGGTTGTGCAGC
KX815125             ATTCCTGGCCTGGTTT
MT568786             ATTCCTGGCCTGGTTT
MK482055             ATTCCTGGCCTGGTTC
MW361240             ATTCCTGGCCTGGTTT
AY222157             ATTCTCGGCTTGGTTT
MZ768805             ATTCTCGGCTTGGTTT
MZ787582             ATTCTCGGCTTGGTTT
MZ410795             ATTCTGGGCTTGGTTT
MZ798276             ATTCTCGACTTGGTCA
MZ753784             ATTCTCGACTTGGTTA
MZ751051             ATTCTCGACTTGGTTA
MZ753903             ATTCTCGACTTGGTTA
AY222158             ACTCGTGGCTTGGTTT
AY222160             ATTCATGGCTTGGTTA
JQ886404             ATTCTTGGTTTGGTTA
```

AY222159 A T T C T T G G C T T G G T T A