Identification and classification of endogenous bacteria within mole-rats of the Family Bathyergidae

By

James Henry du Toit van Sandwyk

Submitted in partial fulfillment of the requirements for the degree of

Magister Scientiae

(Zoology)

in the Faculty of Natural and Agricultural Science

University of Pretoria

Pretoria

March 2007

© University of Pretoria

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Identification and classification of endogenous bacteria within mole-rats of the Family Bathyergidae

By

J.H.d.T. van Sandwyk

J.H.d.T van Sandwyk: Mammal Research Institute
Department of Zoology and Entomology
University of Pretoria
Pretoria
0002
South Africa

Supervisors:

Prof N.C. Bennett: Mammal Research Institute
Department of Zoology and Entomology
University of Pretoria
Pretoria
0002
South Africa

Dr A.D.S. Bastos: Mammal Research Institute
Department of Zoology and Entomology
University of Pretoria
Pretoria
0002
South Africa

Email: jvsandwyk@zoology.up.ac.za

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

DECLARATION

I, James Henry du Toit van Sandwyk, declare that this thesis which I hereby submit for the degree of Magister Scientiae at the University of Pretoria is my own work and has not previously been by me for a degree at this or any other tertiary institution.

Signature

2 March 2007

Date

ACKNOWLEDGEMENTS

I would like to express my thanks my supervisors Nigel Bennett and Amanda Bastos for their help with all the unexpected problems which arose during my thesis. Nigel for all his help with sample collection and funding for the project, and Amanda for all her help with the molecular work, both of you have provided me with a solid foundation for my future pursuits in the field of science. I would also like to thank Dr. Fanus Venter for providing access to his micro-biology laboratory for all my bacterial culturing and for his help with understanding and interpreting the results of the bacterial study. Glen Malherbe and Babsie Potgieter are thanked for all their help with the technical details of my project as well as all the people in the Zoology Molecular Laboratory and Dr. Venter’s Microbiology Laboratory. Special thanks go to all my friends at the university and those in the real world, especially Leanne Hart and Lia Rotherham for all their help in all the various aspects of my project. Leanne and Craig Jackson assisted with the mole-rat pictures.

A special word of thanks is given to my best “bud” Brian R. Smith for his help in my project and for helping me get over some hard times when nothing seemed to be going right by always having a spare ear and helping me to take a fresh look at the problem and find a solution.

Finally I express my deep gratitude to my family, and in particular my Mom for all her help.

"Omnia possum in eo qui me confortat"

TABLE OF CONTENTS

Chapter 1: General Introduction

Chapter 2: Identification of associated bacteria in three species of South African Mole-rats (Bathyergidae)

Chapter 4: General Conclusion

LIST OF FIGURES

CHAPTER 1: General Introduction

CHAPTER 2: Identification of associated bacteria in three species of South African Mole-rats (Bathyergidae)

CHAPTER 3: The recorded incidence of *Bartonella* infection amongst solitary and social species of South African bathyergids: a multi-gene approach

LIST OF TABLES

Chapter 3: The recorded incidence of *Bartonella* infection amongst solitary and social species of South African bathyergids: a multi-gene approach

DISCLAIMER

This thesis comprises a series of chapters, some of which have been prepared as stand alone manuscripts that will subsequently be submitted for publication purposes. As a consequence there may be some unavoidable repetition arising between the chapters.

CHAPTER 1

General Introduction

Contributing Authors: J. H. d. T. van Sandwyk, N. C. Bennett & A. D. Bastos

Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa

1. The Family Bathyergidae and distributional range of the five genera

The family Bathyergidae is a monophyletic group of Hystricomorph rodents which occurs south of the Sahara (Faulkes *et al*. 2004, Ingram *et al*. 2004). The family is divided into two sub families, the Bathyerginae consisting solely of the genus *Bathyergus* (Bennett & Faulkes 2000) and Georychinae (Bennett & Faulkes 2000). *Bathyergus* is one of two polytypic genera along with the genus *Cryptomy*s. The Bathyerginae comprises two species which are distributed along the western coast of Namibia and South Africa (Jarvis & Bennett 1990, 1991). The first, *Bathyergus janetta* (the Namaqua Dune mole-rat) is endemic to Namaqualand and the coastal dunes south of Namibia (Herbst & Bennett 2006) and the second, *Bathyergus suillus* (the Cape Dune mole-rat) occurs in the southern, southwestern and western regions of the Cape Province of South Africa (Bennett & Faulkes 2000). The other four genera within the family Bathyergidae (Figure 1) belong to the subfamily Georychinae (Figure 1, 2 and 3) (Bennett & Faulkes 2000), three of which are monotypic; these include the genus *Georychus*, which is endemic to the Republic of South Africa, the only species within this genus is *Georychus capensis* (the Cape Mole-rat), secondly *Heterocephalus* has its distribution in eastern Africa, the only species being *Heterocephalus glaber* (the Naked mole-rat). The third monotypic genus is *Heliophobius*, the only representative being *Heliophobius argenteocinereus* (the silvery mole-rat), the silvery mole-rat is distributed within the eastern and southeastern regions of Africa. The fifth and final genus within the Bathyergidae, *Cryptomys* is the most diverse in terms of distribution and species richness; it is coincidently the most controversial with much debate going on within the scientific community on classification of some species within this genus and furthermore whether this genus should be divided into *Fukomys* and *Cryptomys* (Koch *et al*. 2006). There are currently 12 recognized species and 4 subspecies, *C. damarensis* (the Damaraland mole-rat), *C. hottentotus* (the common mole-rat) containing four sub species *C. h. pretoriae* (the highveld mole-rat), *C. h. hottentotus* (the common mole-rat), *C. h. natalensis* (the Natal mole-rat) and *C. h. nimrodi* (the Matabeleland mole-rat). Other species within the genus are *C. darlingi* (the Mashona mole-rat), *C. mechowi* (the giant mole-rat), *C. zechi* (the Ghana mole rat), *C. foxi* (the Nigerian Mole rat), *C. ochrecinereus* (the ochre mole rat), *C. anselli* (Ansell's mole-rat), *C. kafuensis* (Kafue mole-rat), *C. whytei* (Whyte's mole-rat), *C. mickelmi* (Caprivi mole-rat), *C. amatus* (Zambian mole-rat) and *C. bocagei* (Bocage's mole rat). This genus has a very broad distributional range from the southern Sudan region in the east through to Ghana and Nigeria in the west, from the Congo and Tanzania in the north all the way down to the tip of Africa in the South (Faulkes *et al*.1997, Bennett & Faulkes, 2000; Ingram *et al.* 2004).

2. The subterranean environment and its effects on the bathyergids and their levels of sociality and the models explaining it

The subterranean niche has long been assumed to be conserved with a characteristic microclimate that is more muted and specialized when compared to the ambient environment (Nevo 1979, Lacey *et al*. 2000). Mammals that occur in burrows share many convergent morphological characteristics, including reduction of the eye, a cylindrical body shape, reduced ear pinnae and enlarged incisors or forefeet for excavation (Stein

2000, McNab 1979). The microenvironment within these burrow systems is generally hypoxic, hypercapnic and with a high humidity (Stein 2000, Roper *et al.* 2001).

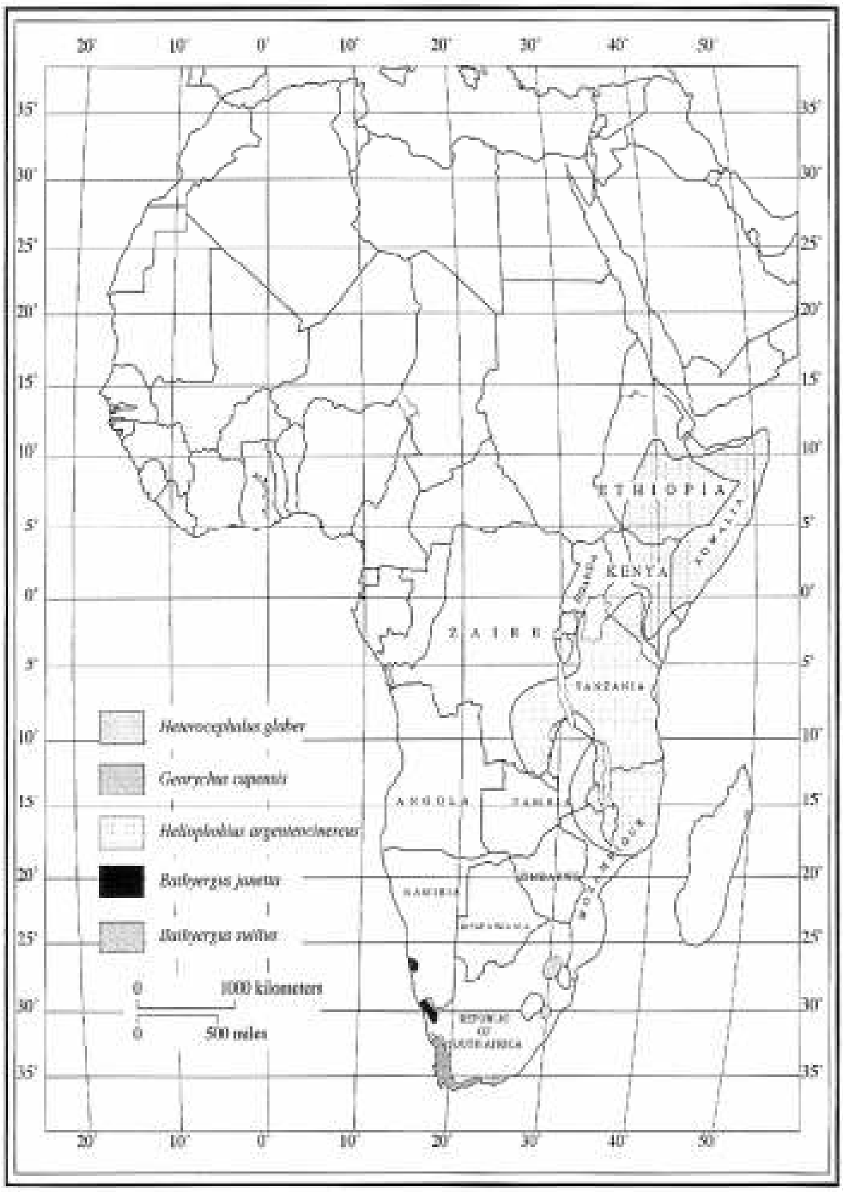

Figure 1 The distribution of the *H. glaber, G. capensis, H. argenteocinereus, B, janetta* and *B. suillus* (Bennett & Faulkes 2000)

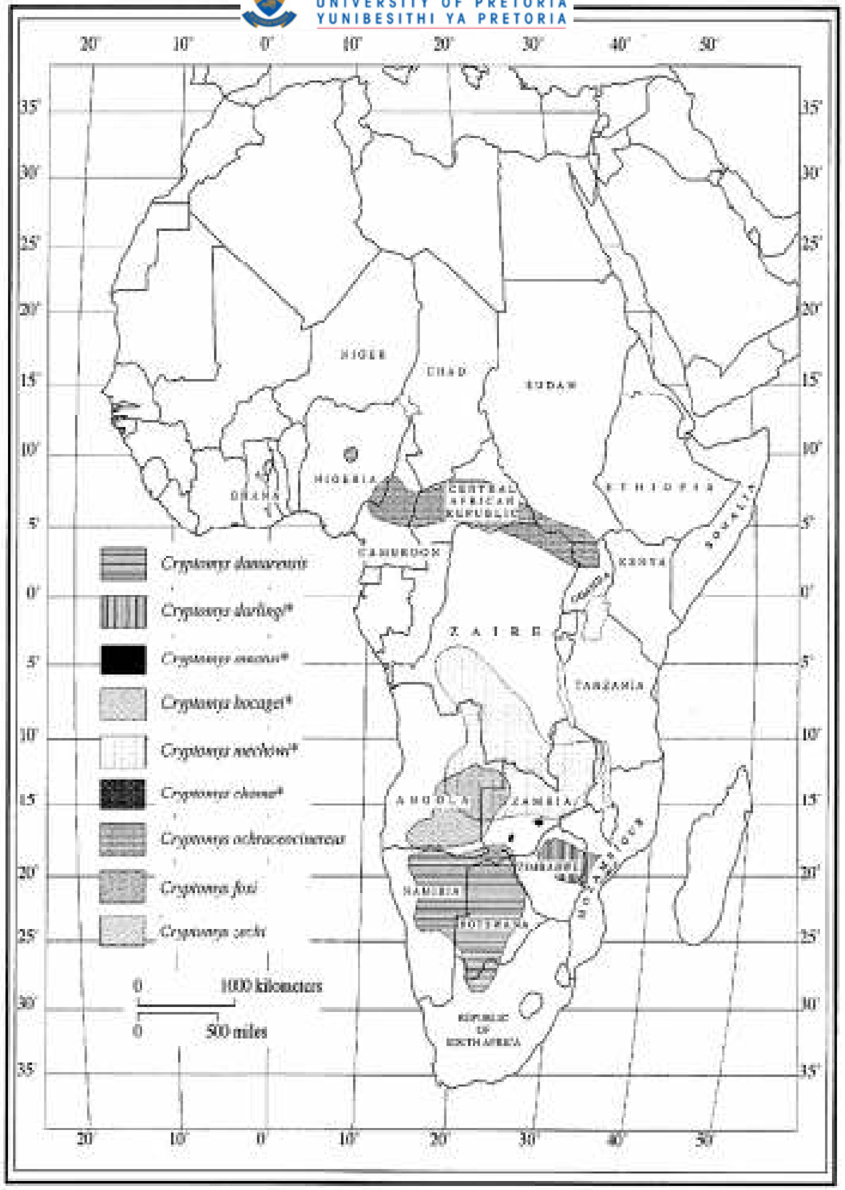

Figure 2 The distribution of nine *Cryptomys* species, approximate distributions denoted by and * (Bennett & Faulkes 2000)

Mole-rats spend their entire lives in the confines of their burrow system. The burrow system serves as a protective residence and the tunnels as foraging bases (Jarvis & Bennett 1990). A mole-rat burrow system comprises deep tunnels, which include the nest, food stores and defecation sites, as well as the shallower foraging tunnels (Bennett & Faulkes 2000). Burrows are typically hypoxic and hypercapnic (Roper *et al*. 2001) and the mole-rats are adapted to these conditions by possessing lower than predicted metabolic rates, low body temperatures and a high thermal conductance (Bennett *et al*. 1992, 1993, 1994). Habitat type is reflected in burrow architecture and it appears that large social groups of mole-rats explore the surrounding area much more efficiently than solitary species (Le Comber *et al*. 2002). The burrow system is also a place of safety where the temperature is equable and protected from above ground predators such as jackal, heron and mole-snakes.

The excavation of burrow systems can provide light into the colony size, burrow architecture, numbers of nests, food stores and toilet areas and the relative dimensions and home ranges of mole-rats. Indeed, there have been a number of studies that have reported the burrow architecture of rodent moles (Hickman 1977, Davies & Jarvis 1986, Zuri & Terkel 1996, Rosi *et al*. 2000, Spinks *et al.* 2000, Sumbera *et al*. 2003, Herbst & Bennett 2006). The majority of these studies have concentrated on solitary species in which the only time there is plural occupancy of the burrow is when there is a mate, during the breeding season or when the mother has young. There are relatively few studies that report the burrow dimensions and architecture of social dwelling mole-rats (Spinks *et al.* 2000) and usually the sample sizes are small.

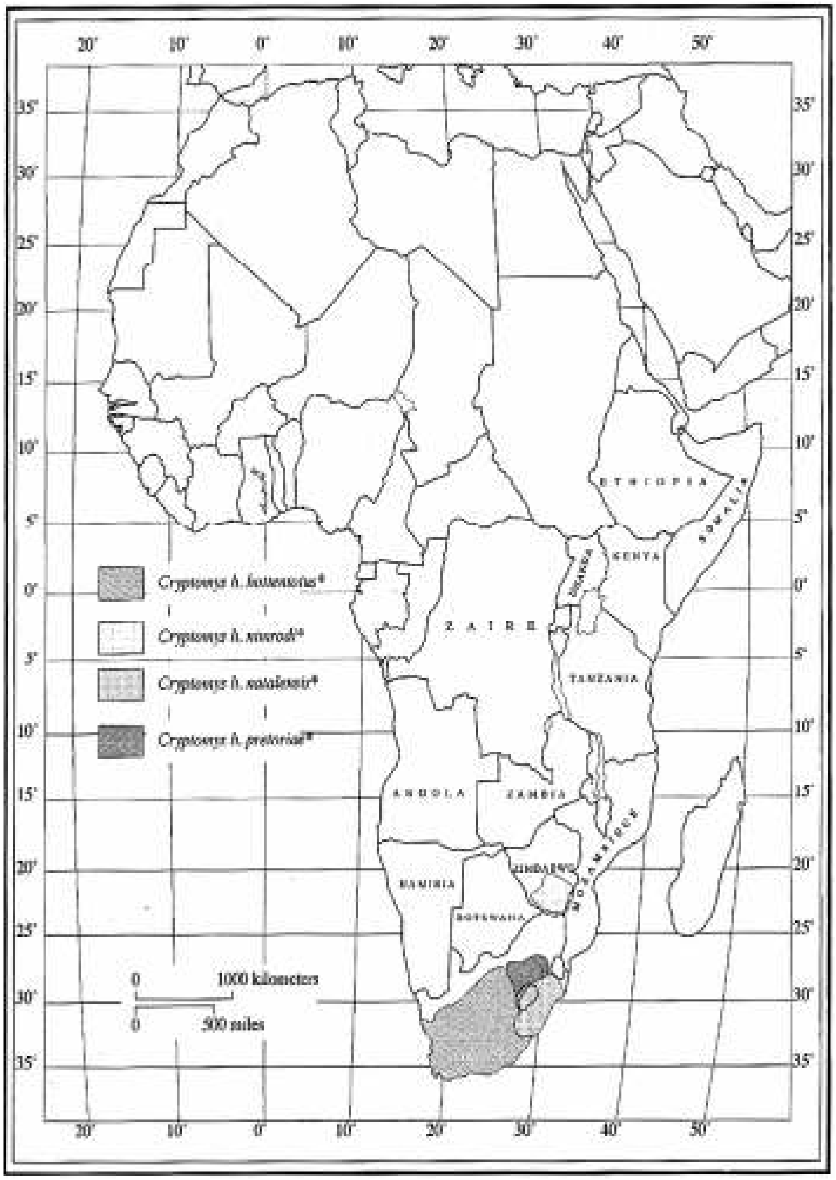

Figure 3 Distribution of *C. hottentotus* related species (Bennett & Faulkes 2000)

African mole-rats inhabit a range of environments ranging from typically mesic through to xeric climate types. They occur in many different biomes and soil types. As a general rule, solitary species are restricted to those areas that experience higher rainfall, in excess of 400mm per annum, whereas social species are common in both xeric and mesic areas of sub-Saharan Africa (Bennett & Faulkes 2000). The Aridity Food Distribution hypothesis posits that sociality in the African mole-rats evolved as a consequence of the frequency and predictability of the rainfall and the associated food resource availability (Jarvis *et al.* 1994, Faulkes *et al*. 1997, Bennett & Faulkes 2000, Herbst & Bennett 2006).

The foraging models of Lovegrove & Wissel (1988) and Spinks & Plaganyi (1999) demonstrate that colony size is important in foraging risk. Increased group size reduces the risk of starvation, and this is particularly true for mole-rat species occurring in arid environments. It logically follows therefore that mole-rats in arid habitats should have generally larger mean colony sizes than those of mesic regions (Jarvis *et al*. 1994, Faulkes *et al.* 1997). Furthermore, adaptations that might reduce colony energy costs include a reduction in mean body mass (Jarvis & Bennett 1991).

Mole-rats are unique in that they display a range of sociality within the family, ranging from solitary through to social and ultimately truly social or eusocial. The solitary representatives of the family comprise the entire sub-family Bathyerginae, *B. suillus* and *B. janetta*, as well as some representatives from the sub-family Georychinae, *G. capensis* and *H. argenteocinereus* (Bennett & Faulkes 2000). Within the Georychinae we also find the social and eusocial members of the family, within the genus *Cryptomys*, all the sub-species of *C. hottentotus* and *C. darlingi* have been shown to be social whereas *C. damarensis* and *H. glaber* (Jarvis & Bennett 1991) are the only mole-rats to have been accepted into the eusocial fold (Jarvis & Bennett, 1993) interestingly enough they are phylogenetically distant with *H. glaber* being ancestral in the molecular phylogeny

(Faulkes *et al*. 1997) and eusociality evolving separately in the two species (Jarvis & Bennett 1993, Faulkes *et al.* 1997). *Bathyergus suillus* and *B. janetta* are found in sandy soils such as sand dunes, sandy loams and sandy alluvium in the coastal regions of the southern, western and Northern Cape and southern Namibia. The Namaqua dune mole-rat is of special interest because it is the only solitary species found in a xeric environment with precipitation below 100mm per annum (Bennett & Faulkes 2000, Herbst & Bennett 2006). However, the distribution is closely linked to seepage channels from high lying areas, which provides moist soils and the necessary geophytic flora. The second sub-family Georychinae shows the greatest variety of habitat usage with members occurring in more compacted soil types than the Bathyerginae, ranging from sandy loams and clay soils in the case of the Cape mole-rat, *G. capensis* to the fine sandy soils which become rock hard during the dry season in which the naked mole-rat occurs (Brett 1991, Bennett & Faulkes 2000). Representatives of the genus *Cryptomys* occupy the greatest range of soil types with the Damaraland mole-rat occurring in loose alluvial sands to the opposite extreme of clay and stony soils in which some of the sub-species of *C. hottentotus* reside. Members of *C. hottentotus* are found in most soil types but seem to prefer granitic soils and sandy alluvium, they generally do not occur in heavy clay soils or highly brecciated soil types (Bennett & Faulkes 2000).

Due to the similar ecological constraints imposed by the subterranean environment on those animals choosing to make their homes there, many of these animals demonstrate parallel evolutionary traits. Amongst the seven families of rodents making use of this habitat the Batherygidae are truly unique, being the only family to display different degrees of sociality (Bennett & Faulkes 2000). The solitary species occur in individual burrow systems and only come into contact with con-specifics during the breeding season or for the period before the juveniles are weaned by the mother (Bennett & Jarvis 1988, Altuna *et al*. 1991, Bennett & Faulkes 2000). This limits the spread of parasites and microorganisms amongst members of the same species through direct contact between individuals, whilst social and eusocial members of the family are in constant contact with other members of their species greatly increasing the spread of these organisms. In a study conducted by Ezenwa (2004) it was shown the prevalence of strongyle nematodes was higher in social bovids than in solitary species. The behaviour of an animal plays an important role in the spread of parasites and pathogens (Ezewa 2004, Hart 1990). Hart (1990) identified 5 contributing factors to the reasons behind host parasite interactions these being “1) avoidance of parasites; 2) controlled exposure to parasites to potentiate the immune system; 3) behaviour of sick animals including anorexia and depression to overcome systemic febrile infections; 4) helping sick animals; 5) sexual selection for mating partners with the genetic endowment for resistance to parasites.” Parasite host relationships take on another dimension when sociality and grouping behaviour are taken into account, Cote’ & Poulin (1995) found that as group size increased so did the incidence of and severity of parasite load, however the severity of free living parasite showed a negative correlation with increasing group size.

The naked mole-rat and the Damaraland mole-rat display a high reproductive skew, with few, but usually a single individual within the group who monopolizes reproduction (Jarvis & Bennett 1991), this phenomenon is unique amongst mammals but has convergent evolutionary links with colonies of eusocial ants (Johnstone & Cant

1999a, b). There are two main classes of models put forward in an attempt to explain reproductive skew, the first being the *Transactional mo*dels which deal with group stability and the various constraints that are placed on reproductive division (Johnstone 2000). The second group is referred to as the *Compromise models***,** these models ignore the effects of group stability but instead conclude that the division of reproduction to be a result of the conflict that exists as a result of the fractional ability of each group member to impose its own optimal reproductive fitness (Johnstone 2000). The social bathyergids live in smaller groups but show no division of labour amongst the various individuals making up the colony (Bennett & Faulkes 2000).

The subterranean environment introduces a variety of constraints on the animals that choose to make their home in it (Lacey *et al*. 2000) and raises the possibility of the existence of unique endogenous organisms associated with these animals. It also implies the recovery of a high proportion common soil bacterial species, such as those belonging to the genera *Acinetobacter* (Tower 1992) and *Bacillus* (Rondon *et al.* 1999). Members of the genus *Acinetobacter* are expected to comprise up to 0.001% of the aerobic microbial communities of soils and water (Tower 1992).

3. Main Aims of the Project, Thesis structure and Content

The main aim of this preliminary study was to investigate the diversity and types of micro-organisms present both externally and internally in African mole-rats. In particular the various types of bacteria which are endogenous to this family and their immediate environment were investigated. Bacteria were selected to give a basic representation of the microscopic fauna and flora that occur in close relationship with these subterranean rodents. Two types of techniques were used in an attempt to identify the micro-organisms, the first being by culturing and the second by targeted Polymerase Chain Reaction amplification.

Chapter 2: This chapter of the thesis discusses the first technique that is used to address the main aim of the project by isolating different bacteria from internal and external regions of the mole-rats and then to culture these bacteria for identification via sequence analysis. External swabs were taken from the nose, mouth and urino-genital area, internal sampling occurred from the caecum, lung, and internal genital tract to collect bacteria. The external regions were chosen because they represent the most likely transmission routes for intra-specific transmission of disease causing pathogens, the internal sites were chosen since they represent the most likely organs to be infected by microbes, through ingestion and respiration and once again social-communal transmission through sexual activity. I selected three species of mole-rat from two different genera. From the genus *Cryptomys*, the species *Cryptomys hottentotus pretoriae* and *Cryptomys damarensis* were obtained, from the genus *Georychus*, the monotypic *Georychus capensis* was investigated. These three mole-rats were chosen because they are representative of the three different levels of sociality which occur within this remarkable family, they also inhabit three uniquely different environments namely the mesic Cape, the savanna of the highveld and the desert environments of Namibia and South Africa.

Chapter 3: This chapter discusses the second method used to identify the endogenous bacteria which employed a more direct method using a specific Polymerase Chain Reaction based test for bacteria belonging to the genus *Bartonella.*

This is the first study of its kind to be conducted on the family Bathyergidae and will enable an inventory of potential zoonotic diseases that these animals may carry to be compiled and further aid our understanding of the complex relationships between mole-rats and the various micro-organisms which coexist with them in their natural environment.

4. Literature Cited

ALTUNA C. A. ; FRANCESCOLI G.; & IZQUIERDO, G. 1991. Copulatory pattern of *Ctenomys pearsoni* (Rodentia, Octodontidae) from Balneario Solis, Uruguay. *Mammalia* **55:** 440-442.

BENNETT, N.C.; & FAULKES, C.G. 2000. African mole-rats ecology and eusociality. Cambridge University Press, Cambridge. U.K.

BENNETT, N.C.; & JARVIS, J.U.M. 1988. The reproductive biology of the Cape mole-rat, *Georychus capensis* (Rodentia: Bathyergidae). *Journal of Zoology, London* **214:** 95-106.

BENNETT, N.C.; CLARKE, B.C.; & JARVIS, J.U.M. 1992. A comparison of metabolic acclimation in two species of social mole-rats (Rodentia: Bathyergidae) in southern Africa. *Journal of Arid Environments* **22:** 189-198.

BENNETT, N.C.; TAYLOR, P.J.; & AGUILAR, G.H. 1993. Thermoregulation and metabolic acclimation in the Natal mole-rat (*Cryptomys hottentotus natalensis*) (Rodentia: Bathyergidae). *Zeitschrift fur Saugetierkunde (Germany)* **58:** 362-367.

BENNETT, N.C.; AGUILAR, G.H.; JARVIS, J.U.M.; & FAULKES, C.G. 1994. Thermoregulation in three species of Afrotropical subterranean mole-rats (Rodentia: Bathyergidae) from Zambia and Angola and scaling within the genus *Cryptomys*. *Oecologia* **97:** 222-228.

BRETT, R. A. 1991. *In:* The biology of the naked mole-rat (Sherman, P.W., Jarvis, J.U.M. and Alexander, R.D., eds). Princeton, New Jersey: Princeton University Press.

COTE´, I.; & POULIN R, 1995. Parasitism and group size in social animals: a meta-analysis. *Behavioral Ecology* **6:** 159–165.

DAVIES, K.C.; & JARVIS, J.U.M. 1986. The burrow system and burrowing dynamics of the mole-rats *Bathyergus suillus* and *Cryptomys hottentotus* in the fynbos of the South-Western Cape, South Africa. *Journal of Zoology, London* **209:** 125-147.

EZENWA, V.O. 2004. Host social behavior and parasitic infection: a multifactorial approach. *Behavioral Ecology* **15:** 446-454.

FAULKES, C.G.; BENNETT, N.C.; BRUFORD, M.W.; O'BRIEN, H.P.; AGUILAR, G.H.; & JARVIS, J.U.M. 1997. Ecological constraints drive social evolution in the African mole-rats. *Proceedings of the Royal Society, London, Series B* **264:** 1619–1627.

FAULKES, C.G.; VERHEYEN, E.; VERHEYEN, W.; JARVIS, J. U. M.; & BENNETT, N.C. 2004. Phylogeographical patterns of genetic divergence and speciation in African mole-rats (Family: Bathyergidae). *Molecular Ecology* **13:** 613–629.

HART, B. 1990. Behavioral adaptations to pathogens and parasites: five strategies. *Neuroscioence Biobehavior Review* 1**4:** 273–294.

HERBST, M.; & BENNETT, N.C. 2006. Burrow architecture and burrowing dynamics of the endangered Namaqua dune mole rat (*Bathyergus janetta*) (Rodentia: Bathyergidae). *Journal of Zoology, London* **270:** 420-428.

HICKMAN, G.C. (1977) Burrow System Structure of *Pappogeomys castanops* (Geomyidae) in Lubbock-County, Texas. *American Midland Naturalist* **97:** 50-58.

INGRAM, C. M.; BURDA, H.; & HONEYCUTT, R. L. 2004. Molecular phylogenetics and taxonomy of the African mole-rats, genus *Cryptomys* and the new genus *Coetomys* Gray, 1864. *Molecular Phylogenetics and Evolution* **31:** 997–1014.

JARVIS, J.U.M.; & BENNETT, N.C. 1990. The evolutionary history, population biology and social structure of African mole-rats: family Bathyergidae. *Progress in Clinical and Biological Research* **335**: 97-128.

JARVIS, J.U.M.; & BENNETT, N.C. 1991. Ecology and behaviour of the family Bathyergidae. *In:* The biology of the naked mole-rat: 66–96. Sherman, P.W., Jarvis, J.U.M. & Alexander, R.D. (Eds). New Jersey: Princeton University Press.

JARVIS, J.U.M.; & BENNETT, N.C. 1993. Eusociality has evolved independently in two genera of bathyergid mole-rats - but occurs in no other subterranean mammal. *Behavioral Ecology Sociobiology* **33:** 253 260.

JARVIS, J.U.M.; O'RIAIN, M.J.; BENNETT, N.C.; & SHERMAN, P.W. 1994. Mammalian eusociality: a family affair. *Trends in Ecology and Evolution* **9:** 47–51.

JOHNSTONE, R.A. 2000. Models of reproductive skew: a review and synthesis. *Ethology* **7:** 243-249.

JOHNSTONE, R.A.; & CANT, M.A. 1999a. Reproductive skew and indiscriminant infanticide. *Animal Behavior* **57:** 243-249.

JOHNSTONE, R.A.; & CANT, M.A. 1999b. Reproductive skew and the threat of eviction: A new perspective. *Proceedings of the Royal Society, London, Series B* **266:** 275-279

KOCK, D.; INGRAM, C.M.; FRABOTTA, L.J.; HONEYCUTT, R.L.; & BURDA, H.

2006. On the nomenclature of Bathyergidae and *Fukomys* n. gen. (Mammalia: Rodentia). *Zootaxa* **1142:**51–55.

LACEY, E.A.; PATTON, J. A.; & CAMERON, G. N. 2000. Introduction. *In:* Life Underground the biology of subterranean rodents: 11-12. Edited by E.A. Lacey, J.L. Patton and G.N. Cameron. Chicago: University Of Chicago Press.

LE COMBER, S.C.; SPINKS, A.C.; BENNETT, N.C.; JARVIS, J.U.M.; & FAULKES, C.G. 2002. Fractal dimensions of African mole-rat burrows. *Canadian Journal of Zoology* **80:** 436-441.

LOVEGROVE, B. G.; & WISSEL, C. 1988. Sociality in mole–rats: metabolic scaling and the role of risk sensitivity. *Oecologia* **74:** 600–606.

MCNAB, B.K. 1979. The Influence of Body Size on the Energetics and Distribution of Fossorial and Burrowing Mammals. *Ecology* **60:** 1010-1021.

NEVO, E. 1979. Adaptive convergence and divergence of subterranean mammals. *Annual Revue of Ecology and Systematics* **10:** 269-308.

ROPER, T.J.; BENNETT, N.C.; CONRADT, L.; & MOLTENO, A. J. 2001. Environmental conditions in burrows of two species of African mole-rat, *Georhychus capensis* and *Cryptomys damarensis*. *Journal of Zoology, London* **254:** 101-107.

RONDON, M.R.; GOODMAN, R.M.; & HANDELSMAN, J. 1999. The Earth's bounty: assessing and accessing soil microbial diversity. *Trends in Biotechnology* **17:**403-409.

ROSI, M.I.; CONA, M.I.; VIDELA, F.; PUIG, S.; & ROIG, V.G. 2000 Architecture of *Ctenomys mendocinus* (Rodentia) burrows from two habitats differing in abundance and complexity of vegetation. *Acta Theriologica* **45:** 491-505.

SPINKS, A.C.; & PLAGANYI, E.E. 1999. Reduced starvation risks and habitat constraints promote cooperation in the common mole-rat, *Cryptomys hottentotus hottentotus*: a computer simulated model *Oikos* **85:** 435-444.

SPINKS, A.C.; BENNETT, N.C.; & JARVIS, J.U.M. 2000. A comparison of the ecology of two populations of the common mole-rats, *Cryptomys hottentotus hottentotus*: the effect of aridity on food, foraging and body mass. *Oecologia* **125:** 341-349.

STEIN, B.R. 2000. Morphology of subterranean rodents. *In:* Life Underground the biology of subterranean rodents: 19-61. Edited by E.A. Lacey, J.L. Patton and G.N. Cameron. Chicago: University Of Chicago Press.

SUMBERA, R.; BURDA, H.; CHITAUKALI, W.N.; & KUBOVA, J. 2003. Silvery mole-rats (*Heliophobius argenteocinereus*, Bathyergidae) change their burrow architecture seasonally. *Naturwissenschaften* **90**: 370-3.

TOWER, K.J. 1992. The genus *Acinetobacter. In:* The Prokaryotes (Balows, A.; Trüper, H.G.; Dworkin, M.; Harder, W.; & SchleiferK-Z; Eds.). Springer-Verlag, New York, N.Y.

ZURI, I.; & TERKEL, J. 1996. Locomotor patterns, territory, and tunnel utilization in the mole-rat *Spalax ehrenbergi Journal of Zoology* **240**: 123-140.

CHAPTER 2

Identification of bacteria associated with three species of South African Mole-rats (Bathyergidae)

Contributing Authors: J. H. d. T. van Sandwyk*, A. D. Bastos*, S.N. Venter# and N. C. Bennett*.

**Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa*

#Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa

Abstract

Rodents are synonymous with disease and are known to carry a wide variety of pathogenic bacteria. However, little is known about the role of the subterranean rodent fauna in this regard. To address this current dearth of knowledge, bacteria were cultured from external orifices and internal organs of three species of bathyergids namely *Georychus capensis*, *Cryptomys damarensis* and *Cryptomys hottentotus pretoriae*, that have markedly different ecotype habitats and levels of sociality. The 16S rRNA gene was targeted for amplification and sequencing as it is taxonomically informative and is readily amplified with universal primers. Bacteria belonging to three Phyla, the Proteobacteria, Actinobacter and Firmicutes were genetically characterized. This study represents the first attempt to uncover the diversity of bacteria cultured by non-selective means in this African rodent family.

1. Introduction

Rodents are synonymous for carrying and transmitting a broad spectrum of microbial agents, a number of which have zoonotic potential. Examples in the literature include (i) a study conducted by Anderson *et al*. (1985) that reported the prevalence of Lyme disease infection in wild rodents in the north-eastern United States, (ii) infection of various rodent species with the plague bacteria, *Yersinia pestis* (Butler *et al.* 1982) and more recently (iii), infection of various rodent species with the bacteria *Bartonella*, which can either be spread by arthropod vectors or be directly transmitted by the bite or scratch of an infected animal (Hansmann *et al.* 2005). In an earlier study conducted by Kosoy *et al.* (1997) it was shown that rodents in the southeastern United States have high infection rates (as much as 42.2 %) with *Bartonella* bacteria.

The subterranean environment introduces a variety of constraints on the animals that choose to make their home in it (Lacey *et al*. 2000) and raises the possibility of the existence of unique endogenous organisms associated with these animals. It also implies the recovery of a high proportion of common soil bacterial species, such as those belonging to the genera *Acinetobacter* (Tower 1992) and *Bacillus* (Rondon *et al.* 1999).

Due to the general lack of knowledge with regard to subterranean rodents, members of the family Bathyergidae whose biology, behaviour and ecology have been intensively studied in South Africa (see Bennett & Faulkes 2000 for overview) were selected as a representative of this rodent guild. Three species with differing degrees of sociality and inhabiting variable ecotypes were selected, namely *Georychus capensis*, *Cryptomys damarensis* and *Cryptomys hottentotus pretoriae*.

The development of sociality in the bathyergids is believed to have arisen as a result of the Food Aridity Hypothesis that bases its premise on the degree of aridity of natural environment in which the mole-rat occurs as well as the subsequent food resource availability, predominantly tubers and geophytes (Jarvis *et al.* 1994, Faulkes *et al*. 1997, Bennett & Faulkes 2000). Most solitary species are confined to areas with a high rainfall, with the exception of *Bathyergus janetta* (Herbst & Bennett 2006), while social and eusocial species occur across mesic and xeric habitat types (Bennett & Faulkes 2000). The bathyergids occur in a variety of soil types ranging from fine clays to stony sands and can be found at a variety of altitudes and vegetation types (Bennett & Faulkes 2000, Faulkes *et al*. 2004).

The solitary Cape mole-rat, *Georychus capensis* (Pallas 1778) inhabits areas with high rainfall, more than 500mm per annum and has two main distributions, namely (i) the Western Cape Province and (ii) south-western Kwa-Zulu Natal. It prefers sandy loams, alluvial sediments and clay soil types, but does not often occur in sand dunes, which are the habitat of choice of the Cape Dune mole-rat, *Bathyergus suillus* (Schreber 1782), (Bennett & Faulkes 2000).

The social highveld Mole-rat, *Cryptomys hottentotus pretoriae* (Lesson 1826) occurs across the highveld of the Gauteng and Free State Province. They occur in a variety of compacted soils but prefer granitic and brecciated soils. The highveld mole-rat occurs in colonies with an average group size of 12 members with a single breeding female (Moolman *et al.* 1998, Bennett & Faulkes 2000).

The eusocial Damaraland mole-rat, *Cryptomys damarensis* (Ogilby 1838) is widely distributed over the Kalahari biosphere of southern Africa including South Africa, Botswana and Namibia. The regions it inhabits have a mean annual rainfall of less than 400mm and on average only exceeding 25mm of rain for four months of the year. *C. damarensis* occurs in colonies with an average size of 16 individuals and a maximum recorded size of 41 (Jarvis & Bennett 1993). It prefers the red Kalahari arenosols and alluvial sand of the valleys and dune slopes of the Kalahari sand complex (Bennett & Faulkes 2000).

Mole-rats are unique amongst mammalian taxa as they are the only family in which a eusocial breeding system has evolved. In the naked mole-rat *Heterocephalus* *glaber* and the Damaraland mole-rat (*Cryptomys damarensis*), reproduction within colonies is restricted to a single breeding pair (Bennett & Falukes 2000).

Another factor to consider in any study that attempts to address relationships between mole-rats and the endemic microbes within a species, is that of transmission between con-specifics. Transmission opportunities vary greatly with the different levels of sociality. A study conducted by Burland *et al*. (2004) on *Cryptomys damarensis* colonies revealed multiple instances of unknown paternity as well as the presence of immigrants of both sexes. Similar results have also been observed in the semi social common mole-rat (*Cryptomys hottentotus sp.*) (Bishop *et al*. 2004). Although no such study has been conducted *Georychus capensis,* to date, contact with con-specifics during breeding and the subsequent weaning of the young by the mother is unavoidable in this solitary bathyergid. Pairing of opposite sex con-specifics during breeding and the subsequent plural occupancy of burrows with young create contact and transmission opportunities. Studies have shown this animal to be highly xenophobic to contact with con-specifics for periods excluding those previously mentioned (Bennett & Jarvis 1988, Bennett & Faulkes 2000). It has also been recorded that these animals occasionally venture onto the surface (Bennett & Faulkes 2000).

The use of molecular techniques over more traditional methods for conducting microbial studies has gained strong support over the last few decades (Felske *et al*. 1998). To conduct the present study the 16S rRNA gene was selected, as this gene has been widely used for comparative studies involving sequence data (Woese 1987, Priest & Austin 1993). Variation between species based on sequences derived from 16S rRNA amplification is between 20-25 %, for example the difference in the 16S rRNAs of *E. coli* and *Bacillus subtilis* is approximately 23 % (Hugenholtz 1998). Bacterial species resolution by means of the 16S rRNA gene is not however without its limitations. In a study conducted by La Scola *et al*. (2003a) on various species within the genus *Bartonella* it was shown that 16S rRNA amplification alone was not sensitive enough to discriminate between closely related species. Similar concerns have also been raised over members of the genera *Bacillus* and *Bosea* (Amman *et al.* 1995, La Scola *et al*. 2003b). However, 16S rRNA remains a valuable primary identification tool as a large database of sequences exists which includes representatives of all known genera. Another unavoidable restriction associated with the non-specific culturing approach used in this study is that bacterial diversity will be severely under-estimated. Direct observation of the microbial diversity of a sample through the use of microscopy and staining generates a bacteria population count one to two orders of magnitude higher than through culturing (Amman *et al.* 1995, Rondon *et al.* 1999). This implies that only 1 % of the expected species diversity present will be available for study (Amman *et al.* 1995), though not implying that only 1 % can in fact be cultured (Rondon *et al*. 1999).

Despite these recognized but unavoidable short-comings this study is extremely valuable as it represents the first attempt to identify bacteria associated with bathyergids, *albeit* only to the genus level.

2. Materials and Methods

2.1. Sample collecting

Samples were collected from naturally occurring mole-rat populations in South Africa. Ten Damaraland mole-rats, *Cryptomys damarensis* from the southern Kalahari region; 5 from Tswalu Nature Reserve and 5 from Hotazel, Northern Cape Province (27°17'S 22°58'E) were sampled. Ten Cape mole-rats, *Georychus capensis* from the Darling region of the Western Cape (S33°23'S E18°21') and 10 highveld mole-rats, *Cryptomys hottentotus pretoriae* from the suburbs of Pretoria, Gauteng (S25°43'S E28°13'E) were also included. Animals were euthanized by an over dose of Halothane under clearance from the Animal Ethics Committee at the University of Pretoria (EC20624–012). Swabs were taken from internal organs and external orifices of the mole-rats. External samples included swabs taken from the nose, mouth and urino-genital area, as well as faecal and urine samples, which were collected when available. Internal samples were taken from the caecum, lung, and internal genital tract. All samples were placed in 1.5mL tubes containing Dulbecco's PBS containing Mg^{2+} and Ca^{2+} and stored at 4°C.

2.2. Bacterial culture and DNA extraction

Samples were initially streaked out with a sterile inoculating loop on a variety of agar media, namely, R2A, MaConkey, MaConkey without salt, Tryptone Soya, Nutrient and Blood (Bovine BTA). However as results were similar with the different non-sanguine media, only Nutrient and Blood agar were used in the latter part of the study.

All plates were incubated at 37°C for 24 hours. Morphologically distinct individual colonies were then obtained in pure culture, and were resuspended in 200µL of Dulbecco's PBS containing Mg^{2+} and Ca^{2+}. DNA was extracted from all samples by means of the High Pure PCR Template Preparation Kit (Roche Applied Science), following the DNA extraction protocol prescribed by the supplier for bacteria and yeast. The DNA was then eluted in a final volume of 200 µl and stored at –20°C until further use.

2.3. 16S rRNA amplification

A region of approximately 1.5 kbp of the 16S gene was amplified with primers 16f27^1 (PA)(5^1-AGAGTTTGATCCTGGCTCAG-3^1) and 16R1522 (pH) (5^1-AAGGAGGTGATCCAGCCGCA-3^1) as described by Hauben *et al.* (1997).

PCRs targeting the 16S region contained 5µL of 10x Buffer, 4µL of 2.5mM dNTP, 30 pmol of each primer, 1U of *Taq* DNA polymerase and 100-200ng of DNA template in a final volume of 50µl. Primer annealing was performed at 4 °C below the Tm of the primer with the lowest Tm, and was determined to be 58°C for 16S rRNA target amplified with this primer pair.

The results of the PCR were assessed by electrophoresis on a 1.5 % agarose gel, with product size being estimated against a 100 bp ladder (Promega). Positive PCRs were purified directly from the tube using a High Pure PCR Product Purification Kit (Roche Applied Science).). This purified product was cycle sequenced in a final volume of 10µl with the forward and/or reverse primer, in separate reactions. Sodium acetate precipitated products were run on an ABI377 automated sequencer (Applied Biosystems, California), the results of which were viewed with CHROMAS (v. 1.43). Forward and reverse sequences were aligned using DAPSA v. 4.91 (Harley 2001) and the resulting full-length sequences were blasted against the Genbank database (www.ncbi.nlm.nih.gov/blast) in order to identify all closely related sequences that have been deposited in this public database (Altschul *et al.* 1997). Finally a p-distance neighbour-joining tree was inferred with sequences generated in this study and those downloaded from Genbank. The latter were restricted (where possible) to sequences reported in published peer-reviewed journals with preference being given to sequences from type specimens. Nodal support was assessed by 10000 bootstrap replications in MEGA v. 3 (Kumar *et al.* 2004). A parsimony tree was inferred for each phylum, using PAUP* version 4.0 (Swofford 2002) with nodal support being assessed by 1000, 250 and 100 bootstrap replications for the Acinetobacter, Firmicutes and γ-Proteobacteria datasets, respectively. Successive (*a posteriori*) weighting with the rescaled consistency (RC) was performed in order to reduce the contribution of homoplasious characters in the parsimony analysis.

3. Results

A total of 54 bacteria, 43 from external samples and 11 from internal samples, were cultured. 16S BLAST similarity searches revealed that they were representative of three different phyla namely the Proteobacteria, Firmicutes and Actinobacteria, with more than half (59.3 %) belonging to the Proteobacteria.

3.1. Actinobacteria

Actinobacteria sequence data derived from the 16S rRNA gene comprised a homologous partial sequence, 672 nucleotides in length (as seen in Appendix II), of which 73 sites were variable and 62 were parsimony informative sites. The base composition frequencies revealed a G-C bias, with individual average base pair composition determined to be: T = 23.3 %, C = 30.9 %, A = 21.0 %, G = 25.0 %. The transition:transversion ratio (R) was 1.2, indicating a slight transitional bias. Parsimony analyses performed in PAUP* included equal weighting followed by successive weighting with the rescaled consistency index (RC) in order to reduce the contribution of homoplasious characters. Equal weighting resulted in 8 equally parsimonious trees with a length of 102, a retention index (RI) = 0.983, consistency index (CI) = 0.941 and rescaled consistency (RC) = 0.925. Reweighting with the RC, did not result in fewer trees (8 equally parsimonious trees were again obtained with a length of 95.567), but the retention index (RI) = 0.991, consistency index (CI) = 0.968 and rescaled consistency (RC) = 0.960 values were higher.

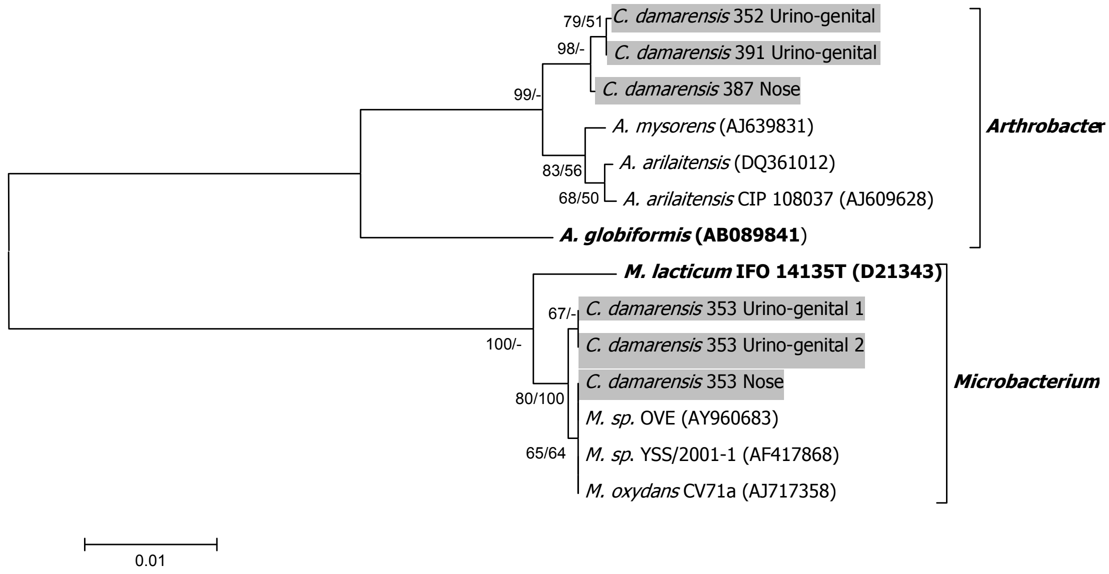

Figure 1 Neighbor-joining tree showing the recovery of two bacterial genera from mole-rats; *Microbacterium* and *Arthrobacter*, within the Actinobacteria phylum and their relatedness to bacterial sequences in the Genbank database. Bacterial sequences generated in this study from mole-rat samples are indicated with grey shading and type species/ strains are indicated in bolded text. Bootstrap support values > 50 % from NJ and parsimony are indicated NJ/ Parsimony. A '-' denotes a node that had less than 50 % support following bootstrap resampling.

Two genera belonging to the phylum Actinobacteria, namely *Arthrobacter* and *Microbacterium* (Figure 1, Appendix I and Appendix II) were cultured from swabs taken from external orifices and are possibly representative of environmental contaminants. The samples clustering in the genus *Arthrobacter* formed a lineage that was genetically distinct from published sequences present on Genbank, however possessed >97 % sequence similarity with unpublished sequences. Similarly, *Microbacterium* bacteria from mole-rats were either identical to previously published sequenced or differed from these at just one site.

3.2. Firmicutes

Five bacterial genera recovered from mole-rat samples were recorded in the Firmicutes namely *Leuconostoc, Paenibacillus, Bacillus, Staphylococcus* and *Aerococcus* (Appendix I). A single unnamed mole-rat bacteria cultured from *Cryptomys hottentotus pretoriae* 3, internal genital tract swab had a 97 % similarity to and un-described bacteria (Bacterium 9-gw3-9, DQ990068), as seen in Appendix I. Bacteria of the phylum Firmicutes were the most common type cultured from internal sampling sites, and corresponded to 7 of the 12 morphologically distinct colonies obtained. Sequence data derived from the 16S rRNA gene varied in length making it necessary to compile four datasets (I-IV) that correspond to different regions of the gene. However, for each of the phylogenies inferred, the same Genbank reference sequences were used throughout in order to ensure constancy across the different datasets. The first dataset (I) comprised 563 bp of which 118 were variable sites and 106 were parsimony informative. The base composition was slightly G-C rich, with the average base pair composition being: T = 21.4 %, C = 25.0 %, A = 25.2 %, G = 28.5 %. The transition:transversion ratio (R) was 1.7. Parsimony analyses performed in PAUP* included equal weighting followed by successive weighting with the rescaled consistency index (RC) in order to reduce the contribution of homoplasious characters. Equal weighting resulted in 13 equally parsimonious trees for 16S rRNA each with a length of 219 and a retention index (RI) = 0.894, consistency index (CI) = 0.721 and rescaled consistency (RC) = 0.645. Reweighting with the RC again resulted in 13 trees each with a length of 145.92. However, the retention index (RI), consistency index (CI) and rescaled consistency (RC) values were higher (0.941, 0.839 and 0.790, respectively). The second dataset (II) comprised 772 bp of which 240 sites were variable and 225 parsimony informative. The base composition was slightly G-C rich, with the average base pair composition being: T = 20.8 %, C = 21.3 %, A = 26.8 %, G = 31.1 %. The transition:transversion ratio (R) was 1.3. Parsimony analyses performed in PAUP* included equal and successive weighting with the rescaled consistency index (RC). Equal weighting resulted in a single tree with a length of 466 and a retention index (RI) = 0.872, consistency index (CI) = 0.768 and rescaled consistency (RC) = 0.670. Reweighting with the RC again resulted in a single tree with a length of 324.67 and a retention index (RI) = 0.932, consistency index (CI) = 0.878 and rescaled consistency (RC) = 0.819 values. The third dataset (III) comprised 359 bp of which 78 were variable sites and 67 were parsimony informative sites. The base composition was slightly G-C rich, with the average base pair composition being: T = 21.7 %, C = 25.9 %, A = 26.0 %, G = 26.3 %. The transition:transversion ratio (R) was 1.6. Parsimony analyses performed in PAUP* included equal weighting and successive weighting with the rescaled consistency index (RC) in order to reduce the contribution of homoplasious characters. Equal weighting resulted in a single tree with a length of 141 and a retention index (RI) = 0.879, consistency index (CI) = 0.716 and rescaled consistency (RC) = 0.630. Reweighting with the RC again resulted in a single tree with a length of 91.35. The noise in the reweighted dataset, as assessed by the RI, CI and RC values (0.933, 0.841 and 0.785, respectively) was lower. Finally the fourth homologous dataset (IV) comprised 929 bp of which 267 were variable sites and 236 were parsimony informative sites. The base composition was slightly G-C rich, with the average base pair composition being: T = 20.5 %, C = 21.7 %, A = 26.9 %, G = 30.8 %, however, the transition:transversion ratio (R) was 1.3, indicating a bias towards transitional changes. Parsimony analyses performed in PAUP* included equal and successive weighting with the rescaled consistency index (RC). Equal weighting resulted in a single tree with a length of 511 and a retention index (RI) = 0.856, consistency index (CI) = 0.767 and rescaled consistency (RC) = 0.657. Reweighting with the RC again resulted in a single tree with length 347.49, however the values obtained for the retention index (RI) = 0.932, consistency index (CI) = 0.887 and rescaled consistency (RC) = 0.827 were higher, following reweighting. The neighbor-joining trees for each of these phylogenies are represented in figures 2 to 5, together with the bootstrap values from NJ and parsimony. Nucleotide sequence alignments of all four datasets are included in Appendices III through VI. The relative positions of the different dataset are represented by Figure 2.

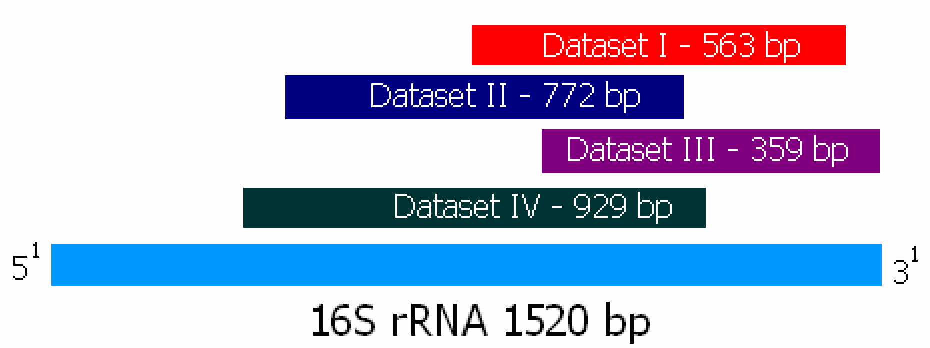

Figure 2 Relative positions of the four Firmicutes dataset on the 16S rRNA gene.

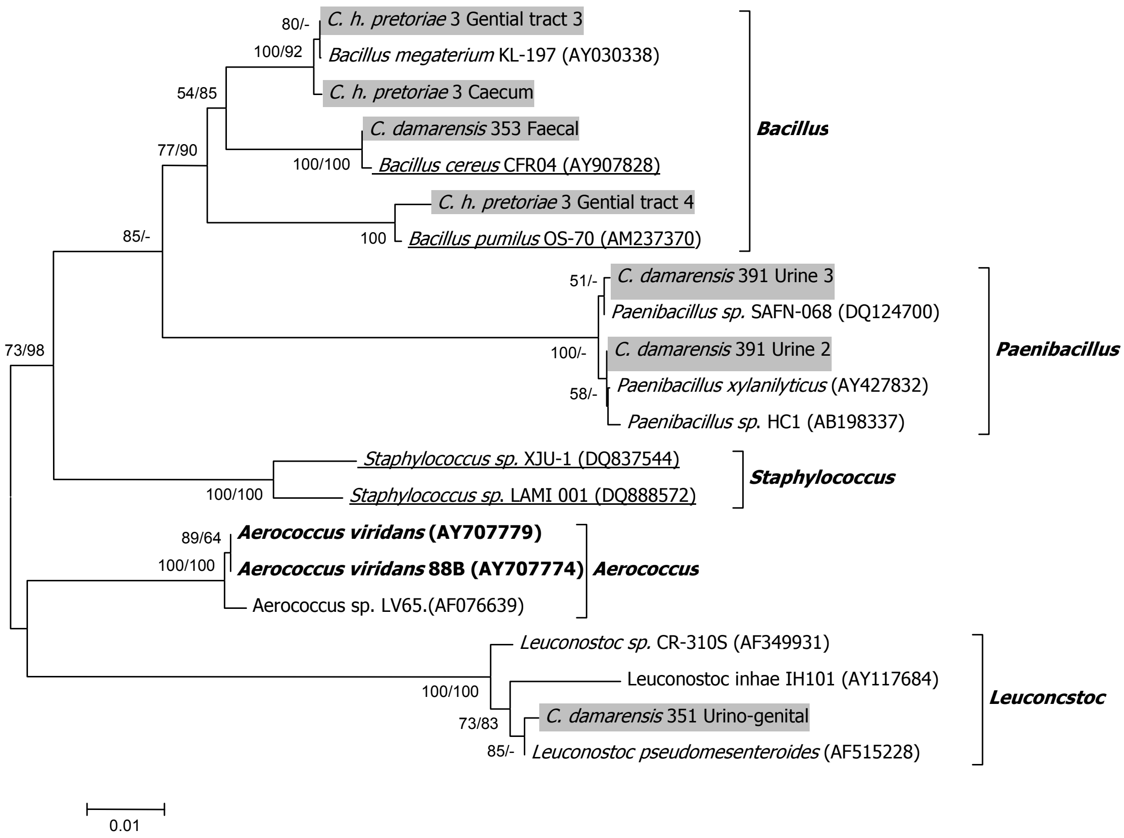

Figure 3 Neighbour-joining tree based on partial 16S data (Dataset I) depicting bacterial relationships within the Firmicutes (Dataset I). Bacterial sequences generated in this study from mole-rat samples are indicated with grey shading, whilst type species/ strains are indicated in bolded text together with the relevant Genbank accession number in brackets. Nodes supported by bootstrap values > 50 % from NJ and MP are indicated next to each of the relevant nodes and are separated by a /. A '-' denotes those nodes that were not supported by parsimony. Unpublished Genbank sequences are indicated as being underlined.

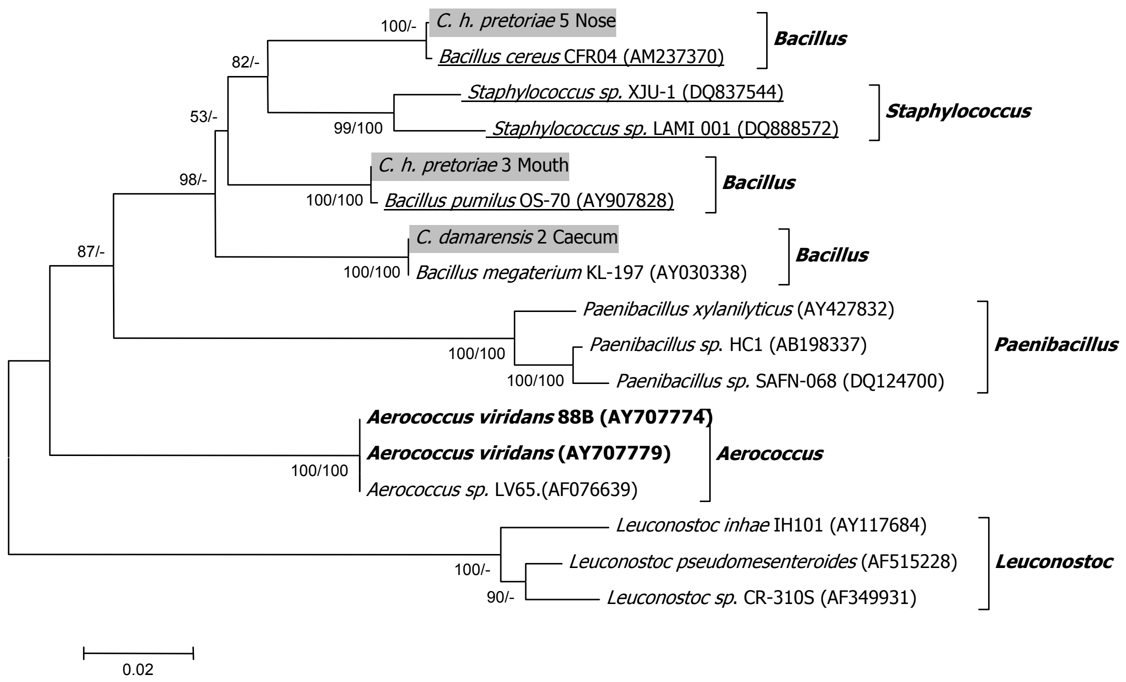

Figure 4 Neighbour-joining tree based on partial 16S data depicting bacterial relationships within the Firmicutes (Dataset II). Bacterial sequences generated in this study from mole-rat samples are indicated with grey shading, whilst type species/ strains are indicated in bolded text together with the relevant Genbank accession number in brackets. Nodes supported by bootstrap values > 50 % from NJ and MP are indicated next to each of the relevant nodes and are separated by a /. A ‘-‘ denotes those nodes that were not supported by parsimony. Unpublished Genbank sequences are indicated as being underlined.

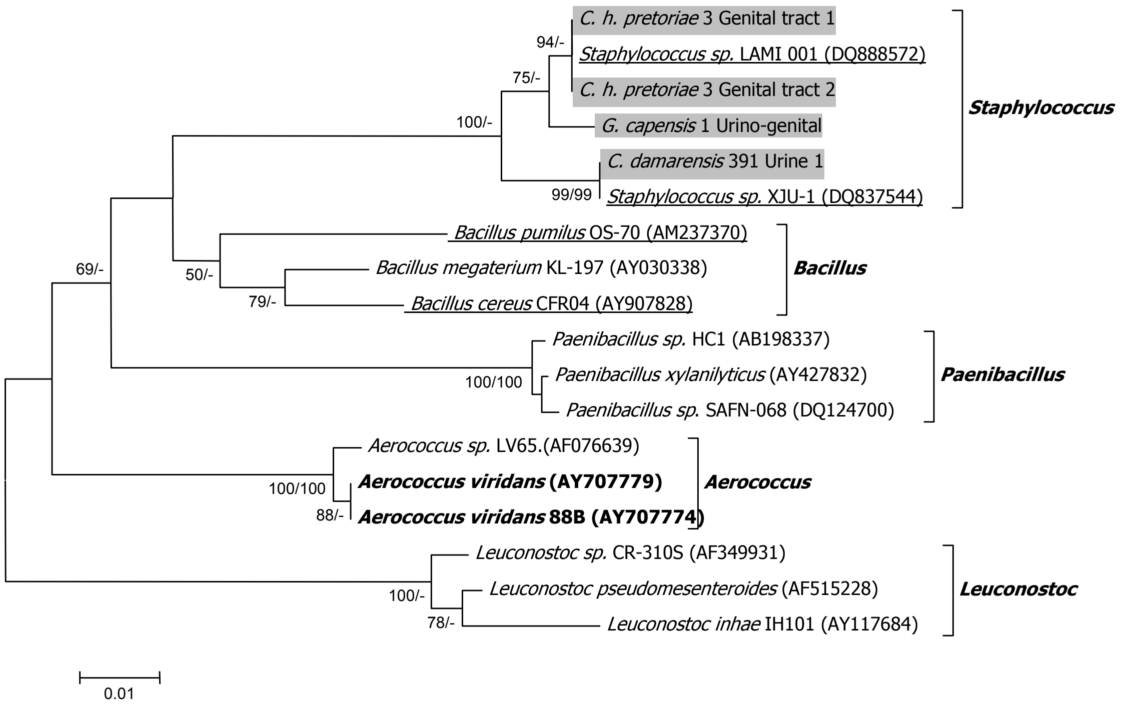

Figure 5 Neighbour-joining tree based on partial 16S data depicting bacterial relationships within the Firmicutes (Dataset III). Bacterial sequences generated in this study from mole-rat samples are indicated with grey shading, whilst type species/ strains are indicated in bolded text together with the relevant Genbank accession number in brackets. Nodes supported by bootstrap values > 50 % from NJ and MP are indicated next to each of the relevant nodes and are separated by a /. A '-' denotes those nodes that were not supported by parsimony. Unpublished Genbank sequences are indicated as being underlined.

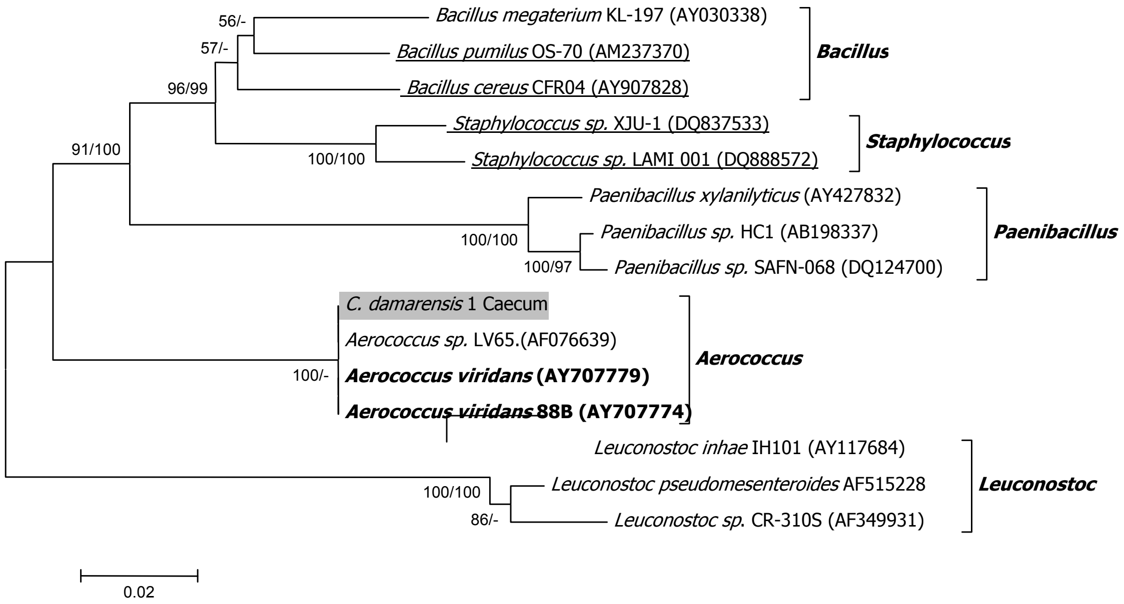

Figure 6 Neighbor-joining tree based on partial 16S data depicting bacterial relationships within the Firmicutes (Dataset IV). Bacterial sequences generated in this study from mole-rat samples are indicated with grey shading, whilst type species/ strains are indicated in bolded text together with the relevant Genbank accession number in brackets. Nodes supported by bootstrap values > 50 % from NJ and MP are indicated next to each of the relevant nodes and are separated by a /. A '-' denotes those nodes that were not supported by parsimony. Unpublished Genbank sequences are indicated as being underlined.

Cultured bacteria forming dataset I all were all shown to be nearly identical to sequences available on Genbank. Sequences obtained were identified as to belonging to three genera namely *Bacillus*, *Paenibacillus* and *Leuconcstoc*. All sequences had sequence similarity of >97 % to sequences available on Genbank. All cultured bacteria in dataset II were identical to previously published sequences on Genbank, all three mole-rat bacteria belong to the genus *Bacillus*. The placement of the *Staphylococcus* sequences within the

Bacillus clade reinforces the weakness of the 16S rRNA gene for resolving finer level taxonomic differences. All mole-rat derived bacteria in dataset III were identified as belonging to the genus *Staphylococcus*, and were identical to sequences downloaded from Genbank. *G. capensis* 1 Urino-genital sample showed 99 % sequence identity to an unpublished bacterial sequence, *Staphylococcus sp.* M210 (EF061907), which was not included in the analysis. Interestingly bacteria of the *Staphylococcus* genus were present in samples taken from the urino-genital area of all three mole-rat species. The only bacteria cultured from mole-rat origin in Dataset IV of the Firmicutes was found to be identical to *Aerococcus viridians* downloaded from Genbank, all three of which being from published origins and can with relative certainty be identified as such.

3.3. Proteobacteria

Sequence data derived from the 16S rRNA gene for the γ sub-class comprised a partial sequence of 326 bp (Appendix VII), of which 74 were variable sites and 72 were parsimony informative sites. The base composition was G-C rich, with the average base pair composition being: T = 26.7 %, C = 29.9 %, A = 19.0 %, G = 24.4 %. The transition:transversion ratio (R) was 1.1, indicating a slight transitional bias. Parsimony analyses performed in PAUP* included equal and successive weighting with the rescaled consistency index (RC). Equal weighting resulted in 8064 equally parsimonious trees with a length of 117 and a retention index (RI) = 0.974, consistency index (CI) = 0.744 and rescaled consistency (RC) = 0.724. Reweighting with the RC reduced the number of trees and resulted in 896 equally parsimonious trees each with a length of 94.94 and a retention index (RI) = 0.982, consistency index (CI) = 0.816 and rescaled consistency (RC) = 0.802.

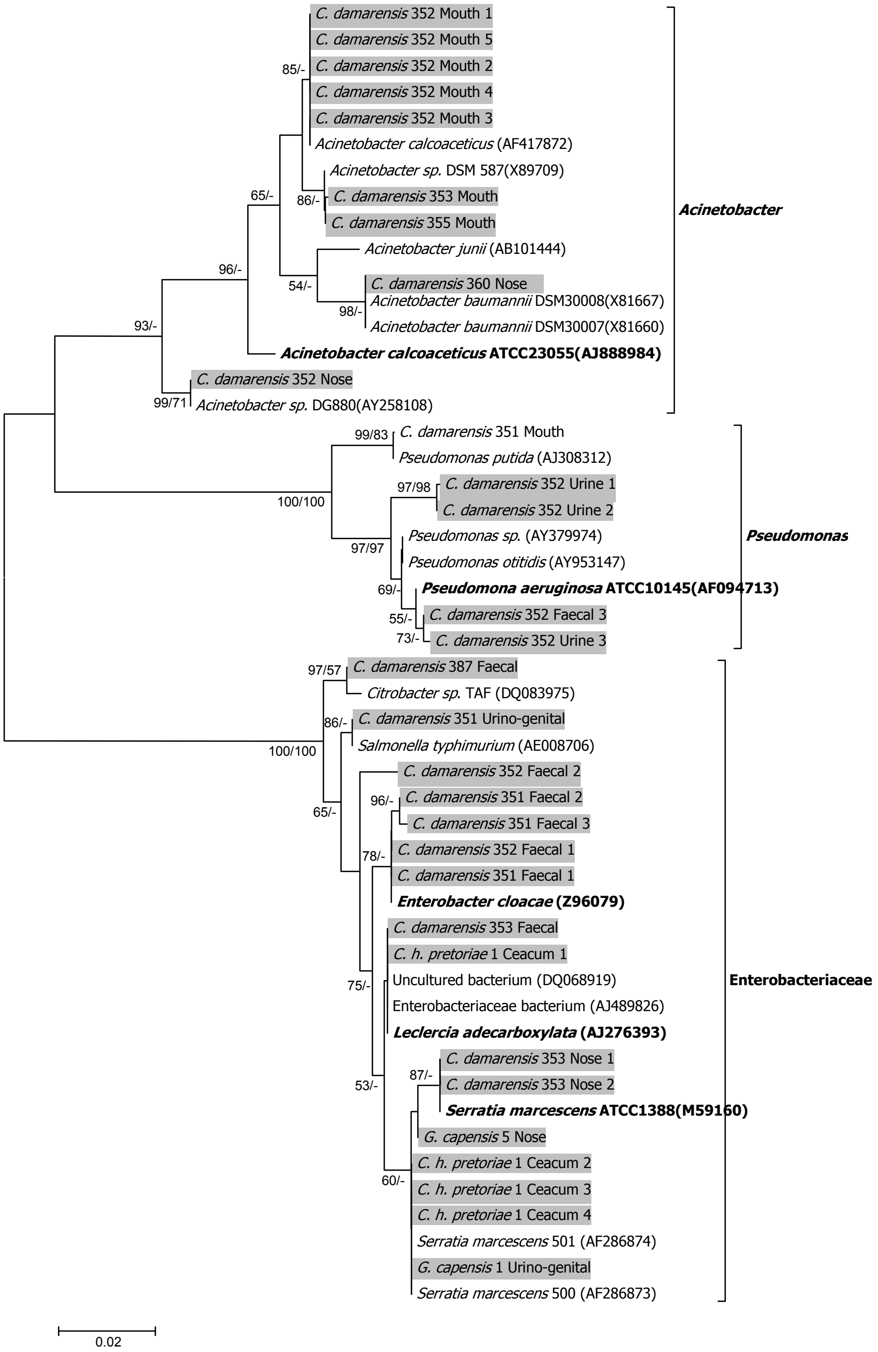

Figure 7 Neighbour-joining tree based on partial 16S data depicting bacterial relationships within the sub-class γ-Proteobacteria. Bacterial sequences generated in this study from mole-rat samples are indicated with grey shading, whilst type species/ strains are indicated in bolded text together with the relevant Genbank accession number in brackets. Nodes supported by bootstrap values > 50 % from NJ and MP are indicated next to the relevant nodes and are separated by a /. Nodes not supported by Parsimony are indicated with a "-".

Within the Proteobacteria, the γ-Proteobacteria were by far the most numerous recorded class of mole-rat bacteria belonging to this phylum (93.7 %) and contained species belonging to the genera *Serratia, Enterobacter, Citrobacter, Acinetobacter, Microbacterium, Pseudomonas* and *Escherichia.*

All bacteria cultured from mole-rat swabs identified to the γ-subclass of the Proetobacteria were found to be nearly identical (> 95 % sequence identity) to sequences available on the Genbank database. Interestingly a pattern exists in that most bacteria cultured from external swab localities were identified as belonging to the genus *Acinetobacter*, a common soil bacteria, while the majority of those isolated from internal samples belonged to the *Pseudomonas* and *Enterobacteriaceae* genera, which are common gut microbes. The only exceptions to this trend were the three bacteria cultured from nasal swabs (*C. damerensis* 353 Nose 1 & 2, *G. capensis* 5 Nose). This apparent discrepancy is however readily accounted for since the bathyergids are coprophagous allowing transmission between faecal and nasal/ mouth surfaces. The only mole-rat derived sample not forming a clear clade with a downloaded sequence was *G. capensis* 5

Nose which was found to have a 95 % sequence similarity with an unpublished Genbank sequence *Serratia marcescens* (EF415649), that was not included in the analyses.

Single instances of bacteria belonging to the α and β classes were recorded, and included one representative of the genus *Agrobacterium* belonging to the α-subclass, as well as a bacteria of unknown generic status, but most likely belonging to the β-subclass.

A single instance of a bacteria of unknown phylum grouping was recorded, this organism most closely matched 'benzo[a]pyrene-degrading bacterium BPC8' isolated by Kanaly *et al.* (2002) (sequence similarity of 99%), Genbank accession number AF494539. The results of the Blast search against the Genbank database are summarised in Appendix I.

4. Discussion

Studies attempting to address the microbial diversity in a given environment face two daunting tasks, firstly, a paradox exists that only 1 % of microorganisms in a given sample are represented through standard culturing techniques (Amman *et al*. 1995), and secondly, studies based on culturing have shown that while some bacteria are widespread in their distribution others are restricted to certain habitat types (Schlegel & Jannasch 1992). These studies are however of the utmost importance as they lay the foundation for future studies which can address the same problems but at a finer scale. In the present study bacteria belonging to three phyla were successfully cultured and sequenced. Although this is probably only representative of approximately 1 % of the possible bacteria (Amman *et al.* 1995) endogenous to mole-rats, and only incorporates results from three species, it provides an important baseline estimate for future studies.

The most prolific phylum recorded was that of the Proteobacteria, traditionally referred to as the 'purple bacteria' (Woese 1987) and which are named after the Greek god Proteus. It is one of the most diverse groups of bacteria in the natural world, and the phylum is divided into five classes named after successive letters of the Greek alphabet commencing at α and ending at ε. Many species within the class are gram negative (Woese 1987) and possess an outer membrane composed of lipopolysaccharides (Madigan & Martinko 2005). These bacteria vary greatly in their metabolic characteristics with some being autotrophic, yet grouping with heterotrophic species. The only common characteristic amongst members of this phylum is that of the purple photosynthetic phenotype which is distributed throughout (Woese 1987). Bacteria belonging to three out of the possible five classes comprising this phylum were identified from the cultured samples namely, the α, β and γ-Proteobacteria.

The γ-Proteobacteria are the most diverse class in the phylum and may be photosynthetic or non-photosynthetic; aerobic or anaerobic; heterotrophic or chemolithotrophic (Woese 1987). The two most common families recorded in this class were the Enterobacteriaceae and the Moraxellaceae. The Enterobacteriaceae are one of the best characterized families of bacteria (Gordon & FitzGibbon 1999), consisting of at least 20 genera (Farmer *et al.* 1985, Gordon & FitzGibbon 1999). The bacteria of this family are usually rod-shaped, and measure 1-5 µm in length. They are common gut flora of many warm blooded species and not surprisingly were often found in association with mole-rat faecal samples, either directly from these samples or from areas that come into contact with the faecal pellets such as the urino-genital surface and the nasal surface and mouth. Mole-rats being coprophagous ingest their faecal pellets to obtain valuable nutrients (Bennett and Faulkes 2000). The family Moraxellaceae has a variety of species known to be pathogenic to humans, and comprises 11 genera (Pettersson *et al.* 1998). The most frequently recorded genus belonging to this family was *Acinetobacter,* which consists of at least 30 species. Although common, it is considered to be of clinical importance as several species have been identified as being opportunistic, multi-antibiotic resistant, nosocomial human pathogens (Van Looveren *et al.* 2004). All bacteria isolated from the mole-rats belonging to this family were cultured from external surface swabs and are most likely as a result of contamination from the soil environment.

The class α-Proteobacteria is of biological importance because of the close relationships formed between these micro-organisms and eukaryotes (Woese 1987). The Rhizobia are important for nitrogen fixing in legume species while members of the *Agrobacterium* are important plant pathogens (Woese 1987, Madigan & Martinko 2005). Other members of the phylum include the families Bartonellacea and Rickettsiaceae both containing notable pathogens to a variety of animal species (Woese 1987, Pretorius *et al.* 2004, Madigan & Martinko 2005, Garrity *et al*. 2005). In a study conducted by Yang *et al.* (1985) utilizing sequence analysis of Cytochrome C it was shown that the mitochondrial precursors of all eukaryotic cells have ancestry in the α sub-class of the Proteobacteria. Of agricultural importance, the family Rhizobiaceae contains the plant pathogen *Agrobacterium tumefaciens*. Infection with this pathogen causes the formation of gall tumors on the plant. *Agrobacterium tumefaciens* was cultured from mole-rat saliva and was most likely present in ingested food. Members of the β-Protobacteria class are aerobic or facultatively-aerobic. These bacteria vary in their degrading abilities and are common in environmental samples (Madigan & Martinko 2005).

The phylum Firmicutes contains many of the gram-positive bacteria, and originally included all of the gram-positive bacteria. However, after taxonomic revision only those with a low G+C content were retained in this phylum while those with a high G+C content were moved to the phylum Actinobacteria (Woese 1987, Stackebrandt *et al.* 1997). Members of the phylum form a phylogenetically deep clade and can thus be considered to be an ancient group (Fuchs & Stupperich 1986, Woese 1987). The Actinobacteria are common soil type bacteria that play an important role in decomposition. As a general trend their genome consists of a G+C content of greater than 50 % with few exceptions (Stackebrandt *et al.* 1997). The two most common families recorded in the phylum Firmicutes, that were cultured from mole-rat samples were the Staphylococcaceae and the Bacillaceae. The family Staphylococcaceae contains gram positive bacteria that include the genus Staphylococcus. This genus consists of thirty-one different species (Boone & Carstenholtz 2001), most being harmless. They are commonly found in association with other animals, and are of recent medical concern due to the increase in hospital infections caused by Methicillin-resistant *Staphylococcus aureus* (Mulligan *et al.* 1995). The Bacillaceae are gram positive bacteria, and most notably contain the genus *Bacillus* which are rod shaped and obligate or facultatively aerobic, as well as being catalase positive bacteria (Boone & Carstenholtz 2001). Under conditions of nutritional stress the bacteria undergo various developmental changes and form endospores (Errrington 1993). Three species of *Bacillus* are considered to be of medical importance, these are *Bacillus anthracis*, *B. cereus* and *B. thuringiensis* (Ash 1991).

These three species vary greatly in their pathogenic effects with *B. anthracis* being the causative agent of anthrax, *B. cereus*, a common soil bacteria, being responsible for cases of food poisoning and *B. thuringiensis* a pathogen of insect larvae, used in biological control (Helgason *et al.* 2000).

The distribution of bacteria amongst the mole-rat species tested is most likely influenced by the environmental conditions of the host animal (Gorrdon & FitzGibbon 1999). This study opens the door for future research which by focusing on a finer level of taxonomy may yield as yet undescribed species. Further study in this regard is needed to form a comprehensive picture of the diversity of bacterial life associated with these remarkable subterranean rodents.

5. Acknowledgements

We wish to thank Leanne Hart and Brian Smith for help with collection of samples, Lia Rotherham with her help with the lab work and data analysis, as well as colleagues in the Zoology Molecular Laboratory and Dr. Venter's Microbiology Laboratory. The collection of samples and the research conducted was approved by the Animal Ethics Committee at the University of Pretoria EC20624–012. The research was funded by the National Research Foundation GUN 2047282 to NCB.

6. Literature cited

ALTSCHUL, S. F.; MADDEN, T.L.; SCHÄFFER, A.A.; ZHANG, J.; ZHANG, Z.; MILLER, W.; & LIPMAN, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25:** 3389-3402.

AMANN, R. I.; LUDWIG, W.; & SCHLEIFER, K.H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews* **59:**143–169.

ANDERSON, J.F.; JOHNSON, R.C.; MAGNARELLI, L.A.; & HYDE, F.W. 1985. Identification of Endemic Foci of Lyme Disease: Isolation of *Borrelia burgdorferi* from Feral Rodents and Ticks (*Dermacentor variabilis*). *Journal of Clinical Microbiology* **22:** 36-38.

ASH, C.; FARROW, J. A. E.; DORSCH, M.; STACKEBRANDT, E.; & COLLINS, M. D. 1991. Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. *International Journal of Systematic Bacteriology* **41:** 343–346.

BENNETT, N.C**.;** & JARVIS, J.U.M. 1988. The reproductive biology of the Cape mole-rat, *Georychus capensis* (Rodentia, Bathyergidae). *Journal of Zoology, London* **214**: 95-106.

BENNETT, N.C.; & FAULKES, C.G. 2000. *In:* African mole-rats ecology and eusociality. Cambridge University Press, Cambridge. U.K. 273pp.

BISHOP, J.M.; O'RYAN, C.; SPINKS, A.C.; BENNETT, N.C.; & JARVIS, J.U.M.

2004. Molecular insight into patterns of colony composition and paternity in the common mole-rat, *Cryptomys hottentotus hottentotus*: a subterranean social mammal. *Molecular Ecology* **13:** 1217-1229.

BOONE, D.R.; & CARSTENHOLTZ, R.W. 2001. *In:* Bergey's Manual of Systematic Bacteriology, 2nd edn, vol. 1 (The Archaea and the Deeply Branching and Phototrophic Bacteria). (Staley, J.T.; Boone, D.R.; Brenner, D.J.; Carstenholtz, R.W.; Garrity, G.M.; Goofellow, M.; Krieg, N.R.; Rainey, F.A. & Schleifer, K.-H. Eds). Springer, New York, N.Y.

BURLAND, T.M.; BENNETT, N.C.; JARVIS, J.U.M.; & FAULKES, C.G. 2004. Colony structure and parentage in wild colonies of cooperatively breeding Damaraland mole-rats suggest incest avoidance alone may not maintain reproductive skew. *Molecular Ecology* **13:** 2371–2379.

BUTLER, T.; FU, Y.; FURMAN, L.; ALMEIDA, C.; & ALMEIDA, A. 1982. Experimental *Yersinia pestis* Infection in Rodents After Intragastric Inoculation and Ingestion of Bacteria. *Infection and Immunity* **32:** 1160-1167.

ERRINGTON, J. 1993. *Bacillus subtilis* sporulation: regulation of gene expression and control of morphogenesis. *Microbiology and Molecular Biology Reviews* **57:**1-33.

FARMER, J.J. 3rd; DAVIS, B.R.; HICKMAN-BRENNER, F.W.; MCWHORTER, A.; HUNTLEY-CARTER, G.P.; ASBURY, M.A.; RIDDLE, C.; WATHEN-GRADY, H.G.; ELIAS, C.; FANNING, G.R.; STEIGERWALT, A.G.; O'HARA, C.M.; MORRIS, G.K.; SMITH, P.B.; & BRENNER, D.J. 1985. Biochemical identification of new species and biogroups of Enterobacteriaceae isolated from clinical specimens. *Journal of Clinical Microbiology* **21:** 46–76.

FAULKES, C.G.; BENNETT, N.C.; BRUFORD, M.W.; O'BRIEN, H.P.; AGUILAR, G.H.; & JARVIS, J.U.M. 1997. Ecological constraints drive social evolution in the African mole-rats. *Proceedings of the Royal Society, London Series B* **264:** 1619–1627.

FAULKES, C.G.; VERHEYEN, E.; VERHEYEN, W.; JARVIS, J. U. M.; & BENNETT, N.C. 2004. Phylogeographical patterns of genetic divergence and speciation in African mole-rats (Family: Bathyergidae). *Molecular Ecology* **13:** 613–629.

FELSKE, A.; WOLTERINK, A.; VAN LIS, R.; & AKKERMANS, A.D.L. 1998. Phylogeny of the Main Bacterial 16S rRNA Sequences in Drentse A Grassland Soils (The Netherlands). *Applied and Environmental Microbiology* **64:** 871–879.

FUCHS, G.; & E. STUPPERICH. 1986. Carbon assimilation pathways in archaebacteria. *Systematic and Applied Microbiology* **7:** 364-369.

GARRITY, G. M.; BELL, J. A.; & LILBURN, T. 2005. *In:* Bergey's Manual of Systematic Bacteriology, 2nd edn, vol. 2 (The Proteobacteria). (Brenner, D.J.; Krieg, N.R.; Staley, J.T.; & Garrity, G.M. Eds). Springer, New York, N.Y.

GORDON, D. M.; & F. FITZGIBBON. 1999. The distribution of enteric bacteria from Australian mammals: host and geographical effects. *Microbiology* **145:** 2663-2671.

HANSMANN, Y.; DEMARTINO, S.; PIEMONT, Y.; MEYER, N.; MARIET, P.; HELLER, R.; CHRISTMANN, D.; & JAULHAC, B. 2005. Diagnosis of cat scratch disease with detection of *Bartonella henselae* by PCR: a study of patients with lymph node enlargement. *Journal of Clinical Microbiology* **43:** 3800-3806.

HARLEY, E.H. 2001. DNA and protein sequence analysis. Version 4.91. Department of Chemical pathology, University of Cape Town.

HAUBEN, L., VAUTERIN, L., SWINGS, J.; & MOORE, E. R. B. 1997. Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. *International Journal of Systematic Bacteriology* **47:** 328-335.

HELGASON, E.; OKSTAD, O. A.; CAUGANT, D. A.; JOHANSEN, H. A.; FOUET, A.; MOCK, M.; HEGNA, I.; & KOLSTO, A-B. 2000. *Bacillus anthracis, Bacillus cereus*, and *Bacillus thuringiensis* – one species on the basis of genetic evidence. *Applied and Environmental Microbiology* **66:** 2627–2630.

HERBST, M.; & BENNETT, N.C. 2006. Burrow architecture and burrowing dynamics of the endangered Namaqua dune mole rat (*Bathyergus janetta*) (Rodentia: Bathyergidae). *Journal of Zoology* **1:** 1-9.

HUGENHOLTZ, P.; GOEBEL, B.M.; & PACE, N.R. 1998. Impact of Culture-Independent Studies on the Emerging Phylogenetic View of Bacterial Diversity. *Journal of Bacteriology* **180:** 4765–4774.

JARVIS, J.U.M.; & BENNETT, N.C. 1993. Eusociality has evolved independently in two genera of bathyergid mole-rats - but occurs in no other subterranean mammal. *Behavioral Ecology and Sociobiology* **33:** 353-360.

JARVIS, J.U.M.; O'RIAIN, M.J.; BENNETT, N.C.; & SHERMAN, P.W. 1994. Mammalian eusociality: a family affair. *Trends in Evolution and Ecology* **9:** 47–51.

KANALY, R.A.; HARAYAMA, S.; & WATANABE, K. 2002. Rhodanobacter sp. Strain BPC1 in a Benzo[a]pyrene-Mineralizing. Bacterial Consortium. *Applied Environmental Microbiology* **68:** 5826-5833.

KOSOY, M. Y.; REGNERY, R.L.; TZIANABOS, T; MARSTON, E.L; JONES, D.C.;

GREEN, D.; MAUPIN, G.O.; OLSON, J.G.; & CHILDS, J.E. 1997. Distribution, diversity, and host specificity of *Bartonella* in rodents from the southeastern United States. *American Journal of Tropical Medicine and Hygiene* **57:**578–588.

KUMAR, S.; TAMURA, K.; & NEI, M. 2004. MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5:** 150-163.

LACEY, E.A.; PATTON, J. A.; & CAMERON, G. N. 2000. *In:* Introduction. In Life Underground the biology of subterranean rodents. Edited by E.A. Lacey, J.L. Patton and G.N. Cameron.pp 11-12.

LA SCOLA, B.; ZEAITER, Z.; KHAMIS, A.; & RAOULT, D. 2003a. Gene sequence-based criteria for species definition in bacteriology: the *Bartonella* paradigm. *Trends in Microbiology* **11:** 318–321.

LA SCOLA, B.; MALLET, M. N.; GRIMONT, P. A. D. ; & RAOULT, D. 2003b. *Bosea eneae sp. nov., Bosea massiliensis sp. nov*. and *Bosea vestrisii sp. nov*., isolated from water supplies, and emendation of the genus Bosea (Das 1996). *International Journal of Systematic and Evolutionary Microbiology*. **53:** 15–20.

LESSON, R.P. 1826. Voyage autour du monde sur la Coquille pendant 1822-1825. *Zoologie*.**1:** 166.

MADIGAN, M. & MARTINKO, J. (editors) 2005. *In:* Brock Biology of Microorganisms, 11th ed., Prentice Hall.

MOOLMAN, M., BENNETT, N.C.; & SCHOEMAN, A.S. 1998. The social structure and dominance hierarchy of the highveld mole-rat, *Cryptomys hottentotus pretoriae*, (Rodentia: Bathyergidae). *Journal of Zoology, London* **246:** 193-201

MULLIGAN, M.E.; MURRAY-LEISURE, K.A.; RIBNER, B.S.; STANDIFORD, H.C.; JOHN, J.F.; KORVICK, J.A.; KAUFFMAN, C.A.; & YU, V.L. 1995. Methicillin-resistant *Staphylococcus aureus*: A consensus review of the microbiology, pathogenesis, and epidemiology with implications for prevention and management. *The American Journal of Medicine* **94:** 313-28.

OGILBY. 1838. *Proceedings of the Zoological Society of London* **6:** 5.

PALLAS. 1778. *In:* Novae Species Quadrupendum e Glirum Ordine. Erlagen: Wolfgang Walter, Part 2, pp. 172.

PETTERSSON, B.; KODJO, A.;RONAGHI, M.; UHLEN, M.; & TONJUM, T. 1998. Phylogeny of the family Moraxellaceae by 16S rDNA sequence analysis, with special emphasis on differentiation of *Moraxella* species. *International Journal of Systematic Bacteriology* **48:** 75-89.

PRETORIUS, A.M.; BEATI, L.; & BIRTLES, R.J. 2004. Diversity of *Bartonella* associated with small mammals inhabiting Free State province, South Africa. *International Journal of Systematic and Evolutionary Microbiology* **54:** 1959–1967.

PRIEST. F.; & AUSTIN, B. 1993. *In:* Modern Baterial Taxonomy. Chapman and Hall, New York, N.Y.

RONDON, M.R.; GOODMAN, R.M.; & HANDELSMAN, J. 1999. The Earth's bounty: assessing and accessing soil microbial diversity. *Trends in Biotechnology* **17:**403-409.

SCHLEGEL, H. G.; & JANNASCH. H.W. 1992. Prokaryotes and their habitats, p. 75–125. *In:* The prokaryotes, vol. I. (Balows, A.; Truper, H.G.; Dworkin, M.; Harder, W.; & Schleifer, K.H.; Eds.). Springer-Verlag, New York, N.Y.

SCHREBER, J.C.D. 1782. *In:* Die Ssugethiere, Part 4. Wolfgang Walter, 1792, pp. 593-936.

STACKEBRANDT, E.; RAINEY, F.A.; & WARD-RAINEY, N.L. 1997. Proposal for a new hierarchic classification system, Actinobacteria classis nov. *International Journal of Systematic Bacteriology* **47:** 479-491.

SWOFFORD, D.L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.

TOWER, K.J. 1992. The genus *Acinetobacter. In:* The Prokaryotes (Balows, A.; Trüper, H.G.; Dworkin, M.; Harder, W.; & SchleiferK-Z; Eds.). Springer-Verlag, New York, N.Y.

WILSON, K.H.; BLITCHINGTON, R.B.; & GREENE, R.C. 1990. Amplification of Bacterial 16S Ribosomal DNA with Polymerase Chain Reaction. *Journal of Clinical Microbiology* **28:** 1942-1946.

WOESE, C. R. 1987. Bacterial evolution. *Microbiological reviews* **51:** 221–271.

VAN LOOVEREN, M.; GOOSSENS, H.; & the ARPAC Steering Group**.** 2004. Antimicrobial resistance of *Acinetobacter* spp. in Europe. *Clinical Microbiology and Infection* **10:**684–704.

YANG, D.; OYAIZU, Y.; OYAIZU, H.; OLSEN, G.J.; & WOESE, C.R. 1985. Mitochondrial origins. *Proceedings of the National Academy of Sciences,* USA **82:** 4443-4447.

Appendix I

Results of the BLAST homology searches of bacteria cultured from internal and external sampling sites in *Cryptomys damaerensis*, *Crytomys hottentotus pretoriae* and *Georychus capensis*. Genera of bacteria present in mole-rat samples over multiple levels of sociality indicated in bolded text.

Host Species and animal number	Degree of sociality	Location of sample	Closest Organism match in Genbank	% match to closest Genbank sequence	Genbank accession number of the closest match	Expected Taxonomy
EXTERNAL						
***Cryptomys damarensis* 351 1**	**Eusocial**	**Faecal**	***Enterobacter sp.* D1**	**99**	**AY756180**	**γ-Proteobacteria**
***Cryptomys damarensis* 351 2**	**Eusocial**	**Faecal**	***Enterobacter sp.* D2**	**96**	**AY756180**	**γ-Proteobacteria**
***Cryptomys damarensis* 351 3**	**Eusocial**	**Faecal**	***Enterobacter sp.* D3**	**95**	**AY756180**	**γ-Proteobacteria**
Cryptomys damarensis 351	Eusocial	Urino-genital	*Escherichia coli*	99	AB210981	γ-Proteobacteria
Cryptomys damarensis 351	Eusocial	Urino-genital	*Leuconostoc esenteroides*	99	CP000414	Firmicutes
Cryptomys damarensis 351	Eusocial	Mouth	*Pseudomonas fluorescens*	99	CP000094	γ-Proteobacteria
Cryptomys damarensis 351	Eusocial	Urine	*Paenibacillus pabali*	98	AB045094	Firmicutes
Cryptomys damarensis 351	Eusocial	Urine	*Paenibacillus pabali*	99	AB045094	Firmicutes
***Cryptomys damarensis* 352 1**	**Eusocial**	**Faecal**	***Enterobacter sp.* B901-2**	**98**	**AB114268**	**γ-Proteobacteria**
***Cryptomys damarensis* 352 2**	**Eusocial**	**Faecal**	***Enterobacter sp.* D1**	**99**	**AY756180**	**γ-Proteobacteria**
Cryptomys damarensis 352 3	Eusocial	Faecal	*Pseudomonas sp.* AHL 2	99	AY379974	γ-Proteobacteria
Cryptomys damarensis 352	Eusocial	Urino-genital	*Arthrobacter sp.* EP04	97	AM398213	Actinobacteria
Cryptomys damarensis 352 1	Eusocial	Mouth	*Acinetobacter sp.* 3-SL3	100	EF010990	γ-Proteobacteria
Cryptomys damarensis 352 2	Eusocial	Mouth	*Acinetobacter sp.* 3-SL4	100	EF010990	γ-Proteobacteria
Cryptomys damarensis 352 3	Eusocial	Mouth	*Acinetobacter sp.* 3-SL5	100	EF010990	γ-Proteobacteria
Cryptomys damarensis 352 4	Eusocial	Mouth	*Acinetobacter sp.* 3-SL6	100	EF010990	γ-Proteobacteria
Cryptomys damarensis 352	Eusocial	Mouth	*Acinetobacter sp.* 3-SL7	100	EF010990	γ-Proteobacteria
Cryptomys damarensis 352	Eusocial	Nose	*Acinetobacter sp.* DG880	100	AY258108	γ-Proteobacteria
Cryptomys damarensis 352 1	Eusocial	Urine	*Pseudomonas sp.* AHL 2	100	AY379974	γ-Proteobacteria

Host Species and animal number	Degree of sociality	Location of sample	Closest Organism match in Genbank	% match to closest Genbank sequence	Genbank accession number of the closest match	Expected Taxonomy
Cryptomys damarensis 352 2	Eusocial	Urine	*Pseudomonas sp*. AHL 3	99	AY379974	γ-Proteobacteria
Cryptomys damarensis 352 3	Eusocial	Urine	*Pseudomonas sp*. AHL 4	99	AY379974	γ-Proteobacteria
***Cryptomys damarensis* 353**	**Eusocial**	**Faecal**	***Bacillus sp*. BFF-3**	**98**	**EF031071**	**Firmicutes**
Cryptomys damarensis 353	Eusocial	Faecal	**Enterobacteriaceae**	97	AY803950	γ-Proteobacteria
Cryptomys damarensis 353	Eusocial	Mouth	*Acinetobacter sp*. Dui-5	99	EF031061	γ-Proteobacteria
Cryptomys damarensis 353	Eusocial	Urino-genital	*Microbacterium sp*. PHD-5	100	DQ227343	Actinobacteria
Cryptomys damarensis 353	Eusocial	Urino-genital	*Microbacterium sp*. PHD-5	99	DQ227343	Actinobacteria
Cryptomys damarensis 353	Eusocial	Nose	*Microbacterium sp*. Cr1	100	AM396495	Actinobacteria
***Cryptomys damarensis* 353 1**	**Eusocial**	**Nose**	***Serratia marcescens* isolate**	**99**	**DQ207558**	**γ-Proteobacteria**
***Cryptomys damarensis* 353 2**	**Eusocial**	**Nose**	***Serratia marcescens* isolate**	**99**	**DQ207558**	**γ-Proteobacteria**
Cryptomys damarensis 355	Eusocial	Faecal	Uncultured soil bacterium	99	AB 097171	β-Proteobacteria
Cryptomys damarensis 355	Eusocial	Mouth	*Agrobacterium tumefaciens*	100	AB247616	α-Proteobacteria
Cryptomys damarensis 355	Eusocial	Mouth	*Acinetobacter sp*. 3-SL3	100	EF010990	γ-Proteobacteria
Cryptomys damarensis 360	Eusocial	Nose	*Acinetobacter sp*. AzoR-3	99	DQ279750	γ-Proteobacteria
Cryptomys damarensis 387	Eusocial	Faecal	*Citrobacter farmeri*	98	AF025371	γ-Proteobacteria
Cryptomys damarensis 387	Eusocial	Nose	*Arthrobacter sp*. EP04	98	AM398213	Actinobacteria
Cryptomys damarensis 391	Eusocial	Urino-genital	benzo[a]pyrene-degrading	99	AF494539	Unknown
Cryptomys damarensis 391	Eusocial	Urino-genital	*Arthrobacter sp*. EP04	98	AM398213	Actinobacteria
***Cryptomys damarensis* 391**	**Eusoicial**	**Urino-genital**	***Staphylococcus sp*. SBT120**	**99**	**AY918868**	**Firmicutes**
Cryptomys hottentotus pretoriae E3	**Social**	**Mouth**	***Bacillus pumilus***	**98**	**DQ988522**	**Firmicutes**
Cryptomys hottentotus pretoriae E5	**Social**	**Nose**	***Bacillus sp*. MHS001**	**97**	**DQ993325**	**Firmicutes**
***Georychus capensis* 1**	**Solitary**	**Urino-genital**	***Staphylococcus equorum***	**99**	**AM237374**	**Firmicutes**
***Georychus capensis* 5**	**Solitary**	**Urino-genital**	***Serratia marcescens***	**98**	**AB270613|**	**γ-Proteobacteria**
***Georychus capensis* 5**	**Solitary**	**Nose**	***Serratia marcescens***	**96**	**DQ832185**	**γ-Proteobacteria**
INTERNAL						
Cryptomys damarensis 1	Eusocial	Caecum	*Aerococcus viridans* strain	99	AY707774	Firmicutes
***Cryptomys damarensis* 2**	**Eusocial**	**Caecum**	***Bacillus sp*. MHS001**	**97**	**DQ993325**	**Firmicutes**
Cryptomys hottentotus pretoriae 1 1	**Social**	**Caecum**	***Enterobacter sp*. WAB1959**	**100**	**AM184298**	**γ-Proteobacteria**
Cryptomys hottentotus pretoriae 1 2	**Social**	**Caecum**	***Serratia marcescens***	**99**	**AB270613**	**γ-Proteobacteria**

Host Species and animal number	Degree of sociality	Location of sample	Closest Organism match in Genbank	% match to closest Genbank sequence	Genbank accession number of the closest match	Expected Taxonomy
Cryptomys hottentotus pretoriae 1 3	**Social**	**Caecum**	***Serratia marcescens***	**99**	**AB270613**	**γ-Proteobacteria**
Cryptomys hottentotus pretoriae 1 4	**Social**	**Caecum**	***Serratia marcescens***	**100**	**AB270613**	**γ-Proteobacteria**
Cryptomys hottentotus pretoriae 3	**Social**	**Caecum**	***Bacillus sp*****. BCL13-1**	**97**	**EF026993**	**Firmicutes**
Cryptomys hottentotus pretoriae 3 4	**Social**	**Genital tract**	***Bacillus sp*****. BCL13-1**	**98**	**EF026993**	**Firmicutes**
Cryptomys hottentotus pretoriae 3 3	Social	Genital tract	Bacterium 9-gw3-9	97	DQ990068	Firmicutes (expected)
Cryptomys hottentotus pretoriae 3 1	**Social**	**Genital tract**	***Staphylococcus sp*****. Ap-9**	**99**	**DQ644501**	**Firmicutes**
Cryptomys hottentotus pretoriae 3 2	**Social**	**Genital tract**	***Staphylococcus sp*****. Ap-9**	**99**	**DQ644501**	**Firmicutes**

Appendix II

Nucleotide sequence alignment of the Actinobacteria created in MEGA v. 3.1 (Kumar *et al.* 2004). Dots indicate sites identical to that of the reference sequence (*C. damarensis* 387 Nose). ? indicates missing data and – indicates gaps.

```
C._damarensis_387_Nose            TCCCAATCGC CAGATCCCAC CTTCGACGAC TCCCCCC-AC ACAAGGTGGT TAGGCCATCG GCTTCGGGTG TTACCAACTT  [ 80]
C._damarensis_352_Urino-genital   .......... .......... .......... .......-.. .......... .......... .......... ..........  [ 80]
C._damarensis_391_Urino-genital   .......... .......... .......... .......-.. .......... .......... .......... ..........  [ 80]
C._damarensis_353_Nose            -T..T.ATTA .C........ ........G. ....T..--- ......--.. .......C.. .....A.... .....G....  [ 80]
C._damarensis_353_Urino-genital_  -T..T.ATTA .C........ ........G. ....T..--- ......--.. .......C.. .....A.... .....G....  [ 80]
C._damarensis_353_Urino-geneta1_  -T..T.ATTA .C........ ........G. ....T..--- ......--.. .......C.. .....A.... .....G....  [ 80]
M._sp._YSS/2001-1_(AF417868)      -T..T.ATTA .C........ ........G. ....T..--- ......--.. .......C.. .....A.... .....G....  [ 80]
M._sp._OVE_(AY960683)             -T..T.ATTA .C........ ........G. ....T..--- ......--.. .......C.. .....A.... .....G....  [ 80]
A._arilaitensis_(DQ361012)        -T........ ...-...... .......... .......-.. .......... .......... .......... ..........  [ 80]
A._mysorens_(AJ639831)            .......... ...-...... .......... .......-.. .......... .......... .......... ..........  [ 80]
M._oxydans_CV71a_(AJ717358)       -T..T.ATTA .C........ ........G. ....T..--- ......--.. .......C.. .....A.... .....G....  [ 80]
A._arilaitensis_CIP_108037_(AJ60  .......... ...-...... .......... .......C.. .......... .......... .......... ..........  [ 80]
M._lacticum_IFO_14135T_(D21343)   -------TTA .G........ ........G. ....T..--- ......--.. .G.....C.. .....A.... .....G....  [ 80]
A._globiformis_(AB089841)         .......... ...-...... ........G. ....T..--. ......-... .......C.. .......... ..........  [ 80]

C._damarensis_387_Nose            TCGTGACTTG ACGGGCGGTG TGTACAAGGC CCGGGAACGT ATTCACCGCA GCGTTGCTGA TCTGCGATTA CTAGCGACTC  [160]
C._damarensis_352_Urino-genital   .......... .......... .......... .......... .......... ?......... .......... ..........  [160]
C._damarensis_391_Urino-genital   .......... .......... .......... .......... .......... .......... .......... ...?......  [160]
C._damarensis_353_Nose            ..A....... .......... ........A. .......... .......... .......... .......... ..........  [160]
C._damarensis_353_Urino-genital_  ..A....... .......... ........A. .......... .......... .......... .......... ..........  [160]
C._damarensis_353_Urino-geneta1_  ..A....... .......... ........A. .......... .......... .......... .......... ..........  [160]
M._sp._YSS/2001-1_(AF417868)      ..A....... .......... ........A. .......... .......... .......... .......... ..........  [160]
M._sp._OVE_(AY960683)             ..A....... .......... ........A. .......... .......... .......... .......... ..........  [160]
A._arilaitensis_(DQ361012)        .......... .......... .......... .......... .......... .......... .......... ..........  [160]
A._mysorens_(AJ639831)            .......... .......... .......... .......... .......... .......... .......... ..........  [160]
M._oxydans_CV71a_(AJ717358)       ..A....... .......... ........A. .......... .......... .......... .......... ..........  [160]
A._arilaitensis_CIP_108037_(AJ60  .......... .......... .......... .......... .......... .......... .......... ..........  [160]
M._lacticum_IFO_14135T_(D21343)   ..A....... .......... ........A. .......... .......... .......... .......... ..........  [160]
A._globiformis_(AB089841)         .......... .......... .......... .......... .......... .......... .......... ..........  [160]

C._damarensis_387_Nose            CGACTTCATG GGGTCGAGTT GCAGACCCCA ATCCGAACTG AGACCGGCTT TTAGGGATTA GCTCCACCTC ACAGTATCGC  [240]
C._damarensis_352_Urino-genital   .......... .......... ...?...... .......... .......... .......... .......... ..........  [240]
C._damarensis_391_Urino-genital   .......... .......... .......... .......... .......... .......... .......... ..........  [240]
C._damarensis_353_Nose            .......... A......... .......T.. .......... G......... ..T......C .......... G.G....T..  [240]
C._damarensis_353_Urino-genital_  .......... A......... .......T.. .......... G......... ..T......C .......... ..G....T..  [240]
C._damarensis_353_Urino-geneta1_  .......... A......... .......T.. .......... G......... ..T......C .......... ..G....T..  [240]
M._sp._YSS/2001-1_(AF417868)      .......... A......... .......T.. .......... G......... ..T......C .......... G.G....T..  [240]
M._sp._OVE_(AY960683)             .......... A......... .......T.. .......... G......... ..T......C .......... G.G....T..  [240]
A._arilaitensis_(DQ361012)        .......... .......... .......... .......... .......... .......... .......... ..........  [240]
A._mysorens_(AJ639831)            .......... .......... .......... .......... .......... .......... .......... ..........  [240]
```

```
M._oxydans_CV71a_(AJ717358)      .......... A......... .......T.. .......... G......... ..T......C .......... G.G....T..  [240]
A._arilaitensis_CIP_108037_(AJ60 .......... .......... .......... .......... .......... .......... .......... ..........  [240]
M._lacticum_IFO_14135T_(D21343)  .......... A......... .......T.. .......... G......... ..T......C .........T G.G....T..  [240]
A._globiformis_(AB089841)        .......... .......... .......... .......... .......... ..T....... .......... ..........  [240]

C._damarensis_387_Nose           AACCCATTGT ACCGGCCATT GTAGCATGCG TGAAGCCCAA GACATAAGGG GCATGATGAT TTGACGTCAT CCCCACCTTC  [320]
C._damarensis_352_Urino-genital  .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_391_Urino-genital  .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_353_Nose           .G...T.... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_353_Urino-genital_ .G...T.... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_353_Urino-geneta1_ .G...T.... .......... .......... .......... .......... .......... .......... ..........  [320]
M._sp._YSS/2001-1_(AF417868)     .G...T.... .......... .......... .......... .......... .......... .......... ..........  [320]
M._sp._OVE_(AY960683)            .G...T.... .......... .......... .......... .......... .......... .......... ..........  [320]
A._arilaitensis_(DQ361012)       .......... .......... .......... .......... .......... .......... .......... ..........  [320]
A._mysorens_(AJ639831)           .......... .......... .......... .......... .......... .......... .......... ..........  [320]
M._oxydans_CV71a_(AJ717358)      .G...T.... .......... .......... .......... .......... .......... .......... ..........  [320]
A._arilaitensis_CIP_108037_(AJ60 .......... .......... .......... .......... .......... .......... .......... ..........  [320]
M._lacticum_IFO_14135T_(D21343)  .G........ .......... .......... .......... .......... .......... .......... ..T.......  [320]
A._globiformis_(AB089841)        .....T.... .......... .......... .......... .......... .......... ........G. ..........  [320]

C._damarensis_387_Nose           CTCCGAGTTG ACCCCGGCAG TCTCCCATGA GTCCCCACCA CTACGTGCTG GCAACATGGA ACGAGGGTTG CGCTCGTTGC  [400]
C._damarensis_352_Urino-genital  .......... .......... .......... .......... .......... .......... .......... ..........  [400]
C._damarensis_391_Urino-genital  .......... .......... .......... .......... .......... .......... .......... ..........  [400]
C._damarensis_353_Nose           .......... .......... .A........ ..T....... T......... .......A.. .......... ..........  [400]
C._damarensis_353_Urino-genital_ .......... .......... .A........ ..T....... T......... .......A.. .......... ..........  [400]
C._damarensis_353_Urino-genetal_ .......... .......... .A........ ..T....... T......... .......A.. .......... ..........  [400]
M._sp._YSS/2001-1_(AF417868)     .......... .......... .A........ ..T....... T......... .......A.. .......... ..........  [400]
M._sp._OVE_(AY960683)            .......... .......... .A........ ..T....... T......... .......A.. .......... ..........  [400]
A._arilaitensis_(DQ361012)       .......... .......... .......... .......... .......... .......... .......... ..........  [400]
A._mysorens_(AJ639831)           .......... .......... .......... .......... .......... .......... .......... ..........  [400]
M._oxydans_CV71a_(AJ717358)      .......... .......... .A........ ..T....... T......... .......A.. .......... ..........  [400]
A._arilaitensis_CIP_108037_(AJ60 .......... .......... .......... .......... .......... .......... .......... ..........  [400]
M._lacticum_IFO_14135T_(D21343)  .......... .......... .A.T...... ..T....... T......... .......A.. .......... ..........  [400]
A._globiformis_(AB089841)        .......... .......... .......... ......G... T...C..... .......... .......... ..........  [400]

C._damarensis_387_Nose           GGGACTTAAC CCAACATCTC ACGACACGAG CTGACGACAA CCATGCACCA CCTGTGAACC AGCCCCGAAG GGAAAGACCA  [480]
C._damarensis_352_Urino-genital  .......... .......... .......... .......... .......... .......... .......... ..........  [480]
C._damarensis_391_Urino-genital  .......... .......... .......... .......... .......... .......... .......... ..........  [480]
C._damarensis_353_Nose           .......... .......... .......... .......... .......... .....TT..G ..TGT.C..A .AGTT.....  [480]
C._damarensis_353_Urino-genital_ .......... .......... .......... .......... .......... .....TT..G ..TGT.C..A .AGTT.....  [480]
C._damarensis_353_Urino-genetal_ .......... .......... .......... .......... .......... .....TT..G ..TGT.C..A .AGTT.....  [480]
M._sp._YSS/2001-1_(AF417868)     .......... .......... .......... .......... .......... .....TT..G ..TGT.C..A .AGTT.....  [480]
M._sp._OVE_(AY960683)            .......... .......... .......... .......... .......... .....TT..G ..TGT.C..A .AGTT.....  [480]
A._arilaitensis_(DQ361012)       .......... .......... .......... .......... .......... .......... .......... .....CT...  [480]
A._mysorens_(AJ639831)           .......... .......... .......... .......... .......... .......... .......... .....CC...  [480]
M._oxydans_CV71a_(AJ717358)      .......... .......... .......... .......... .......... .....TT..G ..TGT.C..A .AGTT.....  [480]
A._arilaitensis_CIP_108037_(AJ60 .......... .......... .......... .......... .......... .......... .......... .....CT...  [480]
```

```
M._lacticum_IFO_14135T_(D21343)   .......... .......... .......... .......... .......... .....TT..G ..TGT.C..A .AGTT.....  [480]
A._globiformis_(AB089841)         .......... .......... .......... .......... .......... .......... G.....A..A ...G.A....  [480]

C._damarensis_387_Nose            --TCTCTGAT CCGGTCTGGC ACATGTCAAG CCTTGGTAAG GTTCTTCGCG TTGCATCGAA TTAATCCGCA TGCTCCGCCG  [560]
C._damarensis_352_Urino-genital   --........ .......... .......... .......... .......... .......... .......?.. ..........  [560]
C._damarensis_391_Urino-genital   --........ .......... .......... .......... .......... .......... .......... ..........  [560]
C._damarensis_353_Nose            --.T....GC ...T...C.T .T........ .......... .......... .......... .......... ..........  [560]
C._damarensis_353_Urino-genital_  --.T....GC ...T...C.T .T........ .......... .......... .......... .......... ..........  [560]
C._damarensis_353_Urino-geneta1_  --.T....GC ...T...C.T .T........ .......... .......... .......... .......... ..........  [560]
M._sp._YSS/2001-1_(AF417868)      --.T....GC ...T...C.T .T........ .......... .......... .......... .......... ..........  [560]
M._sp._OVE_(AY960683)             --.T....GC ...T...C.T .T........ .......... .......... .......... .......... ..........  [560]
A._arilaitensis_(DQ361012)        --.......A G......... .......... .......... .......... .......... .......... ..........  [560]
A._mysorens_(AJ639831)            --......GA G......... .......... .......... .......... .......... .......... ..........  [560]
M._oxydans_CV71a_(AJ717358)       --.T....GC ...T...C.T .T........ .......... .......... .......... .......... ..........  [560]
A._arilaitensis_CIP_108037_(AJ60  --.......A G......... .......... .......... .......... .......... .......... ..........  [560]
M._lacticum_IFO_14135T_(D21343)   --.T....GC ...T...C.T .T........ .......... .......... .......... .......... ..........  [560]
A._globiformis_(AB089841)         CA.T....CG G.....C..T C......... .......... .......... .......... .......... ..........  [560]

C._damarensis_387_Nose            CTTGTGCGGG C?CCCGTCAA TTCCTTTGAG T?TTAGCCTT GCGGGCGTAC TCCCCA?GCG GGGCACTTAA TGCGTTA?CT  [640]
C._damarensis_352_Urino-genital   .......... .?..?...?. ?........? .T........ ....C..... ......A... .....?...? ...?...A..  [640]
C._damarensis_391_Urino-genital   .......... .?........ .......... .T........ ....C..... ......A... .....?.... .......A..  [640]
C._damarensis_353_Nose            .......... TC........ .......... .T........ ....C..... ......G... ...A...... .......G..  [640]
C._damarensis_353_Urino-genital_  .......... TC........ .......... .T........ ....C..... ......G... ...A...... .......G..  [640]
C._damarensis_353_Urino-geneta1_  .......... TC........ .......... .T........ ....C..... ......G... ...A...... .......G..  [640]
M._sp._YSS/2001-1_(AF417868)      .......... TC........ .......... .T........ ....C..... ......G... ...A...... .......G..  [640]
M._sp._OVE_(AY960683)             .......... TC........ .......... .T........ ....C..... ......G... ...A...... .......G..  [640]
A._arilaitensis_(DQ361012)        .......... .C........ .......... .T........ ....C..... ......G... .......... .......G..  [640]
A._mysorens_(AJ639831)            .......... .C........ .......... .T........ ....C..... ......G... .......... .......G..  [640]
M._oxydans_CV71a_(AJ717358)       .......... TC........ .......... .T........ ....C..... ......G... ...A...... .......G..  [640]
A._arilaitensis_CIP_108037_(AJ60  .......... .C........ .......... .T........ ....C..... ......G... .......... .......G..  [640]
M._lacticum_IFO_14135T_(D21343)   .......... TC........ .......... .T........ ....C..... ......G... ...A...... .......G..  [640]
A._globiformis_(AB089841)         .......... .C........ .......... .T........ ....C..... ......G... .......... .......G..  [640]

C._damarensis_387_Nose            ACGGCGCG?A AAACGTGGAA TGTCCCCCAC AC [672]
C._damarensis_352_Urino-genital   ........G. ..?....... .........? .. [672]
C._damarensis_391_Urino-genital   ........?. .......... ...?...... .. [672]
C._damarensis_353_Nose            G..T.A..G. .TC....... ..GA...... .A [672]
C._damarensis_353_Urino-genital_  G..T.A..G. .TC....... ..GA...... .A [672]
C._damarensis_353_Urino-geneta1_  G..T.A..G. .TC....... ..GA...... .A [672]
M._sp._YSS/2001-1_(AF417868)      G..T.A..G. .TC....... ..GA...... .A [672]
M._sp._OVE_(AY960683)             G..T.A..G. .TC....... ..GA...... .A [672]
A._arilaitensis_(DQ361012)        ........G. .......... .......... .. [672]
A._mysorens_(AJ639831)            ........G. .......... .......... .. [672]
M._oxydans_CV71a_(AJ717358)       G..T.A..G. .TC....... ..GA...... .A [672]
A._arilaitensis_CIP_108037_(AJ60  ........G. .......... .......... .. [672]
M._lacticum_IFO_14135T_(D21343)   G..T.A..G. .TC....... ..GA...... .A [672]
A._globiformis_(AB089841)         ........G. .......... .......... .. [672]
```

Appendix III

Nucleotide sequence alignment of dataset I of the Firmicutes created in MEGA v. 3.1 (Kumar *et al.* 2004). Dots indicate sites identical to that of reference sequence (*C. damarensis* 391 Urine 2) , where ? indicates missing data and – indicates gaps.

```
C._damarensis_391_Urine_2        CGCCTGGGGA GTACGGTCGC -AAGACTGAA ACTCAAAGGA ATTGACGGGG ACCCGCACAA GCAGTGGAGT ATGTGGTTTA  [ 80]
C._damarensis_391_Urine_3        .......... .......... -......... .......... .......... .......... .......... ..........  [ 80]
C._damarensis_351_Urino-genital  .......... .....AC.C. C...GT.... .......... .......... .......... ..G......C ..........  [ 80]
C._h._pretoriae_3_Gential_tract_ .......... .......?.. -...?..... .......... .......... G......... ..G......C ..........  [ 80]
C._h._pretoriae_3_Gential_tract_ .......... ....?..... -......... .......... .......... G......... ..G......C ..........  [ 80]
C._h._pretoriae_3_Caecum         .......... .......... -...?..... .......... .......... G..?....?. ..G......C ..........  [ 80]
C._damarensis_353_Faecal         .......... ......C... -...G..... .......... .......... G?......?. ..G..?...C ......?...  [ 80]
Aerococcus_viridans_(AY707779)   .......... .....AC... -...GT.... .......... .......... .......... ..G......C ..........  [ 80]
Aerococcus_sp._LV65.(AF076639)   .......... .....AC... -...GT.... .......... .......... .......... ..G......C ..........  [ 80]
Aerococcus_viridans_88B_(AY70777 .......... .....AC... -...GT.... .......... .......... .......... ..G......C ..........  [ 80]
Bacillus_megaterium_KL-197_(AY03 .......... .......... -......... .......... .......... G......... ..G......C ..........  [ 80]
Leuconostoc_pseudomesenteroides_ .......... .....AC... -...GT.... .......... .......... .......... ..G......C ..........  [ 80]
Leuconostoc_sp._CR-310S_(AF34993 .......... .....AC... -...GT.... .......... .......... .......... ..G......C ..........  [ 80]
Leuconostoc_inhae_IH101_(AY11768 .......... .....AC... -...GT.... .......... .......... ...-...... ..G......C ..........  [ 80]
Paenibacillus_xylanilyticus_(AY4 .......... .......... -......... .......... .......... .......... .......... ..........  [ 80]
Paenibacillus_sp._HC1_(AB198337) .......... .......... -......... .......... .......... .......... .......... ..........  [ 80]
Paenibacillus_sp._SAFN-068_(DQ12 .......... .....?.... -......... .?........ .......... .........? ?......... ..........  [ 80]
Bacillus_cereus_CFR04_(AY907828) .......... ......C... -...G..... .......... .......... G......... ..G......C ..........  [ 80]
Bacillus_pumilus_OS-70_(AM237370 .......... .......... -......... .......... .......... G......... ..G......C ..........  [ 80]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .....AC... -...GT.... .......... .......... .......... ..G......C ..........  [ 80]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .....AC... -...GT.... .......... .......... .......... ..G......C ..........  [ 80]

C._damarensis_391_Urine_2        ATTCGAAGCA ACGCGAAGAA CCTTACCAGG TCTTGACATC CCTCTGACCG GTACAGAGAT GTACCTT--T CCTTCGGGAC  [160]
C._damarensis_391_Urine_3        .......... .......... .......... .......... .......... .......... .......--. ..........  [160]
C._damarensis_351_Urino-genital  .......... .......... .......... .......... .-.T...AGC T.TT...... AG.AG.GTTC T......AG-  [160]
C._h._pretoriae_3_Gential_tract_ .......... .........? .......... .......... ?-......AA C.CT...... AG.G.G.TCC ........GG  [160]
C._h._pretoriae_3_Gential_tract_ ...G...... .......... .?........ .......... .-......AA CCCT...... AGGG.?.TTC ........GA  [160]
C._h._pretoriae_3_Caecum         .......... .......... .......... .......... .-......AA C.CT...... AG.G.G.TCC .?......GG  [160]
C._damarensis_353_Faecal         .......... .......... .......... .......... .-.....AAA CCCT...... AGGG.?.TC. .........G  [160]
Aerococcus_viridans_(AY707779)   .......... .......... ........A. .......... .-.T.....A CCCT...... AGGG...T-C ........G-  [160]
Aerococcus_sp._LV65.(AF076639)   .......... .......... ........A. .......... .-.T.....A CCCT...... AGGG...T-C ........G-  [160]
Aerococcus_viridans_88B_(AY70777 .......... .......... ........A. .......... .-.T.....A CCCT...... AGGG...T-C ........G-  [160]
Bacillus_megaterium_KL-197_(AY03 .......... .......... .......... .......... .-......AA C.CT...... AG.G.G.TCC ........GG  [160]
Leuconostoc_pseudomesenteroides_ .......... .......... .......... .......... .-.T...AGC T.TT...... AG.AG.GTTC T......AG-  [160]
Leuconostoc_sp._CR-310S_(AF34993 .......... .......... .......... .......... .-.T...AGC T.TT...... AG.AG.GTTC T......AG-  [160]
Leuconostoc_inhae_IH101_(AY11768 .......... .......... .......... .......... .-.T...AGC T.TT...... AG.AG.GTTC T......AG-  [160]
Paenibacillus_xylanilyticus_(AY4 .......... .......... .......... .......... .......... .......... .......--. ..........  [160]
Paenibacillus_sp._HC1_(AB198337) .......... .......... .......... .......... .......... .......... .......--. ..........  [160]
```

```
Paenibacillus_sp._SAFN-068_(DQ12 .......... .......... .......... .......... .......... .??....... .......--. ..........  [160]
Bacillus_cereus_CFR04_(AY907828) .......... .......... .......... .......... .-.....AAA CCCT...... AGGG...-C. .........G  [160]
Bacillus_pumilus_OS-70_(AM237370 .......... .......... .......... .......... .-......AA CCCT...... AGGG...T-C ........GA  [160]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......... ........AA .......... .-.T...... C.CT...... AG.GTC.TCC ........GG  [160]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......... ........AA .......... .-.T...AAA C.CT...... AG.G.C.TCC ........GG  [160]

C._damarensis_391_Urine_2        AGAG-GAGAC AGGTGGTGCA TGGTTGTCGT CAGCTCGTGT CGTGAGATGT TGGGTTAAGT CCCGCAACGA GCGCAACCCT  [240]
C._damarensis_391_Urine_3        ....-..... .......... .......... .......... .......... .......... .......... ..........  [240]
C._damarensis_351_Urino-genital  .C.AA.T... .......... ....C..... .......... .......... .......... .......... ..........  [240]
C._h._pretoriae_3_Gential_tract_ .C..A.T... .......... .......... .......... .......... .......... .......... ..........  [240]
C._h._pretoriae_3_Gential_tract_ ?C..A.T... .......... .......?.. .......... .......... .......... .......... ..........  [240]
C._h._pretoriae_3_Caecum         .C..A.T... .......... ....A..... .......... .......... .......... .......... ..........  [240]
C._damarensis_353_Faecal         ?C..A.T... .......... .......... .......... .......... .......... .......... ..........  [240]
Aerococcus_viridans_(AY707779)   .C.AA.T... .......... .......... .......... .......... .......... .......... .........C  [240]
Aerococcus_sp._LV65.(AF076639)   .C.AA.T... .......... .......... .......... .......... .......... .......... .........C  [240]
Aerococcus_viridans_88B_(AY70777 .C.AA.T... .......... .......... .......... .......... .......... .......... .........C  [240]
Bacillus_megaterium_KL-197_(AY03 .C..A.T... .......... .......... .......... .......... .......... .......... ..........  [240]
Leuconostoc_pseudomesenteroides_ .C.AA.T... .......... ....C..... .......... .......... .......... .......... ..........  [240]
Leuconostoc_sp._CR-310S_(AF34993 .C.AA.T... .......... ....C..... .......... .......... .......... .......... ..........  [240]
Leuconostoc_inhae_IH101_(AY11768 .C.AA.T... ........AC ....C..... .......... .......... .......... .......... ..........  [240]
Paenibacillus_xylanilyticus_(AY4 ....-..... .......... .......... .......... .......... .......... .......... ..........  [240]
Paenibacillus_sp._HC1_(AB198337) ....-..... .......... .......... .......... .......... .......... .......... ..........  [240]
Paenibacillus_sp._SAFN-068_(DQ12 ....-..... .......... .......... .......... .......... .......... .......... ..........  [240]
Bacillus_cereus_CFR04_(AY907828) C-..A.T... .......... .......... .......... .......... .......... .......... ..........  [240]
Bacillus_pumilus_OS-70_(AM237370 C-..A.T... .......... .......... .......... .......... .......... .......... ..........  [240]
Staphylococcus_sp._XJU-1_(DQ8375 .C.AA.T... .......... .......... .......... .......... .......... .......... ..........  [240]
Staphylococcus_sp._LAMI_001_(DQ8 .C.AA.T... .......... .......... .......... .......... .......... .......... ..........  [240]

C._damarensis_391_Urine_2        TGATCTTAGT TGCCAGCACT T?GGGTGGGC ACTCTAAGGT GACTGCCGGT GACAAACCGG AGGAA-GGTG GGGATGACGT  [320]
C._damarensis_391_Urine_3        .......... ........T. .C........ .......... .......... .......... .....-.... ..........  [320]
C._damarensis_351_Urino-genital  .AT.G..... ........-. .CA.A..... ......GC.A .......... .......... .....-..C. ....C.....  [320]
C._h._pretoriae_3_Gential_tract_ ....?..... ........-. .TA.T..... .?........ .......... .......... .....-.... ....?.....  [320]
C._h._pretoriae_3_Gential_tract_ ....?..... ........-. .TA.T..... .......... .......... .?........ .....-.... ..........  [320]
C._h._pretoriae_3_Caecum         .......... ........-. .?A.T..... .?........ ........?. .......... .....-.... ..........  [320]
C._damarensis_353_Faecal         ....?..... .....T..-. .AA.T..... .......... .......... .......... .....-.... ..........  [320]
Aerococcus_viridans_(AY707779)   .AT.A..... ........-. .TA.T..... .......T.A .......... .......... .....-.... ..........  [320]
Aerococcus_sp._LV65.(AF076639)   .AT.A..... ........-. .TA.T..... .......T.A .......... .......... .....A.... ..........  [320]
Aerococcus_viridans_88B_(AY70777 .AT.A..... ........-. .TA.T..... .......T.A .......... .......... .....-.... ..........  [320]
Bacillus_megaterium_KL-197_(AY03 .......... ........-. .TA.T..... .......... .......... .......... .....-.... ..........  [320]
Leuconostoc_pseudomesenteroides_ .AT.G..... ........-. .CA.A..... ......GC.A .......... .......... .....-..C. ....C.....  [320]
Leuconostoc_sp._CR-310S_(AF34993 .AT.G..... ........-. .CA.A..... ......GC.A .......... .......... .....-..C. ....C.....  [320]
Leuconostoc_inhae_IH101_(AY11768 .AT.G..... ........-. .CA.A..... ......GC.A .......... .......... .....-..C. ....C.....  [320]
Paenibacillus_xylanilyticus_(AY4 .......... .......... .C........ .......... .......... .......... .....-.... ..........  [320]
Paenibacillus_sp._HC1_(AB198337) .......... .......... .C........ .......... .......... .......... .....-.... ..........  [320]
Paenibacillus_sp._SAFN-068_(DQ12 .......... ........T. .C........ ...?...... .......... .......... .....-.... ..........  [320]
Bacillus_cereus_CFR04_(AY907828) .......... .....T..-. .AA.T..... .......... .......... .......... .....-.... ..........  [320]
Bacillus_pumilus_OS-70_(AM237370 .......... ........-. .TA.T..... .......... .......... .......... .....-.... ..........  [320]
```

```
Staphylococcus_sp._XJU-1_(DQ8375 .A.G...... .....T..-. .AA.T..... ......G.T. .......... .......... .....-.... ..........  [320]
Staphylococcus_sp._LAMI_001_(DQ8 .A.G...... .....T..-. .AA.T..... ......G.T. .......... .......... .....-.... ..........  [320]

C._damarensis_391_Urine_2        CAAATCATCA TGCCCCTTAT GACCTGGGCT ACACACGTAC TACAATGGCC GGTACAACGG G?TGTGAAGC CGCGAGGTGG  [400]
C._damarensis_391_Urine_3        .......... .......... .......... .......... .......... .......... .C........ ..........  [400]
C._damarensis_351_Urino-genital  ..G....... .......... .......... ........G. .........G TA.......A .T..CC..C. .......GT.  [400]
C._h._pretoriae_3_Gential_tract_ .......... .......... .......... ........G? ........AT .......A.. .C..CA.GA. ........CA  [400]
C._h._pretoriae_3_Gential_tract_ .......... .......... ...T...... ........G. ........A. A.A....A.. .C..C..GA. ...A....TT  [400]
C._h._pretoriae_3_Caecum         .......... .......... .......... ........G? ........AT .......A.. .C..CA.GA. ........CA  [400]
C._damarensis_353_Faecal         .......... .......... .......... ........G. ........A. .......A.A .C..CA.GA. ..........  [400]
Aerococcus_viridans_(AY707779)   ......G... .......... ...T...... ........G. ........AT .........A .TC.CA..C. .......GCA  [400]
Aerococcus_sp._LV65.(AF076639)   ......G... .......... ...T...... ........G. ........AT .........A .TC.CA..C. .......GYA  [400]
Aerococcus_viridans_88B_(AY70777 ......G... .......... ...T...... ........G. ........AT .........A .TC.CA..C. .......GCA  [400]
Bacillus_megaterium_KL-197_(AY03 .......... .......... .......... ........G. ........AT .......A.. .C..CA.GA. ........CA  [400]
Leuconostoc_pseudomesenteroides_ ..G....... .......... .......... ........G. .........G TA.......A .T..CC..C. .......GT.  [400]
Leuconostoc_sp._CR-310S_(AF34993 ..G....... .......... .......... ........G. .........G TA.......A .T..CC..C. ..........  [400]
Leuconostoc_inhae_IH101_(AY11768 ..G....... .......... .......... ........G. .........G TA.......A .T..CC..C. T.T..A.GT.  [400]
Paenibacillus_xylanilyticus_(AY4 .......... .......... .......... .......... .......... .......... .C........ ..........  [400]
Paenibacillus_sp._HC1_(AB198337) .......... .......... .......... .......... .......... .......... .CA....... ..........  [400]
Paenibacillus_sp._SAFN-068_(DQ12 .......... .......... .......... .......... .......... .......... .C........ ..........  [400]
Bacillus_cereus_CFR04_(AY907828) .......... .......... .......... ........G. ........A. .......A.A .C..CA.GA. ..........  [400]
Bacillus_pumilus_OS-70_(AM237370 .......... .......... .......... ........G. ........A. A.A....A.. .C..C..GA. ...A....TT  [400]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......... ..TT...... ........G. ........AT AA.....A.. .CA.C...T. .......CCA  [400]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......... ..TT...... ........G. ........A. AA.....A.. .CA.CT..A. ........CA  [400]

C._damarensis_391_Urine_2        AACGAATCTC TAAAAAGCCG GT?TCAGTTC GGATT?CAGG CTGCAA?TCG CCTGCATGAA GTCGGAATTG ?TAGTAATCG  [480]
C._damarensis_391_Urine_3        .......... .......... ..C....... .....G.... ......C... .......... .......... C.........  [480]
C._damarensis_351_Urino-genital  .G.T....-T CTT....TAC ..C....... .....GT..T ......C... A..A...... ........C. C.........  [480]
C._h._pretoriae_3_Gential_tract_ .G.C....-. C.T...A..A T.C....... .....GT... ......C... ...A...... .CT.....C. C.........  [480]
C._h._pretoriae_3_Gential_tract_ .G.C....-. C.T...T.T. T.C....... ....CG...T ?.....C... AA...G.... .C?.....C. C.?.......  [480]
C._h._pretoriae_3_Caecum         .G.C....-. C.T...A..A T.C....... .....GT... ......C.?. ...A...... .C?.....C. C.?.......  [480]
C._damarensis_353_Faecal         .G.T....-T C.T...A... T.C....... .....GT... ......C... ...A...... .C?.....C. C.........  [480]
Aerococcus_viridans_(AY707779)   .G.A....-T CTT......A T.C....... .....G.... ......C... .......... .C......C. C.........  [480]
Aerococcus_sp._LV65.(AF076639)   .G.A....-T CTT......A T.C....... .....G.... ......C... .......... .C......C. C.........  [480]
Aerococcus_viridans_88B_(AY70777 .G.A....-T CTT......A T.C....... .....G.... ......C... .......... .C......C. C.........  [480]
Bacillus_megaterium_KL-197_(AY03 .G.C....-. C.T...A..A T.C....... .....GT... ......C... ...A...... .CT.....C. C.........  [480]
Leuconostoc_pseudomesenteroides_ .G.T....-T CTT....TAC ..C....... .....GT..T ......C... A..A...... ........C. C.........  [480]
Leuconostoc_sp._CR-310S_(AF34993 .G.T....-T CTT....TAC ..C....... .....GT..T ......C... A..A...... ........C. C.........  [480]
Leuconostoc_inhae_IH101_(AY11768 .G.T....-T CTT....TAC ..C....... ...C.G...T ......C... A.....C... ........C. C.........  [480]
Paenibacillus_xylanilyticus_(AY4 ........-. .......... ..C....... .....G.... ......C... .......... .......... C.........  [480]
Paenibacillus_sp._HC1_(AB198337) ........-. .......... ..C....... .....G.... ......C... .......... .......... C.........  [480]
Paenibacillus_sp._SAFN-068_(DQ12 ..?.....-. .......... ..?....... .....G.... ......C... .......... .......... C.........  [480]
Bacillus_cereus_CFR04_(AY907828) .G.T....-T C.T...A... T.C....... .....GT... ......C... ...A...... .CT.....C. C.........  [480]
Bacillus_pumilus_OS-70_(AM237370 .G.C....-. C.T...T.T. T.C....... ....CG...T ......C... A....G.... .CT.....C. C.........  [480]
Staphylococcus_sp._XJU-1_(DQ8375 .G.A....-. C.T...ATTA T.C....... .....GT..T ......C... A..A...... .CT.....C. C.........  [480]
Staphylococcus_sp._LAMI_001_(DQ8 TG.A....-. C.T....TT. T.C....... .....GT..T ......C... A..A...... .CT.....C. C.........  [480]
```

```
C._damarensis_391_Urine_2        CGGATCAGCA TGCCGCGG?G AATACGTTCC CGGGTCTTGT ACACACCGCC CGTCACACCA CGAGAGTTTA TAACACCCGA  [560]
C._damarensis_391_Urine_3        .......... ........T. .......... .......... .......... .......... .......... ..........  [560]
C._damarensis_351_Urino-genital  .......... C.......T. .......... .......... .......... .......... T.G......G ...TG...A.  [560]
C._h._pretoriae_3_Gential_tract_ .......... ........T. .......... ....C..... .......... .......... .........G ..........  [560]
C._h._pretoriae_3_Gential_tract_ .......... ........T. .......... ....C..... .......... .......... .........G ..........  [560]
C._h._pretoriae_3_Caecum         .......... ........T. .......... ....C..... .......... .......... .........G ..........  [560]
C._damarensis_353_Faecal         .......... ........T. .......... ....C..... .......... .......... .........G ..........  [560]
Aerococcus_viridans_(AY707779)   T......... C...A...T. .......... .......... .......... .......... .........G ..........  [560]
Aerococcus_sp._LV65.(AF076639)   T......... C...A...T. .......... .......... .......... .......... .........G ..........  [560]
Aerococcus_viridans_88B_(AY70777 T......... C...A...T. .......... .......... .......... .......... .........G ..........  [560]
Bacillus_megaterium_KL-197_(AY03 .......... ........T. .......... ....C..... .......... .......... .........G ..........  [560]
Leuconostoc_pseudomesenteroides_ .......... C.......T. .......... .......... .......... .......... T.G......G ...TG...A.  [560]
Leuconostoc_sp._CR-310S_(AF34993 .......... C.......T. .......... .......... .......... .......... A.G......G ...TG...A.  [560]
Leuconostoc_inhae_IH101_(AY11768 .......... C.......T. .......... .......... .......... .......... T.G......G ...TG...A.  [560]
Paenibacillus_xylanilyticus_(AY4 .......... ........T. .......... .......... .......... .......... .......... ..........  [560]
Paenibacillus_sp._HC1_(AB198337) .......... ........T. .......... .......... .......... .......... .......... ..........  [560]
Paenibacillus_sp._SAFN-068_(DQ12 .......... ........T. .......... .......... ?......... .......... .......... ..........  [560]
Bacillus_cereus_CFR04_(AY907828) .......... ........T. .......... ....C..... .......... .......... .........G ..........  [560]
Bacillus_pumilus_OS-70_(AM237370 .......... ........T. .......... ....C..... .......... .......... .........G ..........  [560]
Staphylococcus_sp._XJU-1_(DQ8375 TA........ ...TA...T. .......... .......... .......... .......... .........G ..........  [560]
Staphylococcus_sp._LAMI_001_(DQ8 TA........ ...TA...T. .......... .......... .......... .......... .........G ..........  [560]

C._damarensis_391_Urine_2        AGT [563]
C._damarensis_391_Urine_3        ... [563]
C._damarensis_351_Urino-genital  ..C [563]
C._h._pretoriae_3_Gential_tract_ ... [563]
C._h._pretoriae_3_Gential_tract_ ... [563]
C._h._pretoriae_3_Caecum         ... [563]
C._damarensis_353_Faecal         ... [563]
Aerococcus_viridans_(AY707779)   ... [563]
Aerococcus_sp._LV65.(AF076639)   .TC [563]
Aerococcus_viridans_88B_(AY70777 ... [563]
Bacillus_megaterium_KL-197_(AY03 ... [563]
Leuconostoc_pseudomesenteroides_ ..C [563]
Leuconostoc_sp._CR-310S_(AF34993 ..C [563]
Leuconostoc_inhae_IH101_(AY11768 ..C [563]
Paenibacillus_xylanilyticus_(AY4 ... [563]
Paenibacillus_sp._HC1_(AB198337) ... [563]
Paenibacillus_sp._SAFN-068_(DQ12 ... [563]
Bacillus_cereus_CFR04_(AY907828) ... [563]
Bacillus_pumilus_OS-70_(AM237370 ... [563]
Staphylococcus_sp._XJU-1_(DQ8375 ..C [563]
Staphylococcus_sp._LAMI_001_(DQ8 ..C [563]
```

Appendix IV

Nucleotide sequence alignment of dataset II of the Firmicutes created in MEGA v. 3.1 (Kumar et al. 2004). Dots indicate sites identical to that of reference sequence (*C. h. pretoriae* 5 Nose), where ? indicates missing data and – indicates gaps.

```
C ._h._pretoriae_5_Nose           TAACACGTGG GTAACCTGCC CATAAGACTG GGATAAC?CC ?GGAAACCGG GGCTAATACC GGATAACATT TTGAACYGCA  [ 80]
C._damarensis_2_Caecum            .......... .C........ TG........ .......TT. G........A A......... .....GG..C ..CTC.TT..  [ 80]
C._h._pretoriae_3_Mouth           .......... .......... TG........ .......T.. ?......... A......... .....GTTCC ......C...  [ 80]
Aerococcus_viridans_(AY707779)    ........AA .G..T..A.. T.....CGG. .......ATT C......G.. T......... .C....T..C ..CTT.C...  [ 80]
Aerococcus_sp._LV65.(AF076639)    ........AA .G..T..A.. T.....CGG. .......ATT C......G.. T......... .C....T..C ..CTT.C...  [ 80]
Aerococcus_viridans_88B_(AY70777 ........AA .G..T..A.. T.....CGG. .......ATT C......G.. T......... .C....T..C ..CTT.C...  [ 80]
Bacillus_megaterium_KL-197_(AY03 .......... .C........ TG........ .......TT. G........A A......... .....GG..C ..CTC.TT..  [ 80]
Leuconostoc_pseudomesenteroides_ .......... AC........ TCA.G.CTG. .......ATT T......A.A T......... .A....A.C. CA.TGTC...  [ 80]
Leuconostoc_sp._CR-310S_(AF34993 ..?.....C- ACCC...C.T TCA.G.CTG. .....C.ATT T......A.A T......C.. .A....A.A. .A.TGTC...  [ 80]
Leuconostoc_inhae_IH101_(AY11768 .......... A......A.. TCA.G.TTG. .......ATT T......A.A T......... .A..G.G.C. .CTTGTT...  [ 80]
Paenibacillus_xylanilyticus_(AY4 ........A. .C........ .TC...CT.. ...C...TA. C......G.T A......... .A...CTTGC ..CTTTC..C  [ 80]
Paenibacillus_sp._HC1_(AB198337) ........A. .C........ .TC...CT.. ...C...TA. C......G.T A......... .A...GTTG. ..TCTTC..C  [ 80]
Paenibacillus_sp._SAFN-068_(DQ12 ........A. .C........ .TC...TT.. ...C...TA. C......G.T A......... .A...GTTG. ..TCTTC..C  [ 80]
Bacillus_pumilus_OS-70_(AY907828 .......... .......... TG........ .......T.. G......... A......... .....GTTCC ......C...  [ 80]
Bacillus_cereus_CFR04_(AM237370) .......... .......... .......... .......T.. G......... .......... .......... ......C...  [ 80]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......A.. T......... .......T.. G......... .......... ......T... ......C...  [ 80]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......A.. T......... .......T.- G......... A......... .......... .G....C...  [ 80]

C._h._pretoriae_5_Nose           TGGTTCGAAA TTGAAAGGCG GCTTCGGCTG TCACTTATGG ATGGACCCGC GTCGCATTAG CTAGTTGGTG AGGTAACGGC  [160]
C._damarensis_2_Caecum           ...GAGATG. .......AT. .T.......A .......CA. ....G..... .GT....... .......... ..........  [160]
C._h._pretoriae_3_Mouth          ......A.GG A......A.. .T........ .......CA. .......... .G........ .......... G.....T...  [160]
Aerococcus_viridans_(AY707779)   ...AAGA.G. .......A.. ...CT.-... ........A. ...AC.TT.. .GT....... T......... G.........  [160]
Aerococcus_sp._LV65.(AF076639)   ...AAGA.G. .......A.. ...CT.-... ........A. ...AC.TT.. .GT....... T......... G.........  [160]
Aerococcus_viridans_88B_(AY70777 ...AAGA.G. .......A.. ...CT.-... ........A. ...AC.TT.. .GT....... T......... G.........  [160]
Bacillus_megaterium_KL-197_(AY03 ...GAGATG. .......AT. .T.......A .......CA. ....G..... .GT....... .......... ..........  [160]
Leuconostoc_pseudomesenteroides_ ..ACA.A..G ..A....... CT..G.--C. ....C..GA. .....T.... .GT....... T......... G.....A...  [160]
Leuconostoc_sp._CR-310S_(AF34993 ..ACA.A..G ..A....... CT.CG.--C. ....C..GA. .....T.... .GT....... T......... G.....A...  [160]
Leuconostoc_inhae_IH101_(AY11768 ..ACAG...G ..A....... CTACG.--C. ..G.C..GA. .....T.... .GT....... T......... G.....A...  [160]
Paenibacillus_xylanilyticus_(AY4 ..AAGGA.GC .G.....A.. .AGCAAT... ......GA.. ....G..T.. .G........ .......... ..........  [160]
Paenibacillus_sp._HC1_(AB198337) ..AAGA...C .G.....A.. .AGCAAT... ......GG.. ....G..T.. .G........ .......... G.........  [160]
Paenibacillus_sp._SAFN-068_(DQ12 ..AAGGA..C .G.....A.. .AGCAAT... ......GG.. ....G..T.. .G.......? .......... G..?......  [160]
Bacillus_pumilus_OS-70_(AY907828 ......A.GG A......A.. .T........ .......CA. .......... .G........ .......... G.....T...  [160]
Bacillus_cereus_CFR04_(AM237370) .......... .......... .......... .......... .......... .......... .......... ..........  [160]
Staphylococcus_sp._XJU-1_(DQ8375 ......A.T. G......A.. .T........ ........A. .......... .C..T..... .........A .....?....  [160]
Staphylococcus_sp._LAMI_001_(DQ8 ......T... G......AT. .T..T.-..A ........A. .......... .C..T..... .........A ..........  [160]

C._h._pretoriae_5_Nose           TCACCAAGGC AACGATGCGT AGCCGACCTG AGAGGGTGAT CGGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG  [240]
C._damarensis_2_Caecum           .......... ........A. .......... .......... .......... .......... .......... ..........  [240]
```

```
C._h._pretoriae_3_Mouth          .......... G......... .......... .......... .......... .......... .......... ..........  [240]
Aerococcus_viridans_(AY707779)   CT......A. G.T.....A. .......... .......... ........T. .......... .........A ..........  [240]
Aerococcus_sp._LV65.(AF076639)   CT......A. G.T.....A. .......... .......... ........T. .......... .........A ..........  [240]
Aerococcus_viridans_88B_(AY70777 CT......A. G.T.....A. .......... .......... ........T. .......... .........A ..........  [240]
Bacillus_megaterium_KL-197_(AY03 .......... ........A. .......... .......... .......... .......... .......... ..........  [240]
Leuconostoc_pseudomesenteroides_ CT......A. ..T.....A. ......GT.. ....AC.... ........T. .......... .........A ..........  [240]
Leuconostoc_sp._CR-310S_(AF34993 CT......A. ..T.....A. ......GT.. ....AC.... ........T. .......... .........A ....-.....  [240]
Leuconostoc_inhae_IH101_(AY11768 CT......A. ..T.....A. ......GT.. ....AC.... ........T. .......... .........A ..........  [240]
Paenibacillus_xylanilyticus_(AY4 .......... G......... .......... .......... .......... .......... .......... ..........  [240]
Paenibacillus_sp._HC1_(AB198337) .......... G......... .......... .......... .......... .......... .......... ..........  [240]
Paenibacillus_sp._SAFN-068_(DQ12 .......... G......... .......... .......... .......... .......... .........? ..........  [240]
Bacillus_pumilus_OS-70_(AY907828 .......... G......... .......... .......... .......... .......... .......... ..........  [240]
Bacillus_cereus_CFR04_(AM237370) .......... .......... .......... .......... .......... .......... .......... ..........  [240]
Staphylococcus_sp._XJU-1_(DQ8375 .T........ G.....A... .......... .......... .......... ..A....... .....T.... ..........  [240]
Staphylococcus_sp._LAMI_001_(DQ8 .T........ G.....A... .......... .......... .......... ..A....... .....T.... ..........  [240]

C._h._pretoriae_5_Nose           GAGGCAGCAG TAGGGAATCT TCCGCAATGG ACGAAAGTCT GACGGAGCAA CGCCGCGTGA GTGATGAAGG CTTTCGGGTC  [320]
C._damarensis_2_Caecum           .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._h._pretoriae_3_Mouth          .......... .......... .......... .......... .......... .......... .......... T......A..  [320]
Aerococcus_viridans_(AY707779)   .......... .......... .......... G......C.. .......... T......... ....A..... .C........  [320]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... G......C.. .......... T......... ....A..... .C........  [320]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... G......C.. .......... T......... ....A..... .C........  [320]
Bacillus_megaterium_KL-197_(AY03 .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Leuconostoc_pseudomesenteroides_ .......... .......... ...A...... G......C.. ..T....... .........T .......... ..........  [320]
Leuconostoc_sp._CR-310S_(AF34993 .....T.... .......... ...A...... G....G.C.. ..T....... .........T .......... ..........  [320]
Leuconostoc_inhae_IH101_(AY11768 .....T.... .......... ...A...... G......C.. ..T....... .........T .......... ..........  [320]
Paenibacillus_xylanilyticus_(AY4 .......... .......... .......... G......C.. .......... T......... .......... T......A..  [320]
Paenibacillus_sp._HC1_(AB198337) .......... .......... .......... G......C.. .......... T......... .......... T......A..  [320]
Paenibacillus_sp._SAFN-068_(DQ12 .......... .......... .......?.. ?......C.. ........G. T......... .......... T......A..  [320]
Bacillus_pumilus_OS-70_(AY907828 .......... .......... .......... .......... .......... .......... .......... T......A..  [320]
Bacillus_cereus_CFR04_(AM237370) .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......... .......... G......C.. .......... .......... .......... TC.....A..  [320]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......... .......... G......C.. .......... .......... .......... G......C..  [320]

C._h._pretoriae_5_Nose           GTA????A?T ?TTGTTAGGG AAGAACAAGT GC-TAGTTGA ATAA?C?G-C ACCTTGACGG TACCTAA-CC AGAAAGCCAC  [400]
C._damarensis_2_Caecum           ...AAACTC. G......... .......... A.AAGAG.A. C.GCTTGT-- .......... .......-.. ..........  [400]
C._h._pretoriae_3_Mouth          ...AA??T?. G......... .......... ..GAGAG.A. C.GCY.SC-- M......... .......-.. ..........  [400]
Aerococcus_viridans_(AY707779)   ...AAACTC. G..A.A..A. ........A. TGTAGAG.A. C.GCTACA-- GT........ ..T..T.-T. ..........  [400]
Aerococcus_sp._LV65.(AF076639)   ...AAACTC. G..A.A..A. ........A. TGTAGAG.A. C.GCTACA-- GT........ ..T..T.-T. ..........  [400]
Aerococcus_viridans_88B_(AY70777 ...AAACTC. G..A.A..A. ........A. TGTAGAG.A. C.GCTACA-- GT........ ..T..T.-T. ..........  [400]
Bacillus_megaterium_KL-197_(AY03 ...AAACTC. G......... .......... A.AAGAG.A. C.GCTTGT-- .......... .......-.. ..........  [400]
Leuconostoc_pseudomesenteroides_ ...AAGC.C. G....AT... .......GC. AGAATAGG.. ..G.TTTT-- .GT....... ....AT.-.. ......GG..  [400]
Leuconostoc_sp._CR-310S_(AF34993 ...AAGC.C. G....AT... .......GC. AGAATAGGA. ..G.TTTT-- .GT....... ....AT.-.. ......GG..  [400]
Leuconostoc_inhae_IH101_(AY11768 ...AAGC.C. G....AT... .......GC. AGAGTAGG.. ..G.CTTT-- .GT....... ....AT.-.. ......GG..  [400]
Paenibacillus_xylanilyticus_(AY4 ...AAGCTC. G...CC.... ......GTCC TTGAGAG.A. C.GCT.AA-- GGAG...... .....G.-GA ........C.  [400]
Paenibacillus_sp._HC1_(AB198337) ...AAGCTC. G...CC.... ......GCT. .GGAGAG.A. C.GCT.CC-- .AGG...... .....G.-GA ........C.  [400]
Paenibacillus_sp._SAFN-068_(DQ12 ...AAGCTC. G...CC.... ......GCT. .GGAGAG.A. C.GCT.TC-- .AGG...... .....G?AGA ........C.  [400]
```

```
Bacillus_pumilus_OS-70_(AY907828 ...AAGCTC. G......... .......... ..GAGAG.A. C.GCT.GC-- .......... .......-.. ..........  [400]
Bacillus_cereus_CFR04_(AM237370) ...AAACTC. G......... .......... ..-....... ....G.T.G. .......... .......-.. ..........  [400]
Staphylococcus_sp._XJU-1_(DQ8375 ...AAACTC. G......... ........A. TTG.TAG.A. C.G.A.AA-- GT........ .......-.. ..........  [400]
Staphylococcus_sp._LAMI_001_(DQ8 ...AAACTC. G..A...... ........AC .TG..AG.A. C.GTG.AC-- GT........ .......-T. ..........  [400]

C._h._pretoriae_5_Nose           GGCTAACTAC GTGCCAGCAG CCGCGGTAAT ACGTAGGTGG C-AAGCGTTA TCCGGAATTA TTGGGCGTAA AGCGCGCGCA  [480]
C._damarensis_2_Caecum           .......... .......... .......... .......... .-........ .......... .......... ..........  [480]
C._h._pretoriae_3_Mouth          .......... .......... .......... .......... .-.......G .......... .......... ..G..T....  [480]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... .-.......G ......T... .......... ..G.A.....  [480]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... .-.......G ......T... .......... ..G.A.....  [480]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... .-.......G ......T... .......... ..G.A.....  [480]
Bacillus_megaterium_KL-197_(AY03 .......... .......... .......... .......... .-........ .......... .......... ..........  [480]
Leuconostoc_pseudomesenteroides_ ......A... .......... .......... .....T..CC .-G....... ......T... .......... ....A.....  [480]
Leuconostoc_sp._CR-310S_(AF34993 ......A... .......... .......... .....T..CC .-G....... ......T... .......... ....A.....  [480]
Leuconostoc_inhae_IH101_(AY11768 ......A... .......... .......... .....T..CC .-G....... ......T... .......... ....A.....  [480]
Paenibacillus_xylanilyticus_(AY4 .......... .......... .......... .......G.. .-.......G .......... .......... ..........  [480]
Paenibacillus_sp._HC1_(AB198337) .......... .......... .......... .......G.. .-.......G .......... .......... ..........  [480]
Paenibacillus_sp._SAFN-068_(DQ12 .......... .......... ...?.?.... .......G.. ?C.......G ......W... .......... ..........  [480]
Bacillus_pumilus_OS-70_(AY907828 .......... .......... .......... .......... .-.......G .......... .......... ..G..T....  [480]
Bacillus_cereus_CFR04_(AM237370) .......... .......... .......... .......... .-........ .......... .......... ..........  [480]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......... .......... .......... .-........ .......... .......... ........T.  [480]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......... .......... .......... .-........ .......... .......... ........T.  [480]

C._h._pretoriae_5_Nose           GGTGGTTTCT TAAGTCTGAT GTGAAAGCCC ACGGCTCAAC CGTGGAGGGT CATTGGAAAC TGGGAGACTT GAGTGCAGAA  [560]
C._damarensis_2_Caecum           ..C....... .......... .......... .......... .......... .......... ....GA.... ..........  [560]
C._h._pretoriae_3_Mouth          ..C....... .......... .......... C......... ..G....... .......... .....A.... ..........  [560]
Aerococcus_viridans_(AY707779)   .......... .......... .......... ......T... .......... .......... .....A.... ....A.....  [560]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... ......T... .......... .......... .....A.... ....A.....  [560]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... ......T... .......... .......... .....A.... ....A.....  [560]
Bacillus_megaterium_KL-197_(AY03 ..C....... .......... .......... .......... .......... .......... ....GA.... ..........  [560]
Leuconostoc_pseudomesenteroides_ .AC....GA. .......... .......... GGA....... TCC...AT.G .......... ...TTA.... ........T.  [560]
Leuconostoc_sp._CR-310S_(AF34993 .AC.....A. .......... .......... GGA....... TCC...AT.G .......... ...TTA.... ........T.  [560]
Leuconostoc_inhae_IH101_(AY11768 .AC....GA. .......... .......... GGA....... TCC...AA.G .......... ...TCA.... ........T.  [560]
Paenibacillus_xylanilyticus_(AY4 ..C...CAT. ........G. ..TT..T... GG........ .CC...TC.- ..C....... ....T..... ..........  [560]
Paenibacillus_sp._HC1_(AB198337) ..C...CAT. ........G. ..TT..T... GG........ .CC...TC.- ..C....... ....T..... ..........  [560]
Paenibacillus_sp._SAFN-068_(DQ12 S.C...CAT. ........G. ..TT..T... GG........ .CC...TC.- ..C....... ....T...YY ..........  [560]
Bacillus_pumilus_OS-70_(AY907828 ..C....... .......... .......... C......... ..G....... .......... .....A.... ..........  [560]
Bacillus_cereus_CFR04_(AM237370) .......... .......... .......... .......... .......... .......... .......... ..........  [560]
Staphylococcus_sp._XJU-1_(DQ8375 ..C....... .......... .......... .......... .......... .......... .....A.... ..........  [560]
Staphylococcus_sp._LAMI_001_(DQ8 ..C....... .......... .......... .......... .......... .......... .....A.... ..........  [560]

C._h._pretoriae_5_Nose           GAGGAAAGTG GAATTCCATG TGTAGCGGTG AAATGCGTAG AGATATGGAG GAACACCA?T GGCGAA?GCG ACTTT-CTGG  [640]
C._damarensis_2_Caecum           ...A....C. ........C. .......... .......... ....G..... ........G. ......G... G....-T...  [640]
C._h._pretoriae_3_Mouth          .....G.... ........C. .......... .......... ....G..... ........G. ......G... ...C.-....  [640]
Aerococcus_viridans_(AY707779)   ......T... ...C...... .......... G......... .T.......A ........G. ......G... ..A..-....  [640]
Aerococcus_sp._LV65.(AF076639)   ......T... ...C...... .......... G......... .T.......A ........G. ......G... ..A..-....  [640]
Aerococcus_viridans_88B_(AY70777 ......T... ...C...... .......... G......... .T.......A ........G. ......G... ..A..-....  [640]
```

```
Bacillus_megaterium_KL-197_(AY03 ...A....C. ........C. .......... .......... ....G..... ........G. ......G... G....-T...  [640]
Leuconostoc_pseudomesenteroides_ ....T..... ...C...... .......... G......... .T.......A ........G- ......G... G...A-....  [640]
Leuconostoc_sp._CR-310S_(AF34993 ....T..... ...C...... .......... G......... .T.......A ........G. ......G... G...AA....  [640]
Leuconostoc_inhae_IH101_(AY11768 ....T..... ...C...... .......... G......... .T.......A ........GC ......G... G...A-....  [640]
Paenibacillus_xylanilyticus_(AY4 .....G.... ........C. .......... .......... .T..G..... ........G. ......G... ...C.-....  [640]
Paenibacillus_sp._HC1_(AB198337) .....G.... ........C. .......... .......... .T..G..... ........G. ......G... ...C.-....  [640]
Paenibacillus_sp._SAFN-068_(DQ12 .....G.... .R......C. Y.Y....... .....?...? .T..G....R .M......G. ..Y...R... ...C.-..S.  [640]
Bacillus_pumilus_OS-70_(AY907828 .....G.... ........C. .......... .......... ....G..... ........G. ......G... ...C.-....  [640]
Bacillus_cereus_CFR04_(AM237370) .......... .......... .......... .....----- --------.. ........G. ......G... .....-....  [640]
Staphylococcus_sp._XJU-1_(DQ8375 .....G.... .......... .......... .......C.. .......... ........G. ......G... G..C.-....  [640]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......... .......... .......C.. .......... ........G. ......G... .....-....  [640]

C._h._pretoriae_5_Nose           TCTGT?ACTG ACACTGAGGC GCGAAAGCGT GGGGAGCAAA CA?GATTAGA TACCCTGGTA GTCCACG?CG TAAACGATGA  [720]
C._damarensis_2_Caecum           .....A.... ..G....... .......... .......... ..G....... .......... .......C.. ..........  [720]
C._h._pretoriae_3_Mouth          .....A.... ..G......A .......... .......G.. ..?....... .......... ......?C.. ..........  [720]
Aerococcus_viridans_(AY707779)   .....T.... .......... T......... .......... ..G....... .......... .......C.. ..........  [720]
Aerococcus_sp._LV65.(AF076639)   .....T.... .......... T......... .......... ..G....... .......... .......C.. ..........  [720]
Aerococcus_viridans_88B_(AY70777 .....T.... .......... T......... .......... ..G....... .......... .......C.. ..........  [720]
Bacillus_megaterium_KL-197_(AY03 .....A.... ..G....... .......... .......... ..G....... .......... .......C.. ..........  [720]
Leuconostoc_pseudomesenteroides_ A...-A.... ..GT-..... T......T.. ...T...... .--....... .......... .------C.. ...C--....  [720]
Leuconostoc_sp._CR-310S_(AF34993 A...CA.... ..GT...... T......T.. ...T...... ..G....... .......... ......AC.. ..........  [720]
Leuconostoc_inhae_IH101_(AY11768 A....A.... ..GT...... T......T.. ...T...... ..G....... .......... ......AC.. ..........  [720]
Paenibacillus_xylanilyticus_(AY4 G....A.... ..G....... .......... .......... ..G....... .......... .......C.. ..........  [720]
Paenibacillus_sp._HC1_(AB198337) G....A.... ..G....... .......... .......... ..G....... .......... .......C.. ..........  [720]
Paenibacillus_sp._SAFN-068_(DQ12 G...KA.... .MG....... .......... .......?.. ..G....... .......... .......C.. ...?......  [720]
Bacillus_pumilus_OS-70_(AY907828 .....A.... ..G......A .......... .......G.. ..G....... .......... .......C.. ..........  [720]
Bacillus_cereus_CFR04_(AM237370) .....A.... .......... .......... .......... ..G....... .......... .......C.. ..........  [720]
Staphylococcus_sp._XJU-1_(DQ8375 .....A.... ..G....T.T .......... .....T.... ..G....... .......... .......C.. ..........  [720]
Staphylococcus_sp._LAMI_001_(DQ8 .....A.... ..G....T.T .......... .....T.... ..G....... .......... .......C.. ..........  [720]

C._h._pretoriae_5_Nose           GTGCT?AGTG TTAGAGGGT? TCCGCCCTTT AGTGCTGAAG TT?ACGCATT AA [772]
C._damarensis_2_Caecum           .....A.... .........T .......... .......C.. C.A....... .. [772]
C._h._pretoriae_3_Mouth          ....?A.... ....G....T .......C.. ..?....C.. C.?....... .. [772]
Aerococcus_viridans_(AY707779)   .....AG... ..G......T .........C .....C.C.. ..A....... .. [772]
Aerococcus_sp._LV65.(AF076639)   .....AG... ..G......T .........C .....C.C.. ..A....... .. [772]
Aerococcus_viridans_88B_(AY70777 .....AG... ..G......T .........C .....C.C.. ..A....... .. [772]
Bacillus_megaterium_KL-197_(AY03 .....A.... .........T .......... .......C.. C.A....... .. [772]
Leuconostoc_pseudomesenteroides_ ACA..AG... ....GA...T ......TC.. .....C.... C.A....... .. [772]
Leuconostoc_sp._CR-310S_(AF34993 ACA..AG... ....GA...T ......TC.. .....C.... C.A....... .. [772]
Leuconostoc_inhae_IH101_(AY11768 ACA..AG... ....GA...T ......TC.. .....C...C G.A....... .. [772]
Paenibacillus_xylanilyticus_(AY4 .....AG... ....G..T.T CGATA..C.. G....C.... ..A..A.... .. [772]
Paenibacillus_sp._HC1_(AB198337) .....AG... ....G..T.T CGATA..C.. G....C.... ..A..A.... .. [772]
Paenibacillus_sp._SAFN-068_(DQ12 ...G.AG... ....G..T.T CGATA..C.. G....C.... ..A..A.... .. [772]
Bacillus_pumilus_OS-70_(AY907828 .....A.... ....G....T .......C.. .......C.. C.A....... .. [772]
Bacillus_cereus_CFR04_(AM237370) .....A.... .........T .......... .......... ..A....... .. [772]
Staphylococcus_sp._XJU-1_(DQ8375 .....A.... ....G....T .......C.. .......C.. C.A....... .. [772]
Staphylococcus_sp._LAMI_001_(DQ8 .....A.... ....G....T .......C.. .......C.. C.A....... .. [772]
```

Appendix V

Nucleotide sequence alignment of dataset III of the Firmicutes created in MEGA v. 3.1 (Kumar et al. 2004). Dots indicate sites identical to that of reference sequence (*G. capensis* 1 Urino-genital), where ? indicates missing data and – indicates gaps.

```
G._capensis_1_Urino-genital      AGATGTTGGG TTAAGTCCCG CAACGAGCGC AACCCTTAAA CTTAGTTGCC AGCA-TTTAG TTGGGCACTC TAGGTTGACT  [ 80]
C._damarensis_391_Urine_1        .......... .......... .......... .........G .......... .T..-..A.. .......... ..........  [ 80]
C._h._pretoriae_3_Genital_tract_ .......... .......... .......... .........G .......... .T..-..A.. .......... ..........  [ 80]
C._h._pretoriae_3_Genital_tract_ .......... .......... .......... .........G .......... .T..-..A.. .......... ..........  [ 80]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .....C..TT A......... ....-..... .......... ..ATGA....  [ 80]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .....C..TT A......... ....-..... .......... ..ATGA....  [ 80]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .....C..TT A......... ....-..... .......... ..ATGA....  [ 80]
Bacillus_megaterium_KL-197_(AY03 .......... .......... .......... .......G.T .......... ....-..... .......... ..A.G.....  [ 80]
Leuconostoc_pseudomesenteroides_ .......... .......... .......... ........TT G......... ....-..C.. A......... ...CGA....  [ 80]
Leuconostoc_sp._CR-310S_(AF34993 .......... .......... .......... ........TT G......... ....-..C.. A......... ...CGA....  [ 80]
Leuconostoc_inhae_IH101_(AY11768 .......... .......... .......... ........TT G......... ....-..C.. A......... ...CGA....  [ 80]
Paenibacillus_xylanilyticus_(AY4 .......... .......... .......... .......G.T .......... ....C..CG. G......... ..A.G.....  [ 80]
Paenibacillus_sp._HC1_(AB198337) .......... .......... .......... .......G.T .......... ....C..CG. G......... ..A.G.....  [ 80]
Paenibacillus_sp._SAFN-068_(DQ12 .......... .......... .......... .......G.T .......... ....T..CG. G........? ..A.G.....  [ 80]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......... .......... .........G .......... .T..-..A.. .......... ..........  [ 80]
Bacillus_pumilus_OS-70_(AM237370 .......... .......... .......... .......G.T .......... ....-..... .......... ..A.G.....  [ 80]
Bacillus_cereus_CFR04_(AY907828) .......... .......... .......... .......G.T .......... .T..-..A.. .......... ..A.G.....  [ 80]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......... .......... .........G .......... .T..-..A.. .......... ..........  [ 80]

G._capensis_1_Urino-genital      GCCGGTGACA AACCGGAGGA A-GGTGGGGA TGACGTCAAA TCATCATGCC CCTTATGATT TGGGCTACAC ACGTGCTACA  [160]
C._damarensis_391_Urine_1        .......... .......... .-........ .......... .......... .......... .......... ..........  [160]
C._h._pretoriae_3_Genital_tract_ .......... .......... .-........ .......... .......... .......... .......... ..........  [160]
C._h._pretoriae_3_Genital_tract_ .......... .......... .-........ .......... .......... .......... .......... ..........  [160]
Aerococcus_viridans_(AY707779)   .......... .......... .-........ .......... ...G...... ........C. .......... ..........  [160]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .A........ .......... ...G...... ........C. .......... ..........  [160]
Aerococcus_viridans_88B_(AY70777 .......... .......... .-........ .......... ...G...... ........C. .......... ..........  [160]
Bacillus_megaterium_KL-197_(AY03 .......... .......... .-........ .......... .......... ........CC .......... ..........  [160]
Leuconostoc_pseudomesenteroides_ .......... .......... .-..C..... C.......G. .......... ........CC .......... ..........  [160]
Leuconostoc_sp._CR-310S_(AF34993 .......... .......... .-..C..... C.......G. .......... ........CC .......... ..........  [160]
Leuconostoc_inhae_IH101_(AY11768 .......... .......... .-..C..... C.......G. .......... ........CC .......... ..........  [160]
Paenibacillus_xylanilyticus_(AY4 .......... .......... .-........ .......... .......... ........CC .......... ....A.....  [160]
Paenibacillus_sp._HC1_(AB198337) .......... .......... .-........ .......... .......... ........CC .......... ....A.....  [160]
Paenibacillus_sp._SAFN-068_(DQ12 .......... .......... .-........ .......... .......... ........CC .......... ....A.....  [160]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......... .-........ .......... .......... .......... .......... ..........  [160]
Bacillus_pumilus_OS-70_(AM237370 .......... .......... .-........ .......... .......... ........CC .......... ..........  [160]
Bacillus_cereus_CFR04_(AY907828) .......... .......... .-........ .......... .......... ........CC .......... ..........  [160]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......... .-........ .......... .......... .......... .......... ..........  [160]
```

```
G._capensis_1_Urino-genital       ATGGACAATA CAAAGGGCAG CTAAACCGCG AGGTCATGCA AATCCCATAA AGTTGTTCTC AGTTCGGATT GTAGTCTGCA  [240]
C._damarensis_391_Urine_1         .....T.... .......... ?G..T..... ...C..A... .......... .A..A..... .......... ..........  [240]
C._h._pretoriae_3_Genital_tract_  .......... .......... .......... .......... .......... .......... .......... ..........  [240]
C._h._pretoriae_3_Genital_tract_  .......... .......... .......... .......... .......... .......... .......... ..........  [240]
Aerococcus_viridans_(AY707779)    .....TGG.. ...C.A.TC. .A..C..... ...G..A... ....T.T... ..CCA..... .......... .C..G.....  [240]
Aerococcus_sp._LV65.(AF076639)    .....TGG.. ...C.A.TC. .A..C..... ...GY.A... ....T.T... ..CCA..... .......... .C..G.....  [240]
Aerococcus_viridans_88B_(AY70777  .....TGG.. ...C.A.TC. .A..C..... ...G..A... ....T.T... ..CCA..... .......... .C..G.....  [240]
Bacillus_megaterium_KL-197_(AY03  .....TGG.. ........T. .A.G...... ......A..C .......... .ACCA..... .......... ....G.....  [240]
Leuconostoc_pseudomesenteroides_  ....CGT... ...C.A.TT. .C..C..... ...GTGA..T ....T.T... ...ACG.... .......... ..........  [240]
Leuconostoc_sp._CR-310S_(AF34993  ....CGT... ...C.A.TT. .C..C..... ....GGA..T ....T.T... ...ACG.... .......... ..........  [240]
Leuconostoc_inhae_IH101_(AY11768  ....CGT... ...C.A.TT. .C..C.T.T. .A.GTGA..T ....T.T... ...ACG.... ........C. .C........  [240]
Paenibacillus_xylanilyticus_(AY4  ....C.GG.. ...C....T. TG..G..... ....GGAA.G .....T.A.. ..CC.G.... .......... .C..G.....  [240]
Paenibacillus_sp._HC1_(AB198337)  ....C.GG.. ...C...... TG..G..... ....GGAA.G .....T.A.. ..CC.G.... .......... .C..G.....  [240]
Paenibacillus_sp._SAFN-068_(DQ12  ....C.GG.. ...C....T. TG..G..... ....GGAA?G .....T.A.. ..CC.G.?.. .......... .C..G.....  [240]
Staphylococcus_sp._XJU-1_(DQ8375  .....T.... .......... .G..T..... ...C..A... .......... .A..A..... .......... ..........  [240]
Bacillus_pumilus_OS-70_(AM237370  .......GA. ........T. .G.G.....A ....TTA..C .......... .TC....... .........C .C........  [240]
Bacillus_cereus_CFR04_(AY907828)  ......GG.. .....A..T. .A.G...... ....GGA..T ....T..... .ACC...... .......... ....G.....  [240]
Staphylococcus_sp._LAMI_001_(DQ8  .......... .......... .......... .......... .......... .......... .......... ..........  [240]

G._capensis_1_Urino-genital       ACTCGACTAC ATGAAGCTGG AATCGCTAGT AATCGTAGAT CAGCATGCTA CGGTGAATAC GTTCCCGGGT CTTGTACACA  [320]
C._damarensis_391_Urine_1         .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._h._pretoriae_3_Genital_tract_  .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._h._pretoriae_3_Genital_tract_  .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Aerococcus_viridans_(AY707779)    .....C..G. .......C.. .......... ......G... .....C..C. .......... .......... ..........  [320]
Aerococcus_sp._LV65.(AF076639)    .....C..G. .......C.. .......... ......G... .....C..C. .......... .......... ..........  [320]
Aerococcus_viridans_88B_(AY70777  .....C..G. .......C.. .......... ......G... .....C..C. .......... .......... ..........  [320]
Bacillus_megaterium_KL-197_(AY03  .....C.... .......... .......... .....CG... ........CG .......... .........C ..........  [320]
Leuconostoc_pseudomesenteroides_  .......... ......TC.. .......... .....CG... .....C..CG .......... .......... ..........  [320]
Leuconostoc_sp._CR-310S_(AF34993  .......... ......TC.. .......... .....CG... .....C..CG .......... .......... ..........  [320]
Leuconostoc_inhae_IH101_(AY11768  ........G. .C....TC.. .......... .....CG... .....C..CG .......... .......... ..........  [320]
Paenibacillus_xylanilyticus_(AY4  .....C..G. ......TC.. ...T...... .....CG... ........CG .......... .......... ..........  [320]
Paenibacillus_sp._HC1_(AB198337)  .....C..G. ......TC.. ...T...... .....CG... ........CG .......... .......... ..........  [320]
Paenibacillus_sp._SAFN-068_(DQ12  .....C..G. ......TC.. ...T...... .....CG... ........CG .......... .......... .....?....  [320]
Staphylococcus_sp._XJU-1_(DQ8375  .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Bacillus_pumilus_OS-70_(AM237370  ........G. G......... .......... .....CG... ........CG .......... .........C ..........  [320]
Bacillus_cereus_CFR04_(AY907828)  .....C.... .......... .......... .....CG... ........CG .......... .........C ..........  [320]
Staphylococcus_sp._LAMI_001_(DQ8  .......... .......... .......... .......... .......... .......... .......... ..........  [320]

G._capensis_1_Urino-genital       CCGCCCGTCA CACC?CGAGA GTTTGTAACA CCCGAAGC [358]
C._damarensis_391_Urine_1         .......... ....A..... .......... ........ [358]
C._h._pretoriae_3_Genital_tract_  .......... ....A..... .......... ........ [358]
C._h._pretoriae_3_Genital_tract_  .......... ....A..... .......... ........ [358]
Aerococcus_viridans_(AY707779)    .......... ....A..... .......... .......T [358]
Aerococcus_sp._LV65.(AF076639)    .......... ....A..... .......... ......T. [358]
Aerococcus_viridans_88B_(AY70777  .......... ....A..... .......... .......T [358]
Bacillus_megaterium_KL-197_(AY03  .......... ....A..... .......... .......T [358]
Leuconostoc_pseudomesenteroides_  .......... ....AT.G.. ........TG ...A.... [358]
```

```
Leuconostoc_sp._CR-310S_(AF34993 .......... ....AA.G.. ........TG ...A.... [358]
Leuconostoc_inhae_IH101_(AY11768 .......... ....AT.G.. ........TG ...A.... [358]
Paenibacillus_xylanilyticus_(AY4 .......... ....A..... ....A..... .......T [358]
Paenibacillus_sp._HC1_(AB198337) .......... ....A..... ....A..... .......T [358]
Paenibacillus_sp._SAFN-068_(DQ12 .......... ....A..... ....A..... .......T [358]
Staphylococcus_sp._XJU-1_(DQ8375 .......... ....A..... .......... ........ [358]
Bacillus_pumilus_OS-70_(AM237370 .......... ....A..... .......... .......T [358]
Bacillus_cereus_CFR04_(AY907828) .......... ....A..... .......... .......T [358]
Staphylococcus_sp._LAMI_001_(DQ8 .......... ....A..... .......... ........ [358]
```

Appendix VI

Nucleotide sequence alignment of dataset IV of the Firmicutes created in MEGA v. 3.1 (Kumar et al. 2004). Dots indicate sites identical to that of reference sequence (*C. damarensis* 1 Caecum), where ? indicates missing data and – indicates gaps.

```
C._damarensis_1_Caecum           AGCGGCGAAC GGGTGAGTAA CACGTAAGGA ATCTACCTAT AAGCGGGGGA TAACATTCGG AAACGGGTGC TAATACCGCA  [ 80]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... .......... .......... .......... ..........  [ 80]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... .......... .......... .......... ..........  [ 80]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... .......... .......... .......... ..........  [ 80]
Bacillus_megaterium_KL-197_(AY03 .......G.. .......... .....GG.C. .C..G...G. ...ACT.... ....T.CG.. ....C.AA.. ........G.  [ 80]
Leuconostoc_pseudomesenteroides_ ..T....... .......... .....GGAC. .C..G...CA .G..T..... .......T.. ....A.A... ........A.  [ 80]
Leuconostoc_sp._CR-310S_(AF34993 T.TT?.A... ..T.?T-..? .....C-ACC CC..C.T.CA .G..T..... ..C....T.. ....A.A... ....C...A.  [ 80]
Leuconostoc_inhae_IH101_(AY11768 ..T....... .......... .....GGAT. .C......CA .G.TT..... .......T.. ....A.A... ........A.  [ 80]
Paenibacillus_xylanilyticus_(AY4 .......G.. .......... ......G.C. .C..G..CTC ....TT.... C...TAC... ......TA.. ........A.  [ 80]
Paenibacillus_sp._HC1_(AB198337) .......G.. .......... ......G.C. .C..G..CTC ....TT.... C...TAC... ......TA.. ........A.  [ 80]
Paenibacillus_sp._SAFN-068_(DQ12 .......G.. .......... ......G.C. .C..G..CTC ...TTT.... C...TAC... ......TA.. ........A.  [ 80]
Bacillus_pumilus_OS-70_(AM237370 .......G.. .......... .....GG.T. .C..G...G. ...ACT.... ....TCCG.. ....C..A.. ........G.  [ 80]
Bacillus_cereus_CFR04_(AY907828) .......G.. .......... .....GG.T. .C..G..C.. ...ACT.... ....TCCG.. ....C..G.. ........G.  [ 80]
Staphylococcus_sp._XJU-1_(DQ8375 .......G.. .......... .....GG.T. .C........ ...ACT.... ....TCCG.. ....C..G.. ........G.  [ 80]
Staphylococcus_sp._LAMI_001_(DQ8 .......G.. .......... .....GG.T. .C........ ...ACT.... ....TC-G.. ....C..A.. ........G.  [ 80]

C._damarensis_1_Caecum           TAATATCTTC TTCCGCATGG AAGAAGATTG AAAGACGGCT CTG-CTGTCA CTTATAGATG ACCTTGCGGT GCATTAGTTA  [160]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... ...-...... .......... .......... ..........  [160]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... ...-...... .......... .......... ..........  [160]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... ...-...... .......... .......... ..........  [160]
Bacillus_megaterium_KL-197_(AY03 ..GG...... .C.TT..... G...T..... .....T..T. TC.G..A... ....C..... GG.CC..... .......C..  [160]
Leuconostoc_pseudomesenteroides_ ...A.CTCAG .GT......A C.C..AG..A ....G..CT. TG.--C.... .C..G..... GATCC..... ..........  [160]
Leuconostoc_sp._CR-310S_(AF34993 ...A.AT.AG .GT......A C.C..AG..A ....G..CT. .G.--C.... .C..G..... GATCC..... ..........  [160]
Leuconostoc_inhae_IH101_(AY11768 .G.G.CT.CT .GTT.....A C..G.AG..A ....G..CTA .G.--C...G .C..G..... GATCC..... ..........  [160]
Paenibacillus_xylanilyticus_(AY4 ..C.TG.... ..T...C..A .G....C.G. ........AG .AAT...... ...GAG.... GG.C.....C .......C..  [160]
Paenibacillus_sp._HC1_(AB198337) ..G.TGT..T C.T...C..A .GAG.AC.G. ........AG .AAT...... ...GGG.... GG.C.....C .......C..  [160]
Paenibacillus_sp._SAFN-068_(DQ12 ..G.TGT..T C.T...C..A .G...AC.G. ........AG .AAT...... ...GGG.... GG.C.....C ......?C..  [160]
Bacillus_pumilus_OS-70_(AM237370 ..G.TC...G AA........ TTC...GA.. ........T. TC.G...... ....C..... GA.CC....C .......C..  [160]
Bacillus_cereus_CFR04_(AY907828) ...C..T..G AA........ TTCG.A.... ....G..... TC.G...... .....G.... GA.CC...TC .......C..  [160]
Staphylococcus_sp._XJU-1_(DQ8375 ......T..G AA........ TTC..T.G.. ........T. TC.G...... .......... GA.CC...CC .T.....C..  [160]
Staphylococcus_sp._LAMI_001_(DQ8 ...C..T.GG AA........ TTCT.A.G.. .....T..T. T..-..A... .......... GA.CC...CC .T.....C..  [160]

C._damarensis_1_Caecum           GTTGGTGGGG TAACGGCCTA CCAAGACGAT GATGCATAGC CGACCTGAGA GGGTGATCGG CCACATTGGG ACTGAGACAC  [240]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... .......... .......... .......... ..........  [240]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... .......... .......... .......... ..........  [240]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... .......... .......... .......... ..........  [240]
Bacillus_megaterium_KL-197_(AY03 .......A.. .......TC. .....G.A.C .......... .......... .......... .....C.... ..........  [240]
Leuconostoc_pseudomesenteroides_ .......... ...A...... .......A.. .......... ...GT..... .AC....... .......... ..........  [240]
```

```
Leuconostoc_sp._CR-310S_(AF34993 .......... ...A...... .......A.. .......... ...GT..... .AC....... .......... ..........  [240]
Leuconostoc_inhae_IH101_(AY11768 .......... ...A...... .......A.. .......... ...GT..... .AC....... .......... ..........  [240]
Paenibacillus_xylanilyticus_(AY4 .......A.. .......TC. .....G...C .....G.... .......... .......... .....C.... ..........  [240]
Paenibacillus_sp._HC1_(AB198337) .......... .......TC. .....G...C .....G.... .......... .......... .....C.... ..........  [240]
Paenibacillus_sp._SAFN-068_(DQ12 .......... ?......TC. .....G...C .....G.... .......... .......... .....C.... ..........  [240]
Bacillus_pumilus_OS-70_(AM237370 .......... ...T...TC. .....G...C .....G.... .......... .......... .....C.... ..........  [240]
Bacillus_cereus_CFR04_(AY907828) .......A.. .......TC. .....G.A.C .....G.... .......... .......... .....C.... ..........  [240]
Staphylococcus_sp._XJU-1_(DQ8375 ......AA.. ..?....T.. .....G...C ...A.G.... .......... .......... .....C...A ..........  [240]
Staphylococcus_sp._LAMI_001_(DQ8 ......AA.. .......T.. .....G...C ...A.G.... .......... .......... .....C...A ..........  [240]

C._damarensis_1_Caecum           GGCCCAAACT CCTACGGGAG GCAGCAGTAG GGAATCTTCC GCAATGGGCG AAAGCCTGAC GGAGCAATGC CGCGTGAGTG  [320]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Bacillus_megaterium_KL-197_(AY03 .....G.... .......... .......... .......... .......A.. ....T..... .......C.. ..........  [320]
Leuconostoc_pseudomesenteroides_ .......... .......... .......... .......... A......... .........T .......C.. ......T...  [320]
Leuconostoc_sp._CR-310S_(AF34993 .......... .-........ ..T....... .......... A......... ..G......T .......C.. ......T...  [320]
Leuconostoc_inhae_IH101_(AY11768 .......... .......... ..T....... .......... A......... .........T .......C.. ......T...  [320]
Paenibacillus_xylanilyticus_(AY4 .....G.... .......... .......... .......... .......... .......... .......... ..........  [320]
Paenibacillus_sp._HC1_(AB198337) .....G.... .......... .......... .......... .......... .......... .......... ..........  [320]
Paenibacillus_sp._SAFN-068_(DQ12 .....?.... .......... .......... .......... ....?..?.. .......... .....G.... ..........  [320]
Bacillus_pumilus_OS-70_(AM237370 .....G.... .......... .......... .......... .......A.. ....T..... .......C.. ..........  [320]
Bacillus_cereus_CFR04_(AY907828) .....G.... .......... .......... .......... .......A.. ....T..... .......C.. ..........  [320]
Staphylococcus_sp._XJU-1_(DQ8375 ..T..G.... .......... .......... .......... .......... .......... .......C.. ..........  [320]
Staphylococcus_sp._LAMI_001_(DQ8 ..T..G.... .......... .......... .......... .......... .......... .......C.. ..........  [320]

C._damarensis_1_Caecum           AAGAAGGCCT TCGGGTCGTA AAACTCTGTT ATAAGAGAAG AACAAATTGT AGAGTAACT- GCTACAGTCT TGACGGTATC  [400]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... .......... .........- .......... ..........  [400]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... .......... .........- .......... ..........  [400]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... .......... .........- .......... ..........  [400]
Bacillus_megaterium_KL-197_(AY03 .T......T. .......... .......... G.T..G.... .....G.ACA .........- ...TGTAC.. ........C.  [400]
Leuconostoc_pseudomesenteroides_ .T......T. .......... ..G.A..... G..T.G.... ....GC.A.A .T..GG.A.- .A.TTTAGT. ........C.  [400]
Leuconostoc_sp._CR-310S_(AF34993 .T......T. .......... ..G.A..... G..T.G.... ....GC.A.A .T..G..A.- .A.TTTAGT. ........C.  [400]
Leuconostoc_inhae_IH101_(AY11768 .T......T. .......... ..G.A..... G..T.G.... ....GC.A.A GT..GG.A.- .ACTTTAGT. ........C.  [400]
Paenibacillus_xylanilyticus_(AY4 .T.....TT. ....A..... ..G....... GCC..G.... ...GTCC.TG .........- ...CA..GAG ........C.  [400]
Paenibacillus_sp._HC1_(AB198337) .T.....TT. ....A..... ..G....... GCC..G.... ...GCT.G.G .........- ...C.CAAGG ........C.  [400]
Paenibacillus_sp._SAFN-068_(DQ12 .T.....TT. ....A..... ..G....... GCC..G.... ...GCT.G.G .........- ...CTCAAGG ........C.  [400]
Bacillus_pumilus_OS-70_(AM237370 .T.....TT. ....A..... ..G....... G.T..G.... .....G.GCG .........- ...CGCAC.. ........C.  [400]
Bacillus_cereus_CFR04_(AY907828) .T......T. .......... .......... G.T..G.... .....G.GC. ..TTG..TAA ...GGCAC.. ........C.  [400]
Staphylococcus_sp._XJU-1_(DQ8375 .T.....T.. ....A..... .......... G.T..G.... ........TG TT.......- .AACA..... ........C.  [400]
Staphylococcus_sp._LAMI_001_(DQ8 .T.....GT. ....C..... .......... ..T..G.... ......CGTG TA.......- .TGCAC.... ........C.  [400]

C._damarensis_1_Caecum           TTA-TCAGAA AGCCACGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT AGGTGGC-AA GCGTTGTCCG GATTTATTGG  [480]
Aerococcus_viridans_(AY707779)   ...-...... .......... .......... .......... .......... .......-.. .......... ..........  [480]
Aerococcus_sp._LV65.(AF076639)   ...-...... .......... .......... .......... .......... .......-.. .......... ..........  [480]
Aerococcus_viridans_88B_(AY70777 ...-...... .......... .......... .......... .......... .......-.. .......... ..........  [480]
Bacillus_megaterium_KL-197_(AY03 .A.-C..... .......... .......... .......... .......... .......-.. .....A.... ..A.......  [480]
```

```
Leuconostoc_pseudomesenteroides_ A..-C..... ..GG...... ..A....... .......... .......... .T..CC.-G. .....A.... ..........  [480]
Leuconostoc_sp._CR-310S_(AF34993 A..-C..... ..GG...... ..A....... .......... .......... .T..CC.-G. .....A.... ..........  [480]
Leuconostoc_inhae_IH101_(AY11768 A..-C..... ..GG...... ..A....... .......... .......... .T..CC.-G. .....A.... ..........  [480]
Paenibacillus_xylanilyticus_(AY4 .G.-GA.... ....C..... .......... .......... .......... ...G...-.. .......... ..A.......  [480]
Paenibacillus_sp._HC1_(AB198337) .G.-GA.... ....C..... .......... .......... .......... ...G...-.. .......... ..A.......  [480]
Paenibacillus_sp._SAFN-068_(DQ12 .G?AGA.... ....C..... .......... .........? .?........ ...G..?C.. .......... ..W.......  [480]
Bacillus_pumilus_OS-70_(AM237370 .A.-C..... .......... .......... .......... .......... .......-.. .......... ..A.......  [480]
Bacillus_cereus_CFR04_(AY907828) .A.-C..... .......... .......... .......... .......... .......-.. .....A.... ..A.......  [480]
Staphylococcus_sp._XJU-1_(DQ8375 .A.-C..... .......... .......... .......... .......... .......-.. .....A.... ..A.......  [480]
Staphylococcus_sp._LAMI_001_(DQ8 .A.-...... .......... .......... .......... .......... .......-.. .....A.... ..A.......  [480]

C._damarensis_1_Caecum           GCGTAAAGGG AGCGCAGGTG GTTTCTTAAG TCTGATGTGA AAGCCCACGG CTTAACCGTG GAGGGTCATT GGAAACTGGG  [560]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... .......... .......... .......... ..........  [560]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... .......... .......... .......... ..........  [560]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... .......... .......... .......... ..........  [560]
Bacillus_megaterium_KL-197_(AY03 ........C. C.......C. .......... .......... .......... ..C....... .......... ..........  [560]
Leuconostoc_pseudomesenteroides_ ........C. .......AC. ...GA..... .......... ......GGA. ..C...TCC. ..AT.G.... .........T  [560]
Leuconostoc_sp._CR-310S_(AF34993 ........C. .......AC. ....A..... .......... ......GGA. ..C...TCC. ..AT.G.... .........T  [560]
Leuconostoc_inhae_IH101_(AY11768 ........C. .......AC. ...GA..... .......... ......GGA. ..C...TCC. ..AA.G.... .........T  [560]
Paenibacillus_xylanilyticus_(AY4 ........C. C.......C. ..CAT..... ....G...TT ..T...GG.. ..C....CC. ..TC.-..C. ..........  [560]
Paenibacillus_sp._HC1_(AB198337) ........C. C.......C. ..CAT..... ....G...TT ..T...GG.. ..C....CC. ..TC.-..C. ..........  [560]
Paenibacillus_sp._SAFN-068_(DQ12 ........C. C.....S.C. ..CAT..... ....G...TT ..T...GG.. ..C....CC. ..TC.-..C. ..........  [560]
Bacillus_pumilus_OS-70_(AM237370 .......... CT......C. .......... .......... ......C... ..C.....G. .......... ..........  [560]
Bacillus_cereus_CFR04_(AY907828) ........C. C......... .......... .......... .......... ..C....... .......... ..........  [560]
Staphylococcus_sp._XJU-1_(DQ8375 ........C. C...T...C. .......... .......... .......... ..C....... .......... ..........  [560]
Staphylococcus_sp._LAMI_001_(DQ8 ........C. C...T...C. .......... .......... .......... ..C....... .......... ..........  [560]

C._damarensis_1_Caecum           AAACTTGAGT ACAGAAGAGG AATGTGGAAC TCCATGTGTA GCGGTGGAAT GCGTAGATAT ATGGAAGAAC ACCAGTGGCG  [640]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... .......... .......... .......... ..........  [640]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... .......... .......... .......... ..........  [640]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... .......... .......... .......... ..........  [640]
Bacillus_megaterium_KL-197_(AY03 G......... G........A ..A.C....T ....C..... ......A... .......G.. G....G.... ..........  [640]
Leuconostoc_pseudomesenteroides_ T......... G...T..... T.A....... .......... .......... .......... .......... .....-....  [640]
Leuconostoc_sp._CR-310S_(AF34993 T......... G...T..... T.A....... .......... .......... .......... .......... ..........  [640]
Leuconostoc_inhae_IH101_(AY11768 C......... G...T..... T.A....... .......... .......... .......... .......... .....C....  [640]
Paenibacillus_xylanilyticus_(AY4 TG........ G......... .GA......T ....C..... ......A... .......... G....G.... ..........  [640]
Paenibacillus_sp._HC1_(AB198337) TG........ G......... .GA......T ....C..... ......A... .......... G....G.... ..........  [640]
Paenibacillus_sp._SAFN-068_(DQ12 TG..YY.... G......... .GA....R.T ....C.Y.Y. ......A... .?...?.... G....R.M.. ........Y.  [640]
Bacillus_pumilus_OS-70_(AM237370 .......... G......... .GA......T ....C..... ......A... .......G.. G....G.... ..........  [640]
Bacillus_cereus_CFR04_(AY907828) .G........ G......... ..A......T .......... ......A... .--------- ----.G.... ..........  [640]
Staphylococcus_sp._XJU-1_(DQ8375 .......... G......... .GA......T .......... ......A... ...C...G.. .....G.... ..........  [640]
Staphylococcus_sp._LAMI_001_(DQ8 .......... G......... ..A......T .......... ......A... ...C...G.. .....G.... ..........  [640]

C._damarensis_1_Caecum           AAGGCGACAT T-CTGGTCTG TTACTGACAC TGAGGCTCGA AAGCGTGGGG AGCAAACAGG ATTAGATACC CTGGTAGTCC  [720]
Aerococcus_viridans_(AY707779)   .......... .-........ .......... .......... .......... .......... .......... ..........  [720]
Aerococcus_sp._LV65.(AF076639)   .......... .-........ .......... .......... .......... .......... .......... ..........  [720]
Aerococcus_viridans_88B_(AY70777 .......... .-........ .......... .......... .......... .......... .......... ..........  [720]
```

```
Bacillus_megaterium_KL-197_(AY03 ......G.T. .-T....... .A......G. ......G... .......... .......... .......... ..........  [720]
Leuconostoc_pseudomesenteroides_ ......G.T. A-....A... -A......GT -......... ...T.....T .......--. .......... .......---  [720]
Leuconostoc_sp._CR-310S_(AF34993 ......G.T. AA....A... CA......GT .......... ...T.....T .......... .......... ..........  [720]
Leuconostoc_inhae_IH101_(AY11768 ......G.T. A-....A... .A......GT .......... ...T.....T .......... .......... ..........  [720]
Paenibacillus_xylanilyticus_(AY4 ........TC .-....G... .A......G. ......G... .......... .......... .......... ..........  [720]
Paenibacillus_sp._HC1_(AB198337) ........TC .-....G... .A......G. ......G... .......... .......... .......... ..........  [720]
Paenibacillus_sp._SAFN-068_(DQ12 ..R.....TC .-..S.G... KA.....MG. ......G... .......... ...?...... .......... ..........  [720]
Bacillus_pumilus_OS-70_(AM237370 ........TC .-........ .A......G. .....AG... .......... ...G...... .......... ..........  [720]
Bacillus_cereus_CFR04_(AY907828) ........T. .-........ .A........ ......G... .......... .......... .......... ..........  [720]
Staphylococcus_sp._XJU-1_(DQ8375 ......G.TC .-........ .A......G. ...T.TG... .......... .T........ .......... ..........  [720]
Staphylococcus_sp._LAMI_001_(DQ8 ........T. .-........ .A......G. ...T.TG... .......... .T........ .......... ..........  [720]

C._damarensis_1_Caecum           ACGCCGTAAA CGATGAGTGC TAGGTGTTGG AGGGTTTCCG CCCTTCAGTG CCGCAGTTAA CGCATTAAGC ACTCCGCCTG  [800]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... .......... .......... .......... ..........  [800]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... .......... .......... .......... ..........  [800]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... .......... .......... .......... ..........  [800]
Bacillus_megaterium_KL-197_(AY03 .......... .......... ..A.....A. .......... .....T.... .T....C... .......... ..........  [800]
Leuconostoc_pseudomesenteroides_ ---......C --....ACA. ........A. GA........ ..TC.T.... ...A..C... .........T GT........  [800]
Leuconostoc_sp._CR-310S_(AF34993 ..A....... ......ACA. ........A. GA........ ..TC.T.... ...A..C... .........T GT........  [800]
Leuconostoc_inhae_IH101_(AY11768 ..A....... ......ACA. ........A. GA........ ..TC.T.... ...A.CG... .........T GT........  [800]
Paenibacillus_xylanilyticus_(AY4 .......... .......... ........A. G..T..CGAT A..C.TG... ...A...... .A........ ..........  [800]
Paenibacillus_sp._HC1_(AB198337) .......... .......... ........A. G..T..CGAT A..C.TG... ...A...... .A........ ..........  [800]
Paenibacillus_sp._SAFN-068_(DQ12 .........? .........G ........A. G..T..CGAT A..C.TG... ...A...... .A.....?.. ..........  [800]
Bacillus_pumilus_OS-70_(AM237370 .......... .......... ..A.....A. G......... ...C.T.... .T....C... .......... ..........  [800]
Bacillus_cereus_CFR04_(AY907828) .......... .......... ..A.....A. .......... .....T.... .T.A...... .......... ..........  [800]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......... ..A.....A. G......... ...C.T.... .T....C... .......... ..........  [800]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......... ..A.....A. G......... ...C.T.... .T....C... .......... ..........  [800]

C._damarensis_1_Caecum           GGGAGTACGA CCGCAAGGTT GAAACTCAAA GGAATTGACG GGGACCCGCA CAAGCGGTGG AGCATGTGGT TTAATTCGAA  [880]
Aerococcus_viridans_(AY707779)   .......... .......... .......... .......... .......... .......... .......... ..........  [880]
Aerococcus_sp._LV65.(AF076639)   .......... .......... .......... .......... .......... .......... .......... ..........  [880]
Aerococcus_viridans_88B_(AY70777 .......... .......... .......... .......... .......... .......... .......... ..........  [880]
Bacillus_megaterium_KL-197_(AY03 .........G T......AC. .......... .......... ...G...... .......... .......... ..........  [880]
Leuconostoc_pseudomesenteroides_ .......... .......... .......... .......... .......... .......... .......... ..........  [880]
Leuconostoc_sp._CR-310S_(AF34993 .......... .......... .......... .......... .......... .......... .......... ..........  [880]
Leuconostoc_inhae_IH101_(AY11768 .......... .......... .......... .......... ......-... .......... .......... ..........  [880]
Paenibacillus_xylanilyticus_(AY4 .........G T......AC. .......... .......... .......... .....A.... ..T....... ..........  [880]
Paenibacillus_sp._HC1_(AB198337) .........G T......AC. .......... .......... .......... .....A.... ..T....... ..........  [880]
Paenibacillus_sp._SAFN-068_(DQ12 .........? T......AC. ....?..... .......... .......... ..??.A.... ..T....... ..........  [880]
Bacillus_pumilus_OS-70_(AM237370 .........G T......AC. .......... .......... ...G...... .......... .......... ..........  [880]
Bacillus_cereus_CFR04_(AY907828) .........G ........C. .......... .......... ...G...... .......... .......... ..........  [880]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......... .......... .......... .......... .......... .......... ..........  [880]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......... .......... .......... .......... .......... .......... ..........  [880]

C._damarensis_1_Caecum           GCAACGCGAA GAACCTTTAC CAAGTCTTGA CATCC-TTTG ACCACCCTA [929]
Aerococcus_viridans_(AY707779)   .......... .......-.. .......... .....-.... ......... [929]
Aerococcus_sp._LV65.(AF076639)   .......... .......-.. .......... .....-.... ......... [929]
```

```
Aerococcus_viridans_88B_(AY70777 .......... .......-.. .......... .....-.... ......... [929]
Bacillus_megaterium_KL-197_(AY03 .......... .......-.. ..G....... .....-.C.. ..A..T... [929]
Leuconostoc_pseudomesenteroides_ .......... .......-.. ..G....... .....-.... .AGCTTT.. [929]
Leuconostoc_sp._CR-310S_(AF34993 .......... .......-.. ..G....... .....-.... .AGCTTT.. [929]
Leuconostoc_inhae_IH101_(AY11768 .......... .......-.. ..G....... .....-.... .AGCTTT.. [929]
Paenibacillus_xylanilyticus_(AY4 .......... .......-.. ..G....... .....C.C.. ...GGTAC. [929]
Paenibacillus_sp._HC1_(AB198337) .......... .......-.. ..G....... .....C.C.. ...GGTAC. [929]
Paenibacillus_sp._SAFN-068_(DQ12 .......... .......-.. ..G....... .....C.C.. ...GG??C. [929]
Bacillus_pumilus_OS-70_(AM237370 .......... .......-.. ..G....... .....-.C.. ..A...... [929]
Bacillus_cereus_CFR04_(AY907828) .......... .......-.. ..G....... .....-.C.. .AA...... [929]
Staphylococcus_sp._XJU-1_(DQ8375 .......... .......-.. ...A...... .....-.... ...G.T... [929]
Staphylococcus_sp._LAMI_001_(DQ8 .......... .......-.. ...A...... .....-.... .AA..T... [929]
```

Appendix VII

Nucleotide sequence alignment of the γ-Proteobacteria created in MEGA v. 3.1 (Kumar *et al.* 2004). Dots indicate sites identical to that of reference sequence (*C. damarensis* 352 Mouth 4), where ? indicates missing data and – indicates gaps.

```
C._damarensis_352_Mouth_4         CCGCCCTCTT TGCAGTTAGG CTAGCTAC-T TCTGGTGCAA CAAACTCCCA TGGTGTGACG GGCGG-TGTG TACAAGGCCC  [ 80]
C._damarensis_353_Mouth           .......... .......... ........-. .......... .......... ...?...... .....-.... ..........  [ 80]
G._capensis_5_Nose                G.......CC GAAG....A? ???C??..-. ...TT..... .CC....... ...?.....? ?.?..-.... ..........  [ 80]
G._capensis_Uro-genital           G.?.....CC GAAG....A? ??????.?C. ...TT..... .CC....... ...?.....? ?....A.... ..........  [ 80]
C._damarensis_352_Nose            G..T..C.CC .AAG.....A ...C....-. .......... .......... .......... .....-.... ..........  [ 80]
C._damarensis_352_Mouth_1         .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
C._damarensis_352_Mouth_2         .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
C._damarensis_352_Mouth_3         .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
C._damarensis_352_Mouth_5         .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
C._damarensis_355_Mouth           .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
C._damarensis_360_Nose            .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
C._damarensis_387_Feacal          G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_353_Feacal          G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_352_Feacal_1        G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._h._pretoriae_1_Ceacum_1        G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_351_Feacal_1        G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_351_Feacal_2        G.......CC ?AAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_351_Feacal_3        G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_352_Feacal_2        A.A.....CC ?AAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... .....?....  [ 80]
C._damarensis_352_Urine_1         .GT...C... ...G.....A ........-. .....A.... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_352_Feacal_3        .?.T..C.C. ...G.....A ........-. .....A.... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_351_Mouth           .?.T....CC GAAG.....A ........-. .......... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_352_Urine_2         .GT...C... ?..G.....A ........-. .....A.... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_352_Urine_3         .A.T..C.C. ...G.....A ........-. .....A.... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_351_Uro-genital     G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._h._pretoriae_1_Ceacum_2        G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._h._pretoriae_1_Ceacum_3        G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._h._pretoriae_1_Ceacum_4        G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_353_Nose_1          G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
C._damarensis_353_Nose_2          G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
Acinetobacter_sp._DSM_587(X89709 .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
Acinetobacter_baumannii_DSM30008 .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
Acinetobacter_calcoaceticus_(AF4 .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
Acinetobacter_baumannii_DSM30007 .......... .......... ........-. .......... .......... .......... .....-.... ..........  [ 80]
Acinetobacter_junii_(AB101444)   G..T....C. ...G.....A ...C....-. .......... .......... .......... .....-.... ..........  [ 80]
Acinetobacter_sp._DG880(AY258108 G..T..C.CC .AAG.....A ...C....-. .......... .......... .......... .....-.... ..........  [ 80]
Citrobacter_sp._TAF_(DQ083975)   G.......CC GAAG....A. ...C....-. ...TT..A.. .CC....... .......... .....-.... ..........  [ 80]
Enterobacter_cloacae_(Z96079)    G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
Salmonella_typhimurium_(AE008706 G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
```

```
Leclercia_adecarboxylata_(AJ2763 G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
Uncultured_bacterium_(DQ068919)  G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
Enterobacteriaceae_bacterium_(AJ G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
Pseudomonas_putida_(AJ308312)    ...T....CC GAAG.....A ........-. .......... .CC....... .......... .....-.... ..........  [ 80]
Pseudomonas_sp._(AY379974)       ...T....C. ...G.....A ........-. .....A.... .CC....... .......... .....-.... ..........  [ 80]
Pseudomonas_otitidis_(AY953147)  ...T....C. ...G.....A ........-. .....A.... .CC....... .......... .....-.... ..........  [ 80]
Serratia_marcescens_501_(AF28687 G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
Serratia_marcescens_500_(AF28687 G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]
Acinet_calcoaceticus_ATCC23055(A G..T....C. ...G.....A ...C....-. .......... .......... .......... .....-.... ..........  [ 80]
Pseudomona_aeruginosaATCC10145(A ...T..C.C. ...G.....A ........-. .....A.... .CC....... .......... .....-.... ..........  [ 80]
Serratia_marcescens_ATCC1388(M59 G.......CC GAAG....A. ...C....-. ...TT..... .CC....... .......... .....-.... ..........  [ 80]

C._damarensis_352_Mouth_4        GGGAACGTAT TCACCGCGGC ATTCTGATCC GCGATTACTA GCGATTCCGA CTTCATGGAG TCGAGTTGCA GACTCCAATC  [160]
C._damarensis_353_Mouth          .......... .......... .......... .......... ?......... .......... .......... ..........  [160]
G._capensis_5_Nose               .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
G._capensis_Uro-genital          .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
C._damarensis_352_Nose           .......... .......... .......... .......... .......... .......... .......... ..........  [160]
C._damarensis_352_Mouth_1        .......... .......... .......... .......... .......... .......... .......... ..........  [160]
C._damarensis_352_Mouth_2        .......... .......... .......... .......... .......... .......... .......... ..........  [160]
C._damarensis_352_Mouth_3        .......... .......... .......... .......... .......... .......... .......... ..........  [160]
C._damarensis_352_Mouth_5        .......... .......... .......... .......... .......... .......... .......... ..........  [160]
C._damarensis_355_Mouth          .......... .......... .......... .......... .......... .......... .......... ..........  [160]
C._damarensis_360_Nose           .......... .......... .......... .......... .......... .......... .......... ..........  [160]
C._damarensis_387_Feacal         .......... ......T... ?......... A......... .......... .......... .......... ..........  [160]
C._damarensis_353_Feacal         .......... ......TA.. .........T A......... .......... .......... ..?....... ..........  [160]
C._damarensis_352_Feacal_1       .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
C._h._pretoriae_1_Ceacum_1       .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
C._damarensis_351_Feacal_1       .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
C._damarensis_351_Feacal_2       .......... ......TA.. .........T A......... .......... .......... ?.?....... ..........  [160]
C._damarensis_351_Feacal_3       .......... ......TA.. .........T A.?....... ........?. .......... ?.?....... ..........  [160]
C._damarensis_352_Feacal_2       .......... ......T... .......... A......... .......... .......... .......... ..........  [160]
C._damarensis_352_Urine_1        .......... ......T.A. ........T. A......... .......... .....C.C.. .......... ....G.G...  [160]
C._damarensis_352_Feacal_3       .......... ......T.A. ........T. A......... .......... .....C.C.. .......... ....G.G...  [160]
C._damarensis_351_Mouth          .......... ........A. ........T. .......... .......... .....C.C.. .......... ....G.G...  [160]
C._damarensis_352_Urine_2        .......... ......T.A. ........T. A......... .......... .....C.C.. .......... ....G.G...  [160]
C._damarensis_352_Urine_3        .......... ......T.A. ........T. A......... .......... .....C.C.. .......... ....G.G...  [160]
C._damarensis_351_Uro-genital    .......... ......T... .......... A......... .......... .......... .......... ..........  [160]
C._h._pretoriae_1_Ceacum_2       .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
C._h._pretoriae_1_Ceacum_3       .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
C._h._pretoriae_1_Ceacum_4       .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
C._damarensis_353_Nose_1         .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
C._damarensis_353_Nose_2         .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
Acinetobacter_sp._DSM_587(X89709 .......... .......... .......... .......... .......... .......... .......... ..........  [160]
Acinetobacter_baumannii_DSM30008 .......... .......... .......... .......... .......... .......... .......... ..........  [160]
Acinetobacter_calcoaceticus_(AF4 .......... .......... .......... .......... .......... .......... .......... ..........  [160]
Acinetobacter_baumannii_DSM30007 .......... .......... .......... .......... .......... .......... .......... ..........  [160]
Acinetobacter_junii_(AB101444)   .......... .......... .......... .......... .......... .......... .......... ..........  [160]
```

```
Acinetobacter_sp._DG880(AY258108 .......... .......... .......... .......... .......... .......... .......... ..........  [160]
Citrobacter_sp._TAF_(DQ083975)   .......... ......T... .......... A......... .......... .......... .......... ..........  [160]
Enterobacter_cloacae_(Z96079)    .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
Salmonella_typhimurium_(AE008706 .......... ......T... .......... A......... .......... .......... .......... ..........  [160]
Leclercia_adecarboxylata_(AJ2763 .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
Uncultured_bacterium_(DQ068919)  .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
Enterobacteriaceae_bacterium_(AJ .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
Pseudomonas_putida_(AJ308312)    .......... ........A. ........T. .......... .......... .....C.C.. .......... ....G.G...  [160]
Pseudomonas_sp._(AY379974)       .......... ......T.A. ........T. A......... .......... .....C.C.. .......... ....G.G...  [160]
Pseudomonas_otitidis_(AY953147)  .......... ......T.A. ........T. A......... .......... .....C.C.. .......... ....G.G...  [160]
Serratia_marcescens_501_(AF28687 .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
Serratia_marcescens_500_(AF28687 .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]
Acinet_calcoaceticus_ATCC23055(A .......... .......... .......... .......... .......... .......... .......... ..........  [160]
Pseudomona_aeruginosaATCC10145(A .......... ......T.A. ........T. A......... .......... .....C.C.. .......... ....G.G...  [160]
Serratia_marcescens_ATCC1388(M59 .......... ......TA.. .........T A......... .......... .......... .......... ..........  [160]

C._damarensis_352_Mouth_4        CGGACTACGA TCGGCTTTTT GAGATTAGCA TCCTATCGCT AGGTAGCAAC CCTTTGTACC GACCATTGTA GCACGTGTGT  [240]
C._damarensis_353_Mouth          .......... .......... .......... .......... .....?.... .......... .......... ..........  [240]
G._capensis_5_Nose               .......... CGTA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA ?G........ ..........  [240]
G._capensis_Uro-genital          .......... CGTA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA CG........ ..........  [240]
C._damarensis_352_Nose           .......... .......... .......... ....C....G .......... .......... .......... ..........  [240]
C._damarensis_352_Mouth_1        .......... .......... .......... .......... .......... .......... .......... ..........  [240]
C._damarensis_352_Mouth_2        .......... .......... .......... .......... .......... .......... .......... ..........  [240]
C._damarensis_352_Mouth_3        .......... .......... .......... .......... .......... .......... .......... ..........  [240]
C._damarensis_352_Mouth_5        .......... .......... .......... .......... .......... .......... .......... ..........  [240]
C._damarensis_355_Mouth          .......... .......... .......... .......... .......... .......... .......... ..........  [240]
C._damarensis_360_Nose           .......... .......... .......... ..AC...... GT........ .......... .......... ..........  [240]
C._damarensis_387_Feacal         .......... CATA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA TG........ ..........  [240]
C._damarensis_353_Feacal         .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
C._damarensis_352_Feacal_1       .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
C._h._pretoriae_1_Ceacum_1       .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
C._damarensis_351_Feacal_1       .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
C._damarensis_351_Feacal_2       .......... CGCA....A. ...G.CC..T .G..C..?.G ....C..TT. T.......TG CG........ ?.........  [240]
C._damarensis_351_Feacal_3       .......... CGCA....A. ...G.CC..T .G..C..?.G ....C..TT. T.......TG CG........ A.........  [240]
C._damarensis_352_Feacal_2       .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ?.........  [240]
C._damarensis_352_Urine_1        .......... ....T...A. .G.......T C.ACC....G GCT.G..... .......... .......... ..........  [240]
C._damarensis_352_Feacal_3       .......... ....T...A. .G.......T C.ACC....G GCT.G..... .......... .......... ..........  [240]
C._damarensis_351_Mouth          .......... ....T...A. .G.......T C.ACC....G GCT.G..... .......... .......... ..........  [240]
C._damarensis_352_Urine_2        .......... ....T...A. .G.......T C.ACC....G GCT.G..... .......... .......... ..........  [240]
C._damarensis_352_Urine_3        .......... ....T...A. .G.......T C.ACC....G GCT.G..... .......... .......... ..........  [240]
C._damarensis_351_Uro-genital    .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
C._h._pretoriae_1_Ceacum_2       .......... CGTA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA CG........ ..........  [240]
C._h._pretoriae_1_Ceacum_3       .......... CGTA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA CG........ ..........  [240]
C._h._pretoriae_1_Ceacum_4       .......... CGTA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA CG........ ..........  [240]
C._damarensis_353_Nose_1         .......... CATA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA TG........ ..........  [240]
C._damarensis_353_Nose_2         .......... CATA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA TG........ ..........  [240]
Acinetobacter_sp._DSM_587(X89709 .......... .......... .......... .......... .......... .......... .......... ..........  [240]
```

```
Acinetobacter_baumannii_DSM30008 .......... .......... .......... ..AC...... GT........ .......... .......... ..........  [240]
Acinetobacter_calcoaceticus_(AF4 .......... .......... .......... .......... .......... .......... .......... ..........  [240]
Acinetobacter_baumannii_DSM30007 .......... .......... .......... ..AC...... GT........ .......... .......... ..........  [240]
Acinetobacter_junii_(AB101444)   .......... .......... .......... ..AC...... GT........ .......... .......... ..........  [240]
Acinetobacter_sp._DG880(AY258108 .......... .......... .......... ....C....G .......... .......... .......... ..........  [240]
Citrobacter_sp._TAF_(DQ083975)   .......... CATA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA TG........ ..........  [240]
Enterobacter_cloacae_(Z96079)    .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
Salmonella_typhimurium_(AE008706 .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
Leclercia_adecarboxylata_(AJ2763 .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
Uncultured_bacterium_(DQ068919)  .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
Enterobacteriaceae_bacterium_(AJ .......... CGCA....A. ...G.CC..T .G..C....G ....C..TT. T.......TG CG........ ..........  [240]
Pseudomonas_putida_(AJ308312)    .......... ....T...A. .G.......T C.ACC....G GCT.G..... .......... .......... ..........  [240]
Pseudomonas_sp._(AY379974)       .......... ....T...A. .G.......T C.ACC....G GCT.G..... .......... .......... ..........  [240]
Pseudomonas_otitidis_(AY953147)  .......... ....T...A. .G.......T C.ACC....G GCT.G..... .......... .......... ..........  [240]
Serratia_marcescens_501_(AF28687 .......... CGTA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA CG........ ..........  [240]
Serratia_marcescens_500_(AF28687 .......... CGTA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA CG........ ..........  [240]
Acinet_calcoaceticus_ATCC23055(A .......... .......... .......... .......... .......... .......... .......... ..........  [240]
Pseudomona_aeruginosaATCC10145(A .......... ....T...A. .G.......T C.ACC....G GCT.G..... .......... .......... ..........  [240]
Serratia_marcescens_ATCC1388(M59 .......... CATA....A. ...G.CC..T .G..C....G ....C..TT. T.......TA TG........ ..........  [240]

C._damarensis_352_Mouth_4        AGCCCTGGCC GTAAGGGCCA TGATGACTTG ACGTCGTCCC CGCCTTCCTC CAGTTTGTCA CTGGCAGTAT CCTTAAAGTT  [320]
C._damarensis_353_Mouth          .......... .......... .......... .......... .......... .......... .......... ..........  [320]
G._capensis_5_Nose               ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
G._capensis_Uro-genital          ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._damarensis_352_Nose           ........T. .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_352_Mouth_1        .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_352_Mouth_2        .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_352_Mouth_3        .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_352_Mouth_5        .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_355_Mouth          .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_360_Nose           .......... .......... .......... .......... .......... .......... .......... ..........  [320]
C._damarensis_387_Feacal         .?......T. .......... .......... .....A.... .A........ ......A... ....?...C. ....TG....  [320]
C._damarensis_353_Feacal         .?....ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._damarensis_352_Feacal_1       ........T. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._h._pretoriae_1_Ceacum_1       ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._damarensis_351_Feacal_1       ........T. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._damarensis_351_Feacal_2       ........T. .?........ .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._damarensis_351_Feacal_3       ........T. .......... .......... .....A.... .A........ ......A... ....?...C. ....TG....  [320]
C._damarensis_352_Feacal_2       ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._damarensis_352_Urine_1        .......... .......... .......... .....A.... .A........ .G........ .C......C. .....G...G  [320]
C._damarensis_352_Feacal_3       .......... .......... .......... .....A.... .A........ .G........ .C......C. .....G...G  [320]
C._damarensis_351_Mouth          .....A.... .......... .......... .....A.... .A........ .G........ .C......C. .....G...G  [320]
C._damarensis_352_Urine_2        .......... .......... .......... .....A.... .A........ .G........ .C......C. .....G...G  [320]
C._damarensis_352_Urine_3        .......... .......... .......... .....A.... .A........ .G........ .C......C. .....G...G  [320]
C._damarensis_351_Uro-genital    ........T. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._h._pretoriae_1_Ceacum_2       ......ACT. .......... .......... .....A.... .A........ .?....A... .?......C. ....TG....  [320]
C._h._pretoriae_1_Ceacum_3       ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
```

```
C._h._pretoriae_1_Ceacum_4       ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._damarensis_353_Nose_1         ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
C._damarensis_353_Nose_2         ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
Acinetobacter_sp._DSM_587(X89709 .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Acinetobacter_baumannii_DSM30008 .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Acinetobacter_calcoaceticus_(AF4 .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Acinetobacter_baumannii_DSM30007 .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Acinetobacter_junii_(AB101444)   .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Acinetobacter_sp._DG880(AY258108 ........T. .......... .......... .......... .......... .......... .......... ..........  [320]
Citrobacter_sp._TAF_(DQ083975)   ........T. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
Enterobacter_cloacae_(Z96079)    ........T. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
Salmonella_typhimurium_(AE008706 ........T. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
Leclercia_adecarboxylata_(AJ2763 ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
Uncultured_bacterium_(DQ068919)  ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
Enterobacteriaceae_bacterium_(AJ ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
Pseudomonas_putida_(AJ308312)    .....A.... .......... .......... .....A.... .A........ .G........ .C......C. .....G...G  [320]
Pseudomonas_sp._(AY379974)       .......... .......... .......... .....A.... .A........ .G........ .C......C. .....G...G  [320]
Pseudomonas_otitidis_(AY953147)  .......... .......... .......... .....A.... .A........ .G........ .C......C. .....G...G  [320]
Serratia_marcescens_501_(AF28687 ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
Serratia_marcescens_500_(AF28687 ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]
Acinet_calcoaceticus_ATCC23055(A .......... .......... .......... .......... .......... .......... .......... ..........  [320]
Pseudomona_aeruginosaATCC10145(A .......... .......... .......... .....A.... .A........ .G........ .C......C. .....G...G  [320]
Serratia_marcescens_ATCC1388(M59 ......ACT. .......... .......... .....A.... .A........ ......A... ........C. ....TG....  [320]

C._damarensis_352_Mouth_4        CCCGAC [326]
C._damarensis_353_Mouth          ...AT. [326]
G._capensis_5_Nose               ....G. [326]
G._capensis_Uro-genital          ....G. [326]
C._damarensis_352_Nose           ....G. [326]
C._damarensis_352_Mouth_1        ...... [326]
C._damarensis_352_Mouth_2        ...... [326]
C._damarensis_352_Mouth_3        ...... [326]
C._damarensis_352_Mouth_5        ...... [326]
C._damarensis_355_Mouth          ...AT. [326]
C._damarensis_360_Nose           ...AT. [326]
C._damarensis_387_Feacal         ....G. [326]
C._damarensis_353_Feacal         ....G. [326]
C._damarensis_352_Feacal_1       ....G. [326]
C._h._pretoriae_1_Ceacum_1       ....G. [326]
C._damarensis_351_Feacal_1       ....G. [326]
C._damarensis_351_Feacal_2       ....G. [326]
C._damarensis_351_Feacal_3       ....G. [326]
C._damarensis_352_Feacal_2       ....G. [326]
C._damarensis_352_Urine_1        ...AC. [326]
C._damarensis_352_Feacal_3       ...AC. [326]
C._damarensis_351_Mouth          ...AC. [326]
C._damarensis_352_Urine_2        ...AC. [326]
```

```
C._damarensis_352_Urine_3         ...AC. [326]
C._damarensis_351_Uro-genital     ....G. [326]
C._h._pretoriae_1_Ceacum_2        ....?. [326]
C._h._pretoriae_1_Ceacum_3        ....G. [326]
C._h._pretoriae_1_Ceacum_4        ....G. [326]
C._damarensis_353_Nose_1          ....G. [326]
C._damarensis_353_Nose_2          ....G. [326]
Acinetobacter_sp._DSM_587(X89709 ...AT. [326]
Acinetobacter_baumannii_DSM30008 ...AT. [326]
Acinetobacter_calcoaceticus_(AF4 ...... [326]
Acinetobacter_baumannii_DSM30007 ...AT. [326]
Acinetobacter_junii_(AB101444)   ...AT. [326]
Acinetobacter_sp._DG880(AY258108 ....G. [326]
Citrobacter_sp._TAF_(DQ083975)   ....G. [326]
Enterobacter_cloacae_(Z96079)    ....G. [326]
Salmonella_typhimurium_(AE008706 ....G. [326]
Leclercia_adecarboxylata_(AJ2763 ....G. [326]
Uncultured_bacterium_(DQ068919)  ....G. [326]
Enterobacteriaceae_bacterium_(AJ ....G. [326]
Pseudomonas_putida_(AJ308312)    ...AC. [326]
Pseudomonas_sp._(AY379974)       ...AC. [326]
Pseudomonas_otitidis_(AY953147)  ...AC. [326]
Serratia_marcescens_501_(AF28687 ....G. [326]
Serratia_marcescens_500_(AF28687 ....G. [326]
Acinet_calcoaceticus_ATCC23055(A ...... [326]
Pseudomona_aeruginosaATCC10145(A ...AC. [326]
Serratia_marcescens_ATCC1388(M59 ....G. [326]
```

CHAPTER 3

Bartonella incidence in solitary and social species of South African bathyergids: a multi-gene approach

Contributing Authors: J. H. d. T. van Sandwyk, N. C. Bennett & A. D. Bastos

Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa

Abstract

Five species of southern African bathyergids were screened for the presence of the blood parasite *Bartonella* in liver samples. Two regions were amplified by PCR for identification and verification of infection, namely the Citrate synthase (*gltA*) gene and the 12S-23S inter-generic region. Infection was found to be present in only one mole-rat species, the Cape dune mole-rat (*Bathyergus suillus*) with an infection rate of 38.7 % (n= 31samples). Hystrichopsyllidid fleas collected from the pelages of 8 individuals of *B. suillus* obtained on a different sampling occasion but same geographic locality were shown to have a 25 % infection rate. Phylogenetic analysis of the nucleotide sequences revealed that the fleas possessed a new, previously undescribed species of *Bartonella* that was not detected in the Dune mole-rats, whilst the mammalian host species possessed a *Bartonella* species related to that previously described from a South African rodent.

1. Introduction

The family Bartonellaceae was first recognised by Gieszczykiewicz (1939), and the genus *Bartonella* was coined by Strong *et al.* (1915) and subsequently acknowledged by Skreman *et al.* (1980) (Garrity *et al.* 2005). There are currently 16 recognized species within this genus; of which seven have been shown to cause disease in humans (Jacomo *et al*. 2002, Molia *et al.* 2004). These Gram-negative bacteria are part of the alpha subclass of the Proteobacteria (Garrity *et al.* 2005). On infection the bacteria invades the erythrocytes of the host animal resulting in a chronic infection. *Bartonella* infection of the natural host usually results in a haemotrophic infection but does not cause any immediately observable detrimental effects to the mammalian reservoir host (Birtles 2005). However, opportunistic infection of a foreign host or an individual of the reservoir host species possessing a compromised immune system can result in the presentation of clinical effects (Birtles 2005). The transmission of all *Bartonella* species between different mammalian hosts is facilitated by a variety of blood-feeding, haematophagous arthropods (Breitschwerdt & Kordick 2000, Pretorius *et al.* 2004, Birtles 2005).

The *Bartonella* bacteria acts more like a parasite given its mode of transmission. Recent studies have shown a variety of mammalian species to be susceptible to this parasitic infection including rodents, carnivores and ungulates (Ying *et al*. 2002, Molia *et al*. 2004, Pretorius *et al.* 2004).

Many bacterial phylogenetic studies conducted to date have made use of the 16S rRNA gene. There has however been concern that if a recent transfer of genetic material between organisms has occurred that the phylogeny might reveal the organism to be more closely related to the donor of the material than to the organism's true relative (Sneath 1993, Birtles & Raoult 1996). Studies conducted on the methyl-coenzyme M reductase subunit A gene (*mcrI*) from closely related species within the family Methanosarcinaceae by Springer *et al*. (1995) revealed that trees obtained from this data closely resembled those obtained from 16S rRNA data (Birtles & Raoult 1996). It was also shown that *mcrI* gene sequences mutate approximately three times more rapidly than those obtained from the more conserved 16S rRNA region, thus making it possible to phylogenetically resolve relationships of bacterial species that are closely related (Birtles & Raoult 1996). The use of the multimeric, existing in the form of a dimer and a sexmer, enzyme Citrate synthase (*gltA*) to identify different species of bacteria was first proposed by Regnery *et al.* (1992) to identify members within the genus *Rickettsia*. This was later applied to studies of the genus *Bartonella* in which Joblet *et al.* (1995) obtained levels of similarity between derived sequences of 80-90 %. These levels of similarity were lower than those obtained for 16S rRNA which where greater than 97.8 % (Birtles & Raoult 1996). A review by La Scola *et al.* 2003 of the most widely used genes for the identification of species within the genus *Bartonella*, namely 16S rDNA, *glt*A, *gro* EL, *rpo*B, *fts*Z, *rib*C and the 16S-23S internal transcribed spacer, reveled that *glt*A and *rpo*B were the most effective in terms of their discriminatory abilities.

Recent studies conducted by Pretorius *et al.* (2004) have shown that a large variety of South African rodents are infected with diverse and in some cases previously undescribed strains of *Bartonella*, in the Free State Province. In the current study we focus solely on subterranean mammalian taxa in an attempt to diagnose for the first time the extent to which this emerging pathogen is present in the South African members of the family Bathyergidae.

2. Material and Methods

2.1 Sampling

Liver samples were collected from freshly captured mole-rats. Thirty four Damaraland mole-rats, *Cryptomys damarensis* from the southern Kalahari, Hotazel Northern Cape Province (25°58'S 21°49'E); 18 Cape mole-rats, *Georychus capensis* from the Darling region of the Western Cape (33°23'S 18°21'E); 19 Natal mole-rats, *Cryptomys hottentotus natalensis* from Glengarry, KwaZulu Natal (25^{o}58’S 21^{o}49’E); 31 Cape dune mole-rats, *Bathyergus suillus* from Cape Town international airport (33^{o}58’S 18^{o}35’E) and 12 highveld mole-rats , *Cryptomys hottentotus pretoriae* from the suburbs of Pretoria, Gauteng (25^{o}43’S 28^{o}13’E). All liver samples were placed in 1.5mL tubes containing Dulbecco’s PBS (with Mg^{2+} and Ca^{2+}) and stored at 4°C. Lice and fleas belonging to the family Hystrichopsyllidae were collected from the Cape dune mole-rats sampled on a separate occasion, but from the same locality, and stored in 90 % ethanol.

2.2. Extraction of Bartonella genetic material for screening

DNA was extracted from all samples by means of the High Pure PCR Template Preparation Kit (Roche Applied Science) following the protocol prescribed by the supplier for mammalian tissue extraction. The DNA was then eluted in a final volume of 200 µl and stored at –20°C until further use.

2.3. Primer design and target amplification region

A 513 base pair (bp) region corresponding to the central region of the *gltA* (Citrate synthase) gene, was targeted for amplification and sequencing. Group-specific primers Bart-EF (5^{1}-CACGACTCYATTGATATTACAGA-3^{1}) and Bart-ER (5^{1}-CACGTGGRTCATAATTTTTATA-3^{1}) were used for PCR amplification (A.D. Bastos, unpublished). A second 453 bp region targeting the 16S-23S inter-genenic region was also amplified with primers 321s (5^{1}-AGATGATGATCCCAAGCCTTCTGG-3^{1}) and 983as (3^{1}TGTTCTYACAACAATGATGATG-5^{1}), (Breitschwerdt & Maggi 2005), in order to independently verify the positive results obtained with the *gltA* primers, and in accordance with the recommendations of Fenollar & Raoult (2004). Species identification was based solely on the results of the *gltA* gene.

2.4. Amplification of the gltA and inter-generic region

PCRs targeting the *gltA* gene contained 5µL of 10x Buffer, 10mM dNTP, 0.6 µM of each of the primers, 1U of *Taq* DNA polymerase and 3µl of DNA template in a final volume of 50µl. Reaction conditions were identical for the inter-generic region, with the exception of the primer concentration, which was 0.4µM. Primer annealing temperatures were 53°C and 54°C for gltA and 16S-23S inter-generic region amplification, respectively

2.5. Infection rate and genetic characterization of positive samples

The results of the PCR were then analyzed by agarose gel electrophoresis on a 1.5% gel, against a 100 bp ladder (Promega). Positives, of the expected band size were purified directly from the tube using a High Pure PCR Product Purification Kit (Roche Applied Science). This purified product was cycle sequenced in a final volume of 10µl with both the forward and reverse primers separately. Following the removal of unincorporated primers, ddNTPs and dNTPs by sodium acetate precipitation, the fluorescently labeled DNA products were run on an ABI377 automated sequencer (Applied Biosystems, California), and viewed with CHROMAS (v. 1.43). Sequence data were then collated using DAPSA v. 4.91 (Harley 2001) and blasted against the Genbank database (www.ncbi.nlm.nih.gov/blast) in order to identify all closely related sequences that have been deposited in this public database (Altschul *et al*. 1997). A final homologous dataset 420 nucleotides in length and comprising 76 taxa was identified for phylogenetic analysis. MODELTEST (Posada & Crandall 1998) was used to select the model that best fits the data. An initial p-distance neighbor-joining tree was inferred, to assess species-designations based on genetic distance alone. This was followed by more rigorous phylogenetic approaches and bootstrap resampling in order to evaluate the reliability of the resulting phylogeny. Minimum evolution was the phenetic approach of choice, with the Kimura 3-parameter (Kimura 1981) model being used for tree inference in MEGA v. 3 (Kumar *et al.* 2004). Nodal support for all phenetic trees was assessed by 10000 bootstrap replications. Parsimony and Maximum Likelihood phylogenies were inferred using PAUP* version 4.0 (Swofford 2002) with nodal support being assessed by 10000 and 200 bootstrap replications, respectively. Successive (*a posteriori*) weighting with the rescaled consistency (RC) was performed in order to reduce the contribution of homoplasious characters to the parsimony analysis.

3. Results

A total of 104 samples representative of five and three different mole-rat species and genera, respectively and one flea genus were screened in this study. Twelve of a total of 31 liver samples from *B. suillus* were positive with the *gltA* primer-set, corresponding to an infection rate of 38.7 % (Table 1). No evidence of *Bartonella* infection was found in the remaining four mole-rat species screened. Interestingly 2 of 8 *B. suillus* fleas obtained from the pelage of the dune mole-rats, collected on a prior occasion from the same locality (infection rate of 25 %.), were positive for the presence of *Bartonella*. The results from the *gltA* PCR screening are summarised in Table 1 and subsequent BLAST search are summarised in Table 2.

TABLE 1 Summary of *Bartonella* positives identified with the *gltA* primer-set

Species analysed	**Number screened**	**% infected**
Cryptomys damarensis	34	0
Georychus capensis	18	0
Cryptomys hottentotus natalensis	19	0
Cryptomys hottentotus pretoriae	12	0
Bathyergus suillus	31	38.7
Fleas (Hystrichopsyllidae)	8	25

TABLE 2 List of the most closely related *Bartonella* sequences in Genbank to those obtained from *B. suillus*

***Bartonella* positive mole-rat sample**	**Closest species match in Genbank**	**% match to closest Genbank sequence**	**Gene Bank accession number of the closest match**
B. suillus 3*	*Bartonella sp.* RP-tr109	92	AJ583119
B. suillus 4	*Bartonella sp.* RP-tr109	98	AJ583119
B. suillus 5	*Bartonella sp.* RP-tr109	98	AJ583119
B. suillus 6	*Bartonella sp.* RP-tr109	98	AJ583119
B. suillus 10*	*Bartonella sp.* RP-tr109	87	AJ583119
B. suillus 894*	*Bartonella sp.* RP-tr109	97	AJ583119
B. suillus 944*	*Bartonella sp.* RP-tr109	98	AJ583119
B. suillus 952*	*Bartonella sp.* RP-tr109	97	AJ583119
B. suillus 953*	*Bartonella sp.* RP-tr109	98	AJ583119
B. suillus 955*	*Bartonella sp.* RP-tr109	98	AJ583119
B. suillus Flea 7*	*Bartonella sp.* af82up	94	AY454539
B. suillus Flea 13*	*Bartonella sp.* RP-tr109	98	AJ583119

* indicates those amplicons only sequenced with forward primer (Bart-EF)

3.1. Nucleotide sequence analysis

Sequence data derived from the *gltA* gene comprised a homologous region of 420 bp, of which 190 sites were variable and 150 were parsimony informative. An A-T bias in base composition was observed, with individual bases having the following an average composition across taxa: T = 31.0 %, C = 21.8 %, A = 26.1 %, G =21.1 %. The transition:transversion ratio (R) was 1.6, indicating slight transitional bias.

The K81uf + G model selected under the Akaike Information Criterion (Posada & Buckley 2004) in ModelTest (AICc =7915.4878, Akaike weight =0.2773, Gamma distribution shape parameter =0.2644) was used for Maximum Likelihood inference in PAUP* version 4.0 (Swofford 2002).

Parsimony analyses performed in PAUP* included equal weighting followed by successive weighting with the rescaled consistency index (RC) in order to reduce the contribution of homoplasious characters. Equal weighting resulted in 579 equally parsimonious trees for *gltA* with a length of 678 and a retention index (RI) = 0.809, consistency index (CI) = 0.431 and rescaled consistency index (RC) = 0.348. Successive weighting with the RC resulted in 3 parsimonious trees with a length of 228.88 and a retention index (RI) = 0.858, consistency index (CI) = 0.624 and rescaled consistency index (RC) = 0.536.

3.2. 16S-23S intergeneric region results

BLAST identification results obtained from the 16S-23S intergeneric region did not match with *Bartonella* species available on the data base and were thus excluded from phylogenetic analyses.

3.3. Phylogenetic analysis and species identification

After performing homology searches against the Genbank database with the BLASTn online tool (Altschul *et al.* 1997) it was apparent that most of the *Bartonella* sequences from mole-rats were most closely related to *Bartonella* sp. RP-tr109 (AJ583119).The only sequence that differed was obtained from *B. suillus* Flea 7, and was most closely related to *Bartonella* sp. af82up (AY454539). these findings were confirmed though the construction of a complete summary of all Bartonella isolates see Table 1. The neighbour-joining tree generated by MEGA (v. 3) with uncorrected p-distances (Figure 1) clearly demonstrates that all the *B. suillus* samples together with *B. suillus* flea 13 form a clade with *Bartonella* sp. RP-tr109 (AJ583119) supported by boot strap values of 94 % for p-distance, 88 % for parsimony, 93 % for minimum evolution and 88 % for maximum likelihood. The second *Bartonella* species found in a flea, namely *B. suillus* flea 7 forms a clade with *Bartonella* sp. af82up (AY454539) supported by boot strap values of 100 % for p-distance, 99 % for parsimony, 100 % for minimum evolution and 100 % for maximum likelihood (Appendix I). However, the genetic distance between these two taxa was 5 % across the gene region sequenced (Figure 1), indicating that the *Bartonella* species in flea 7 represents a distinct and novel species.

Complete alignment generated by MEGA (v. 3) is seen in Appendix II.

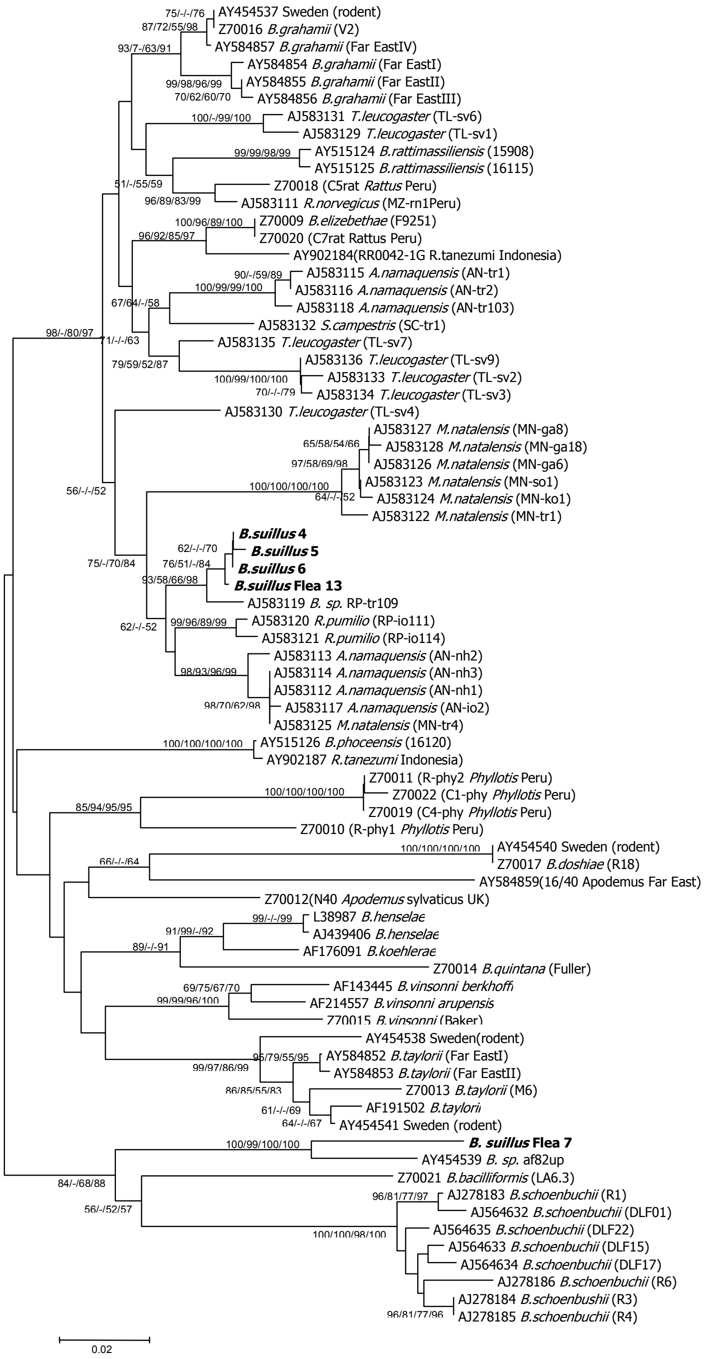

Figure 1 Neighbour Joining (NJ) tree based on partial *gltA* sequences inferred using uncorrected p-distances. Bootstrap values from Minimum Evolution (ME), Maximum Parsimony (MP) and Maximum likelihood (ML) analysis that were > 50 % are indicated next to the relevant nodes as follows (NJ/ MP/ ML / ME).

4. Discussion

The accurate identification of *Bartonella* species infecting host animals has recently received some criticism. Pretorious *et al.* (2004) proved the short comings of using sequence similarity alone to assign the rank of species to individual organisms within the genus *Bartonella*. In their study sequence dissimilarity between individual organisms was sufficiently high to assign them to distinct species. However, a phylogenetic analysis of the data revealed that they formed a monophyletic clade and therefore shared a common ancestor. Validation of the taxonomic status of individuals within this genus can only be achieved through the use of a variety of methods (Lan & Reeves 2001), a finding echoed by Pretorious *et al*. (2004).

Of all the southern African bathyergids tested in this study only samples collected from or in association with *Bathyergus suillus* (Cape dune mole-rat), sub-family Bathyerginae proved to be positive for the presence of the bacterium *Bartonella*. *Bathyergus suillus* is a solitary mole-rat occurring in the southern, southwestern and western regions of the Cape Province of South Africa (Bennett & Faulkes 2000). The Cape dune mole-rat is consumed as a food source by the local inhabitants of the area both historically (Bennett & Faulkes 2000) and during the present. Activities associated with capture of these animals facilitates the opportunity for zoonotic transmission of the bacterium from *B. suillus* to humans, either through infection by a bite or a scratch, or alternatively via an invertebrate vector such as fleas, ticks or lice (Jacomo *et al*. 2002). The latter is possible as the presence of 2 different strains of *Bartonella* in the Hystrichopsyllidid fleas (Table 1) was verified in this study.

The first species of *Bartonella* found to be present in *B. suillus* was identified through a BLAST search (Altschul *et al.* 1997) as *Bartonella* sp. RP-tr109, originally identified by Pretorius *et al.* (2004) from a four striped grass mouse *Rhabdomys pumilio* collected from Gariep in the Free State province of South Africa. The presence of this bacterial species in another species of South African rodent confirms their conclusions regarding the low level of host specificity amongst rodent related *Bartonella* species. Identification of this strain of *Bartonella* in a flea collected from *B. suillus* may point to the Hystrichopsyllidid flea being the likely transmission vector to mole-rats and perhaps other mammal species

The second sequence derived from a haematophagus vector *B. suillus* Flea 7, was shown to be closely related to *Bartonella* sp. af82up (AY454539) originally described by Ehrenborg *et al.* (unpublished). A phylogenetic analysis revealed a monophyletic lineage based on bootstrap support of 100 % in nearly all methods of inference, indicating that the clade is meaningful (Hillis and Bull 1993). Whilst the phylogeny supports common ancestry, genetic distance (> 4 %) indicates that they represent two distinct *Bartonella* species as this value is greater than the 96% sequence identity value proposed by La Scola *et al*. (2003), to distinguish between species on the basis of *gltA* gene sequences.

Based on this, it is concluded that the *Bartonella* present in *B. suillus* flea 7 represents a previously undescribed species.

5. Acknowledgements

We wish to thank Dr. Justin O'Riain from the University of Cape Town for providing us with twenty of the *Bathyergus suillus* samples and Gundula Gutjahr for the flea samples. The collection of samples and the research conducted was approved by the Animal Ethics Committee at the University of Pretoria EC20624–012. The research was funded by the National Research Foundation GUN 2047282 to NCB.

6. Literature cited

ALTSCHUL, S. F.; MADDEN, T.L.; SCHÄFFER, A.A.; ZHANG, J.; ZHANG, Z.; MILLER, W.; & LIPMAN, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25:** 3389-3402.

BENNETT, N.C.; & FAULKES, C.G. 2000. *In:* African mole-rats ecology and eusociality. Cambridge University Press, Cambridge. U.K. 273pp.

BIRTLES, R.J. 2005. Bartonellae as Elegant Hemotropic Parasites. *Annals of the New York Academy of Sciences* **1063:** 270–279.

BIRTLES, R. J.; & RAOULT, D. 1996. Comparison of partial citrate synthase gene (*gltA*) sequences for phylogenetic analysis of *Bartonella* species. *International Journal of Systematic Bacteriology* **46:** 891–897.

BREITSCHWERDT, E.B.; & KORDICK, D.L. 2000. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clinical Microbiology Reviews* **13:** 428-438.

BREITSCHWERDT, E.B.; & MAGGI, R.G. 2005. Potential Limitations of the 16S-23S rRNA Intergeneric Region for Molecular Detection of *Bartonella* Species. *Journal of Clinical Microbiology* **43:** 4921-4922.

EHRENBORG, C.; HANDLEY, S.; ELLIS, B.A.; MILLS, J.N.; & HOLMBERG, M. Genetic diversity over short geographic distances and lack of host specificity among *Bartonella grahamii* infecting Swedish woodland rodents of central Sweden. *Unpublished data*.

FENOLLAR, F.; & RAOULT, D. 2004. Molecular genetic methods for the diagnosis of fastidious microorganisms. *APMIS* **112:** 785–807.

GARRITY, G. M.; BELL, J. A.; & LILBURN, T. 2005. *In:* Bergey's Manual of Systematic Bacteriology, 2nd edn, vol. 2 (The Proteobacteria). (Brenner, D.J.; Krieg, N.R.; Staley, J.T.; & Garrity, G.M. Eds). Springer, New York, N.Y.

GIESZCZYKIEWICZ, M. 1939. Zagadniene systematihki w bakteriologii - Zür Frage der Bakterien-Systematic. *Bulletin de l'Académie Polonaise des Sciences Série des Sciences Biologiques* **1:** *9-27.*

HARLEY, E.H. 2001. DNA and protein sequence analysis. Version 4.91. Department of Chemical pathology, University of Cape Town.

HILLIS, D.M.; & BULL, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42:** 182-192.

JACOMO, V.; KELLY, P.J.; & RAOULT, D. 2002. Natural history of *Bartonella* infections (an exception to Koch's postulate). *Clinical and Diagnostic Laboratory Immunology* **9:** 8–18.

JOBLET, C.; ROUX, V.; DRANCOURT, M; GOUVERNET, J.; & RAOULT, D. 1995. Identification of *Bartonella* (Rochalimaea) species among fastidious gramnegative bacteria on the basis of the partial sequence of the citrate synthase gene. *Journal of Clinical Microbiology* **33:** 1879–1883.

KIMURA, M., 1981. Estimation of evolutionary distances between homologues nucleotide sequences. *Proceedings of the National Academy of Sciences USA* **78:** 454–458.

KUMAR, S.; TAMURA, K.; & NEI, M. 2004. MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5:** 150-163.

LAN, R.; & REEVES, P.R. 2001. When does a clone deserve a name? A perspective on bacterial species based on population genetics. *Trends in Microbiology* **9:** 419–424.

LA SCOLA, B.; ZEAITER, Z.; KHAMIS, A.; & RAOULT, D. 2003. Gene sequence-based criteria for species definition in bacteriology: the Bartonella paradigm. *Trends in Microbiology* **11:** 318–321.

MOLIA, S.; CHOMELA, B.B.; KASTEN, R.W.; LEUTENEGGER, C.M.; STEELE, B.R.; MARKER, L.; MARTENSON, J.S.; KEET, D.F.; BENGIS, R.G.; PETERSON, R.P.; MUNSONG, L.; & O'BRIEN, S.J. 2004. Prevalence of Bartonella infection in wild African lions (*Panthera leo*) and cheetahs (*Acinonyx jubatus*). *Veterinary Microbiology* **100:** 31–41.

PRETORIUS, A.M.; BEATI, L.; & BIRTLES, R.J. 2004. Diversity of *Bartonella* associated with small mammals inhabiting Free State province, South Africa. *International Journal of Systematic and Evolutionary Microbiology* **54:** 1959–1967.

POSADA, D.; & CRANDALL, K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14:** 817-818.

POSADA, D.; & BUCKLEY, T. 2004. Model Selection and Model Averaging in Phylogenetics: Advantages of Akaike Information Criterion and Bayesian Approaches Over Likelihood Ratio Tests. *Systematic Biology* **53:**793 – 808.

REGNERY, R.L.; ANDERSON, B.E.; CLARRIDGE, J.E. III; RODRIGUEZ-

BARRADAS, M.C.; JONES, D.C.; & CARR, J.H. 1992. Characterization of a novel Rochalimaea species, *R. henselae sp.* nov., isolated from blood of a febrile, human immunodeficiency virus-positive patient. *Journal of Clinical Microbiology* **30:** 265–274.

SKERMAN, V.B.D., MCGOWAN, V. & SNEATH, P.H.A. (editors). 1980. Approved Lists of Bacterial Names. *International Journal of Systematic Bacteriology* **30:** 225-420.

SNEATH, P.H.A. 1993. Evidence from Aeromonas for genetic crossing-over in ribosomal sequences. *International Journal of Systematic Bacteriology* **43:** 626–629.

SPRINGER, E.; SACHS, M.S.; WOESE, C.R.; & BOONE, D.R. 1995. Partial gene sequences for the A subunit of methyl-coenzyme M reductase (*mcrI*) as a phylogenetic tool for the family Methanosarcinaceae. *International Journal of Systematic Bacteriology* **45:** 554–559.

STRONG, R.P.; TYZZER, E.E.; BRUES, C.T.; SELLARDS, A.W.; & GASTIABURU, J.C. 1915. *In:* Harvard School of Tropical Medicine. Report of the first expedition to South America, 1913, pp. I-XIV, 1-220, Cambridge, Massachusetts.

SWOFFORD, D.L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.

YING, B., KOSOY, M.Y., MAUPIN, G.O., TSUCHIYA, K.R.; & GAGE, K.L. 2002. Genetic and ecologic characteristics of Bartonella communities in rodents in Southern China. *American Journal of Tropical Medicine and Hygiene* **66:** 622–627.

Appendix I

The p-distance table calculated in MEGA v. 3.1 (Kumar *et al. 2005*) using only closest matches to unknown *B. suillus* and flea samples.

[1] #Z70021_B.bacilliformis(LA6.3)
[2] #AJ278183_B.schoenbuchii(R1)
[3] #AJ278185_B.schoenbuchii(R4)
[4] #AJ278186_B.schoenbuchii(R6)
[5] #AJ564632_B.schoenbuchii(DLF01)
[6] #AJ564633_B.schoenbuchii(DLF15)
[7] #AJ564634_B.schoenbuchii(DLF17)
[8] #AJ564635_B.schoenbuchii(DLF22)
[9] #AJ583131_T.leucogaster(TL-sv6)
[10] #AJ583111_R.norvegicus(MZ-rn1Peru)
[11] #AJ583112_A.namaquensis(AN-nh1)
[12] #AJ583113_A.namaquensis(AN-nh2)
[13] #AJ583114_A.namaquensis(AN-nh3)
[14] #AJ583115_A.namaquensis(AN-tr1)
[15] #AJ583116_A.namaquensis(AN-tr2)
[16] #AJ583117_A.namaquensis(AN-io2)
[17] #AJ583118_A.namaquensis(AN-tr103)
[18] #AJ583120_R.pumilio(RP-io111)
[19] #AJ583121_R.pumilio(RP-io114)
[20] #B._suillus_4
[21] #B._suillus_6
[22] #B._suillus_5
[23] #B._suillus_Flea_7
[24] #B._suillus_Flea_13
[25] # AY454539 B._sp._af82up_af82up_gltA
[26] # AJ583119 B._sp._RP-tr109_RP-tr109_gltA

```
[     1       2     3     4     5     6     7     8     9    10    11    12    13    14    15    16    17    18    19    20    21    22    23    24    25    26 ]
[ 1]
[ 2]  0.110
[ 3]  0.114 0.024
[ 4]  0.119 0.026 0.021
[ 5]  0.114 0.007 0.031 0.033
[ 6]  0.112 0.014 0.010 0.021 0.021
[ 7]  0.114 0.024 0.019 0.026 0.031 0.010
[ 8]  0.110 0.017 0.017 0.029 0.024 0.007 0.017
[ 9]  0.136 0.146 0.146 0.160 0.148 0.148 0.148 0.141
[10]  0.129 0.143 0.145 0.150 0.145 0.143 0.145 0.138 0.053
[11]  0.145 0.152 0.150 0.160 0.157 0.150 0.152 0.148 0.064 0.069
[12]  0.143 0.143 0.140 0.150 0.148 0.140 0.143 0.138 0.064 0.067 0.010
[13]  0.145 0.152 0.150 0.160 0.157 0.150 0.152 0.148 0.064 0.069 0.000 0.010
[14]  0.131 0.148 0.150 0.152 0.150 0.148 0.150 0.143 0.069 0.060 0.086 0.079 0.086
[15]  0.133 0.145 0.148 0.150 0.148 0.145 0.148 0.140 0.067 0.057 0.083 0.076 0.083 0.002
[16]  0.148 0.155 0.152 0.162 0.160 0.152 0.155 0.150 0.067 0.071 0.002 0.012 0.002 0.088 0.086
[17]  0.131 0.148 0.150 0.152 0.150 0.148 0.150 0.143 0.069 0.055 0.086 0.079 0.086 0.010 0.007 0.083
[18]  0.124 0.131 0.133 0.143 0.136 0.133 0.136 0.126 0.057 0.064 0.033 0.038 0.033 0.079 0.076 0.036 0.079
[19]  0.126 0.133 0.136 0.145 0.138 0.136 0.138 0.129 0.060 0.067 0.036 0.040 0.036 0.081 0.079 0.038 0.081 0.007
[20]  0.129 0.145 0.145 0.155 0.150 0.145 0.148 0.143 0.057 0.062 0.033 0.038 0.033 0.076 0.074 0.036 0.076 0.026 0.029
[21]  0.129 0.145 0.145 0.155 0.150 0.145 0.148 0.143 0.057 0.062 0.033 0.038 0.033 0.076 0.074 0.036 0.076 0.026 0.029 0.000
[22]  0.134 0.151 0.151 0.156 0.156 0.151 0.153 0.149 0.062 0.064 0.035 0.040 0.035 0.077 0.074 0.037 0.077 0.030 0.032 0.002 0.002
[23]  0.131 0.136 0.138 0.138 0.138 0.136 0.133 0.136 0.146 0.140 0.148 0.148 0.148 0.150 0.148 0.150 0.145 0.133 0.131 0.138 0.138 0.136
[24]  0.126 0.143 0.143 0.152 0.148 0.143 0.145 0.140 0.055 0.060 0.036 0.040 0.036 0.074 0.071 0.038 0.074 0.029 0.031 0.002 0.002 0.005 0.138
[25]  0.114 0.121 0.129 0.126 0.126 0.126 0.119 0.126 0.148 0.133 0.133 0.131 0.133 0.138 0.136 0.136 0.133 0.126 0.124 0.136 0.136 0.134 0.055 0.136
[26]  0.131 0.152 0.152 0.162 0.155 0.152 0.155 0.148 0.060 0.060 0.040 0.045 0.040 0.074 0.071 0.043 0.074 0.033 0.036 0.012 0.012 0.015 0.143 0.014 0.140
```

Appendix II

The alignment created in MEGA v. 3.1 (Kumar *et al. 2005*). Dots indicate base pair similarity with the first entry, whereas ? indicates missing data.

```
B.henselae                       GCC TAT AAA TAT AGC ATT GGA CAA GCA TTT GTT TAT CCA CGT AAT GAT CTT AGT TAC GCT GCA AAT TTT CTC CGT ATG  [ 99]
AF143445_B.vinsonni_berkhoffi    ..T ... ... ..C ..T ..C ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ...  [ 99]
AF191502_B.taylorii              ..T ... ... ... ..T ..A ..G ... ..C ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ...  [ 99]
AF176091_B.koehlerae             ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... .A. ...  [ 99]
AF214557_B.vinsonni_arupensis    ..T ... ... ..C ..T ..C ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ..C ... ... ...  [ 99]
AJ278184_B.schoenbushii(R3)      ..T ... ... ..C ... ..C ... ..G C.T ... ... ... ... ... ..C ... ... ..C ..T ... ... ... ..C ..T .A. ...  [ 99]
AJ439406_B.henselae              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...  [ 99]
AY454537_Sweden(rodent)          ... ... ... ..C ..T ..C ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
AY454538_Sweden(rodent)          ..A ... ... ... ..T ..C ..G ... ..C ... ... ... ... ..A ... ... ... ... ... ... ..T ... ... ... ... ...  [ 99]
AY454540_Sweden(rodent)          ..T ..C ... ... ... ... ... ... ... ... ..G ... ..C .A. ... AG. ... ..C ..T ..C ... ... ..C ..T ... ...  [ 99]
AY454541_Sweden(rodent)          ..T ... ... ... ..T ..A ..G ... ..C ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ...  [ 99]
AY515124_B.rattimassiliensis(159 ... ... ... ..C ..T ..C ... ... C.. ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
AY515125_B.rattimassiliensis(161 ... ... ... ..C ..T ..C ... ... C.. ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
AY515126_B.phoceensis(16120)     ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ..C ... ..T ..C ... ... ... ..T ... ...  [ 99]
AY584852_B.taylorii(Far_EastI)   ..T ... ... ... ..T ..A ..G ... ..C ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ...  [ 99]
AY584853_B.taylorii(Far_EastII)  ..T ... ... ... ..T ..A ..G ... ..C ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ...  [ 99]
AY584854_B.grahamii(Far_EastI)   ... ... ... ..C ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..C ... .A. ...  [ 99]
AY584855_B.grahamii(Far_EastII)  ... ... ... ..C ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..C ... .A. ...  [ 99]
AY584856_B.grahamii(Far_EastIII) T.. ... ... ..C ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..C ... .A. ...  [ 99]
AY584857_B.grahamii(Far_EastIV)  ... ... ... ..C ..T ..C ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
AY584859(16/40_Apodemus_Far_East ..T ... ... ... ..T ..C ... ... ... ... ... ... ..T .A. ... ... ... ..C ... A.. .A. ... ... ..T ... ...  [ 99]
Z70009_B.elizebethae(F9251)      ... ... ... ... ..T ... ... ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
Z70010(R-phy1_Phyllotis_Peru)    ... ... ... ..C ..T ..C ..G ... ..T ... A.. ... ..G ... ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
Z70011(R-phy2_Phyllotis_Peru)    ... ... ... ..C ..T ..C ... ... ..T ... ..C ... ..G ... ... ... ... ... ... ... ..G ... ..C ... ... ...  [ 99]
Z70012(N40_Apodemus_sylvaticus_U ..T ... ... ... ..T ... ... ... ... ... ..G ... ... ..A ... ... ... ... ..T ... ... ... ... ..T ... ...  [ 99]
Z70013_B.taylorii(M6)            ..T ... ... ... ..T ..A ..G ... ..C ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ...  [ 99]
Z70014_B.quintana(Fuller)        ..G ... ... ... ... ..C ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... .AC ...  [ 99]
Z70015_B.vinsonni(Baker)         ..T ... ... ..C ..T ..C ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ..C ... ... ...  [ 99]
Z70016_B.grahamii(V2)            ... ... ... ..C ..T ..C ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
Z70017_B.doshiae(R18)            ..T ..C ... ... ... ... ... ... ... ... ..G ... ..C .A. ... AG. ... ..C ..T ..C ... ... ..C ..T ... ...  [ 99]
Z70018(C5rat_Rattus_Peru)        ... ... ... ... ..T ... ... ... C.. ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
Z70019(C4-phy_Phyllotis_Peru)    ... ... ... ..C ..T ..C ... ... ..T ... ..C ... ..G ... ... ... ... ... ... ... ..G ... ..C ... ... ...  [ 99]
Z70020(C7rat_Rattus_Peru)        ... ... ... ... ..T ... ... ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
Z70021_B.bacilliformis(LA6.3)    ..A ... ... ... ..T ... ..G ... C.T ... ... ... ... ... ... ..C ... .A. ... ... A.. ... ... ..T .A. ...  [ 99]
Z70022(C1-phy_Phyllotis_Peru)    ... ... ... ..C ..T ..C ... ... ..T ... ..C ... ..G ... ... ... ... ... ... ... ..G ... ..C ... ... ...  [ 99]
AJ278183_B.schoenbuchii(R1)      ..T ... ... ..C ... ..C ... ... C.T ... ... ... ... ... ..C ... ... G.. ..T ... ... ... ..C ..T .AC ...  [ 99]
AJ278185_B.schoenbuchii(R4)      ..T ... ... ..C ... ..C ... ..G C.T ... ... ... ... ... ..C ... ... ..C ..T ... ... ... ..C ..T .A. ...  [ 99]
AJ278186_B.schoenbuchii(R6)      ..T ... ... ..C ... ... ... ..G C.T ... ... ... ... ... ..C ... ... G.. ..T ... ... ... ..C ..T .AC ...  [ 99]
AJ564632_B.schoenbuchii(DLF01)   ..T ... ... ..C ... ..C ... ... C.T ... ... ... ... ... ..C ... ... G.. ..T ... ... ... ..C ..T .AC ...  [ 99]
AJ564633_B.schoenbuchii(DLF15)   ..T ... ... ..C ... ..C ... ..G C.T ... ... ... ... ... ..C ... ... ..C ..T ... ... ... ..C ..T .AC ...  [ 99]
AJ564634_B.schoenbuchii(DLF17)   ..T ... ... ..C ... ..C ... ..G C.T ... ... ... ... ... ..C ... ... ..C ..T ... ... ... ..C ..T .AC ...  [ 99]
AJ564635_B.schoenbuchii(DLF22)   ..T ... ... ..C ... ..C ... ..G C.T ... ... ... ... ... ..C ... ... ..C ..T ... ... ... ..C ..T .AC ...  [ 99]
```

```
AJ583131_T.leucogaster(TL-sv6)   ... ... ... ..C ..T ..C ... ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ... ... ... ...  [ 99]
AJ583111_R.norvegicus(MZ-rn1Peru ... ... ... ... ..T ... ... ... C.. ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
AJ583112_A.namaquensis(AN-nh1)   ... ... ... ..C ..T ..C ..G ... ... ... ... ... ... ..C ... ... ..C ... ..T ... ... ... ..C ... ... ...  [ 99]
AJ583113_A.namaquensis(AN-nh2)   ..T ... ... ..C ..T ..C ... ... ... ... ... ... ... ..C ... ... ..C ... ..T ... ... ... ..C ... ... ...  [ 99]
AJ583114_A.namaquensis(AN-nh3)   ... ... ... ..C ..T ..C ..G ... ... ... ... ... ... ..C ... ... ..C ... ..T ... ... ... ..C ... ... ...  [ 99]
AJ583115_A.namaquensis(AN-tr1)   ..T ... ... ... ..T ... ... ... ..G ... ... ... ... T.. ... ... ... ... ..T ... ... ... ... ... ... ...  [ 99]
AJ583116_A.namaquensis(AN-tr2)   ..T ... ... ... ..T ... ... ... ..G ... ... ... ... T.. ... ... ... ... ..T ... ... ... ... ... ... ...  [ 99]
AJ583117_A.namaquensis(AN-io2)   ... ... ... ..C ..T ..C ..G ... ... ... ... A.. ... ..C ... ... ..C ... ..T ... ... ... ..C ... ... ...  [ 99]
AJ583118_A.namaquensis(AN-tr103) ..T ... ... ... ..T ... ... ... ..G ... ... A.. ... T.. ... ... ... ... ..T ... ... ... ... ... ... ...  [ 99]
AJ583120_R.pumilio(RP-io111)     ... ... ... ..C ..T ..C ..G ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ..T ... ...  [ 99]
AJ583121_R.pumilio(RP-io114)     ... ... ... ..C ..T ..C ..G ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ..T ... ...  [ 99]
AJ583122_M.natalensis(MN-tr1)    ... ... ... ..C ..T ..C ... ... ... ... ... ..C ... ... ... ... ... ... ..T ..C ... ..C ..C ..T ... ...  [ 99]
AJ583123_M.natalensis(MN-so1)    ..T ... ... ..C ..T ..C ... ... ... ... ... ..C ... ... ... ... ... ... ..T ..C ... ..C ..C ..T ... ...  [ 99]
AJ583124_M.natalensis(MN-ko1)    ..T ... ... ..C ..T ..C ... ... ... ... ... ..C ... ... ... ... ... ... ..T ..C ... ..C ..C .CT ... ...  [ 99]
AJ583125_M.natalensis(MN-tr4)    ... ... ... ..C ..T ..C ..G ... ... ... ... ... ... ..C ... ... ..C ... ..T ... ... ... ..C ... ... ...  [ 99]
AJ583126_M.natalensis(MN-ga6)    ..T ... ... ..C ..T ..C ... ... ... ... ... ..C ... ... ... ... ... ... ..T ..C ... ..C ..C ..T ... ...  [ 99]
AJ583127_M.natalensis(MN-ga8)    ..T ... ... ..C ..T ..C ... ... ... ... ... ..C ... ... ... ... ... ... ..T ..C ... ..C ..C ..T ... ...  [ 99]
AJ583128_M.natalensis(MN-ga18)   ..T ... ... ..C ..T ..C ... ... ... ... ... ..C ... ... ... ... ... ... ..T ..C ... ..C ..C ..T ... ...  [ 99]
AJ583129_T.leucogaster(TL-sv1)   ... ... ... ..C ..T ..C ... ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ... ... ... ...  [ 99]
AJ583130_T.leucogaster(TL-sv4)   ..T ... ... ..C ..T ..C ..G ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
AJ583132_S.campestris(SC-tr1)    ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ...  [ 99]
AJ583133_T.leucogaster(TL-sv2)   ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ..C ..C ... ... ...  [ 99]
AJ583134_T.leucogaster(TL-sv3)   ... ... G.. ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ..C ..C ... ... ...  [ 99]
AJ583135_T.leucogaster(TL-sv7)   ... ... ... ... ..T ... ... ... ... ... ... ... ... ..C ... ... ... ... ..T ..G ... ... ..C ... ... ...  [ 99]
AJ583136_T.leucogaster(TL-sv9)   ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ..C ..C ... ... ...  [ 99]
AY902184(RR0042-1G_R.tanezumi_In ... ... ... ... ..T ... ... ... ... ... ... ... ..T ... ... ... ... ... ..T ... ... ... ..C ..T ... ...  [ 99]
AY902187(RR0037-2_GR.tanezumi_In ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ..C ... ..T ..C ... ... ... ..T ... ...  [ 99]
B._suillus_4                     ... ... ... ..C ..T ..C ..G ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
B._suillus_6                     ... ... ... ..C ..T ..C ..G ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
B._suillus_5                     ... ... ... ..C ..T ..C ..G ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
B._suillus_Flea_7                ..A ... ... ..C ..T ... ..G ... C.. ... A.. ... ..C ... ..C ... ..C ... ... ... ... ..C ..C ..T .A. ...  [ 99]
B._suillus_Flea_13               ... ... ... ..C ..T ..C ..G ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]
AY454539_B._sp._af82up           ..A ... ... ... ..T ... ..G ... C.. ... ... ... ... ... ..C ... ..A ... ... ... ... ... ..C ..T .A. ...  [ 99]
AJ583119_B._sp._RP-tr109         ... ... ... ... ..T ..C ..G ... ... ... ... ... ... ..C ... ... ... ... ..T ... ... ... ..C ... ... ...  [ 99]

L38987_B.henselae                TGT TTT TCT GTT CCT TGT GAA GAA TAT AAA ATT AAT CCG GTG CTG ACT CGA GCT ATG GAT AGA ATC TTT ACT CTT CAT  [177]
AF143445_B.vinsonni_berkhoffi    ... ... ... ... ..A ... ... ... ..C ... .CC ..C ..T ..A ..C ..A ... ..A ... ... C.. ... ... .TC ... ...  [177]
AF191502_B.taylorii              ..C ... .GC ... ... ... ... ... ..C ... G.C ... T.T ..T ..T ... ..G ..A ... ... C.. ... ... .TC ..G ...  [177]
AF176091_B.koehlerae             ... ... ... A.C ... ... ... ... ... ... ... ... ..A ... ..A ... ... ... ... ... C.. ... ... ..C ... ...  [177]
AF214557_B.vinsonni_arupensis    ..C ... ... ... ... ... ... ... ..C ... .CC ..C ..T ..A ..C ..A ... ..A ... ... C.. ... ... .TC ... ...  [177]
AJ278184_B.schoenbushii(R3)      ..C ... ... ... ... ... ... ..G ..C ... G.. ... ..A ..T ..T G.G ... ... ... ..C C.. ... ... ... ..G ...  [177]
AJ439406_B.henselae              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ...  [177]
AY454537_Sweden(rodent)          ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..T G.. ..G ..G ... ... CA. ... ... .TC ... ...  [177]
AY454538_Sweden(rodent)          ..C ... .GC ... ... ... ... ... ..C ... ..C ... ..C ..T ..T ... ... ..A ... ... C.. ..T ... .T. ... ...  [177]
AY454540_Sweden(rodent)          ..C ... G.. ..C ... ... ... ... ..C C.. G.. ... ..A ... ..C ... ... ... ... ... ... ..T ... .TC ... ...  [177]
AY454541_Sweden(rodent)          ..C ... .GC ... ... ... ... ... ..C ... G.C ... ..C ..T ..T ... ..G ..A ... ... C.. ... ... .TC ..G ...  [177]
AY515124_B.rattimassiliensis(159 ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ..A ..T G.. ... ..G ... ... CA. ... ... .TC ... ...  [177]
AY515125_B.rattimassiliensis(161 ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ..A ..T G.. ... ..G ... ... CA. ... ... .TC ... ...  [177]
AY515126_B.phoceensis(16120)     ..C ... ... ... ... ... ... ... ... ... ..C ..C ..T ... ..C GT. ..G ..G ... ... C.. ... ... .TC ... ...  [177]
AY584852_B.taylorii(Far_EastI)   ..C ... .GC ... ... ... ... ... ..C ... ..C ... ..C ..T ..C ... ..G ..A ... ... C.. ... ... .TC ..G ...  [177]
AY584853_B.taylorii(Far_EastII)  ..C ... .GC ... ... ... ... ... ..C ... ..C ... ..C ..T ..C ... ..G ..A ... ... C.. ... ... .TC ..G ...  [177]
AY584854_B.grahamii(Far_EastI)   ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..T G.. ..G ..G ... ... CA. ..A ... ..C ... ...  [177]
AY584855_B.grahamii(Far_EastII)  ... ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..T G.. ..G ..G ... ... CA. ..A ... ..C ... ...  [177]
AY584856_B.grahamii(Far_EastIII) ... ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..T G.. ..G ..G ... ... CA. ..A ... ..C ... ...  [177]
```

```
AY584857_B.grahamii(Far_EastIV)  ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..T G.. ..G ..G ... ... CA. ... ... .TC ... ...  [177]
AY584859(16/40_Apodemus_Far_East ..C ... .G. ... ... ... ... ... ... ... ..A ... ..T ... ..C G.. ... ..A ... ... C.. ... ... .TC ..C ...  [177]
Z70009_B.elizebethae(F9251)      ... ... G.. ... ... ... ... ... ... ... ..A ..C ..T ... ..T G.C ... ..A ... ... CA. ... ... .TC ... ...  [177]
Z70010(R-phy1_Phyllotis_Peru)    ..C ... ... ... ... ... ... ... ..C ... .CC ... ..C ... ..C ... ... ..A ... ... C.. ... ... .T. ... ...  [177]
Z70011(R-phy2_Phyllotis_Peru)    ... ... ... ..C ... ... ... ... ..C ... .CC ... ..C ... ..T ... ... ..A ... ... C.. ... ... .TC ... ...  [177]
Z70012(N40_Apodemus_sylvaticus_U ..C ... ... ... ... ... ... ... ... ... .CA ... ..T ..T ..T ... ... ..A ... ..C CA. ... ... .TC ..C ...  [177]
Z70013_B.taylorii(M6)            ..C ... ..C ... ... ... ... .G. ..C ..T ..C ... T.C ..T ..C ... ..G ..A ... ... C.. ... ... ..C ..G ...  [177]
Z70014_B.quintana(Fuller)        ... ... ..C ... ... ... ... ... ..C ... ... ... ..A ..A ..T .G. ... ... ... ... C.. ... .G. ... ... ...  [177]
Z70015_B.vinsonni(Baker)         ... ... ... ... ..G ... ... ... ..C ... .CC ..C ..T ..A ..C ..A ... ..A ... ... C.. ... ... .TC ... ...  [177]
Z70016_B.grahamii(V2)            ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..T G.. ..G ..G ... ... CA. ... ... .TC ... ...  [177]
Z70017_B.doshiae(R18)            ..C ... G.. ..C ... ... ... ... ..C C.. G.. ... ..A ... ..C ... ... ... ... ... ... ..T ... .TC ... ...  [177]
Z70018(C5rat_Rattus_Peru)        ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..T T.. ..G ..A ... ... CA. ... ... .TC ... ...  [177]
Z70019(C4-phy_Phyllotis_Peru)    ... ... ... ..C ... ... ... ... ..C ... .CC ... ..C ... ..T ... ... ..A ... ... C.. ... ... .TC ... ...  [177]
Z70020(C7rat_Rattus_Peru)        ... ... G.. ... ... ... ... ... ... ... ..A ..C ..T ... ..T G.C ... ..A ... ... CA. ... ... .TC ... ...  [177]
Z70021_B.bacilliformis(LA6.3)    ..C ..C ... ... ... ... ... ... C.C ... ... .GC ..T ..T A.T G.. ... ... ... ... C.. ... ... ... ... ...  [177]
Z70022(C1-phy_Phyllotis_Peru)    ... ... ... ..C ... ... ... ... ..C ... .CC ... ..C ... ..T ... ... ..A ... ... C.. ... ... .TC ... ...  [177]
AJ278183_B.schoenbuchii(R1)      ..C ... ... ... ... ... ... ... ..C ... G.. ... ..A ..T ..T G.G ... ... ... ..C C.. ... ... ... ..A ...  [177]
AJ278185_B.schoenbuchii(R4)      ..C ... ... ... ... ... ... ..G ..C ... G.. ... ..A ..T ..T G.G ... ... ... ..C C.. ... ... ... ..G ...  [177]
AJ278186_B.schoenbuchii(R6)      ..C ... ... ... ... ... ... ..G ..C ... G.. ... ..A ..T ..T G.A ... ... ... ..C C.. ... ... ... ..G ...  [177]
AJ564632_B.schoenbuchii(DLF01)   ..C ... ... ... ... ... ... ... ..C ... G.. ... ..A ..T ..T G.G ... ... ... ..C C.. ... ... ... ..A ...  [177]
AJ564633_B.schoenbuchii(DLF15)   ..C ... ... ... ... ... ... ... ..C ... G.. ... ..A ..T ..T G.G ... ... ... ..C C.. ... ... ... ..A ...  [177]
AJ564634_B.schoenbuchii(DLF17)   ..C ... ... ... ... ... ... ... ..C ... G.. ... ..A ..T ..T G.A ... ... ... ..C C.. ... ..C ... ..A ...  [177]
AJ564635_B.schoenbuchii(DLF22)   ..C ... ... ... ... ... ... ... ..C ... G.. ... ..T ..T ..T G.G ... ... ... ..C C.. ... ... ... ..A ...  [177]
AJ583131_T.leucogaster(TL-sv6)   ..C ... G.. ... ... ... ... ... ... .T. .CA ... ..T ..A ..T T.. ... ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583111_R.norvegicus(MZ-rn1Peru ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..T T.. ..G ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583112_A.namaquensis(AN-nh1)   ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..T ... ..T ... ... ..G ... ... CA. ... ... .TC ... ...  [177]
AJ583113_A.namaquensis(AN-nh2)   ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..T ... ..T ... ... ..G ... ... CA. ... ... .TC ... ...  [177]
AJ583114_A.namaquensis(AN-nh3)   ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..T ... ..T ... ... ..G ... ... CA. ... ... .TC ... ...  [177]
AJ583115_A.namaquensis(AN-tr1)   ..C ... G.. ... ... ... ... ... ... ... .CA .GC ..T ... ..C G.. ..G ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583116_A.namaquensis(AN-tr2)   ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..C G.. ..G ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583117_A.namaquensis(AN-io2)   ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..T ... ..T ... ... ..G ... ... CA. ... ... .TC ... ...  [177]
AJ583118_A.namaquensis(AN-tr103) ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..C G.. ..G ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583120_R.pumilio(RP-io111)     ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..T ... ..T G.. ... ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583121_R.pumilio(RP-io114)     C.C ... ... ... ... ... ... ..T ... ... TCA ..C ..T ... ..T G.. ... ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583122_M.natalensis(MN-tr1)    ..C ... ... ... ... ... ... ..T ... C.. TCA ... ..C ... ..T G.. ..G ..G ... ... CA. ..T ... .T. ... ..C  [177]
AJ583123_M.natalensis(MN-so1)    ..C ... ... ... ... ... ... ..T ... C.. TCA ... ..C ... ..T G.. ..G ..G ... ... CA. ..T ... .TC ... ..C  [177]
AJ583124_M.natalensis(MN-ko1)    ..C ... ... ... ... ... ... ..T ... C.. TCA ... ..C ... ..T G.. ..G ..G ... ... CA. ..T ... .TC ... ..C  [177]
AJ583125_M.natalensis(MN-tr4)    ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..T ... ..T ... ... ..G ... ... CA. ... ... .TC ... ...  [177]
AJ583126_M.natalensis(MN-ga6)    ..C ... ... ... ... ... ... ..T ... C.. TCA ... ..C ... ..T G.. ..G ..G ... ... CA. ..T ... .TC ... ..C  [177]
AJ583127_M.natalensis(MN-ga8)    ..C ... ... ... ... ... ... ..T ... C.. TCA ... ..C ... ..T G.. ..G ..G ... ... CA. ..T ... .TC ... ..C  [177]
AJ583128_M.natalensis(MN-ga18)   ..C ... ... ... ... ... ... ..T ... C.. TCA ... ..C ... ..T G.. ..G ..G ... ... CA. ..T ... .TC ... ..C  [177]
AJ583129_T.leucogaster(TL-sv1)   ..C ... G.. ... ... ... ... ... ... .C. .CA ..C ..T ..A ..T T.. ... ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583130_T.leucogaster(TL-sv4)   ..C ... ... ... ... ... ... ... ... ... G.A ..C ..T ... ..T G.. ..G ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583132_S.campestris(SC-tr1)    ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ... ..T G.. ..G ..A ... ... .A. ... ... .T. ... ...  [177]
AJ583133_T.leucogaster(TL-sv2)   ..C ... G.. ... ... ... ... ... ... G.. .CA ..C ..T ..T ..T G.. ..G ..G ... ... .A. ... ... .TC ... ...  [177]
AJ583134_T.leucogaster(TL-sv3)   ..C ... G.. ... ... ... ... ... ... G.. .CA ..C ..T ..T ..T G.. ..G ..G ... ... .A. ... ... .TC ... ...  [177]
AJ583135_T.leucogaster(TL-sv7)   ..C ... G.. ... ... ... ... ... ... ... .CA ..C ..T ..A ..T G.. ..G ..A ... ... CA. ... ... .TC ... ...  [177]
AJ583136_T.leucogaster(TL-sv9)   ..C ... G.. ... ... ... ... ... ... G.. .CA ..C ..T ..T ..T G.. ..G ..G ... ... .A. ... ... .TC ... ...  [177]
AY902184(RR0042-1G_R.tanezumi_In ... ... G.. ... ... ... ... ... ... ... ..A ..C ..T ..A ..T G.C ... ..A ... ... CA. ... ... .TC ... ...  [177]
AY902187(RR0037-2_GR.tanezumi_In ..C ... ... ... ... ... ... ... ... ... ..C ..C ..T ..A ..C GT. ..G ..G ... ... C.. ... ... .TC ... ...  [177]
B._suillus_4                     ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..C ... ..T G.. ... ..G ... ... CA. ... ... .TC ... ...  [177]
B._suillus_6                     ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..C ... ..T G.. ... ..G ... ... CA. ... ... .TC ... ...  [177]
B._suillus_5                     ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..C ... ..T G.. ... ..G ... ... CA. ... ... .TC ... ...  [177]
B._suillus_Flea_7                ..C ... ... ... ... ..C C.. ... ... ... ... ... ..A ..A ..T G.. ... ... ... ..C C.. ..T ... ..C ... ...  [177]
```

```
B._suillus_Flea_13               ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..C ... ..T G.. ... ..G ... ... CA. ... ... .TC ... ...  [177]
AY454539_B._sp._af82up           ..C ... ..C ... ... ... ... ... ... ... ... ... ..A ... ..T G.. ... ... ... ..C C.. ..T ..C ..C ... ...  [177]
AJ583119_B._sp._RP-tr109         ..C ... ... ... ... ... ... ..T ... ... TCA ..C ..T ... ..T G.. ... ..G ... ... CA. ... ... .TC ... ...  [177]

L38987_B.henselae                GCA GAT CAT GAA CAA AAT GCT TCG ACA TCC ACT GTA CGT CTT GCA GGT TCA TCA GGT GCT AAT CCT TT GCA TGT ATT G [256]
AF143445_B.vinsonni_berkhoffi    ... ... ..C ... ... ... ... ..T ... ... ... ... ..C ... ..T ..G ... ... ..C ... ... ... .. ... ... ..C . [256]
AF191502_B.taylorii              ... ... ... ... ... ... ... ..T ... ... ... ... ..C ... ..T ..A ... ... ... ... ... ... .. ... ... ..C . [256]
AF176091_B.koehlerae             ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ... ... ... . [256]
AF214557_B.vinsonni_arupensis    ... ... ..C ... ... ... ... ..C ... ... ... ... ..C ... ..T ..A ... ... ..C ... ... ... .. ... ... ..C . [256]
AJ278184_B.schoenbushii(R3)      ... ... ... ... ... ..C ..A ..C ... ..A ..C ... ... ..G ... ... ... ... ..A ..A ... ... .. ..G ... ... . [256]
AJ439406_B.henselae              ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ... ... ... . [256]
AY454537_Sweden(rodent)          ... ... ... ... ... ... ... ..T ... ..T ..G ... ... ... ..T ..A ..C ..G ... ... ... ... .. ..C ... ... . [256]
AY454538_Sweden(rodent)          ... ..C ... ... ... ... ... ..T ... ... ... ... ..C ... ... ..A ... ... ... ... ... ... .. ... ... ..C . [256]
AY454540_Sweden(rodent)          ..C ... ... ... ... ... ... ..T ..C ... ... ... ..C ... ... ..A ... ... ..G ... ... ... .. ... ... ..C . [256]
AY454541_Sweden(rodent)          ... ... ... ... ... ... ... ..T ... ... ... ... ..C ... ..T ..A ... ... ... ... ... ... .. ... ... ..C . [256]
AY515124_B.rattimassiliensis(159 ..T ... ... ..G ... ... ... ..T ... ..T ..G ... ... ... ..T ..A ..T ..G ... ... ... ... .. ... ... ..C . [256]
AY515125_B.rattimassiliensis(161 ..T ... ... ..G ... ... ... ..T ... ..T ..G ... ..C ... ..T ..A ..T ..G ... ... ... ... .. ... ... ..C . [256]
AY515126_B.phoceensis(16120)     ... ... ... ... ... ... ... ..C ... ..T ..C ... ..C ... ... ..A ... ... ..A ... ... ... .. ... ... ..C . [256]
AY584852_B.taylorii(Far_EastI)   ... ... ... ... ... ... ... ..T ... ... ... ... ..C ... ..T ..A ... ... ... ... ... ... .. ... ... ..C . [256]
AY584853_B.taylorii(Far_EastII)  ... ... ... ... ... ... ... ..T ... ... ... ... ..C ... ..T ..A ... ... ... ... ... ... .. ... ... ..C . [256]
AY584854_B.grahamii(Far_EastI)   ... ... ... ... ... ... ... ..T ... ..T ..G ... ... ... ..T ..A ..C ... ... ... ... ... .. ..C ... ... . [256]
AY584855_B.grahamii(Far_EastII)  ... ... ... ... ... ... ... ..T ... ..T ..G ... ... ... ..T ..A ..C ... ... ... ... ... .. ..C ... ... . [256]
AY584856_B.grahamii(Far_EastIII) ... ... ... ... ... ... ... ..T ... ..T ..G ... ... ... ..T ..A ..C ... ... ... ... ... .. ..C ... ... . [256]
AY584857_B.grahamii(Far_EastIV)  ... ... ... ... ... ... ... ..T ... ..T ..G ... ... ... ..T ..A ..C ... ... ... ... ... .. ..C ... ... . [256]
AY584859(16/40_Apodemus_Far_East ... ... ... ... ... ... ..C ..T ..C ... ... ... ..C ... ..G ..A ... ... ... ... ... ... .. ..G ... ... . [256]
Z70009_B.elizebethae(F9251)      ... ... ... ... ... ... ... ..T ... ..G ..A ..C ... ... ..T ..A ... ... ..G ... ... ... .. ..C ... ... . [256]
Z70010(R-phy1_Phyllotis_Peru)    ... ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ..C ..T ..G ... ... ... ... .. ... ... ... . [256]
Z70011(R-phy2_Phyllotis_Peru)    ... ... ... ... ... ... ... ..T ... ... ... ..G ..C ... ... ..A ... ... ..C ... ... ... .. ... ... ... . [256]
Z70012(N40_Apodemus_sylvaticus_U ... ... ..C ... ... ... ... ..T ... ... ... ... ..C ... ... ..A ... ... ..A ... ... ... .. ... ... ... . [256]
Z70013_B.taylorii(M6)            ... ... ... ... ... ... ..C ..T ... ... ... ... ..A ... ..T ..A ... ... ... ... ... ... .. ... ... ..C . [256]
Z70014_B.quintana(Fuller)        ... ... ... ... ... ... ... ..T ... ... ... ... ... ... .T. ..A ... ..G ..A ... ... ... .. ... ... ..C . [256]
Z70015_B.vinsonni(Baker)         ... ... ..C ... ... ... ... ..T ... ... ... ... ..C ... ..T ..G ... ... ..C ... ... ... .. ... ... ..C . [256]
Z70016_B.grahamii(V2)            ... ... ... ... ... ... ... ..T ... ..T ..G ... ... ... ..T ..A ..C ..G ... ... ... ... .. ..C ... ... . [256]
Z70017_B.doshiae(R18)            ..C ... ... ... ... ... ... ..T ..C ... ... ... ..C ... ... ..A ... ... ..G ... ... ... .. ... ... ..C . [256]
Z70018(C5rat_Rattus_Peru)        ..T ... ... ..G ... ... ... ..T ... ..T ..G ... ..A ... ..T ..A ... ..G ..G ..C ... ... .. ..C ... ... . [256]
Z70019(C4-phy_Phyllotis_Peru)    ... ... ... ... ... ... ... ..T ... ... ... ..G ..C ... ... ..A ... ... ..C ... ... ... .. ... ... ... . [256]
Z70020(C7rat_Rattus_Peru)        ... ... ... ... ... ... ... ..T ... ..G ..A ..C ... ... ..T ..A ... ... ..G ... ... ... .. ..C ... ... . [256]
Z70021_B.bacilliformis(LA6.3)    ... ... ... ... ... ... ..A ..T ..G ..A ..A ... ..C ... ... ... ... ... ..A ... ... ... A. ... ... ... . [256]
Z70022(C1-phy_Phyllotis_Peru)    ... ... ... ... ... ... ... ..T ... ... ... ..G ..C ... ... ..A ... ... ..C ... ... ... .. ... ... ... . [256]
AJ278183_B.schoenbuchii(R1)      ... ... ... ... ... ..C ..A ..C ... ..A ..C ... ... ..A ... ... ... ... ..A ..A ... ... .. ..G ... ... . [256]
AJ278185_B.schoenbuchii(R4)      ... ... ... ... ... ..C ..A ..C ... ..A ..C ... ... ..G ... ... ... ... ..A ..A ... ... .. ..G ... ... . [256]
AJ278186_B.schoenbuchii(R6)      ... ... ... ... ... ..C ..A ..C ... ..A ..C ... ... ..G ... ... ... ... ..A ..A ... ... .. ..G ... ... . [256]
AJ564632_B.schoenbuchii(DLF01)   ... ... ... ... ... ..C ..A ..C ... ..A C.G ... ... ..A ... ... ... ... ..A ..A ... ... .. ..G ... ... . [256]
AJ564633_B.schoenbuchii(DLF15)   ... ... ... ... ... ..C ..A ..C ... ..A ..C ... ... ..G ... ... ... ... ..A ..A ... ... .. ..G ... ... . [256]
AJ564634_B.schoenbuchii(DLF17)   ... ... ... ... ... ..C ..A ..C ... ..A ..C ... ... ..G ... ... ... ... ..A ..A ... ... .. ..G ... ... . [256]
AJ564635_B.schoenbuchii(DLF22)   ... ... ... ... ... ..C ..A ..C ... ..A ..C ... ... ..G ... ... ... ... ..A ..A ... ... .. ..G ... ... . [256]
AJ583131_T.leucogaster(TL-sv6)   ..C ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..C ..A ... ..T ..G ... ... ... .. ..T ... ... . [256]
AJ583111_R.norvegicus(MZ-rn1Peru ..T ... ... ..G ... ... ... ..T ... ..T ..G ... ... ... ..T ..A ... ..G ... ..C ... ... .. ..T ... ... . [256]
AJ583112_A.namaquensis(AN-nh1)   ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ... ... ... .. ..C ... ..C . [256]
AJ583113_A.namaquensis(AN-nh2)   ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ... ... ... .. ..C ... ..C . [256]
AJ583114_A.namaquensis(AN-nh3)   ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ... ... ... .. ..C ... ..C . [256]
AJ583115_A.namaquensis(AN-tr1)   ..G ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..T ..A ... ... ..G ... ... ... .. ..C ... ... . [256]
AJ583116_A.namaquensis(AN-tr2)   ..G ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..T ..A ... ... ..G ... ... ... .. ..C ... ... . [256]
AJ583117_A.namaquensis(AN-io2)   ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ... ... ... .. ..C ... ..C . [256]
```

```
AJ583118_A.namaquensis(AN-tr103) ..G ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..T ..A ... ... ..G ... ... ... .. ..C ... ... . [256]
AJ583120_R.pumilio(RP-io111)     ... ... ... ... ... ... ... ..T ... ... ..A ..C ... ... ..T ..G ... ..G ..G ... ... ... .. ..C ... ..C . [256]
AJ583121_R.pumilio(RP-io114)     ... ... ... ... ... ... ... ..T ... ... ..A ..C ... ... ..T ..G ... ..G ..G ... ... ... .. ..C ... ..C . [256]
AJ583122_M.natalensis(MN-tr1)    ... ..C ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ..A ... ... .. ..C ... ..C . [256]
AJ583123_M.natalensis(MN-so1)    ... ..C ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ..A ... ... .. ..C ... ..C . [256]
AJ583124_M.natalensis(MN-ko1)    ... ..C ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ..A ... ... .. ..C ... ..C . [256]
AJ583125_M.natalensis(MN-tr4)    ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ... ... ... .. ..C ... ..C . [256]
AJ583126_M.natalensis(MN-ga6)    ... ..C ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ..A ... ... .. ..C ... ..C . [256]
AJ583127_M.natalensis(MN-ga8)    ... ..C ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ..A ... ... .. ..C ... ..C . [256]
AJ583128_M.natalensis(MN-ga18)   ... ..C ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ..G ..G ..A ... ... .. ..C ... ..C . [256]
AJ583129_T.leucogaster(TL-sv1)   ..C ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..C ..A ... ..T ... ... ... ... .. ..T ..C ... . [256]
AJ583130_T.leucogaster(TL-sv4)   ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..A ... ... ... ... ... ... .. ..C ... ..C . [256]
AJ583132_S.campestris(SC-tr1)    ... ... ... ... ... ... ... ..T ... ..T ..G ..C ..C ... ..T ..A ... ... ..A ..C ... ... .. ..C ... ... . [256]
AJ583133_T.leucogaster(TL-sv2)   ... ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..T ..A ... ... ..A ... ... ... .. ..C ... ... . [256]
AJ583134_T.leucogaster(TL-sv3)   ... ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..T ..A ... ... ..A ... ... ... .. ..C ... ... . [256]
AJ583135_T.leucogaster(TL-sv7)   ... ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..T ..A ... ... ..G ... ... ... .. ..C ... ... . [256]
AJ583136_T.leucogaster(TL-sv9)   ... ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..T ..A ... ... ..A ... ... ... .. ..C ... ... . [256]
AY902184(RR0042-1G_R.tanezumi_In ..C ... ... ... ... ... ... ..T ... ..G ..A ..C ... ... ..T ..A ... ... ..G ... ... ... .. ..C ... ... . [256]
AY902187(RR0037-2_GR.tanezumi_In ... ... ... ... ... ... ... ..C ... ..T ..C ... ..C ... ... ..A ... ... ..A ... ... ... .. ... ... ..C . [256]
B._suillus_4                     ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..G ... ..G ..G ... ... ... .. ..C ... ..C . [256]
B._suillus_6                     ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..G ... ..G ..G ... ... ... .. ..C ... ..C . [256]
B._suillus_5                     ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..G ... ..G ..G ... ... ... .. ..C ... ..C . [256]
B._suillus_Flea_7                ... ... ... ... ... ... ..A ..T ... ..A ..G ... ... ... T.T ... ... ... ..A ... ... ... .. ..G ... ..A . [256]
B._suillus_Flea_13               ... ... ... ... ... ... ... ..T ... ..T ..A ..C ... ... ..T ..G ... ..G ..G ... ... ... .. ..C ... ... . [256]
AY454539_B._sp._af82up           ... ... ... ... ... ... ..A ..T ... ..A ..A ... ... ... ..T ... ... ... ..A ... ... ... .. ..G ... ..A . [256]
AJ583119_B._sp._RP-tr109         ... ... ... ... ... ... ... ..T ... ..T ..G ..C ... ... ..T ..G ... ..G ..G ... ... ... .. ..C ... ..C . [256]

L38987_B.henselae                CA GCA GGT GTT GCA TGC CTT TCT GTT GAA AGA ATT CCT GAA TTC ATT GCA CGT GCA AAA GAT AAA AAT GAT TCT TTC C [394]
AF143445_B.vinsonni_berkhoffi    .T ... ... ... ..T ..T ... ... ... A.G ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AF191502_B.taylorii              .. ... ... ... ..G ... ... ..C ... ..G ... ..C ... ... ... ... ... ... ... ... ... ... ... ... C.. ... . [394]
AF176091_B.koehlerae             .. ... ... ... ... ... ... ... ... ... ... ..C ... C.. ... ... ... ... ... ... ... ... ... ... ... ... . [394]
AF214557_B.vinsonni_arupensis    .T ... ... ..C ..T ..T ... ... ... A.G ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ278184_B.schoenbushii(R3)      .. ... ... ... ..G ... ... ... A.. ... .A. ... ... ... ..T ..C ... ... ... ... ... ... ... ... C.. ... . [394]
AJ439406_B.henselae              .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... . [394]
AY454537_Sweden(rodent)          .. ... ... ... ... ... ... ... A.. ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AY454538_Sweden(rodent)          .. ... ... ... ..G ... ... ..C ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... . [394]
AY454540_Sweden(rodent)          .. ... ... ... ... ..T ... ... ..G A.. .A. ... ... ... ..T ... ..T ... ... ... ... ... ... ... C.C ... . [394]
AY454541_Sweden(rodent)          .. ... ... ... ..G ... ... ..C ... ..G ... ..C ... ... ... ... ... ... ... ... ... ... ... ... C.. ... . [394]
AY515124_B.rattimassiliensis(159 .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ..C ... ... ... ... ... ... C.. ... . [394]
AY515125_B.rattimassiliensis(161 .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ..C ... ... ... ... ... ... C.. ... . [394]
AY515126_B.phoceensis(16120)     .T ... ... ... ..C ... ... ... ... ..G ... ... ... ... ... ..C ... ..G ... ... ... ... ... ... C.. ... . [394]
AY584852_B.taylorii(Far_EastI)   .. ... ... ... ..G ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... . [394]
AY584853_B.taylorii(Far_EastII)  .. ... ... ... ..G ... ... ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... . [394]
AY584854_B.grahamii(Far_EastI)   .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ... . [394]
AY584855_B.grahamii(Far_EastII)  .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AY584856_B.grahamii(Far_EastIII) .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AY584857_B.grahamii(Far_EastIV)  .. ... ... ... ... ... ... ... A.. ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AY584859(16/40_Apodemus_Far_East .. ... ... ... ... ..T ... ..A ... ..G CA. ... ..A A.. ... ... ... ..A ... ... ..C ... ... ..C C.. ... . [394]
Z70009_B.elizebethae(F9251)      .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ..T ... ... ... ... ... ... ... C.. ... . [394]
Z70010(R-phy1_Phyllotis_Peru)    .T ... ... ... ..C ... ... ..C ... ... ... ... ... ... ..T ... ..C ... ..C ... ... ... ... ... C.. ... . [394]
Z70011(R-phy2_Phyllotis_Peru)    .T ... ... ... ..T ..T ... ..C ... A.. ... ... ... ... ..T ... ..G ... ..C ... ..C .G. ... ... C.. ... . [394]
Z70012(N40_Apodemus_sylvaticus_U .. ... ... ... ..G ... ... ... ... A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
Z70013_B.taylorii(M6)            .. ... ... ... ..G ... ... ..C ... ..G ... ..C ... ... ... ... ... ... ... ... ... ... ... ... C.. ... . [394]
Z70014_B.quintana(Fuller)        .. ... ... ... ... ... ... ... ..C ... ... ... ..G ... ... ... ..A ... ... ... ... ... ... ... C.. ... . [394]
```

```
Z70015_B.vinsonni(Baker)         .C ... ..A ... ..T ... ... ... ... A.G ... ... ... ... ..T ... ... ... ... ... ... ... ... ..C C.. ..T . [394]
Z70016_B.grahamii(V2)            .. ... ... ... ... ... ... ... A.. ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
Z70017_B.doshiae(R18)            .. ... ... ... ... ..T ... ... ..G A.. .A. ... ... ... ..T ... ..T ... ... ... ... ... ... ... C.C ... . [394]
Z70018(C5rat_Rattus_Peru)        .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
Z70019(C4-phy_Phyllotis_Peru)    .T ... ... ... ..T ..T ... ..C ... A.. ... ... ... ... ..T ... ..G ... ..C ... ..C .G. ... ... C.. ... . [394]
Z70020(C7rat_Rattus_Peru)        .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ..T ... ... ... ... ... ... ... C.. ... . [394]
Z70021_B.bacilliformis(LA6.3)    .. ... ... ... ... ... ... ... ... A.. .A. ... ... ... ..T ... ..G ... ... ... ... ... ... ... C.. ..T . [394]
Z70022(C1-phy_Phyllotis_Peru)    .T ... ... ... ..T ..T ... .AC ... A.. ... ... ... ... ..T ... ..G ... ..C ... ..C .G. ... ... C.. ... . [394]
AJ278183_B.schoenbuchii(R1)      .. ... ... ... ..G ... ... ... A.. ... .A. ... ... ..G ..T ..C ... ... ... ... ... ... ... ... C.. ... . [394]
AJ278185_B.schoenbuchii(R4)      .. ... ... ... ..G ... ... ... A.. ... .A. ... ... ... ..T ..C ... ... ... ... ... ... ... ... C.. ... . [394]
AJ278186_B.schoenbuchii(R6)      .. ... ... ... ..G ... ... ... A.. ... .A. ... ... ... ..T ..C ... ... ... ... ... ... ... ... C.. ... . [394]
AJ564632_B.schoenbuchii(DLF01)   .. ... ... ... ..G ... ... ... A.. ... .A. ... .G. ..G ..T ..C ... ... ... ... ... ... ... ... C.. ... . [394]
AJ564633_B.schoenbuchii(DLF15)   .. ... ... ... ..G ... ... ... A.. ... .A. ... ... ... ..T ..C ... ... ... ... ... ... ... ... C.. ... . [394]
AJ564634_B.schoenbuchii(DLF17)   .. ... ... ... ..G ... ... ... A.. ... .A. ... ... ... ..T ..C ... ... ... ... ... ... ... ... C.. ... . [394]
AJ564635_B.schoenbuchii(DLF22)   .. ... ... ... ..G ... ... ... A.. ... .A. ... ... ... ..T ..C ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583131_T.leucogaster(TL-sv6)   .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583111_R.norvegicus(MZ-rn1Peru .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583112_A.namaquensis(AN-nh1)   .. ..T ... ... ... ..T ... ... A.. ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583113_A.namaquensis(AN-nh2)   .. ..T ... ... ... ..T ... ... A.. ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583114_A.namaquensis(AN-nh3)   .. ..T ... ... ... ..T ... ... A.. ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583115_A.namaquensis(AN-tr1)   .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583116_A.namaquensis(AN-tr2)   .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583117_A.namaquensis(AN-io2)   .. ..T ... ... ... ..T ... ... A.. ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583118_A.namaquensis(AN-tr103) .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583120_R.pumilio(RP-io111)     .. ... ... ... ... ... ... ... A.. ... .A. ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583121_R.pumilio(RP-io114)     .. ... ... ... ... ... ... ... A.. ... .A. ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583122_M.natalensis(MN-tr1)    .. ... ... ... ... ... ... ..G A.. A.. ... ... ... ... ..T ... ..G ... ... ... ... ... ... ... C.. ... . [394]
AJ583123_M.natalensis(MN-so1)    .. ... ... ... ... ... ... ..A A.. A.. ... ... ... ... ..T ... ..G ... ... ... ... ... ... ... C.. ... . [394]
AJ583124_M.natalensis(MN-ko1)    .. ... ... ... ... ... ... ..A A.. A.. ... ... ... ... ..T ... ..G ... ... ... ... ... ... ... C.. ... . [394]
AJ583125_M.natalensis(MN-tr4)    .. ..T ... ... ... ..T ... ... A.. ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583126_M.natalensis(MN-ga6)    .. ... ... ... ... ... ... ..A A.. A.. ... ... ... ... ..T ... ..G ... ... ... ... ... ... ... C.. ... . [394]
AJ583127_M.natalensis(MN-ga8)    .. ... ... ... ... ... ... ..A A.. A.. ... ... ... ... ..T ... ..G ... ... ... ... ... ... ... C.. ... . [394]
AJ583128_M.natalensis(MN-ga18)   .. ... ... ... ... ... ... ..A A.. A.. ... ... ... ... ..T ... ..G ... ... ... ... ... ... ... C.. ... . [394]
AJ583129_T.leucogaster(TL-sv1)   .. ... ... ... ... ... ... ... A.. A.. ... ... ... ..G ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583130_T.leucogaster(TL-sv4)   .. ... ... ... ... ... ... ... A.. ... ... ... ... ..G ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583132_S.campestris(SC-tr1)    .. ... ... ... ... ... ... ... A.. A.. .A. ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583133_T.leucogaster(TL-sv2)   .. ... ... ..C ... ... ... ... AC. A.. ... ... ... ..G ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583134_T.leucogaster(TL-sv3)   .. ... ... ..C ... ... ... ... A.. A.. ... ... ... ..G ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583135_T.leucogaster(TL-sv7)   .. ... ... ..C ... ... ... ... A.. A.. ... ... ... ... ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AJ583136_T.leucogaster(TL-sv9)   .. ... ... ..C ... ... ... ... A.. A.. ... ... ... ..G ..T ... ... ... ... ... ... ... ... ... C.. ... . [394]
AY902184(RR0042-1G_R.tanezumi_In .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... ..T ... ... ... ... ... ... ... C.. ... . [394]
AY902187(RR0037-2_GR.tanezumi_In .T ... ... ... ..C ... ... ... ... ..G ... ... ... ... ... ..C ... ..G ... ... ... ... ... ... C.. ... . [394]
B._suillus_4                     .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... .A. ... ... ... ... ... ... ... C.. ..T . [394]
B._suillus_6                     .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... .A. ... ... ... ... ... ... ... C.. ..T . [394]
B._suillus_5                     .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... .A. ... ... ... ... ... ... ... C.. ..T . [394]
B._suillus_Flea_7                .. ... ..C ..A ... ... ... ... ... C.. .A. ... ... ... ..T ... ... ..A ... ... A.. ... ... ... CG. ..T . [394]
B._suillus_Flea_13               .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... .A. ... ... ... ... ... ... ... C.. ..T . [394]
AY454539_B._sp._af82up           .. ... ... ..A ... ..T ... ... ... C.. .A. ... ... ..G ..T ... ..G ... ... ... A.. ... ... ... CG. ... . [394]
AJ583119_B._sp._RP-tr109         .. ... ... ... ... ... ... ... A.. A.. ... ... ... ... ..T ... .A. ... ... ... ... ... ... ... C.. ..T . [394]

L38987_B.henselae                GC CTT ATG GGT TTT GGT CAT CGA GTC  [420]
AF143445_B.vinsonni_berkhoffi    .T ... ... ..A ... ... ... ... ...  [420]
AF191502_B.taylorii              .. ... ... ..A ... ... ..C A.. ...  [420]
AF176091_B.koehlerae             .. ... ... ... ... ... ..C ... ...  [420]
```

```
AF214557_B.vinsonni_arupensis    .. ... ... ..A ... ... ..C ... ..G   [420]
AJ278184_B.schoenbushii(R3)      .T ... ... ..C ... ..C ..C A.. ..T   [420]
AJ439406_B.henselae              .. ... ... ... ... ... ... ... ...   [420]
AY454537_Sweden(rodent)          .. ... ... ..C ... ..G ..C ..T ...   [420]
AY454538_Sweden(rodent)          .. ... ... ..G ... ..G ..C A.. ...   [420]
AY454540_Sweden(rodent)          .. ... ... ..A ... ... ... A.. ...   [420]
AY454541_Sweden(rodent)          .. ... ... ..A ... ... ..C A.. ...   [420]
AY515124_B.rattimassiliensis(159 .. ... ..A ..C ... ..C ... ..T ...   [420]
AY515125_B.rattimassiliensis(161 .. ... ..A ..C ... ..C ... ..T ...   [420]
AY515126_B.phoceensis(16120)     .. ... ... ..A ... ..C ..C ... ..T   [420]
AY584852_B.taylorii(Far_EastI)   .. ... ... ..A ... ... ..C A.. ...   [420]
AY584853_B.taylorii(Far_EastII)  .. ... ... ..A ... ..G ..C A.. ...   [420]
AY584854_B.grahamii(Far_EastI)   .. ... ... ..C ... ..G ..C ..T ...   [420]
AY584855_B.grahamii(Far_EastII)  .. ... ... ..C ... ..G ..C ..T ...   [420]
AY584856_B.grahamii(Far_EastIII) .. ... ... ..C ... ..G ..C ..T ...   [420]
AY584857_B.grahamii(Far_EastIV)  .. ... ... ..C ... ..G ..C ..T ...   [420]
AY584859(16/40_Apodemus_Far_East .. ... ... ..A ... ..C ... ... ...   [420]
Z70009_B.elizebethae(F9251)      .. ... ... ..C ... ... ..C ..T ...   [420]
Z70010(R-phy1_Phyllotis_Peru)    .. ..C ... ..A ... ... ... ..T ...   [420]
Z70011(R-phy2_Phyllotis_Peru)    .A ..G ... ..A ... ... ... ..T ...   [420]
Z70012(N40_Apodemus_sylvaticus_U .. ... ... ..A ... ... ... ... ...   [420]
Z70013_B.taylorii(M6)            A. ... ... ..A ... ... ..C A.. ...   [420]
Z70014_B.quintana(Fuller)        .T ... ... ... ... ... ..C ... ..T   [420]
Z70015_B.vinsonni(Baker)         .. ... ... ..A ... ... ..C A.. ...   [420]
Z70016_B.grahamii(V2)            .. ... ... ..C ... ..G ..C ..T ...   [420]
Z70017_B.doshiae(R18)            .. ... ... ..A ... ... ... A.. ...   [420]
Z70018(C5rat_Rattus_Peru)        A. ... ... ..C ... ..C A.C ..T ...   [420]
Z70019(C4-phy_Phyllotis_Peru)    .A ..G ... ..A ... ... ... ..T ...   [420]
Z70020(C7rat_Rattus_Peru)        .. ... ... ..C ... ... ..C ..T ...   [420]
Z70021_B.bacilliformis(LA6.3)    .T ... ... ..C ..C ..C ..C A.. ...   [420]
Z70022(C1-phy_Phyllotis_Peru)    .A ..G ... ..A ... ... ... ..T ...   [420]
AJ278183_B.schoenbuchii(R1)      .T ... ... ..C ... ..C ..C A.. ..T   [420]
AJ278185_B.schoenbuchii(R4)      .T ... ... ..C ... ..C ..C A.. ..T   [420]
AJ278186_B.schoenbuchii(R6)      .T ... ... ..C ... ..C ..C A.. ..T   [420]
AJ564632_B.schoenbuchii(DLF01)   .T ... ... ..C ... ..C ..C A.. ..T   [420]
AJ564633_B.schoenbuchii(DLF15)   .T ... ... ..C ... ..C ..C A.. ..T   [420]
AJ564634_B.schoenbuchii(DLF17)   .T ... ... ..C ... ..C ..C A.. ..T   [420]
AJ564635_B.schoenbuchii(DLF22)   .T ... ... ..C ... ... ..C A.. ..T   [420]
AJ583131_T.leucogaster(TL-sv6)   .. ..C ... ..C ... ... ..C ..T ...   [420]
AJ583111_R.norvegicus(MZ-rn1Peru .. ... ... ..C ... ... ..C ..T ...   [420]
AJ583112_A.namaquensis(AN-nh1)   .. ... ... ..A ... ... ..C ..T ...   [420]
AJ583113_A.namaquensis(AN-nh2)   .. ... ... ..A ... ... ..C ..T ...   [420]
AJ583114_A.namaquensis(AN-nh3)   .. ... ... ..A ... ... ..C ..T ...   [420]
AJ583115_A.namaquensis(AN-tr1)   .T ... ... ..C ... ... ..C ..C ..T   [420]
AJ583116_A.namaquensis(AN-tr2)   .T ... ... ..C ... ... ..C ..C ..T   [420]
AJ583117_A.namaquensis(AN-io2)   .. ... ... ..A ... ... ..C ..T ...   [420]
AJ583118_A.namaquensis(AN-tr103) .T ... ... ..C ... ... ..C ..C ...   [420]
AJ583120_R.pumilio(RP-io111)     .. ... ... ..C ... ... ..C ..T ...   [420]
AJ583121_R.pumilio(RP-io114)     .. ... ... ..C ... ... ..C ..T ...   [420]
AJ583122_M.natalensis(MN-tr1)    .. ... ... ..C ... ... ..C ..T ...   [420]
AJ583123_M.natalensis(MN-so1)    .. ... ... ..C ... ... ..C ..T ...   [420]
AJ583124_M.natalensis(MN-ko1)    .. ... ... ..C ... ... ..C ..T ...   [420]
AJ583125_M.natalensis(MN-tr4)    .. ... ... ..A ... ... ..C ..T ...   [420]
AJ583126_M.natalensis(MN-ga6)    .. ... ... ..C ... ... ..C ..T ...   [420]
```

```
AJ583127_M.natalensis(MN-ga8)    .. ... ... ..C ... ... ..C ..T ...  [420]
AJ583128_M.natalensis(MN-ga18)   .. ... ... ..C ... ... ..C ..T ...  [420]
AJ583129_T.leucogaster(TL-sv1)   .. ..C ... ..C ... ... ..C ..T ...  [420]
AJ583130_T.leucogaster(TL-sv4)   .. ... ... ..C ... ... ..C ..T ..T  [420]
AJ583132_S.campestris(SC-tr1)    .T ... ... ..C ... ... ..C ..T ...  [420]
AJ583133_T.leucogaster(TL-sv2)   .. ..C ... ... ... ... ..C ..C ..T  [420]
AJ583134_T.leucogaster(TL-sv3)   .. ... ... ... ... ... ..C ..C ..T  [420]
AJ583135_T.leucogaster(TL-sv7)   .. ... ... ..C ... ... ..C ..T ..T  [420]
AJ583136_T.leucogaster(TL-sv9)   .. ... ... ... ... ... ..C ..C ..T  [420]
AY902184(RR0042-1G_R.tanezumi_In .. ... ... ..C ... ... ..C ... ...  [420]
AY902187(RR0037-2_GR.tanezumi_In .. ... ... ..A ... ..C ..C ... ..T  [420]
B._suillus_4                     .. ... ... ..C ... ... ..C ..T ...  [420]
B._suillus_6                     .. ... ... ..C ... ... ..C ..T ...  [420]
B._suillus_5                     .. ... ... ..C ... ... ..C ..T ...  [420]
B._suillus_Flea_7                .. ... ... ..C ... ... ..C ..T ...  [420]
B._suillus_Flea_13               .. ... ... ..C ... ... ..C ..T ...  [420]
AY454539_B._sp._af82up           .T ... ... ..C ... ... ..C ..T ...  [420]
AJ583119_B._sp._RP-tr109         .. ... ... ..C ... A.. ... ..T ...  [420]
```

CHAPTER 4

General Conclusion

Contributing Authors: J. H. d. T. van Sandwyk, N. C. Bennett & A. D. Bastos

Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa

The species that comprise the Bathyergidae inhabit a wide range of habitats, occurring in geographical areas ranging from mesic to arid areas. This range in distribution is associated with differences in the degree of sociality displayed by the Bathyergidae and includes representatives that are solitary through to those species displaying a eusocial lifestyle. The current research was conduced to investigate the array of bacteria that are associated with the external orifices and internal organs of three species of the South African Bathyergidae. These particular species were selected to best reflect the variety of environmental conditions inhabited by the representative species of the family as well as the different degrees of sociality. Five solitary Cape mole-rats, *Georychus capensis* were collected from the mesic Darling region of the Western Cape (S33°23'S E18°21'), five social highveld mole-rats, *Cryptomys hottentotus* pretoriae from the temperate savanna biome within the suburbs of Pretoria, Gauteng (S25°43'S E28°13'E) and ten eusocial Damaraland mole-rats, *Cryptomys damarensis* from the arid southern Kalahari desert, Hotazel, Northern Cape Province (25°58'S 21°49'E).

1. Identified bacteria associated with three species of bathyergids

Bacteria cultured and identified based on 16S ribosomal RNA (rRNA) sequencing included representatives belonging to 3 Phyla, the Proteobacteria, Actinobacteria and Firmicutes. Due to the inclusion of samples from external orifices, contamination with environmental bacteria was unavoidable and was confirmed by the ubiquitous presence of members of the genus *Acinetobacter* which are known to comprise up to 0.001% of the aerobic microbial communities of soils and water (Tower 1992). Despite this drawback, strong correlations between the location of a sample and the resulting bacteria were uncovered. For example, the presence of the plant pathogen *Agrobacterium tumefaciens* in a saliva sample suggests infection of the animal's native food source with this bacteria. Whilst it was not possible to determine the exact pathogenicity of the various bacteria cultured in the study, it is unlikely that any are pathogenic as all animals captured were in good health. It is however possible that under conditions of stress that they may cause disease and even result in mortality as healthy, field-caught animals have been known to rapidly succumb to disease following their capture and transfer to a laboratory (N.C. Bennett, pers comm.).

These results provide a preliminary overview of the bacterial diversity of readily and non-selectively cultured bacteria that are associated with mole-rats. Despite the fact that they most likely only represent 1 % of the actual bacterial species diversity present (Amman *et al.* 1995), they provide important baseline information future studies in this field.

2. *Bartonella* prevalence in mole-rat species: Variation with degree of sociality and possible implications

Liver samples from five species of southern African Bathyergidae, *Cryptomys damarensis, Georychus capensis, Cryptomys hottentotus natalensis, Bathyergus suillus, Cryptomys hottentotus pretoriae* were screened for the presence of the blood parasite *Bartonella*. The *Bartonella* parasite was found to be present only in the Cape dune mole-rat, *Bathyergus suillus*. In an attempt to find the vector of this parasite, Hystrichopsyllidid fleas collected from the pelages of *B. suillus* were screened for the presence of *Bartonella*. Unfortunately the fleas used in this part of the study were not collected from the same *B. suillus* individuals screened for the presence of *Bartonella* in the initial phase of the study, but were collected from dune mole-rats collected from the same locality. Fleas subsequently tested positive for the presence of *Bartonella* and also possessed a new species of *Bartonella* that is not present in the Cape dune mole-rat. Identification of the *Bartonella* parasite was based on sequences obtained from amplification of the Citrate synthase gene and 16S-23S intergenic region. The presence of the *Bartonella* bacteria in the fleas found on the mole-rat may point to them as the invertebrate vector for spread of the bacteria amongst the Dune mole-rat population, with the inability to detect the novel flea-associated species of *Bartonella* in *B. suillus* being due to the relatively small sample size. The fleas belonging to the family Hystrichopsyllidae are specific to *B. suillus* (De Graaff 1981), and may explain the detection of *Bartonella* in *B. suillus* but not in *G. capensis,* which occurs sympatrically with *B. suillus*. Future studies should be directed at the common mole-rat, *C. hottentotus hottentous* which also occurs in the same locality as *B. suillus*, but which was not included in the present study.

The presence of *Bartonella* in *B. suillus* may result from a combination of factors, namely the presence of distinct, species-restricted haematophagous insect vectors and the fact that this mole-rat, in contrast to most other species, spends some time at the surface where it may come into contact terrestrial rodents and/or their ticks and fleas.

The presence of an opportunistic zoonotic pathogen in an animal used as a food source by the local inhabits of the Western Cape is of clinical importance due to the high HIV infection rates of the human population. Whilst bacterial infection through the oral route has not been demonstrated, the capture and handling of animals is likely to result in either a bite or scratch. As the latter is a recognized mode of transmission for *B. henselae* the causative agent of catch scratch disease (Margileth 1968, Hansmann *et al.* 2005), it is possible that transmission of *Bartonella* from *B. suillus* to humans is may be effected in a similar manner.

Bacillary angiomatosis first reported by Stolrer *et al.* (1983) in AIDS (acquired immune deficiency syndrome) patients is a potentially severe disease leading to the development of multiple subcutaneous nodules. In a study conducted by Frean *et al.* (2002) it was shown that 10 % of patients attending HIV clinics in Johannesburg, South Africa were infected with *Bartonella*. Though the disease is not restricted to people infected by HIV the clinical manifestations of the disease are closely linked to immune-suppression (Frean *et al.* 2002).

3. Pilot studies and continuing research

Two pilot studies were conducted to assess the prevalence of other microbial agents present in the Southern African bathyergids. The first of these studies attempted to assess the degree of infection of different mole-rat species with the Apicomplexan parasite *Eimeria*. Previous studies conducted on the Silvery mole-rat *Heliophobius argenteocinereus* have shown a diversity of coccidians to be present within these subterranean rodents (Koudela *et al.* 2000, Modrý *et al.* 2005). The second study attempted to address the possibility of infection of the South African mole-rat species with the fungus *Emmonsia* previously shown to be present in Ansell's mole-rats

(*Cryptomys anselli*) and blind mole rats (*Spalax galili* and *Spalax golani*), sister taxa belonging to the family Spalacidae (Hubálek *et al.* 2005). The sample size for both of the above-mentioned projects consisted of ten *Cryptomys damarensis* collected from Hotazel, Northern Cape Province, and are ongoing, although to date no definitive results have been obtained.

This study provided the first overview of bacterial diversity in this African rodent family, using both a general 16S sequencing-culturing approach, as well as a genus-directed approach, which was not reliant on culturing. Whilst each approach had its own intrinsic drawbacks and advantages, both provided valuable starting information that may lead to future studies aimed at assessing the complex web of interactions between these subterranean rodents and the micro-organisms found in their environment.

4. Literature Cited

AMANN, R. I.; LUDWIG, W.; & SCHLEIFER, K.H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological Reviews **59:**143–169.

DE GRAAF, G. 1981. *In:* The rodents of southern Africa. Butterworth Press, Pretoria, South Africa.

FREAN, J., ARNDT, S.; & SPENCER, D. 2002. High rate of *Bartonella henselae* infection in HIV-positive outpatients in Johannesburg, South Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **96:** 549–550.

HUBÁLEK, Z., BURDA, H., SCHARFF, A., HETH, G., NEVO, E., SUMBERA, R., J. PESKO, J.; & ZIMA. 2005. Emmonsiosis of subterranean rodents (Bathyergidae, Spalacidae) in Africa and Israel. *Medical Mycology* **43:** 691-697.

HANSMANN, Y.; DEMARTINO, S.; PIEMONT, Y.; MEYER, N.; MARIET, P.; HELLER, R.; CHRISTMANN, D.; & JAULHAC, B. 2005. Diagnosis of cat scratch disease with detection of *Bartonella henselae* by PCR: a study of patients with lymph node enlargement. *Journal of Clinical Microbiology* **43:** 3800-3806.

KOUDELA, B.; ŜUMBERA, R.; & SEDLÁČEK, F. 2000. *Eimeria burdai* sp. n. (Apicomplexa: Eimeriidae), a new parasite species from subterranean African silvery mole-rat, *Heliophobius argenteocinereus*. *Folio Parasitologica* **47:** 97-99.

MARGILETH, A. M. 1968. Cat scratch disease: nonbacterial regional lymphadenitis: the study of 145 patients and a review of the literature. *Pediatrics* **42:** 803-818.

MODRÝ, D.; JIRKŮ, M.; & ŠUMBERA, R. Three new species of Eimeria

(Apicomplexa: Eimeriidae) from the Silvery Mole Rat *Heliophobius argenteocinereus* Peters, 1846 (Rodentia: Bathyergidae) from Malawi. *Journal of Parasitology* **91:** 1200–1203

STOLER, M. H.; BONFIGLIO, T. A.; STEIGBIGEL, R. T.; & PEREIRA, M. 1983. An atypical subcutaneous infection associated with acquired immune deficiency syndrome. *American Journal of Clinical Pathology* **80:** 714-718.

TOWER, K.J. 1992. The genus *Acinetobacter. In:* The Prokaryotes (Balows, A.; Trüper, H.G.; Dworkin, M.; Harder, W.; & SchleiferK-Z; Eds.). Springer-Verlag, New York, N.Y.