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Evidence for Disease Mediated Extinction: Correlation Between an Introduced Pathogen and Extinction of *Rattus Macleari* on Christmas Island

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EVIDENCE FOR DISEASE MEDIATED EXTINCTION: CORRELATION BETWEEN AN INTRODUCED PATHOGEN AND EXTINCTION OF *RATTUS MACLEARI* ON CHRISTMAS ISLAND

by

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ABSTRACT

EVIDENCE FOR DISEASE MEDIATED EXTINCTION: CORRELATION BETWEEN AN INTRODUCED PATHOGEN AND EXTINCTION OF *RATTUS MACLEARI* ON CHRISTMAS ISLAND

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The Durham Collection and the Cambridge and Oxford University Museums provided the materials to investigate the possibility that the extinction of indigenous rats of Christmas Island was a result of disease introduced by infected ship rats (*R. rattus*) in 1899. The collections of H.E. Durham in 1901-1902 reveal that *R. macleari* was present on Christmas Island up to then and includes specimens of *R. rattus* together with specimens that exhibit characteristics of both *R. rattus* and *R. macleari*. Durham's notes indicate both *R. rattus* and *R. macleari* specimens were heavily infected with trypanosomes at the time of collection. In addition, documentation from a visit to the island by K.R. Hanitsch of the Raffles Museum in Singapore suggests that by 1904 *R. macleari* was no longer present. Thus, the invasion of the island by ship rats, the presence of a pathogenic organism and the extinction of an endemic rat species all coincide at the same time. Portions of nuclear gene sequences were analyzed from skin samples of the Durham collection to determine if *R. macleari* was a unique species and if hybridization occurred when ship rats were introduced to the island. Specimens were also analyzed to determine the presence of trypanosome infection.

The morphologically described *R. macleari* samples revealed sequences different from those of known rats, suggesting *R. macleari* was in fact a unique, endemic rat species that is now extinct. The molecular evidence thus far does not suggest that hybridization occurred between *R. macleari* and *R. rattus*. In addition, four of the rats showed a clear signal for rat specific trypanosomes, indicating that the pathogen was present. An independent laboratory has confirmed the results. Although the data are correlative, this is the first confirmed example of a known disease-causing agent coincident with an extinction event in an endemic species and could serve as a model for first contact followed by extinction as may have occurred to multiple species at the end of the Pleistocene era over 10,000 years ago.

This thesis is dedicated to my parents and husband who have supported me all the way since the beginning of my studies.

Also, this thesis is dedicated to the memory of my father, Walter Smith.

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CHAPTER I

INTRODUCTION

Christmas Island

Christmas Island is a small territory of Australia located in the Indian Ocean 2,000 kilometers northwest of Perth and 1,300 kilometers south of Singapore (Fig. 1). [1] Captain William Mynors, of the *Royal Mary*, named the island when he arrived on Christmas Day in 1643. The island remained uninhabited until 1888 when the Clunies-Ross brothers from the neighboring Cocos-Keeling Islands established the first settlement at Flying Fish Cove (2). A joint lease between George Clunies-Ross and naturalist Dr. John Murray of England was granted in 1891 and small phosphate shipments began to be exported in 1895. By 1897 the Christmas Island Phosphate Company was formed. After World War II, Christmas Island came under the jurisdiction of Singapore and in 1948 the mining was taken over by the Australian and New Zealand Governments in a partnership with the British Phosphate Commissioners. Due to the effects of drought and low phosphate prices, the government closed the phosphate mine in December of 1987.

Today, over 60% of Christmas Island is National Park with large areas of pristine and ancient rainforests. The flora and fauna of the island are of particular interest because they have evolved independently of human interference. There are over 200 species of native flowering plants, 16 of which are endemic to the island. Red crabs can be found all over the forest floor and their annual mass migration to the sea to spawn has been Christmas Island's claim to fame. The island is also a nesting site for various sea bird species.

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[1] The model for this thesis is the Journal of Bacteriology.

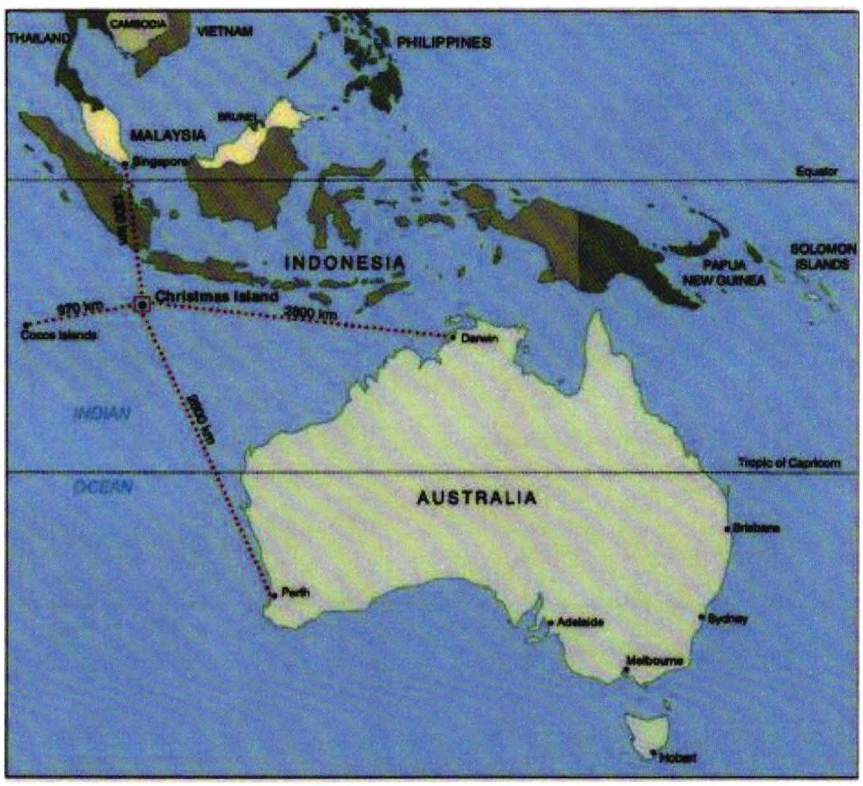

FIG. 1. Location of Christmas Island. Christmas Island is located at 10° south in the Indian Ocean. Illustration is taken from Shire of Christmas Island website at http://www.christmas.shire.gov.cx.

Disappearance of Christmas Island Rats

At the time of human colonization on Christmas Island, five mammals, including two rodents, were endemic to the island. The mammals included the Christmas Island fruit bat (*Pteropus natalis*), the Christmas Island insect bat (*Pipistrellus murrayi*) and the Christmas Island shrew (*Crocidura trichura*) (2). *Rattus macleari* and *Rattus nativitatis* were the two endemic species of rats (2). These two species became extinct within the first few years of the twentieth century. The extinction occurred quickly, with confirmation of extinction in 1908 by the naturalist C.W. Andrews following several visits to the Island between 1897 and 1908 (18).

Many expeditions provide pre-extinction accounts of the rodent population, including the rise and decline of native species. In 1887, the *H.M.S. Flying Fish* visited Christmas Island to collect scientific specimens of rock and coral. A holotype of *R. macleari* was included in this collection (18). In 1887, J.J. Lister collected 7 specimens of *R. macleari* and 2 larger specimens of *R. nativitatis*. He described the rats as nocturnal and abundant, and both ground dwelling and arboreal. After mining began, C.W. Andrews conducted a study of Christmas Island's natural history. He compiled "A Monograph of Christmas Island" in which he describes *R. macleari* as being "the commonest of the mammals found in the island; in every part I visited, it occurred in swarms. During the day nothing is to be seen of it, but soon after sunset numbers may be seen running about in all directions and the whole forest is filled with its peculiar querulous squeaking and the noise of frequent fights. These animals, like most of those found on the island, are almost completely devoid of fear, and in the bush if a lantern be held out they will approach to examine the new phenomenon. Their natural food appears

to be mainly fruits and young shoots and to obtain the former they ascend trees to a great height ... and frequently come into conflict with the fruit bats on the tops of the papaya-trees ... In daytime these rats live in holes among the roots of trees, in decaying logs, and shallow burrows. They seem to breed all the year round" (3, 18).

Dr. Herbert Durham, a pathologist, arrived on the island November 25, 1901 as part of the London School of Tropical Medicine's "Beri-beri Expedition" investigating outbreaks of disease among the Christmas Island Phosphate Company workers. In his official reports he mentions rats destroying vegetable gardens grown to feed the mineworkers. The mine manager, Captain Vincent, told Durham that the *S.S. Hindustan* had introduced *R. rattus* in a cargo of hay in 1899. By his visit in 1901-02, the ship rat population had multiplied (18). Durham collected 19 specimens of both ship rats and endemic rats on Christmas Island and his collection was presented to the Zoology Museum in June of 1910.

Scientists continued to visit Christmas Island and from these visits, a number of accounts addressed the disappearance of the native rats (18). K. R. Hanitsch from the Raffles Museum in Singapore wrote they had expected the rats to be a nuisance and were equipped with traps but found none. "The rats had disappeared, at least those two species, *M. nativitatis* and *M. macleari* which Andrews had found in such abundance only 7 years before." Andrews visited the island again in 1908 and confirmed the extinction of *R. macleari* and *R. nativitatis*. A medical officer reported to him that about 1902-03 he had seen "individuals of the native species of rats crawling about the paths in the daytime, apparently in a dying condition" (18).

The Durham Collection

The Durham Collection is split between the Zoological Collections, University Museum, Oxford and the University Museum of Zoology, Cambridge (18). The collection contains three distinct morphologies, including rats with characteristics of *R. macleari*, *R. rattus* and possible hybrids (18). *R. nativitatis* specimens were not included in the collection because these rats were already scarce on the island. *R. macleari* were described as large with a pelage that is cream ventrally and chestnut brown dorsally with long dark guard hairs. The ear pinna (flaps) are small and the facial vibrissae (whiskers) are coarse and dark. The tail is bicolored, being dark proximally but white distally. The hind foot is elongated as well. The ship rats, *R. rattus*, were described as smaller rats with a dark gray pelage dorsally and pale gray ventrally. There are small amounts of pale guard hairs concentrated laterally and caudally. The ear pinna are relatively large and the facial vibrissae are very fine. The tail is uniformly dark. The possible hybrids were described as large rats with a variable pelage, cream or gray ventrally, dorsally ranging from mid to dark brown with some chestnut and many long pale guard hairs dorsally. The ear pinna are large and the facial vibrissae are coarse and dark. The tail is uniformly colored but paler than the ship rats. The hind foot is relatively short. The third type of rat is described many times as a possible cross between *R. rattus* and *R. macleari* (18).

Durham also published notes on blood parasites in Christmas Island rats. *R. macleari* from around the settlement appeared to have abundant trypanosomes, unlike the rats collected at the top of Phosphate Hill. He also collected a number of *R. rattus* and found they harbored a similar trypanosome and proposed that the ship rats had introduced the infections (18).

Pathogen Effects on Populations

Every organism is host to many kinds of parasites, from viruses and bacteria to fungi and metazoans. Typically these organisms cause little or no harm, but under certain circumstances parasites can become pathogenic, causing severe illness and death. Over the last decade a number of epidemics have caused large-scale declines in several wildlife species including North Sea seals, the Serengeti lions and a wide variety of frog species in Australia, Central America and the western United States (6). In each of these examples, parasitic organisms crossed species barriers or geographical boundaries and became pathogenic to the highly susceptible and immunologically naïve host populations. Therefore parasites can indirectly regulate the population density of their hosts, and affect the dynamics of a community, causing extinction of local populations and, potentially, of whole species (4).

Contact with new species brings the danger of contracting new disease agents, likely to be spread from the invader to a native species that has not evolved effective countermeasures (15). A good example is the brainworm of white-tailed deer of northeastern North America. As forests have become fragmented, the white-tailed deer have invaded areas occupied by moose (*Alces alces*), wapiti (*Cervus elephus*) and caribou (*Rangifer tarandus*). Each of these mammals can be killed by brainworm infections and populations have been exterminated in areas where large populations of white-tailed deer have moved in (15).

Pathogenic organisms have been involved in a number of declines and extinctions of endemic species on oceanic and land-bridge islands as well (25). Direct disease induced extinctions are relatively rare; however, a few thoroughly documented cases do

exist. The introduction of avian poxvirus and avian malaria, *Plasmodium relictum*, into the avifauna of the Hawaiian Islands is one such example (18). When the Hawaiian Islands were first discovered, the endemic avifauna inhabited all parts of the islands. When the tropical subspecies of the night mosquito, *Culex pipiens fatigans*, was introduced to Maui it allowed for the spread of avian poxvirus and avian malaria. These diseases swept through the native bird populations, causing many species to become extinct; the remainder were restricted to the upper elevations of the islands (24).

The Christmas Island rats likely went extinct between 1902-03 (18). Pickering and Norris suggest that the extinction of *R. macleari* goes beyond extinction related to invading competitors. The varied description of the putative hybrid rats along with the uncertainty in both Durham's and Hanitsch's accounts suggest hybridization between native and introduced species occurred. Durham and Hanitsch note that the type 3 morphology of *R. rattus* is variable in size and coat color. Also, it is extremely rare for such hybridization events to be successful unless direct competition occurs or there is a major change in physical environment. With *R. macleari*, the selection pressure may have been the introduction of disease by ship rats from the *Hindustan,* perhaps the infection by trypanosomes identified by Durham (18). Leigh Van Valen developed the Red Queen Hypothesis model of evolution, which suggests that the extinction of a species is determined by its ability to keep up with a deteriorating environment and depends on variation within the gene pool (18). Species have a greater potential to adapt to a changing environment, such as the introduction of a parasitic infection, when there is more genetic variation. For *R. macleari*, hybridization may have been the only possibility to acquire new genomic variability (18).

Trypanosomes

Trypanosomes are flagellated protozoans of the Order Kinetoplastida and parasitize diverse species from humans to plants (20). Members are characterized by the kinetoplast, found within the single mitochondrion, and is a DNA-containing disc-shaped organelle with DNA organized into a complex network of linked mini- and maxi-circles. In addition, trypanosomes have a cytoskeleton consisting of microtubules arranged beneath the plasma membrane, a flagellar pocket, a paraxial rod that connects to the flagellum, an undulating membrane and occasionally a glycocalyx (20) (Fig.2).

Within the Order Kinetoplastida, three families are recognized: Bodonidae (free-living), Cryptobiidae (parasites of fish and invertebrates) and Trypanosomatidae. All species of Trypanosomatidae have a single nucleus and are either elongated with a single flagellum or rounded with a short, non-protruding flagellum. Most trypanosomatids pass through different morphological stages depending on the phase of their life cycle and the host they are parasitizing (14, 20) (Fig. 3). The trypomastigotes stage is characteristic of bloodstream forms of the genus *Trypanosoma*. In trypomastigotes the kinetoplast and kinetosome are near the posterior end of the body and the flagellum runs along the surface and continues as a free whip anterior to the body. The flagellular membrane and flagellum constitute the undulating membrane.

Other body forms differ in shape, position of the kinetosome and kinetoplast, development of the flagellum or the shape of the undulating membrane. An amastigote occurs in the life cycles of some species and is a definitive characteristic of the genus *Leishmania*. The flagellum is very short, projecting only slightly beyond the flagellar pocket. In the promastigote stage the elongated body has the flagellum extending

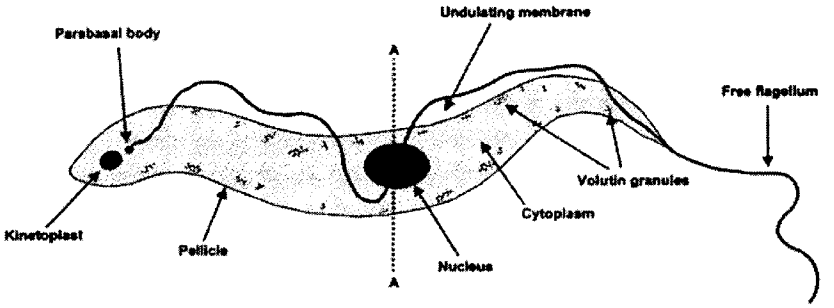

FIG. 2. Fundamental features of a trypanosome (trypomastigote). Figure is modified from (23).

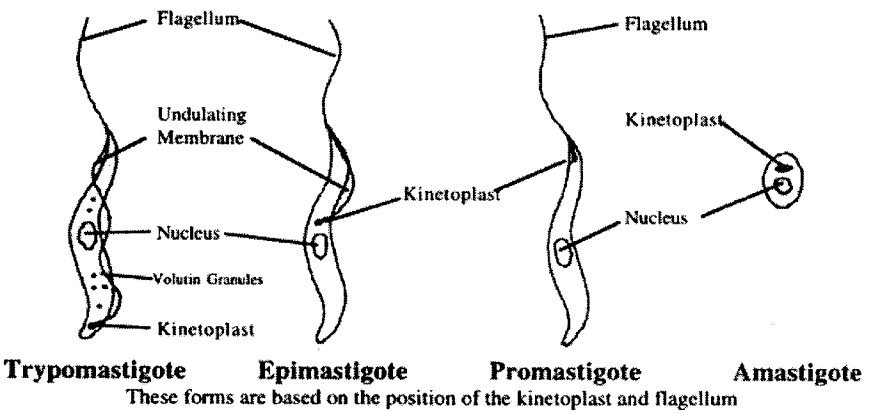

FIG. 3. Four major morphological forms of trypanosomes. Figure taken from the University of Cambridge, Department of Pathology, http://teaching.path.cam.ac.uk./partIB_pract/P14/.

forward as a functional organelle. The kinetosome and kinetoplast are located in front of the nucleus, near the anterior end of the body. The promastigote form is found in the life cycles of several species while they are in their insect hosts. The epimastigotes form occurs in some life cycles. The kinetoplast and kinetosome are still located between the nucleus and the anterior end, but a short undulating membrane lies along the proximal part of the flagellum (20).

Members of the genus *Trypanosoma* exhibit the greatest diversity of forms during their life cycles, changing into multiple epimastigotes in the insect vector's midgut and then into infective trypomastigotes in either the hindgut or foregut. Metacyclic trypomastigotes are either passed via feces to contaminate a wound or injected with saliva during feeding. Many members of the Trypanosome family are heteroxenous, meaning that during one stage of their lives they live in the blood and/or fixed tissue of vertebrates. During other stages they live in the intestines of bloodsucking invertebrates (20).

The trypanosomes of mammals are divided into two groups, Stercoraria and Salivaria. The distinctions are based on the precise characteristics of their development in their insect hosts (13, 16). If a species develops in the anterior portions of the digestive tract (i.e. salivary glands) it is classified Salivaria. When a species develops in the hindgut of its invertebrate host, it is grouped within the Stercoraria. Mammalian trypanosomes are associated with the causative agents of serious diseases of man and domestic animals such as African sleeping sickness in tropical Africa, Chagas' disease in the Americas, and the trypanosomiasis of livestock, Nagana, in Africa and in tropical and subtropical areas throughout Asia, and Central and South America (14).

Trypanosoma brucei is the causative agent of African sleeping sickness in humans and Nagana in livestock and is a member of the Salivaria group (14, 16, 20). There are three subspecies of *T. brucei*, including *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense*. All are morphologically indistinguishable, but have been treated as separate species. *T.b. gambiense* causes a chronic form of sleeping sickness occurring in west central and central Africa, while *T. b. rhodesiense* occurs in central and east central Africa and causes a more acute type of infection. The insect vectors of *T. b. brucei* and *T. b. rhodesiense* are in the genus *Glossina*, better known as the tsetse fly. When feeding, the tsetse fly may inoculate a host with up to several thousand flagellates with a single bite (20).

In human infections with *T. b. rhodesiense* and *T. b. gambiense*, a small sore often develops at the inoculation site of metacyclic trypanosomes. This is followed by a rapid increase of parasite numbers and invasion of all organs of the body. The lymph nodes become swollen and intermittent periods of fever accompany the beginning stages of the disease as the number of trypanosomes in the blood increases. *T. b. rhodesiense* does not invade the central nervous system because the host usually dies before nervous disorders develop. When trypanosomes of *T. b. gambiense* invade the central nervous system, they initiate the chronic, sleeping sickness stage of infection along with disturbances in coordination, tremor of tongue, hands and trunk, paralysis and finally the onset of coma and death. The mechanism of pathogenesis is unclear (20).

Trypanosoma cruzi is the etiological agent for Chagas' disease in humans and unlike *T. brucei*, is a member of the group Stercoraria. *T. cruzi* is found throughout most of South and Central America as well as in the United States. Many kinds of wild and

domestic mammals serve as reservoirs, with animals that live in close proximity to humans being particularly important in the epidemiology of the disease. For *T. cruzi* the insect vector is the reduviid bug, which often defecates on the skin of its host when it feeds. The reduviid bug feces may contain metacyclic trypanosomes, which gain entry into the body of the vertebrate host through the bite, scratched skin, or mucous membranes that are rubbed with fingers contaminated with feces (20).

Chagas' disease manifests in both the acute phase and chronic phases. The acute phase develops rapidly and is initiated by inoculation into the wound of the trypanosomes from the insect vector's feces. The local inflammation produces a small red nodule, known as a chagoma, with swelling of the regional lymph nodes. Trypanosomes can also enter through the conjunctiva of the eye, causing edema of the eyelid and conjunctiva with swelling of the preauricular lymph nodes. As the acute phase progresses, pockets of parasites, or pseudocysts, may be found in almost every organ of the body. The heart muscle is usually invaded with symptoms of anemia, loss of strength, nervous disorders, chills, muscle and bone pain, and varying degrees of heart failure. Death may occur three to four weeks after infection. The acute stage is most common and severe among children less than five years old. The chronic stage is most often seen in adults with central and nervous dysfunction. The recurring infection may last for years and symptoms are relatively non-severe although progressive (20).

Rodent Trypanosomes

Rodent trypanosomes are members of the group Stercoraria and are highly specific for their vertebrate hosts where they live extracellularly, primarily in the

bloodstream (1). These trypanosomes are transmitted in the feces of the insect vector to the rodent host during grooming (1). Fleas become infected upon ingesting a blood meal from an infected rodent. The trypanosomes undergo morphological changes in the alimentary tract of the insect, followed by accumulation of infective metacyclic forms in the rectum. Upon ingestion of the trypanosomes by the rodent, the metacyclic forms enter the bloodstream through the oral mucosal membranes. The metacyclic trypomastigotes convert to epimastigotes, which are the reproductively competent form of the parasite in the vertebrate host (1).

Trypanosoma lewisi is a trypanosome of the sub-genus *Herpetosoma*, a common blood parasite of Black and Brown rats (*R. rattus* and *R. norvegicus*), and is found in all parts of the world where these rats occur (5). Under natural conditions, the intermediate hosts or vectors of *T. lewisi* are rat-fleas, the most common being *Nosopsyllus fasciatus* in temperate areas and *Xenopsylla cheopis* in tropical and subtropical areas. *T. lewisi* has a stringent rat specificity and cannot grow in mice but can develop in the dog flea (*Ctenocephalides canise)*, mouse flea (*Leptophsylla segnis)* and human flea (*Pulex irritans*) (5). Transmission of *T. lewisi* from rat to rat by fleas was first demonstrated by Rabinowitsch & Kempner (1899) and later confirmed by others (14).

After infection, trypanosomes appear in the blood following an incubation period of about 6 days (14). After the incubation period, the trypanosomes multiply rapidly (up to 300,000 per µl of blood) then decrease progressively to zero (crisis). The reproductive period lasts for about 10 days and is followed by a period of infection. Only the non-dividing adult trypanosomes remain in the blood. These persist for several weeks or months with a gradual decrease in the number of trypanosomes, followed by a crisis

(zero), when all forms of the trypanosome suddenly disappear from the blood and the rat recovers from the infection. The course of development of *T. lewisi* in its host is governed by acquired immunity development during infection and the appearance of two types of proteins, ablastin and trypanolysin. Ablastin inhibits growth and multiplication of the trypanosomes, but does not affect the adult forms. Trypanolysin kills the adult organisms, bringing the infection to an end (14).

Ancient DNA

Many obstacles are faced when working to extract DNA from old or ancient samples including molecular damage, potential contamination issues and nuclear insertions of mitochondrial sequences. The enzymatic repair processes that continually maintain the DNA molecules of living cells break down after cell death. Consequently, enzymes such as lysosomal nucleases rapidly degrade the DNA. *Post mortem* modification of DNA includes oxidative lesions, strand breakage, cross link formation and hydrolytic lesions (12, 26). After death, the irreversible DNA damage accumulates and results in the gradual loss of endogenous DNA and nucleotide sequence information. Contamination with "modern" human DNA can be a problem, particularly when studying human remains, because the samples have often been handled during excavation or curation in museums. In addition, molecular biology labs are often contaminated with previously amplified DNA that can remain stable for long periods of time. Nuclear insertions of mitochondrial sequences are also an issue, although not in the current study, because they can be confused with ancient mitochondrial DNA sequences but may in fact, be modern nuclear DNA contamination (12, 17, 26).

Standards have been proposed to avoid problems associated with low copy DNA amplification and to authenticate ancient DNA sequences (10, 17, 26). To avoid laboratory contamination, the handling of ancient samples, DNA extractions and PCR setup should be in dedicated laboratory facilities separated by room, or ideally by building, from other molecular laboratories. Each set of extractions should include at least one extraction control that does not contain any sample material but is otherwise treated identically. Also for each set of PCR reactions, negative controls should be performed to differentiate between contamination that occurs during the extraction and during set up of the PCR. Repeated amplifications from the same or different extracts from the same specimen are useful to detect contamination, to determine if the extract contains useful DNA and it allows for the detection of consistent changes due to miscoding DNA lesions. Cloning and sequencing of multiple clones from multiple PCR reactions is also necessary to correct for DNA damage, jumping PCR and contamination (17). In addition, it is helpful to amplify short overlapping fragments to detect nuclear insertions of mitochondrial sequences. The suggested standards of authenticity also include quantitative PCR analysis to determine the number of molecules of endogenous DNA in a given sample. Biochemical assays such as amino acid analysis are also helpful to determine macromolecular preservations. Also, it is best to send a representative sample to a second independent laboratory for extraction, PCR amplification and sequence determination. Replication in a second laboratory serves to detect contamination of chemicals or samples during handling in the original laboratory (10, 17, 26).

The suggested standards for authenticating ancient DNA results are continuously changing as new materials are studied. Cloning and independent replication of results have been widely accepted, however, reports are still being published without the basic authentication procedures. Studies of pathogen DNA have been reported in a number of ancient animal and human remains. Tuberculosis studies have reported the retrieval of *Mycobacterium tuberculosis* from skeletal material (7, 8) although soil bacteria could genetically resemble *M. tuberculosis* and may be a possible contamination source (17). In another study of historical outbreaks, DNA specific for *Yersinia pestis* was reportedly amplified from the dental pulp of French plague victims (9) and 14th century French Black Death victims (19), however, the strict criteria of ancient DNA authentication were not followed in this study. Amplified products were directly sequenced and results were not independently reproduced by another laboratory. Gilbert et al. (11) failed to replicate the reports of specific *Y. pestis* DNA amplification from dental pulp residues extracted from plague victims.

Analysis of ancient pathogens has the potential to make significant contributions to the study of disease causing agents. For instance, recent studies of the 1918 flu pandemic from fixed tissue samples and permafrost preserved corpses allowed for the sequence of the entire genome to be determined (21, 22). RNA was not expected to exist in the remains due to the instability of RNA when compared to double-stranded DNA; therefore, the suggested standards for ancient DNA authentication were followed.

Durham's notes on blood parasites in the Christmas Island rats reveal that in 1901-1902 both *R. rattus* and some specimens of *R. macleari* were heavily infected with trypanosomes. Hanitsch's notes show that *R. macleari* was no longer present on

Christmas Island after 1904. As a result of these observations, it is proposed that the extinction of *R. macleari* occurred between 1901 and 1904. In samples collected during this period, there is morphological evidence for interspecific hybridization between *R. macleari* and *R. rattus*. The selective pressure for such hybridization may have resulted from the introduction of trypanosome- infected individuals of *R. rattus* in a cargo of hay in 1899. The hypothesis to be examined in the present study is that the introduction of trypanosomes by *R. rattus* led to the extinction of *R. macleari* on Christmas Island. Ancient DNA methods were used to test the Durham collection for evidence that *R. macleari* was a distinct rat species, for evidence of hybridization between ship rats and *R. macleari*, and to test for the presence of rat specific trypanosomes.

CHAPTER II

MATERIALS AND METHODS

Sample Collection

Skin samples of *Rattus macleari* and *Rattus rattus* were obtained from the H.E. Durham Collection, Zoological Collections of Oxford University Museum and Cambridge University Museum and were provided by Ross MacPhee (American Museum of Natural History, New York, New York) and Alex Greenwood (Old Dominion University, Norfolk, Virginia).

Media Specifications

All agar plates were made with Luria-Bertani (LB) media prepared as per manufacturer's instructions (Beckton Dickinson, Franklin Lakes, New Jersey, USA). The pH was adjusted to 7.0 and the mixture autoclaved at 15 psi for 20 min. After autoclaving, the agar media was cooled to 55°C and ampicillin was added to a final concentration of 100 μg/ml. LB agar plates containing ampicillin, IPTG and X-Gal (Sigma-Aldrich, St. Louis, Missouri, USA) were made as above and supplemented with 0.5 mM IPTG and 80 μg/ml X-Gal. The solution was poured into 15x100 mm plates, allowed to solidify and stored at 4°C.

Ancient DNA Conditions

All ancient DNA extractions and PCR set-up were performed in a PCR workstation located in a designated ancient DNA room to prevent contamination of samples and reagents with modern DNA. Solutions and reagents used in extractions and

PCR set up were stored at the appropriate temperature in the ancient room. All pipettors, filtered pipette tips, microcentrifuge tubes and materials were subjected to short wave ultraviolet light prior to and after work in the ancient DNA room. Protective clothing including lab coats and gloves were worn at all times when working in the ancient DNA room and removed before exiting.

Ancient DNA Extraction

DNA was isolated using the GeneClean Kit for Ancient DNA (QBiogene, Vista, California, USA). This procedure is designed for isolation of DNA from samples of bone, preserved tissue or animal by-products. Skin samples, approximately 0.5 cm^2 in size, were added directly to an overnight soaking solution containing 0.5 M EDTA, 10% SDS and 20 mg/ml Proteinase K using sterile forceps. Samples were rotated and incubated at 37°C for 12-15 hrs. After addition of DeHybernation Solution A, the mixture was rotated for an additional 2-4 hrs at 60°C. Samples were centrifuged at 14 000 x *g* for 5 min to pellet the DNA and supernatant transferred to sterile microcentrifuge tube. Ancient DNA Glassmilk and DeHybernation Solution A were added and samples were rotated for an additional 2 hrs at 37°C and subsequently centrifuged at 14 000 x *g* for 2 min to pellet the Glassmilk. The supernatant was discarded and Salton Wash #1 was added to resuspend the pellet, which was transferred to a spin filter. Salton Wash #2 was added to the mixture and centrifuged at 14 000 x *g* for 2 min to wash the Glassmilk/DNA complex. A 1:1 solution of acetone:ethanol was added to the pellet and centrifuged at 14 000 x *g* to further wash out impurities. Ancient DNA Alcohol Wash was added and centrifuged to empty the filter of wash solution. The

Glassmilk was dried in the spin filter for 2 min by centrifugation at 14 000 x *g* and the filter placed into a DNA-free Elution Catch tube. The pellet was resuspended in 50 µl of DNA-free Elution Solution and centrifuged for 2 min, then the spin filter was removed and discarded. The DNA was stored at -20°C until needed.

Primers

Oligonucleotide primers used in PCR, screening of transformants and sequencing are given in Table 1. All gene specific primers and plasmid specific primers were obtained from Integrated DNA Technologies, Inc. (Coralville, Iowa, USA). Primers were received lyophilized and resuspended in ultra-pure water to 100 µM concentration. From these, primer stocks were made at 10 µM and 2 µM concentrations for PCR and sequencing, respectively.

Polymerase Chain Reaction

Preliminary analysis of rat DNA presence in the samples was performed using the cytochrome B specific primers CytB.L1/CytB.R1. The PCR program used was: initial denaturation 5 min at 95°C; denaturation 20 sec at 92°C; annealing 1 min at 55°C; extension 1 min at 72°C; 40 cycles of denaturation, annealing and extension; final extension 10 min at 72°C.

To amplify portions of the RAG1 gene from *Rattus* samples, the following PCR program was performed using primers RAG1.L1/RAG1.R1 and RAG1.L2/RAG1.R2: initial denaturation 2 min at 95°C; denaturation 35 sec at 95°C; annealing 1 min at 51°C; extension 1 min at 72°C; 40 cycles of denaturation, annealing and extension; final

TABLE 1. Primers used in PCR and sequencing

Primer	Sequence
CytB.L1	5' AAAAAGCTTTCCATCCAACATCTCAGCATGAGA 3'
CytB.R1	5' AAACTGCAGCCCCTCAGAATGATATTTGTCCTC 3'
RAG1 - L1	5' TGCCGCATCTGTGGCAATCA 3'
RAG1 - R1	5' TCTTTCGGAAAAGGCTTTGA 3'
RAG1 - L2	5' AGCACCTGTTCTGTAGAATA 3'
RAG1 - R2	5' TGCTCAGAAAGGACTTGACC 3'
GHR1 - L1	5' CTTCCCTTGGCTCTCTGCAC 3'
GHR1 - R1	5' GCATAAAAGTCAATGTTTGC 3'
GHR1 - L2	5' AATGTCCGAGACAGCAGATA 3'
GHR1 - R2	5' AAGCAGTCGCGTTGAGTATA 3'
Tryp1 - L1	5' AATTCATTCCGTGCGAAAGC 3'
Tryp1 - R1	5' GCTGATAGGGCAGTTGTTCG 3'
Tryp4 - L1	5' ATCAATTTACGTGCATATTC 3'
Tryp4 - R1	5' CAGATAACGTGCTGAGGATA 3'
M13F	5' CGCCAGGGTTTTCCCAGTCACGAC 3'
M13R	5' TCACACAGGAAACAGCTATGAC 3'

extension 10 min at 72°C. The same PCR program was used to amplify portions of the GHR1 gene from *Rattus* samples, but using primers GHR.L1/GHR.R1 and GHR.L2/GHR.R2, respectively.

To determine if trypanosome DNA was present in samples, the following PCR programs were run using primers Tryp1.L1/Tryp1.R1 and Tryp4.L1/Tryp4.R1: initial denaturation 2 min at 95°C; denaturation 30 sec at 95°C; annealing 1 min at 55°C for Tryp1 primers or 57.1°C for Tryp4 primers; extension 1 min at 72°C; 40 cycles of denaturation, annealing and extension; final extension 10 min at 72°C.

All PCR programs were performed on the BioRad MyCycler thermocycler (Hercules, California, USA). Platinum Supermix HiFi (Invitrogen, Carlsbad, California, USA) was used in PCR reactions where the products were to be cloned. Platinum Supermix (Invitrogen) was used for PCR screenings. PCR reactions were performed with 1 µl each primer at 10 µM, 1 µl template DNA and 23 µl of the appropriate Supermix.

All PCR products were electrophoresed on 3% (w/v) agarose gels. Gels were prepared by melting Agarose Low EEO Electrophoresis Grade (Fisher Scientific, Pittsburgh, Pennsylvania, USA) in 0.5x TBE buffer. When cool, but before solidification, ethidium bromide (50-75 µg/ml, final conc.) (Sigma) was added. The mixture was poured into a gel-casting tray and allowed to solidify. The solidified gel was placed in the electrophoresis chamber and covered with 0.5x TBE buffer. The samples were electrophoresed at 150V with a 50 bp DNA Ladder (MBI Fermentas, Hanover, Maryland, USA) was used as a molecular weight marker. Gels were photographed under

UV illumination using a Gel Logic 200 Imaging System (Kodak, Rochester, New York, USA).

Cloning and Transformation

Cloning and transformation reactions were performed as described for either the pGEM®-T Vector System (Promega, Madison, Wisconsin, USA) using JM109 High Efficiency Competent Cells (Promega) or for pCR4-TOPO Cloning Kit for Sequencing (Invitrogen) using One Shot TOP 10 chemically competent *E. coli* cells (Invitrogen). Uptake of the plasmid was by heat shocking in a dry bath incubator for both protocols. Transformed cells were transferred to SOC medium and incubated between 1-1.5 hrs in a 37°C shaking water bath before plating. The JM109 cultures were spread onto LB agar plates containing 100 μg/ml ampicillin, 0.5 mM IPTG and 80 μg/ml X-Gal, while the One Shot TOP 10 cultures were spread on LB agar plates containing 100 μg/ml ampicillin. Plates were incubated overnight at 37°C.

Transformants were screened for the presence of an insert by PCR using M13F and M13R primers. The following PCR program was used: initial denaturation 2 min at 94°C; denaturation 30 sec at 94°C; annealing 30 sec at 60°C; extension 2 min at 72°C; 30 cycles of denaturation, annealing and extension; final extension 10 min at 72°C. Single colonies were placed into the PCR mixture directly from spread plates using sterile toothpicks. After completion, PCR screening products were electrophoresed on 3% agarose gels.

PCR Purification and Sequencing

PCR amplified DNA was purified using the Wizard® PCR Preps DNA Purification System and the Vac-Man® Laboratory Vacuum Manifold (Promega, Madison, Wisconsin, USA). Minicolumns and Syringe Barrel Luer-Lok® Extensions were assembled and inserted into the vacuum manifold. Briefly, PCR product was added to Direct Purification Buffer and vortexed. After the addition of resin, the mixture was vortexed 3 times over a one-minute period to ensure the binding of DNA to the resin. The resin/DNA mixture was loaded into syringe barrels and a vacuum was applied. The column was washed with 80% isopropanol and the vacuum was reapplied to draw the solution through the minicolumn. The resin was dried for an additional 30 sec, the minicolumn was then transferred to a 1.5 ml microcentrifuge tube and centrifuged to remove any residual isopropanol. The minicolumn was transferred to a new microcentrifuge tube and the DNA fragment eluted with water and stored at -20°C.

Purified DNA was sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA). The following reagents were combined in a PCR tube: 5.4 µl 5X Big Dye® Terminator v3.1 sequencing buffer, 2.0 µl primer, 2.6 µl Big Dye Terminator v3.1, 8 µl purified DNA and 2 µl reagent grade water. The following sequencing program was used to incorporate dye terminators with ramping at 1°C/sec: denaturation 30 sec at 96°C; annealing 15 sec at 50°C; extension 4 min at 60°C; 25 cycles of denaturation, annealing and extension.

Unincorporated dye terminators were removed using ethanol and sodium acetate precipitation (0.125 M EDTA, 3 M sodium acetate and 95% ethanol). The precipitated pellet was washed with 70% ethanol and dried in a Minicycler at 90°C. Dried pellets

were resuspended in 20 µl Hi Di Formamide, denatured for 2 min in a Minicycler at 95°C and returned to ice. Sample reactions were run on an ABI Prism 3130xl Genetic Analyzer. Results were analyzed and compared with data available in the National Center for Biotechnology Information GenBank database. Sequence analysis and alignments were performed using Vector NTI Suite (Invitrogen) of programs and BioEdit (Ibis Biosciences, Carlsbad, California, USA).

Samples 2075, 2077, 2079, 18607 and 18846 were sent to Dr. Thomas Gilbert, Centre for Ancient Genetics & Centre for Comparative Genomics, University of Copenhagen, Denmark and primer sets RAG1.L1/R1, GHR.L1/R1, Tryp1.L1/R1 and Tryp4.L1/R1 were used for independent verification of results.

CHAPTER III

RESULTS

Nuclear Sequences

DNA was extracted from skin samples of described *R. rattus*, *R. macleari* and hybrids from the Durham collection following ancient DNA protocols. Polymerase chain reactions (PCR) were performed using gene-specific primers for portions of RAG1 (recombination activating gene 1) and GHR (growth hormone regulator) genes. PCR results are shown in Figures 4 and 5. A 100-bp PCR product was present in all 18 samples from RAG1.L1/R1 amplifications (Fig. 4). Negative control DNA extractions and negative PCR controls were satisfactory, with no DNA amplification evident after PCR products were visualized using gel electrophoresis. Representatives from each morphologically characterized group were selected for further genetic analysis. Sample 2078 and 2079 represent the *R. rattus* collection, samples 2074, 2075, 18606 and 18607 represent the hybrid collection and samples 18841, 18845 and 18846 represent the *R. macleari* collection. A 110-bp PCR product was present in representative samples from GHR.L2/R2 amplifications (Fig. 5, lanes B and C) as well as a 123-bp fragment from RAG1.L2/R2 amplifications (Fig. 5, lanes E and F) and a 148-bp fragment from GHR.L1/R1 amplifications (Fig. 5, lanes H and I). Products were cloned, PCR screened and sequenced. Sequence data was obtained from 2 separate PCR reactions and sequence analysis of at least 2 clones per reaction. All RAG1, GHR and trypanosome clone sequences are in Appendix A – G.

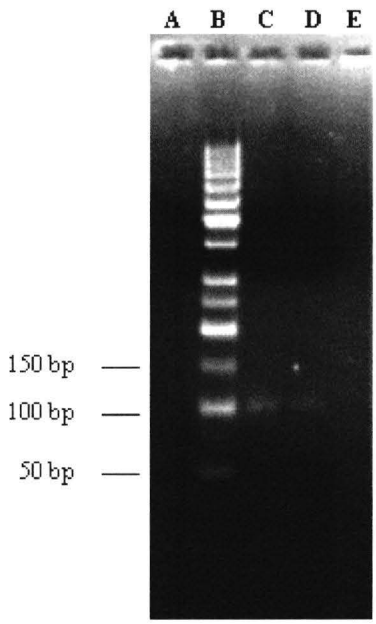

FIG. 4. RAG1.L1/R1 PCR products from sample 2079. Agarose gel (3%) stained with ethidium bromide showing 100-bp amplified products with primer set RAG1.L1/R1. Lane A contains a mock extraction control. Lane B displays a 50-bp ladder. Lanes C and D contain DNA extract while lane E contains the PCR negative control.

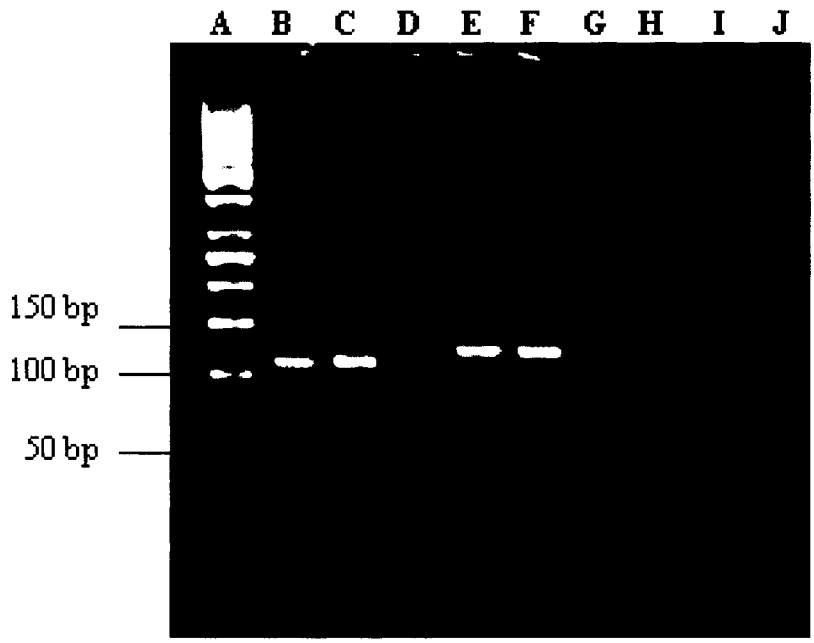

FIG. 5. RAG1.L2/R2, GHR.L1/R1 and GHR.L2/R2 PCR products from sample 18606. Agarose gel (3%) stained with ethidium bromide showing amplified products from DNA extracted from sample 18606. The molecular weight marker (Lane A) is a 50-bp ladder. Lanes B and C contain a 110-bp product from primer set GHR.L2/R2 with lane D as the PCR negative control. Lanes E and F contain a 123-bp product from primer set RAG1.L2/R2 with lane G as PCR negative control. Lanes H and I contain a 148-bp product from primer set GHR.L1/R1 and lane J is the PCR negative control.

Consensus sequence alignments for RAG1.L1/R1 are shown in Fig. 6. Table 2 shows the nucleotide similarities between the Durham collection relative to *R. norvegicus* (XM001079242) and *R. exulans* (DQ023455). *R. rattus* samples 2073, 2076 and 2078 show 100% homology to the *R. norvegicus* RAG1 gene, while samples 2072, 2079 and 2080 illustrate a single base pair change at position 34. The hybrid collection also displays a unique site at position 34, however, sample 18607 shows 100% homology to the *R. norvegicus* RAG1 gene. Sample 2077 displays base pair changes at positions 33 and 72. Described *R. macleari* samples 18841, 18843, 18844, 18845 and 18846 also show base pair changes at positions 33 and 72.

Alignments for consensus sequences of RAG1.L2/R2 representative samples are shown in Fig. 7. Described *R. rattus* samples and hybrids show base pair changes in the *R. norvegicus* RAG1 gene at positions 56 and 96 in RAG.L2/R2 alignments with the exception of hybrid sample 18607, which has base pair changes at positions 32, 56 and 96. Described *R. macleari* samples display base pair changes at positions 65 and 96. Table 2 shows the RAG1 nucleotide similarities between the collection relative to *R. norvegicus* (XM001079242) and *R. exulans* (DQ023455).

```
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         5         15         25         35         45         55
R.norvegicus R1     TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACGGGCACA ACCGGAGATA CCCAGTCCAC
R.exulans R1        .......... .......... T......... .......... .......... ...G.....T

2072R1con           .......... .......... .......... ...A...... .......... ..........
2073R1Con           .......... .......... .......... .......... .......... ..........
2076R1con           .......... .......... .......... .......... .......... ..........
2077R1con           .......... .......... .......... ..T....... .......... ..........
2078R1con           .......... .......... .......... .......... .......... ..........
2079R1con           .......... .......... .......... ...A...... .......... ..........
2080R1con           .......... .......... .......... ...A...... .......... ..........

2074R1con           .......... .......... .......... ...A...... .......... ..........
2075R1con           .......... .......... .......... ...A...... .......... ..........
18606R1con          .......... .......... .......... ...A...... .......... ..........
18607R1con          .......... .......... .......... .......... .......... ..........
18608R1con          .......... .......... .......... ...A...... .......... ..........
18842R1con          .......... .......... .......... ...A...... .......... ..........

18841R1con          .......... .......... .......... ..T....... .......... ..........
18843R1con          .......... .......... .......... ..T....... .......... ..........
18844R1con          .......... .......... .......... ..T....... .......... ..........
18845R1con          .......... .......... .......... ..T....... .......... ..........
18846R1con          .......... .......... .......... ..T....... .......... ..........

                    ....|....| ....|....| ....|....| ....|....|
                         65         75         85         95
R.norvegicus R1     GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
R.exulans R1        .......... .T........ .......... ..........

2072R1con           .......... .......... .......... ..........
2073R1Con           .......... .......... .......... ..........
2076R1con           .......... .......... .......... ..........
2077R1con           .......... .T........ .......... ..........
2078R1con           .......... .......... .......... ..........
2079R1con           .......... .......... .......... ..........
2080R1con           .......... .......... .......... ..........

2074R1con           .......... .......... .......... ..........
2075R1con           .......... .......... .......... ..........
18606R1con          .......... .......... .......... ..........
18607R1con          .......... .......... .......... ..........
18608R1con          .......... .......... .......... ..........
18842R1con          .......... .......... .......... ..........

18841R1con          .......... .T........ .......... ..........
18843R1con          .......... .T........ .......... ..........
18844R1con          .......... .T........ .......... ..........
18845R1con          .......... .T........ .......... ..........
18846R1con          .......... .T........ .......... ..........
```

FIG. 6. Consensus sequence data for cloned RAG1.L1/R1 PCR products. Sequences are aligned relative to *R. norvegicus* (XM001079242). A 'dot' represents homology. All samples from the Durham collection are represented. Primers are highlighted in red.

```
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                       5         15         25         35         45         55         65
R.norvegicus R2     AGCACCTGTT CTGTAGAATA TGCATTCTCC GGTGCCTCAA GGTCATGGGC AGCTATTGTC CCTCGTGCCG
R.exulans R2        .......... .......... ........G. .......... .......... .......... ....T.....

2078R2con           .......... .......... .......... .......... .......... .....C.... ..........
2079R2con           .......... .......... .......... .......... .......... .....C.... ..........

2074R2con           .......... .......... .......... .......... .......... .....C.... ..........
2075R2con           .......... .......... .......... .......... .......... .....C.... ..........
18606R2con          .......... .......... .......... .......... .......... .....C.... ..........
18607R2con          .......... .......... .......... .A........ .......... .....C.... ..........

18841R2con          .......... .......... .......... .......... .......... .......... ....T.....
18845R2con          .......... .......... .......... .......... .......... .......... ....T.....
18846R2con          .......... .......... .......... .......... .......... .......... ....T.....

                    ....|....| ....|....| ....|....| ....|....| ....|....| ...
                       75         85         95         105        115
R.norvegicus R2     ATATCCATGC TTCCCTACTG ACCTGCAGAG TCCGGTCAAG TCCTTTCTGA GCA
R.exulans R2        ......G... .......... .....G.... .......... .......... ...

2078R2con           .......... .......... .....G.... .......... .......... ...
2079R2con           .......... .......... .....G.... .......... .......... ...

2074R2con           .......... .......... .....G.... .......... .......... ...
2075R2con           .......... .......... .....G.... .......... .......... ...
18606R2con          .......... .......... .....G.... .......... .......... ...
18607R2con          .......... .......... .....G.... .......... .......... ...

18841R2con          .......... .......... .....G.... .......... .......... ...
18845R2con          .......... .......... .....G.... .......... .......... ...
18846R2con          .......... .......... .....G.... .......... .......... ...
```

FIG. 7. Consensus sequence data for cloned RAG1.L2/R2 PCR products. Sequences are aligned relative to *R. norvegicus* (XM001079242). A 'dot' represents homology. Primers are highlighted in red. Representatives of described *R. rattus* (2087 and 2079), hybrid (18606, 18607, 2074 and 2075) and *R. macleari* (18841, 18845 and 18846) samples are included in alignments.

Table 2. Comparison of Christmas Island samples' RAG1 nucleotide similarity

Sample	Morphological Description[e]	RAG1.L1/R1		RAG1.L2/R2	
		Nucleotide similarity (%)		Nucleotide similarity (%)	
		R. norvegicus[a]	*R. exulans*[a]	*R. norvegicus*[a]	*R. exulans*[a]
2072	*R. rattus*	99	95	-	-
2073	*R. rattus*	100	96	-	-
2076	*R. rattus*	100	96	-	-
2077	*R. rattus*	98	96	-	-
2078[b]	*R. rattus*	100	96	99	96
2079[b]	*R. rattus*	99	95	99	96
2080	*R. rattus*	99	95	-	-
2074[c]	hybrid	99	95	99	96
2075[c]	hybrid	99	95	99	96
18606[c]	hybrid	99	95	99	96
18607[c]	hybrid	100	96	97	95
18608	hybrid	99	95	-	-
18842	hybrid	99	95	-	-
18841[d]	*R. macleari*	98	96	99	98
18843	*R. macleari*	98	96	-	-
18844	*R. macleari*	98	96	-	-
18845[d]	*R. macleari*	98	96	99	98
18846[d]	*R. macleari*	98	96	99	98

[a] Accession numbers are: *R. norvegicus* (XM001079242), *R. exulans* (DQ023455)

[b] Representatives of *R. rattus* collection (2078 and 2079)

[c] Representatives of hybrid collection (2074, 2075, 18606 and 18607)

[d] Representatives of *R. macleari* collection (18841, 18845 and 18846)

[e] Descriptions adapted from Pickering and Norris (18)

Consensus sequence alignments for GHR.L1/R1 are shown in Fig. 8. The described *R. rattus* and hybrid collections display base pair changes at positions 80, 96, 97 and 115 compared to *R. norvegicus* (NM017094), while the described *R. macleari* samples show nucleotide changes at positions 96, 97, 115 and 128. Fig. 9 shows a similar pattern with GHR.L2/R2 consensus sequence alignments relative to *R. norvegicus* (NM017094). The described *R. rattus* and hybrid collection demonstrate nucleotide changes at positions 38-40, 55, 74 and 79. The described *R. macleari* samples differ at positions 38-40, 70, 74 and 79. Table 3 shows GHR nucleotide similarities relative to *R. norvegicus* (NM017094) and *R. exulans* (DQ019074).

```
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        5         15         25         35         45         55
R.norvegicus G1     CTTCCCTTGG CTCTCTGCAC CCCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
R.exulans G1        .......... .......... ..T...G... .......... .......... T.........

2078Glcon           .......... .......... .......... .......... .......... ..........
2079Glcon           .......... .......... .......... .......... .......... ..........

2074Glcon           .......... .......... .......... .......... .......... ..........
2075Glcon           .......... .......... .......... .......... .......... ..........
18606Glcon          .......... .......... .......... .......... .......... ..........
18607Glcon          .......... .......... .......... .......... .......... ..........

18841Glcon          .......... .......... .......... .......... .......... ..........
18845Glcon          .......... .......... .......... .......... .......... ..........
18846Glcon          .......... .......... .......... .......... .......... ..........


                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        65         75         85         95        105        115
R.norvegicus G1     TTCTGGGCAG TGAAACTGAG TCAACCCACC AACTCCCCTC TACACCAATG AGCAGTCCCG
R.exulans G1        ......A... .......... .......... .....GT... .......... ....A....A

2078Glcon           .......... .........A .......... .....GT... .......... ....A.....
2079Glcon           .......... .........A .......... .....GT... .......... ....A.....

2074Glcon           .......... .........A .......... .....GT... .......... ....A.....
2075Glcon           .......... .........A .......... .....GT... .......... ....A.....
18606Glcon          .......... .........A .......... .....GT... .......... ....A.....
18607Glcon          .......... .........A .......... .....GT... .......... ....A.....

18841Glcon          .......... .......... .......... .....GT... .......... ....A.....
18845Glcon          .......... .......... .......... .....GT... .......... ....A.....
18846Glcon          .......... .......... .......... .....GT... .......... ....A.....


                    ....|....| ....|....| ....|...
                       125        135        145
R.norvegicus G1     TGTCACTGGC AAACATTGAC TTTTATGC
R.exulans G1        .......... .......... ........

2078Glcon           .......... .......... ........
2079Glcon           .......... .......... ........

2074Glcon           .......... .......... ........
2075Glcon           .......... .......... ........
18606Glcon          .......... .......... ........
18607Glcon          .......... .......... ........

18841Glcon          .......A.. .......... ........
18845Glcon          .......A.. .......... ........
18846Glcon          .......A.. .......... ........
```

FIG. 8. Consensus sequence data for cloned GHR.L1/R1 PCR products. Sequences are aligned relative to *R. norvegicus* (NM017094). A 'dot' represents homology. Representatives of described *R. rattus* (2087 and 2079), hybrid (18606, 18607, 2074 and 2075) and *R. macleari* (18841, 18845 and 18846) are included. Primers are highlighted in red.

```
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        5         15         25         35         45         55
R.norvegicus G2     AATGTCCGAG ACAGCAGATA CCGCTCCAGA TGCTGAG~~~ CCTGTCCCAG ACTACACCAC
R.exulans G2        .......... .......... .......... .......ATG .......... ..........

2078G2con           .......... .......... .......... .......ATG .......... ....T.....
2079G2con           .......... .......... .......... .......ATG .......... ....T.....

2074G2con           .......... .......... .......... .......ATG .......... ....T.....
2075G2con           .......... .......... .......... .......ATG .......... ....T.....
18606G2con          .......... .......... .......... .......ATG .......... ....T.....
18607G2con          .......... .......... .......... .......ATG .......... ....T.....

18841G2con          .......... .......... .......... .......ATG .......... ..........
18845G2con          .......... .......... .......... .......ATG .......... ..........
18846G2con          .......... .......... .......... .......ATG .......... ..........

                    ....|....| ....|....| ....|....| ....|....| ....|....|
                        65         75         85         95        105
R.norvegicus G2     GGTTCACACC GTGAAGTCCC CAAGGGGCCT TATACTCAAC GCGACTGCTT
R.exulans G2        .......... ...C....T. .......... .......... ..........

2078G2con           .......... ...C....T. .......... .......... ..........
2079G2con           .......... ...C....T. .......... .......... ..........

2074G2con           .......... ...C....T. .......... .......... ..........
2075G2con           .......... ...C....T. .......... .......... ..........
18606G2con          .......... ...C....T. .......... .......... ..........
18607G2con          .......... ...C....T. .......... .......... ..........

18841G2con          .........T ...C....T. .......... .......... ..........
18845G2con          .........T ...C....T. .......... .......... ..........
18846G2con          .........T ...C....T. .......... .......... ..........
```

FIG. 9. Consensus sequence data for cloned GHR.L2/R2 PCR products. Sequences are aligned relative to *R. norvegicus* (NM017094). A 'dot' represents homology and (~) indicates a gap in the alignment at this position. Primers are highlighted in red. Representatives of described *R. rattus* (2087 and 2079), hybrid (18606, 18607, 2074 and 2075) and *R. macleari* (18841, 18845 and 18846) samples are included in alignments.

Table 3. Comparison of Christmas Island samples' GHR nucleotide similarity

Sample	Morphological Description[e]	GHR.L1/R1		GHR.L2/R2	
		Nucleotide similarity (%)		Nucleotide similarity (%)	
		R. norvegicus[a]	*R. exulans*[a]	*R. norvegicus*[a]	*R. exulans*[a]
2072	*R. rattus*	-	-	-	-
2073	*R. rattus*	-	-	-	-
2076	*R. rattus*	-	-	-	-
2077	*R. rattus*	-	-	-	-
2078[b]	*R. rattus*	97	95	94	99
2079[b]	*R. rattus*	97	95	94	99
2080	*R. rattus*	-	-	-	-
2074[c]	hybrid	97	95	94	99
2075[c]	hybrid	97	95	94	99
18606[c]	hybrid	97	95	94	99
18607[c]	hybrid	97	95	94	99
18608	hybrid	-	-	-	-
18842	hybrid	-	-	-	-
18841[d]	*R. macleari*	97	95	94	99
18843	*R. macleari*	-	-	-	-
18844	*R. macleari*	-	-	-	-
18845[d]	*R. macleari*	97	95	94	99
18846[d]	*R. macleari*	97	95	94	99

[a] Accession numbers are: *R. norvegicus* (NM017094), *R. exulans* (DQ019074)

[b] Representatives of *R. rattus* collection (2078 and 2079)

[c] Representatives of hybrid collection (2074, 2075, 18606 and 18607)

[d] Representatives of *R. macleari* collection (18841, 18845 and 18846)

[e] Descriptions adapted from Pickering and Norris (18)

Trypanosome Sequences

DNA was extracted from skin samples of described *R. rattus*, *R. macleari* and hybrids from the Durham collection following ancient DNA protocols. Polymerase chain reactions (PCR) were performed using gene-specific primers for portions of the 18S ribosomal RNA gene of *Trypanosoma lewisi*. Control DNA extractions and negative PCR controls were always negative. A 64-bp fragment was amplified from samples 2079, 18607 and 18846 using primer set Tryp1.L1/R1 (Fig. 10). Primer set Tryp4.L1/R1 yielded a 74-bp product for samples 2077, 2079, 18607 and 18846 (Fig. 11, lanes B, F and K; sample 2077 amplification not shown). Products were cloned, PCR screened and sequenced.

Consensus sequence alignments for Tryp1.L1/R1 are shown in Fig. 12. Samples 2079 (*R. rattus*), 18607 (hybrid) and 18846 (*R. macleari*) show 100% similarity to *T. lewisi* (AJ009156). Samples 2077 (*R. rattus*), 2079 (*R. rattus*), 18607 (hybrid) and 18846 (*R. macleari*) consensus sequence alignments for Tryp4.L1/R1 primer set are displayed in Figure 13 and match *T. lewisi* (AJ009156) sequence data with 100% similarity. The sequence data was reproduced in a separate laboratory and used in generating the consensus sequence, thus fulfilling an essential criterion of ancient DNA authentication. All consensus sequences, including independent verification sequence data are in Appendices F and G.

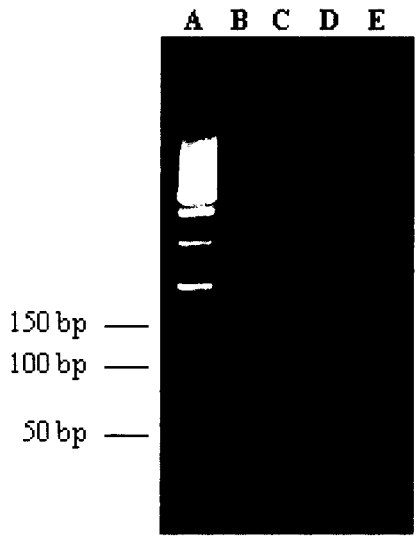

FIG. 10. Tryp1.L1/R1 PCR product from sample 2079. Agarose gel (3%) stained with ethidium bromide showing a 64-bp amplified product. Lane A contains a 50-bp ladder. Lanes B through D contain DNA extract while lane E is the PCR negative control. The band intensity is weak and is less visible in print than in digital form.

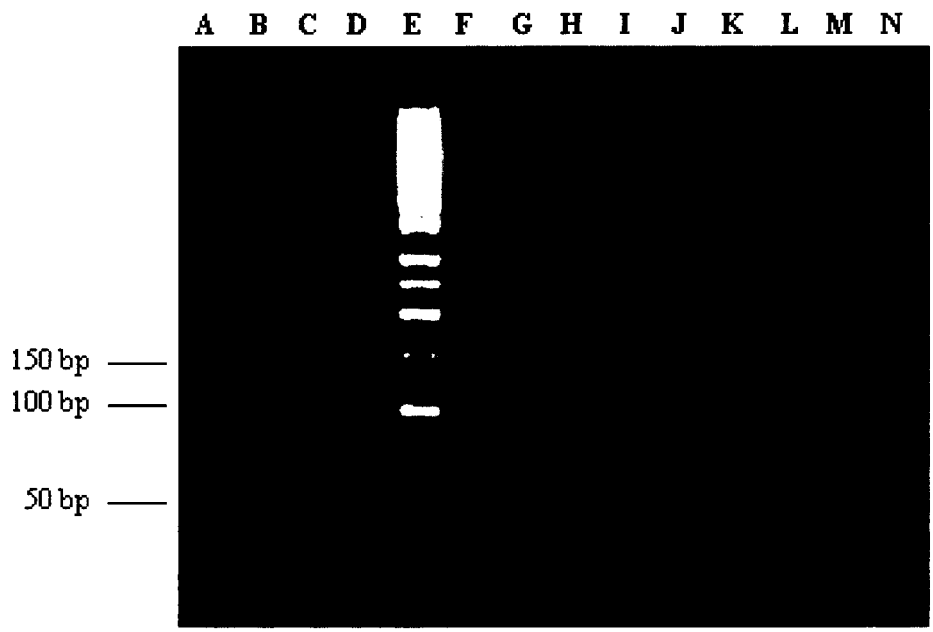

FIG. 11. Tryp4.L1/R1 PCR products from samples 2079, 18607 and 18846. Agarose gel (3%) stained with ethidium bromide showing 74-bp amplified products. Products resulting from samples 2079, 18607 and 18846 (sample 2077 is missing from gel photo). The molecular weight marker is a 50-bp ladder. Lanes A through M contain DNA extracts but only lanes B (2079), F (18607) and K (18846) show a 74-bp amplified product. Lane N is the PCR negative control.

```
                    ....|....| ....|....| ....|....| ....|....| ....|....|
                        5          15         25         35         45
T. lewisi T1        AATTCATTCC GTGCGAAAGC CGGATTCTTT CCGGCGTCTT TTGACGAACA
T. cruzi T1         .......... .......... ...G..T... A.CCG.CG.C ..TTGACGA.

2079T1con           .......... .......... .......... .......... ..........
18607T1con          .......... .......... .......... .......... ..........
18846T1con          .......... .......... .......... .......... ..........

                    ....|....| ....|.
                        55         65
T. lewisi T1        ACTGCCCTAT CAGC..
T. cruzi T1         CAACTG.CC. ATCAGC

2079T1con           .......... ......
18607T1con          .......... ......
18846T1con          .......... ......
```

FIG. 12. Consensus sequence data for cloned Tryp1.L1/R1 PCR products. Sequences are aligned relative to *T. lewisi* (AJ009156). A 'dot' represents homology. Primers are highlighted in red.

```
                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        5          15         25         35         45         55
T. lewisi T4        ATCAATTTAC GTGCATATTC TTTTTGGTCC TCGCAAGAGG TCCTTTTACG GGAATATCCT
T. cruzi T4         T.A....... .......... .........T ...TTTTTAC GG.GAGGG.C TTT.ACGGGA

2077T4con           .......... .......... .......... .......... .......... ..........
2079T4con           .......... .......... .......... .......... .......... ..........
18607T4con          .......... .......... .......... .......... .......... ..........
18846T4con          .......... .......... .......... .......... .......... ..........

                    ....|....| ....|....| .
                        65         75
T. lewisi T4        CAGCACGTTA TCTG..... .
T. cruzi T4         ATATC.TCAG CAC.TTATCT G

2077T4con           .......... .......... .
2079T4con           .......... .......... .
18607T4con          .......... .......... .
18846T4con          .......... .......... .
```

FIG. 13. Consensus sequence data for cloned Tryp4.L1/R1 PCR products. Sequences are aligned relative to *T. lewisi* (AJ009156). A 'dot' represents homology. Primers are highlighted in red.

CHAPTER IV

DISCUSSION

Nuclear Sequence Contrasts and Comparisons

The key to investigating the disappearance of the endemic rat, *R. macleari*, from Christmas Island, lies in the H. E. Durham Collection's skin samples. Because these skins are over 100-years old, ancient DNA protocols were followed to determine species' associations and parasitism by trypanosomes. Most ancient DNA work relies heavily on mitochondrial DNA, due to the abundance of mitochondria in a cell, in comparison to nuclear DNA (12). Ancient DNA is highly degraded and often considerably damaged in comparison to modern DNA. Therefore, the relatively higher copy of mitochondrial gene DNA is more likely to be retrieved than single copy nuclear gene DNA. Consequently, primers were designed to amplify a portion of the cytochrome B gene to determine if any DNA could be retrieved from the Durham collection samples. Preliminary analyses of the Christmas Island specimens using CytB.L1/R1 primers were conducted in this study and in a separate facility. Products as large as 377-bp were amplified. However, sequencing analysis not only yielded rat mitochondrial sequences, human mitochondrial sequences but also a combination of rat and human mitochondrial sequences. These results are typical in ancient DNA work due in part to the fact that samples have been handled during excavation and curation in museums. Thus, human contamination issues are a critical issue.

The retrieval of DNA with non-species specific primers was problematic; therefore, rat-specific nuclear DNA markers (RAG1.L1/R1) were used to determine if quality DNA remained in the samples. Of the samples, 17 of the 18 specimens yielded

rat DNA of reasonable quality; the one exception was sample 2080. Repeated attempts were made to amplify a portion of the RAG1 gene from this sample; 3 clones from a single PCR reaction were obtained and sequenced. Therefore, the consensus sequence for sample 2080 is from a single PCR reaction.

Although the sequence databases do not contain a great abundance of Indonesian rat representatives, portions of nuclear RAG1 and GHR gene sequences obtained in this study suggest that *R. macleari* was a unique species distinct from *R. rattus* and the known Indonesian rats. Genetic analysis with the RAG1.L2/R2 primer set and GHR primers was conducted on representatives from each of the described types of rats. Table 4 shows a summary of sequencing results from the H. E. Durham Collection. Sequence alignments were compared to published *R. norvegicus* (Norway rat) and *R. exulans* (Pacific rat) due to lack of *R. rattus* (ship rat) sequences in NCBI GenBank database. Consensus sequence data was generated from two separate PCR reactions and sequence analysis of at least 2 clones per reaction (except sample 2080). This served to detect heterogeneity in the amplified products due to contamination, DNA damage or jumping PCR (10, 17). In addition, several samples were sent to an independent laboratory, and the data were reproduced, fulfilling a critical verification step for ancient DNA.

The rats collected from Christmas Island and morphologically described as *R. rattus* demonstrate between 99-100% similarity to portions of the *R. norvegicus* RAG1 gene. Three samples illustrate two nucleotide differences relative to *R. norvegicus* while three additional samples show three nucleotide differences in their RAG1 sequence.

Table 4. Summary of sequencing results from the H. E. Durham collection

Sample #	Morphological Description [a]	RAG1 L1/R1 (100 bp)	RAG1 L2/R2 (123 bp)	GHR L1/R1 (148 bp)	GHR L2/R2 (110 bp)	Tryp1 L1/R1 (64 bp)	Tryp4 L1/R1 (74 bp)
E2072	*R. rattus*	positive	*	*	*	negative	negative
E2073	*R. rattus*	positive	*	*	*	negative	negative
E2076	*R. rattus*	positive	*	*	*	negative	negative
E2077	*R. rattus*	positive	*	*	*	negative	positive
E2078	*R. rattus*	positive	positive	positive	positive	negative	negative
E2079	*R. rattus*	positive	positive	positive	positive	positive	positive
E2080	*R. rattus*	positive	*	*	*	negative	negative
E2074	hybrid	positive	positive	positive	positive	negative	negative
E2075	hybrid	positive	positive	positive	positive	negative	negative
18606	hybrid	positive	positive	positive	positive	negative	negative
18607	hybrid	positive	positive	positive	positive	positive	positive
18608	hybrid	positive	*	*	*	negative	negative
18842	hybrid	positive	*	*	*	negative	negative
18841	*R. macleari*	positive	positive	positive	positive	negative	negative
18843	*R. macleari*	positive	*	*	*	negative	negative
18844	*R. macleari*	positive	*	*	*	negative	negative
18845	*R. macleari*	positive	positive	positive	positive	negative	negative
18846	*R. macleari*	positive	positive	positive	positive	positive	positive

[a] Descriptions adapted from Pickering and Norris (18)

All described *R. rattus* samples, with the exception of sample 2077, show between 8 and 9 nucleotide differences in comparison to portions of the *R. exulans* RAG1 gene and demonstrate between 95-96% similarity to the Pacific rat. The GHR gene sequence data display a total of 10 nucleotide differences in comparison to portions of the *R. norvegicus* GHR gene with 94% sequence similarity. The *R. rattus* samples demonstrate 7 nucleotide changes when compared to portions of the *R. exulans* GHR gene and are 99% homologous to *R. exulans*.

The hybrid collection displays similar sequence characteristics to the morphologically described *R. rattus* group, demonstrating a total of three nucleotide differences with 99% similarity to portions of the *R. norvegicus* RAG1 gene. The group displays 9 sequence differences relative to portions of the *R. exulans* RAG1 gene sequence and shares 95% nucleotide similarity. Much like the *R. rattus* group, the hybrid collection demonstrates 10 nucleotide differences when compared to portions of the GHR gene of *R. norvegicus* while illustrating 7 differences in comparison to *R. exulans* GHR gene. Sample 18607 of the hybrid collection demonstrates similar GHR gene nucleotide sequences to the rest of the collection with the exception of RAG1 gene data, in which this sample displays 100% homology to *R. norvegicus* as in three of the seven morphologically described *R. rattus* samples. However, the second portion of the RAG1 gene data contains a single base pair change unlike any other sample in the collection.

The Christmas Island rats morphologically described as *R. macleari* display 98% similarity to portions of the *R. norvegicus* RAG1 gene and show 4 sequence differences with 3 nucleotide differences unlike those described for the *R. rattus* and hybrid collection sequence data. Of the 3-nucleotide differences, 2 nucleotides are identical to

that of *R. exulans* RAG1 sequence. Overall, the group demonstrates 6 nucleotide changes in comparison to portions of the *R. exulans* RAG1 gene and is 96-98% similar to *R. exulans*. The *R. macleari* group demonstrates 10 nucleotide differences and shares 94-97% sequence similarity to portions of the *R. norvegicus* GHR gene. The group displays 7 nucleotide differences and displays 95-99% similarity to portions of the *R. exulans* GHR gene. All differences in GHR sequence data, however, are not identical to the nucleotide changes illustrated in the *R. rattus* and hybrid collection data. *R. macleari* demonstrates a unique GHR sequence for the two portions of the gene relative to *R. norvegicus*, *R. exulans* and both morphologically described *R. rattus* and hybrid groups.

Sample 2077 is described in the Pickering and Norris article (18) as exhibiting *R. rattus* characteristics. Interestingly, the appendix of the article includes Durham's notes and classifies sample 2077 as *R. macleari*. RAG1.L1/R1 sequence data suggests that sample 2077 be grouped with the described *R. macleari* samples. Unfortunately, the discrepancy between the article and the appendix was discovered late into the project and further analysis with the remaining nuclear gene primer combinations was not conducted.

Although nucleotide homologies are relatively similar among the different groups of rats, the consensus sequence alignments suggest otherwise. *R. macleari* samples are notably different and display more genetic variation to *R. norvegicus* and *R. exulans* than the *R. rattus* and hybrid collections. The relational tree located in Appendix E illustrates the differences among the groups of rats, particularly *R. macleari* from other rats in the Durham Collection and is based on nuclear sequence data to date. Clearly, the molecular evidence thus far supports *R. macleari* as a different species distinct from ship rats and Indonesian rats of Southeast Asia.

Trypanosome Presence

Four of the rat samples yielded murid specific trypanosome species sequences indicating that the pathogen in question was present (Table 4). A region of 100% homology relative to the 18s rRNA gene of *Trypanosoma lewisi* was sequenced from samples 2077, 2079, 18607 and 18846 of Tryp4.L1/R1 amplified products. Only three of those samples were positive for the Tryp1.L1/R1 region of the 18S rRNA gene of *T. lewisi*, but also show 100% homology to the rodent trypanosome. Primers were designed to amplify rodent trypanosomes specifically; however, this did not prevent the amplification of a free-living family of kinetoplastids, Bodonidae, and proved to be particularly problematic in Tryp1.L1/R1 amplifications. Tryp4.L1/R1 primer specificity was greater than Tryp1.L1/R1, however, these primers also amplified Bodonidae from the collection samples but less frequently. This could explain the discrepancy between the Tryp4 and Tryp1 retrieval rates. In addition, trypanosome DNA is expected to be less than single copy nuclear DNA, which makes detection from both modern and ancient specimens challenging. Each PCR is subject to stochastic variation in amplification i.e. there are so few surviving pathogen DNA molecules that trypanosome DNA does not amplify in every round of PCR, unlike nuclear DNA sequences. Compounded with the interference from Bodonidae sequences, this may explain why the Tryp1.L1/R1 primer combination was not quite as successful in detecting trypanosome DNA as the Tryp4. L1/R1 combination. Importantly, the trypanosome sequence data was reproduced in a separate laboratory, thus fulfilling an essential criterion of ancient DNA authentication and greatly increases the confidence in the validity of the data.

According to Pickering and Norris (18), Durham's notes on blood parasites in the Christmas Island rats reveal that in 1901-1902 both *R. rattus* and some specimens of *R. macleari* were heavily infected with trypanosomes. In the appendix of the Pickering and Norris article, sample 18846 is specifically noted as having been "infected by trypanosomes" (18). Sample 18846 trypanosome sequence data demonstrates 100% homology to *T. lewisi* 18S rRNA gene for both trypanosome primer sets. Encouragingly, a representative from each type of rat from Durham's collection tested positive for the presence of trypanosome infection. Typically trypanosome infection in rodents is self-limiting, however, *R. macleari* may have been immunologically naïve and highly susceptible to such infection with *T. lewisi*. Christmas Island rats would not have evolved control methods to combat the parasitic infection. Whether hybridization occurred or proved to be a successful strategy is still unanswered. The molecular evidence to date does not suggest hybridization between *R. macleari* and *R. rattus*.

The introduction of trypanosome-infected ship rats in 1899 to Christmas Island appears to have driven the endemic species to extinction. Although the data are correlative, this is the first example of the arrival of an invasive species bearing a known pathogen coincident with the extinction of a related endemic species. Such a first contact scenario and its consequences could serve as a model for the extirpation of mammals in other first contact situations such as the extinctions that occurred 10,000 years ago at the end of the Pleistocene.

CHAPTER V

CONCLUSIONS

Material from the collections and archives of the Cambridge and Oxford University Museums shed new light on the disappearance of the endemic rat *Rattus macleari* from Christmas Island. The rats are thought to have gone extinct between 1898 and 1908 as a result of trypanosome disease introduced by infected individuals of *R. rattus* in 1899. The H.E. Durham collection dates back to 1901-1902 and reveals that *R. macleari* was present on the island at that time, although in lesser numbers than years before. The collection contains specimens of *R. rattus* and *R. macleari*, together with a number of rats that exhibit a combination of morphological characteristics from both *R. rattus* and *R. macleari*. Durham's notes indicate the specimens were heavily infested with trypanosomes and in 1904 Hanitsch noted the endemic rats were no longer present on the island. There is morphological evidence for interspecific hybridization between *R. macleari* and *R. rattus*. The selection pressure for such an event may have been the introduction of trypanosomes to *R. macleari* of Christmas Island.

The museum skin samples were genetically analyzed to determine if Christmas Island rats (*R. macleari*) were a different species and had hybridized with ship rats (*R. rattus*). All skin samples of the Durham collection yielded rat DNA. The morphologically described *R. macleari* samples revealed sequences different from known rats, suggesting *R. macleari* was in fact a unique endemic rat species that is now extinct. The skin samples were also analyzed for the presence of trypanosome DNA. Four of the rats showed a clear signal for rat specific trypanosomes, indicating that the pathogen was present. However, the molecular evidence thus far does not indicate that hybridization

occurred between *R. macleari* and *R. rattus*. The results were confirmed by an independent laboratory and fulfill an important criterion of ancient DNA authentication. This is the first confirmed example of an introduced pathogen coincident with an extinction event of an endemic species and could serve as a model for first contact situations such as the late Pleistocene extinctions.

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APPENDIX A

RAG1.L1/R1 CLONE SEQUENCE DATA

R. rattus specimens from the Durham Collection

Specimen 2072

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
2072R1.3.4       TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACAGGCACA ACCGGAGATA CCCAGTCCAC
2072R1.3.5       ---------- ---------- ---------- ---------- ---------- ----------
2072R1.3.6       ---------- ---------- ---------- ---G------ ---A..A... ----------
2072R1.1.1       ---------- ---------- ---------- ---------- ---------- ----------
2072R1.1.4       ---------- ---------- ---------- ---------- ---------- ----------
2072R1.1.5       ---------- ---------- ---------- ---------- ---------- ----------

                 ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95
2072R1.3.4       GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
2072R1.3.5       ---------- ---------- ---------- ----------
2072R1.3.6       ---------- ---------- ---------- ----------
2072R1.1.1       ---------- ---------- ---------- ----------
2072R1.1.4       ---------- ---------- ---------- ----------
2072R1.1.5       ---------- ---------- ---------- ----------
```

Specimen 2073

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
2073R1.7.10      TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACAGGCACA ACCGGAGATA CCCAGTCCAC
2073R1.7.11      ---------- ---------- ---------- ---------- ---------- ----------
2073R1.7.12      ---------- ---------- ---------- ---------- ---------- ---------T
2073R1.1.1       ---------- ---------- ---------- ---------- ---------- ----------
2073R1.1.2       ---------- ---------- --------A- ---------- ---------- ----------

                 ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95
2073R1.7.10      GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
2073R1.7.11      ---------- ---------- ---------- ----------
2073R1.7.12      ---------- ---------- ---------- ----------
2073R1.1.1       ---------- .T-------- ---------- ----------
2073R1.1.2       ---------- ---------- ---------- ----------
```

Specimen 2076

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     5          15         25         35         45         55
2076R1.11.4      TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACGGGCACA ACCGGAGATA CCCAGTCCAC
2076R1.11.5      .......... .......... .......... .......... .......... ..........
2076R1.11.6      .......... .......... .......... .......... .......... ..........
2076R1.12.7      .......... .......... .......... .......... .......... --T.......
2076R1.12.8      .......... .......... .......... .......... .......... --T.......
2076R1.12.9      .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....|
                     65         75         85         95
2076R1.11.4      GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
2076R1.11.5      .......... .......... .......... ..........
2076R1.11.6      .......... .......... .......... ..........
2076R1.12.7      .......... .......... .......... ..........
2076R1.12.8      .......... .......... .......... ..........
2076R1.12.9      .......... .......... .......... ..........
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Specimen 2077

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     5          15         25         35         45         55
2077R1.14.11     TGCCGCATCT GTGGCAATCA CTTCAAGAGT GATGGGCACA ACCGGAGATA CCCAGTCCAC
2077R1.14.12     .......... .......... .......... ..CA...... .......... ..........
2077R1.37G       .......... ....N..... .......... ..CA...... .......... ..........
2077R1.38G       .......... .......... .......... .......... .......... ..........
2077R1.44G       .......... .......... .......... ......T... .......... .T........
2077R1.45G       .......... .......... .......... .......... .......... ..........
2077R1.46G       .......... ...-...... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....|
                     65         75         85         95
2077R1.14.11     GGGCCCGTGG ATGCTAAAAC TCAAAGCCTT TTCCGAAAGA
2077R1.14.12     .......... .C........ .......... ..........
2077R1.37G       .......... .C........ .......... ..........
2077R1.38G       .......... .......... .......... ..........
2077R1.44G       .......... .......... .......... ..........
2077R1.45G       .......... .......... .......... ..........
2077R1.46G       .......... .......... .......... ..........
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Specimen 2078

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     5          15         25         35         45         55
2078R1.7         TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACGGGCACA ACCGGAGATA CCCAGTCCAC
2078R1.8         .......... .......... .......... .......... .......... ..........
2078R1.9.13      .......... .......... .......... ---A...... .......... ..........
2078R1.9.14      .......... .......... .......... ---A...... .......... ..........
2078R1.9.15      .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....|
                     65         75         85         95
2078R1.7         GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
2078R1.8         A......... .......... .......... ..........
2078R1.9.13      .......... .......... .......... ..........
2078R1.9.14      .......... .......... .......... ..........
2078R1.9.15      .......... .......... .......... ..........
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Specimen 2079

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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         5         15         25         35         45         55
2079.R1.D1          TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACAGGCACA ACCGGAGATA CCCAGTCCAC
2079.R1.D2          .......... .......... .......... .......... .......... ..........
2079.R1.D3          .......... .......... .......... .......... .......... ..........
2079.R1.U2          .......... .......... .......... .......... .......... ..........
2079.R1.U4          .......... .......... .......... .......... .......... ..........
2079.R1.U5          .......... .......... .......... .......... .......... ..........
2079.R1.G50         .......... .......... .......... .......... .......... ..........
2079.R1.G51         .......... .......... .......... .......... .......... ..........
2079.R1.G52         .......... .......... .......... .......... .......... ..........
2079.R1.G53         .......... .......... .......... .......... .......... ..........
2079.R1.G54         .......... .......... .......... .......... .......... ..........

                    ....|....| ....|....| ....|....| ....|....|
                         65         75         85         95
2079.R1.D1          GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
2079.R1.D2          .......... .......... .......... ..........
2079.R1.D3          .......... .......... .......... ..........
2079.R1.U2          .......... .......... .......... ..........
2079.R1.U4          .......... .......... .......... ..........
2079.R1.U5          .......... G......... .......... ..........
2079.R1.G50         .......... .......... .......... .........-
2079.R1.G51         ..T....... .......... .......... ..........
2079.R1.G52         .......... .......... .......... ..........
2079.R1.G53         .......... .......... .......... ..........
2079.R1.G54         .......... .......... .......... ..........
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Specimen 2080

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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         5         15         25         35         45         55
2080R1.11.12        TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACAGGCACA ACCGGAGATA CCCAGTCCAC
2080R1.11.16        .......... .......... .......... .......... .......... ..........
2080R1.11.17        .......... .......... .......... .......... .......... ..........

                    ....|....| ....|....| ....|....| ....|....|
                         65         75         85         95
2080R1.11.12        GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
2080R1.11.16        .......... .......... .......... ..........
2080R1.11.17        .......... .......... .......... ..........
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Hybrid specimens from the Durham collection

Specimen 2074

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                ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                     5          15         25         35         45         55
2074R1.5        TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACAGGCACA ACCGGAGATA CCCAGTCCAC
2074R1.6        ---------- ---------- ---------- ---------- ---------- ----------
2074R1.1.1      ---------- ---------- ---------- ---------- ---------- ----------
2074R1.1.2      ---------- ---------- ---------- ---------- ---------- ----------
2074R1.1.3      ---------- ---------- ---------- ---G------ ---------- ----------

                ----|----| ----|----| ----|----| ----|----|
                     65         75         85         95
2074R1.5        GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
2074R1.6        ---------- ---------- ---------- ----------
2074R1.1.1      ---------- ---------- ---------- ----------
2074R1.1.2      ---------- ---------- ---------- ----------
2074R1.1.3      ---------- ---------- ---------- ----------
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Specimen 2075

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                ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                     5          15         25         35         45         55
2075R1.4.6      TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACAGGCACA ACCGGAGATA CCCAGTCCAC
2075R1.4.13     ---------- ---------- ---------- ---------- ---------- ----------
2075R1.5.11     ---------- ---------- ---------- ---G------ ---------- ----------
2075R1.5.18     ---------- ---------- ---------- ---G------ ---------- ----------
2075R1.33G      ---------- ---------- ---------- ---------- ---------- ----------
2075R1.34G      .........M ---------- ---------- ---------- ---------- ----------
2075R1.35G      ..M....... ---------- ---------- ---------- ---------- ----------

                ----|----| ----|----| ----|----| ----|----|
                     65         75         85         95
2075R1.4.6      GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAAT
2075R1.4.13     ---------- ---------- ---------- ----------
2075R1.5.11     ---------- ---------- ---------- ----------
2075R1.5.18     ---------- ---------- ---------- ----------
2075R1.33G      ---------- ---------- ---------- --------GA
2075R1.34G      ---------- ---------- ---------- --------GA
2075R1.35G      ---------- ---------- ---------- --------GA
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Specimen 18606

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                ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                     5          15         25         35         45         55
18606R1.1.1     TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACAGGCACA ACCGGAGATA CCCAGTCCAC
18606R1.1.3     ---------- ---------- ---------- ---------- ---------- ----------
18606R1.2.4     ---------- ---------- ---------- ---------- ------A--- ----------
18606R1.2.5     ---------- ---------- ---------- ---------- ---------- ----------
18606R1.2.6     ---------- ---------- ---------- ---G------ ---------- ----------

                ----|----| ----|----| ----|----| ----|----|
                     65         75         85         95
18606R1.1.1     GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
18606R1.1.3     ---------- ---------- ---------- ----------
18606R1.2.4     ---------- ---------- ---------- ----------
18606R1.2.5     ---------- ---------- ---------- ----------
18606R1.2.6     ---------- ---------- ---------- ----------
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Specimen 18607

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
18607.R1.1       TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACGGGCACA ACCGGAGATA CCCAGTCCAC
18607.R1.2       .......... .......... .......... .......... .......... ..........
18607.R1.3       .......... .......... .......... ....T..... .......... ..........
18607.R1.4       .......... .......... .......... .......... .......... ..........
18607.R1.5       .......... .......... .......... .......... .......... ..........
18607.R1.7       .......... .......... .......... .......... .......... ..........
18607.R1.8       .......... .......... .......... .......... .......... ..........
18607.R1.9       .......... .......... .......... .......... .......... ..........
18607R1.57G      .......... ...-...... .......... ..T....... .......... ..........
18607R1.61G      .......... .......... .......... ...A...... .......... ..........
18607R1.62G      .......... .......... .......... ...A...... .......... ..........

                 ----|----| ----|----| ----|----| ----|----|
                      65         75         85         95
18607.R1.1       GGACCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
18607.R1.2       ..G....... .......... .......... ..........
18607.R1.3       ..G..T.... .......... .......... ..........
18607.R1.4       ..G....... .......... .......... ..........
18607.R1.5       ..G....... .......... .......... ..........
18607.R1.7       ..G....... .......... .......... ..........
18607.R1.8       ..G....... .......... .......... ..........
18607.R1.9       .......... .......... .......... ..........
18607R1.57G      ..G....... .T........ .......... .........-
18607R1.61G      ..G....... .......... .......... ..........
18607R1.62G      ..T....... .......... .......... ..........
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Specimen 18608

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
18608R1.1.1      TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACAGGCACA ACCGGAGATA CCCAGTCCAC
18608R1.1.2      .......... .......... .......... ...G...... .......... ..........
18608R1.1.3      .......... .......... .......... .......... .......... ..........
18608R1.2.4      .......... .......... .......... .......... .......... ..........
18608R1.2.5      .......... .......... .......... .......... .......... ..........
18608R1.2.6      .......... .......... .......... ...G...... .......... ..........

                 ----|----| ----|----| ----|----| ----|----|
                      65         75         85         95
18608R1.1.1      GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
18608R1.1.2      .......... .......... .......... ..........
18608R1.1.3      .......... .......... .......... ..........
18608R1.2.4      .......... .......... .......... ..........
18608R1.2.5      .......... .......... .......... ..........
18608R1.2.6      .......... .......... .......... ..........
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Specimen 18842

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5          15         25         35         45         55
18842R1.3.7      TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACAGGCACA ACCGGAGATA CCCAGTCCAC
18842R1.3.8      .......... .......... .......... .......... .......... ..........
18842R1.3.9      .......... .......... .......... .......... .......... ..........
18842R1.1.2      .......... .......... .......... .......... .......... ..........
18842R1.1.3      .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95
18842R1.3.7      GGGCCCGTGG ACGCTAAAAC TCAAAGCCTT TTCCGAAAGA
18842R1.3.8      .......... .......... .......... ..........
18842R1.3.9      .......... .......... .......... ..........
18842R1.1.2      .......... .......... .......... ..........
18842R1.1.3      .........A .......... .......... ..........
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R. macleari specimens of the Durham collection

Specimen 18841

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5          15         25         35         45         55
18841R1.1.1      TGCCGCATCT GTGGCAATCA CTTCAAGAGT GATGGGCACA ACCGGAGATA CCCAGTCCAC
18841R1.1.2      .......... .......... .......... .......... .......... .........T
18841R1.1.3      .......... .......... .......... ....A..... .......... .........T
18841R1.2.4      .......... .......... .......... --C....... .......... ..........
18841R1.2.5      .......... .......... .......... ........T. .......... .........T
18841R1.2.6      .......... .......... .......... .........G .......... ..........
18841R1.8.1      .......... .......... .......... .......... ....A..... ..........
18841R1.8.2      .......... .......... .......... .......... .......... ..........
18841R1.8.3      .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95
18841R1.1.1      GGGTCCGTGG ATGCTAAAAC TCAAAGCCTT TTCCGAAAGA
18841R1.1.2      ...C...... .......... .......... ..........
18841R1.1.3      ...C...... .......... .......... ..........
18841R1.2.4      ...C...... .......... .......... ..........
18841R1.2.5      ...C...... .......... .......... ..........
18841R1.2.6      ...C...... .......... .......... ..........
18841R1.8.1      ...C...... .......... .......... ..........
18841R1.8.2      ...C...... .......... .......... ..........
18841R1.8.3      ...C...... .......... .......... ..........
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Specimen 18843

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
18843R1.5.10     TGCCGCATCT GTGGCAATCA CTTCAAGAGT GGTGGGCACA ACCGGAGATA CCCAGTCCAC
18843R1.5.11     .......... .......... .......... .A........ .......... ..........
18843R1.5.1      .......... .......... .......... .A........ .......... ..........
18843R1.5.2      .......... .......... .......T.. .A........ .......... ..........
18843R1.5.3      .......... .......... .......... .A........ .......... ..........
18843R1.6.4      .......... .......... .......... .A........ .......... ..........
18843R1.6.5      .......... .......... .......... .A........ .......... ..........

                 ----|----| ----|----| ----|----| ----|----|
                      65         75         85         95
18843R1.5.10     GGGCCTGTGG ATGCTAAAAC TCAAAGCCTT TTCCGAAAGA
18843R1.5.11     .....C.... .......... .......... ..........
18843R1.5.1      .....C.... .......... .......... ..........
18843R1.5.2      .....C.... .......... .......... ..........
18843R1.5.3      .....C.... .......... .......... ..........
18843R1.6.4      .....C.... .......... .......... ..........
18843R1.6.5      .....C.... .......... .......... ..........
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Specimen 18844

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
18844R1.7.7      TGCCGCATCT GTGGCAATCA CTTCAAGAGT GATGGGCACA ACCGGAGATA CCCAGTCCAC
18844R1.7.8      .......... .......... .......... .......... .......... ..........
18844R1.7.9      .......... .......... .......... .......... .......... ..........
18844R1.1.4      .......... .......... .......... .......... .......... ..........
18844R1.1.5      .......... .......... .......... .......... ..T....... ..........
18844R1.1.6      .......... .......... .......... .......... .......... ..........

                 ----|----| ----|----| ----|----| ----|----|
                      65         75         85         95
18844R1.7.7      GGGCCCGTGG ATGCTAAAAC TCAAAGCCTT TTCCGAAAGA
18844R1.7.8      .......... .......... .......... ..........
18844R1.7.9      .......... .......... .......... ..........
18844R1.1.4      .......... .......... .......... ..........
18844R1.1.5      .......... .......... .......... ..........
18844R1.1.6      .......... .......... .......... ..........
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Specimen 18845

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
18845R1.4.7      TGCCGCATCT GTGGCAATCA CTTCAAGAGT GATGGGCACA ACCGGAGATA CCCAGTCCAC
18845R1.4.8      .......... .......... .......... .......... .......... ..........
18845R1.4.9      .......... .......... .......... .......... .......... .........T
18845R1.13       .......... .......... .......... .......... .......... ..........
18845R1.14       .......... .......... .......... ..CA...... .......... ..........

                 ----|----| ----|----| ----|----| ----|----|
                      65         75         85         95
18845R1.4.7      GGGCCCGTGG ATGCTAAAAC TCAAAGCCTT TTCCGAAAGA
18845R1.4.8      .......... ...T...... .......... ..........
18845R1.4.9      .......... .......... .......... ..........
18845R1.13       .......... .......... .......... ..........
18845R1.14       .......C.. .C........ .......... ..........
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Specimen 18846

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                ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                     5          15         25         35         45         55
18846.R1.4.8    TGCCGCATCT GTGGCAATCA CTTCAAGAGT GACGGGCACA ACCGGAGATA CCCAGTCCAC
18846.R1.4.9    .......... .......... .......... ..T......G .......... ..........
18846.R1.4.10   .......... .......... .......... ..T....... .......... ..........
18846.R1.9.9    .......... .......... .......... ..T.....T. .......... ..........
18846.R1.9.10   .......... .......... .......... ..T.A..... .......... .........T

                ----|----| ----|----| ----|----| ----|----|
                     65         75         85         95
18846.R1.4.8    GGGCCCGTGG ATGCTAAAAC TCAAAGCCTT TTCCGAAAGA
18846.R1.4.9    .......... .......... .......... ..........
18846.R1.4.10   .......... .......... .......... ..........
18846.R1.9.9    .......... .......... .......... ..........
18846.R1.9.10   .......... .......... .......... ..........
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APPENDIX B

RAG1.L2/R2 CLONE SEQUENCE DATA

R. rattus specimens from the Durham Collection

Specimen 2078

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55         65
2078R2.1.2       AGCACCTGTT CTGTAGAATA TGCATTCTCC GGTGCCTCAA GGTCATGGGC AGCTACTGTC CCTCGTGCCG
2078R2.1.3       .......... .......... .......... .......... .......... .......... ..........
2078R2.2.4       .......... .......... .......... .......... .......... .......... ..........
2078R2.2.5       .......... .......... .......... .......... .......... .......... ..........
2078R2.2.6       .......... .......... .......... .......... .......... .......... ..........

                 ----|----| ----|----| ----|----| ----|----| ----|----| ---
                      75         85         95        105        115
2078R2.1.2       ATATCCATGC TTCCCTACTG ACCTGGAGAG TCCGGTCAAG TCCTTTCTGA GCA
2078R2.1.3       .......... .......... .......... .......... .......... ---
2078R2.2.4       .......... .......... .......... .......... .......... ---
2078R2.2.5       .......... .......... .......... .......... .......... ---
2078R2.2.6       .......... .......... .......... .......... .......... ---
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Specimen 2079

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55         65
2079R2.D1        AGCACCTGTT CTGTAGAATA TGCATTCTCC GGTGCCTCAA GGTCATGGGC AGCTACTGTC CCTCGTGCCG
2079R2.D3        .......... .......... .......... .......... .......... .......... ..........
2079R2.D4        .......... .......... .......... .......... .......... .......... ..........
2079R2.D5        .......... .......... .......... .......... .......... .......... ..........
2079R2.U1        .......... .......... .......... .......... .......... .......... ..........
2079R2.U4        .......... .......... .......... .......... .......... .......... ..........

                 ----|----| ----|----| ----|----| ----|----| ----|----| ---
                      75         85         95        105        115
2079R2.D1        ATATCCATGC TTCCCTACTG ACCTGGAGAG TCCGGTCAAG TCCTTTCTGA GCA
2079R2.D3        .......... .......... .......... .......... .......... ---
2079R2.D4        .......... .......... .......... .......... .......... ---
2079R2.D5        .......... .......... .......... .......... .......... ---
2079R2.U1        .......... .......... .......... .......... .......... ---
2079R2.U4        .......... .......... .......... .......... .......... ---
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Hybrid specimens from the Durham Collection

Specimen 2074

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5         15         25         35         45         55         65
2074R2.2.1       AGCACCTGTT CTGTAGAATA TGCATTCTCC GGTGCCTCAA GGTCATGGGC AGCTACTGTC CCTCGTGCCG
2074R2.2.2       ---------- ---------- ---------- ---------- ---------- ---------- ----------
2074R2.2.3       ---------- ---------- ---------- ---------- ---------- ---------- ----------
2074R2.12.10     ---------- ---------- ---------- ---------- ---------- -------C-- ----------
2074R2.12.15     ---------- ---------- ---------- ---------- ---------- ---------- ----------

                 ----|----| ----|----| ----|----| ----|----| ----|----| ---
                      75         85         95        105        115
2074R2.2.1       ATATCCATGC TTCCCTACTG ACCTGGAGAG TCCGGTCAAG TCCTTTCTGA GCA
2074R2.2.2       ---------- ---------- ---------- ---------- ---------- ---
2074R2.2.3       ---------- ---------- ---------- ---------- ---------- ---
2074R2.12.10     ---------- ---------- ---------- ---------- ---------- ---
2074R2.12.15     ---------- ---------- ---------- ---------- ---------- ---
```

Specimen 2075

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5         15         25         35         45         55         65
2075R2.1         AGCACCTGTT CTGTAGAATA TGCATTCTCC GGTGCCTCAA GGTCATGGGC AGCTACTGTC CCTCGTGCCG
2075R2.2         -------T-- ---------- ---------- ---------- --A------- ---------- ----------
2075R2.11        ---------- ---------- ---------- ---------- ---------- ---------- ----------
2075R2.12        ---------- ---------- ---------- ---------- ---------- ---------- ----------

                 ----|----| ----|----| ----|----| ----|----| ----|----| ---
                      75         85         95        105        115
2075R2.1         ATATCCATGC TTCCCTACTG ACCTGGAGAG TCCGGTCAAG TCCTTTCTGA GCA
2075R2.2         ---------- ---------- ---------- ---------- ---------- ---
2075R2.11        ---------- ---------- ---------- ---------- ---------- ---
2075R2.12        ---------- ---------- ---------- ---------- ---------- ---
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Specimen 18606

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5         15         25         35         45         55         65
18606R2.4.4      AGCACCTGTT CTGTAGAATA TGCATTCTCC GGTGCCTCAA GGTCATGGGC AGCTACTGTC CCTCGTGCCG
18606R2.4.5      ---------- ---------- ---------- ---------- ---------- ---------- ----------
18606R2.4.6      ---------- ---------- ---------- ---------- ---------- ---------- ----------
18606R2.5.7      ---------- ---------- ---------- ---------- ---------- ---------- ----------
18606R2.5.8      ---------- ---------- ---------- ---------- ---------- ---------- ----------
18606R2.5.9      ---------- ---------- -A-------- ---------- ---------- ---------- ----------

                 ----|----| ----|----| ----|----| ----|----| ----|----| ---
                      75         85         95        105        115
18606R2.4.4      ATATCCATGC TTCCCTACTG ACCTGGAGAG TCCGGTCAAG TCCTTTCTGA GCA
18606R2.4.5      ---------- ---------- ---------- ---------- ---------- ---
18606R2.4.6      ---------- ---------- ---------- ---------- ---------- ---
18606R2.5.7      ---------- ---------- ---------- ---------- ---------- ---
18606R2.5.8      ---------- ---------- ---------- ---------- ---------- ---
18606R2.5.9      ---------- ---------- ---------- ---------- ---------- ---
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Specimen 18607

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     5         15         25         35         45         55         65
18607R2.1.6      AGCACCTGTT CTGTAGAATA TGCATTCTCC GATGCCTCAA GGTCATGGGC AGCTACTGTC CCTCGTGCCG
18607R2.1.7      .......... .......... .......... .......... .......... .......... ..........
18607R2.2.8      .......... .......... .......... .......... .......... .......... ..........
18607R2.2.9      .......... .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....| ...
                     75         85         95        105        115
18607R2.1.6      ATATCCATGC TTCCCTACTG ACCTAGAGAG TCCGGTCAAG TCCTTTCTGA GCA
18607R2.1.7      .......... .......... .......... .......... .......... ---
18607R2.2.8      ....T..... .......... .......... .......... .......... ---
18607R2.2.9      .......... .......... .......... .......... .......... ---
```

R. macleari specimens from the Durham Collection

Specimen 18841

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     5         15         25         35         45         55         65
18841R2.1.2      AGCACCTGTT CTGTAGAATA TGCATTCTCC GGTGCCTCAA GGTCATGGGC AGCTATTGTC CCTCTTGCCG
18841R2.1.3      .......... .......... ......T... .......... .......... .......... ..........
18841R2.2.4      .......... .......... .......... .......... .......... .......... ..........
18841R2.2.5      .......... .......... .......... .......... .......... .......... ..........
18841R2.2.6      .......... .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....| ...
                     75         85         95        105        115
18841R2.1.2      ATATCCATGC TTCCCTATTG ACCTGGAGAG TCCGGTCAAG TCCTTTCTGA GCA
18841R2.1.3      .......... .......C.. .......... .......... .......... ---
18841R2.2.4      .......... .......C.. .......... .......... .......... ---
18841R2.2.5      .......... .......C.. .......... .......... .......... ---
18841R2.2.6      .......... .......C.A .......... .......... .......... ---
```

Specimen 18845

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     5         15         25         35         45         55         65
18845R2.1.2      AGCACCTGTT CTGTAGAATA TGCATTCTCC GGTGCCTCAA GGTCATGGGC AGCTATTGTC CCTCTTGCCG
18845R2.1.3      .......... .......... .......... .......... .......... .......... ..........
18845R2.2.4      .......... .......... .......... .......... .......... .......... ..........
18845R2.2.5      .......... .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....| ...
                     75         85         95        105        115
18845R2.1.2      ATATCCATGC TTCCCTACTG ACCTGGAGAG TCCGGTCAAG TCCTTTCTGA GCA
18845R2.1.3      .......... .......... .......... .......... .......... ---
18845R2.2.4      .......... .......... .......... .......... .......... ---
18845R2.2.5      .......... .......... .......... .......... .......... ---
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Specimen 18846

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55         65
18846R2.14.16    AGCACCTGTT CTGTAGAATA TGCATTCTCT TGGTGCCTCA AGGTCATGGG CAGCTATTGT CCCTCTTGCC
18846R2.14.17    .......... .......... .......... C......... .......... .......... ..........
18846R2.15.18    .......... .......... .......... C......... .......... .......... ..........
18846R2.15.19    .......... .......... .......... C......... .......... .......... ..........

                 ----|----| ----|----| ----|----| ----|----| ----|----| ----
                      75         85         95        105        115
18846R2.14.16    GATATCCATG CTTCCCTACT GACCTGGAGA GTCCGGTCAA GTCCTTTCTG AGCA
18846R2.14.17    .......... .......... .......... .......... .......... ....
18846R2.15.18    .......... .......... .......... .......... .......... ....
18846R2.15.19    .......... .......... .......... .......... .......... ....
```

APPENDIX C

GHR.L1/R1 CLONE SEQUENCE DATA

R. rattus specimens from the Durham Collection

Specimen 2078

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
2078G1.4.8       CTTCCCTTGG CTCTCTGCAC CCCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
2078G1.4.9       .......... .......... .......... .......... .......... ..........
2078G1.5.10      .......... .......... .......... .......... .......... ..........
2078G1.5.11      .......... .......... .......... .......... .......... ..........
2078G1.5.12      .......... .......... T......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95        105        115
2078G1.4.8       TTCTGGGCAG TGAAACTGAA TCAACCCACC AACTCGTCTC TACACCAATG AGCAATCCCG
2078G1.4.9       .......... .......... .......... .......... .......... ..........
2078G1.5.10      .......... .......... .......... .......... .......... ..........
2078G1.5.11      .......... .......... .......... .......... .......... ..........
2078G1.5.12      .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|...
                     125        135        145
2078G1.4.8       TGTCACTGGC AAACATTGAC TTTTATGC
2078G1.4.9       .......... .......... ........
2078G1.5.10      .......... .......... ........
2078G1.5.11      .......... .......... ........
2078G1.5.12      .......... .......... ........
```

Specimen 2079

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
2079.G1.D6       CTTCCCTTGG CTCTCTGCAC CCCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
2079.G1.D8       .......... .......... .......... .......... .......... ..........
2079.G1.D10      .......... .......... .......... .......... .......... ..........
2079.G1.U1       .......... .......... .......... .......... .......... ..........
2079.G1.U2       .......... .......... .......... .......... .......... ..........
2079.G1.U3       .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95        105        115
2079.G1.D6       TTCTGGGCAG TGAAACTGAA TCAACCCACC AACTCGTCTC TACACCAATG AGCAATCCCG
2079.G1.D8       .......... .......... .......... .......... .......... ..........
2079.G1.D10      .......... .......... .......... .......... .......... ..........
2079.G1.U1       .......... .......... ........T. .......... .......... ..........
2079.G1.U2       .......... .......... .......... .......... .......... ..........
2079.G1.U3       .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|...
                     125        135        145
2079.G1.D6       TGTCACTGGC AAACATTGAC TTTTATGC
2079.G1.D8       .......... .......... ........
2079.G1.D10      ......C... .......... ........
2079.G1.U1       .......... .......... ........
2079.G1.U2       .......... .......... ........
2079.G1.U3       .......... .......... ........
```

Hybrid specimens from the Durham Collection

Specimen 2074

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
2074G1.5.9       CTTCCCTTGG CTCTCTGCAC CCCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
2074G1.4.4       .......... .......... .......... .......... .......... ..........
2074G1.4.6       .......... .......... .......... .......... .......... ..........
2074G1.5.19      .......... .......... .......... .......... .......... ..........
2074G1.5.20      .......... .......... .......... .......... .......... ....G.....

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95        105        115
2074G1.5.9       TTCTGGGCAG TGAAACTGAA TCAACCCACC AACTCGTCTC TACACCAATG AGCAATCCCG
2074G1.4.4       .......... .......... .......... .......... .......... ..........
2074G1.4.6       .......... .......... .......... .......... .......... ..........
2074G1.5.19      .......... .......... .......... .......... .......... ..........
2074G1.5.20      .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|...
                    125        135        145
2074G1.5.9       TGTCACTGGC AAACATTGAC TTTTATGC
2074G1.4.4       .......... .......... ........
2074G1.4.6       .......... .......... ........
2074G1.5.19      .......... .......... ........
2074G1.5.20      .......... .......... ........
```

Specimen 2075

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
2075.G1.1.1      CTTCCCTTGG CTCTCTGCAC CCCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
2075.G1.1.2      .......... .......... .......... .......... .......... ..........
2075.G1.2.1      .......... .......... .......... .......... .......... ..........
2075.G1.2.2      .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95        105        115
2075.G1.1.1      TTCTGTGCAG TGAAACTGAA TCAACCCACC AACTCGTCTC TACACCAATG AGCAATCCCG
2075.G1.1.2      .....G.... .......... .......... .......... .......... ..........
2075.G1.2.1      .....G.... .......... .......... .......... .......... ..........
2075.G1.2.2      .....G.... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|...
                    125        135        145
2075.G1.1.1      TGTCACTGGC AAACATTGAC TTTTATGC
2075.G1.1.2      .......... .......... ........
2075.G1.2.1      .......... .......... ........
2075.G1.2.2      .......... .......... ........
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Specimen 18606

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                     5          15         25         35         45         55
18606G1.7.12     CTTCCCTTGG CTCTCTGCAC CCCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
18606G1.8.13     ---------- ---------- ---------- ---------- ---------- ----------
18606G1.7.5      ---------- ---------- ---------- ---------- ---------- ----------
18606G1.7.6      ---------- ---------- ---------- ---------- ---------- ---------T
18606G1.8.8      ---------- ---------- ---------- ---------- ---------- ----------
18606G1.8.9      ---------- ---------- ---------- T--------- ---------- ----------

                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                     65         75         85         95         105        115
18606G1.7.12     TTCTGGGCAG TGAAACTGAA TCAACCCACC AACTCGTCTC TACACCAATG AGCAATCCCG
18606G1.8.13     ---------- ---------- ---------- ---------- ---------- ----------
18606G1.7.5      ---------- ---------- ---------- ---------- ---------- --------T.
18606G1.7.6      ---------- -----T---- ---------- ---------- ---------- ----------
18606G1.8.8      ---------- ---------- ---------- ---------- ---------- ----------
18606G1.8.9      ---------- ---------- ---------- ---------- ---------- ----------

                 ----|----| ----|----| ----|...
                    125        135        145
18606G1.7.12     TGTCACTGGC AAACATTGAC TTTTATGC
18606G1.8.13     ---------- ---------- --------
18606G1.7.5      ---------- ---------- --------
18606G1.7.6      ---------- ---------- --------
18606G1.8.8      ---------- ---------- --------
18606G1.8.9      ---------- ---------- --------
```

Specimen 18607

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                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                     5          15         25         35         45         55
18607G1.2.1      CTTCCCTTGG CTCTCTGCAC CTCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
18607G1.2.2      ---------- ---------- .C-------- ---------- ---------- ----------
18607G1.1        ---------- ---------- .C-------- ---------- ---------- ----------
18606G1.2        ---------- ---------- .C-------- ---------- ---------- ----------
18607G1.3        ---------- ---------- .C-------- ---------- ---------- ----------

                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                     65         75         85         95         105        115
18607G1.2.1      TTCTGGGCAG TGAAACTGAA TCAACCCACC AACTCGTCTC TACACCAATG AGCAATCCTG
18607G1.2.2      ---------- ---------- ---------- ---------- ---------- --------C.
18607G1.1        ---------- ---------- ---------- ---------- ---------- --------C.
18606G1.2        ---------- ---------- ---------- ---------- ---------- --------C.
18607G1.3        ---------- ---------- ---------- ---------- ---------- --------C.

                 ----|----| ----|----| ----|...
                    125        135        145
18607G1.2.1      TGTCACTGGC AAACATTGAC TTTTATGC
18607G1.2.2      ---------- ---------- --------
18607G1.1        ---------- ---------- --------
18606G1.2        ---------- ---------- --------
18607G1.3        ---------- ---------- --------
```

R. macleari specimens from the Durham Collection

Specimen 18841

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
18841G1.4.7      CTTCCCTTGG CTCTCTGCAC CCCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
18841G1.4.8      ---------- ---------- ---------- ---------- ---------- ----------
18841G1.5.10     ---------- ---------- ---------- ---------- ---------- ----------
18841G1.5.11     ---------- ---------- ---------- ---------- ---------- ----------
18841G1.5.12     ---------- ---------- ---------- ---------- ---------- ----------

                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      65         75         85         95        105        115
18841G1.4.7      TTCTGGGCAG TGAAACTGAG TCAACCCACC AACTCGTCCC TACACCAATG AGCAATCCCG
18841G1.4.8      ---------- ---------- ---------- --------T- ---------- ----------
18841G1.5.10     ---------- ---------- ---------- --------T- ---------- ----------
18841G1.5.11     ---------- ---------- ---------- --------T- ---------- ----------
18841G1.5.12     ---------- ---------- ---------- --------T- ---------- ----------

                 ----|----| ----|----| ----|---
                    125        135        145
18841G1.4.7      TGTCACTAGC AAACATTGAC TTTTATGC
18841G1.4.8      ---------- ---------- --------
18841G1.5.10     ---------- ---------- --------
18841G1.5.11     ---------- ---------- --------
18841G1.5.12     ---------- ---------- --------
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Specimen 18845

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
18845G1.4.8      CTTCCCTTGG CTCTCTGCAC CCCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
18845G1.4.9      ---------- ---------- ---------- ---------- ---------- ----------
18845G1.5.10     ---------- ---------- ---------- ---------- ---------- ----------
18845G1.5.12     ---------- ---------- ---------- ---------- ---------- ----------

                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      65         75         85         95        105        115
18845G1.4.8      TTCTGGGCAG TGAAACTGAG TCAACCCACC AACTCGTCTC TACACCAATG AGCAATCCCG
18845G1.4.9      ---------- ---------- ---------- ---------- ---------- ----------
18845G1.5.10     ---------- ---------- ---------- ---------- ---------- ----------
18845G1.5.12     ---------- ---------- ---------- ---------- ---------- ----------

                 ----|----| ----|----| ----|---
                    125        135        145
18845G1.4.8      TGTCACTAGC AAACATTGAC TTTTATGC
18845G1.4.9      ---------- ---------- --------
18845G1.5.10     ---------- ---------- --------
18845G1.5.12     ---------- ---------- --------
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Specimen 18846

```
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    5          15         25         35         45         55
18846.G1.7.1    CTTCCCTTGG CTCTCTGCAC CCCTCCATTA CCCTGACAAT GGAAGACAAA CCACAGCCAC
18846.G1.7.2    .......... .......... .......... .......... .......... ..........
18846.G1.7.3    .......... .......... .......... .......... .......... ..........
18846.G1.8.4    .......... .......... .......... .......... .......... ..........
18846.G1.8.6    .......... .......... .......... .......... .......... ..........

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                    65         75         85         95        105        115
18846.G1.7.1    TTCTGGGCAG TGAAACTGAG TCAACCCACC AACTCGTCTC TACACCAATG AGCAATCCCG
18846.G1.7.2    C......... .......... .......... .......... .......... ..........
18846.G1.7.3    .......... .......... .......... .......... .......... ..........
18846.G1.8.4    .......... .......... .......... .......... .......... ..........
18846.G1.8.6    .......... .......... .......... .......... .......... ..........

                ....|....| ....|....| ....|...
                   125        135        145
18846.G1.7.1    TGTCACTAGC AAACATTGAC TTTTATGC
18846.G1.7.2    .......... .......... ........
18846.G1.7.3    .......... .......... ........
18846.G1.8.4    .......... .......... ........
18846.G1.8.6    G......... .......... ........
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APPENDIX D

GHR.L2/R2 CLONE SEQUENCE DATA

R. rattus specimens from the Durham Collection

Specimen 2078

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
2078G2.7.13      AATGTCCGAG ACAGCAGATA CCGCTCCAGA TGCTGAGATG CCTGTCCCAG ACTATACCAC
2078G2.7.14      .......... .......... .......... .......... .......... ..........
2078G2.7.15      .......... .......... T......... .......... .......... ..........
2078G2.8.16      .......... .......... .......... .......... .......... ..........
2078G2.8.17      .......... .......... .......... .......... .......... ..........
2078G2.8.18      .......... .......... .......... .......... ..C....... ..........

                 ----|----| ----|----| ----|----| ----|----| ----|----|
                      65         75         85         95        105
2078G2.7.13      GGTTCACACC GTGCAGTCTC CAAGGGGCCT TATACTCAAC GCGACTGCTT
2078G2.7.14      .......... .......... .......... .......... ..........
2078G2.7.15      .......... .......... .......... .......... ..........
2078G2.8.16      .......... .......... .......... .......... ..........
2078G2.8.17      .......... .......... .......... .......... ..........
2078G2.8.18      .......... .......... .......... .......... ..........
```

Specimen 2079

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
2079G2.1.7       AATGTCCGAG ACAGCAGATA CCGCTCCAGA TGCTGAGATG CCTGTCCCAG ACTATACCAC
2079G2.1.8       .......... .......... .......... .......... .........A ..........
2079G2.1.9       .......... .......... .......... .......... .......... ..........
2079G2.2.10      .......... .......... .......... .......... .......... ..........
2079G2.2.1       .......... .......... .......... .......... .......... ..........
2079G2.2.2       .......... .......... .......... .......... .......... ..........

                 ----|----| ----|----| ----|----| ----|----| ----|----|
                      65         75         85         95        105
2079G2.1.7       GGTTCACACC GTGCAGTCTC CAAGGGGCCT TATACTCAAC GCGACTGCTT
2079G2.1.8       .......... .......... .......... .......... ..........
2079G2.1.9       .......... .......... .......... .......... ..........
2079G2.2.10      .......... .......... .......... .......... ..........
2079G2.2.1       .......... .......... .......... .......... ..........
2079G2.2.2       .......... .......... .......... .......... ..........
```

Hybrid specimens from the Durham Collection

Specimen 2074

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     5          15         25         35         45         55
2074G2.7.10      AATGTCCGAG ACAGCAGATA CCGCTCCAGA TGCTGAGATG CCTGTCCCAG ACTATACCAC
2074G2.7.11      .......... .......... .......... .......... .......... ..........
2074G2.7.12      .......... .......... .......... .......... .......... ..........
2074G2.8.13      .......... .......... .......... .......... .......... ..........
2074G2.8.14      .......... .......... .......... .......... .......... ..........
2074G2.8.15      .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                     65         75         85         95        105
2074G2.7.10      GGTTCACACC GTGCAGTCTC CAAGGGGCCT TATACTCAAC GCGACTGCTT
2074G2.7.11      .......... .......... .......... .......... ..........
2074G2.7.12      .......... .......... .......... .......... ..........
2074G2.8.13      .......... .......... .......... .......... ..........
2074G2.8.14      .......... .......... .......... .......... ..........
2074G2.8.15      .......... .......... .......... .......... ..........
```

Specimen 2075

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                     5          15         25         35         45         55
2075G2.1.1       AATGTCCGAG ACAGCAGATA CCGTTCCAGA TGCTGAGATG CCTGTCCCAG ACTATACCAC
2075G2.2.1       .......... .......... ...C...... .......... .......... ..........
2075G2.2.2       .......... .......... ...C...... .......... .......... ..........
2075G2.1.8       .......... .......... ...C...... .......... .......... ..........
2075G2.1.7       .......C.. .......... ...C...... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                     65         75         85         95        105
2075G2.1.1       GGTTCACACC GTGCAGTCTC CAAGGGGCCT TATACTCAAC GCGACTGCTT
2075G2.2.1       .......... .......... .......... .......... ..........
2075G2.2.2       .......... .......... .......... .......... ..........
2075G2.1.8       .......... .......... .......... .......... ..........
2075G2.1.7       .......... .......... .......... .......... ..........
```

Specimen 18606

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
18606G2.10.14    AATGTCCGAG ACAGCAGATA CCGCTCCAGA TGCTGAGATG CCTGTCCCAG ACTATACCAC
18606G2.10.15    .......... .......... .......... .......... .......... ..........
18606G2.10.16    .......... .......... .......... .......... .......... ..........
18606G2.11.10    .......... .......... .......... .......... .......... ..........
18606G2.11.11    .......... .......... .......... .......... .T...T.... ..........
18606G2.11.12    .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95        105
18606G2.10.14    GGTTCACACC GTGCAGTCTC CAAGGGGCCT TATACTCAAC GCGACTGCTT
18606G2.10.15    .......... .......... .......... .......... ..........
18606G2.10.16    .......... .......... .......... .......... ..........
18606G2.11.10    .......... .......... .......... .......... ..........
18606G2.11.11    .......... .......... .......... .......... ..........
18606G2.11.12    .......... .......... .......... .......... ..........
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Specimen 18607

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
18607G2.1.1      AATGTCCGAG ACAGCAGATA CCGCTCCAGA TGCTGAGATG CCTGTCCCAG ACTATACCAC
18607G2.4.4      .......... .......... .......... .......... .......... ..........
18607G2.4.5      .......... .......... .......... .......... .......... ..........
18607G2.2.1      .......... .......... .......... .......... .......... ..........
18607G2.2.2      .......... .......... .......... .......... .......... ..........
18607G2.2.3      .......... .......... .......... .......... .......... ..........
18607G2.2.1A     .......... .......... .......... .......... .......... ..........
18607G2.2.2A     .......... .......... ..A....... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95        105
18607G2.1.1      GGTTCACACC GTGCAGTCTC CAAGGGGCCT TATACTCAAC GCGACTGCTT
18607G2.4.4      .......... .......... .......... .......... ..........
18607G2.4.5      .......... .......... .......... .......... ..........
18607G2.2.1      .......... .......... .......... .......... ..........
18607G2.2.2      .......... .......... .......... .......... ..........
18607G2.2.3      .......... .......... .......... .......... ..........
18607G2.2.1A     .......... .......... .......... .......... ..........
18607G2.2.2A     .......... .......... .......... .......... ..........
```

R. macleari specimens from the Durham Collection

Specimen 18841

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
18841G2.7.13     AATGTCCGAG ACAGCAGATA CCGCTCCAGA TGCTGAGATG CCTGTCCCAG ACTACACCAC
18841G2.7.14     .......... .......... .......... .......... .......... ..........
18841G2.7.15     .......... .......... .......... .......... .......... ..........
18841G2.8.17     .......... .......... .......... .......... .......... ..........
18841G2.8.18     .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95        105
18841G2.7.13     GGTTCACACT GTGCAGTCTC CAAGGGGCCT TATACTCAAC GCGACTGCTT
18841G2.7.14     .......... .......... .......... .......... ..........
18841G2.7.15     .......... .......... .......... .......... ..........
18841G2.8.17     ...C...... .......... .......... .......... ..........
18841G2.8.18     .......... .......... .......... .......... ..........
```

Specimen 18845

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
18845G2.7.13     AATGTCCGAG ACAGCAGATA CCGCTCCAGA TGCTGAGATT CCCGTCCCAG ACTACACCAC
18845G2.7.14     .......... .......... .......... .........G ..T....... ..........
18845G2.7.15     .......... .......... .......... .........G ..T.....G. ..........
18845G2.8.16     .......... .......... .......... .........G ..T....... ..........
18845G2.8.17     .......... .......... .......... .........G ..T....... ..........
18845G2.8.18     .......... .......... .......... .........G .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95        105
18845G2.7.13     GGTTCACACT GTGCAGTCTC CAAGGGGCCT TATACTCAAC GCGACTGCTT
18845G2.7.14     .......... .......... .......... .......... ..........
18845G2.7.15     .......... .......... .......... .......... ..........
18845G2.8.16     .......... .......... .......... .......... ..........
18845G2.8.17     .......... .......... .......... .......... ..........
18845G2.8.18     .......... .......... .......... .......... ..........
```

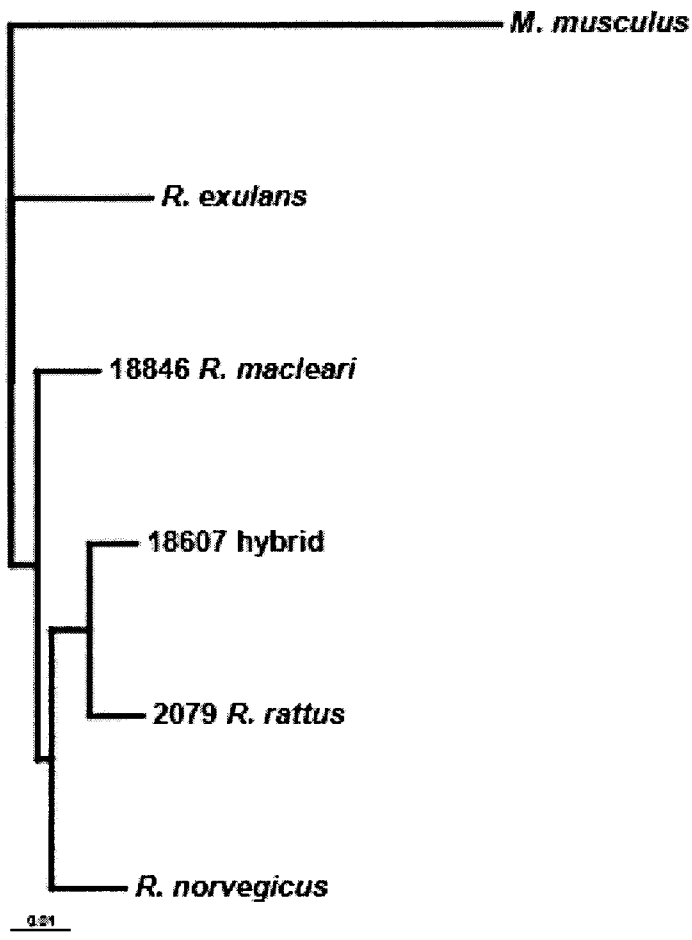
Specimen 18846

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                      5         15         25         35         45         55
18846G2.10.7     AATGTCCGAG ACAGCAGATA CCGCTCCAGA TGCTGAGATG CCTGTCCCAG ACTACACCAC
18846G2.10.8     .......... .......... .......... .......... .......... ..........
18846G2.10.9     .......... .......... .......... .......... .......... ..........
18846G2.11.11    .......... .......... .......... .......... .......... ..........
18846G2.11.12    .......... .......... .......... .......... .......... ..........

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                      65         75         85         95        105
18846G2.10.7     GGTTCACACT GTGCAGTCTC CAAGGGGCCT TATACTCAAC GCGACTGCTT
18846G2.10.8     .......... .......... .......... .......... ..........
18846G2.10.9     .......... .......... .......... .......... ..........
18846G2.11.11    .......... .......... .......... .......... ..........
18846G2.11.12    .......... .......... .......... .......... ..........
```

APPENDIX E

RELATIONSHIP OF *R. MACLEARI* TO OTHER RATS



Neighbor Joining Tree constructed in Vector NTI (Invitrogen) from nuclear sequence data (to date) of Durham collection and published *R. norvegicus* and *R. exulans* on GenBank NCBI database.

APPENDIX F

TRYP1.L1/R1 CLONE SEQUENCE DATA

Specimen 2079

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                    5          15         25         35         45
2079T1.U4       AATTCATTCC GTGCGAAAGC CGGATTCTTT CCGGCGTCTT TTGACGAACA
2079T1.U11      .......... .......... .......... .......... ..........
2079T1.1.1      .......... .......... .......... .......... ..........
2079T1.1.1      .......... .......... .......... ....T..... ..........
2079T1.G1       .......... .......... .......... .......... ..........
2079T1.G2       .......... .......... .......... .......... ..........
2079T1.G3       .......... .......... .......... .......... ..........

                ....|....| ....
                    55
2079T1.U4       ACTGCCCTAT CAGC
2079T1.U11      .......... ....
2079T1.1.1      .......... ....
2079T1.1.1      .......... ....
2079T1.G1       .......... ....
2079T1.G2       .......... ....
2079T1.G3       .......... ....
```

Specimen 18607

```
                ....|....| ....|....| ....|....| ....|....| ....|....|
                    5          15         25         35         45
18607T1.1.1     AATTCATTCC GTGCGAAAGC CGGATTCTTT CCGGCGTCTT TTGACGAACA
18607T1.1.2     .......... .......... .......... .......... ..........
18607T1.1.3     .......... .......... .......... .......... ..........
18607T1.2.4     .......... .......... .......... .......... ..........
18607T1.2.5     .......... .......... .......... .......... ..........
18607T1.G1      .......... .......... .......... .......... ..........
18607T1.G2      .......... .......... .......... .......... ..........

                ....|....| ....
                    55
18607T1.1.1     ACTGCCCTAT CAGC
18607T1.1.2     .......... ....
18607T1.1.3     .......... ....
18607T1.2.4     .......... ....
18607T1.2.5     .......... ....
18607T1.G1      .......... ....
18607T1.G2      .......... ....
```

Specimen 18846

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45
18846T1.1.1      AATTCATTCC GTGCGAAAGC CGGATTCTTT CCGGCGTCTT TTGACGAACA
18846T1.1.4      .......... .......... .......... .......... ..........
18846T1.2.5      .......... .......... .......... .......... ..........
18846T1.2.6      .......... .......... .......... .......... ..........
18846T1.G1       .......... .......... .......... .......... ..........
18846T1.G2       .......... .......... .......... .......... ..........

                 ----|----| ----
                      55
18846T1.1.1      ACTGCCCTAT CAGC
18846T1.1.4      .......... ....
18846T1.2.5      .......... ....
18846T1.2.6      .......... ....
18846T1.G1       .......... ....
18846T1.G2       .......... ....
```

APPENDIX G

TRYP4.L1/R1 CLONE SEQUENCE DATA

Specimen 2077

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
2077T4.1.1       ATCAATTTAC GTGCATATTC TTTTTGGTCC TCGCAAGAGG TCCTTTTACG GGAATATCCT
2077T4.1.2       ---------- ---------- ---------- ---------- ---------- ----------
2077T4.1.3       ---------- ---------- ---------- ---------- ---------- ----------
2077T4.1.4       ---------- ---------- ---------- ---------- ---------- ----------

                 ----|----| ----
                      65
2077T4.1.1       CAGCACGTTA TCTG
2077T4.1.2       ---------- ----
2077T4.1.3       ---------- ----
2077T4.1.4       ---------- ----
```

Specimen 2079

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
2079T4.2.1       ATCAATTTAC GTGCATATTC TTTTTGGTCC TCGCAAGAGG TCCTTTTACG GGAATATCCT
2079T4.2.3       ---------- ---------- ---------- ---------- ---------- ----------
2079T4.7.1       ---------- ---------- ---------- ---------- ---------- ----------
2079T4.7.2       ---------- ---------- ---------- ---------- ---------- ----------
2079T4.7.3       ---------- ---------- ---------- ---------- ---------- ----------
2079T4.7.4       ---------- ---------- ---------- ---------- ---------- ----------
2079T4.7.5       ---------- ---------- ---------- ---------- ---------- ----------

                 ----|----| ----
                      65
2079T4.2.1       CAGCACGTTA TCTG
2079T4.2.3       ---------- ----
2079T4.7.1       ---------- ----
2079T4.7.2       ---------- ----
2079T4.7.3       ---------- ----
2079T4.7.4       ---------- ----
2079T4.7.5       ---------- ----
```

Specimen 18607

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
18607.T4.4       ATCAATTTAC GTGCATATTC TTTTTGGTCC TCGCAAGAGG TCCTTTTACG GGAATATCCT
18607.T4.4F      .......... .......... .......... .......... .......... ..........
18607.T4.5       .......... .......... .......... .......... .......... ..........
18607.T4.A       .......... .......... .......... .......... .......... ..........
18607.T4.C       .......... .......... .......... .......... .......... ..........
18607.T4.E       .......... .......... .......... .......... .......... ..........
18607.T4.F       .......... .......... .......... .......... .......... ..........
18607.T4.G1      .......... .......... .......... .......... .......... ..........
18607.T4.G2      .......... .......... .......... .......... .......... ..........
18607.T4.G3      .......... .......... .......... .......... .......... ..........

                 ----|----| ----
                      65
18607.T4.4       CAGCACGTTA TCTG
18607.T4.4F      .......... ....
18607.T4.5       .......... ....
18607.T4.A       .......... ....
18607.T4.C       .......... .TA.
18607.T4.E       .......... ....
18607.T4.F       .......... ....
18607.T4.G1      .......... ....
18607.T4.G2      .......... ....
18607.T4.G3      .......... ....
```

Specimen 18846

```
                 ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
                      5          15         25         35         45         55
18846T4.2        ATCAATTTAC GTGCATATTC TTTTTGGTCC TCGCAAGAGG TCCTTTTACG GGAATATCCT
18846T4.3        .......... .......... .......... .......... .......... ..........
18846T4.4        .......... .......... .......... .......... .......... ..........
18846T4.5        .......... .......... .......... .......... .......... ..........
18846T4.6        .......... .......... .......... .......... .......... ..........
18846T4.7        .......... .......... .......... .......... .......... ..........
18846T4.8        .......... .......... .......... .......... .......... ..........
18846T4.G1       .......... .......... .......... .......... .......... ..........

                 ----|----| ----
                      65
18846T4.2        CAGCACGTTA TCTG
18846T4.3        .......... ....
18846T4.4        .......... ....
18846T4.5        .......... ....
18846T4.6        .......... ....
18846T4.7        .......... ....
18846T4.8        .......... ....
18846T4.G1       .......... ....
```

VITA

KELLY B. WYATT

EDUCATION:

Old Dominion University
Department of Biological Sciences — Norfolk, VA
Master of Science in Biology — August 2007

Westminster College
Department of Biological Sciences — New Wilmington, PA
Bachelor of Science in Biology — May 2001

RESEARCH EXPERIENCE:

- Investigated the phylogenetic relationship between *Pfiesteria piscicida* and *P. shumwayae* through DNA sequencing analysis of ribosomal RNA genes. Published sequences on National Center for Biotechnology Information GenBank database, accession numbers DQ344043, DQ344042, DQ344041, DQ344040, DQ344039, DQ344038, DQ344037, DQ344036, DQ344035, DQ344034.
- Conducted Master's research on trypanosome presence and the extinction event of *Rattus macleari* from Christmas Island through sequencing analysis of ribosomal RNA genes. Analyzed nuclear genetic sequences to determine if *R. macleari* is a different species.

TECHNICAL SKILLS:

- Modern and ancient DNA extractions, PCR, various cloning methodologies, electrophoresis, sequencing analysis, real-time PCR.

PUBLICATIONS:

Marshall, H.G., Hargraves, P.E., Burkholder, J.A., Parrow, M.W., Elbrachter, M., Allen, E.H., Knowlton, V.M., Rublee, P.A., Hynes, W.L., Egerton, T.A., Remington, D.L., **Wyatt, K.B.**, Lewitus, A.J., and Henrich, V.C. Taxonomy of *Pfiesteria* (Dinophyceae). *Harmful Algae* 5 (2006) 481-496.

WORK EXPERIENCE:

Department of Biological Sciences — 8/02-present
Old Dominion University, Norfolk, VA
Laboratory and Research Specialist

- Manage university's genetic analyzing facility (Applied Biosystems 3130xl Genetic Analyzer), responsible for maintenance, loading samples, data collection, troubleshooting and ordering supplies
- Prepare media, chemical solutions, stains and cultures for microbiology laboratory and research needs