Cytosystematics of Gerbils

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Thesis presented in partial fulfilment of the requirements for the degree Masters of Science in Zoology at Stellenbosch University

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March 2013

Declaration

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March 2013

Abstract

The objectives of this research were to: (1) accurately identify chromosome homology, (2) identify chromosome rearrangements leading to diploid number variation and chromosome evolution to formulate the ancestral gerbil karyotype and distinguish homoplasy from hemiplasy, and (3) construct a phylogeny using chromosomal characters based on G-banding and fluorescence *in situ* hybridization (FISH) of ten gerbil species representing five genera (*Gerbilliscus*, *Desmodillus*, *Psammomys*, *Meriones*, *Taterillus*), with probes derived from flow-sorted chromosomes of *G. paeba* (GPA, 2n = 36), and polarised with the murid outgroup, *Micaelamys namaquensis*.

All paints successfully hybridized to all ingroup and outgroup taxa. Three of the 19 *G. paeba* painting probes (GPA 7, 9 and X [including the X; autosome translocation in *T. pygargus*]) were conserved as whole chromosomes, and 16 were rearranged (GPA 1-6, 8, 10-17). Chromosome painting correctly identified the homology of the heterochromatic GPA 7, which was conserved as whole chromosomes in all gerbils. Thirteen previously misidentified G-band homologies were correctly identified with FISH; one in *D. auricularis*, and six each in *G. kempi* and *G. gambianus*. Homology maps identified 57 syntenic associations and that 19 rearrangements are responsible for diploid number differentiation among species. Parsimony analysis of the two matrices (syntenic association and rearrangements) retrieved a sister-species relationship between *G. gambianus* and *G. kempi*, and *P. obesus* and *M. persicus* (syntenic associations), an unresolved clade that included *D. auricularis*, *G. gambianus*, *P. obesus* and *M. persicus* (chromosome rearrangements) and a basal position for *T. pygargus*.

Phylogenies derived from chromosomal data failed to resolve the deeper nodes. Consequently, characters were subsequently mapped on a molecular consensus tree (including a chronogram). This allowed inferences on the rate of chromosome evolution, which indicates that the basal *D. auricularis* is separated from *Gerbilliscus* by nine rearrangements (four Robertsonian fusions, five inversions), at a rate of 1.25/Myr. *Gerbilliscus* species evolved with an average of 10 Robertsonian rearrangements involving GPA 1–6, 8, 10 – 12, of which four are homoplasies (GPA 1-3, 5),

one a potential hemiplasy (GPA 5) to southern African taxa, one a synapomorphy to *G. paeba* and *G. tytonis* (GPA 6), two synapomorphies in *G. kempi* and *G. gambianus* (GPA 11, 12), and three are synapomorphic to *Gerbilliscus* (GPA 4, 8, 10). Homoplasic characters across the two clades include GPA 3 (*T. pygargus*, *G. paeba* and *G. tytonis*) and GPA 5p-q prox (*D. auricularis*, *P. obesus* and *M. persicus*). *Gerbilliscus* (excluding *G. paeba* and *G. tytonis*) had the slowest chromosome evolutionary rate of < 1/Myr; *G. paeba* and G. *tytonis* were slightly faster at 2/Myr. The clade comprised of *M. persicus*, *P. obesus* and *T. pygargus* evolved faster, at a rate of 4/Myr (seven fissions, five fusions, two inversions), 2.3/Myr (seven fissions, two fusions, four inversions) and 16/Myr (eight fusions), respectively, indicating heterogeneity among Gerbillinae: A slow rate in *Desmodillus* and *Gerbilliscus*, and a fast evolutionary rate in *Psammomys*, *Meriones* and *Taterillus*. The putative ancestral karyotype was postulated to be 2n = 56, and included five biarmed autosomes and X chromosome, and 22 acrocentrics. This is provisional, since *Brachiones*, *Desmodilliscus*, *Pachyuromys*, *Sekeetamys*, *Gerbillus* and *Rhombomys* were not analysed.

This work forms part of a peer-reviewed research publication: Knight, LI, Ng, B.L., Cheng , W., Yang, F., Rambau, R.V. Tracking chromosome evolution in southern African gerbils using flow-sorted chromosome paints. 2013. *Cytogenetic and Genome Research* (In Press)

Opsomming

Die objektief van hierdie navorsing was om: (1) akkurate chromosoom homologie te identifiseer, (2) chromosoom herranskikkings te identifiseer wat mag lei tot diploide chromosoom getal variasie en chromosoom evolusie ten einde te formuleer die voorouer karyotiepe van "gerbils" sowel as om te onderskei tussen homoplasie en hemiplasie, en (3) die konstruksie van 'n filogenetiese boomstam gebasseer op chromosoom karakters verkry vanaf G-banding en FISH (fluoressensie *in situ* hibridisasie) van tien "gerbil" spesies wat vyf genera verteenwoordig (*Gerbilliscus*, *Desmodillus*, *Psammomys*, *Meriones*, *Taterillus*), deur van geskikte sondes gebruik te maak wat verkry in deur floei-sorteerde chromosome van *G. peaba* (GPA, 2n = 36), wat gepolariseerd was met die Murid buitegroep, *Micealamys namaquensis*.

Alle chromosoom verwe het suksesvolgehibridiseer aan al die ingroep en buitegroep taxa. Drie van die 19 *G.peaba* verfwe (GPA 7, 9 and X (including the X; autosome translocation in *T. pygargus*) was bewaar as heel chromosome, en 16 herrangskik (GPA 1-6, 8, 10-17). Chromosoom verfwing kon suksesvol die homologie van die heterochromatise GPA7 identifiseer wat gekonserveerd was as heel chromosome in al die "gerbils", wat moontlik aandui die teenwoordigheid van funksionele gene. Dertien voorheen mis geidentifiseerde G-band homologieë was gekorregeer deurmiddel van FISH, een in *D. auricularis*, en ses elk in *G. kempi* en *G. gambianus*. Homologie kaarte het 57 sintesiese assosiasies geidentifiseer en dat 19 herrangskikings verantwoordelik was vir diploied nommer differensiasies tussen spesies. Parsimonie analises van die twee matrikse (sinteniese assosiasies en herrangskikings) wys 'n suster-spesie verwantskap tussen *G. gambianus* en *G. kempi*, en *P. obesus* en *M .persicus* (sinteniese assosiasies), 'n unopgeloste klade wat *D. auricularis*, *G. gambianus*, *P. obesus* en *M. persicus* (chromosoom herrangskikkings) opmaak vorm die basale posisie vir *Taterillus pygargus*.

Filogenetise boomstamme verkry vanaf die chromosomale data misluk egter om die dieper nodes op te los. Karakters was daarna geplot op 'n konsensus boom (insluitend 'n chronogram). Dit het dieper insigte toegelaat soos die tempo van chromosoom evolusie, wat aandui dat die basale *D. auricularis* geskei is vanaf *Gerbilliscus* met nege herrangskikkings (vier Robertsonian, vyf inversies) teen 'n tempo van 1.25/Mja. *Gerbilliscus* spesies het verander met 13 herranskikinge

(11 saamsmeltings en twee inversies), waarvan vier potensiele homoplasies/hemiplasies (GPA 1-3, 5). Met die uitsluitsel van *G. paeba* en *G. tytonis*, het *Gerbilliscus* die laagste chromosoom evolutionêre tempo van al die "gerbils" < 1 /Mja, *G. paeba* en *G. tytonis* was ietwat vinniger met 'n tempo van 2/Mja. The klade wat bestaan uit *M. persicus*, *P. obesus* en *T. pygargus* verander vinniger as *Desmodillus* en *Gerbilliscus*, met 'n evolutionêre tempo van 4/Mja (sewe fissies, vyf samesmeltings, twee inversies) en 2.3 Mja (sewe fissies, twee samesmeltings, vier inversies) onderskeidelik, wat grootendeels tandem was. Die karyotiepe van *Taterillus pygarus* het agt samesmeltings gehad wat predominant tandem was, teen 'n tempo van 16/Mja.

Terwyl meeste van die herrangskikinge synapomorfies was, was sommige homoplasties of hemiplasties. Homoplastiese karakters wat gedeel was tussen die twee klades sluit in GPA 3 (in *T pygargus* en *G. paeba* en *G. tytonis*) en GPA 5p-q prox (*D. auricularis*, *P. obesus* en *M. persicus*). GPA 5 was hemiplasties aan alle suider Afrikaanse taxa. Die analise van sinteniese assosiasies en chromosoom herrangskikings was geanaliseer in PAUP, en gepolariseer met die murid *Micealamys namaquensis*. *Taterillus pygarus* het 'n basale posisie in beide filogenetiese boomstamme. Die data stel voor dat FISH meer akkurate resultate lewer op chromosoom homologie as die streng gebruik making van banding patrone. Verder het die tempo van chromosoom evolusie gevarieër vanaf stadig (*Desmodillus* en *Gerbilliscus*) tot vinnig (*Psammomys*, *Meriones* en *Taterillus*), chromosoom karakters egter was nie sterk genoeg om dieper filogenetiese verwantskappe te ondersoek nie. Die voornemende voorouerlike karytiepe van "gerbils" was hier gehipotiseer as 2n = 56. Drie bevindinge resoneer uit hierdie studie. Eerstens, chromosoom verwing kon chromosoom homologieë wat voorheen deur banding studies mis ge-identifiseer was korrek identifiseer: hierdie sluit in een konflik in *D. auricularis*, en ses elk vir *G. kempi* en *G. gambianus*. Tweedens, die homologie van die heterochromatiese of C-positiewe autosome, GPA 7, was gedemonstreer as bewaar as 'n heel chromosoom as beide heterochromaties en euchromatiese chromosome in alle "gerbils", wat aandui dat dit functionele gene dra. Derdens gebasseer op simpleisiomorfe wat geidentifiseer was vanaf die homologie kaarte en vergelykbare opleidings, hipotiseer ek dat die voorgestelde voorouer karyotiepe bestaan uit ses autosome (GPA 7, 9, 13, 15, 16, 17) en die X chromosoom, wat onveranderd gebly het tussen alle suider Afrikaanse taxa. Met die uitsluitsel van GPA 7p/7q, was almal behoue as twee-armige chromosome in die voor ouer karyotiepe. In lyn met hierdie is, 21 akrosentries GPA1p,1q, 2p, 2q, 3p, 3q, 4p, 4q, 5p, 5q, 6p, 6q, 8p, 8q, 10q, 10p, 11p,11q, 12p, 12q en 14, wat

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Acknowledgements

I would not have successfully completed my MSc thesis had it not been for the support and encouragement of many, whom I would like to express my gratitude. My supervisor, Dr. R.V Rambau, for providing me with the much-needed guidance and assistance, and always being on hand to help me. A special thank you to Prof. T.J. Robinson for helping me to analyse and interpret parts of my data and the many lab meetings.

I wish to thank Dr. Fengtang Yang who provided the flow-sorted chromosomes of *G. paeba* that were used in the study. The study also received numerous contributions of specimens. I am thankful to Drs G. Dobigny, V. Volobouev, L. Granjon and T. Wassenaar, and Mr. J. Watson and K. Bâ for providing the gerbils used in the study. To my friends and colleagues Adriaan Engelbrecht, Madeleine Ramantswana and Leigh Richards who helped me with fieldwork and specimen collection.

I am grateful for the support of the Evolutionary Genomics Group and all who assisted me, especially Adriaan Engelbrecht, Madeleine Ramantswana, Ethel Phiri, Leigh Richards and Daleen Badenhorst. I would like to express a heartfelt thank you to my family (mom, Jennifer, dad, sister and brother) and my best friends at home who continue to encourage me.

This study was kindly funded by the National Research Foundation (NRF).

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Chapter 1

General Introduction

1.1 Preamble

The subfamily Gerbillinae (Rodentia, Muridae) is the second largest subfamily among murids, and contains 15 extant genera and 116 described species (Musser & Carleton 2005). Systematic relationships among these species have been investigated using a variety of tools including allozymes, cranial morphology, chromosomes (reviewed in Chevret & Dobigny 2005; Pavlinov 2008). More recently, a molecular sequence phylogeny based on representatives of 11 genera and 28 species has provided a clearer picture about evolutionary relationships among gerbils (Chevret & Dobigny 2005). The availability of a more representative phylogeny of gerbils provides an ideal opportunity to test a variety of hypotheses. Of particular interest is the role of chromosome evolution among gerbils. However, cytogenetic data of gerbils are generally limited to conventional (G- and -C) banding; while these techniques are very useful their utility is rather limited when making comparisons between taxa that differ as a result of extensive chromosomal rearrangements (Benazzou *et al*. 1982, 1984; Qumsiyeh & Chesser 1988; Engelbrecht *et al*. 2011). In fact, some gerbil genera are known to be characterised by high levels of genome reshuffling, such as *Taterillus* (Dobigny *et al*. 2005), and thus comparative analyses with other gerbils (particularly intergeneric relationships) may be problematic.

Identification of homeology and subsequently description of chromosomal characters is best achieved using fluorescence *in situ* hybridization. This approach has been widely used to explore karyotype differentiation among vertebrates (e.g., fish, Mazzuchelli *et al*. 2012; birds, Nishida *et al*. 2008, de Oliveira *et al*. 2010; mammals, Wienberg 2004), but has not yet been fully exploited in understanding genome evolution among gerbils. This study attempts to refine and provide karyotype comparisons among gerbils using flow-sorted chromosomes of *G. paeba* representing five genera and 10 species: *Gerbilliscus* (*G. tytonis*. *G. leucogaster*, *G. afra*, *G. kempi*, *G. gambianus*), *Desmodillus auricularis*, *Psammomys obesus*, *Meriones persicus*, *Taterillus pygargus*, and the outgroup *Micaelamys namaquensis*. This approach (coupled with the DNA sequence phylogeny) provided useful insights on: (1) rate of chromosome evolution among gerbils, (2) working hypothesis on the ancestral karyotype of gerbils, (3) resolving and

correcting previous homeologies inferred from G-banding, and (4) a detailed assessment of convergence, i.e., true homoplasy (shared character states due to convergence and reversals) *vs.* hemiplasy (topological discordance between a gene and species tree due to the phylogenetic sorting of chromosome polymorphisms across successive nodes in species trees, Avise & Robinson 2008; Robinson *et al.* 2008).

1.2 Taxonomy and distribution

The Gerbillinae are distinguished from other muroid rodents by their molars and incisors (Pavlinov 2008), and two to three ovate rings of enamel enclosing the molar central cement (Chimimba & Bennett 2005). They are further characterised by an inflated mastoid and auditory bulla (a character that improves hearing in open, xeric environments; Lay 1972), nasals that project over the incisors, a broad brain case, slanted zygomatic arch and a slightly narrow rostrum (Petter 1971; Ellerman 1941). External post-cranial characteristics of gerbils include long limbs (an adaptation for saltatorial locomotion in desert terrains) and a tufted tail (Ellerman 1941).

The Gerbillinae subfamily consist of nine monotypic genera: *Ammodillus*, *Brachiones*, *Desmodilliscus*, *Desmodillus*, *Microdillus*, *Pachyuromys*, *Rhombomys*, *Sekeetamys* and *Tatera*. Polytypic genera include *Gerbillurus*, *Gerbilliscus*, *Gerbillus*, *Meriones*, *Psammomys*, and *Taterillus*. Of these genera, five were the main focus of this study: *Desmodillus*, *Gerbilliscus*, *Gerbillurus*, *Meriones*, *Psammomys*, and *Taterillus*. Anatomical features that distinguish genera used in the study include dentition (plain upper incisors in *Psammomys* and grooved incisors in *Meriones*, see Table 1.1; length of palatal foramina in *Gerbilliscus* [< 3 mm] and *Taterillus* [> 3 mm, Petter 1971]. Gerbil genera and species are further differentiated from each others using a collection of morphological and phenotypic traits, such as bulla and dental characteristics and body size (Table 1.1). While features defining species may be clear, the distinction between *G. paeba* and *G. tytonis* (Griffin 1990; Perrin a, b), *G. kempi* and *G. gambianus* (Granjon *et al.* 2012), *Gerbillus* (Abu Baker & Amr 2003) and *Taterillus* (Dobigny *et al.* 2002), species may be problematic because of phenotype convergence in overlapping distribution areas, as in the case of *G. paeba* and *G. tytonis* (Perrin a, b)

Table 1.1: Summary of morphological and phenotypic features that differentiate gerbils used in this investigation (Ellerman 1941; Nowak 1999; Stuart & Stuart 2001; Chimimba & Bennett 2005; Musser & Carleton 2005; Pavlinov 2008; Dobigny *et al.* 2005; Darvish 2009, 2011; Hoath 2009).

Genus	Species	Bulla	Dentition	Total length (mm)	Tail length (mm)	Mass (g)
Gerbilliscus	*G. leucogaster*	Tympanic portion of the bulla more inflated than in *Tatera*	Upper incisors are grooved Lower incisors ungrooved.	280	150	70
	G. afra	Ear bulla well-developed	Upper incisors are grooved Lower incisors plain	300	150	91-103
	G. kempi	Inflated tympanic portion of the bulla	Advanced horseshoe type	138.8	147	73.3
	G. gambianus	Inflated tympanic portion of the bulla	Advanced horseshoe type	153.3	144.8	114.5
Gerbillurus	*G. paeba*	Ear bulla inflated; undeveloped occipital region Posterior bulla not well developed	Upper incisors are grooved Lower incisors ungrooved.	200	110	< 30
	G. tytonis	Tympanic bulla larger than *G. paeba*	Upper incisors are grooved.	220	120	35
Taterillus	*T. pygargus*	Tympanic portion of bullae well inflated	Molar crowns are lophodont	113	150	No data
Desmodillus	*D. auricularis*	Bulla is greatly inflated	Upper incisors long, slender and grooved. Upper molars are small with three transverse laminae	200	75-80 % of body length	53
Meriones	*M .persicus*	Mastoid bulla large	Upper incisors each with one groove. Molars prismatic	127	136 % of total body length	100
Psammomys	*P. obesus*	Mastoid fully enlarged	Plain upper incisors. Molars are prismatic	251-356	100-157	32-43

Gerbils are widely distributed in Africa and Asia over three broad geographic areas (Schlitter & Ågren 2006). These are the savannas and Namib and Kalahari deserts of Africa, which are occupied by *Gerbillurus, Gerbilliscus, Desmodillus* and *Taterillus*; the deserts and semi-deserts in North Africa which are inhabited by *P. obesus*; and the deserts, semi-deserts and steppes in Central Asia, a native habitat of *M. persicus* that extends between Asia and Transcaucasia, to the Middle East (Darvish 2011).

1.3 Phylogenetic relations of the Gerbillinae

Numerous attempts to elucidate the phylogenetic relationships of gerbils employing cranial morphology, various molecular markers and conventional cytosystematics retrieved incongruent evolutionary relationships. For instance, the close relationship of the East African *Gerbilliscus* and *Gerbillurus* is confined to chromosome banding data analyses (Volobouev *et al*. 2007), and rejected by all molecular-based studies (Colangelo *et al*. 2007; Granjon *et al*. 2012). Similarly, Pavlinov (2008) described the two morphologically distinct genera, *Gerbillurus* and *Gerbilliscus*, whereupon following molecular-based studies, *Gerbillurus* is now regarded as a synonym of *Gerbilliscus* (Chevret & Dobigny 2005; Colangelo *et al*. 2007; Granjon *et al*. 2012). Inconsistencies in the most recent morphological, molecular sequence data and conventional cytosystematics are summarised in the following sections:

(a) Morphological systematics of the Gerbillinae

According to Pavlinov (2001, 2008) *Tatera*, *Taterillus*, *Desmodillus* and *Gerbilliscus* are differentiated into two tribes, namely Taterillini (*Tatera*, *Taterillus, Gerbilliscus*) and Gerbillurini (*Desmodillus* and *Gerbillurus*), defined by bullae and dental traits (node A, Figure 1.1). Further, *D. auricularis* and *G. paeba* have a similar mastoid structure (a deep dorsal penetration of the tympanic cavity into the mastoid) and horseshoe pattern in the anteroconid portion of the first molar (M1; Pavlinov 2001). Although the mastoid in *Gerbilliscus* and *Taterillus* are not enlarged to the same degree as in *D. auricularis* and *G. paeba*, they do have a (more advanced) horseshoe dental pattern in the lower M1. Similarly, the genera *Dipodillus, Gerbillus, Monodia Microdillus, Desmodilliscus* and *Pachyuromys*, and *Sekeetamys, Meriones, Brachiones, Psammomys* and *Rhombomys*, comprise a monophyletic assemblage characterized by prismatic tooth crown (Pavlinov 2001, 2008). However, these phylogenetic relationships were retrieved without the use of outgroups.

Additional morphological descriptions of these gerbils indicate that the monotypic *Tatera* (*T. indica*) and *Gerbilliscus* (formerly *Tatera*), each a monophyletic genus, are confined to Asia and Africa, respectively. The Asian *Tatera* appeared to be more distant from the African taxa based on size of the bullae; *T. indica* has a more advanced bulla (small; shallow penetration by the tympanic cavity into the mastoid) while *Gerbilliscus* has a more primitive bulla (characterized by a large deeper tympanic cavity into the mastoid, Pavlinov 2001; 2008). Alternatively, the African *Tatera* has a more advanced anteroconid M1 dental morphology than the Asian *Tatera*. The genus has alternatively been separated into two broad taxonomic groups; the African species comprise the genus *Gerbilliscus*, and the Asian one represent *Tatera sensu stricto*. This subdivision is further supported by the difference in humerus length between the two genera (Musser & Carleton 2005).

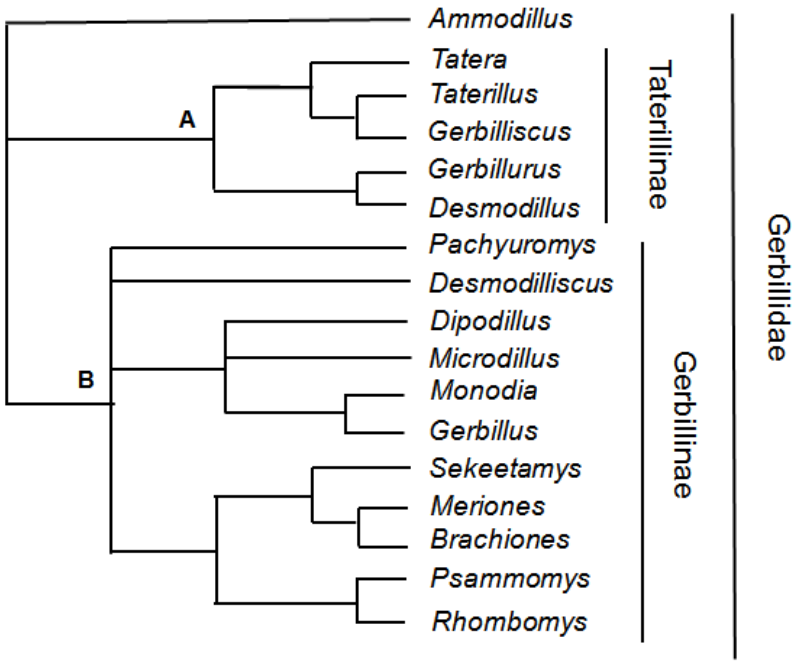

Figure 1.1: The morphologically-based phylogeny of gerbils using cranial and dental characteristics (Pavlinov 2001, 2008). Taterillinae and Gerbillinae are defined based on shared dental and middle-ear characteristics (tree redrawn from Chevret & Dobigny 2005).

However, using morphological features to infer systematic relations is problematic, since they are prone to convergence and may vary according to diet and the local environment (Darvish

2011; Lecompte *et al*. 2005). Further, morphological convergence due to environmental pressures has been demonstrated in East African *Gerbilliscus* (Colangelo *et al*. 2010) and *Meriones* (Darvish 2011), thus demonstrating the disadvantage of using morphological and phenotypic characteristics only.

(b) Molecular-based systematics of the Gerbillinae

Molecular studies using nuclear genes, the LCAT gene (Michaux & Catzeflis 2000; Michaux *et al*. 2001), vWF gene (Michaux *et al*. 2001) and the GHR, BRCA 1, RAG 1 and c-myc nuclear genes (Steppan *et al*. 2004) found the Gerbillinae to have diverged from the Deomyinae almost 20 Myr ago. This is also supported by cytochrome *b* (1140 bp) and 12S rRNA (950 bp) sequence data based on 28 representative species of 11 genera (Chevret & Dobigny 2005). These sequence data also retrieved four main lineages that were African (*Taterillus*), Asian (*Rhombomys*) and Middle Eastern and African (*Sekeetamys*), or Middle Eastern, African and Asian (*Gerbillus*, *Psammomys* and *Meriones*) origin – the latter originated in Africa and subsequently migrated to Asia. *Desmodillus, Gerbilliscus* and *Gerbillurus* are a monophyletic assemblage to the exclusion of *Tatera.* Similarly, the phylogeny indicates that *Tatera* is a monotypic genus confined to Asia, while *Gerbilliscus* (formerly *Tatera*) is strictly African. In turn, *Gerbilliscus* is further differentiated into geographical clades. These include a southern clade (*G. brantsii*, *G. afra*, *G. leucogaster*), western clade (*G. kempi*, *G. gambianus*, *G. guinea*, *Gerbilliscus* sp.) and eastern clade (*G. robusta*, *G. vicinus*, *G. nigricaudus*; Colangelo *et al*. 2007) , as well as a "*Gerbillurus* group", which diverged first from the southern and West African *Gerbilliscus* 7.5 Myr ago, following the origin of *Gerbilliscus* 8.5 Myr ago (Colangelo *et al*. 2007). *Gerbilliscus* species are separated with a maximum sequence divergence of 25.7 % between *G. vicinus* and *G. kempi*, to a minimal divergence of 9.6 % between the southern African *G. afra* and *G. brantsii* (Colangelo *et al*. 2007). Clearly, the relationships within *Gerbilliscus* are paraphyletic; *Gerbillurus* is basal to the southern and West African *Gerbilliscus*, to the exclusion of the East African *Gerbilliscus* (Colangelo *et al*. 2007; Granjon *et al*. 2012). Due to the paraphyly of *Gerbilliscus*, the two genera were synonymised and designated as *Gerbilliscus.* Consequently, I followed this taxonomic treatment throughout this study.

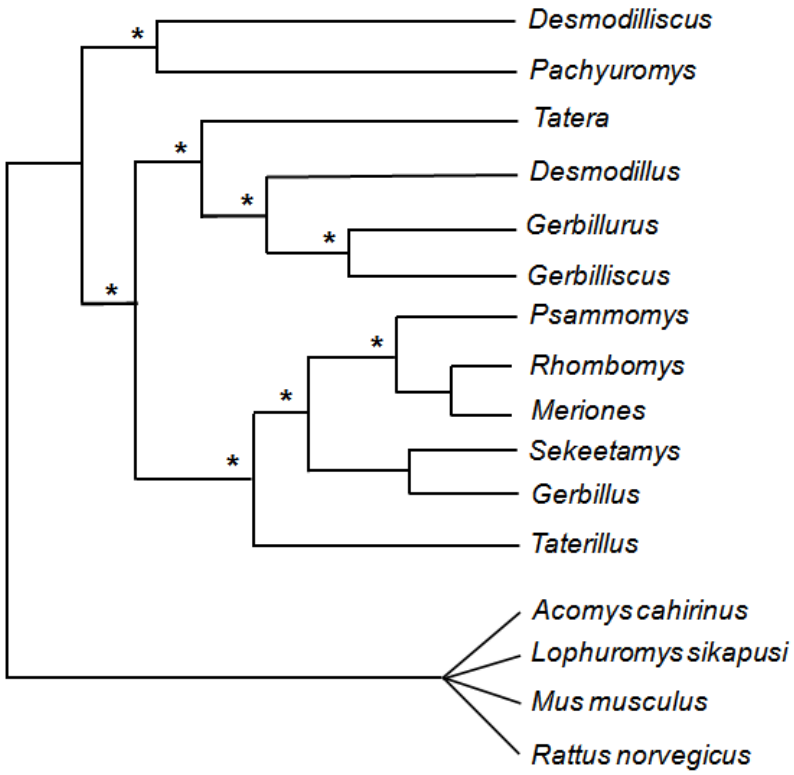

Figure 1.2: A simplified phylogeny of Gerbillinae established using the mitochondrial genes cytochrome *b* and 12S rRNA showing three clades (modified from Chevret & Dobigny 2005). Clade 1 consists of *Desmodilliscus* plus *Pachyuromys*, Clade 2 is comprised of *Gerbillurus*, *Gerbilliscus* and *Desmodillus*, with *Tatera* at the base, and Clade 3 contains *Psammomys, Meriones, Sekeetamys* and *Gerbillus* with *Taterillus* at the base. The phylogeny was polarised with *Acomys cahirinus*, *Lophuromys sikapusi*, *Mus musculus* and *Rattus norvegicus*. Asterisks indicate bootstrap support greater than 90 %.

(c) Cytosystematics of the Gerbillinae

Chromosomal data has been used extensively to elucidate relations among gerbils. The cytogenetic data of gerbils are limited to conventional (G- and -C) banding which were partly employed to differentiate between species (e.g., *Gerbillus*, Granjon *et al*. 2002; *Psammomys*, Mostafa *et al*. 2006), and construct phylogenetic relationships among species within a genus (e.g. *Taterillus*, Dobigny *et al*. 2005; *Gerbilliscus*, Volobouev *et al*. 2007), subtribe (*Psammomys* and *Meriones*; Qumsiyeh & Chesser 1988) or among gerbils originating from the same subregion (southern African *Desmodillus* and *Gerbilliscus*; Qumsiyeh 1986b).

The comparative G-band analysis of the eight southern African gerbils (*Gerbillurus paeba*, *Gerbillurus tytonis*, *Gerbillurus setzeri*, *Gerbillurus vallinus*, *Gerbilliscus leucogaster*, *Gerbilliscus afra*, *Gerbilliscus brantsii*, *D. auricularis*) revealed that the majority of chromosomal rearrangements separating *Gerbilliscus* species are Robertsonian fissions and fusions. For example, two fusions of *G. brantsii* and *G. afra* 1 + 2, and 7 + 8, both 2n = 44, differentiate the two species from *G. leucogaster* (2n = 40; Qumsiyeh 1986b; Qumsiyeh *et al.* 1991). G-banding comparisons further suggest that *Gerbilliscus* species may have evolved from a 2n = 44 karyotype (present in both *G. afra* and *G. brantsii*; Qumsiyeh 1986b). Comparisons between *G. paeba* and *G. tytonis* have shown the two to have identical diploid numbers of 2n = 36, and few structural rearrangements (pericentric inversions in the GPA 11/12 and the absence of the heterochromatic GPA 33 in *G. tytonis*) which suggests a sister-species relationship (Qumsiyeh 1986).

However, the G- and C-banding data revealed more detailed relations within *Gerbilliscus*/*Gerbillurus* (*Gerbilliscus* sp., *G. guineae*, *G. gambianus*, *G. kempi*, *G. leucogaster*, *G. robusta*, and *Gerbillurus tytonis*; Volobouev *et al*. 2007). According to these comparisons, the southern African endemic *Gerbillurus* has a closer relationship to the East African *Gerbilliscus* than it did to the southern African *Gerbilliscus*. *Gerbilliscus robusta* and *G. tytonis* were found to have a sister-species relationship, since they are separated only by a single inversion and have a bootstrap support (maximum parsimony analysis) value of 99% (Volobouev *et al*. 2007). The overall comparative analysis of the six *Gerbilliscus* species and *G. tytonis* revealed that Robertsonian rearrangements are the dominant chromosome mutation. Similarly, the West African *Gerbilliscus* species are separated by 17 rearrangements containing seven fissions, five translocations and five inversions. However, Robertsonian rearrangements appear to be homoplasic (Qumsiyeh *et al*. 1987; Britton-Davidian *et al*. 2005). A combination of allozymes and G-C banding results retrieved a clade of southern African *Gerbillurus* (*G. vallinus* and *G. paeba*) and southern + eastern *Gerbilliscus* (*G. robusta*, *Gerbilliscus* sp., *G. nigricauda*, *G. leucogaster*, *G. afra* and *G. brantsii*) that were based largely of homoplasic characters (Qumsiyeh *et al*. 1987). In fact, instances wherein Robertsonian fusion and fission events occurred frequently have resulted in homoplasy (e.g., *Gerbilliscus*, Qumsiyeh *et al*. 1987; *Meriones*, Qumsiyeh 1989). However, these prior gerbil cytosystematics have largely focused on intrageneric relationships and less on intergeneric relationships (*Gerbilliscus*, Volobouev *et al*. 2007; *Gerbillus*, Aniskin *et al*. 2006; *Meriones*, Qumsiyeh 1989 and *Taterillus*, Dobigny 2002a; 2004).

1.4 Rationale

Chromosome rearrangements may be considered as rare genomic changes (RGCs), since a change or a mutation in a chromosome is large and occurs slowly (1-10 rearrangements/Myr; Burt *et al*. 1999) and rarely (Rokas & Holland 2000; Murphy *et al*. 2004a). Further, these large-scale mutations are uncommon and are less likely to undergo convergent or parallel evolution than nucleotide sequences (Rokas & Holland 2000; Granjon & Montgelard 2012). The chromosome mutation will accumulate in a population and promote reproductive isolation with other populations, thus causing speciation (Ayala & Coluzzi 2005). Therefore chromosome rearrangements are valid characters that can be used to infer phylogenetic relations (see Dobigny *et al*. 2004). Cytosystematics of gerbils has only been conducted using primary banding homology to date. However, the conclusions based solely on banding data may be inadequate since they are more effective when identifying regions of homology among closely related taxa (Robinson 2001). In particular, banding patterns are useful in detecting Robertsonian fusions and fissions, common in the Gerbillinae (Qumsiyeh 1986), but may not adequately address instances wherein extensive rearrangements have occurred, especially when these result in huge differences in diploid number. This is partly applicable to the Gerbillinae, which have extensive diploid number variation ranging between 2n = 14/15 (Dobigny *et al*. 2003) to 2n = 76-78 (Dobigny *et al*. 2002b; Granjon *et al*. 1992), and have since speciated over a period spanning more than 10 Myr (Chevret & Dobigny 2005) across regions in Africa, Asia and the Middle East (Nowak 1999; Musser & Carleton 2005). Nonetheless, G-banding comparisons demonstrates that Robertsonian rearrangements were prevalent during these extended periods which raises questions about the possibility of ancient polymorphisms or hemiplasy (Robinson & Avise 2008), which has to date not yet been investigated in gerbils.

Hemiplasy is defined as characters that resemble homoplasy (Avise & Robinson 2008). It occurs when ancestral polymorphic traits, such as fusion/fission variants of a chromosome, are fixed only in a few descendants due to lineage sorting (Avise & Robinson 2008). It is more likely to occur in instances of Robertsonian rearrangements and in phylogenies with short internodal distances (short evolutionary time; Robinson *et al*. 2008). When the polymorphism persists from an ancestral lineage into a descendent lineage following rapid speciation events, the polymorphic state may sort into numerous species nodes. Hemiplasy has been observed in bovids (Robinson & Ropiquet 2011) and possibly bats (Mao *et al*. 2008), which have also undergone frequent Robertsonian rearrangements through the course of their respective evolution. Hence, interpretation of chromosome data in conjunction with a molecular based phylogeny with

cladogenic dates allows evaluation of the role of homoplasy and hemiplasy in evolution and speciation of gerbils.

In the present study, detailed karyotype comparison of gerbils was undertaken using FISH in an attempt to provide new insights on the evolutionary relationships among these rodents. Karyotype comparisons of rodent (e.g., Sciuridae, Richard *et al*. 2003, Li *et al*. 2004; Castoridae, Pedetidae and Dipodidae, Graphodatsky *et al*. 2008a) and non-rodent species (see Ferguson-Smith & Trifonov 2007) have traditionally been undertaken using *Homo sapiens* (HSA) painting probes as a reference point across numerous representative mammalian taxa. Among rodents, painting probes derived from *Mus musculus* (MMU) are preferred because the species has a complete genetic map (Scalzi & Hozier 1998), and has been used as a reference point to compare the karyotypes of Muroid rodents (e.g. *Rhabdomys pumilio*, Rambau & Robinson 2003; *Otomys irroratus*, Engelbrecht *et al*. 2006; Romanenko *et al*. 2012; Chaves *et al*. 2012). However *M. musculus* chromosomes are highly rearranged, which may hamper the comparison of chromosome homologies (Graphodatsky *et al*. 2008a). Consequently, probes have been generated for specific taxa, and to-date almost a hundred mammals have been flow-sorted (Murphy *et al*. 2001). The development of flow-sorts from closely related taxa provides a more detailed analysis among conspecifics and has proved successful in understanding the karyotypic relationships within taxonomic groups (Romanenko *et al*. 2010). For example, the karyotypic relationships in the genera *Microtus* (Sitnikova *et al*. 2007; Lemskaya *et al*. 2010) and *Akodon* (Ventura *et al*. 2009) were described using painting probes derived from conspecifics. Other examples include comparative painting at higher taxonomic levels including order (Chiroptera, Richards *et al.* 2010) or family (Canidae, Graphodatsky *et al.* 2008b). These taxon specific probes provide a more refined comparison of chromosomes between conspecifics and congenerics.

Gerbils are an ideal group to study chromosome evolution - the interspecific karyotype diversity shows diploid number variation to range from 2n = 14/15 in *Taterillus tranieri* (Dobigny *et al.* 2003) to 2n = 76-78 in *Desmodilliscus braueri* and species distinctions are often accompanied by karyotype differences (Dobigny *et al.* 2002b; Granjon *et al.* 1992). Further, the phylogeny of the Gerbillinae subfamily (including a chronograph) using 11 of 15 representative genera has identified three main clades/lineages and periods of divergence among species (Chevret & Dobigny 2005). A more detailed comparative analysis interpreted in the context of the published molecular phylogeny should enable us to identify chromosomal rearrangements/characters

defining the clades. These include the complex relationship within *Gerbilliscus* (the paraphyly of southern + West African and East African *Gerbilliscus* clades retrieved using DNA sequences) and the relationship between the African *Gerbilliscus* (formerly *Tatera*) and the Asian *Tatera.*

To adequately determine the role of chromosome change in the phylogeny of gerbils require chromosome painting data derived from a wide representation of several gerbil genera and species that represent the various phylogenetic clades and nodes identified by Chevret & Dobigny (2005). These include *Psammomys* (*P. obesus*), *Meriones* (*M. persicus*), *Desmodillus*, *Taterillus* (*T. pygargus*) and *Gerbilliscus* (*G. paeba*, *G. tytonis*, *G. leucogaster*, *G. afra*, *G. kempi*, *G. gambianus*). Furthermore, gerbils have large heterochromatin content (Qumsiyeh 1988), and it is uncertain whether it is phylogenetically informative (Qumsiyeh 1988). For instance, the homology of *G. paeba* chromosome 7 of its published karyotype (therein referred to as *Gerbillurus paeba* 33; Qumsiyeh 1986) was described as being comprised completely of diffused heterochromatin (Qumsiyeh 1988; Qumsiyeh *et al*. 1991) and the homology thereof is unclear. This will be determined by establishing whether there is homology among C-positive autosomes which characterise most gerbil taxa (Qumsiyeh 1988) that hybridize to other gerbil species. Furthermore, the extent of chromosome homology and karyotype reorganisation between *Gerbilliscus*, *Taterillus*, *Psammomys* and *Meriones* are not completely known, and it is unclear how they are related to the southern African and West African *Gerbilliscus*, and this is partly attributable to limitation of conventional banding techniques.

FISH localises nucleic acids and DNA sequences using non-radioactive biotinylated probes, which are visualised with the use of fluorescently-labelled antibodies (Sessions 1996; Wienberg 2004). Flow-sorted chromosomes indiscriminately amplify the entire length of a chromosome (Telenius *et al*. 1992). The FISH protocol is based on nucleotide similarity at a resolution of 5-10 mbp (Rens *et al.* 2006). Furthermore, FISH can identify regions of homeology across taxa that diverged at least ~90 million years ago (Murphy *et al*. 2004b), as this time frame includes the divergence of gerbils which spans 20 Myr (Michaux *et al*. 2001; Steppan *et al*. 2004).

1.5 Aims and Objectives

This study focuses on comparative analysis and cytosystematics of five representative gerbil genera and 10 species: five southern African taxa *D. auricularis* (2n = 50), *G. afra* (2n = 44), *G. leucogaster* (2n = 40), *G. paeba* (2n = 36), *G. tytonis* (2n = 36); two extralimital *Gerbilliscus* specimens from outside the southern African sub-region, *G. kempi* (2n = 48) and *G. gambianus* (2n = 52), as well as *M. persicus* (2n = 42), *P. obesus* (2n = 48) and *T. pygargus* (2n = 22). According to molecular sequence data these species evolved over an evolutionary period spanning approximately 10 Myr and represent five lineages (Chevret & Dobigny 2005). The aims and objectives of the study are:

1) To accurately identify chromosome homology, particularly in instances where homologous banding patterns could not successfully be established.

2) Identify chromosome rearrangements and causes leading to diploid number variation and chromosome evolution in gerbils, and herewith formulate the ancestral gerbil karyotype.

3) Construct a phylogeny among the gerbil species using chromosomal characters based on G-banding and FISH results.

Chapter 2

Materials and Methods

2.1. Introduction

Chromosomal data have contributed to distinguishing populations, chromosome races and species in vertebrates (White 1973), including gerbils. The early cytogenetic studies of gerbils included mainly non-differential staining, which subjectively paired chromosomes and arrange karyotypes based on size and morphology. These provided limited information such as diploid number (2n) variation and number of chromosomal arms (e.g., *Meriones*, Nadler & Lay 1969; Korobitsyna & Korablev 1980). The advent of chromosome digestion using enzymes and subsequent staining produced banding patterns that made it possible to identify homologues and rearrangements separating species such as inversions, translocations and other rearrangements (Caspersson *et al*. 1968). Differential G, R and Q-banding each produces characteristic bands along the length of homologues, which are used to construct karyotypes (Bickmore & Sumner 1989; Seabright 1971; Sumner 1990). The G-band technique uses trypsin digestion and Giemsa stain to produce the distinctive bands along the euchromatic regions of chromosomes (Sumner 1990). R-banding produces the reverse staining intensity of G-banding - light bands are dark and dark bands are light. Q-banding produces fluorescent bands from quinacrine mustard or quinacrine (Caspersson *et al*. 1968), while C-banding stains the heterochromatic regions of chromosomes using barium hydroxide (Sumner 1990). These banding patterns are useful in cytotaxonomy to identify and differentiate species (e.g., *Gerbilliscus*, Colangelo *et al*. 2001; *Gerbillus*, Granjon *et al*. 2002; *Psammomys*, Mostafa *et al*. 2006); however they are generally incapable of detecting regions of homeology among distantly related taxa, particularly in cases where extensive genomic changes have occurred; a case in point being comparisons between *Taterillus* and other gerbils (Benazzou *et al*. 1982, 1984). During the past two decades advances in cytogenetics, particularly in the field of molecular cytogenetics where FISH is routinely used (Trask 2002; Dobigny & Yang 2008), have made it possible to successfully hybridize probes between and among distantly related taxa, and consequently reconstruct phylogenies. Chromosomes are useful markers in establishing phylogenetic relationships (Rokas & Holland 2000), and have been used for reconstructing phylogenies of a variety of mammalian taxa (Ferguson-Smith & Trifonov 2007) for several reasons.

Firstly, changes at the chromosome level occur at a large scale, and since they occur infrequently (Rokas & Holland 2000), it reduces instances of homoplasy. Further, chromosome rearrangements or mutations (fusions, fissions, translocations, inversions, heterochromatin amplification) can be coded into binary format (present = 1 or absent = 0), and analysed in a similar fashion to molecular sequences. With the use of an outgroup and parsimony, phylogenetic relationships can be inferred between and among taxa (reviewed in Dobigny *et al.* 2004). The present study employs the molecular cytogenetic technique (FISH) to identify regions of homeology among gerbils, and the inclusion of an outgroup to polarize the chromosomal data allows synapomorphies to be distinguished from symplesiomorphies, and in doing so enabled the generation of an ancestral gerbil karyotype.

2.2 Materials

(a) Specimens [a]

A total of 10 species representing five gerbil genera were examined (Table 2.1). These include the southern African taxa (*D. auricularis*, *G. paeba*, *G. tytonis*, *G. leucogaster* and *G. afra*), three West African (*G. kempi*, *G. gambianus* and *T. pygargus*), one North African (*P. obesus*), and the Middle Eastern/Asian *M. persicus* (Table 2.1). The outgroup used in the study was the murine rodent *M. namaquensis*, since molecular sequence data of murids indicates that the Gerbillinae (and Deomyinae) is a sister taxon to the Murinae (Michaux & Catzeflis, 2000; Michaux *et al.* 2001; Steppan *et al.* 2004; Granjon & Montgelard 2012).

[a] The study was initially restricted to include only the southern African taxa.

Table 2.1: Species used in the study with collection localities, geographic coordinates, published diploid numbers (with reference of karyotype description), and sample size (N).

Species	Locality	Coordinates	2n	N
Desmodillus auricularis (DAU)	Tankwa Karoo, Northern Cape, South Africa	-32° 2108' S 19° 8195' E	52, XY	2[a]
Gerbilliscus gambianus (GGA)	Mbour, Senegal	14°4215'-S 16°9640'-E	52, XX	1[b]
[c]*Gerbilliscus kempi* (GKE)	_	_	48, XX	1[b]
Gerbilliscus afra (GAF)	Clanwilliam, Western Cape, South Africa	- 32° 1842' S 18° 9097' E	44, XY	1[a]
	Vensterklip, Western Cape, South Africa	-32° 5622' S 18° 5925' E	44, XX	3[a]
Gerbilliscus leucogaster (GLE)	Windhoek, Namibia	-22° 33.572' S 17° 0824' E	40, XY	1[b]
	Windhoek, Namibia	-22° 33.572' S 17° 0824' E	40, XX	1[b]
Gerbilliscus paeba (GPA)	Anysberg NR, Northern Cape, South Africa	-33° 4763' S 20° 6057' E	36, XY	1[a]
	Sutherland, Northern Cape, South Africa	- 33° 3931' S 20° 6555' E	36, XY	1[a]
	Gobabeb, Namibia	-22°.4553'S 18°.9632' E	36, XY	1[a]
Gerbilliscus tytonis (GTY)	Gobabeb, Namibia	-22°.4553'S 18°.9632' E	36, XY	1[b]
[c]*Meriones persicus*(MPE)	_	_	42, XY	1; This study
Psammomys obesus (POB)	Tunisia	_	48, XX	1; Qumsiyeh & Chesser 1988
Taterillus pygargus (TPY)	Mbour, Senegal	14°.4215'-S -16°9640'-E	22, XX	1; Dobigny *et al.* 2002a
Micaelamys namaquensis (MNA)	Sir Lowry's Pass, South Africa	- 32° 1166' S 18° 9130' E	24; XY	1; Baker *et al.* 1988

[a] Karyotype was reconstructed according to Qumsiyeh (1986)
[b] Karyotype was reconstructed according to Volobouev *et al*. (2007)
[c] Specimen of unknown locality was provided by Dr. Vitaly Volobouev

(b) Tissue culture

Tissue biopsies (tail or rib muscle) were used to establish fibroblast cell cultures following conventional cytogenetic techniques. Primary tissue biopsies were cleaned with 70% ethanol and incubated overnight in growth chambers maintained at 37°C and 5% CO_2 to monitor possible contamination. After a 24 hour incubation period the biopsies were cleaned and minced into small pieces and transferred to tissue flasks to allow attachment and growth of primary cells (Schwarzacher & Wolf, 1974). Cells were grown in media comprising Dulbecco's Modified Eagle Medium (DMEM, GIBCO) enriched with 15% foetal calf serum (FCS; GIBCO) and 15% amniomax (GIBCO with Glutamate) in incubation chambers maintained at 37°C and 5% CO_2 (Schwarzacher & Wolf, 1974). Cell division was arrested at metaphase stage using Colcemid (10 µg/ml; GIBCO BRL) and cells were harvested using the hypotonic treatment (0.075 M) followed immediately by fixation in modified Carnoy's methanol: glacial acetic acid (3:1) fixative (Östergren & Heneen 1962).

2.3 Methods

(a) Giemsa banding (GTG-Banding)

Chromosomes were G-banded by trypsin and stained with Giemsa following Seabright (1971). Briefly, this entailed aging slides for 1-2 hours (species-dependent) at 65°C, and digested with trypsin (0.25% in 1XPBS) for a minimum of 30 seconds to a maximum of 1 minute (species-dependent). This was followed by a rinse in calf serum buffer (500 µl FCS and 50 ml 0.025 M KH_2PO_4) for three minutes. Slides were stained with 2% Giemsa solution (diluted in 0.025 M KH_2PO_4).

(b) Constitutive heterochromatin banding (CBG-banding)

C-banding was used to localise the position of constitutive heterochromatin on the chromosomes. This was done either on freshly prepared (*G. paeba*, *G. tytonis*, *M. persicus*, *P. obesus*) or aged slides (*Gerbilliscus*, *D. auricularis*, *T. pygargus*) following Sumner (1972). This entailed treatment in 0.2 M HCl solution for three minutes and incubation in a saturated 5% Ba $(OH)_2$ solution for 1-2 minutes at 55°C (species-dependent). This was followed by a second incubation in 2XSSC for 30 minutes at 55°C. Aged slides were similarly incubated in a 5% Ba $(OH)_2$ solution for 2 minutes at 55°C, and in 2XSSC for 1 hour at 65°C, and finally stained with 2% Giemsa for a minimum of 5 minutes (species-dependent).

(c) Standardisation of karyotypes

A standard numbering system based on the homology of *G. leucogaster* chromosomal arms was initially used as a template to construct the karyotypes of the southern African gerbils (Qumsiyeh 1986b; Qumsiyeh *et al.* 1991). *Gerbilliscus paeba* and *D. auricularis* were arranged based on shape (biarmed to acrocentric chromosomes) and size (large to small; Qumsiyeh 1986), and *G. afra* was compiled according to the karyotype of *G. leucogaster* to illustrate the centric fissions (Qumsiyeh *et al.* 1986). The karyotypes of *G. tytonis*, *G. kempi*, *G. gambianus* were constructed following Volobouev *et al.* (2007), *P. obesus* was arranged following Qumsiyeh & Chesser (1988) and *M. namaquensis* was constructed according to Baker *et al.* (1988); karyotypes were generally ordered based on shape (biarmed to acrocentric chromosomes) and size (large to small).

2.4 Fluorescence *in situ* hybridization (FISH)

(a) Painting with index species: Human and mouse painting probes

Probes derived from *H. sapiens* (HSA) and *M. musculus* (MMU) were initially chosen to paint gerbils, with the intention to compare and extend the homology maps to those species analysed with these two paints (Ferguson-Smith & Trifonov 2007; Romanenko *et al.* 2010). Of the 23 human paints, only two human painting probes (HSA 3 and 6) successfully hybridized and a single mouse probe (MMU 4) from a possible 20 mouse paints produced hybridization signal, and the remainder failed to consistently hybridize. This was due either to the deep divergence time between human and gerbils (Murphy *et al.* 2004b) or to the fact that the mouse genome has been extensively rearranged, making painting to other species difficult (Graphodatsky *et al.* 2008a). Consequently, *G. paeba* was flow-sorted and used as an index species in this study (see below).

(b) Flow-sorting and DOP-PCR

Gerbilliscus paeba was chosen partly because it is a derived species among southern African gerbils (Chevret & Dobigny 2005; Colangelo *et al.* 2007). *Gerbilliscus paeba* chromosomes were sorted on a MoFlo dual-laser cell sorter and isolated based on size and base-pair composition (Yang *et al.* 2009)[a]. Mitotic cells were stained with base-pair specific fluorescent DNA-binding dyes; Hoechst 33258 binds preferentially to AT complementary pairs while chromomycin A3 to GC pairs. Chromosome flow-sorts were amplified with the 6 MW primer (5'CCGACTCGAGNNNNNNATGTGG 3') using DOP-PCR (degenerate oligonucleotide primed PCR; Telenius *et al.* 1992). The primary PCR amplification programme involved an

initial denaturation at 94°C for 9 minutes, a second denaturation at the same temperature for 1 minute (9 cycles), annealing at 30°C for 90 seconds and an extension at 72°C for 3 minutes. This was followed by 30 cycles of 94°C for 1 minute and 62°C for 1 minute.

The final extension step was at 72°C for 1 minute. Probes were labelled with either Biotin-16-2'-deoxy-uridine-5'-triphosphate-dUTP (Roche) or Digoxigenin-11-2'deoxy-uridine-5'-triphosphate, alkali-stable-dUTP (Roche) using a second PCR programme. It entailed an initial denaturation at 94°C for 3 minutes, a second denaturation for 1 minute (30 cycles), annealing at 62°C for 90 seconds and an extension at 72°C for 2 minutes and 45 seconds. This was followed by a final extension step at 72°C for 8 minutes.

(c) ZOO-FISH

Fluorescence *in situ* hybridization experiments followed the protocols of Rens *et al.* (2006) and Yang & Graphodatsky (2009), with minor modifications. *Gerbilliscus paeba* chromosome flow-sorts were assigned by hybridizing the labelled probes onto the species of origin to identify each peak in the flow-karyotype. Slides were aged for one hour and briefly dehydrated in an ethanol series (70-100%) for 2 minutes each. Dehydrated slides were denatured in 70% formamide for 1-2 minutes at 60-66°C, and placed in 70% ice-cold ethanol for three minutes to stop denaturation. Slides were dehydrated again using an ethanol series (see above) for 1 minute each.

Gerbilliscus paeba chromosomes were flow-sorted at the Wellcome Trust Sangers Institute (Cambridge) by Dr. Fengtang Yang, Ng BL., Cheng W. and Fu, B.

The DNA probe mixtures were comprised of 3-6 µl DOP-PCR product (species-dependent), 4 µl salmon sperm, 4-8 µl mouse *cot*-1 (species-dependent), 2 µl sodium acetate (3 M) and 54 µl of 100% ethanol, and allowed to precipitate overnight in a -80°C freezer. Thereafter the precipitate was centrifuged at high speed (13000 rpm) for 30 minutes and the resulting pellet was washed twice in 80% ethanol and air-dried at 37°C. Dried DNA probes were eluted in 15 µl hybridization buffer (50%, deionised formamide, 10% dextran sulphate, 0.5 M phosphate buffer pH 7.3, 1X Denhardt's solution). Probes were denatured at 72-75°C for 10 minutes and re-annealed for 15 minutes at room temperature. Probes were applied to denatured metaphase chromosome spreads and sealed with rubber cement. Hybridization times were species-dependent and varied between 12 hours (*G. paeba* and *G. tytonis*) and three nights (*G. leucogaster*, *G. afra*, G. *kempi*, *gambianus*, *D. auricularis*, *P. obesus*, *M. persicus*, *T. pygargus*).

(d) Detection and post hybridization washes

Detection of signals involved rinsing slides twice in 50% formamide for five minutes each and twice in 2XSSC (pH 7) for five minutes each at 43°C, followed by a rinse in 4XT (100 ml 20X SSC, 400 ml dH_2O, Tween 20) for three minutes. Biotin-labelled chromosomes were detected with the antibody Cy3-streptavidin (1:500 µl), and digoxigenin-labelled probes detected with FITC (Fluorescein Isothiocyanate)-conjugated anti-digoxigenin IgG made in sheep (1:1000 µl). All slide preparations were incubated for 30 minutes at 37°C, counterstained with DAPI (4'6-diamidino-2-phenylindole) and mounted using V-1000 Vectashield medium.

(e) Image Capture

Brightfield and fluorescent images were captured using a CCD (charge-coupled device) camera attached to an Olympus BX60 epifluorescent microscope equipped with DAPI, FITC and Cy3 fluorescence filters. Images were captured using the Genus software system version 3.7 (Applied Imaging Corp., Newcastle, UK). In order to facilitate identification of hybridized chromosomes, DAPI stained images were inverted into G-bands.

2.5 Phylogenetic Reconstructions

2.5.1 Coding of characters

The FISH signals obtained on the chromosomes basically formed the raw data which were then converted into chromosome characters. These were coded using two basic standard approaches – as chromosome rearrangements and syntenic associations as defined in Dobigny *et al*. (2004). The characters were converted or transformed into binary characters (present = 1, absent = 0). Adjacent syntenies are defined as whole chromosomes (or both chromosome arms, p/q), and where multiple signals were obtained the chromosome segments were labeled according to their proximity to the centromere: proximal (a), median (b) and distal (c). Rearrangements are defined as the change in chromosome structure (with the use of either the probe or homologous banding patterns). Both banding patterns and FISH data were used to determine intrachromosomal (inversions) and interchromosomal (conservation/rearrangement of GPA chromosomes) rearrangements. Variations in type of rearrangements were further distinguished as a fission into three or more segments (*1), inverted (^{a}1), inverted but fused to a separate chromosome segment ($^{a\#}$1), or a fission (1). Characters were then compiled into two binary matrices: one based on syntenic associations and the other based on rearrangements.

2.5.2 Mapping chromosome rearrangements onto a DNA sequence consensus tree

While using syntenic associations and rearrangements to reconstruct phylogenies is standard practice (Dobigny & Yang 2008), both methods have inherent limitations. Syntenic characters are not independent from each other and may result in overweighting (Dobigny *et al*. 2004), while using chromosome rearrangements may be prone to convergent or reversal events when breakpoints are reused (Mlynarski *et al*. 2010; Skinner & Griffin 2012). Furthermore, previous studies on gerbils have indicated that Robertsonian rearrangements may be homoplasic and using them in isolation may lead to erroneous conclusions (Qumsiyeh *et al*. 1987; Qumsiyeh 1989) as demonstrated in bats (Mao *et al*. 2008) and the house mouse (Britton-Davidian *et al*. 2005). Consequently, rather than rely solely on phylogenies generated from chromosome characters, the rearrangements/syntenies were mapped on a consensus phylogeny generated from DNA sequence data obtained from the more comprehensive gerbil DNA-based phylogenies of Chevret & Dobigny (2005), Colangelo *et al*. (2005; 2007) and Granjon *et al*. (2012). This phylogeny includes the species under examination (i.e., *G. paeba*, *G. tytonis*, *G. leucogaster*, *G. afra*, *G. kempi*, *G. gambianus*, *D. auricularis*, *P. obesus*, *M. persicus*, *T. pygargus*). In effect, the tree emphasises the monophyly of *Gerbilliscus*, the basal position of *Desmodillus* to *Gerbilliscus* and

the clustering of *P. obesus* and *M. persicus* outside the *Desmodillus* and *Gerbilliscus* genera, and the association of *T. pygargus* outside this assemblage, all well-established evolutionary associations.

2.5.3 Phylogenetic analysis

Maximum Parsimony (MP) was used to analyse the evolutionary associations in the ten gerbil species. Phylogenetic trees were reconstructed using PAUP v 4b10 (Swofford 2002). The data were polarised *a posteriori* using an outgroup, *M. namaquensis*. For each data matrix, a branch and bound search option and global tree statistics were calculated (consistency index, retention index), and nodal support was evaluated using 1000 bootstrap replicates (Swofford 2002).

Chapter 3

Results

3.1. Karyotype and FISH analyses

(a) *Gerbilliscus paeba*

The karyotype of *G. paeba* is comprised of a diploid number of 2n = 36, with all chromosomes biarmed (autosomes and sex chromosomes, Fig. 1a). The sex chromosomes are comprised of a large metacentric X and a small metacentric Y. The C-band analyses revealed that constitutive heterochromatin occurs largely around the centromeric regions of the autosomes, with a single pair of autosomes, *G. paeba* 7 (GPA 7) that stains entirely C-positive (Fig. 3.1b).

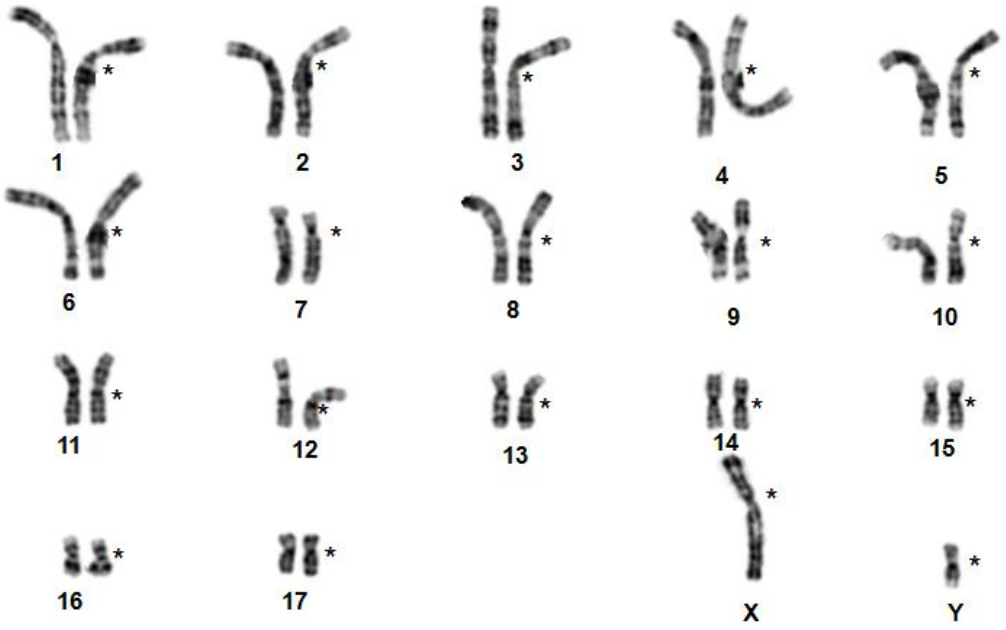

Figure 3.1: (a) The G-banded karyotype of the index species, a male *G. paeba* (2n = 36). Asterisks indicate the position of the centromere.

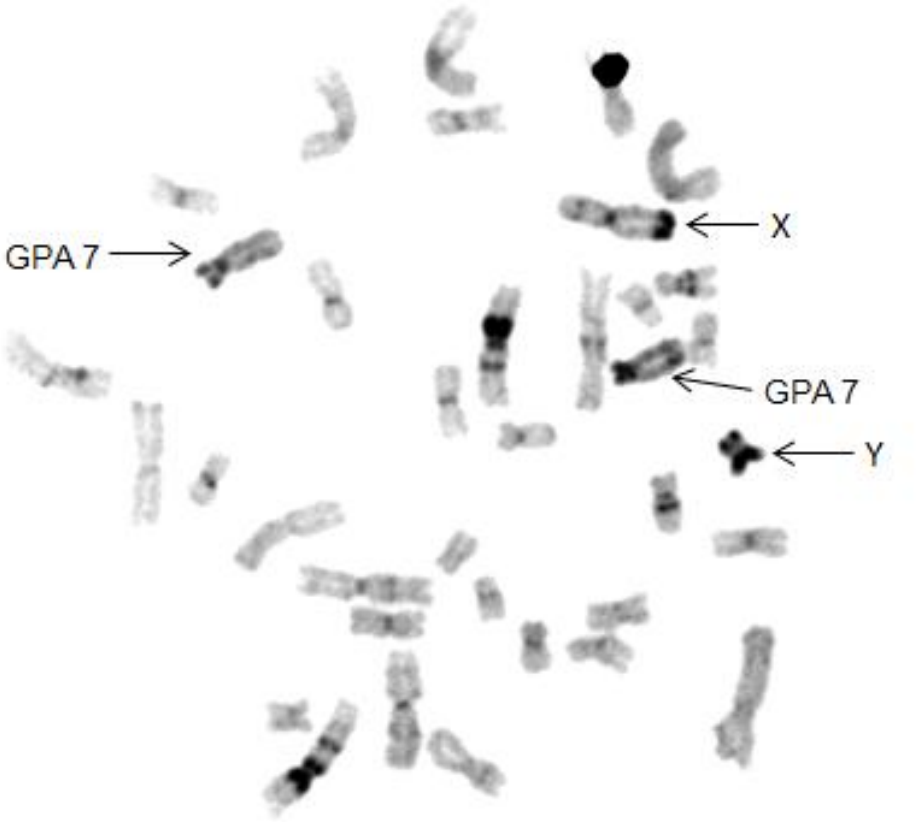

Figure 3.1: (b) The C-banded metaphase chromosome spread of *G. paeba* (2n = 36). Arrows indicate position of the sex chromosomes and the C-positive autosome GPA 7.

Flow-sorting resolved the chromosomes of *G. paeba* (2n = 36) into 16 peaks, of which 14 contained a single type of chromosomes (1, 4-6, 7-8, 12-17, X, Y), one peak contained two chromosomes (2 and 3) and one contained four pairs of autosomes (7, 9 - 11; Fig. 3.2). The homologues of GPA 7, which are C-positive, were sorted into two peaks, likely as a result of variation in the amount of heterochromatin.

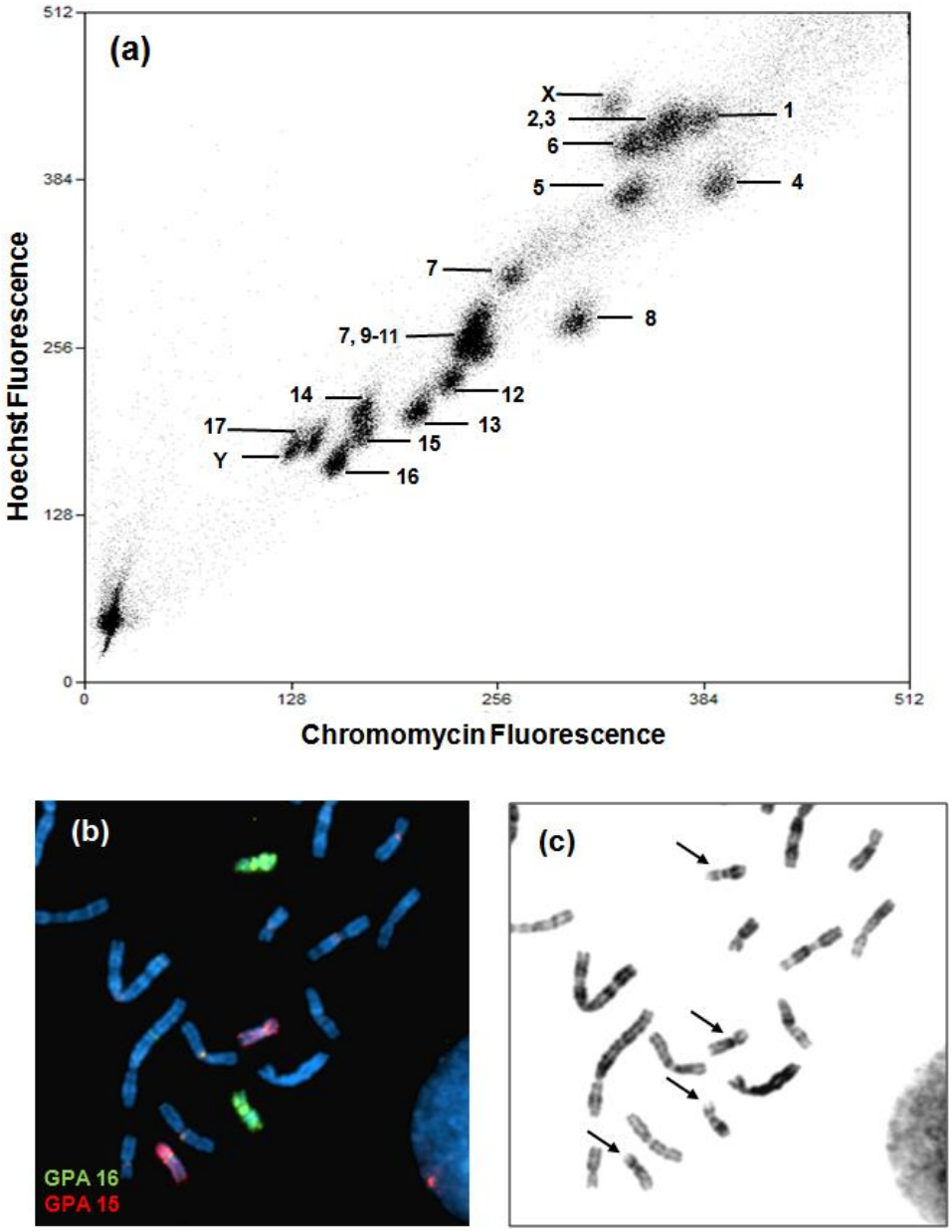

Figure 3.2: (a) The bivariate flow karyotype of *G. paeba*. (b) Chromosomes were assigned by hybridizing the DNA peak onto *G. paeba* metaphase chromosome preparations. (c) Digoxigenin-labelled probe GPA 16 (green) and biotin-labelled GPA 15 (red) was identified by inverting the DAPI-stained images to produce chromosome-specific bands.

(a) *Gerbilliscus tytonis*

The specimens analysed had a diploid number of 2n = 36, and the autosomes are entirely biarmed (Fig. 3.3). The X chromosome is a large metacentric while the Y is a small acrocentric. Heterochromatin is restricted to the centromeric regions of all autosomes (Fig. 1-A, Appendix; the C-bands are in the appendix). The Y chromosome is heterochromatic while the heterochromatin in the X occurs in the terminal region of the q arm and the centromeric region.

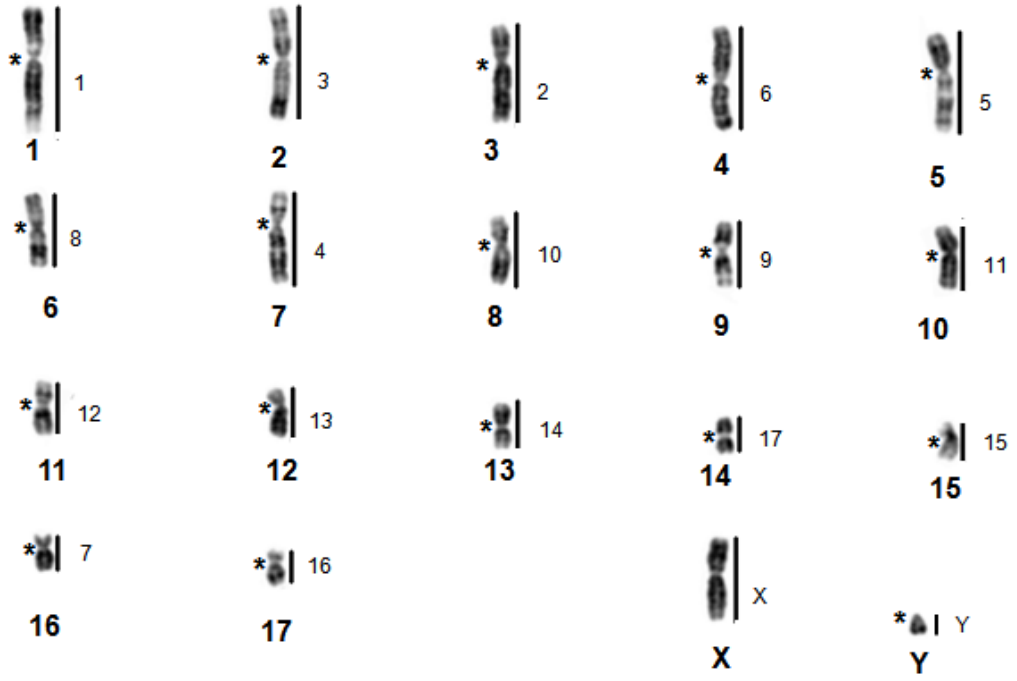

Figure 3.3: The G-banded haploid karyotype of a male *G. tytonis* (2n = 36). The vertical lines to the right indicate regions of homology determined by cross-species chromosome painting (FISH) using *G. paeba* whole chromosome painting probes. Asterisks indicate position of the centromere.

Gerbilliscus paeba painting probes identified 19 homologous regions in *G. tytonis* (Fig. 3.3). Each GPA chromosome was retained as a single chromosome in *G. tytonis*. GPA 1-17, X and Y were homologous to GTY 1, 3, 2, 7, 5, 4, 16, 6, 9, 8, 10, 11, 12, 13, 15, 17, 14, X and Y respectively. In summary, the karyotype of *G. tytonis* was conserved intact, which reflects the close evolutionary association of the two taxa (Colangelo *et al*. 2007; Qumsiyeh *et al*. 1991).

(b) *Gerbilliscus leucogaster*

The G-band karyotype of *G. leucogaster* has a diploid number of 2n = 40. It is characterised by 14 pairs of biarmed (metacentric and submetacentric) chromosomes and five pairs of acrocentric chromosomes (Fig. 3.4). The X chromosomes were metacentric and the Y-chromosome is a small metacentric. Heterochromatin is restricted to the centromeric regions of the autosomes. One pair of autosomes, GLE 11, stained entirely C-positive. The X chromosome has heterochromatin restricted to the terminal ends of the arms while the Y chromosome is entirely heterochromatic (Fig. 2-A in Appendix).

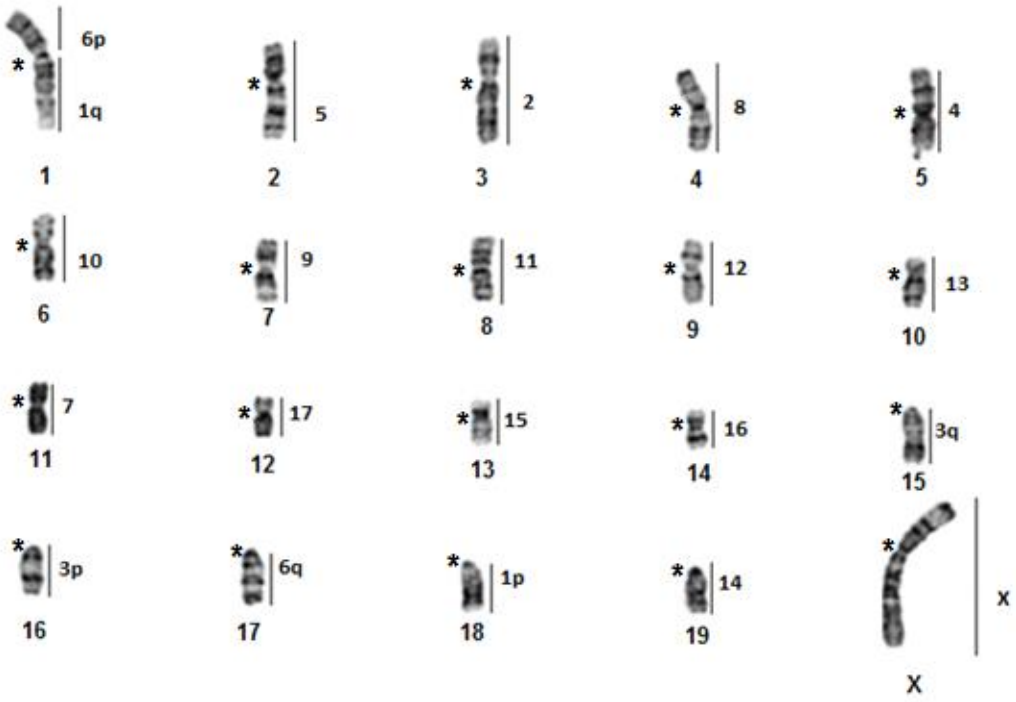

Figure 3.4: The G-banded haploid karyotype *G. leucogaster* (2n = 40)**.** The vertical lines to the right indicate regions of homology determined by cross-species FISH using *G. paeba* flow-sorts, represented by numbers on the right of each line. Asterisks indicate position of the centromere.

Gerbilliscus paeba painting probes identified 21 regions of homology in *G. leucogaster*. Fifteen GPA chromosomes were conserved intact to single *G. leucogaster* (GLE; Fig. 3.3): GPA 2, 4, 5, 7- 17 and X hybridized the entire lengths of GLE 3, 5, 2, 11, 4, 7, 6, 8, 9, 10, 19, 13, 14, 12 and X respectively. The remainder of the *G. paeba* probes produced two signals: GPA 1 hybridized to GLE 1q and GLE 18, while GPA 6 is homologous to GLE 1p and 17. GPA 3 is homologous to two acrocentric chromosomes, GLE 15 + 16.

(c) *Gerbilliscus afra*

Gerbilliscus afra has a diploid number of 2n = 44, and the karyotype has 12 pairs of biarmed (metacentric and submetacentric) autosomes and nine acrocentric autosomal pairs, as well as a large metacentric X and medium-sized metacentric Y chromosome (Fig. 3.5). Heterochromatin occurred predominantly around the centromeric regions of the autosomes, and the terminal regions of the X chromosome. *Gerbilliscus afra* also had a C-positive autosome element, GAF 15. The Y chromosome was entirely heterochromatic (Fig. 3 - A in Appendix).

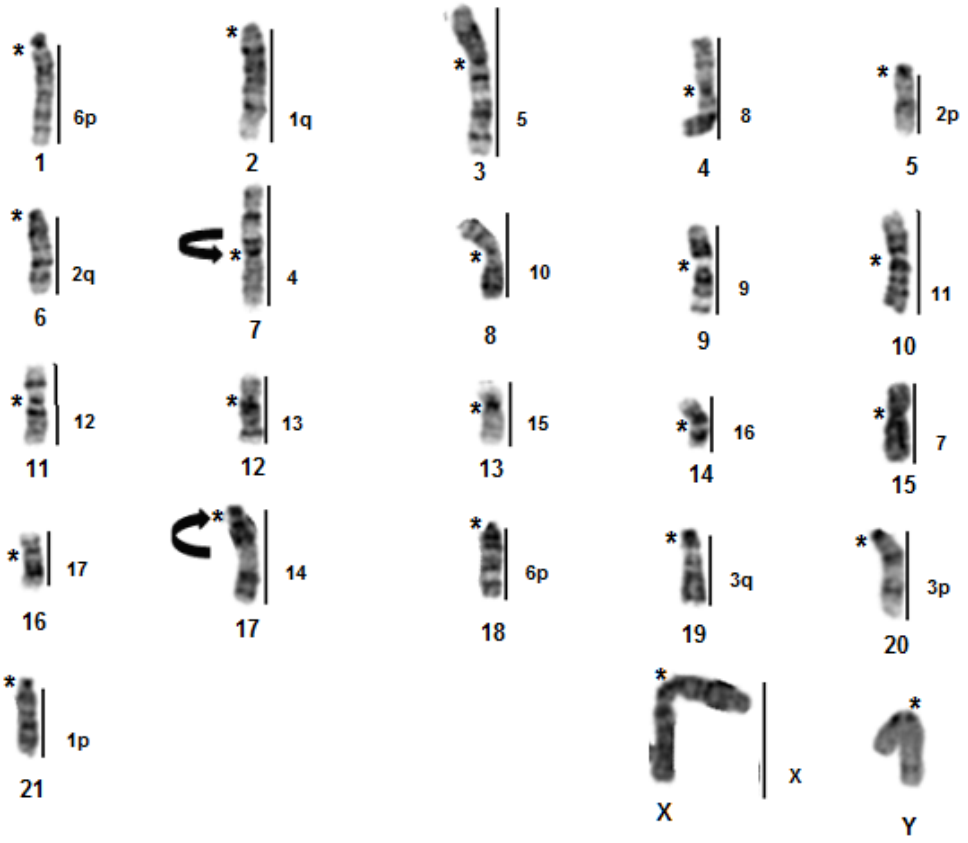

Figure 3.5: The G-banded haploid karyotype of *G. afra* (2n = 44). The vertical lines to the right indicate regions of homology determined by cross-species FISH using *G. paeba* flow-sorts, represented by numbers on the right of each line. Asterisks indicate position of the centromere and arrows indicate inversions in relation to the paint (chromosome).

Gerbilliscus paeba painting probes identified 22 regions of homology within *G. afra* (GAF). Fourteen homologous regions were conserved intact (whole-chromosome homology). GPA 4, 5, 7-17 and the X were homologous to GAF 7, 3, 15, 4, 9, 8, 10, 11, 12, 17, 13, 14, 16 and X

respectively (Fig. 3.5). Four *G. paeba* chromosomes (GPA 1, 2, 3 and 6) each produced two signals in *G. afra*. GPA 1 is homologous to GAF 2 + 21, GPA 2 is homologous to 5 + 6, GPA 3 is homologous to 19 + 20 and GPA 6 corresponds to 1 + 18.

(d) *Gerbilliscus kempi*

Gerbilliscus kempi has a diploid number of 2n = 48 with nine pairs of biarmed (metacentric and submetacentric) and 14 pairs of acrocentric autosomal pairs (Fig. 3.6). The X chromosomes were large acrocentrics. Heterochromatin occurred around the centromeric regions of all autosomes and the proximal regions of the X chromosomes (Fig. 4-A in Appendix).

Figure 3.6: The G-banded haploid karyotype of *G. kempi* (2n = 48). The vertical lines to the right indicate regions of homology determined by cross-species FISH using *G. paeba* flow-sorts, represented by numbers on the right of each line. Asterisks indicate position of the centromere.

Gerbilliscus paeba probes identified 25 homologous regions in *G. kempi* (GKE; Fig. 3.6). Ten conserved chromosomes hybridized to single chromosomes in *G. kempi* (whole-chromosome conservation); GPA 7, 4, 8-10, 13, 15, 16, 17 and X were homologous to GKE 8, 2, 1, 4, 3, 5, 7, 9, 6 and X (Fig. 3.6). One chromosome, GPA 14, was conserved intact but inverted and fused with an arm of a separate autosomal segment, GPA 12q, which corresponds to GKE 10. Seven chromosomes, GPA 1, 2, 3, 5, 6, 11 and 12, produced double signals in *G. kempi*, that correspond to GKE 11 + 19, 12 + 23, 15 + 17, 13 + 21, 16 + 18, 14 + 22 and 10 prox + 20, respectively. The proximal portion of the X chromosome that failed to hybridize contains heterochromatin.

(e) *Gerbilliscus gambianus*

Gerbilliscus gambianus has a diploid number of 2n = 52; it includes seven pairs of biarmed (metacentric and submetacentric) autosomes and 18 pairs of acrocentric autosomes (Fig. 3.7). The X chromosome is a large submetacentric and Y a small submetacentric. C-banding indicates that heterochromatin occurs in the centromeric regions of all autosomes (Fig. 5-A in Appendix). Heterochromatin also occurs in the p arm of the X chromosome, and interstitially on the distal portion of the q arm. The Y chromosome is entirely heterochromatic (Fig. 5-in Appendix).

Gerbilliscus paeba painting probes identified a total of 28 regions of homology in *G. gambianus* (GGA), including seven conserved autosomes which hybridized intact to single GGA chromosomes (Fig. 3.7). These include GPA 7, 9, 10, 13, 15-17, X and Y, which were homologous to GGA 6, 2, 1, 3, 5, 7 and 4. GPA X and Y were homologous to GGA Xq and Yq respectively. Hybridization signal is absent on the p arm of GGA X due to the heterochromatin content. As in *G. kempi*, GPA 14 was conserved intact, although it is inverted and fused with GPA 12q, corresponding to GKE 8. A further six chromosomes produced two hybridization signals, which included GPA 4, 5, 6, 8, 11, 12 corresponding to GKA 16 + 17, 11 + 21, 14 + 19, 18 + 24, 22 + 23 and 8 prox + 25, respectively.

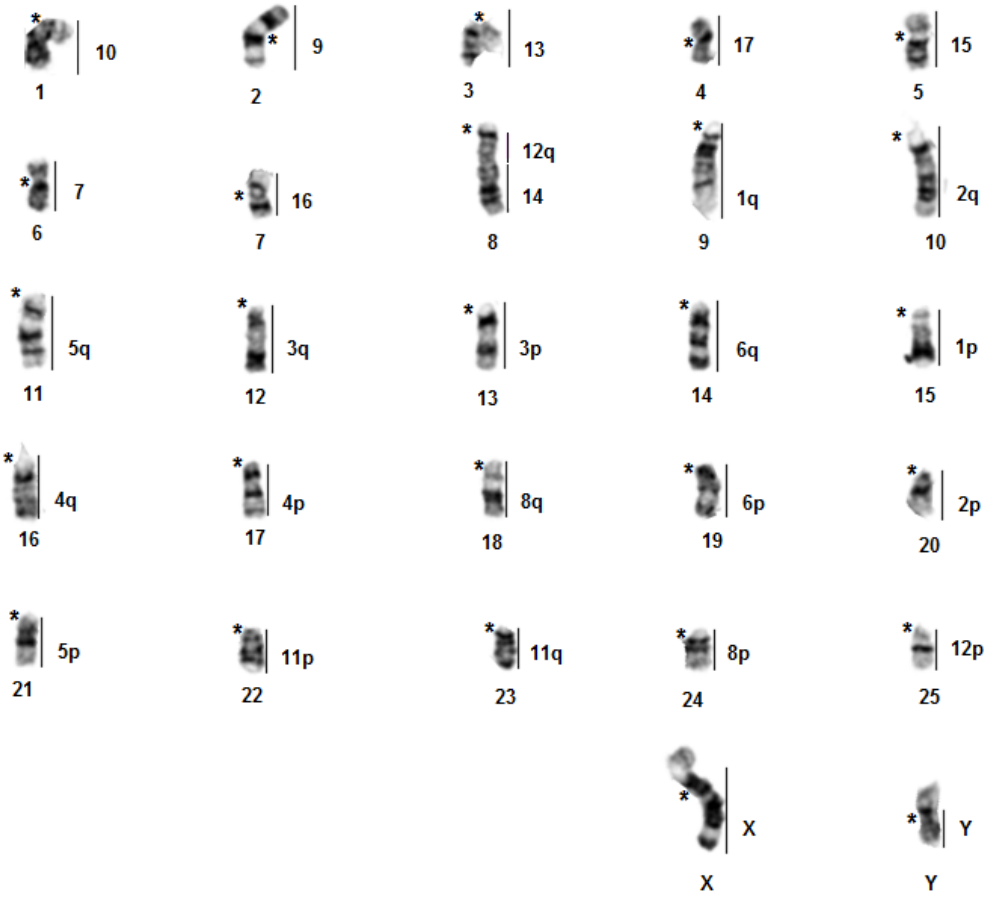

Figure 3.7: The G-banded haploid karyotype of *G. gambianus* (2n = 52)**.** The vertical lines to the right indicate regions of homology determined by cross-species FISH using *G. paeba* flow-sorts, represented by numbers on the right of each line. Asterisks indicate position of the centromere.

(f) *Desmodillus auricularis*

Desmodillus auricularis specimens examined in the study have a diploid number of 2n = 50, which is comprised of 14 biarmed (metacentric and submetacentric) and 10 acrocentric autosomal pairs. The sex chromosomes are both metacentric, with a large X and medium-sized Y (Fig. 3.8). Constitutive heterochromatin is restricted around the centromeric regions of all chromosomes (Fig. 6-A in Appendix).

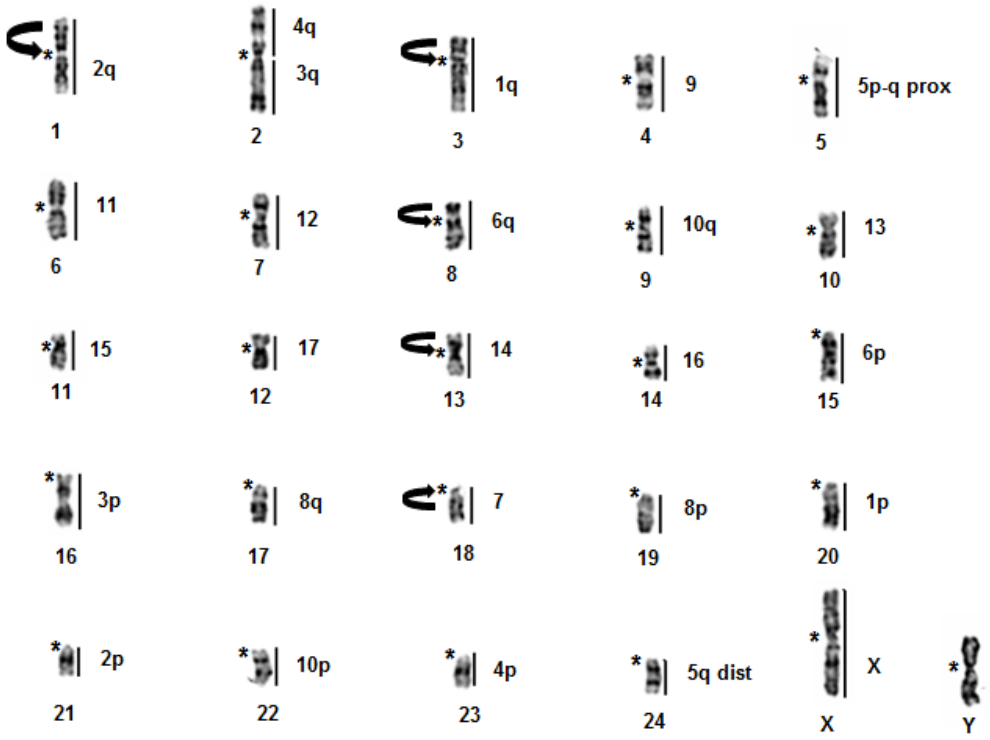

Figure 3.8: The G-banded haploid karyotype of *D. auricularis* (2n = 50)**.** The vertical lines to the right indicate regions of homology determined by cross-species FISH using *G. paeba* flow-sorts, represented by numbers on the right of each line. Asterisks indicate position of the centromere and arrows indicate inversions in relation to the paint (chromosome).

A total of 26 regions of homology were painted on *D. auricularis* (DAU) karyotype, including all autosomes and the X chromosome. Ten chromosomes were conserved intact and delineated only single, homologous regions in *D. auricularis* (Fig. 3.8). GPA 7, 9, 11-17 and X hybridized to DAU 18, 4, 6, 7, 10, 13, 11, 14, 12 and X, respectively. Eight GPA probes produced more than one hybridization signal. GPA 1, 2, 3, 4, 5, 6, 8 and 10 hybridized to DAU 3 + 20, 1 + 21, 2q + 16, 2p + 23, 5 + 24, 8 + 15, 17 + 19 and 9 + 22, respectively.

(g) *Meriones persicus*

Meriones persicus has a diploid number of 2n = 42, which is comprised of 17 pairs of biarmed (metacentric and submetacentric) and three pairs of acrocentric autosomes. The X was a large metacentric and Y a small metacentric (Fig. 3.9). The C-band analyses showed constitutive heterochromatin to occur predominantly within the centromeric regions (Fig. 7-A in Appendix),

although there is also the presence of a C-positive autosome (MPE 7), and interstitial heterochromatin on MPE 13.

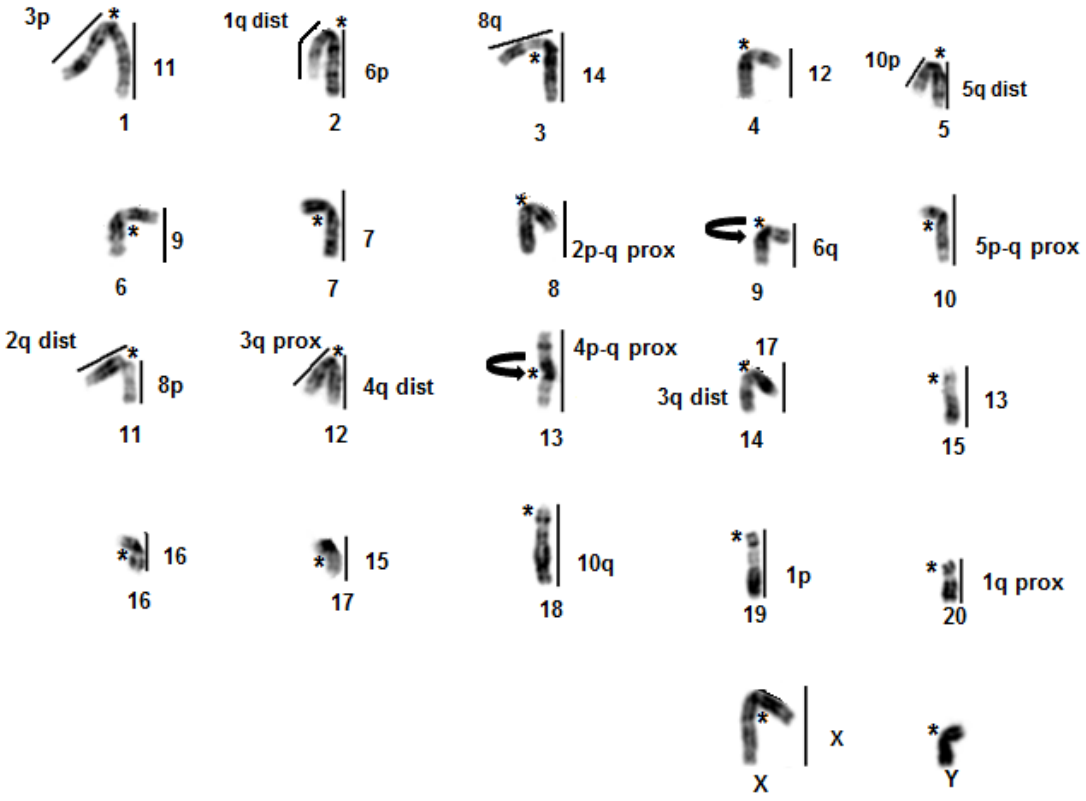

Figure 3.9: The G-banded haploid karyotype of *M. persicus* (2n = 42). The vertical lines to the right indicate regions of homology determined by cross-species FISH using *G. paeba* flow-sorts, represented by numbers on the right of each line. Asterisks indicate position of the centromere and arrows indicate inversions in relation to the paint (chromosome).

The hybridization of GPA probes onto *M. persicus* (MPE) delineated 28 regions of homology. Seven GPA chromosomes were homologous to whole chromosomes within *M. persicus* (Fig. 3.9). GPA 9, 7, 12, 13, 15, 16 and X were homologous to MPE 6, 7, 4, 15, 17, 16 and X respectively. Three chromosomes, GPA 11, 14 and 17, were conserved intact but fused to segments of other autosomes. GPA 11, 14 and 17 were homologous to MPE 1q, 3q and 14p, respectively. Six double signals were produced by GPA 2, 4, 5, 6, 8 and 10, and corresponded to MPE 8 + 11p, 12q + 13, 5q + 10, 2q + 9, 3p + 11q and 5p + 18, respectively. GPA 1 and 3 each produced three hybridization signals; GPA 1 is homologous to MPE 2p + 19 + 20 while GPA 3 is homologous to 1p + 12p + 14q.

(h) *Psammomys obesus*

Psammomys obesus has a diploid number of 2n = 48. It is comprised of 14 biarmed (metacentric and submetacentric) and nine pairs of acrocentrics autosomes (Fig. 3.10). The X chromosomes were large metacentrics. The C-banding analyses show that heterochromatin occurs predominantly around the centromeric regions (Fig. 8-A in Appendix), but also in the centromeric region, terminal regions of the q arm and the proximal regions in the p arm of the X chromosome.

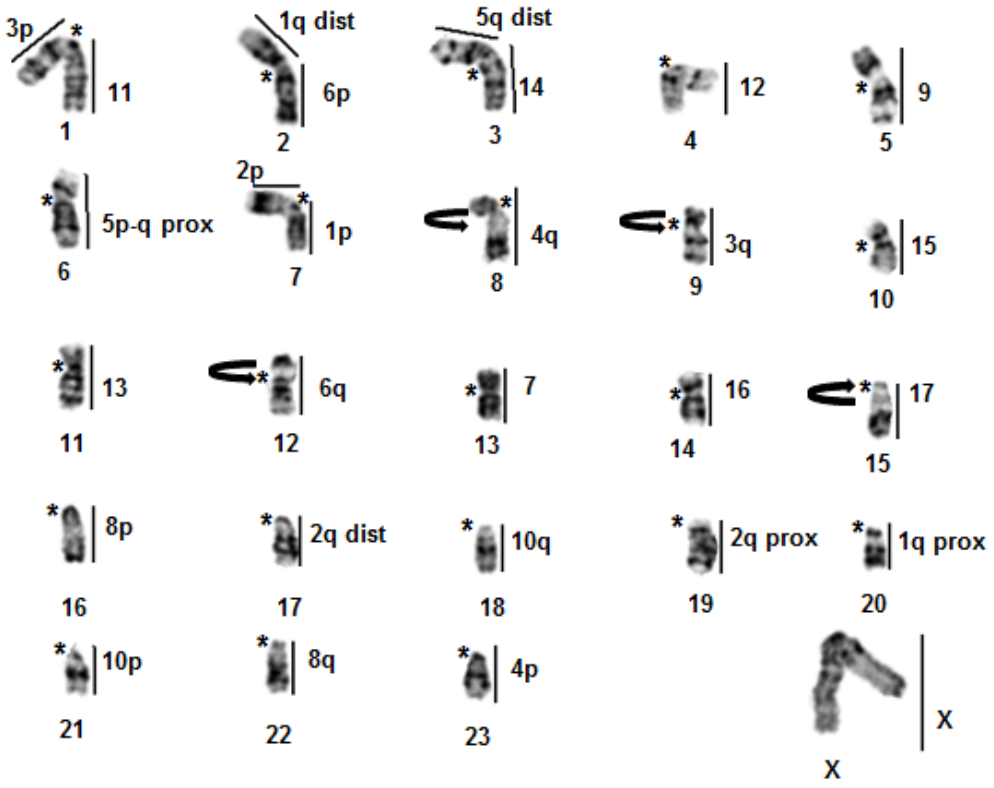

Figure 3.10: The G-band haploid karyotype of *P. obesus* (2n = 48). The vertical lines to the right indicate regions of homology determined by cross-species chromosome painting. Asterisks indicate position of the centromere and arrows indicate inversions in relation to the paint (chromosome).

GPA painting probes delineated a total of 28 regions of homology. Eight GPA chromosomes hybridized intact to single *P. obesus* (POB) chromosomes. GPA 7, 9, 12, 13, 15, 16, 17 and X, hybridized to POB 13, 5, 4, 11, 10, 14, 15 and X respectively (Fig. 3.10). Two chromosomes, GPA 11 and 14, although conserved, fused to separate autosome segments. GPA 11 and 14 were

homologous to POB 1q and 3q, respectively. Six GPA chromosomes produced two hybridization signals; GPA 3, 4, 5, 6, 8 and 10 hybridized to POB 1p + 9, 8 + 23, 3p + 6, 2q + 12, 16 + 22 and 18 + 21, respectively. Two GPA probes produced three signals; GPA 1 and 2 hybridized to POB 2p + 7q + 20, and POB 7p + 17 + 19, respectively.

(i) ***Taterillus pygargus***

Taterillus pygargus has a diploid of 2n = 22. It includes 10 biarmed (metacentric and submetacentric) autosomes and a large X chromosomes (Fig. 3.11a). The C-banding analyses show constitutive heterochromatin to be minimal and confined to the centromeric regions of the autosomes, as well as interstitial heterochromatin of the proximal and distal regions of the X (Fig. 9-A in Appendix).

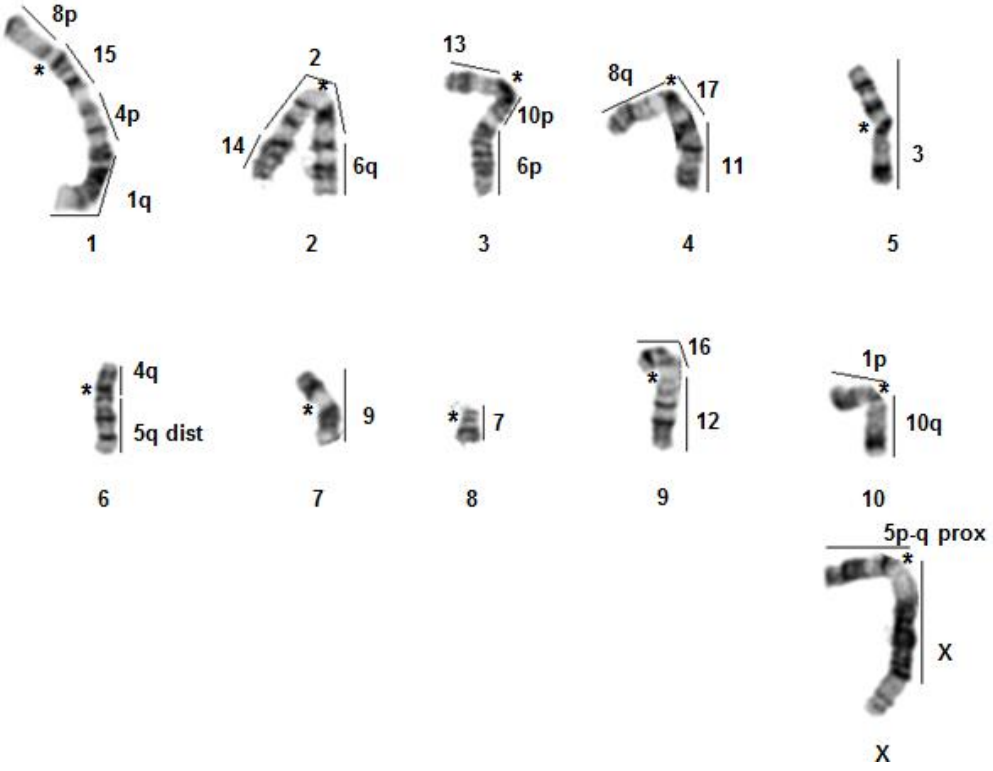

Figure 3.11: (a) The G-banded haploid karyotype of *T. pygargus* (2n = 22). The vertical lines to the right indicate regions of homology determined by cross-species FISH using *G. paeba* flow-sorts represented by numbers on the right of each line. Asterisks indicate position of the centromere.

A total of 24 regions of homology were conserved in *T. pygargus* (TPY). The hybridization of GPA probes to *T. pygargus* delineated only three chromosomes that hybridized to single TPY chromosomes. GPA 3, 7 and 9 are homologous to TPY 5, 8 and 7, respectively (Fig. 3.11a). Nine *G. paeba* chromosomes were conserved but occur fused to other GPA elements; GPA 11-17 and X are homologous to TPY 4q prox, 9q, 3p, 2p prox, 1q prox, 9p, 4q prox respectively, as well as Xq, the sex-autosome translocation (Fig. 3.11b). GPA 2 failed to hybridize successfully. Its homology is assumed as 2p dist-q prox, since it is the only chromosome segment that did not produce a hybridization signal. Fragmented hybridization signals in *T. pygargus* were as a result of six GPA chromosomes GPA 1, 4, 5, 6, 8 and 10 which were homologous to TPY 1q dist + 10p, 1q med + 6p, 6q + Xp, 3q dist + 2q dist, 1p + 4p, 3q prox + 10q, respectively.

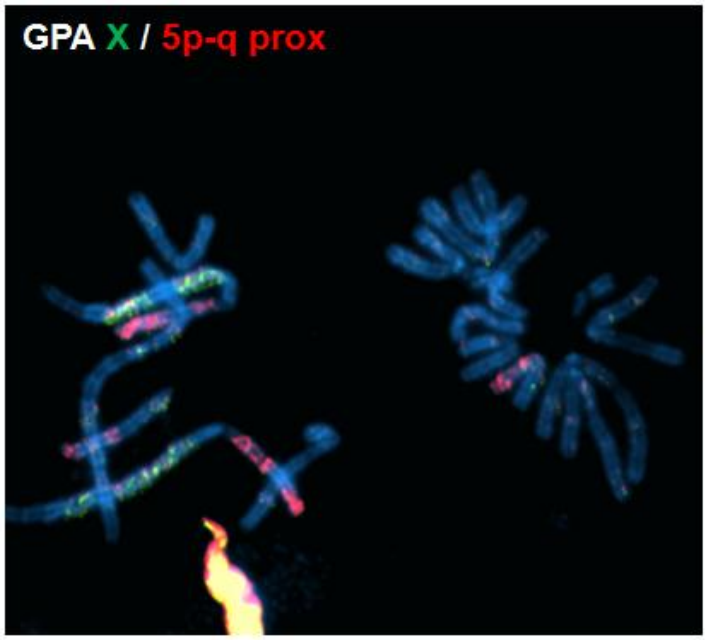

Figure 3.11: (b) The sex/autosome translocation in *T. pygargus*, GPA X/5p-q prox

(j) *Micaelamys namaquensis*

Micaelamys namaquensis has a diploid number of 2n = 24. It is comprised of five pairs of biarmed (metacentric and submetacentric) autosomes, six acrocentric autosomes, and acrocentric sex chromosomes (Fig. 3.12). Constitutive heterochromatin occurs in the centromeric regions of the autosomes and sex chromosomes (Fig. 10-A in Appendix).

A total of 31 regions of homology were conserved in *M. namaquensis* (MNA). Chromosome painting with *G. paeba* paints produced fragmented signals and fewer conserved chromosomes, either intact as single chromosomes or fused to separate autosomal segments. GPA 13 and 14 were conserved but were fused to segments of other GPA autosomes: GPA 13 was fused with GPA 12q and 8p, which corresponds to MNA 4, while GPA 14 fused with GPA 11 p prox, 9q and 8q prox, the equivalent of MNA 5. The hybridizations of GPA 4 and 6 to MNA 1 and 2 were disrupted (Fig. 3.12). GPA 4 and 6 were disrupted, and homologous to MNA 1q dist + 1q prox and MNA 2q prox + 2q dist respectively. The larger autosomes particularly failed to hybridize completely. GPA 1, 2, 3 and 11 were homologous to MNA 3q dist, 9prox + 9 dist, 3p prox + 6 prox, 5p + 10 respectively; only GPA 1q dist, 2p, 3p med+3q med and 11p prox+11q dist hybridized successfully. Complete hybridization occurred with GPA 5, 10, 12, 15, 16, 17 and X. These were homologous to 1p prox + 1q prox (disrupted)+2q dist + 7, 2p prox+2 p dist (disrupted), 2p med + 4p prox, 3p dist + 6 dist, 8 + 9 med, 1p med + 3q prox and X respectively. Unlike the ingroup, GPA 7 failed to hybridize with *M. namaquensis.*

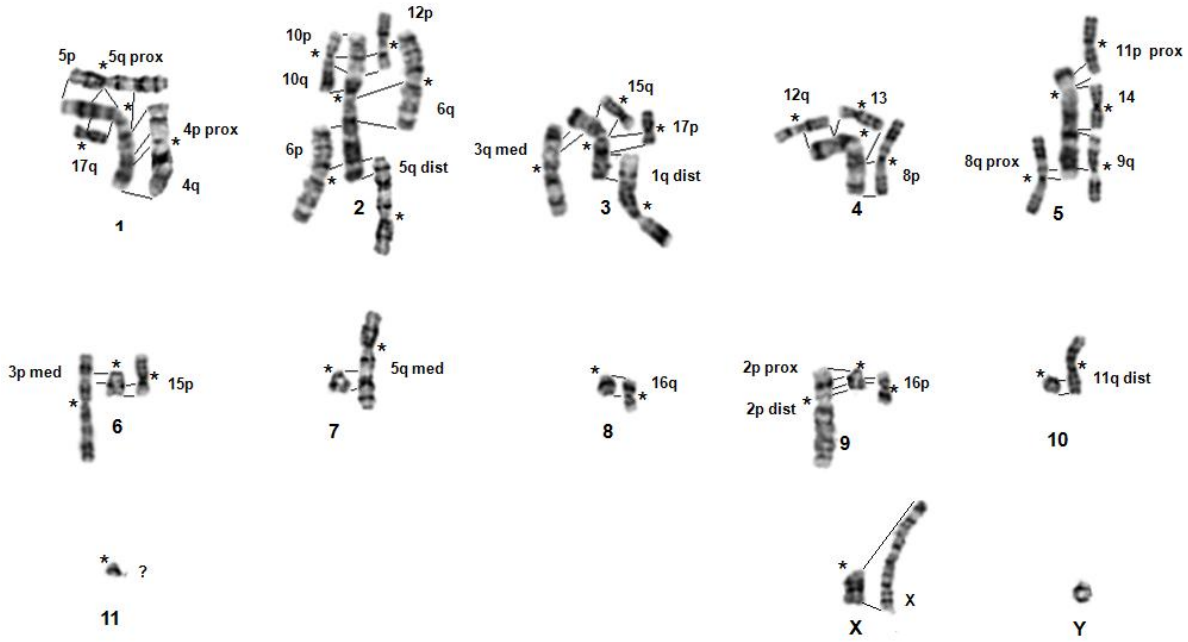

Figure 3.12: The G-banded haploid karyotype of *M. namaquensis* (2n = 24)**.** The lines to the right indicate regions of homology determined by cross-species FISH using *G. paeba* flow-sorts represented by numbers on the right of each line. Asterisks indicate position of the centromere.

3.2 Comparative G-bands directed by ZOO-FISH

Whole-chromosome *G. paeba* painting probes hybridized to all the examined gerbil species and enabled unequivocal identification of regions of homology and homeology across the board (Fig. 3.13). Using *G. paeba* as the index species two types or levels of chromosome conservation was obtained:

(a) Conserved chromosomes that were retained intact as whole chromosomes in the subfamily include GPA 7, 9 and X (including the X; autosome translocation in *T. pygargus*). It can be postulated these were also present in the ancestral karyotype.

(b) Rearranged chromosomes involved GPA 1-6, 8, 10-17, and (with the exception of *G. tytonis*) include fissions, fusions and inversions. For instance, GPA 1 is homologous to acrocentric chromosomes GLE 18 + 1, GAF 21 + 2, GLE 19 + 11, GGA 15 + 19 and TPY 10p + 1q dist (right), indicative of Robertsonian rearrangement events. GPA 1 is homologous to DAU 20 + 3, POB 7q + 20 + 2p, MPE 10 + 20 + 2p (left), indicative of tandem fissions, as illustrated in table 3.1 and Fig. 3.13.

The change in karyotype between *Desmodillus* and *G. afra* required nine rearrangements comprised of four Robertsonian fusions and five inversions. Robertsonian rearrangements of GPA 1, 2, 3 and 6 occurred in the southern African *Gerbilliscus*. Although these Robertsonian rearrangements are also present in the West African *Gerbilliscus*, G, *kempi* and *G. gambianus* also shared a fission of GPA 5, which remained conserved in the southern African *Gerbilliscus*. Furthermore, GPA 14, which was single in *G. leucogaster* and *G. afra*, are fused to separate autosomal segments in the West African *Gerbilliscus*. In contrast, rearrangements in the karyotypes of *P. obesus* and *M. persicus* required several fissions and fusions - GPA 1 and 2 in *P. obesus*, and GPA 1 and 3 in *M. persicus*, fissioned into three segments, contrasting the Robertsonian rearrangements in *Desmodillus* and *Gerbilliscus*. Several fissions involving GPA 1 – 6, 8 and 10 were followed by successive fusions to other autosomal segments. GPA 5p-q prox, which is homologous to DAU 5, POB 6, MPE 10, forms part of a sex autosome translocation in *T. pygargus*. It is the only species examined with a sex-autosome translocation, hence an autapomorphy to the species.

The hybridization of all GPA probes with the outgroup, *M. namaquensis*, produced multiple hybridization signals. More importantly, some of these paints were retained intact (GPA 15, 16,

17) in all the ingroup taxa, or it failed to demonstrate complete hybridization and homology (GPA 1, 2, 3, 11; table 3.1). Other GPA probes (GPA 4, 6) produced disrupted signal (Fig. 3.13). On the other hand, the X chromosome was conserved intact, as with the ingroup taxa. *Gerbilliscus paeba* clearly distinguishes between the Robertsonian rearrangements present in *Gerbilliscus* and *Desmodillus* and the more complex rearrangements present in *P. obesus*, *M. persicus* and *T. pygargus*. The FISH data also demonstrate that the type of karyotypic changes in *Gerbilliscus* and *Desmodillus*, *Meriones* and *Psammomys*, and *Taterillus*, were notably different from each other.

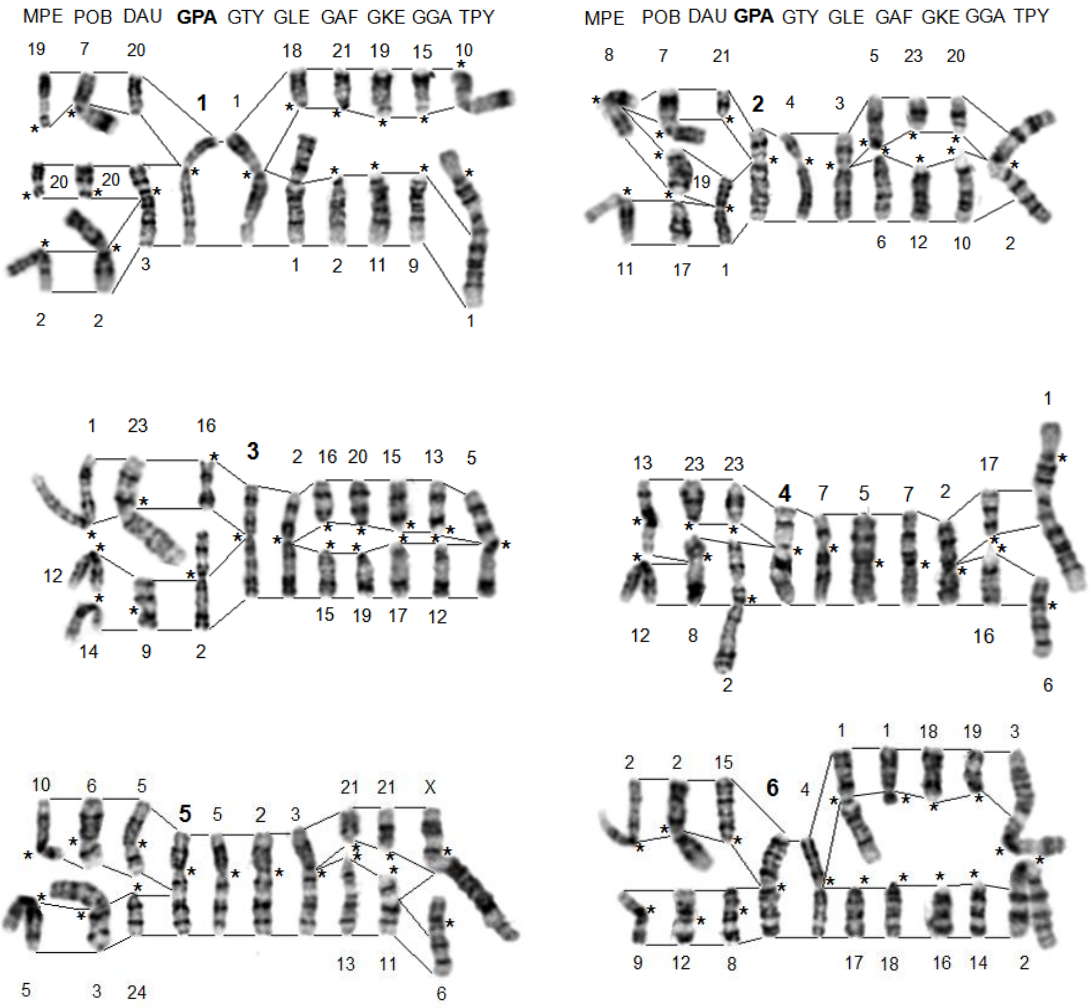

Figure 3.13: Comparative G-band alignment directed by FISH with *G. paeba* paints. The gerbil chromosome comparisons are of (left to right) *M. persicus* (MPE), *P. obesus* (POB), *D. auricularis* (DAU), *G. paeba* (GPA, in bold), *G. tytonis* (GTY), *G. leucogaster* (GLE), *G. afra* (GAF), *G. kempi* (GKE), *G. gambianus* (GGA) and *T. pygargus* (TPY). These comparisons identified a total of 221 regions of homeology among the ten gerbil species. Asterisks indicate position of the centromere.

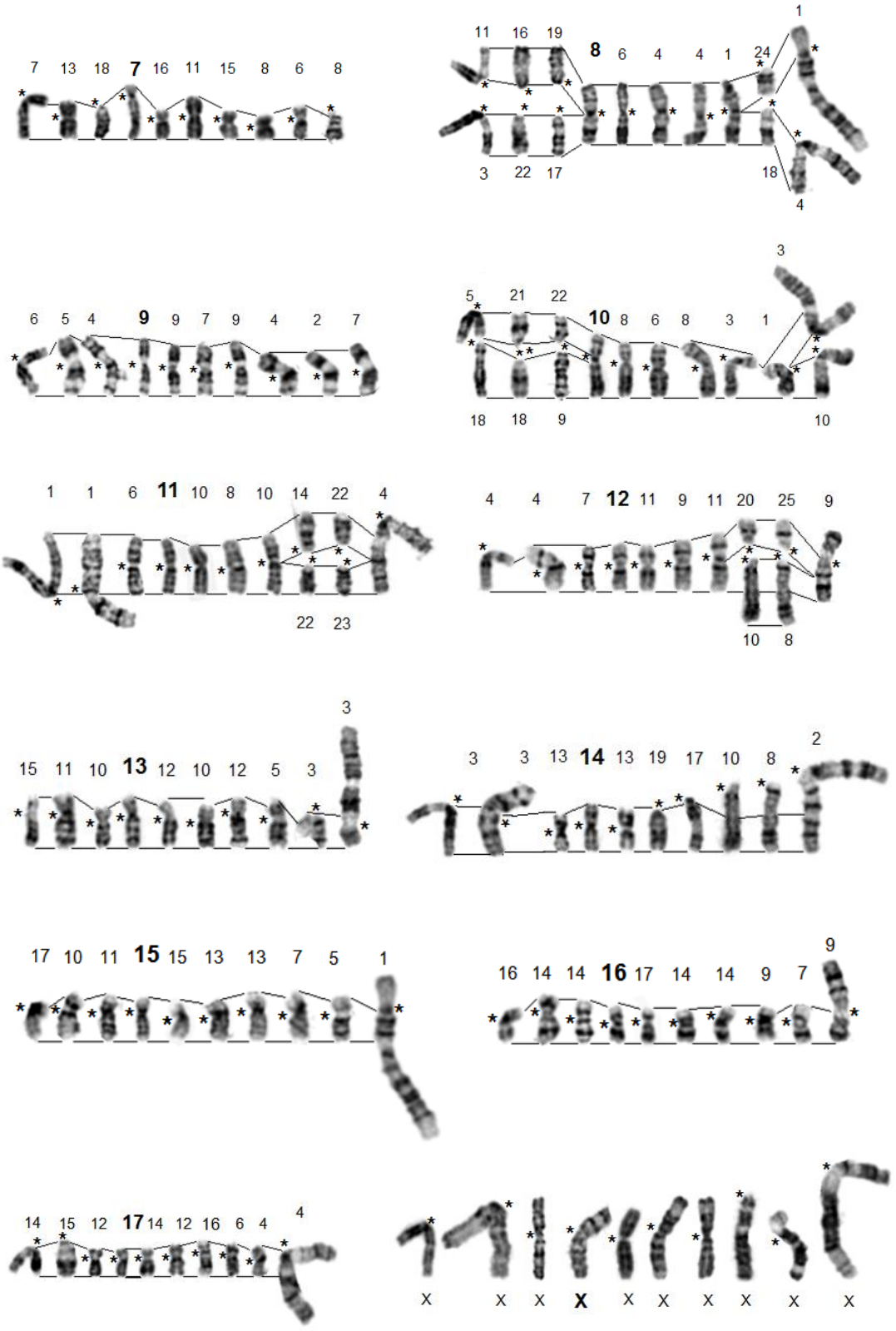

Figure 3.13 (continued)

3.3 Phylogenetic reconstructions using chromosomal characters

(a) Data matrices: Syntenic associations

A total of 57 syntenic chromosome associations were derived from the homology maps of all the species analysed. Within the ingroup, 19 characters - 1, 9, 15, 21, 25, 33, 36, 37, 42, 43, 44, 46, 50, 51, 52, 53, 55, 56 and 57 - were syntenies that contained whole chromosomes (chromosome segments comprised of both arms, p/q), GPA 1p/1q, 2p/2q, 3p/3q, 4p/4q, 5p/5q, 6p/6q, 7p/7q, 8p/8q, 9p/9q, 10p/10q, 11p/11q, 12p/12q, 13p/13q, 14p/14q, 15p/15q, 16p/16q, 17p/17q, Xp/Xq, Yp/Yq. Characters 2, 10, 11, 27 and 47 included chromosome segments or arms (GPA 1qa, 2qa, 2qc, 5qc, and 12p); characters 3, 4, 5, 6, 12, 13, 16, 19, 22, 23, 26 and 28 comprised of two chromosome segments (GPA 1p/2p, 1q/6p, 1qc/6p, 1p/10q, 2p/2qa, 2qc/8p, 3qa/4qc, 3q/4q, 4p/4qa, 4q/5qc, 5p/5qa and 5qc/5qa). With the exception of the X chromosome, *M. namaquensis* comprised strictly of autapomorphic syntenies, a total of 10 (Table 3.1)

The characters 17, 20, 29, 32, 38 and 48 were comprised of three chromosomal segments (GPA 3p/11q/11p, 3qc/17p/17q, 5qc/14p/14q, 5p/5qa/Xq, 8q/14p/14q and 12q/14p/14q); characters 34 and 49 were comprised of four segments (GPA 6p/10p/13p/13q and 12q/12p/16p/16q), and three characters (8, 35, 40) contained five segments (GPA 1q/4p/15q/15p/8p, 6q/2p/2q/14q/14p, 8q/17p/17q/11p/11q). Overall, 35 characters were parsimony uninformative (GPA 7p/7q, 9p/9q, Xp/Xq) and 22 were parsimony informative.

Table 3.1: Chromosome homologies established between the ingroup and outgroup taxa using *G. paeba* whole chromosome painting probes.

Gerbilliscus paeba	***Gerbilliscus tytonis***	***Gerbilliscus leucogaster***	***Gerbilliscus afra***	***Gerbilliscus kempi***	***Gerbilliscus gambianus***	***Desmodillus auricularis***	***Meriones persicus***	***Psammomys obesus***	***Taterillus pygargus***	***Micaelamys namaquensis* (OG)**
1	1	1q+18	2+21	11+19	9+15	3 $_{inv}$ +20	2p+19+20	2p+7q+20	1q dist+ 10p	3q dist
2	3	3	5+6	12+23	10+20	1 $_{inv}$ +21	8+11p	7p+17+19	2p dist-q prox	9 prox+9 dist
3	2	15+16	19+20	15+17	12+13	2q+16	1p+12p+14q	1p+9 $_{inv}$	5	3p prox+6 prox
4	7	5 $_{inv}$	7 $_{inv}$	2$_{inv}$	16 $_{inv}$+17	2p+23	12q+13$_{inv}$	8 $_{inv}$+23	1q med+6p	1q med +1q dist
5	5	2	3	13+21	11+21	5+24	5q+ 10	3p+6	Xp+6q	1p prox+1q prox+2q dist
6	4	1p+17	1+18	16+18	14+19	8 $_{inv}$ +15	2q+9 $_{inv}$	2q+12 $_{inv}$	2q dist+3q dist	2q prox+2 q med
7	16	11	15	8	6	18 $_{inv}$	7	13	8	-
8	6	4	4	1	18+24	17+19	3p+11q	16+22	1p+4p	4q dist+5q prox
9	9	7	9	4	2	4	6	5	7	5q med
10	8	6	8	3	1	9 $_{inv}$ +22	5p+18	18 +21	3q prox+10q	2p prox+2 p dist
11	10	8	10	14+22	22+23	6	1q	1q	4q dist	5p+10
12	11	9	11	10 prox+ 20	8 prox, 25	7	4	4	9q	2p med+4p prox
13	12	10	12	5	3	10	15	11	3p	4p dist-4q prox
14	13	19 $_{inv}$	17 $_{inv}$	10 dist	8 dist	13	3q	3q	2p prox	5q prox
15	15	13	13	7	5	11	17	10	1q prox,	3p dist+6 dist
16	17	14	14	9	7	14	16	14	9p	8+9 med
17	14	12	16	6	4	12	14p	15 $_{inv}$	4q prox	1p med+ 3q prox
X	X	X	X	X dist	Xq	X	X	X	Xq	X
Y	Y	-	-	-	Yq	-	-	-	-	-

Standard cytogenetic abbreviations were used: “p” and “q” indicates the short and long arm respectively. The abbreviations “prox”, “med” and “dist” indicates the proximal, median and distal positions, respectively, relative to the centromere. “inv” indicates a pericentric inversion.

The tree retrieved from shared syntenic associations was 67 steps long, and had a consistency index (CI) of 0.851, retention index (RI) of 0.873 and homoplasy index (HI) of 0.149 (Table 3.2). The phylogenetic tree retrieved a basal position *T. pygargus*, with a bootstrap support of 98% (Fig. 3.14a). *Psammomys obesus* and *M. persicus*, and *G. gambianus* and *G. kempi* had sister-species relationships, with a bootstrap support value of 91% and 69% respectively. The sister-species relationships and *Desmodillus* were unresolved at the basal node. The southern African *Gerbilliscus* (*G. paeba*, *G. tytonis*, *G. leucogaster*, *G. afra*) had a bootstrap support value of 94%. The clade uniting *Gerbilliscus leucogaster* with *G. paeba* and *G. tytonis* have the weakest support, with a bootstrap support value of 64%, closely followed by *Gerbilliscus kempi* and *Gerbilliscus gambianus* with a bootstrap support value of 69%.

(b) Chromosome rearrangements

Comparative G-band analyses enabled the identification of 19 rearrangements. Of these 17 were variable and 15 parsimony informative (Table 3.3). A single most parsimonious tree obtained had a tree length (L) of 26 steps, CI = 0.692, RI = 0.778 and HI = 0.308. The Gerbillinae comprised a monophyletic assemblage (Fig. 3.14b), and the sister relationship between *G. tytonis* and *G. paeba* had the highest statistical support (98% bootstrap support). The clade including *G. kempi*, *G. afra*, *G. leucogaster*, *G. paeba* and *G. tytonis* was monophyletic, and had the lowest bootstrap support value of 60%, while the relationship between *G. leucogaster*, *G. paeba* and *G. tytonis* had the second-lowest value at 62%. The southern African *G. afra*, *G. leucogaster*, *G. paeba* and *G. tytonis* assemblage had a bootstrap support value of 82%. *Gerbilliscus gambianus*, *D. auricularis*, *M. persicus* and *P. obesus* formed a polytomy with a bootstrap support of 96%. *Taterillus pygargus* was basal to the subfamily.

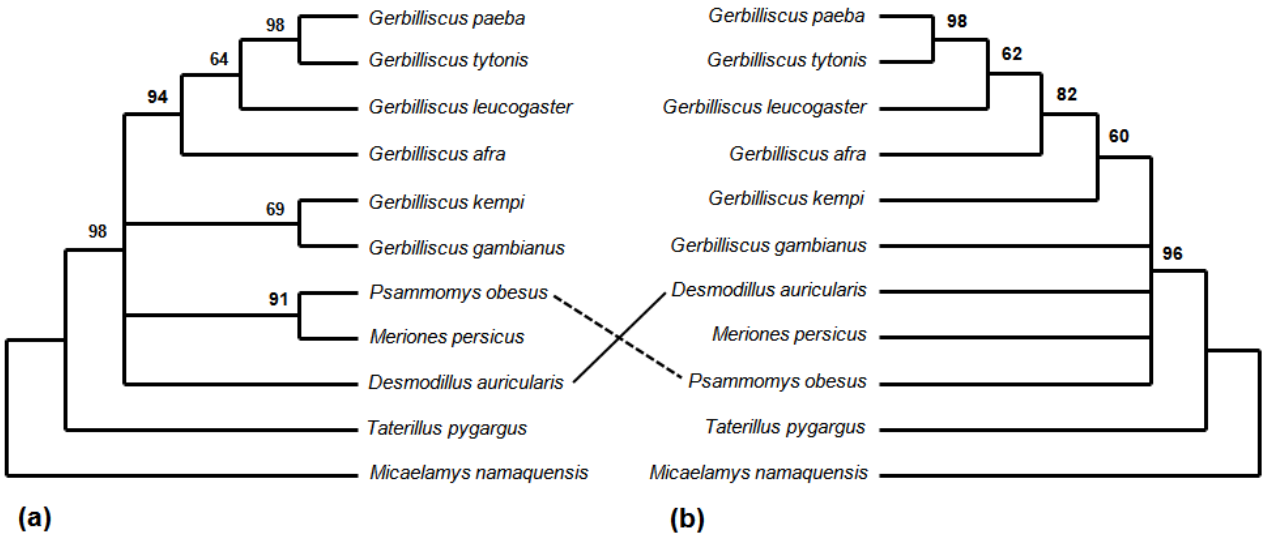

Figure 3.14: Phylogenies that were retrieved using parsimony as implemented in PAUP using two data matrices: (a) Analysis of the binary matrix of syntenic associations retrieved a tree comprised of monophyletic clade consisting of the southern African *Gerbilliscus* (*G. paeba*, *G. tytonis*, *G. leucogaster*, *G. afra*), and one which was unresolved at the basal node (*D. auricularis*, *G. kempi* and *G. gambianus*, and *P. obesus* and *M. persicus*). *Taterillus pygargus* was basal to the ingroup taxa. The tree had a tree length (L) of 67, consistency index (CI) = 0.851 and (RI) = 0.873. (b) Analysis of the chromosome rearrangement matrix retrieved a phylogeny also comprised of a monophyletic clade (*G. paeba*, *G. tytonis*, *G. leucogaster*, *G. afra*, *G. kempi*), and one which was unresolved (*G. gambianus*, *D. auricularis*, *M. persicus*, *P. obesus*). *Taterillus pygargus* had a basal position to the ingroup taxa. Tree characteristics are: L = 26, CI = 0.692, RI = 0.778.

Table 3.2: Matrix of chromosomal characters based on syntenic associations [a].

Character no.	Character	GPA	GTY	GLE	GAF	GKE	GGA	DAU	MPE	POB	TPY	MNA
1	1p/1q	1	1	0	0	0	0	0	0	0	0	0
2	1qa	0	0	0	0	0	0	0	1	1	0	0
3	1p/2p	0	0	0	0	0	0	0	0	1	0	0
4	1q/6p	0	0	1	0	0	0	0	0	0	0	0
5	1qc/6p	0	0	0	0	0	0	0	1	1	0	0
6	1p/10q	0	0	0	0	0	0	0	0	0	1	0
7	1qc/17p/15q/3qb	0	0	0	0	0	0	0	0	0	0	1
8	1q/4p/15q/15p/8p	0	0	0	0	0	0	0	0	0	1	0
9	2p/2q	1	1	1	0	0	0	0	0	0	0	0
10	2qa	0	0	0	0	0	0	0	0	1	0	0
11	2qc	0	0	0	0	0	0	0	0	1	0	0
12	2p/2qa	0	0	0	0	0	0	0	1	0	0	0
13	2qc/8p	0	0	0	0	0	0	0	1	0	0	0
14	2pa/16p/2pc	0	0	0	0	0	0	0	0	0	0	1
15	3p/3q	1	1	0	0	0	0	0	0	0	1	0
16	3qa/4qc	0	0	0	0	0	0	0	1	0	0	0
17	3p/11q/11p	0	0	0	0	0	0	0	1	1	0	0
18	3pb/15p	0	0	0	0	0	0	0	0	0	0	1
19	3q/4q	0	0	0	0	0	0	1	0	0	0	0
20	3qc /17p/17q	0	0	0	0	0	0	0	1	0	0	0
21	4p/4q	1	1	1	1	1	0	0	0	0	0	0
22	4p/4qa	0	0	0	0	0	0	0	1	0	0	0
23	4q/5qc	0	0	0	0	0	0	0	0	0	1	0
24	4q/4pa/5qa/17q/5p	0	0	0	0	0	0	0	0	0	0	1
25	5p/5q	1	1	1	1	0	0	0	0	0	0	0
26	5p/5qa	0	0	0	0	0	0	1	1	1	0	0
27	5qc	0	0	0	0	0	0	1	0	0	0	0
28	5qc/10p	0	0	0	0	0	0	0	1	0	0	0
29	5qc/14p/14q	0	0	0	0	0	0	0	0	1	0	0
30	5qb	0	0	0	0	0	0	0	0	0	0	1
31	5qc/6p/6q/10q/12p/10p	0	0	0	0	0	0	0	0	0	0	1
32	5p/5qa/X	0	0	0	0	0	0	0	0	0	1	0
33	6p/6q	1	1	0	0	0	0	0	0	0	0	0
34	6p/10p/13p/13q	0	0	0	0	0	0	0	0	0	1	0
35	6q/2p/2q/14q/14p	0	0	0	0	0	0	0	0	0	1	0
36	7p/7q	1	1	1	1	1	1	1	1	1	1	0
37	8p/8q	1	1	1	1	1	0	0	0	0	0	0
38	8q/14p/14q	0	0	0	0	0	0	0	1	0	0	0
39	8p/13q/13p/12q	0	0	0	0	0	0	0	0	0	0	1
40	8q/17p/17q/11p/11q	0	0	0	0	0	0	0	0	0	1	0
41	8qa/9q/14q/14p/11pa	0	0	0	0	0	0	0	0	0	0	1
42	9p/9q	1	1	1	1	1	1	1	1	1	1	0
43	10p/10q	1	1	1	1	1	1	0	0	0	0	0
44	11p/11q	1	1	1	1	0	0	1	0	0	0	0
45	11qc	0	0	0	0	0	0	0	0	0	0	1
46	12p/12q	1	1	1	1	0	0	1	1	1	0	0
47	12p	0	0	0	0	1	1	0	0	0	0	0
48	12q/14p/14q	0	0	0	0	1	1	0	0	0	0	0
49	12q/12p/16p/16q	0	0	0	0	0	0	0	0	0	1	0
50	13p/13q	1	1	1	1	1	1	1	1	1	0	0
51	14p/14q	1	1	1	1	0	0	1	0	0	0	0
52	15p/15q	1	1	1	1	1	1	1	1	1	0	0
53	16p/16q	1	1	1	1	1	1	1	1	1	0	0
54	16q	0	0	0	0	0	0	0	0	0	0	1
55	17p/17q	1	1	1	1	1	1	1	0	1	0	0
56	Xp/Xq	1	1	1	1	1	1	1	1	1	0	1
57	Yp/Yq	1	1	0	0	0	1	0	0	0	0	0

[a] The presence of adjacent syntenies were marked as present (1) or absent (0) in *G. paeba* (GPA; index species), *G. tytonis* (GTY), *G. leucogaster* (GLE), *G. afra* (GAF), *G. kempi* (GKE), *G. gambianus* (GGA), *D. auricularis* (DAU), *P. obesus* (POB), *M. persicus* (MPE), *T. pygargus* (TPY) and the outgroup *M. namaquensis* (MNA). Chromosome segments are marked as proximal (a), median (b) or distal (c), relative to the centromere.

Table 3.3: The binary characters based on rearrangement of *G. paeba* (GPA) chromosomes. It was derived from a combination of FISH and G-C banding. Rearrangements are further characterised by a fission into three segments (*), inversions ([a]), an inversion plus fusion to a chromosome/chromosome segment ([a#]), the absence of a hybridization signal (?) and instances wherein GPA chromosomes did not hybridize completely ([b]).

	Fissions, Fusions and Inversions																		
	GPA 1	**GPA 2**	**GPA 3**	**GPA 4**	**GPA 5**	**GPA 6**	**GPA 8**	**GPA 9**	**GPA 10**	**GPA 11**	**GPA 12**	**GPA 7**	**GPA 15**	**GPA 16**	**GPA 17**	**GPA 13**	**GPA 14**	**GPA X**	**GPA Y**
Taxa																			
G. paeba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G. tytonis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G. leucogaster	1	0	1	[a]1	0	1	0	0	0	0	0	0	0	0	0	0	[a]1	0	0
G. afra	1	1	1	[a]1	0	1	0	0	0	0	0	0	0	0	0	0	[a]1	0	0
G. kempi	1	1	1	0	1	1	0	0	0	1	1	0	0	0	0	0	[a#]1	0	0
G. gambianus	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	[a#]1	0	0
D. auricularis	1	1	1	1	1	1	1	0	1	0	0	[a]1	0	0	0	0	0	0	0
M. persicus	*1	1	*1	1	1	1	1	0	1	[a#]1	0	0	0	0	[a#]1	0	[a#]1	0	0
P. obesus	*1	*1	1	1	1	1	1	0	1	[a#]1	0	0	0	0	[a]1	0	[a#]1	0	0
T. pygargus	1	[a#]1	0	1	1	1	1	0	1	[a#]1	[a#]1	0	[a#]1	[a#]1	[a#]1	[a#]1	[a#]1	0	0
M. namaquensis	[b]1	[b]1	[b]1	[b]1	*1	1	[b]1	[b]1	1	[b]1	1	?	1	1	1	[a#]1	[a#]1	0	0

The rearrangement (present = 1) of GPA chromosomes was defined as fissions (the breakage of a *G. paeba* probe), fusions (the retention of a *G. paeba* probe) and pericentric inversions (the 180° rotation of a chromosome segment) in *G. paeba* 1-17, and in conserved chromosomes with/without inversions (13-X).

3.4 Mapping of chromosome rearrangements onto the molecular phylogenetic consensus tree

The consensus phylogenetic tree established by Chevret & Dobigny (2005) among 11 genera and 28 species produced three main clades (Fig. 3.15). The clade comprised of *Gerbilliscus* and *Desmodillus* experienced predominantly Robertsonian fusions/fissions and inversions (Table 3.3). Under a parsimonious scenario, *D. auricularis* (2n = 50) separated from *G. afra* (2n = 44) at node B by nine rearrangements (four Robertsonian and five inversions). The clades nested within this clade are, similarly, also separated primarily by Robertsonian rearrangements; the lineage leading to *G. kempi* (2n = 48) and *G. gambianus* (2n = 52) are separated from *G. afra*/*G. leucogaster* by three Robertsonian rearrangements involving GPA 5, 11, 12 and 14. The West African *G. kempi* and *G. gambianus* are further separated by two Robertsonian fissions involving GPA 4 and 8 (node G). Similarly, *G. leucogaster* (2n = 40) is separated from *G. afra* by two Robertsonian fusions involving GPA 1, 2 and 6 (node F). *Gerbilliscus paeba* and *G. tytonis* (both 2n = 36) are separated from *G. afra* by four Robertsonian fusions (GPA 1, 2, 3, 6) and two inversions (GPA 4, 14; node C). The diploid number was retained in *G. paeba* and *G. tytonis* (node D). The second clade encompassing *T. pygargus*, *P. obesus* and *M. persicus*, experienced more complex rearrangements, relative to GPA; *M. persicus* and *P. obesus* had 14 (seven fissions, five fusions, two inversions in GPA 4p-q prox, 6q; node K) and 13 (seven fissions, two fusions, four inversions in GPA 4q, 3q, 6q, 17) rearrangements respectively. Both *M. persicus* and *P. obesus* share the fission of GPA 1 (Table 3.3) and fusion of GPA 3p+ 11 and GPA 1q dist+6p (node J). *Taterillus pygargus* had eight fusions involving various combinations of GPA autosomal arms (excluding GPA 3, 7 and 9).

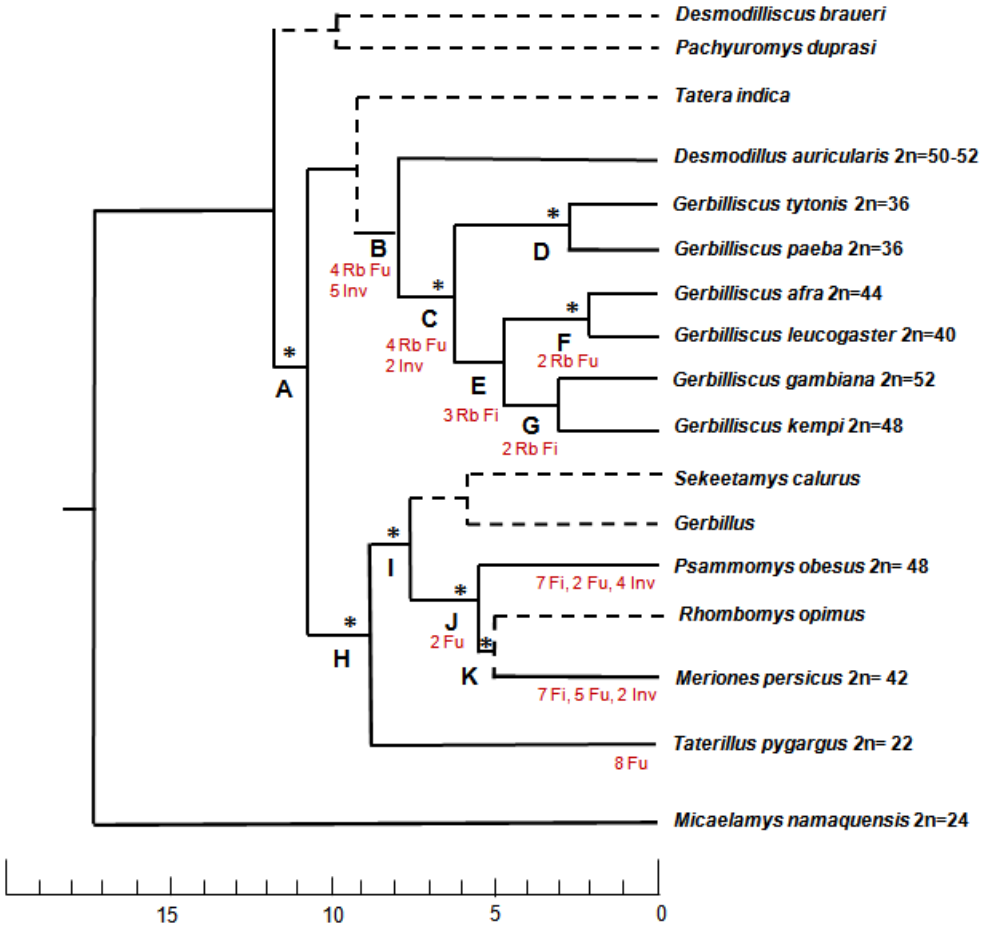

Figure 3.15: The simplified chronogram of gerbils established with cytochrome *b* and 12S rRNA mitochondrial genes (modified form Chevret & Dobigny 2005). Chromosome rearrangements were mapped onto the consensus phylogeny to determine the rate of chromosome evolution within the representative taxa. Alphabetical letters (A-K) indicates nodes at which the representative species diverged and dashed lines indicate excluded data and taxa not examined. Strong relationships are indicated by an asterisk "*" and has bootstrap support values > 90 % (Chevret & Dobigny 2005). The scale below indicates divergence times per million years (Myr). Abbreviations indicate fusions (Fu), fissions (Fi) and inversions (Inv).

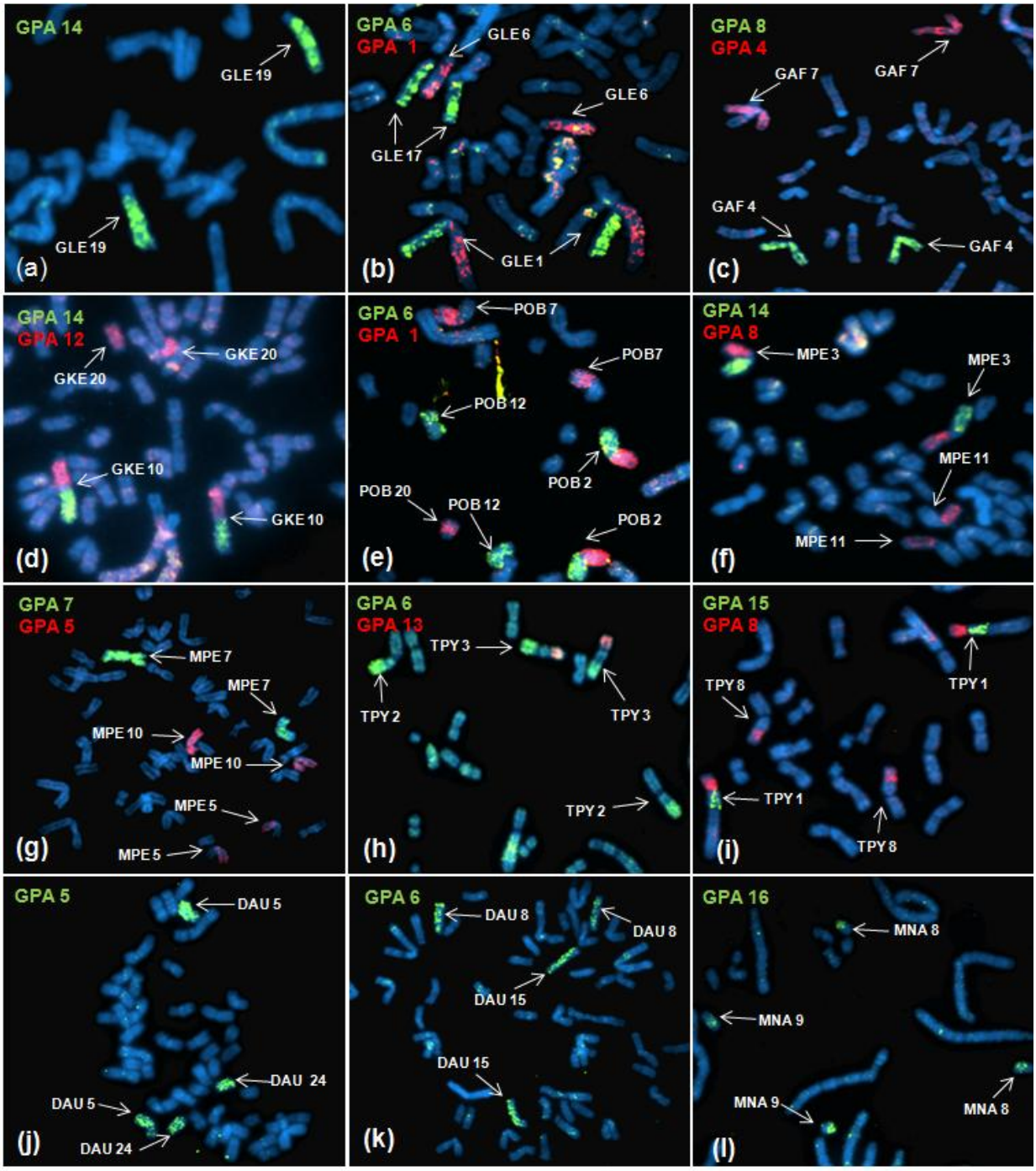

Figure 3.16: Examples of FISH with *G. paeba* painting probes on the ingroup and the outgroup species. The painting probes are indicated on the left corner of each plate while the hybridized chromosomes are numbered with species abbreviation and chromosome number along with an arrow : (a-b) *G. leucogaster*, GLE, (c) *G. afra*, GAF, (d) *G. kempi*, GLE (e) *P. obesus*, POB, (f-g) *M. persicus*, MPE, (h-i) *T. pygargus*, TPY, (j-k) *D. auricularis*, DAU and (l) the outgroup *M. namaquensis*, MNA. Biotin-labelled paints were tagged with CY3-strepavidin fluorochrome (red) and digoxigenin paints were tagged with fluorescence isothiocyanate conjugated anti-digoxigenin (green). Chromosomes were counterstained with DAPI. Images were taken at a magnification of 100 µm.

Chapter 4

Discussion

4.1 Main findings

The karyotypes and diploid numbers of all species analysed in *G. paeba*, *G. tytonis*, *G. afra*, *G. kempi*, *G. gambianus*, *P.obesus*, and *T. pygargus* were nearly identical to those previously identified (Benazzou *et al.*1984; Qumsiyeh 1986a, b; Qumsiyeh & Chesser 1988; Qumsiyeh *et al.* 1991; Volobouev *et al*. 2007). The only differences are the descriptions of the sex chromosomes in *G. paeba*, *G. afra* and *G. leucogaster*. The X and Y chromosomes in *G. leucogaster* were not large, as described by Volobouev *et al*. (2007), but consisted of a large biarmed X and a small acrocentric Y corresponding to that found by Qumsiyeh *et al.* (1986b). Qumsiyeh (1986a, b) also described the Y chromosome in *G. paeba* and *G. leucogaster* as acrocentric, although a p and q arm can clearly be seen. The karyotypes of *D. auricularis* varied to those previously published; *D. auricularis* had a diploid number of 2n = 50; FN = 76 (excluding sex chromosomes), contrasting the 2n = 52; FN = 78 found by Qumsiyeh (1986a, b). The heterochromatin identified with C-banding procedures was identical in all species (Appendix).

4.2 Discrepancies identified by FISH

Although the homologies identified with FISH were largely in agreement with published G-band analyses (Qumsiyeh 1986a, b; Qumsiyeh & Chesser 1988; Qumsiyeh *et al.* 1991; Colangelo *et al.* 2001; Volobouev *et al*. 2007), it was possible to correct several mismatches in the identification of presumptive homologues, and resolve some of the problematic associations reported in previous comparisons. This is most evident in the comparison between *G. kempi*, *G. gambianus* and *G. tytonis* (Volobouev *et al*. 2007) as well as between *Desmodillus* and *G. paeba* (Qumsiyeh 1986a, b). Banding analyses initially suggested GTY 1, 2, 3, 5, 10 and 11 to be homologous to GKE 17 + 11, 15 + 14, 12 + 20, 13 + 19, 21 + 22 and 23 + 10 prox (determined indirectly as homology to *G. paeba*; Volobouev *et al*. 2007). The current data unequivocally identified GTY1 = GKE 11 + 19, GTY2 = GKE 15 + 17, GTY3 = 12 + 23, GTY5 = GPA 13 + 21, GTY10 = 14+ 22, GTY11 = 20 + 10prox.

Similarly, Volobouev *et al*. (2007) reported that five GTY chromosomes, 3, 4, 5, 6, 7 and 10, are each conserved in *G. gambianus* (GGA) as GTY 3 = GGA 21 + 10, GTY 4 = GGA 16 + 14, GTY 5 = GGA 20 + 11, GTY 6 = GGA 19 + 18, GTY 7 = GGA 17 + 22 and GTY 10 =24 + 23. FISH analyses identified the homology of these GTY chromosomes as GTY 3 = GGA 20 + 10, GTY4 = GGA 19 + 14, GTY 5 = GGA 21 + 11, GTY 6 = GGA 24 + 18, GGA 7 = 17 + 16 and GTY 10 = GGA 22 + 23 respectively (Fig. 3.6). Secondly, DAU 2 was initially described as homologous to GPA 3q plus a *de novo* euchromatic addition (Qumsiyeh 1986b). Our results indicate that the "euchromatic addition" is in fact homologous to GPA 4q (Fig. 3.8). The FISH analyses therefore, allow the accurate identification of regions of homology among all 10 species that have been analysed.

4.2.2. Heterochromatin analyses

Previous cytogenetic comparisons (C-banding) among gerbils consistently identified a late replicating chromosome in nearly all gerbils (Qumsiyeh 1988), which corresponds to GPA 7 of the flow-sort. This chromosome has a heterochromatic p-arm with a different staining intensity relative to the remainder of the chromosome, thus indicating differences in repeat elements present (Graphodatsky *et al.* 2011). Qumsiyeh (1988), Qumsiyeh *et al.* (1991) and Qumsiyeh & Schlitter (1991) described GPA 7 as being entirely heterochromatic, and it is unclear as to how it is phylogenetically distributed, and whether it is phylogenetically informative. The hybridization of GPA 7 onto the gerbils revealed that it was conserved intact, and homologous to single chromosomes in all the ingroup taxa. C-positive autosomes identified by C-banding procedures were observed in only four species; the index species *G. paeba, G. leucogaster, G. afra* and *M. persicus* (see Appendix). The West African *Gerbilliscus*, *G. kempi* and *G. gambianus*, *P. obesus* and *T. pygargus* all lacked a C-positive chromosome (Appendix). This implies that GPA 7 hybridized to both heterochromatic and euchromatic chromosomes in all the gerbil species.

The differences among the chromosomes painted by GPA 7 are their sizes and, in one instance in *Desmodillus* (DAU 18, Fig. 3.8), the difference is due to a pericentric inversion resulting in an acrocentric form (in contrast all GPA 7 homologues in other gerbils are biarmed). Banding analyses of *G. paeba* and *G. tytonis* confirmed the two species to have identical karyotypes, with the exception of the C-positive autosome GPA 7 and a biarmed Y chromosome in *G. paeba* (Qumsiyeh *et al.* 1991). This was previously determined using strictly banding analyses (Qumsiyeh *et al.* 1991). Although previous banding comparison suggest GPA 7 is wholly

heterochromatic (Qumsiyeh 1988; Qumsiyeh *et al*. 1991), our data refutes this especially given the fact that it successfully hybridized with euchromatic genomic fractions in all related species, taxa that last shared an ancestor approximately 10 Myr (Chevret & Dobigny 2005).

4.3 Phylogenomic analyses using PAUP

The phylogeny retrieved with PAUP using shared syntenic associations of gerbils derived a monophyletic assemblage to the exclusion of the outgroup. Two syntenic associations, GPA 7p/7q, 9p/9q and Xp/Xq, were conserved in all the examined gerbils. The GPA 5p/5q, 10p/10q, 13p/13q, 15p/15q, 16p/16q and 17p/17q syntenic associations are synapomorphic to the southern African *G. paeba, G. tytonis*, *G. afra* and *G. leucogaster* clade, which is well-supported with a bootstrap support of 94%. The sister-species relationship between *G. kempi* and *G. gambianus* using chromosome characters confirmed that they are evolutionarily close (Colangelo *et al*. 2007), and the two species share two synapomorphic syntenic associations: GPA 12p and 12q/14q/14p. The relationship between *G. kempi* and *G. gambianus* is in contrast to the phylogenetic relationship using mtDNA (Chevret & Dobigny 2005). Chevret & Dobigny (2005) found a monophyletic relationship between the West and southern African *Gerbilliscus* assemblage, that included *G. paeba, G. tytonis*, *G. afra* and *G. leucogaster*, with a bootstrap support value exceeding 90%. Furthermore, the molecular sequence consensus tree found the West and southern African *Gerbilliscus* to have bootstrap support values above 90 and 95% respectively (Colangelo *et al*. 2007; Granjon *et al*. 2012). Further discrepancies include the basal position of *D. auricularis* to *Gerbilliscus*, *Psammomys* and *Meriones*. By contrast, mtDNA placed *Desmodillus* basal only to *Gerbilliscus* (Chevret & Dobigny 2005).

A single similarity obtained using chromosome data and DNA-sequence based is the close relationship between *G. paeba* and *G. tytonis*. The phylogenomic analysis of *P. obesus* and *M. persicus* was also congruent to the close phylogenetic relationship association established using sequence DNA. The 1q prox, 1q dist/6p and 3p/11 syntenic associations are synapomorphic to the species. In addition, *P. obesus* and *M. persicus* have a bootstrap support value of 91 %, one of the highest obtained in the phylogenomic construction. *Taterillus pygargus*, which had eight autapomorphic syntenies (1p/10p, 1q/4p/15/8p, 4q/5q dist, 5p-q prox/X, 6p/10/13, 6q/2/14, 8q/17/11 and 12/16), was basal to all ingroup taxa, with a bootstrap support value of 98%. This contrasts with the basal position *T. pygargus* had to *Meriones* and *Psammomys* using mtDNA (Chevret & Dobigny 2005), which is attributable to the number of characters employed and the

nature of the characters used (macromolecules in the case of chromosomal segments *vs.* nucleotides in DNA sequences as character (Rokas & Holland 2000; Granjon & Montgelard 2012).

The phylogenetic relationship established using chromosome rearrangements, which are defined as fissions, fusions and inversions determined by the hybridization of *G. paeba* painting probes (interchromosomal rearrangements) and matching banding patterns (intrachromosomal rearrangements), produced a monophyletic clade including *G. paeba*, *G. tytonis*, *G. leucogaster*, *G. afra* and *G. kempi*. However, the assemblage was poorly supported, having a bootstrap support value of only 60%. The phylogeny also retrieved a polytomy among *G. gambianus*, *D. auricularis*, *M. persicus* and *P. obesus*. All four species displayed fragmented hybridization signals when painted by GPA 1-3, 5, 6 and 8. This contrasts the sister-species relationship found between *G. gambianus* and *G. kempi* (Colangelo *et al*. 2007), as well as the close association found between *M. persicus* and *P. obesus* using mtDNA (Chevret & Dobigny 2005). The close relationship retrieved between *G. paeba* and *G. tytonis* using chromosome rearrangements was similar to that obtained using DNA-sequences (Colangelo *et al*. 2007; Chevret & Dobigny 2005). It had the highest bootstrap support value of 98%. The karyotypes were conserved entirely intact, with no chromosome rearrangements present. *Taterillus pygargus* was found to be basal to all gerbil species.

The single similarity obtained using DNA-sequence based phylogenies, syntenic associations and chromosome rearrangements is the close relationship between *G. paeba* and *G. tytonis*, which is attributable to the fact that they share the same syntenic associations and have no chromosome rearrangements separating them. When employing both syntenic associations and chromosome rearrangements, *T. pygargus* had a basal position to all the gerbils, thus both approaches contrasted the result found by Chevret & Dobigny (2005), which placed *Taterillus* at a basal position only to *Psammomys* and *Meriones* (as well as *Sekeetamys*, *Gerbillus* and *Rhombomys* when taking into account all gerbils). The level of incongruence among the phylogenetic relationships obtained using chromosomal and DNA data is not uncommon. This variation was also observed in bats - the discordance between chromosome characters, in the form of Robertsonian rearrangements, and DNA-sequence based trees were attributed to the vast majority of rearrangements being autapomorphic, as well as the homoplasic nature of Robertsonian rearrangements, since it produced only two well-supported groupings (Mao *et al.*

2008). The relationship between *G. paeba* and G. *tytonis* was not surprising. In a study on bathyergids by Deauve *et al.* (2008) that employed chromosome characters (rearrangements and syntenies), a sister-species relationship was founded in two *Bathyergus* species with identical diploid numbers and similar karyotypes, similar to its DNA-sequence based phylogeny. The variation in the chromosome and DNA-sequence based topologies likely resulted from differences in the number and type of characters employed and its individual rate of evolution. Chromosomes have a slow rate of mutation in comparison to mitochondrial DNA (Rokas & Holland; Granjon & Montgelard 2012). Furthermore, Chevret & Dobigny (2005), Colangelo *et al* (2007) and Granjon *et al*. (2012) had a larger taxon representation that included additional genera and species.

Syntenic associations provided more resolution in constructing phylogenies due to more characters and variation present, particularly when noting the polytomy formed using chromosome rearrangements; it was sufficient to resolve the relationship between *D. auricularis*, *G. gambianus, P. obesus* and *M. persicus*. The use of chromosome syntenic characters and rearrangements itself does not allow us to infer the rate of chromosome evolution, but does allow the construction of phylogenetic relationships. Although the relationships constructed using chromosome characters are not well-supported, and it differs to that obtained using DNA sequence data, the relationships may alter when including more taxon representatives. The rate of chromosome evolution, however, can only be achieved by mapping the rearrangements onto a molecular phylogenetic consensus tree.

4.4 Chromosome evolution: Mode and tempo of chromosome evolution in Gerbillinae

Molecular phylogenetic analyses show Gerbillinae diverged almost 20 Myr (Michaux *et al*. 2001; Steppan *et al*. 2004), and have since produced three main clades over 11.5 Myr during the cladogenesis of the subfamily (Chevret & Dobigny 2005). The extensive chromosomal homeology established by FISH demonstrates that only two autosomes, GPA 7 and 9, and the X chromosome have been conserved *in toto* across all gerbils - these chromosomes displayed whole-chromosome homology in the ingroup taxa. One of the three main clades identified by Chevret & Dobigny (2005) comprised of *Desmodillus* and the southern and West African *Gerbilliscus*, herein represented by *G. afra, G. leucogaster, G. kempi* and *G. gambianus*. Thus it encompasses a large number of representative species for this clade. The southern African *Gerbilliscus* (*G. paeba, G. tytonis*, G. *leucogaster* and *G. afra*) and *D. auricularis* have seven autosomes that were conserved intact and exhibit whole-chromosome homology – GPA 11 - 17.

The retention of gross chromosomal homology among gerbil species is remarkable. For instance, the karyotypes of *G. paeba* and *G. tytonis* have been conserved *in toto*, yet they have been unable to hybridize, as found in other groups with identical karyotypes (e.g., monotremes, Wrigley & Graves 1988; bears, Nash *et al*. 1998; bats, Richards *et al.* 2010, fish, Terencio *et al*. 2012). *Gerbilliscus paeba* and *G. tytonis* display agonistic behaviour when brought into contact (Dempster & Perrin 1989a, b), and have specific odour preferences (Dempster & Perrin 1990). The level of pre-zygotic isolation may also be a result of the genetic distance between them (Coyne & Orr 1997), since Chevret & Dobigny (2005) and Colangelo *et al*. (2007) found the two to be distinct species. The data also demonstrates that Robertsonian rearrangements were important in chromosomal evolution and cladogenesis in the southern African and West African gerbils.

Interpretation of the rearrangements in the context of DNA sequence divergence times allows us to make inferences about rate of evolution, defined as the ratio of chromosome change by the time of divergence (Badenhorst *et al.* 2011). *Desmodillus* accumulated four Robertsonian rearrangements and five inversions since it separated from *Gerbilliscus*, reflecting a rate of 1.25 rearrangements per million years (even taking the homoplasic rearrangement detected by GPA5 into account); *G. kempi* accumulated three Robertsonian rearrangements following its split from the *G. afra/G. leucogaster* lineage 5.5 million years ago (Colangelo *et al*. 2007), which translates into an evolutionary rate of less than 1 rearrangement per million years. Four probes, GPA 4, 8, 10 and 5, hybridized two separate chromosomes in *Desmodillus* - DAU2p and 23, 17/19, 9/22 and 5/24 - and whole chromosomes in *G. leucogaster* and *G. afra* (Fig. 3.13). In addition to the overwhelming presence of Robertsonian rearrangements in these gerbils, inversion rearrangements were also noted in several species. In a most parsimonious scenario, *D. auricularis* (2n = 50) evolved to the 2n = 44 present in *G. afra*. Side-by-side G-band comparison (directed by FISH) in *Desmodillus* and *G. afra* shows that five autosomal pairs differ due to the position of the centromeres (DAU 1 *vs*. GAF 6, DAU 3 *vs*. GAF 2, DAU 13 *vs*. GAF 17, DAU 8 *vs*. GAF 18, DAU 18 *vs*. GAF 15; Fig. 3.13). Similarly, two GPA autosomes differ from their orthologs in *G. afra* as a result of centromere variability (GAF17 *vs*. GPA14 and GAF7 *vs*. GPA4; Fig. 3.5).

Two Robertsonian rearrangements separate *G. leucogaster* and *G. afra* since they last shared a common ancestor 2.5 Myr (Colangelo *et al*. 2007), representing an evolutionary rate of less than

1 rearrangement/Myr. In fact, *Gerbilliscus afra* (2n = 44) and *G. leucogaster* (2n = 40) have near-identical karyotypes, and display monobrachial homology; GAF 1 + 2, and GAF 5 + 6, is homologous to GLE 1 and 3 respectively. Theoretically, this should not severely affect fertility, since the trivalents formed in hybrids at meiosis are still able to segregate normally (Baker & Bickham 1986). This has been demonstrated in the polymorphic cryptic species complex, *Saccostomus campestris* (2n = 32-46), which yielded successful hybrids (Maputla *et al*. 2011). However, *G. afra* and *G. leucogaster* are two distinct species separated by a 13.5% sequence divergence (Colangelo *et al*. 2007). Furthermore, *G. afra* and *G. leucogaster* are allopatrically distributed, which precludes any potential hybridization between the two species. Assuming a parsimonious scenario, the evolutionary rate in *G. paeba*/*G. tytonis*, evolved from a 2n = 44 (present in *G. afra*) 2.9 Myr (Chevret & Dobigny 2005; Colangelo *et al*. 2007). The change required four Robertsonian fusions and two inversions, indicating an evolutionary rate of 2 changes/Myr, slightly more rapid than what is observed in its congenerics (*G. leucogaster*, *G. afra*, *G. kempi*, *G. gambianus*). The split between *G. kempi* and *G. gambianus* occurred 3.2 Myr, and the species are separated by two fissions (GPA 4 and 8) equating to a chromosome evolutionary rate of 0.6/Myr. *Gerbilliscus gambianus*, which has been referred to as a synonym of *G. kempi* by Musser & Carleton (2005), has been considered numerous times as a distinct species. Examples include Duplantier & Bâ (2001; therein referred to as *Tatera gambiana*), Colangelo *et al*. (2005; 2007), Volobouev *et al*. (2007) and Granjon *et al*. (2012), whom each described *G. gambianus* as a distinct species in their respective studies. *Gerbilliscus kempi* and *G. gambianus* are separated by a genetic divergence of 13.7% (Colangelo *et al*. 2007), thus supporting the distinction of two separate, valid species. Although this level of genetic distance is sufficient for rodent species recognition if variation between taxa exceeds 11% (Bradley & Baker 2001; Ferguson 2002), or more specifically, in the range of 9-27% for *Gerbilliscus* species (Granjon *et al*. 2012), however additional criteria such as morphological, karyological and reproductive isolation characteristics are important for species recognition (Faleh *et al*. 2010; Taylor *et al*. 2009; Ducroz *et al*. 1997; Granjon & Montgelard 2012).

The second major clade retrieved from molecular phylogenetics included the genera *Meriones*, *Psammomys* and *Taterillus* (*Sekeetamys*, *Gerbillus* and *Rhombomys* also present formed part of the clade but were not analysed in the present study; Chevret & Dobigny 2005). *Taterillus pygargus* has undergone the most extensive chromosome rearrangements since its speciation 0.5 Myr (Dobigny *et al*. 2005). This is confirmed by the FISH data which identified eight syntenies

that were comprised of various combinations of GPA chromosomal arms, including the sex-autosome translocation of TPY X (GPA 5p-q prox and GPA X), which is autapomorphic. X; autosome translocations is a diagnostic trait in the genus, contributing to the odd diploid number present in male specimens (Dobigny *et al.* 2002a). The accumulation of these eight rearrangements - fusions of TPY 1 (GPA 1q, 4p, 15, 8p,), TPY 2 (GPA 6q, 2, 14), TPY 3 (GPA 6p, 10p, 12), TPY 4 (GPA8q, 17, 11), TPY 9 (GPA 16 and 12), TPY 6 (GPA 4q and 5q dist), TPY 10 (GPA1p and 10q) and TPY X (GPA 5p-q prox and GPA X - since the speciation of *T. pygargus* 0.5 Myr (Chevret & Dobigny 2005) suggests an evolutionary rate of 16 changes/Myr. This accelerated rate of chromosome evolution is common in *Taterillus* since it has been previously observed using banding pattern homologies (Dobigny *et al*. 2005).

Chromosome painting of *M. persicus* and *P. obesus* indicate that up to three hybridization fragments were produced by GPA 1 and 2, and GPA 1 and 3, respectively. Three rearrangements, the fission of GPA 1 (homologous to MPE 2p + 19 + 20 and POB 2p + 7q+20) and fusions of GPA 3p + 11 (equivalent of MPE 1 and POB 1), and GPA 1q dist + 6p (equivalent of MPE 2 and POB 2) are synapomorphic to *M. persicus* and *P. obesus*. The genera diverged 5.5 Myr, equating to an evolutionary rate of 0.5 changes/Myr. *Meriones persicus* underwent a further seven fissions when painted by GPA 2 – 6, 8 and 10 and five subsequent fusions. The seven fissions are homologous to MPE 8 + 11p, MPE 1p + 12p + 14q, MPE 13 + 12q, MPE 10 + 5q, MPE 9 + 2q, MPE 3p + 11q and MPE 5p + 18, respectively. The subsequent fusions involved GPA 8q + GPA 14 (MPE 3), GPA 10p + GPA 5q dist (MPE 5), GPA 2q dist + GPA 8p (MPE 11), GPA 3q prox + GPA4q dist (MPE 12) and GPA 17 + GPA 3q dist (MPE 14). The G-bands directed by FISH indicated the additional presence of two pericentric inversions (Fig. 3.9); GPA 4p-q prox = MPE 13, GPA 6q = MPE 9, accumulating to 14 rearrangements in total. The events spanned since the divergence of *Meriones* from *Psammomys–Rhombomys* over 3.5 Myr (Chevret & Dobigny 2005), equating to an evolutionary rate of 4 changes/Myr, slightly more rapid than in *Gerbilliscus* and *Desmodillus*. *Psammomys obesus* diverged from *Rhombomys–Meriones* 5.5 Myr (Chevret & Dobigny 2005). The hybridization of GPA 2-6, 8 and 10 produced fragmented signals, indicating seven fissions. These displayed homology to POB 7p + 17 + 19, POB 1p + 9, POB 8 + 23, POB 3p + 6, POB 2q + 12, POB 16 + 22 and POB 18 + 21, respectively, and two subsequent fusions of GPA 5q dist + 14 (POB 3) and GPA 2p + 1p (POB 7). The additional G-band comparisons directed by FISH between *G. paeba* and *P. obesus* show that four autosomal pairs differ by the position of the centromeres, indicating the

presence of inversions; GPA 4q = POB 8, GPA 3q = POB 9, GPA 6q = POB 12 and GPA 17 = POB 15 (Fig. 3.10). The cumulative number of rearrangements (7 fissions, 2 fusions, 4 inversions) culminated to an evolutionary rate of 2.3 changes/Myr in *P. obesus*, i.e. slightly slower than in *M. persicus*. The two species are only separated by one rearrangement (14 in *M. persicus* and 13 in *P. obesus*).

On average, the rearrangement of GPA 1-6, 8, 10, 11 and 12 in *Gerbilliscus* over 8 million years equates to a chromosome evolutionary rate of ~1.25 million years (10 rearrangements over 8 million years), which is considerably slower than values previously obtained in other gerbils (e.g. West African *Taterillus* have a rate of 45 rearrangements per Myr; Dobigny *et al.* 2002a, 2005). *Taterillus pygargus*, *M. persicus* and *P. obesus* have an evolutionary rate of 16/Myr, 4/Myr and 2.3/Myr respectively (present data). Some *Gerbillus* species show a rate of ~12.3 rearrangements per million years (Aniskin *et al.* 2006; Chevret & Dobigny, 2005). Recently, it was shown that six representative genera in the tribe Rattini displayed an evolutionary rate of 0.6-3.33 rearrangements per Myr, which was considered slow for murid rodents (Badenhorst *et al.* 2011). Therefore, the rate of chromosome evolution among the southern African gerbils studied here is unexpectedly slow, while the chromosome evolutionary rate of *M. persicus* and *P. obesus* is moderately faster. *Taterillus pygargus*, by all accounts, had the most extensive rate of chromosome evolution. In comparison to non-murid rodents the evolutionary rates are relatively fast. Sciurid karyotypes are highly conserved relative to human (*Homo sapiens*) – humans are only separated from *Menetes berdmorei* by 20 chromosomal changes (Richard *et al.* 2003). The early divergence of the West African *Desmodilliscus braueri* (Musser & Carleton 2005), with the inclusion of additional analyses, allowed Chevret & Dobigny (2005) to propose an African origin for gerbils, followed by two subsequent dispersal events (*Desmodillus* and *Gerbilliscus*, and *Psammomys*, *Rhombomys* and *Meriones*) across Asia and Africa. *Desmodillus* and *Gerbilliscus* are proposed to have originated in southern Africa, and dispersed from the subregion (*G. paeba*, *G. tytonis*, *G. leucogaster*, *G. afra*) to West Africa (*G. kempi*, *G. gambianus*), following the West African *Gerbilliscus*' divergence from East Africa (when taking into account representative *Gerbilliscus* from this region; Colangelo *et al.* 2005) as inferred by the divergence dates by Colangelo *et al.* (2007). The change in the karyotypes of the *Desmodillus*, and western and southern African gerbil representatives demonstrated predominantly Robertsonian rearrangements and inversions. However, the southern African *G. afra* and *G. leucogaster* had experienced fewer karyotypic changes when painted by *G. paeba*

probes in comparison to the West African *Gerbilliscus*, thus supporting Chevret & Dobigny's (2005) hypothesis of a northward distribution (from southern Africa upward), as well as Colangelo *et al.*'s (2005) hypothesis that chromosomal changes influenced speciation in the West African *Gerbilliscus*, while the stable karyotypes of East African *Gerbilliscus* have been retained due to speciation being driven largely by climatic and not chromosomal changes. Furthermore, *G. leucogaster* and *G. afra* are isolated from each other and have an allopatric distribution. The two species may have experienced a genotypic or phenotypic divergent allopatric speciation, as suggested by Taylor (2000), indicating that the probable inability to hybridize is due to pre-zygotic isolation (genetic distance of 13.5%, Colangelo *et al.* 2007). The West African *Gerbilliscus* had undergone more frequent Robertsonian rearrangements, resulting in the higher diploid numbers. *Gerbilliscus kempi* and *G. gambianus* are also separated by a sequence divergence value of 13.7% (Colangelo *et al.* 2007). *Taterillus* developed a more accelerated rate of chromosome evolution in a short time (0.5 Myr; Chevret & Dobigny 2005). Dobigny *et al.* (2005) proposed that speciation within the West African *Taterillus* was driven by chromosome rearrangements in response to climatic shifts, particularly since *Taterillus* species have low genic divergence, similar morphology (Dobigny *et al.* 2002c), and a recent speciation (Chevret & Dobigny 2005).

4.5 Homoplasy *vs.* Hemiplasy

The chromosomal homology map established by FISH and banding comparison demonstrates that Robertsonian rearrangements were important in chromosomal evolution and cladogenesis in southern African gerbils, as well as in West African *Gerbilliscus* species, *G. kempi* and *G. gambianus*. Early G-band comparisons among gerbils have suggested that Robertsonian rearrangements may be homoplasic and interpreting them in an evolutionary context may be problematic (Qumsiyeh *et al*. 1987) - a finding recently shown for the Bovidae (Robinson & Ropiquet 2011). Using the gerbil sequence-based phylogeny as a scaffold, we show that the rearrangements identified by the GPA painting probes 1-6, 8, 10, 11, and 12, one is a synapomorphy (GPA 6) uniting *G. paeba* and *G. tytonis*; two are synapomorphies in *G. kempi* and *G. gambianus* (GPA 11 and 12), and three are synapomorphies (GPA 4, 8 and 10) for *Gerbilliscus* (with the exclusion of *G. gambianus* in GPA 10). The remainder (GPA 1, 2, 3, 5) are potential homoplasies/hemiplasies in the clade comprising *Desmodillus* and *Gerbilliscus* (Avise & Robinson 2008).

With respect to the first of these, GPA 1, two possible hypotheses may be suggested for the observed patterns when the character is mapped to the tree in Fig. 3.15. The first is that it is an example of hemiplasy, having undergone a fusion at ~6 Myr (node C), and persisted as a polymorphism until the divergence of *G. leucogaster* and *G. afra* at ~2.5 Myr. The alternative explanation (i.e., homoplasy) suggests that the fusion arose at D (the common ancestor to *G. tytonis* and *G. paeba*) and convergently so in *G. leucogaster* (i.e., requiring two changes *vs.* the one for hemiplasy). Although hemiplasy offers the most parsimonious solution in terms of rare genomic changes (RGC, Rokas & Holland 2000), the required persistence time (~3.5 my) is at the upper boundary suggested by Robinson *et al*. (2008) and Robinson & Ropiquet (2011), and we regard homoplasy as the more likely of the two hypotheses. The same reasoning applies to the Robertsonian rearrangements involving GPA 2 and GPA 3. A more convincing case for hemiplasy can be made with respect to GPA 5 (arose at node C and persisted as a polymorphism, at which point it was lost in the lineage leading to *G. kempi* and *G. gambianus*). In the clade comprising *T. pygargus*, *M. persicus* and *P. obesus*, homoplasy may also be argued for in the case of GPA 5p-q prox, particularly if it arose at node A (Fig. 3.15) and retained since the divergence of the two clades leading to H and B for over a period of 10 Myr (Chevret & Dobigny 2005). GPA 5p-q prox is homologous to DAU 5, MPE 10 and POB 6, and although *Desmodillus* diverged approximately 8 Myr, *Meriones* and *Psammomys* diverged 3.5 and 5.5 Myr, respectively. The GPA 5p-q prox segment fused to TPY X, resulting in the X; autosome translocation in the *Taterillus* lineage, thus supports the argument of homoplasy.

Moreover, the whole-chromosome homology of GPA 3 that has been retained in *G. paeba* and *G. tytonis* has also been conserved in *T. pygargus*. *Taterillus* diverged from the *Sekeetamys* - *Gerbillus*, and *Psammomys*-*Rhombomys*-*Meriones* genera 8.5 Myr (Chevret & Dobigny 2005). The fusion of GPA 3 at node C would suggest homoplasy (Robinson *et al.* 2008), particularly since *T. pygargus* only speciated 0.5 Myr (Chevret & Dobigny 2005). This is also notable when examining the congenerics of *T. pygargus*; *T. gracilis* (2n = 36/37) and *T. arenius* (2n = 30/31) speciated 2 Myr and 0.5 Myr ago, respectively (Chevret & Dobigny 2005). The analyses of chromosome banding homologies between *T. pygargus*, *T. gracilis* and *T. arenius* demonstrate that TPY 5 is homologous to *T. gracilis* (TGR) 11 and 12, and *T. arenius* (TAR) 12 and 13 respectively, all acrocentric chromosomes (Dobigny *et al.* 2003), indicating a fusion of GPA 3 in *T. pygargus*. This is also evident in *T. petteri* 5 (TPY 5); GPA 3 displays homology to *T. petteri* (TPE) 8, an acrocentric chromosome, indicating and inversion.

Consequently, the findings of Qumsiyeh *et al*. (1987) that homoplasy is present in the chromosomal evolution of gerbils is partly confirmed by the present study. Our results that show 4 of the 10 rearrangements identified in *Gerbilliscus* are homoplasic when mapped to the tree (three convergences/reversals and 1 probable hemiplasy, all of which contribute to the "noise" evident in the species phylogeny), and a single homoplasy (5p-q prox) in *D. auricularis*, *P. obesus* and *M. persicus* across the two successive clades. Qumsiyeh *et al*. (1987) on the other hand obtained 7 homoplasies in 15 Robertsonian rearrangements among *G. paeba*, *G. vallinus*, *G. robustus*, *G. nigricauda*, *G. leucogaster*, *G. afra* and *G. brantsii*. It should be noted, however, that the study by Qumsiyeh *et al*. (1987) may have over-estimated the number of homoplasic traits due to topological differences in the trees used (allozyme-based in Qumsiyeh *et al.* (1987), and mtDNA and nuclear sequences in our case) and, in some instances, the misidentification of the chromosomes involved. Therefore, our data are in contrast to Qumsiyeh *et al.* (1987) who suggested that Robertsonian rearrangements were homoplasic, at least during cladogenesis in gerbils.

4.6 The ancestral karyotype of the Gerbillinae

The oldest representative taxon in the study, the southern African endemic *Desmodillus*, diverged approximately 8.12 Myr (Chevret & Dobigny 2005), with a diploid number of 2n = 50-52 (Qumsiyeh 1986b, present data). It has been hypothesised that all modern gerbils may have evolved from a 2n = 44 or 2n = 50 (Qumsiyeh & Schlitter 1991), which is close to the diploid number of *Desmodillus*. Furthermore, the extent of homology across species analysed in the present investigation, is such that karyotypes can be reconstructed through simple arm combinations, which makes it possible to propose ancestral chromosomes at the nodes of the consensual tree. The clade comprised of the *Desmodillus* and *Gerbilliscus* assemblage diverged roughly 10.5 Myr from the clade comprised of *Meriones*, *Psammomys* and *Taterillus* (Fig. 3.15; Chevret & Dobigny 2005). We hypothesise that the ancestral Gerbillinae karyotype comprised of six autosomes (GPA 7, 9, 13, 15, 16, 17) and the X chromosomes which remained unchanged among all the southern African *Gerbilliscus* and *Gerbillurus*. With the exception of GPA 7p/7q, these were retained as biarmed chromosomes in the ancestral karyotype (Fig. 4.1). In addition to these, 21 acrocentrics GPA1p,1q, 2p, 2q, 3p, 3q, 4p, 4q, 5p, 5q, 6p, 6q, 8p, 8q, 10q, 10p, 11p,11q, 12p, 12q and 14, resulting in the putative ancestral diploid number of 2n = 56. The use of the DNA sequence phylogeny and an outgroup enabled the construction of a putative ancestral

karyotype by analysing the mode of chromosome evolution between the last common ancestor to the most derived karyotype in Gerbillinae (Dobigny *et al.* 2004; Robinson & Ruiz-Herrera 2008). However this is preliminary, since further refinements are possible (Dobigny *et al.* 2004; Kellogg *et al*. 2007) once representative species of *Brachiones*, *Desmodilliscus*, *Pachyuromys*, *Sekeetamys*, *Gerbillus* and *Rhombomys* are included.

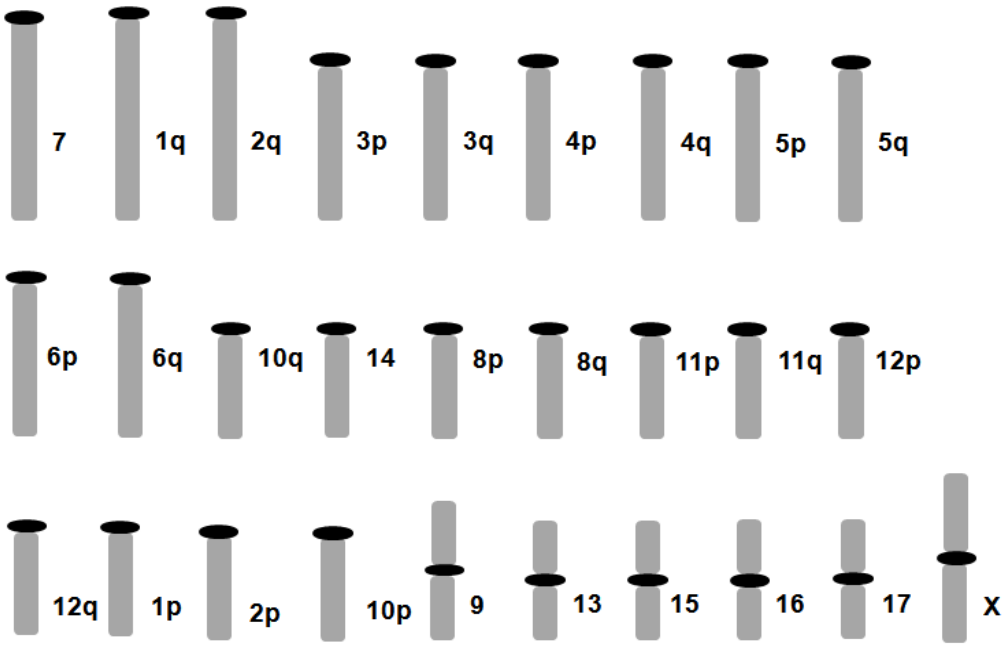

Figure 4.1: The putative ancestral karyotype of Gerbillinae (2n = 56). It comprised of 21 pairs of acrocentric chromosomes. The six autosomes, GPA 7, 9, 13, 15, 16 and 17, and X chromosome, were conserved intact in the ancestral karyotype. With the exception of GPA 7, they were all retained as biarmed chromosomes.

Chapter 5

Conclusions

The present study aimed to determine the phylogenetic relationships of gerbilline rodents using chromosome characters, FISH and whole-chromosome painting probes of *G. paeba* (2n = 36). In the first instance, the ZOO-FISH approach provided detailed assessment of chromosome conservation among 10 gerbil species, and hence allowed identification of synapomorphies and symplesiomorphs among the gerbils analysed. Secondly, the FISH corrects and extends earlier G- banded homologies (in *G. kempi*, *G. gambianus* and *D. auricularis*). Interestingly, hybridization of the late replicating GPA 7 to single autosomes within Gerbillinae, including euchromatic and heterochromatic components, clearly indicates this chromosome contains functional genes. However, the phylogeny established from the chromosome data of the 10 species has shown little congruence to the phylogeny obtained using mtDNA (Chevret & Dobigny 2005). With the exception of *T. pygargus*, the present cytogenetic data places *Desmodillus* at a basal position to the whole subfamily (syntenic associations), or in a polytomy (chromosome rearrangements). Additionally, both methods of coding characters placed *T. pygargus* at a basal position to Gerbillinae which is in contrast to the findings of Chevret & Dobigny (2005). Generally, reconstructions of phylogenetic relationships using chromosome characters (syntenic associations and chromosomal rearrangements) were not successful in resolving deep cladogenic events (i.e. basal polytomies), and consequently chromosome rearrangements were mapped on the molecular sequence phylogeny.

By mapping the chromosome rearrangements onto a consensus molecular phylogenetic tree it was possible to determine the rate of chromosome evolution (mode and tempo), construct a putative ancestral karyotype for Gerbillinae, and determine to what extent the rearrangements are homoplasic or hemiplasic. The rate of chromosome evolution in Gerbillinae ranged from relatively fast (16/Myr in *T. pygargus*) to slow (1.25/Myr in *Desmodillus* and less than1/Myr in *Gerbilliscus*), while *M. persicus* and *P. obesus* experienced a fairly moderate rate of chromosome evolution (4/Myr and 2.3/Myr respectively). The dispersal of gerbils from southern Africa (*Desmodillus, G. paeba, G. tytonis, G. leucogaster, G. afra*) to West Africa (*G. kempi* and *G. gambianus;* Chevret & Dobigny 2005) has shown that the increase in diploid number between the southern African and West African *Gerbilliscus* to be driven by Robertsonian

rearrangements. The clade comprised of *P. obesus*, *M. persicus* and *T. pygargus* dispersed into the Middle East, Africa and Asia, and exhibit a different mode and tempo of chromosome evolution, which is demonstrated by the tandem rearrangements. The Robertsonian chromosome rearrangements prevalent in the *Desmodillus* and *Gerbilliscus* assemblage, however, were found to be homoplasic (GPA 1-3, 5), since these occurred over the course of the evolution spanning approximately 10 Myr. *Taterillus pygargus*, *G. paeba* and *G. tytonis* had one homoplasy (GPA 3), and *D. auricularis*, *T. pygargus*, *M. persicus* and *P. obesus* shared a single homoplasy (GPA 5p-q prox). GPA 5 was a potential hemiplasy to the southern African taxa.

The chromosomal data is in support of the consolidation of *Gerbillurus* within *Gerbilliscus*. In fact, the basal position of *G. paeba* and *G. tytonis* to the West + southern African *Gerbilliscus*, means the southern African *Gerbilliscus* (*G. paeba*, *G. tytonis*, *G. leucogaster*, *G. afra*) is consequently paraphyletic. However, the relationship between the East and southern African *Gerbilliscus* (including *Gerbillurus*) could not be established since no representative East African *Gerbilliscus* were included in the present study, and it remains to be seen how it would fit into the chromosome-derived phylogenetic relationships.

Hybridization of *G. paeba* probes to the outgroup, *M. namaquensis*, produced largely fragmented signals, with some probes not hybridizing completely, possibly indicating it might be too distantly related to Gerbillinae. The spiny mouse of the genus *Acomys* (subfamily Deomyinae) is a sister taxon to the Gerbillinae (Michaux *et al*. 2001; Steppan *et al*. 2004), and would be a more suitable alternative. Nonetheless, the use of an outgroup (for polarising the data) and the molecular phylogeny suggests that the putative ancestral karyotype to be 2n = 56, comprised of GPA 7, 9, 13, 15, 16, 17, and X that were conserved, and 21 acrocentrics, GPA1p, 1q, 2p, 2q, 3p, 3q, 4p, 4q, 5p, 5q, 6p, 6q, 8p, 8q, 10q, 10p, 11p, 11q, 12p, 12q and 14. This ancestral karyotype is provisional, mainly because of the outstanding taxa that were not included in this investigation. This includes genera such as *Pachyuromys*, *Brachiones*, and most importantly, additional species from the speciose genera such as *Meriones* and the chromosomally divergent *Taterillus*.

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Appendix

The G-banded karyotypes and C-bands of Gerbillinae.

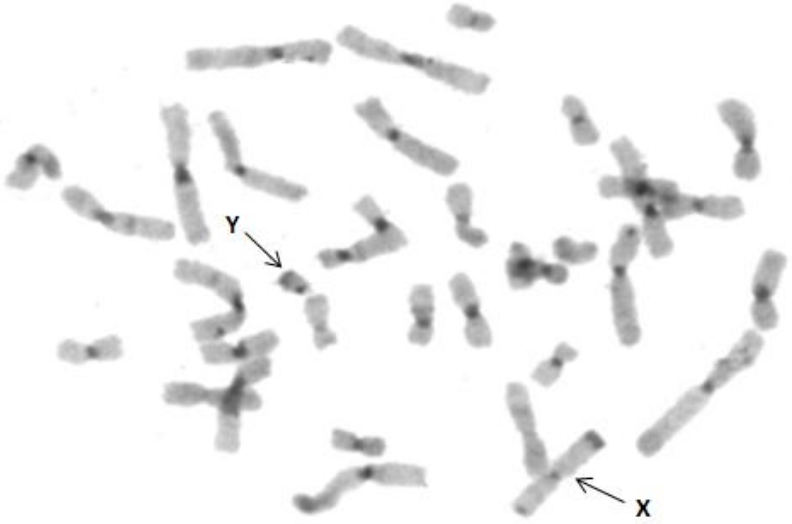

Figure 1-A: The C-banded metaphase spreads of a male *G. tytonis* (2n = 36). Arrows indicate position of the sex chromosomes.

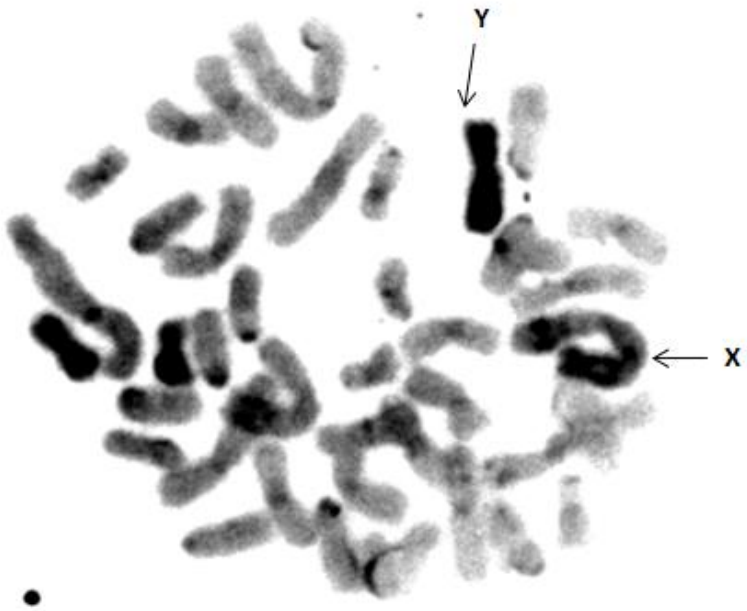

Figure 2-A: The C-banded metaphase spread of a male *G. leucogaster* (2n = 40). The arrows indicate position of the sex chromosomes.

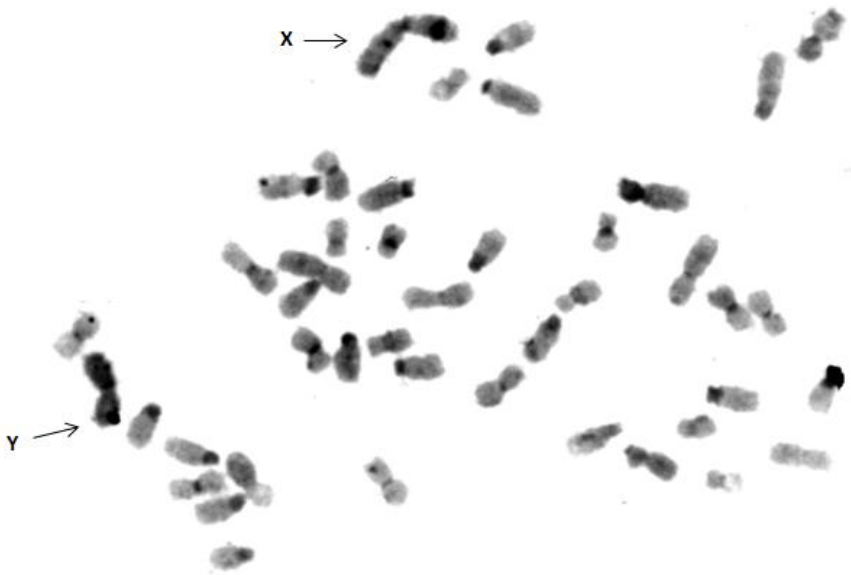

Figure 3-A: The C-banded metaphase spread of *G. afra* (2n = 44). The arrows indicate position of the sex chromosomes.

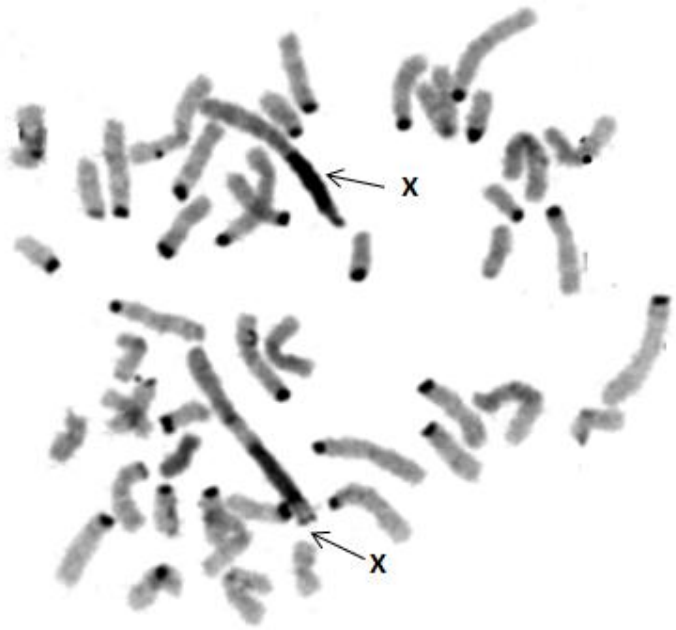

Figure 4-A: The C-banded metaphase spread of a female *G. kempi* (2n = 48). Arrows indicate position of the sex chromosomes

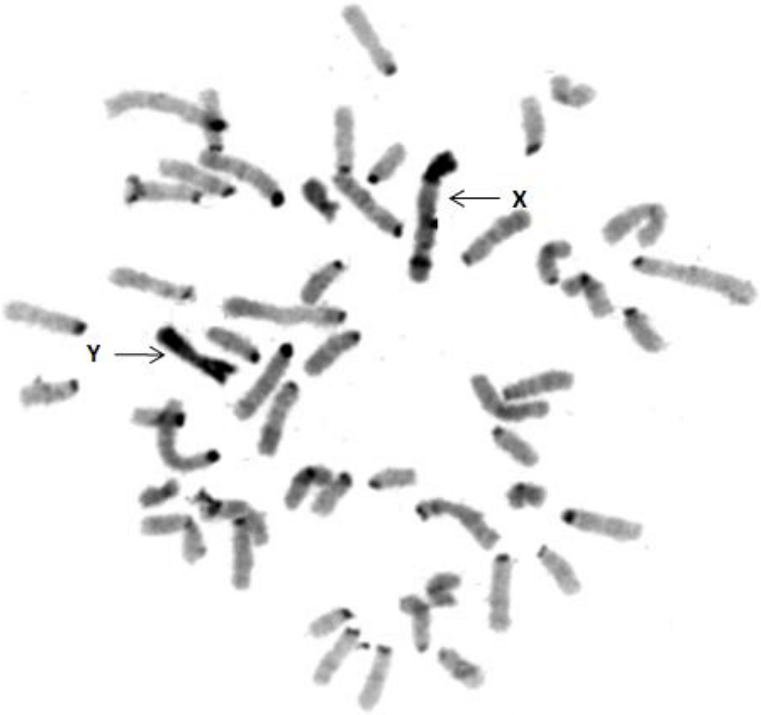

Figure 5-A: The C-banded metaphase spread of a male *G. gambianus* (2n = 52). The arrows indicate the sex chromosomes.

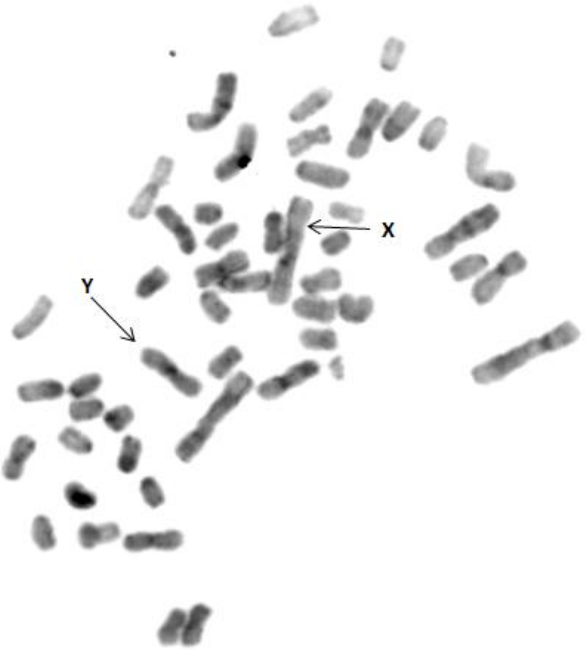

Figure 6-A: The C-banded metaphase spread of *D. auricularis* (2n = 50). The arrows indicate the sex chromosomes.

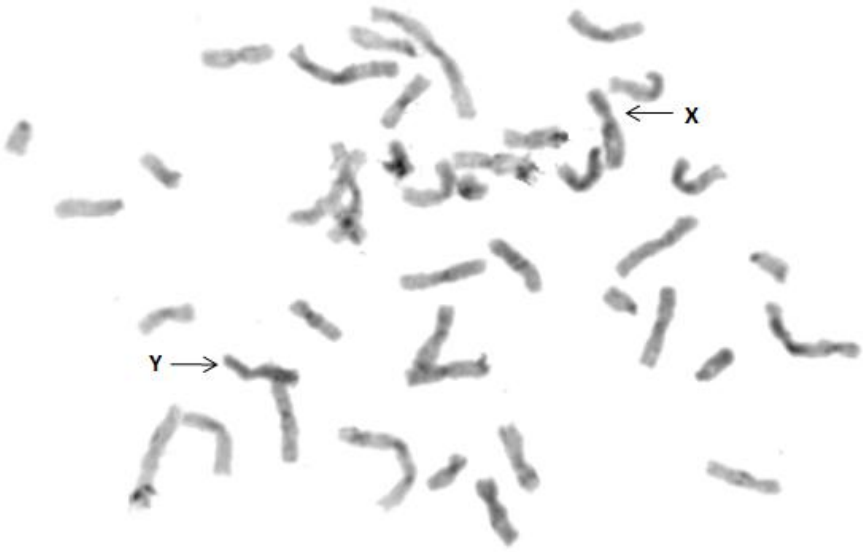

Figure 7-A: The C-banded metaphase spread of male *M. persicus* (2n = 42). The arrows indicate the position of the sex chromosomes.

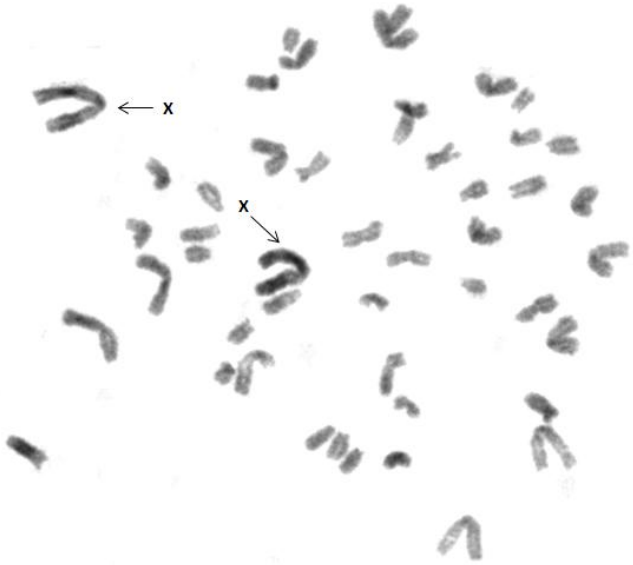

Figure 8-A: The C-banded metaphase spread of *P. obesus* (2n = 48). Arrows indicate the position of the sex chromosomes.

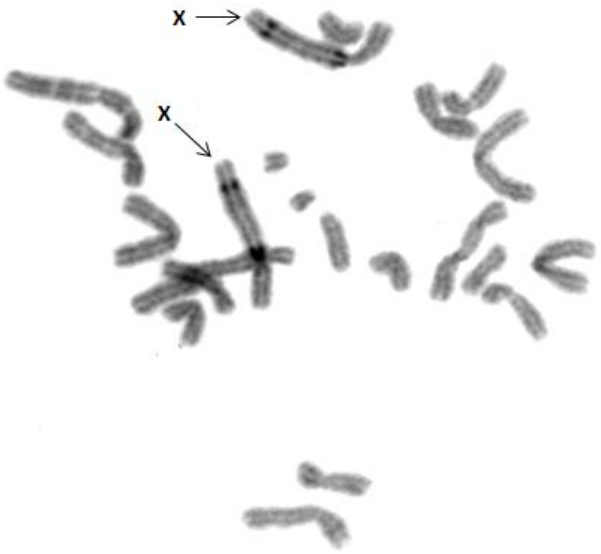

Figure 9-A: The C-banded metaphase spread of *T. pygargus* (2n = 22). The arrows indicate the position of the sex chromosomes.

Figure 10-A: The C-banded metaphase spread of the male outgroup, *M. namaquensis* (2n = 24). The arrows indicate the location of the sex chromosomes.