Proposal to Sequence an Organism of Unique Interest for Research on Aging: *Heterocephalus glaber*, the Naked Mole-Rat

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Summary

Aging is not only a major puzzle of biology but it has a profound impact on medicine with age-related diseases like heart diseases, type II diabetes, cancer, and neurodegenerative diseases among the leading causes of death in modern societies. Recent research has revealed several gene systems that can regulate longevity in model organisms and appear evolutionary conserved. Nonetheless, the longevity effects of these genes are modest when compared to the lengthening of lifespans during evolution. Among mammals there is at least a 40-fold variation in maximum longevity. We still do not know why different species of similar body plan, biochemistry, and physiology can age at such different rates, but these differences must be seated in the genome.

In this white paper, we propose the genome sequencing of a unique long-lived organism, the naked mole-rat (*Heterocephalus glaber*). *Heterocephalus* has a record longevity of 28.3 years which makes it the longest-lived rodent with a much longer lifespan than expected for its relatively small body size. Because *Heterocephalus* can live remarkably longer than similar-sized rodents, such as mice and rats that can only live up to 4-5 years, it can be used as a model of resistance to disease and, in fact, *Heterocephalus* is extremely resistant to neoplasia. Therefore, sequencing its genome will provide the sophisticated molecular biology tools necessary for *Heterocephalus* to be used not only as another model of human biology but primarily as the first model of resistance to chronic diseases of aging.

Because *Heterocephalus* was chosen to dovetail with existing mammalian sequencing projects, studies in *Heterocephalus* can be conducted in parallel in closely-related short-lived species to test hypotheses of aging. Researchers will thus be able to study mechanisms and genes previously associated with aging using a unique comparative approach. Such studies will increase our understanding of the evolution of longevity as well as of the molecular, cellular, and genetic mechanisms of aging. Moreover, insights obtained in *Heterocephalus* will drive experiments in more traditional models, in particular by taking advantage of the mouse as a surrogate system to study the *Heterocephalus* genome.

Overall, sequencing the *Heterocephalus* genome will help establish this organism as the first long-lived model for biomedical research. If we could understand the molecular and genetic basis of differences in aging between mammals, it would open a new paradigm for biomedical research on aging and age-related diseases with countless potential applications for improving human health. In addition to its utility as a model to study the molecular and genetic mechanisms of aging, *Heterocephalus* has other fascinating evolutionary adaptations, such as being virtually poikilothermic which makes it a unique model to study mammalian metabolic regulation. *Heterocephalus* also has a eusocial system that makes it exceptional in studies of growth, development, reproduction, and behavior.

Introduction

Aging is a major biological process with a profound impact on human medicine and society. Age-related diseases such as diseases of the heart and malignant neoplasms are the leading causes of death in modern societies. In fact, the incidence and morbidity of most clinical conditions increases with age (Hoyert et al., 2006). Moreover, neurodegenerative diseases, like Alzheimer's and Parkinson's disease, are among the major health concerns of aging adults and are recognized as important targets of biomedical research. In this white paper, we propose the sequencing of an organism of unique interest for aging research: the naked mole-rat (*Heterocephalus glaber*) whose record longevity of 28.3 years makes it the longest-lived rodent (Buffenstein, 2005). *Heterocephalus* also has a much longer lifespan than expected for its relatively small body size (Figure 1).

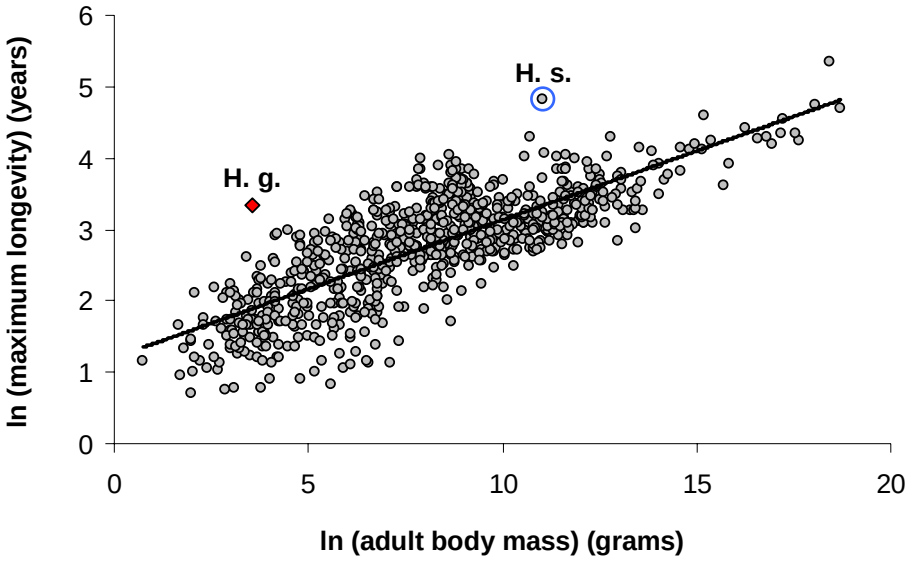

Figure 1: Plot of the relationship between ln-transformed maximum longevity and typical adult body mass for non-volant mammals (*n* = 820). *Heterocephalus glaber* (H. g.) is highlighted by a red square. H. s. = *Homo sapiens* (in blue). Data from de Magalhaes et al., 2007a.

Recent strides have been made concerning our understanding of aging. Research has now elucidated several gene systems that can regulate longevity, and often aging and multiple age-related diseases, in short-lived model organisms including rodents (Guarente and Kenyon, 2000; Finch and Ruvkun, 2001). Interestingly, some of these genes have been shown to determine longevity in very different species, such as in roundworms and in mice (Holzenberger, 2003; Liu et al., 2005). Therefore, at least some mechanisms of aging are evolutionary conserved and have thus potential therapeutic applications (Baur et al., 2006). Several genes have also been associated with human longevity and/or survival in old age (Vijg and Suh, 2005), and the human genome sequence made it possible to identify the gene responsible for progeria (Eriksson et al., 2003).

Despite such breakthroughs in the genetics of aging, the longevity effects of the genes identified so far are modest relative to the lengthening and shortening of lifespans during evolution. Animals of different species age at remarkably different rates (Finch, 1990; Miller, 1999). For instance, among mammals there is at least a 40-fold variation in maximum longevity even for animals in captivity under adequate husbandry conditions (Austad, 1997). Such differences in maximum longevity in captivity reflect mostly differences between species in disease susceptibility, in particular susceptibility to age-related diseases (de Magalhaes, 2006). Gathering insights about the genetic, cellular, and molecular mechanisms by which animals of different species age at different paces would provide important clues about human aging and age-related diseases with multiple implications for biomedical research (Austad, 2005). For example, nearly all vertebrates share the

amyloid-beta peptide sequence associated with Alzheimer's disease in humans, yet the accumulation of Alzheimer-like changes varies widely among species (Finch and Stanford, 2004). What other gene differences, one may ask, modulate the effect of the amyloid gene during aging?

Short-lived model organisms have been typically chosen to study aging and age-related diseases. Indeed, aging research using traditional models has greatly benefited from the high-throughput approaches made possible by genome sequences. For example, several genes have been associated with aging in model organisms by taking advantage of post-genomic methods such as high-throughput gene expression (Murphy et al., 2003) and large-scale RNAi screens (Hamilton et al., 2005). Many have argued, however, the necessity to study aging in long-lived organisms and investigate why they live so long (Strehler, 1986), yet due to technical difficulties few studies have been conducted so far. Some results suggest that cells from longer-lived mammals are more resistant to stress (Kapahi et al., 1999; Harper et al., 2007). Nonetheless, it remains a mystery why different species of similar body plan, biochemistry, and physiology can age at remarkably different rates, yet these differences must be seated in the genome (Miller, 1999). Therefore, as a first (but crucial) step to study the genetic and molecular mechanisms underlying differences in mammalian longevity and aging we propose 6x genome sequencing of *Heterocephalus glaber*.

Rationale

Independently of environmental conditions, mammals of different species age at remarkably different paces (Finch, 1990). For example, no matter how well a mouse is taken care of, it will age at a physiological, pathological, and biochemical level about 25-30 times faster than a human being (Miller, 1999). Consequently, it is clear that genetics plays a major role in longevity and aging differences between species (Cutler, 1979; Miller, 1999; Partridge and Gems, 2006). Furthermore, mammals show major species differences in a host of age-related diseases, like heart diseases, cancer, type II diabetes, and neurodegenerative diseases, which may be traced to specific gene differences (Finch, 1990). If we could understand why different species have different susceptibilities to many age-related diseases, it would open a new paradigm for biomedical research on aging and age-related diseases. In other words, if we could identify even a fraction of the genes that determine differences in the incidence of diseases across species it would provide important insights into the mechanisms of human diseases that could lead to a better diagnosis and treatment.

It is now widely recognized that in species under low hazard conditions—e.g., due to developing the ability to fly or superior intelligence, being isolated in predator-free islands or living underground—selection will favor alleles that confer a slower life history, including a longer lifespan and a delay of the basic process of aging (Kirkwood and Austad, 2000; Miller, 2001). There is still much controversy regarding how many genes are involved with some authors arguing that a vast number of genes must be involved in the evolution of longevity while others have argued that changes in a dozen or fewer genes may suffice (Miller, 2001). The latter arguments are partly based on the extraordinary breakthroughs made on the genetics of aging in model organisms demonstrating that it is possible to delay aging in a coordinated fashion by manipulating single genes. Such aging-related genes are one of the most exciting areas of aging research and it is possible that functional alterations or their differential regulation in long-lived species contribute to species differences in aging, a hypothesis that has practically not been tested because of a lack of adequate paradigms.

While we do not know for a fact that evolution acted on the same mechanism(s) to extend the longevity of *Heterocephalus* as it did to extend human longevity, it is very likely that changes in at least some common genes and/or mechanisms were involved. There is abundant evidence that mechanisms of aging are by and large evolutionary conserved. Genes associated with aging in yeast

and invertebrates have been shown to play a role in aging in higher organisms (Guarente and Kenyon, 2000). For instance, disruption of the insulin/IGF1 pathway has been shown to extend lifespan in yeast, worms, flies, and mice and mutations in *IGF1R* have recently been shown to contribute to human longevity (Suh et al., 2008). Therefore, studies in *Heterocephalus* will undoubtedly provide clues about the molecular and genetic mechanisms of human aging (as studies in mice and rats have) and may even provide genetic tools to fight human age-related diseases.

We did not select *Heterocephalus* solely because of its utility for research but also because it would allow researchers to study closely-related species with different lifespans adept to experimental manipulation. As a result, our rationale for selecting *Heterocephalus* was not just based on the fact it is a long-lived organism, but also because it can be compared to shorter-lived rodents, in particular mice and rats that can only live up to 4-5 years even in protected environments. Indeed, we think mice will be an excellent surrogate system to study the *Heterocephalus* genome, an approach previously suggested by Ray and Capecchi to study the bat genome (http://www.genome.gov/Pages/Research/Sequencing/BACLibrary/BrownBatBAC.pdf). Proof-of-concept has been recently demonstrated by replacing in mice a regulatory sequence of the paired-related homeobox gene *Prx1* by the orthologous sequence from a bat. Not only was *Prx1* expression elevated but the transgenic mice developed significantly longer forelimbs (Cretekos et al., 2008). Similarly, it is possible that the differential regulation of homologous genes, perhaps even genes already associated with aging in rodents, underlie species differences in aging and by generating transgenic mice with homologous genomic regions from *Heterocephalus* we will be able to evaluate how genomic changes affect life history in mammals.

Aging is widely regarded as the result of accumulating damage (Kirkwood and Austad, 2000), and thus *Heterocephalus* must have evolved mechanisms to slow the accumulation of different forms of damage. Many sources of cellular and molecular damage have been proposed to be crucial in aging, including (but not limited to) oxidative damage, senescent cells, protein toxicity, DNA alterations, and changes to mitochondria, yet which one is more important remains controversial. Consequently, researchers will greatly benefit from having *Heterocephalus* as a long-lived paradigm in which to test if different forms of damage accumulate slower than in short-lived rodents and dissect the molecular and genetic pathways involved and identify protective mechanisms (e.g., repair systems) in *Heterocephalus*. Modern molecular biology methods, such as RNAi, microarrays, and tandem mass spectrometry, take advantage of knowledge of the genome and thus sequencing *Heterocephalus* will greatly facilitate studies in these animals, as further detailed below.

Sequencing the *Heterocephalus* genome will also be crucial for researchers to prioritize candidate genomic regions for further studies. For example, it is possible to analyze sequence data from species with different brain sizes to obtain candidate genes by associating rates of molecular evolution with phenotypic changes (Gilbert et al., 2005). In fact, several studies have now identified genes that could be involved in the evolution of unique human traits (Finch and Stanford, 2004; Vallender and Lahn, 2004; Pollard et al., 2006). Given the availability of sequence information for other rodents (one of the reasons for selecting *Heterocephalus* as a long-lived species), analyses of *Heterocephalus* genes, such as homologs of known aging-related genes, will allow the identification of the most promising candidates for follow-up.

We are convinced that understanding the genetic and molecular mechanisms by which *Heterocephalus* evolved such a long lifespan when compared to similar-sized rodents will provide major insights on human aging, longevity, and disease susceptibility. In fact, *Heterocephalus* are very resistant to neoplasia (Buffenstein, 2005 & 2008) and can thus provide key insights into cancer biology that potentially can be used to develop therapies for improving human health. The idea that other organisms possess unique features whose understanding may help alleviate human diseases is

not new, as exemplified by the ample and exciting research conducted in species with a greater regeneration ability than humans (McGann et al., 2001; Hawkins and Lovett, 2004). Lastly, as further described below, *Heterocephalus* have other distinctive adaptations and its genome is likely to be an evolutionary goldmine on thermoregulation, disperser morphs, queen castes and the associated brain and skeletal changes in adults, unmyelinated neurons in juveniles, and visual degeneration in adults.

Proposal

This proposal, written on behalf of a large national and international community of researchers studying aging and age-related diseases as well as experts on *Heterocephalus* biology (Appendix I), proposes the complete sequencing of *Heterocephalus glaber*. This organism was chosen by taking into consideration species already being sequenced (http://www.genome.gov/10002154) and model systems that can be used as surrogates. We think *Heterocephalus* is the long-lived species that best complements current models and has the highest potential to provide insights on the human aging process. The proposal was widely circulated through the scientific community, particularly among biogerontologists but also evolutionary biologists and mammalogists studying *Heterocephalus*, and made available in a website (http://genomics.senescence.info/sequencing/) for further discussion. A summary of our rationale was published to obtain feedback from the gerontological community (de Magalhaes et al., 2007b).

Heterocephalus glaber is an underground dwelling, eusocial rodent native to north Eastern Africa (Sherman et al., 1991). *Heterocephalus* belongs to the Bathyergidae family (Faulkes et al., 2004; Deuve et al., 2008), which in turn is part of the Hystricognathi suborder of rodents that includes the new world caviomorpha and the old world phiomorpha (Figure 2). Fossil evidence suggests that this suborder diverged from the Sciurognathi suborder to which mice and rats belong about 55 million years ago, though estimates based on molecular clocks have produced controversial results (Huchon et al., 2002; Springer et al., 2003). Despite their small size (adult body mass is on average about 35 grams), *Heterocephalus* are the longest-lived rodents with a record longevity of over 28.3 years in captivity—and unpublished data from one of us (Dr. Rochelle Buffenstein) suggests they may live over 30 years. Furthermore, these mouse-sized rodents continue to breed well into their third decade of life and show attenuated age-related changes in physiological function (O'Connor et al., 2002, Buffenstein, 2008), such as maintenance of vascular youthfulness well into old age (Csiszar et al., 2007). Strikingly, cancer has not been observed to date in *Heterocephalus*.

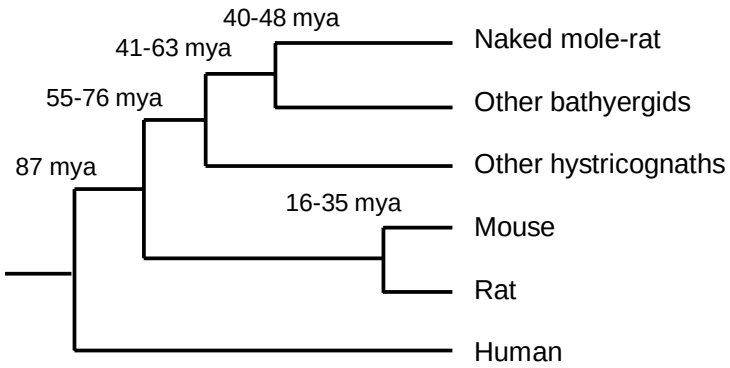

Figure 2: Phylogeny of *Heterocephalus* and related species. *Heterocephalus* diverged from other bathyergids (i.e., other mole-rats) about 40-48 million of years ago and from other hystricognaths (e.g., *Hystrax* and *Cavia*) about 41-63 mya. Phylogeny from Huchon et al., 2002; Springer et al., 2003; Faulkes et al., 2004; Deuve et al., 2008.

Sequencing the genome of *Heterocephalus* will be crucial to study its unique traits. In particular, it will greatly aid in providing information about a major physiological innovation that is the evolution of longevity and resistance to diseases—and age-related diseases like cancer in particular. Because of its unique phylogeny (Figure 2), sequencing *Heterocephalus* will also improve the annotation of other rodent genomes. In fact, our proposal to sequence this species may allow the identification of functional elements in the human genome associated with longevity.

Specific biological/biomedical rationales for the utility of new sequence data

Improving human health and understanding human disease by understanding the biology of aging

Following on the above rationale, we are convinced that studying *Heterocephalus* will yield important clues about the mechanisms of human aging and age-related diseases and sequencing *Heterocephalus* will be essential to develop such studies. Specifically, having the *Heterocephalus* genome sequence will: 1) provide researchers with the molecular biology tools to study mechanisms of aging and aging-related genes in this long-lived organism and possibly in comparison with shorter-lived rodents; 2) allow researchers to prioritize genes for further studies in cells or using a traditional model organism, such as the mouse, as a surrogate.

Even though the precise molecular and cellular processes of aging remain contentious, there is a vast list of mechanisms associated with aging in model organisms and humans. Studying these processes in *Heterocephalus* and how different forms of damage accumulate would allow researchers to gather new clues about the mechanisms underlying the aging process, and eventually dissect the molecular genetics of the mechanisms involved. In fact, numerous researchers are studying (or have demonstrated interest in studying) putative mechanisms of aging and candidate aging-related genes in *Heterocephalus*. The letters of support in Appendix II demonstrate the breadth of aging studies that can be conducted in *Heterocephalus*. These include endothelial function in cardiovascular aging (page 3 in Appendix II), cellular senescence (p 8), membrane composition (p 13), the growth factor heregulin and its role in neurodegeneration (p 1), selenoproteins and redox homeostasis (p 14), mechanisms that maintain genome integrity such as DNA repair (p 19), mitochondria (pp. 5, 26), bone remodeling and integrity (p 2), DNA replication (p 25), protein turnover (p 15), proteases (p 28), tumor suppressors (p 28), thermoregulation and metabolism (pp. 5, 30), and stress resistance (p 29).

The studies conducted so far in *Heterocephalus* demonstrate the high potential for discovery using this species. For example, the free radical theory is one the most influential in biogerontology, arguing that the accumulation of oxidative damage underlies the process of aging and that differences between species in reactive oxygen species production contribute to differences in aging (Harman, 1981; Beckman and Ames, 1998). Surprisingly, young *Heterocephalus* produce similar amounts of reactive oxygen species to shorter-lived rodents (Labinskyy et al., 2006; Lambert et al., 2007) and have high levels of oxidative damage (Andziak et al., 2005). In fact, the studies conducted so far suggest that antioxidant activity is unlikely to explain the extreme longevity of *Heterocephalus* when compared to mice (Andziak et al., 2006). However, *Heterocephalus* do show attenuated age-related changes in accrued oxidative damage (Andziak and Buffenstein, 2006) and exhibit marked resistance to oxidative insults as well as vascular resistance to proapoptotic stimuli (Labinskyy et al., 2006). Taken together, these results suggest that *Heterocephalus* evolved novel mechanisms to cope with oxidative damage and dissecting these mechanisms would provide important clues about aging.

Having the genome sequence will vastly facilitate studies of *Heterocephalus* biology, biochemistry, and physiology by allowing researchers to employ modern molecular biology tools such as transcription profiling and proteomics. For example, cells from long-lived rodents appear to

be more resistant to oxidative stress (Harper et al., 2007). In model organisms, stress response genes identified by high-throughput transcriptional studies can extend longevity when overexpressed (Wang et al., 2004). With the *Heterocephalus* genome sequence researchers will have the sophisticated tools necessary to study stress response pathways and how these diverged from those of short-lived rodents, potentially providing new clues about the mechanisms of aging.

Having the *Heterocephalus* genome will also allow researchers to prioritize genes for further studies. For example, studies of molecular evolution rates, including of aging-related genes, can be used to associate patterns of selection with the evolution of longevity (de Magalhaes and Church, 2007). In another example, Ashur-Fabian et al. (2004) sequenced the p53 gene in *Spalax ehrenbergi*—another mole-rat species—and noticed two amino acids substitutions specific to *Spalax* in the DNA-binding domain identical to tumor-associated mutations. Based on these results, in vitro studies revealed that those specific changes in *Spalax* p53 favor cell arrest and DNA repair with a bias against apoptosis (Avivi et al., 2007). It would be interesting to conduct similar analyses of aging-related genes in *Heterocephalus*, which would be greatly facilitated by having the genome sequence. We would also like to determine if *Heterocephalus* evolved, for example, innovative DNA repair genes or has differences in the content of putative aging-related genes when compared to shorter-lived rodents as well as analyze regulatory sequences. To identify specific changes in the *Heterocephalus* lineage, genome comparisons could employ the mouse and rat genomes, as well as the genomes of other rodents like the guinea pig (*Cavia porcellus*, a hystricognath) that can live up to 12 years despite being considerably bigger than *Heterocephalus*.

Heterocephalus genes of interest obtained from computational or experimental studies (e.g., transcription of proteomic studies of *Heterocephalus* in vivo or in vitro) could also be experimentally tested in traditional model organism, particularly in mice. This concept is exemplified in the skin cancer protection offered by creating transgenic mice expressing one or two CPD-photolyases, repair enzymes evolutionary lost in placental mammals (Jans et al., 2005). In fact, as aforementioned, we think mice would be an excellent surrogate system to study how the *Heterocephalus* genome modulates longevity and aging by replacing mouse genes, including coding and/or regulatory sequences, with *Heterocephalus* homologs.

Expanding our knowledge of cancer biology

Recently, it was shown that similar processes driven by ortholog genetic events underlie malignant evolution in murine and human tumors (Maser et al., 2007). Having an additional paradigm of cancer resistance in which to study these homolog genes would be extremely useful to identify genetic changes that underlie species differences in cancer. It has long been argued that because of the age-related development of cancer, mechanisms that confer resistance to oncogenesis could have evolved in long-lived species, such as changes in DNA repair genes (Sager et al., 1983; Lee et al., 1991). As in studies of mechanisms of aging, having the *Heterocephalus* genome will be a key step to expand research on cancer biology using *Heterocephalus*. For example, whole-genome screens for *Heterocephalus* candidate tumor suppressors will be feasible, similar to those done so far in human cells using RNAi (Westbrook et al., 2005) or by transfecting mouse cells with human genes to find genes responsible for cancer development and progression (Wan et al., 2004). Overall, *Heterocephalus* genes may be major players in the genetic suppression of tumor formation with potential applications in humans.

Informing other aspects of human biology

Although our proposal focuses mostly on longevity, aging, and age-related diseases, the proposed organisms will also be useful to inform about the genetic factors determining other life

history traits such as the rate of growth and development. Despite their small size, *Heterocephalus* reach maturity at about 7 months of age and can grow until about two years of age and because of their eusocial system have a distinct reproductive physiology with a single breeder (queen) per colony (Jarvis, 1981; Clarke and Faulkes, 1997).

Moreover, *Heterocephalus* possesses other unique traits of interest and we are convinced that sequencing its genome will reveal many treasures. *Heterocephalus* has been used to study abnormal lens differentiation and its role in eye morphogenesis (Nikitina et al., 2004), vitamin D (Buffenstein et al., 1993 & 1994), neural plasticity and cortical remodeling in the brain (Catania and Remple, 2002), as well as vomeronasal organs, which may make *Heterocephalus* a unique model to study this sensory system (Smith et al., 2007). *Heterocephalus* exhibits a selective lack of pain-related neurotransmitters (Park et al., 2008), and it would be very useful to have a catalogue of putative *Heterocephalus* neurotransmitters and their promoters to guide experiments. Moreover, because *Heterocephalus* is virtually poikilothermic (Buffenstein and Yahav, 1991), it is an exceptional model for studying thermoregulatory features of circadian cycles (Herold et al., 1998) and mammalian metabolic regulation (Goldman et al., 1999). Although menopause has not been observed to date, *Heterocephalus* exhibits stress-induced infertility (Faulkes et al., 1991) and hence may be a useful model of human stress-related infertility.

As reflected in Appendix II, there are numerous researchers currently studying a multitude of biological processes in *Heterocephalus* who consider sequencing its genome essential to further develop their research. Processes under study include neurobiology and behavior (pages 4, 12, 32 in Appendix II), bone growth and healing (pp. 2, 4), pain and sensory adaptations (pp. 6, 7, 17, 32), respiratory system (p 6), sociality (pp. 10, 12), reproduction and infertility (pp. 10, 12), development (pp. 11, 32), sexual differentiation (p 10), and olfactory systems (p 17).

<u>Providing a long-lived model organism for experimentation and facilitating the ability to do experiments</u>

Mammalian model systems have been typically chosen to study specific human diseases, with an emphasis on short-lived genotypes that show early onset conditions. This is especially true for rodent models such as mice and rats (Finch, 1990). It seems timely for us to consider long-lived models. *Heterocephalus* is an excellent choice for a long-lived model organism due to its small size, extremely long lifespan (when compared to other rodents), and a low incidence of many diseases that typically affect humans and mice. In other words, we do not suggest *Heterocephalus* will be used as a model of human disease, but rather as a model of resistance to certain diseases. Because mice and rats age so much faster than *Heterocephalus*, having the full genome sequence of *Heterocephalus* will provide researchers with similar species with highly divergent lifespans that can be compared with a high degree of sophistication in silico, in vitro, and in vivo. Importantly, *Heterocephalus* can be kept and bred in captivity (Buffenstein, 2005), cell lines have been derived by different groups, and tissues (including from old animals) are available.

Having the *Heterocephalus* genome sequence will greatly facilitate the ability to perform experiments by providing researchers with the broad array of modern molecular biology tools. Knowledge of the genome facilitates, and is often essential, for many high-throughput methods such as microarrays, antibodies, PCR-based methods, and proteomics. Succinctly, having the genome sequence will allow researchers to study gene expression profiles across species, as has been done before (Fraser et al., 2005), some of which may be related to species different in longevity. For example, FOXO1a has been associated with aging in model organisms and shown to be significantly up-regulated in humans when compared to shorter-lived primates. This information led de Candia et al. (2008) to apply a combination of genomic approaches to study the role of FOXO1a in the

regulation of oxidative stress response and show differences between humans and chimpanzees that are consistent with the shorter lifespan of chimpanzees. Similarly, rodent cells express low levels of DDB2 when compared to human cells and overexpression of DDB2 in mice protects against UV-induced cancer (Alekseev et al., 2005). Because recent studies suggest a large effect of sequence divergence in microarrays, even in closely related species (Gilad et al., 2005), species-specific comparisons will be necessary, which require knowledge of *Heterocephalus* coding sequences.

In addition to gene expression studies, having the full genome sequence will allow researchers to gain insights into the evolutionary forces shaping *Heterocephalus* and will provide prioritized targets for experimentation, in particular by using the mouse as a surrogate system to construct transgenic mice with homologous *Heterocephalus* genes or regulatory regions. Having the full genome sequence will also help researchers study regulatory regions, perhaps coupled to gene expression studies, to gain new insights about the regulation of aging and age-related diseases, as has been previously done in mice (Lu et al., 2004).

Strategic issues in acquiring new sequence data

The demand for the new sequence data

As reflected in the list of 79 scientists who supported this proposal (Appendix I), we expect that a large community of researchers working on aging and age-related diseases, as well as researchers studying other interesting features of *Heterocephalus*, will employ the genome sequence of *Heterocephalus* in their work. Our proposal generated ample interest because, as aforementioned, scientists working on the biology and genetics aging will then possess an arsenal of sophisticated molecular biology tools to study these long-lived animals in vivo and in vitro and test hypotheses of aging—including mechanistic hypotheses and aging-associated genes—using a novel and unique comparative biology paradigm. Even researchers that will not directly conduct aging studies in *Heterocephalus* will benefit from the sequencing of the *Heterocephalus* genome by, for example, being able to study in short-lived model organisms or in genetic association studies in humans homologs of genes that are revealed as high-priority from the *Heterocephalus* genome. We also anticipate that having the genome sequence for this long-lived species will encourage many in the community of researchers working on the broad area of genome sciences to focus on the evolution of longevity and mechanisms of aging. Lastly, the work of researchers studying other unique features of *Heterocephalus* will also considerably benefit from this proposal and they have enthusiastically supported it (Appendix II).

The suitability of the organism for experimentation

As detailed above, *Heterocephalus* is already being used as a model for biological/biomedical research and colonies, often with hundreds of animals, already exist. Cell lines and tissues are also available. *Heterocephalus* is easy to breed in captivity, though mutants are not available at present. Having its genome, however, will allow researchers to create transgenic mice with candidate *Heterocephalus* genes, as detailed above.

The rationale for the complete sequence of the organism

The complete sequence of *Heterocephalus* is crucial to prioritize candidate genes for further analysis and develop sophisticated molecular biology tools to study this organism. Despite progress in model organisms, our knowledge of species differences in longevity and disease-resistance is still limited. We know of genes in which genetic manipulations can delay aging in model organisms but

we do not know whether functional changes or differential regulation of these genes contribute to species differences in longevity. The way we do not know whether protein-coding, RNA-encoding, regulatory, or even some unknown genomic feature contributes to species differences in aging highlights the need for complete genome sequencing. Possibly the evolution of longevity involves a combination of regulatory and protein changes and thus it is essential to have the complete sequence of *Heterocephalus* to conduct comprehensive studies. Furthermore, important genes may be missing from EST and cDNA libraries because they are expressed at very low levels or not expressed at all in normal conditions. As highlighted in the recent sequencing of the rhesus macaque genome (Gibbs et al., 2007), the value of finished sequence is well-established not only for microarrays but for many applications such as PCR-based methods and studies of proteins.

The cost of sequencing the genome and the state of readiness of the organism's DNA for sequencing

One of us (Dr. Rochelle Buffenstein) can provide the necessary materials (e.g., tissue samples) for genome sequencing of *Heterocephalus*.

The exact genome size of *Heterocephalus* is unknown but is expected to be around 3,000 Mb, as reported for other rodents (http://www.genome.gov/19516773). We are not aware of any unusual biological feature posing challenges for genome sequencing. We propose the complete sequencing (i.e., 6x or higher coverage) of *Heterocephalus glaber* using a whole genome shotgun strategy including paired sequence reads from large-insert clones for long-range continuity as judged cost-effective at the time. The cost of complete sequencing is estimated to be around $15-20M, though because of progress in new sequencing technologies (Church, 2006) these costs are expected to drop in a near future.

We have contacted Dr. Eric Lander who has expressed a strong interest in pursuing this project and carrying out the sequencing of this organism at the Broad Institute.

Other sources of funding and previous NHGRI proposals

No other sources of funding are currently being sought.

Concluding remarks

Aging is the biggest risk factor for several clinical conditions and understanding its molecular and genetic mechanisms has an unparalleled potential to improve human health. We think the complete sequencing of *Heterocephalus glaber* will be a major step forward in establishing this organism as the first long-lived model of biomedical research. With the genome sequence, numerous mechanisms of aging can be tested in these long-lived organisms (and in comparison to short-lived rodents like mice and rats) with a high degree of sophistication to provide unique insights. Sequencing *Heterocephalus* will also allow the prioritization of candidate genes and will facilitate the use of the mouse as a surrogate system in which to study the genetic programs of *Heterocephalus*. Being cancer-resistant, *Heterocephalus* can also be used to dissect the genetics of cancer. Metabolism, behavior, development, and neurobiology are other processes well-suited for studies in *Heterocephalus*. In conclusion, we think sequencing *Heterocephalus* will provide a genetic roadmap to understand human longevity, aging, and age-related disease.

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Appendix I

The following scientists, mostly biogerontologists but also experts on *Heterocephalus* and evolutionary biologists, support the proposal. A selection of support letters is provided as a separate file in Appendix II.

Name	Institution
Atwood, Craig	University of Wisconsin-Madison
Barja, Gustavo	Complutense University
Bennett, Nigel	University of Pretoria
Brand, Martin	Medical Research Council Dunn Human Nutrition Unit
Bronikowski, Anne	Iowa State University
Brunet, Anne	Stanford University
Burda, Hynek	University of Duisburg-Essen
Burhans, William	Roswell Park Cancer Institute
Campisi, Judith	Lawrence Berkeley National Laboratory
Carey, James	University of California, Davis
Casper, Diana	Montefiore Medical Center
Catania, Kenneth	Vanderbilt University
Coles, L. Stephen	University of California, Los Angeles
Demetrius, Lloyd	Harvard University
Downs, Colleen	University of KwaZulu-Natal
Erwin, Joseph	Foundation for Comparative and Conservation Biology
Estep, Preston	Longenity, Inc.
Faragher, Richard	University of Brighton
Faulkes, Christopher	Queen Mary, University of London
Finkel, Toren	National Heart, Lung, and Blood Institute
Fraifeld, Vadim	Ben Gurion University of the Negev
Gems, David	University College London
Gilad, Yoav	University of Chicago
Gladyshev, Vadim	University of Nebraska-Lincoln
Goldman, Bruce	University of Connecticut
Gorbunova, Vera	University of Rochester
Guerin, John	Centenarian Species and Rockfish Project
Hayflick, Leonard	University of California, San Francisco
Heguy, Adriana	Memorial Sloan Kettering Cancer Center
Holmes, Donna	Washington State University
Hornsby, Peter	University of Texas Health Science Center at San Antonio
Hulbert, A. J.	University of Wollongong
Jepsen, Karl	Mount Sinai School of Medicine
Kapahi, Pankaj	Buck Institute for Age Research
Kenyon, Cynthia	University of California, San Francisco
Khalyavkin, Alexander	Russian Academy of Sciences
Kirkwood, Tom	Newcastle University
Larson, John	University of Illinois at Chicago
Lee, Phyllis	University of Stirling

Lee, Siu	Cornell University
Lee, Virginia	University of Pennsylvania
Levitt, Jonathan	City College of New York
Lewin, Gary	Max Delbrück Center for Molecular Medicine
Lopez-Otin, Carlos	University of Oviedo
Macieira-Coelho, Alvaro	French National Institute of Health
McCarter, Roger	Pennsylvania State University
Moradas-Ferreira, Pedro	University of Porto
Muradian, Khachik	Institute of Gerontology, Academy of Medical Sciences of Ukraine
O'Connor, Timothy	Cornell University
Oliveira, Catarina	University of Coimbra
O'Riain, Justin	University of Cape Town
Park, Thomas	University of Illinois at Chicago
Parker, Joel	University of Southampton
Partridge, Linda	University College London
Platzer, Matthias	Leibniz Institute for Age Research
Ponting, Chris	University of Oxford
Promislow, Daniel	University of Georgia
Richardson, Arlan	University of Texas Health Science Center at San Antonio
Rollo, C. David	McMaster University
Ryazanov, Alexey	University of Medicine and Dentistry of New Jersey
Samuels, David	Virginia Bioinformatics Institute
Schaffler, Mitchell	Mount Sinai School of Medicine
Schmidt, Jennifer	University of Illinois at Chicago
Shay, Jerry	University of Texas Southwestern Medical Center at Dallas
Sherman, Paul	Cornell University
Sinclair, David	Harvard Medical School
Skulachev, Vladimir	Moscow State University
Smith, Tim	Slippery Rock University
Summers, Kyle	East Carolina University
Tissenbaum, Heidi	University of Massachusetts Medical School
Toussaint, Olivier	University of Namur
Towett, Philemon	University of Nairobi
Trojanowski, John	University of Pennsylvania
Ungvari, Zoltan	New York Medical College
Van Remmen, Holly	University of Texas Health Science Center at San Antonio
Vijg, Jan	Buck Institute for Age Research
Warner, Huber	University of Minnesota
Wolkow, Catherine	National Institute on Aging
Zwaan, Bas	Leiden University