Appendices

Image on reverse: Australian Old Endemic rodent, *Leggadina lakedownensis.*
Modified image from Strahan, 2002

Appendix 1: Nucleotide sequence of exon 6, intron 6 and exon 7 of Zp3 from New Guinean and Australasian murine rodents. Exons are indicated in bold. Polymorphic sites are highlighted in grey.

```
                                  *   10    *   20    *   30    *   40    *   50
Mus musculus                : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus norvegicus           : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCCTTCAACAA
Lorentzimys nouhuysi        : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Anisomys imitator           : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Chiruromys vates            : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Coccymys ruemmleri          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Hyomys goliath              : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Macruromys major            : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Mallomys aroaensis          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Mallomys rothschildi        : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Mammelomys lanosus          : CCCGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Mammelomys rattoides        : CCCGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pogonomelomys mayeri        : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pogonomys macrourus         : CCAGCTAACCAGATCCCTGATAAGCTCAACAAAGCCTGTTCATACAACAA
Crossomys moncktoni         : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Hydromys chrysogaster       : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCNTTCAACAA
Parahydromys asper          : CCAGCTAACCAGATCCCCGATAAGCTTAACAAAGCCTGTTCGTTCAACAA
Leptomys elegans            : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudohydromys ellermani    : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCATTCAACAA
Xeromys myoides             : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCATTCAACAA
Melomys burtoni             : CCAGCTAACCAGATCCCCGATAAGCTCCACAAAGCCTGTTCGTTCAACAA
Melomys capensis            : CCAGCTAACCAGATCCCCGATAAGCTCCACAAAGCCTGTTCGTTCAACAA
Melomys cervinipes          : CCAGCTAACCAGATCCCCGATAAGCTCCACAAAGCCTGTTCGTTCAACAA
Melomys rubicola            : CCAGCTAACCAGATCCCCGATAAGCTCCACAAAGCCTGTTCGTTCAACAA
Melomys rufescens           : CCAGCTAACCAGATCCCCGATAAGCTCCACAAAGCCTGTTCGTTCAACAA
Paramelomys levipes         : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Paramelomys platyops        : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Paramelomys rubex           : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCNTTCAACAA
Solomys salebrosus          : CCAGCTAACCAGATCCCCGATAAGCTCCACAAAGCCTGTTCCTTCAACAA
Uromys anak                 : CCAGCTAACCAGATCCCCGATAAACTCAACAAAGCCTGTTCGTTCAACAA
Uromys caudimaculatus       : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Conilurus penicillatus      : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Leggadina forresti          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Leggadina lakedownensis     : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Leporillus conditor         : CCAGCTAACCAGATCCCCGACAAGCTCAACAAAGCCTGTTCGTTCAACAA
Mesembriomys gouldii        : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Mesembriomys macrurus       : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Mastacomys fuscus           : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCATTCAACAA
Notomys alexis              : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCATTCAACAA
Notomys aquilo              : CCAGCTAACCAGATCCCCGATAAGCTCAANAAAGCCTGTTCATTCAACAA
Notomys cervinus            : CCAGCTAGCCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Notomys fuscus              : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCATTCAACAA
Notomys mitchellii          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCATTCAACAA
Pseudomys albocinereus      : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys apodemoides       : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys australis         : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys bolami            : CCAGCTAACCAGATCCCCNATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys calabyi           : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys chapmani          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys delicatulus       : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys desertor          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys fieldi            : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys fumeus            : CCAGCTAACCAGATCCCTGATAAGCTCAACAAAGCCTGTTCATTCAACAA
Pseudomys gracilicaudatus   : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCTTTCAACAA
Pseudomys hermannsburgensis : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys higginsi          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys johnsoni          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys laborifex         : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTNAACAA
Pseudomys nanus             : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCTTTCAACAA
Pseudomys novaehollandiae   : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCTTTCAACAA
Pseudomys occidentalis      : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys oralis            : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys patrius           : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys pilligaensis      : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Pseudomys shortridgei       : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Zyzomys argurus             : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Zyzomys maini               : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Zyzomys palatilis           : CCAGCTAACCAGATCCCGGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Zyzomys pedunculatus        : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Zyzomys woodwardi           : CCAGTTAACCAGATCCCGGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
```

```
                                  *   60    *   70    *   80    *   90    *  100
Mus musculus                : GACTTCCCAGAGGTGAGG-AGACCAGGTGCC---TGTGTGTAGGCACCCG
Rattus norvegicus           : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCAGTGTGTGTAGGCACCCA
Lorentzimys nouhuysi        : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Anisomys imitator           : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCA
Chiruromys vates            : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTTGGTGTGTGTAGGCACCCG
Coccymys ruemmleri          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTNTGTAGGCACCCG
Hyomys goliath              : GACTTCCCAGAGGTAAGG-AGACCAGGCTCT----GTGTGTAGGCACCCG
Macruromys major            : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCA
Mallomys aroaensis          : GACTTCCCAGAGGTGAGG-AGACCAGGTTCTCGGTGTGTGTAGGCACCCG
Mallomys rothschildi        : GACTTCCCAGAGGTGAGG-AGACCAGGTTCTCGGTGTGTGTAGGCACCCG
Mammelomys lanosus          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Mammelomys rattoides        : GACTTCCCAGAGGTGAGGAAGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Pogonomelomys mayeri        : GACTTCCCAGAGGTAAGG-AGACCAGCCTCTCGGTGTGTGTAGGCACCCG
Pogonomys macrourus         : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCT
Crossomys moncktoni         : GACTTCCCAGAGGTGAGG-AGACCAGTCTCTCG--GTGTATAGGCAACCG
Hydromys chrysogaster       : GACTTCCCAGAGGTNAGG-AGACCAGGCTCTCG--GTGTATAGGCAACCN
Parahydromys asper          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCG--GTGTATAGGCAACCG
Leptomys elegans            : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCG--GTGTATAGGCAACCG
Pseudohydromys ellermani    : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCG--GTGTATAGGGAACCG
Xeromys myoides             : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCT--GTGTATAGGCAACCG
Melomys burtoni             : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Melomys capensis            : GACTTCCCAGAGGTGAGG-AGACCAGGCTCACGGTGTGTGTAGGCACCCN
Melomys cervinipes          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGAGTAGGCACCCG
Melomys rubicola            : GACTTCCCAGAGGTGAGG-AGACCAGGCTCACGGTGTGTGTAGGCACCCG
Melomys rufescens           : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGAGTAGGCACCCG
Paramelomys levipes         : GACTTCCCAGAGGTGA----GACCAGGCTCTCAGTGTG--TAGGCACCCG
Paramelomys platyops        : GACTTCCCAGAGGTGA----GACCAGGCTCTCAGTGTG--TAGGCACCGG
Paramelomys rubex           : GACTTCCCAGAGGTGA----GACCAGGCTCTCAGTGTG--TAGGCACCCG
Solomys salebrosus          : GACTTCCCAGAGGTGACG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Uromys anak                 : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCAGTGTGTGTAGGCACCCG
Uromys caudimaculatus       : GACTTCCCAGAGGTGAGGGAGACCAGGCTCTCAGTGTGTGTAGGCACCTG
Conilurus penicillatus      : GACTTCCCAGAGGTGAGG-AGACCAGGCTCACGGTGTGTGTAGGCACCTG
Leggadina forresti          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Leggadina lakedownensis     : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Leporillus conditor         : GACTTCCCAGAGGTGAGG-AGACCACGCTCACGGTGTGTGTAGGCACCCG
Mesembriomys gouldii        : GACTTCCCAGAGGTGAGG-AGACCAGGCTCACGGTGTGTGTAGGCACCCG
Mesembriomys macrurus       : GACTTCCCAGAGGTGAGG-AGACCAGGCTTACGGTGTGTGTAGGCACCCG
Mastacomys fuscus           : GACCTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTGGGCACCCA
Notomys alexis              : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTAGGCACCCG
Notomys aquilo              : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTAGGCACTCG
Notomys cervinus            : GACTTCCCAGAGGTGAGG-AGACCCGGNTCTCCGGGTGTGTGGGCACCCA
Notomys fuscus              : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTAGGCACCCG
Notomys mitchellii          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTAGGCACCCG
Pseudomys albocinereus      : GACTTCCCAGAGGTGAGG-AGACTAGGCTCTCC--------AGGCACCCG
Pseudomys apodemoides       : GACTTCCCAGAGGTGAGG-AGACTAGGCTCTCCGGGTGTATAGGCACCCG
Pseudomys australis         : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTGGGCACCCG
Pseudomys bolami            : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCNGGGTGTGTGGGCACCCG
Pseudomys calabyi           : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTAGGCACCCG
Pseudomys chapmani          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTAGGCACCCG
Pseudomys delicatulus       : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTGGGCACCNG
Pseudomys desertor          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGAGGTGTGTGGGCACCCG
Pseudomys fieldi            : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTGGGCACCCG
Pseudomys fumeus            : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCG--GTGTGTAGGCACCCG
Pseudomys gracilicaudatus   : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGCTGTGTGGGCACCCG
Pseudomys hermannsburgensis : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTGGGCACCCG
Pseudomys higginsi          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTGGGCACCCG
Pseudomys johnsoni          : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTAGGCACCCG
Pseudomys laborifex         : GACTTCCCNGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTAGGCACCCG
Pseudomys nanus             : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGCTGTGTGGGCACCCG
Pseudomys novaehollandiae   : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTGGGCACCCG
Pseudomys occidentalis      : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTGGGCACCCG
Pseudomys oralis            : GACTTCCCAGAGGTGAGG-AGACCNGGCTCTCGGGGTGTGTGGGCACCCG
Pseudomys patrius           : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTAGGCACCCG
Pseudomys pilligaensis      : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGCGTGTGGGCACCCG
Pseudomys shortridgei       : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGGGTGTGTGGGCACCCG
Zyzomys argurus             : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Zyzomys maini               : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Zyzomys palatilis           : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTATGTAGGCACCCG
Zyzomys pedunculatus        : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCCG
Zyzomys woodwardi           : GACTTCCCAGAGGTGAGG-AGACCAGGCTCTCGGTGTGTGTAGGCACCTG
```

```
                                  *  110    *  120    *  130    *  140    *  150
Mus musculus                : G-GGGCTACTCAAATTGA-TTTCT--TCAATTATACAATGGCAAACA---
Rattus norvegicus           : GAAGGCTATTCACATCGA-CCTCTCTTCAATAACACAATGGCAAA-----
Lorentzimys nouhuysi        : G-AGGCTATTCACATCAA-TTTATCTTCAATGATACAATGGCAAAC----
Anisomys imitator           : T-AGGCTATTCACATCAA-TTTCTCTTCAA--------------ACA---
Chiruromys vates            : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Coccymys ruemmleri          : G-AGGGTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Hyomys goliath              : G-AGGCTATTCACATCA--TTTCTCTTCAATTATACAATGGCAAACA---
Macruromys major            : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Mallomys aroaensis          : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Mallomys rothschildi        : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Mammelomys lanosus          : G-AGGCTATTCACATCAA-TTTCTCTTCAATTGTACAATGGCAAGCA---
Mammelomys rattoides        : G-AGGCTATTCACATCAA-TTTCTCTTCAATTGTACAATGGCAAACA---
Pogonomelomys mayeri        : G-AGGCTATTGTCATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pogonomys macrourus         : G-AAGCTATTCACATCGA-TTTCTCTTCAATTATACAATGGTGAACA---
Crossomys moncktoni         : G-AGGCTATTNACATCAA-TTTCTCTTCAATTATATGATGGCAAACA---
Hydromys chrysogaster       : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACGATGGCAAACA---
Parahydromys asper          : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACGATGGCAAACA---
Leptomys elegans            : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACGATGGCAAACA---
Pseudohydromys ellermani    : G-AGGCTATTCACATCAA-TTTCTCTTCAGTTATACGATGGCAAACA---
Xeromys myoides             : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACGATGGCAAACA---
Melomys burtoni             : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Melomys capensis            : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Melomys cervinipes          : G-AGGCTATTCACATCAA-TTTCTCTTCACTTATACAATGGCAAACC---
Melomys rubicola            : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Melomys rufescens           : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Paramelomys levipes         : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Paramelomys platyops        : G-AGGCTATTCACATCAA-TTTCTCTTCGATTATACAATGGCAAACC---
Paramelomys rubex           : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Solomys salebrosus          : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Uromys anak                 : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATANAGTGGCAAACA---
Uromys caudimaculatus       : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Conilurus penicillatus      : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Leggadina forresti          : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAACTGCAAACA---
Leggadina lakedownensis     : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAACGGCAAACA---
Leporillus conditor         : G-AGGCTATTCACATCAA-TTTCTCCTCAATTATACAATGGCAAACA---
Mesembriomys gouldii        : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Mesembriomys macrurus       : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Mastacomys fuscus           : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Notomys alexis              : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Notomys aquilo              : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Notomys cervinus            : G-AGGCTATTCACATCAA-TTTCTCTTCAATTACACAATGGCAAACA---
Notomys fuscus              : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACC---
Notomys mitchellii          : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATTCAATGGCAAACC---
Pseudomys albocinereus      : G-AGGCTATTCACATCAA-TTTCTCTTCAATTAGACAACGGCAAACA---
Pseudomys apodemoides       : G-AGGCTATTCACATCAA-TTTCTCTTCAATTAGACAACGGCAAACA---
Pseudomys australis         : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys bolami            : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCGAACA---
Pseudomys calabyi           : G-AGGCTATTCACATCAA-TTTCTCTACAATTATACAGTGGCAAACA---
Pseudomys chapmani          : G-AGGCTAGTCACATCAA-TTTCTCTTCAATTATACAACGGCAAACA---
Pseudomys delicatulus       : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCGAACA---
Pseudomys desertor          : G-AGGCTATTCTCAGCAA-TTTCTCT------------------------
Pseudomys fieldi            : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys fumeus            : G-AGGCTATTCATATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys gracilicaudatus   : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys hermannsburgensis : G-AGGCTATTCTC------------TTCAGTTATACAATGGCGAACA---
Pseudomys higginsi          : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys johnsoni          : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys laborifex         : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys nanus             : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys novaehollandiae   : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCGAACA---
Pseudomys occidentalis      : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCAAACAAAC
Pseudomys oralis            : G-AGGCTATTCTCAGCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys patrius           : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
Pseudomys pilligaensis      : G-AGGCTATTCTCNGCAA-TTTCTCTTCAATTATACAATGGCGAACA---
Pseudomys shortridgei       : G-AGGCTATTCTCAGCAA-TTTCTCT------------------------
Zyzomys argurus             : G-AGGCTATTCACATCA---------TCAATTATACAATGGCAAACT---
Zyzomys maini               : G-AGGCTATTCACATCAA-TTTCTCTTCAATTGTACAATGGCAAACA---
Zyzomys palatilis           : G-AGGCTATTCACATCAAATTTCTCTTCAACTATACAATGGCAAACT---
Zyzomys pedunculatus        : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACN---
Zyzomys woodwardi           : G-AGGCTATTCACATCAA-TTTCTCTTCAATTATACAATGGCAAACA---
```

```
                                  *  160    *  170    *  180    *  190    *  200
Mus musculus                 : -TCTTCCTGTCCTA-GCTGAGCTAAGT-AAGCTTTTT-GTCTTGTTACTC
Rattus norvegicus            : ---CATTGCCCCTA-TCTGAGCTTAATTAAGCCTTTT-GTCTTGTTACTC
Lorentzimys nouhuysi         : -TCTTCCTGACCTA-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Anisomys imitator            : -TCTTCCTGACCTA-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Chiruromys vates             : -TCTTCCTGACCTA-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Coccymys ruemmleri           : -TCTTCCTGACCTA-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Hyomys goliath               : -TCTTCCTGACCTA-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Macruromys major             : -TCTTCCCGACCTA-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Mallomys aroaensis           : -TCTTCCTGACCTA-TCT-----AAGT-AAACCTTTT-TTCTTGTTACTC
Mallomys rothschildi         : -TCTTCCTGACCTA-TCTGAGCTAAGT-AAACCTTTT-TTCTTGTTACTC
Mammelomys lanosus           : -TCTTCCTGACCTA-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Mammelomys rattoides         : -ACTTCCTGACCTA-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pogonomelomys mayeri         : -TCTTCCTGACCTA-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pogonomys macrourus          : -TCTTCCTGACCTT-CCTGAC-------ATATCTTTT-GTCTTGTTACTC
Crossomys moncktoni          : -TCTTCCTGACCTG-TCTGAGCTAAGC-AAGCCTTTT-GTCTTGTTACTC
Hydromys chrysogaster        : -TCTTCCTGACCTG-TCCGAGCTAAGC-AAGCCTTTT-GTCTTGTTACTC
Parahydromys asper           : -TCTTCCTGACCTG-TCCGAGCTAAGC-AAGCCTTTT-GTCTTGTTACTC
Leptomys elegans             : -TCTTCCTGACCTG-----AGCTAAGC-AAGCCTTTT-GTTTTGTTACTC
Pseudohydromys ellermani     : -TCTTCCTGACCTG-TCTGAGCTAAGC-AAGCCTTTTTGTCTTGTTACTC
Xeromys myoides              : -TCTTCCTGACCTG-TCTGAGCTAAAC-AAGCCTTTT-GTCTTGTTACTC
Melomys burtoni              : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCCTGTTACTC
Melomys capensis             : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Melomys cervinipes           : -TCTTCCTGACCTG-CCTGAGCTAAGT-AAGCCTTTT-GTCCTGTTACTC
Melomys rubicola             : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Melomys rufescens            : -TCTTCTTGACCTG-TCTGAGCTAAGT-AAACCTTTT-GTCCTGTTACTC
Paramelomys levipes          : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTTTGTCTTGTTACTC
Paramelomys platyops         : -TCTTTCTGACCTG-TCTGAGCTAAGT-AAGCCTTTTTGTCTTGTTACTC
Paramelomys rubex            : -TCTTCCTGACCTG-TCTGANCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Solomys salebrosus           : -TCTTCCTGACCTG-CCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Uromys anak                  : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Uromys caudimaculatus        : -TCTTCCTGACCT----------AAGT-AAGCCTTTT-GTCTTGTTACTC
Conilurus penicillatus       : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Leggadina forresti           : -TCTTCCTGACCCT-TCTGAGCTAAGT-ACGCCTTTT-GTCTTGTTATTC
Leggadina lakedownensis      : -TCTTCCTGACCCG-TCTGAGCNAAGT-ACGCCTTTT-GTCTTGTTATTC
Leporillus conditor          : -TCTTCCTGACCTG-TCTGAGCTAAGT-NAGCCTTTT-GTCTTGTTACTC
Mesembriomys gouldii         : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Mesembriomys macrurus        : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Mastacomys fuscus            : -TCTTCCTGACCCG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Notomys alexis               : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Notomys aquilo               : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGNCTTTT-GTCTTGTTACTC
Notomys cervinus             : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Notomys fuscus               : -TCTTNCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Notomys mitchellii           : -TCTTCCTGACCTG-TTTGAGCTAAGT-AAGCCTTTT-GTCATGTTACTC
Pseudomys albocinereus       : -TCTTCCTGACCTG-----AGCTCAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys apodemoides        : -TCTTCCTGACCTG-TCTGAGCTCAGT-AAGCCTTTT-GTCTTGTTACTN
Pseudomys australis          : -TCTTCCTGACCCG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys bolami             : -TCTTNCTGACCNG-NCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys calabyi            : -TCTTCCTGACCTG-TCTGAGCTAAGCCAAGCCTTTT-GTCTTGTTACTC
Pseudomys chapmani           : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys delicatulus        : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys desertor           : --------GACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys fieldi             : -TCTTCCTGACCCG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys fumeus             : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys gracilicaudatus    : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys hermannsburgensis  : -TCTTTCTGACCCG-ACTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys higginsi           : -TCTTCCTGACCCG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys johnsoni           : -TCTTCCTGACCTG-TCTGAGNTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys laborifex          : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys nanus              : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys novaehollandiae    : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys occidentalis       : ATCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys oralis             : -TCTTCCTGACCTG-TTTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys patrius            : -TCTTCCTGACCTG-TCT---------AAGCCTTTT-GTCTTGTTACTC
Pseudomys pilligaensis       : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Pseudomys shortridgei        : --------GACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Zyzomys argurus              : -TCTTCCTGACCTGGTCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Zyzomys maini                : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Zyzomys palatilis            : -TCTTCCTGACCTGGTCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
Zyzomys pedunculatus         : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTAGTTACTC
Zyzomys woodwardi            : -TCTTCCTGACCTG-TCTGAGCTAAGT-AAGCCTTTT-GTCTTGTTACTC
```

```
                                  *  210    *  220    *  230    *  240    *  250
Mus musculus                : AGTTGGTTGCCAGTAGAGGGTGATGCTGACATCTGTGATTGCTGCAGCCA
Rattus norvegicus           : AGTTGGTTGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCAA
Lorentzimys nouhuysi        : AGTTGGTTGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGTCA
Anisomys imitator           : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCAA
Chiruromys vates            : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Coccymys ruemmleri          : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Hyomys goliath              : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCAA
Macruromys major            : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCCA
Mallomys aroaensis          : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Mallomys rothschildi        : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Mammelomys lanosus          : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGC
Mammelomys rattoides        : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGC
Pogonomelomys mayeri        : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pogonomys macrourus         : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGGGA
Crossomys moncktoni         : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Hydromys chrysogaster       : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Parahydromys asper          : AGTTGGTCNCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Leptomys elegans            : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudohydromys ellermani    : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Xeromys myoides             : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Melomys burtoni             : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Melomys capensis            : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCANCGA
Melomys cervinipes          : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Melomys rubicola            : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Melomys rufescens           : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGAGA
Paramelomys levipes         : AGTTGGTCNCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGTGA
Paramelomys platyops        : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGTGA
Paramelomys rubex           : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGTGA
Solomys salebrosus          : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGAGA
Uromys anak                 : AGTTGGTCACCAGTAGAGGGCAGTGCTGACATCTGTGATTGCTGCATCGA
Uromys caudimaculatus       : AGTTGGTCACCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCATCGA
Conilurus penicillatus      : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCATCGA
Leggadina forresti          : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Leggadina lakedownensis     : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Leporillus conditor         : AGTTGGTCACCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCATCGA
Mesembriomys gouldii        : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCACGGA
Mesembriomys macrurus       : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCACGGA
Mastacomys fuscus           : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Notomys alexis              : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Notomys aquilo              : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Notomys cervinus            : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Notomys fuscus              : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Notomys mitchellii          : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys albocinereus      : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys apodemoides       : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys australis         : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys bolami            : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys calabyi           : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys chapmani          : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys delicatulus       : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys desertor          : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys fieldi            : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCAA
Pseudomys fumeus            : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys gracilicaudatus   : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys hermannsburgensis : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys higginsi          : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys johnsoni          : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys laborifex         : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys nanus             : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys novaehollandiae   : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys occidentalis      : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys oralis            : AGCTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys patrius           : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys pilligaensis      : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Pseudomys shortridgei       : AGTTGGTGGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Zyzomys argurus             : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Zyzomys maini               : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Zyzomys palatilis           : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
Zyzomys pedunculatus        : AGTTGGTCGCCAGTAGAGGGCGATGCTAACATCTGTGATTGCTGCAGCGA
Zyzomys woodwardi           : AGTTGGTCGCCAGTAGAGGGCGATGCTGACATCTGTGATTGCTGCAGCGA
```

```
                                  *  260    *  270    *  280    *  290    *  300
Mus musculus                : TGGCAACTGTAGTAATTCAAGCTCTTCACAGTTCCAGATCCATGGACCCC
Rattus norvegicus           : TGGCAACTGTAGTAATTCAAGCTCTTCAGAGTTCGAGACCCATGAACCAG
Lorentzimys nouhuysi        : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTTCCAGATCCATGGACCCC
Anisomys imitator           : CGGCAACTGTAGTAATTCAAGTTCTTCATGGTTCCAGATCCATGGACCCC
Chiruromys vates            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTTCCGGATCCATGGAGCCC
Coccymys ruemmleri          : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTTCCAGATCCATGGACCCC
Hyomys goliath              : TGGCAACTGTAGTAATTCAAGTTCTTCGTGGTTCCAGATCCATGGACCCC
Macruromys major            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTTCCAGATCCATGGACTCC
Mallomys aroaensis          : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTTCCAGATCCATGGACCCC
Mallomys rothschildi        : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTTCCAGATCCATGGACCCC
Mammelomys lanosus          : TGGCAACTGCAGTAATTCCAGTTCTTCGTGGCTCCAGATCCATGGACCCC
Mammelomys rattoides        : TGGCAACTGCAGTAATTCCAGTTCTTCGTGGCTCCAGATCCATGGACCCC
Pogonomelomys mayeri        : TGGCAACTGCAGTAATTCAAGTTCTTCATGGTTCCAGATCCATGGACTCC
Pogonomys macrourus         : GGGCAACTGTAGTAATTCAAGTTCTTCATGGTTCCAGATCCATGGACCCC
Crossomys moncktoni         : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCTC
Hydromys chrysogaster       : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Parahydromys asper          : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Leptomys elegans            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudohydromys ellermani    : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Xeromys myoides             : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Melomys burtoni             : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGAGCCC
Melomys capensis            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAAATCCATGGAGCCC
Melomys cervinipes          : TGGCAACTGTAGTAATTCAAGTTCTTCANGGTCCCGGATCCATGGAGCCC
Melomys rubicola            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Melomys rufescens           : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Paramelomys levipes         : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Paramelomys platyops        : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Paramelomys rubex           : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCT
Solomys salebrosus          : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Uromys anak                 : TGGCAATTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCACGGATCCC
Uromys caudimaculatus       : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Conilurus penicillatus      : TGGCAACTGTAGTAATTCAAGTTCTACATGGTCCCAGATCCATGGATCCC
Leggadina forresti          : CGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCACGGATCCC
Leggadina lakedownensis     : CGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCACGGATCCC
Leporillus conditor         : TGGCAACTGTAGCAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Mesembriomys gouldii        : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Mesembriomys macrurus       : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Mastacomys fuscus           : TGGCAACTGTAGTAATTCAAGTTCTTCGTGGTCCCAGATCCATGGATCCC
Notomys alexis              : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Notomys aquilo              : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Notomys cervinus            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Notomys fuscus              : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Notomys mitchellii          : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys albocinereus      : TGGCAACTGTAGTAATTTGAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys apodemoides       : TGGCAACTGTAGTAATTTGAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys australis         : TGGCAACTGTAGTAATTCAAGTTCTTCNTGGTCCCAGATCCATGGATCCC
Pseudomys bolami            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys calabyi           : TGGCAACTGTAGTAATTCAAGTTCTCCATGGTCCCAGATCCATGGATCCC
Pseudomys chapmani          : TGGCAACTGTAGTAATTCAAGTTCTCCATGGTCCCAGATCCATGGATCCC
Pseudomys delicatulus       : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys desertor          : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys fieldi            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCGC
Pseudomys fumeus            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys gracilicaudatus   : TGGCAACTGTAGTAATTCAAGTTCTTCGTGGTCCCAGATCCATGGATCCC
Pseudomys hermannsburgensis : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys higginsi          : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys johnsoni          : CGGCAACTGTAGTAATTCAAGTTCTCCATGGTCCCAGATCCATGGATCCC
Pseudomys laborifex         : CGGCAACTGTAGTAATTCAAGTTCTCCATGGTCCCAGATCCATGGATCCC
Pseudomys nanus             : TGGCAACTGTAGTAATTCAAGTTCTTCGTGGTCCCAGATCCATGGATCCC
Pseudomys novaehollandiae   : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys occidentalis      : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys oralis            : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys patrius           : CGGCAACTGTAGTAATTCAAGTTCTCCATGGTCCCAGATCCATGGATCCC
Pseudomys pilligaensis      : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Pseudomys shortridgei       : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Zyzomys argurus             : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Zyzomys maini               : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Zyzomys palatilis           : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATCCATGGATCCC
Zyzomys pedunculatus        : TGGCAACTGTAGTAATTCAAGTTCTTCATGGTCCCAGATTCATGGATCCC
Zyzomys woodwardi           : TGGCAATTGTAGTAATTCAAGTTCTTCGTGGTCCCAGATCCATGGATCCC
```

```
                                  *  310
Mus musculus                : GCCAGTGGTCCAA
Rattus norvegicus           : CCCAGTGGTCCAA
Lorentzimys nouhuysi        : CCCAGAGGTCCAA
Anisomys imitator           : CCCAGAGGTCCAA
Chiruromys vates            : CCCAGAGGTCCAA
Coccymys ruemmleri          : CCCAGAGGTCCAA
Hyomys goliath              : CCCAGAGGTCCAA
Macruromys major            : CCCAGAGGTCCAA
Mallomys aroaensis          : CCCAGAGGTCCAA
Mallomys rothschildi        : CCCAGAGGTCCAA
Mammelomys lanosus          : CCCAGAGGTCCAA
Mammelomys rattoides        : CCCAGAGGTCCAA
Pogonomelomys mayeri        : CCCAGAGGTCCAA
Pogonomys macrourus         : CCCAGAGGTCCAA
Crossomys moncktoni         : CCCAGAGGTCCAA
Hydromys chrysogaster       : CCCAGAGGTCCAA
Parahydromys asper          : CCCAGAGGTCCAG
Leptomys elegans            : CCCAGAGGTCCAA
Pseudohydromys ellermani    : CCCAGAGGTCCAA
Xeromys myoides             : CCCAGAGGTCCAA
Melomys burtoni             : CCCAGAGGTCCAA
Melomys capensis            : CCCAGAGGTCCAA
Melomys cervinipes          : CCCAGAGGTCCAA
Melomys rubicola            : CCCAGAGGTCCAA
Melomys rufescens           : CCCAGAGGTCCAA
Paramelomys levipes         : CCCAAAGGTCCAA
Paramelomys platyops        : CCCAGAGGTCCAA
Paramelomys rubex           : CCCAGAGGTCCAA
Solomys salebrosus          : CCCAGAGGTCCAA
Uromys anak                 : CCCAGAGGTCCAA
Uromys caudimaculatus       : CCCAGAGGTCCAA
Conilurus penicillatus      : CCCAGAGGTCCAA
Leggadina forresti          : CCCAGAGGTCCAA
Leggadina lakedownensis     : CCCAGAGGTCCAA
Leporillus conditor         : CCCAGAGGTCCAA
Mesembriomys gouldii        : CCCAGAGGTCCAA
Mesembriomys macrurus       : CCCAGAGGTCCAA
Mastacomys fuscus           : CCCAGAGGTCCAA
Notomys alexis              : CCCAGAGGTCCAA
Notomys aquilo              : CCCAGAGGTCCAA
Notomys cervinus            : CCCAGAGGTCCAA
Notomys fuscus              : CCCAGAGGTCCAA
Notomys mitchellii          : CCCAGAGGTCCAA
Pseudomys albocinereus      : CCCAGAGGTCCAA
Pseudomys apodemoides       : CCCAGAGGTCCAA
Pseudomys australis         : CCCAGAGGTCCAA
Pseudomys bolami            : CCCAGAGGTCCAA
Pseudomys calabyi           : CCCAGAGGTCCAA
Pseudomys chapmani          : CCCAGAGGTCCAA
Pseudomys delicatulus       : CCCAGAGGTCCAA
Pseudomys desertor          : CCCAGAGGTCCAA
Pseudomys fieldi            : CCCAGAGGTCCAA
Pseudomys fumeus            : CCCAGAGGTCCAA
Pseudomys gracilicaudatus   : CCCAGAGGTCCAA
Pseudomys hermannsburgensis : CCCAGAGGTCCAA
Pseudomys higginsi          : CCCAGAGGTCCAA
Pseudomys johnsoni          : CCCAGAGGTCCAA
Pseudomys laborifex         : CCCAGAGGTCCAA
Pseudomys nanus             : CCCAGAGGTCCAA
Pseudomys novaehollandiae   : CCCAGAGGTCCAA
Pseudomys occidentalis      : CCCAGAGGTCCAA
Pseudomys oralis            : CCCAGAGGTCCAA
Pseudomys patrius           : CCCAGAGGTCCAA
Pseudomys pilligaensis      : CCCAGAGGTCCAA
Pseudomys shortridgei       : CCCAGAGGTCCAA
Zyzomys argurus             : CCCAGAGGTACAA
Zyzomys maini               : CCCAGAGGTCCAA
Zyzomys palatilis           : CCCAGAGGTCCAA
Zyzomys pedunculatus        : CCCAGAGGTCCAA
Zyzomys woodwardi           : CCCAGAGGTTCAA
```

Appendix 2: Nucleotide sequence of exon 6, intron 6 and exon 7 of *Zp3* from African, Eurasian and South-east Asian murine rodents. Exons are indicated in bold. Polymorphic sites are highlighted in grey.

```
                               *    10    *    20    *    30    *    40    *    50
Mus musculus              : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus norvegicus         : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCCTTCAACAA
Dasymys incomtus          : CCAGCTAACCAGATTCCCGATAAGCTCAACAAAGCCTGTTCGTTTAACAA
Micaelamys namaquensis    : GCAGCTAGCCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Aethomys chrysophilus     : CCANCTAACCTGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Aethomys ineptus          : CCAGCTAACCTGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Lemniscomys griselda      : CCAGCTAACCAGATCCCNGATAAGGTCAACAAAGCCTGTTCGTTCAACAA
Rhabdomys pumilio         : CCAGCTGACCAGATCCCCGATAAGGTCAACAAAGCCTGTTCGTTCAACAA
Hylomyscus alleni         : CCGGCTAACCAGATCCCCGACAAGCTCAACAAAGCCTGTTCATTCAACAA
Mastomys natalensis       : CCAGCTAACCAGATCCCTGACAAACTTAACAAAGCCTGTTCATTCAACAA
Apodemus chevrieri        : CCTGCTAACCAGATCCCGGNCAAGCTCAACAAAGCCTGTTCGTTCAACAA
Leopoldamys edwardsi      : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Leopoldamys sabanus       : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Niviventer fulvescens     : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Maxomys bartelsii         : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCATTCAACAA
Maxomys hellwaldii        : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCCTTCAACAA
Bandicota indica          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Bunomys andrewsi          : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAG
Paruromys dominator       : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCATTCAACAA
Rattus exulans            : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus steini             : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus niobe              : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus verecundus         : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus mordax             : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus praetor            : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus leucopus           : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus fuscipes           : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus lutreolus          : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus sordidus           : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus tunneyi            : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus villosissimus      : CCNGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA


                               *    60    *    70    *    80    *    90    *   100
Mus musculus              : GACTTCCCAGAGGTGAGGAGACCAG-----GTGCC-----TGTG-TGTAG
Rattus norvegicus         : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Dasymys incomtus          : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--GGTGTG-CATAG
Micaelamys namaquensis    : GACGTCCCAGAGGTGAGGAGACCAG-----GTGCTC--GGTGTG-TGTAG
Aethomys chrysophilus     : GACTTCCCAGAGGTGAGGAGACCAGACGAGGCTCTC--GGTGTG-TGTAG
Aethomys ineptus          : GACTTCCCAGAGGTGAGGAGACCAGACGAGGCTCTC--GGTGTG-TGTAG
Lemniscomys griselda      : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC----GGTG-TGTGG
Rhabdomys pumilio         : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC----AATA-TGTGG
Hylomyscus alleni         : GACCTCCCAGAGGTGAGGAGACCAG-----GCTTTG----CGTG-CGTGG
Mastomys natalensis       : GACTTCGCAGAGGTGAGGAGACCAG-----GCTTTG----TGTG-TGTGG
Apodemus chevrieri        : GACTTCGCAGAGGTGAGGAGACCAG-----GCTCTCTTGGAGTG-TGTAG
Leopoldamys edwardsi      : GACTTCCCAGAGGTGAGGAGACCAG-----GCCCTC--GGTGCGGTGTGT
Leopoldamys sabanus       : GACTTCCCACAGGTGAGGAGACCAG-----GCCCTC--GGTGCGGTGTGG
Niviventer fulvescens     : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--GGTGTG-TGTAG
Maxomys bartelsii         : GACTTCCCAGAGGTGAGGAGACCAG-----ACTCTC--GGTGTG-TGTAG
Maxomys hellwaldii        : GACTTCCCAGAGGTGAGNAGACCAG-----GCTCTC--AGTGTG-TGTAG
Bandicota indica          : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Bunomys andrewsi          : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Paruromys dominator       : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus exulans            : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTCTCAGTGTG-TGTAG
Rattus steini             : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus niobe              : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus verecundus         : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus mordax             : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus praetor            : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--TGTGTG-TGTAG
Rattus leucopus           : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus fuscipes           : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus lutreolus          : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus sordidus           : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus tunneyi            : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
Rattus villosissimus      : GACTTCCCAGAGGTGAGGAGACCAG-----GCTCTC--AGTGTG-TGTAG
```

```
                             *  110    *  120    *  130    *  140    *  150
Mus musculus           : ----GCACCCGG-GGGCTACTCAAATTGATTTCT--TCAATTA---TACA
Rattus norvegicus      : ----GCACCCAGAAGGCTATTCACATCGACCTCTCTTCAATAA---CACA
Dasymys incomtus       : ----GCACCCGG-AGGCTACTCAC-TCGATTTCTCTTCAATTA---TACA
Micaelamys namaquensis : ----GCACCGGG-AGGCTACTCAC-TCGATTTCTCTTCAATTA---TACA
Aethomys chrysophilus  : ----GCACNCGG-AGGCTACTCAC-TCNATTTCTTTTCAAT-A---TNCA
Aethomys ineptus       : ----GCACGCGG-AGGCTACTCAC-TCGATTTCTTTTCAAT-A---TACA
Lemniscomys griselda   : ----GCACCCGG-TGGCTACTCAC-TCGATTTCTCTTCAATTA---TANA
Rhabdomys pumilio      : ----GCACCCCG-TGGCTCCTCCC-CCATTTTCTCTTCAATTA---TACA
Hylomyscus alleni      : ----GCACCCGG-AGGCTATTCACAGCGATTTCTCTTCGAGCA---CACG
Mastomys natalensis    : ----GCACCCGG-AGGCTATTCACATCGATTTCTCTTCGATTA---CACA
Apodemus chevrieri     : ----GCACCCGG-AGGCTATTCACATCCATTTCTCTTCAATTA---TACA
Leopoldamys edwardsi   : GTAAGCACCCCG--------TCANGTCCACCTCTCTTCAATAA---CGCA
Leopoldamys sabanus    : GTAAGCACCCTG--------TCACATCCACCTCTCTTCAATGA---CGCA
Niviventer fulvescens  : ----GCACCGGG AGGCCCGTCACATCCACCTCTCTTCAATAT--ACACA
Maxomys bartelsii      : ----GCACTGGG-AGGTTATTCACATCAACCTCTTTTCAATAA---TACA
Maxomys hellwaldii     : ----GCACCCAGAAGGCTATTCACANCGACCTCTCTTCAATAA---CACA
Bandicota indica       : ----GCACACGGGAGGCTATTCACATCAACCTCTCTTCAATAA---CACA
Bunomys andrewsi       : ----GCACCCTGGAGGCTATTCACATCGACCTCTCTTCAATAA---CACA
Paruromys dominator    : ----GCACCCTGGAGGCTATTCACATCCACCTCTCTTCAATAA---CACA
Rattus exulans         : ----GCACCCGGGAGGCTATTCACATCAACCTCT--TCAATAA---CACA
Rattus steini          : ----GCACCCGGGAGGTTATTCACATCGACCTCTCTTCAATAATAACACA
Rattus niobe           : ----GCACCNGGGAGGTTATTCACATCGACCTCTCTTCAATAATAACACA
Rattus verecundus      : ----GCACCCGGGAGGTTATTCACATCGACCTCTCTTCAATAATAACACA
Rattus mordax          : ----GCACCCGGGAGGTTATTCACATCGACCTCTCTTCAATAATAACACA
Rattus praetor         : ----GCACCCGGGAGGTTATTCACATAGACCTCTCTTCAATAATAACACA
Rattus leucopus        : ----GCACCCGGGAAGTTATTCACATCGACCTCTCTTCAATAATAACACA
Rattus fuscipes        : ----GCACCCGGGAGGTTATTCACATCGACCTCTCTTCAATAATAACACA
Rattus lutreolus       : ----GCACCCGGGAGGTTACTCACATCGACCTCTCTTCAATAATAACACA
Rattus sordidus        : ----GCACCCGGGAGGTTATTCACATCGACCTCTCTTCAATAATAACACA
Rattus tunneyi         : ----GCACCCGGGAGGTTATTCACATCGACCTCTCTTCAATAATAACACA
Rattus villosissimus   : ----GCACCCGGGAGGTTATTCACATCGACCTCTCTTCAATAATAACACA


                             *  160    *  170    *  180    *  190    *  200
Mus musculus           : ATGGC--AAACATCTTCCTGTCC---TAGCTGAGCT-AAG-TAAGCT-TT
Rattus norvegicus      : ATGGC--AAACA----TTGCCCC---TATCTGAGCTTAA-TTAAGCC-TT
Dasymys incomtus       : ATGGC--AAACATCTTCCTGTCC---TATCTGAACT-AAGTTAAGCC-TT
Micaelamys namaquensis : ATGGC--AAACATCTTCCTGTCC---TATCTGAGCT-AAGCTAAGCC-TT
Aethomys chrysophilus  : ATGGC-TAAACATCTTCCTGTCC---TATCTGAGCT-AAGTTAAGCC-TT
Aethomys ineptus       : ATGGC-TAAACATCTTCCTGTCC---TATCTGAGCT-AAGTTAAACC-TT
Lemniscomys griselda   : GTGGC--AAACATCTTCCTGTCT---TATCTGAGCT-AAGTTAGGCC-TT
Rhabdomys pumilio      : ANGGC--AAACATCTTCCTGTCT---TATCT------AAGTTAAGCC-TT
Hylomyscus alleni      : ATGGC--AAACATCTTCCTGACC---TTTCTGAGCT-AAGT-AAGCT-TT
Mastomys natalensis    : ATGGC—-AAACT----TCTGTCC---TTTCTGAGCT-AAGT-AAGCTTTT
Apodemus chevrieri     : ATGGC-AAAACCTCTTCCTGTCC---TATCTGAGCT-AAGA-AAGCC-TT
Leopoldamys edwardsi   : GTGGC--AAACG----CTGCCTC---TATCTGAGCT-AA-TTAAGCC-TT
Leopoldamys sabanus    : GTGGC--AAACG----CTGCCTC---TATCNGAGCT-AA-TTAAGCC-TT
Niviventer fulvescens  : GTGGC--AAACA----TTGCCTC---TATCTGAGCT-AA-TTAAGCC-TT
Maxomys bartelsii      : ATGGC--AAACATCTTCCTCTCCTCCTATCTGAGCT-AA-GTAAGCC-TT
Maxomys hellwaldii     : ATGGC--AAACA----TTGCCCC---TATCTGAGCTTAA-TTAAGCC-TT
Bandicota indica       : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Bunomys andrewsi       : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Paruromys dominator    : ATGGCAAAAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus exulans         : ATAGC--AAACC----TTGCCCC---TAGCTGAGCT-AA-TTAAGCC-TT
Rattus steini          : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus niobe           : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus verecundus      : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus mordax          : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus praetor         : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus leucopus        : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus fuscipes        : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus lutreolus       : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus sordidus        : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus tunneyi         : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
Rattus villosissimus   : ATGGC--AAACA----TTGCCCC---TATCTGAGCT-AA-TTAAGCC-TT
```

```
                             *  210    *  220    *  230    *  240    *  250
Mus musculus           : TTGTCTTGTT---ACTCAGTTGGTTGCCAGTAGAGGGTGATGCTGACATC
Rattus norvegicus      : TTGTCTTGTT---ACTCAGTTGGTTGCCAGTAGAGGGCGATGCTGACATC
Dasymys incomtus       : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Micaelamys namaquensis : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGGCATC
Aethomys chrysophilus  : TTGTCTTGTT---ACTCAGTTGGTTGCCAGTAGAGGGCGATGCTGACATC
Aethomys ineptus       : TTGTCTTGTT---ACTCAGTTGGTTGCCAGTAGAGGGCGATGCTGACATC
Lemniscomys griselda   : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rhabdomys pumilio      : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Hylomyscus alleni      : TTGTCTTGTTT--ACTCAGTTGGCTACCGGTAGAGGGCGATGCTGACATC
Mastomys natalensis    : TTGTCTTGTT---ACTCAGTTGGTTACCAGTAGAGGGCGATGCTGACATC
Apodemus chevrieri     : TTGTCTTGTT---ATTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Leopoldamys edwardsi   : TTGTCTTGTT---ACTCAGTTGGTTGCCAGTAGAGGGCGATGCTGACATC
Leopoldamys sabanus    : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Niviventer fulvescens  : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Maxomys bartelsii      : TTGTCTTGTT---ACTCAGTTGGTCACCAGTAGAGGGCGATGCTGACATC
Maxomys hellwaldii     : TTGTCTTGTT---ACTCAGTTGGTTGCCAGTAGAGGGCGATGCTGACATC
Bandicota indica       : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Bunomys andrewsi       : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Paruromys dominator    : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rattus exulans         : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rattus steini          : TTGTCTTGTT---ACTCAGTTGGTTGCCAGTAGAGGGCGATGCTGACATC
Rattus niobe           : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rattus verecundus      : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rattus mordax          : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rattus praetor         : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rattus leucopus        : TTGTCTTGTTGTTACTCAGTTGGTTGCCAGTAGAGGGCGATGCTGACATC
Rattus fuscipes        : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rattus lutreolus       : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rattus sordidus        : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC
Rattus tunneyi         : TTGTCTTGTT---ACTCAGTTGGTNGCCAGTAGAGGGCGATGCTGACATC
Rattus villosissimus   : TTGTCTTGTT---ACTCAGTTGGTCGCCAGTAGAGGGCGATGCTGACATC

                             *  260    *  270    *  280    *  290    *  300
Mus musculus           : TGTGATTGCTGCAGCCATGGCAACTGTAGTAATTCAAGCTCTTCACAGTT
Rattus norvegicus      : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Dasymys incomtus       : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCACAGTT
Micaelamys namaquensis : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCACAGTT
Aethomys chrysophilus  : TGTGACTGCTGCAGCGATGGCAACTGTAGTAATTCAAGCTCTTCACAGTT
Aethomys ineptus       : TGTGACTGCTGCAGCGATGGCAACTGTAGTAATTCAAGCTCTTCACAGTT
Lemniscomys griselda   : TGTGATTGCTGCAGCGATGGCAATTGTAGTAATTCAAGCTCTTCACAG--
Rhabdomys pumilio      : TGTGATTGCTGCACCGATGGCAACTGTAGTAATTCAAGCTCTTCACAGTT
Hylomyscus alleni      : TGTGATTGCTGCAGCCATAGCAACTGTAGTAATTCAAGCTCTTCATGGTT
Mastomys natalensis    : TGTGATTGCTGCAGCCACGCCAACTGTAGTAATTCAAGCTCTTCACAGTT
Apodemus chevrieri     : TGTGATTGCTGCAGCCATGGCAACTGTAGTAATTCAAGCTCCTCACTGTT
Leopoldamys edwardsi   : TGTGATTGCTGCAGCCATGGCAACTGTAGTAATTCAAGCTCTTCACAGTT
Leopoldamys sabanus    : TGTGATTGCTGCAGCCATGGCAACTGTAGTAATTCAAGCTCTTCACAGTT
Niviventer fulvescens  : TGTGATTGCTGCAGCCATGGCAACTGTAGTAATTCAAGCTCTTCACGGTT
Maxomys bartelsii      : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCACAGTT
Maxomys hellwaldii     : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Bandicota indica       : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Bunomys andrewsi       : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Paruromys dominator    : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus exulans         : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus steini          : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus niobe           : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus verecundus      : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus mordax          : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus praetor         : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus leucopus        : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCCAGCTCTTCAGAGTT
Rattus fuscipes        : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus lutreolus       : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus sordidus        : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus tunneyi         : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
Rattus villosissimus   : TGTGATTGCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTT
```

```
                             *  310    *  320    *  330
Mus musculus           : CCAGATCCAT---GGA---CCCCGCCAGTGGTCC
Rattus norvegicus      : CGAGACCCAT---GAA---CCAGCCCAGTGGTCC
Dasymys incomtus       : CCTGATCCAT---GGA---CCCCACCAGTGGTCC
Micaelamys namaquensis : CCAGATCCAC---GGA---CCCCACCAGTGGTCC
Aethomys chrysophilus  : CCAGATCCAT---GGA---CCCCGCCAGTGGTCC
Aethomys ineptus       : CCAGATCCAT---GGA---CCCCGCCAGTGGTCC
Lemniscomys griselda   : ----ACCCAT---GGA---CTCTACCAGTGGTCC
Rhabdomys pumilio      : CCAGATCCAC---AGA---CCCTACCAGTGGTCC
Hylomyscus alleni      : CCAGATCCAT---GGG---CCCTACCAGTGGTCC
Mastomys natalensis    : CCTGATCCAC---GGA---CCCTACCAGTGGTCC
Apodemus chevrieri     : CAAGATCCAT---GGA---ACCCCCCAGTGGTCC
Leopoldamys edwardsi   : CCAGATCCAT---GGA---CCAGGCCAATGGTCC
Leopoldamys sabanus    : CCAGATCCAT---GGA---CCAGGCCAGTGGTCC
Niviventer fulvescens  : CCAGATCCAT---GGA---CCANGCCAGTGGTCC
Maxomys bartelsii      : CCAGATCCAT---GGA---CCAGGCCAGTGGTCT
Maxomys hellwaldii     : CGAGACCCAT---GGATCATCAGGCCAGTGGTCC
Bandicota indica       : CCAGATCCAT---GAA---CCAGGCCAGTGGTCC
Bunomys andrewsi       : CCAGATCCAT---GAA---CCAGGCCAGTGGTCC
Paruromys dominator    : CCAGATCCAT---GAA---CCAGGCCAGTGGTCC
Rattus exulans         : CGAGACCCAT---GGA---CCAAACCAGTGGTCC
Rattus steini          : CCAGATCCAT---GAA---CAAGGCCAATGGTCC
Rattus niobe           : CCAGATCCAT---GAA---CAAGGCCAGTGGTCC
Rattus verecundus      : CCAGATCCAT---GAA---CAAGGCCAGTGGTCC
Rattus mordax          : CCAGATCCAT---GAA---CAAAGCCAGTGGTCC
Rattus praetor         : CCAGATCCAT---GAA---CAAAGCCAGTGGTCC
Rattus leucopus        : CCAGATCCAT---GAA---CAAGGCCAGTGGTCC
Rattus fuscipes        : CCAGATCCATGATGAA---CAAGGCCAGTGGTCC
Rattus lutreolus       : CCAGATCCATGATGAA---CAAGGCCAGTGGTCC
Rattus sordidus        : CCAGATCCATGATGAA---CAAGGCCAGTGGTCC
Rattus tunneyi         : CCAGATCCATGATGGA---CAAGGCCAGTGGTCC
Rattus villosissimus   : CCAGATCCATGATGAA---CAAGGCCAGTGGTCC
```

Appendix 3: Nucleotide (cDNA) sequence for exon 6 and exon 7 of Zp3 from selected species of mammals. The exon 6/7 boundary is indicated with a line.

```
                            *   10    *   20    *   30    *   40    *   50
Mus musculus          : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCGTTCAACAA
Rattus norvegicus     : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCCTTCAACAA
Rattus rattus         : CCAGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCCTTCAACAA
Rattus tanezumi       : CCGGCTAACCAGATCCCCGATAAGCTCAACAAAGCCTGTTCCTTCAACAA
Lasiopodomys brandtii : CCAGCCAACCAGACCCCAGATAAGCTCAACAAAGCCTGTTCCTTTAACAG
Lagurus lagurus       : CCAGCCAACCAGACCCCAGATAAGCTCAACAAAGCCTGTTCCTTCAACAG
Mesocricetus auratus  : CCAGCCAACCAGACCCCAGATGAGCTCAACAAAGCCTGCTCCTTCAACAG
Onychomys torridus    : CCAGCCAACCAGACCCCAGATGAGCTCAACAAAGCCTGTTCCTACAACAG
Peromyscus polionotus : CCAGCAAACCAGACCCCAGATGAGCTCAATAAAGCCTGCTCCTACAACAG
Homo sapiens          : CTAGCTGAGCAGGACCCAGATGAACTCAACAAGGCCTGTTCCTTCAGCAA
Pan troglodytes       : CTAGCTGAGCAGGACCCAGATGAACTCAACAAAGCCTGTTCCTTCAGCAA
Macaca fascicularis   : CCAGCTGAGCAGGAACCAGACGAACTCAACAAAGCCTGTTCCTTCAGCAA
Macaca mulatta        : CCAGCTGAGCAGGAACCAGACGAACTCAACAAAGCCTGTTCCTTCAGCAA
Macaca radiata        : CCAGCTGAGCAGGAACCAGATGAACTCAACAAAGCCTGTTCCTTCAGCAA
Callithrix jacchus    : CTAGCTGAGCAGGACCCAGATGAACTGAACAAAGCCTGTTCCTTCAGCAA
Canis lupus           : CCGGCTGACCGAGTCCCAGACCAGCTAAACAAAGCTTGTTCCTTCATCAA
Vulpes vulpes         : CCGGCTGACCGAGTCCCAGACCAGCTAAACAAAGCGTGTTCCTTCATCAA
Mustela erminea       : CTGGCAGACCGAGTCCCGGACCAGCTAAACAAAGCCTGTTCCTTTATCAA
Mustela putorius      : CTGGCAGACCGAGTCCCGGACCAGCTAAACAAAGCCTGTTCCTTTATCAA
Felis catus           : CCAGCTAGCCGAGTCCCGGACCAGCTAAACAAAGCCTGTTCCTTCATCAA

                            *   60 |  *   70    *   80    *   90    *  100
Mus musculus          : GACTTCCCAGAG |TTGGTTGCCAGTAGAGGGTGATGCTGACATCTGTGATT
Rattus norvegicus     : GACTTCCCAGAG |TTGGTTGCCAGTAGAGGGCGATGCTGACATCTGTGATT
Rattus rattus         : GACTTCCCAGAG |TTGGTTGCCAGTAGAGGGCGATGCTGACATCTGTGATT
Rattus tanezumi       : GACTTCCCAGAG |TTGGTTGCCAGTAGAGGGCGATGCTGACATCTGTGATT
Lasiopodomys brandtii : GACTTCCAAGAG |CTGGCTGCCAGTAGAGGGCGATACTGATGTCTGTGACT
Lagurus lagurus       : GACTTCCAAGAG |TTGGCAGCCAGTAGAGGGCGATGCTGACGTCTGTGACT
Mesocricetus auratus  : GTCTTCCAAGAG |TTGGTCGCCAGTAGAGGGCGATGCTGAGGTCTGCGGCT
Onychomys torridus    : GACTTCCAATAT |CTGGCTGCCAGTAGAGGGCGACGCTGCCATCTGTGACT
Peromyscus polionotus : GACTTCCAATAG |CTGGCTGCCAGTAGAGGGCGACACTGCCATCTGTGACT
Homo sapiens          : GCCTTCCAACAG |CTGGTTCCCAGTGGAAGGCTCGGCTGACATCTGTCAAT
Pan troglodytes       : GCCTTCCAACAG |CTGGTTCCCAGTGGAAGGCCCGGCTGACATCTGTCAAT
Macaca fascicularis   : GTCTTCCAACAG |CTGGTTCCCAGTGGAAGGCCCAGCTGACATCTGTCAAT
Macaca mulatta        : GTCTTCCAACAG |CTGGTTCCCAGTGGAAGGCCCAGCTGACATCTGTCAAT
Macaca radiata        : GTCTTCCAACAG |CTGGTTCCCAGTGGAAGGCCCAGCTGACATCTGTCAAT
Callithrix jacchus    : GGCTTCCAACAG |CTGGTTCCCGGTGGAAGGCCCGGCTGACATCTGCCAGT
Canis lupus           : GTCTACCAAGAG |GTCCTACCCTGTAGAAGGCTCGGCTGATATTTGTCGCT
Vulpes vulpes         : GTCTACCAAGAG |GTGGTACCCTGTAGAAGGCTCGGCTGATATTTGTCGCT
Mustela erminea       : GTCCAGCAGGAG |GTGGTCCCCCGTAGAAGGCACTGCTGACATCTGTCGCT
Mustela putorius      : GTCCAGCAGGAG |GTGGTCCCCCGTAGAAGGCACTGCTGACATCTGTCGCT
Felis catus           : GTCTTCTAACAG |GTGGTTCCCAGTAGAAGGCCCTGCTGACATCTGTAACT

                            *  110    *  120    *  130    *  140    *  150
Mus musculus          : GCTGCAGCCATGGCAACTGTAGTAATTCAAGCTCTTCACAGTTCCAGATC
Rattus norvegicus     : GCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTTCGAGACC
Rattus rattus         : GCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTTCGAGACC
Rattus tanezumi       : GCTGCAGCAATGGCAACTGTAGTAATTCAAGCTCTTCAGAGTTCGAGACC
Lasiopodomys brandtii : GCTGCACCAAGGGCGACTGTAGCAGTTCCAGATATTCGAGGCCCCGGGCC
Lagurus lagurus       : GCTGCACCAAGGGCGACTGTAGCAGTTCAAGGTATTCCAGGCCCCGGGGC
Mesocricetus auratus  : GCTGCAGCAGTGGCGACTGTGGTAGCTCAAGCCGTTCACGGTACCAGGCC
Onychomys torridus    : GCTGCATTAAGGGTGACTGTAGT---CCCAGCGATTCAAGGAACCAGGCC
Peromyscus polionotus : GCTGCGTTAAGGGCGACTGCAGTAGTTTGAACAACTCGAAGCACCAGGCC
Homo sapiens          : GCTGTAACAAAGGTGACTGTGGCACTCCAAGCCATTCCAGGAGGCAGCCT
Pan troglodytes       : GCTGTAACAAAGGTGACTGTGGCACTCCAAGCCATTCCAGGAGGCAGCCT
Macaca fascicularis   : GCTGTAGCAAGGGTGACTGTGGCACTCCAAGCCATTCCAGGAGGCAGCCC
Macaca mulatta        : GCTGTAGCAAGGGTGACTGTGGCACTCCAAGCCATTCCAGGAGGCAGCCC
Macaca radiata        : GCTGTAGCAAGGGTGACTGTGGCACTCCAAGCCATTCCAGGAGGCAGCCC
Callithrix jacchus    : GCTGTAGCAAGGGTGACTGTGGCACTCCAAGCCATGCCAGGAGGCAGCCC
Canis lupus           : GTTGTAACAAAGGCAGCTGTGGCCTTCCAGGCCGGTCCAGGAGGCTGTCC
Vulpes vulpes         : GTTGTAACAAAGGCAGCTGTGGCCTTCCAGGCCGGTCCAGGAGGCTGTCC
Mustela erminea       : GTTGTAACAAAGGCAGCTGTGGCCTTCCAGGCCGTTCCAGGAGGCTGTCC
Mustela putorius      : GTTGTAACAAAGGCAGCTGTGGCCTTCCAGGCCGTTCCAGGAGGCTGTCC
Felis catus           : GTTGTAACAAAGGTAGCTGTGGCCTTCAGGGCCGTTCCTGGAGGCTGTCC
```

```
                            *  160    *  170
Mus musculus          : CATGGACCCCGCCAGTGGTCC
Rattus norvegicus     : CATGAACCAGCCCAGTGGTCC
Rattus rattus         : CATGAACCAGCCCAGTGGTCC
Rattus tanezumi       : CATGAACCATCCCAGTGGTCC
Lasiopodomys brandtii : CACGCAGTG------------
Lagurus lagurus       : CACGGAGGT------------
Mesocricetus auratus  : CATGGAGTGAGCCAGTGGCCC
Onychomys torridus    : CATGGAGAGAAGCAGTGGCCC
Peromyscus polionotus : CATGGAGAGAAACAGTGGCCC
Homo sapiens          : CATGTCATGAGCCAGTGGTCC
Pan troglodytes       : CATGTCGTGAGCCAGTGGTCC
Macaca fascicularis   : CATGTCGTGAGCCAGTGGTCC
Macaca mulatta        : CATGTCGTGAGCCAGTGGTCC
Macaca radiata        : CATGTCGTGAGCCAGTGGTCC
Callithrix jacchus    : CATGTCGTGAGCCTGGGGTCG
Canis lupus           : CACCTAGAGAGAGGGTGGCGC
Vulpes vulpes         : CACCTAGAGAGAGGGTGGCGC
Mustela erminea       : CGTCTAGAGAGAAGGGGGCGC
Mustela putorius      : CGTCTAGAGAGAAGGGGACGC
Felis catus           : CACCTAGACAGACCGTGGCAC
```

References

Image on reverse;	Australian Old Endemic rodent, *Notomys fuscus.* Modified image from the private collection of Assoc. Prof. Bill Breed

References

Anisimova, M, Bielawski, JP & Yang, Z 2001, 'Accuracy and power of the likelihood ratio test in detecting adaptive molecular evolution', *Molecular Biology and Evolution,* vol. 18, no. 8, pp. 1585-1592.

Aplin, KP 2005,'Ten million years of murine rodent evolution in the Australasian region: a review of the phylogenetic evidence and a speculative historical biogeography', in JR Merrick, M Archer, GM Hickey & MSY Lee (eds), *Evolution and biogeography of Australasian vertebrates,* Australian Scientific Publishing, Sydney, pp. 707-744.

Baba, T, Azuma, S, Kashiwabara, S & Toyoda, Y 1994, 'Sperm from mice carrying a targeted mutation of the acrosin gene can penetrate the oocyte zona pellucida and effect fertilization', *Journal of Biological Chemistry*, vol. 269, no. 50, pp. 31845-31849.

Baibakov, B, Gauthier, L, Talbot, P, Rankin, TL & Dean, J 2007, 'Sperm binding to the zona pellucida is not sufficient to induce acrosome exocytosis', *Development,* vol. 134, pp. 933-943.

Baverstock, PR, Gelder, M & Jahnke, A 1983, 'Chromosome evolution in Australian *Rattus* - G-banding and hybrid meiosis', *Genetica,* vol. 60, pp 93-103.

Bedford, JM 1991,'The coevolution of mammalian gametes', in B Dunbar & MG O'Rand (eds), *A comparative overview of mammalian fertilization*, Blenum Press, New York.

Berlin, S & Smith, NGC 2005, 'Testing for adaptive evolution of the female reproductive protein ZPC in mammals, birds and fishes reveals problems with the M7-M8 likelihood ratio test', *BMC Evolutionary Biology,* vol. 5, pp. 65.

Berry, RJ & Scriven, PN 2005, 'The house mouse: a model and motor for evolutionary understanding', *Biological Journal of the Linnean Society,* vol. 84, pp. 335-347.

Bininda-Emonds, ORP, Gittleman, JL & Purvis, A 1999, 'Building large trees by combining phylogenetic information: a complete phylogeny of the extant Carnivora (Mammalia)', *Biological Reviews of the Cambridge Philosophical Society,* vol. 74, pp. 143-175.

Bleil, JD & Wassarman, PM 1980, 'Structure and function of the zona pellucida: identification and characterization of the proteins of the mouse oocyte's zona pellucida', *Developmental Biology,* vol. 76, pp 185-202.

Bleil, JD & Wassarman, PM 1983, 'Sperm-egg interactions in the mouse: sequence of events and induction of the acrosome reaction by a zona pellucida glycoprotein', *Developmental Biology,* vol. 95, pp. 317-324.

Bleil, JD, Greve, JM & Wassarman, PM 1988, 'Identification of a secondary sperm receptor in the mouse egg zona pellucida: role in maintenance of binding of acrosome-reacted sperm to eggs', *Developmental Biology,* vol. 128, pp. 376-385.

Bleil, JD & Wassarman, PM 1988, 'Galactose at the non-reducing terminus of *O*-linked oligosaccharides of mouse egg zona pellucida glycoprotein ZP3 is essential for the glycoprotein's sperm receptor activity', *Proceedings of the National Academy of Sciences USA,* vol. 85, pp. 6778-6782.

Boja, ES, Hoodbhoy, T, Fales, HM & Dean, J 2003, 'Structural characterization of native mouse zona pellucida proteins using mass spectrometry', *Journal of Biological Chemistry,* vol. 278, pp. 34189-34202.

Bork, P & Sander, C 1992, 'A large domain common to sperm receptors (*Zp2* and *Zp3*) and TGF-beta type II receptor', *FEBS Letters,* vol. 300, pp. 237-240.

Breed, WG 1983, 'Variation in sperm morphology in the Australian rodent genus, *Pseudomys* (Muridae)', *Cell Tissue Res,* vol. 229, pp. 611-625.

Breed, WG 1984, 'Sperm head structure in the Hydromyinae (Rodentia: Muridae): a further evolutionary development of the subacrosomal space in mammals', *Gamete Research,* vol. 10, pp. 31-44.

Breed, WG & Yong, HS 1986, 'Sperm morphology of murid rodents from Malaysia and its possible phylogenetic significance', *American Museum Novitates,* vol. 2856, pp. 1-12.

Breed, WG & Leigh, CM 1991, 'Distribution of filamentous actin in and around spermatids and in spermatozoa of Australian conilurine rodents', *Molecular Reproduction and Development,* vol. 30, pp. 369-384.

Breed, WG & Musser, GG 1991, 'Sulawesi and Philippine Rodents (Muridae): a survey of spermatozoal morphology and its significance for phylogenetic inference', *American Museum Novitates,* vol. 3003, pp. 1-15.

Breed, WG & Aplin, KP 1994, 'Sperm morphology of murid rodents from New Guinea and the Solomon Islands: phylogenetic implications', *Australian Journal of Zoology,* vol. 43, pp. 17-30.

Breed, WG 1995, 'Spermatozoa of murid rodents from Africa: morphological diversity and evolutionary trends', *Journal of Zoology London,* vol. 237, pp. 625-651.

Breed, WG 1997, 'Evolution of the spermatozoon in Australasian rodents', *Australian Journal of Zoology,* vol. 45, pp. 459-478.

Breed, WG & Taylor, J 2000, 'Body mass, testes mass and sperm size in murine rodents', *Journal of Mammalogy,* vol. 81, pp. 758-768.

Breed, WG 2004, 'The spermatozoon of Eurasian murine rodents: its morphological diversity and evolution', *Journal of Morphology,* vol. 261, pp. 52-69.

Breed, WG 2005, 'Evolution of the spermatozoon in muroid rodents', *Journal of Morphology,* vol. 265, pp. 271-290.

Breed, WG & Leigh, CM 2007, ‘Are the Old Endemic rodent lineages of the Philippines and Australasia sister groups?’ In *Proceedings from the 53rd Australian Mammal Society Annual Conference, University of New England*, Armidale, June 2007.

Castle, PE 2002, 'Could multiple low-affinity bonds mediate primary sperm-zona pellucida binding', *Reproduction,* vol. 124, pp. 29-32.

Chapman, NR, Kessopoulos, E, Andrews, PD, Horby, DP & Barratt, CLR 1998, 'The polypeptide backbone of recombinant human zona pellucida glycoprotein-3 initiates acrosomal exocytosis in human spermatozoa *in vitro*', *Biochemical Journal,* vol. 330, pp. 839-845.

Chen, J, Litscher, E & Wassarman, PM 1998, 'Inactivation of the mouse sperm receptor, mZP3, by site-directed mutagenesis of individual serine residues located at the combining site for sperm', *Proceedings of the National Academy of Sciences USA,* vol. 95, pp. 6193-6197.

Chimimba, CT, Dippenaar, NJ & Robinson, TJ 1999, 'Morphometric and morphological delineation of southern African species of *Aethomys* (Rodentia: Muridae)', *Biological Journal of the Linnean Society,* vol. 67, no. 4, pp. 501-527.

Clark, NL, Aagaard, JE & Swanson, WJ 2006, 'Evolution of reproductive proteins from animals and plants', *Reproduction,* vol. 131, no. pp. 11-22.

Conner, SJ, Lefievre, L, Hughes, DC & Barratt, CLR 2005, 'Cracking the egg: increased complexity in the zona pellucida', *Human Reproduction,* vol. 20, no. 5, pp. 1148-1152.

Cummins, J 1990,'Evolution of sperm form: levels of control and competition', in BD Bavister, J Cummins & ERS Roldan (eds), *Proceedings of the International Symposium on fertilization in mammals*, Serono Symposia, Norwell, MA, pp. 51 -64.

Dean, J 2003, 'Reassessing the molecular biology of sperm-egg recognition with mouse genetics.' *BioEssays,* vol. 26, pp. 29-38.

Dell, A, Chalabi, S, Easton, RL, Haslam, SM, Sutton-Smith, M, Patankar, M, Lattanzio, FA, Panico, M, Morris, HR & Clark, GF 2003, 'Murine and human zona pellucida 3 derived from mouse eggs express identical *O*-glycans', *Proceedings of the National Academy of Sciences, USA,* vol. 100, no. 26, pp. 15631-15636.

Donoghue, AM, Howard, JG, Byers, AP, Goodrowe, KL, Bush, M, Blumer, E, Lukas, J, Stover, J, Snodgrass, K & Wildt, DE 1992, 'Correlation of sperm viability with gamete interaction and fertilization in vitro in the cheetah (*Acinonyx jubatus*)', *Biology of Reproduction,* vol. 46, pp. 1047-1056.

Dunbar, BS, Avery, S, Lee, V, Prasad, S, Schwahn, D, Schwoebel, E, Skinner, S & Wilkins, B 1994, 'The mammalian zona pellucida: its biochemistry, immunochemistry, molecular biology, and development expression', *Reproduction, Fertility and Development,* vol. 6, pp. 331-347.

Easton, RL, Patonkar, MS, Lattanzio, FA, Leaven, TJ, Morris, HR, Clark, GF & Dell, A 2000, 'Structural analysis of murine zona pellucida glycans', *Journal of Biological Chemistry,* vol. 275, pp. 7731-7742.

Ensslin, MA & Shur, BD 2003, 'Identification of mouse sperm SED1, a bimotif EGF repeat and discoidin-domain protein involved in sperm-egg binding', *Cell,* vol. 114, pp. 405-417.

Familiari, G, Relucenti, M, Heyn, R, Micara, G & Correr, S 2006, 'Three-dimensional structure of the zona pellucida at ovulation', *Microscopy Research and Technique,* vol. 69, pp. 415-426.

Flannery, TF 1990, *The Mammals of New Guinea,* Robert Brown & Associates, Brisbane Qld.

Florman, HM, Bechtol, KB & Wassarman, PM 1984, 'Enzymatic dissection of the functions of the mouse egg's receptor for sperm', *Developmental Biology,* vol. 106, no. 1, pp. 243-255.

Florman, HM & Wassarman, PM 1985, '*O*-linked oligosaccharides of mouse egg ZP3 account for its sperm receptor activity', *Cell,* vol. 41, pp. 313-324.

Ford, F 2006, 'A splitting headache: relationships and generic boundaries among Australian murids', *Biological Journal of the Linnean Society,* vol. 89, pp. 117-138.

Fournier-Delpech, S, Courtens, JL, Pisselet, CL, Delaleu, B & Courot, M 1982, 'Acquisition of zona binding by ram spermatozoa during epididymal passage, as revealed by interaction with rat oocytes', *Gamete Research,* vol. 5, pp. 403-408.

Gahlay, GK, Srivastava, N, Govind, CK & Gupta, SK 2002, 'Primate recombinant zona pellucida proteins expressed in *Escherichia coli* bind to spermatozoa', *Journal of Reproductive Immunology,* vol. 53, pp. 67-77.

Gahlay, GK, Batra, D & Gupta, SK 2005, 'Baculovirus expressed C-terminal fragment of bonnet monkey (*Macaca radiata*) zona pellucida glycoprotein-3 inhibits ZP3-mediated induction of acrosomal exocytosis', *Molecular Reproduction and Development,* vol. 71, pp. 237-244.

Gardner, AJ & Evans, JP 2006, 'Mammalian membrane block to polyspermy: new insights into how mammalian eggs prevent fertilisation by multiple sperm', *Reproduction, Fertility and Development,* vol. 18, pp. 53-61.

Gavrilets, S 2000, 'Rapid evolution of reproductive barriers driven by sexual conflict', *Nature,* vol. 403, pp. 886-889.

Godthelp, H 1999, 'Diversity, relationships and origins of the tertiary and quarternary rodents of Australia', in M Archer, R Arena, M Bassarova, K Black, J Brammall, B Cooke, P Creaser, K Crosby, A Gillespie, H godthelp, M Gott, SJ Hand, B Kear, A Krikmann, B Mackness, J Muirhead, A Musser, T Myres, N Pledge, Y Wang, S Wroe (eds), *Evolutionary history and diversity of Australian mammals. Australian Mammalogy,* vol. 21, pp. 32-34.

Govind, CK, Hasegawa, A, Koyama, K & Gupta, SK 2000, 'Delineation of a conserved B cell epitope on Bonnet Monkey (*Macaca radiata*) and human zona pellucida glycoprotein-B by monoclonal antibodies demonstrating inhibition of sperm-egg binding', *Biology of Reproduction*, vol. 62, pp. 67-75.

Graur, D & Li, W-H 2000, *Fundamentals of molecular evolution,* Sinauer, Sunderland MA.

Green, DPL 1997, 'Three-dimensional structure of the zona pellucida', *Reviews of Reproduction,* vol. 2, pp. 147-156.

Greve, JM & Wassarman, PM 1985, 'Mouse egg extracellular coat is a matrix of interconnected filaments possessing a structural repeat', *Journal of Molecular Biology,* vol. 181, pp. 253-264.

Hanada, A & Chang, MC 1972, 'Penetration of zona-free eggs by spermatozoa of different species', *Biology of Reproduction,* vol. 6, pp. 300-309.

Hanada, A & Chang, MC 1976, 'Penetration of hamster and rabbit zona-free eggs by rat and mouse spermatozoa with special reference to sperm capacitation', *Journal of Reproduction and Fertility,* vol. 46, pp. 239-241.

Hanada, A & Chang, MC 1978, 'Penetration of the zona-free or intact eggs by foreign spermatozoa and the fertilization of deer mouse eggs *in vitro*', *Journal of Experimental Zoology,* vol. 203, no. 2, pp. 277-285.

Harcourt, AH, Harvey, PH, Larson, SG & Short, RV 1981, 'Testis weight, body weight and breeding systems in primates', *Nature,* vol. 293, pp. 55-57.

Harris, JD, Hibler, DW, Fontenot, GK, Hsu, KT, Yurewicz, EC & Sacco, AG 1994, 'Cloning and characterization of zona pellucida genes and cDNAs from a variety of mammalian species: the ZPA, ZPB and ZPC gene families', *DNA Sequence - The Journal of Sequencing and Mapping,* vol. 4, pp. 361-393.

Hartmann, JF, Gwatkin, RBL & Hutchison, CF 1972, 'Early contact interactions between mammalian gametes *in vitro*: evidence that the vitellus influences adherence between sperm and zona pellucida', *Proceedings of the National Academy of Sciences, USA,* vol. 69, no. 10, pp. 2767-2769.

Hartmann, JF & Hutchison, CF 1974, 'Nature of the pre-penetration of contact interactions between hamster gametes *in vitro*', *Journal of Reproduction and Fertility,* vol. 36, pp. 49-57.

Hennig, L 1999, 'WinGene/WinPep: user-friendly software for the analysis of amino acid sequences', *BioTechniques,* vol. 26, pp. 1170-1172.

Hoodbhoy, T & Dean, J 2004, 'Insights into the molecular basis of sperm-egg recognition in mammals', *Reproduction,* vol. 127, pp. 417-422.

Hoodbhoy, T, Joshi, S, Boja, ES, Williams, SA, Stanley, P & Dean, J 2005, 'Human sperm do not bind to rat zonae pellucidae despite the presence of four homologous glycoproteins', *Journal of Biological Chemistry*, vol. 208, no. 13, pp. 12721-12731.

Hughes, DC & Barratt, CLR 1999, 'Identification of the true human orthologue of the mouse *Zp1* gene: evidence for greater complexity in the mammalian zona pellucida?' *Biochimica et Biophysica Acta,* vol. 1447, pp. 303-306.

Hunter, RHF & Rodriquez-Martinez, H 2002, 'Analysing mammalian fertilization: reservations and potential pitfalls with an *in vitro* approach', *Zygote,* vol. 10, pp. 11-15.

Jaeger, J-J, Tong, H & Denys, C 1986, 'The age of Mus-Rattus devergence: paleontological data compared with the molecular clock', *CR Academie des Sciences Paris II,* vol. 14, pp. 917-922.

Jansa, SA, Lundrigan, BL & Tucker, PK 2003, 'Tests for positive selection on immune and reproductive genes in closely related species of the murine genus *Mus*', *Journal of Molecular Evolution,* vol. 56, pp. 294-307.

Jansa, SA, Barker, FK & Heaney, LR 2006, 'The pattern and timing of diversification of Philippine endemic rodents: evidence from mitochondria and nuclear gene sequences', *Systematic Biology,* vol. 55, no. 1, pp. 73-88.

Johnston, DS, Wright, WW, Shaper, JH, Hokke, CH, Van den Eijnden, DH & Joziasse, DH 1998, 'Murine sperm-zona binding, a fucosyl residue is required for a high affinity sperm-binding ligand', *Journal of Biological Chemistry,* vol. 273, no. 4, pp. 1888-1895.

Jovine, L, Qi, H, Williams, Z, Litscher, E & Wassarman, PM 2002, 'The ZP domain is a conserved module for polymerization of extracellular proteins', *Nature Cell Biology,* vol. 4, pp. 457-461.

Jovine, L, Qi, H, Williams, Z, Litscher, E & Wassarman, PM 2004, 'A duplicated motif controls assembly of zona pellucida domain proteins', *Proceedings of the National Academy of Sciences USA,* vol. 101, no. 16, pp. 5922-5927.

Jungnickel, MK, Sutton, KA & Florman, HM 2003, 'In the beginning: lessons from fertilization in mice and worms', *Cell,* vol. 114, pp. 401-404.

Kenagy, GH & Trombulak, SC 1986, 'Size and function of mammalian testes in relation to body size', *Journal of Mammalogy,* vol. 67, pp. 1-22.

Kimura, M 1980, 'A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences', *Journal of Molecular Evolution,* vol. 16, pp. 111-120.

Kinloch, RA, Ruiz-Seiler, B & Wassarman, PM 1990, 'Genomic organization and polypeptide primary structure of zona pellucida hZP3, the hamster sperm receptor', *Developmental Biology.,* vol. 142, no. 2, pp. 414-421.

Kinloch, RA, Mortillo, S, Stewart, CL & Wassarman, PM 1991, 'Embryonal carcinoma cells transfected with *Zp3* genes differentially glycosylate similar polypeptides and secrete active mouse sperm receptor', *Journal of Cell Biology,* vol. 115, no. 3, pp. 655-664.

Kinloch, RA, Mortillo, S & Wassarman, PM 1992, 'Transgenic mouse eggs with functional hamster sperm receptors in their zona pellucida', *Development,* vol. 115, pp. 937-946.

Kinloch, RA, Sakai, Y & Wassarman, PM 1995, 'Mapping the mouse ZP3 combining site for sperm by exon swapping and site-directed mutagenesis', *Proceedings of the National Academy of Sciences USA,* vol. 92, pp. 263-267.

Kipersztok, S, Osawa, GA, Liang, L-F, Modi, WS & Dean, J 1995, 'POM-ZP3, a bipartite transcript derived from human ZP3 and a POM121 homologue', *Genomics*, vol. 25, pp. 354-359.

Kouba, AJ, Atkinson, MW, Gandolf, AR & Roth, TL 2001, 'Species-specific sperm-egg interaction affects the utility of a heterologous bovine *in vitro* fertilization system for evaluating antelope sperm', *Biology of Reproduction,* vol. 65, pp. 1246-1251.

Kumar, S, Tamura, K & Nei, M 2004, 'MEGA 3: integrated software for molecular evolutionary genetic analysis and sequence alignment', *Briefings in Bioinformatics,* vol. 5, no. 2, pp. 150-163.

Kyte, J & Doolittle, RF 1982, 'A simple method for displaying the hydropathic character of a protein', *Journal of Molecular Biology,* vol. 157, no. 1, pp. 105-132.

Lambert, H 1984, 'Role of sperm-surface glycoproteins in gamete recognition in two mouse species', *Journal of Reproduction and Fertility,* vol. 70, no. 1, pp. 281-284.

Lanzendorf, SE, Holmgren, WJ, Johnson, DE, Scobey, MJ & Jeyendran, RS 1992, 'Hemizona assay for measuring zona binding in the lowland gorilla', *Molecular Reproduction and Development,* vol. 31, pp. 264-267.

Lecompte, E, Denys, C & Granjon, L 2005, 'Confrontation of morphological and molecular data: the *Praomys* group (Rodentia, Murinae) as a case of adaptive convergences and morphological stasis', *Molecular Phylogenetics and Evolution,* vol. 37, pp. 899-919.

Lefievre, L, Conner, SJ, Salpekar, A, Olufowobi, O, Ashton, P, Pavlovic, B, Lenton, W, Afnan, M, Brewis, IA, Monk, M, Hughes, DC & Barratt, CL 2004, 'Four zona pellucida glycoproteins are expressed in the human', *Human Reproduction,* vol. 19, no. 7, pp. 1580-1586.

Leyton, L & Saling, PM 1989, 'Evidence that aggregation of mouse sperm receptors by ZP3 triggers the acrosome reaction', *Journal of Cell Biology,* vol. 108, pp. 2163-2168.

Li, D, Cao, S & Xu, G 2007, 'Polypeptide backbone derived from carboxyl terminal of mouse ZP3 inhibits sperm-zona binding', *Molecular Reproduction and Development*, vol. 74, pp. 1327-1336.

Lin, Y, Mahan, K, Lathrop, WF, Myles, DG & Primakoff, P 1994, 'A hyaluronidase activity of the sperm plasma membrane protein PH-20 enables sperm to penetrate the cumulus cell layer surrounding the egg', *Journal of Cell Biology,* vol. 125, pp. 1157-1163.

Litscher, E, Juntunen, K, Seppo, A, Penttila, L, Niemela, R, Renkonen, O & Wassarman, PM 1995, 'Oligosaccharide constructs with defined structures that inhibit binding of mouse sperm to unfertilized eggs in vitro', *Biochemistry,* vol. 34, pp. 4662-4669.

Litscher, ES, Qi, H & Wassarman, PM 1999, 'Mouse zona pellucida glycoproteins mZP2 and mZP3 undergo carboxy-terminal proteolytic processing in growing oocytes.' *Biochemistry,* vol. 38, pp. 12280-12287.

Liu, C, Litscher, E & Wassarman, PM 1995, 'Transgenic mice with reduced numbers of functional sperm receptors on their eggs reproduce normally', *Molecular Biology of the Cell,* vol. 6, pp. 577-585.

Liu, C, Litscher, ES, Mortillo, S, Sakai, Y, Kinloch, RA, Stewart, CL & Wassarman, PM 1996, 'Targeted disruption of the mZP3 gene results in production of eggs lacking a zona pellucida and infertility in female mice.' *Proceedings of the National Academy of Sciences USA,* vol. 93, pp. 5431-5436.

Liu, DY, Lopata, A, Pantke, P & Gordon Baker, HW 1991, 'Horse and marmoset monkey sperm bind to the zona pellucida of salt-stored human oocytes', *Fertility and Sterility,* vol. 56, pp. 764-767.

Lu, Q & Shur, BD 1997, 'Sperm from ß1,4-galactosyltransferase-null mice are refractory to ZP3-induced acrosome reactions and penetrate the zona pellucida poorly', *Development,* vol. 124, pp. 4121-4131.

Lu, Q, Hasty, P & Shur, BD 1997, 'Targeted mutation in ß1,4-galactosyltransferase leads to pituitary insufficiency and neonatal lethality', *Developmental Biology,* vol. 181, pp. 257-267.

McGregor, L, Flaherty, SP & Breed, WG 1989, 'Structure of the zona pellucida and cumulus oophorus in three species of native Australian rodents', *Gamete Research,* vol. 23, pp. 279-287.

McHugh, JA & Rutledge, JJ 1998, 'Heterologous fertilization to characterize spermatozoa of the genus *Bos*', *Theriogenology,* vol. 50, pp. 185-193.

McLeskey, S, Dowds, C, Carballada, R, White, RR & Saling, PM 1998, 'Molecules involved in mammalian sperm-egg interaction', *International Review of Cytology,* vol. 177, pp. 57-113.

Miller, DJ, Macek, MB & Shur, BD 1992, 'Complementarity between sperm surface β-1,4-galactosyltransferase and egg-coat ZP3 mediates sperm-egg binding.' *Nature,* vol. 357, pp. 589-593.

Miller, DJ, Gong, X & Shur, BD 1993, 'Sperm require ß-N-acetylglucosaminidase to penetrate through the egg zona pellucida', *Development,* vol. 118, pp. 1279-1289.

Miller, DJ, Shi, X & Burkin, H 2002, 'Molecular basis of mammalian gamete binding', *Recent Progress in Hormone Research*, vol. 57, pp. 37-73.

Moller, CC, Bleil, JD, Kinloch, RA & Wassarman, PM 1990, 'Structural and functional relationships between mouse and hamster zona pellucida glycoproteins', *Developmental Biology,* vol. 137, pp. 276-286.

Murphy, WJ, Eizirik, E, Johnson, WE, Zhang, YP, Ryder, OA & O'Brien, SJ 2001, 'Molecular phylogenetics and the origin of placental mammals', *Nature,* vol. 409, pp. 614-618.

Musser, GG & Carleton, MD 2005,'Superfamily Muroidea', in DE Wilson & DM Reeder (eds), *Mammal Species of the World*, vol. 2, 3rd edn, John Hoptkins University Press, Baltimore, MD, pp. 894-1531.

Nagdas, SK, Araki, Y, Chayko, CA, Orgebin-Crist, M-S & Tulsiani, DRP 1994, '*O*-linked trisaccharide and *N*-linked poly-N-acetyllactosaminyl glycans are present on Mouse ZP2 and ZP3', *Biology of Reproduction,* vol. 51, pp. 262-272.

Nehrke, K, Hagen, FK & Tabak, LA 1996, 'Charge distribution of flanking amino acids influences *O*-glycan acquisition *in vivo*', *Journal of Biological Chemistry,* vol. 271, pp. 7061-7065.

Nei, M & Kumar, S 2000, *Molecular Evolution and Phylogenetics,* Oxford University Press, New York.

Nicholas, KB, Nicholas, HBJ & Deerfield, DWI 1997, 'GeneDoc: analysis and visualization of genetic variation', *EMBNET NEWS,* vol. 4, no. 2, pp. 14.

Nielsen, R & Yang, Z 1998, 'Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene', *Genetics,* vol. 148, pp. 929-936.

O'Rand, MG 1988, 'Sperm-egg recognition and barriers to interspecies fertilization', *Gamete Research,* vol. 19, pp. 315-328.

Patra, AK, Gahlay, GK, Reddy, BVV, Gupta, SK & Panda, AK 2000, 'Refolding, structural transition and spermatozoa-binding of recombinant bonnet monkey (*Macaca radiata*) zona pellucida glycoprotein-C expressed in *Escherichia coli'*, *European Journal of Biochemistry,* vol. 267, pp. 7075-7081.

Posada, D & Crandall, KA 1998, 'Modeltest: testing the model of DNA substitution', *Bioinformatics*, vol. 14, no. 9, pp. 817-818.

Prasad, SV, Wilkins, B, Skinner, SM & Dunbar, BS 1996, 'Evaluating zona pellucida structure and function using antibodies to rabbit 55 kDa ZP protein expressed baculovirus expression system', *Molecular Reproduction and Development,* vol. 43, pp. 519-529.

Purvis, A 1995, 'A composite estimate of primate phylogeny', *Philosophical Transactions of the Royal Society London, B Series,* vol. 348, no. 1326, pp. 405-421.

Qi, H, Williams, Z & Wassarman, PM 2002, 'Secretion and assembly of zona pellucida glycoproteins of growing mouse oocytes microinjected with epitope-tagged cDNAs for mZP2 and mZP3', *Molecular Biology of the Cell,* vol. 13, pp. 530-541.

Rankin, T, Familari, M, Lee, E, Ginsberg, A, Dwyer, N, Blanchette-Mackie, J, Drago, J, Westphal, H & Dean, J 1996, 'Mice homozygous for an insertional mutation in the *Zp3* gene lack a zona pellucida and are infertile', *Development,* vol. 122, pp. 2903-2910.

Rankin, T, Tong, Z-B, Castle, PE, Lee, E, Gore-Langton, R & Nelson, LM 1998, 'Human ZP3 restores fertility in *Zp3* null mice without affecting order-specific sperm binding', *Development,* vol. 125, pp. 2415-2424.

Rankin, T, Talbot, P, Lee, E & Dean, J 1999, 'Abnormal zonae pellucidae in mice lacking ZP1 result in early embryonic loss', *Development,* vol. 126, pp. 3847-3855.

Rankin, T & Dean, J 2000, 'The zona pellucida: using molecular genetics to study the mammalian egg coat', *Reviews of Reproduction,* vol. 5, pp. 114-121.

Rankin, T, O'Brien, MO, Lee, E, Wigglesworth, K, Eppig, J & Dean, J 2001, 'Defective zonae pellucidae in *Zp2*-null mice disrupts folliculogenesis, fertility and development', *Development,* vol. 128, pp. 1119-1126.

Rankin, TL, Coleman, JS, Epifano, O, Hoodbhoy, T, Turner, SG, Castle, PE, Lee, E, Gore-Langton, R & Dean, J 2003, 'Fertility and taxon-specific sperm binding persist after replacement of mouse sperm receptors with human homologs', *Developmental Cell,* vol. 5, pp. 33-43.

Roldan, E & and Yanagimachi, R 1989, 'Cross-fertilization between Syrian and Chinese Hamsters', *Journal of Experimental Zoology,* vol. 250, pp. 321-328.

Rosiere, T & Wassarman, PM 1992, 'Identification of a region of mouse zona pellucida mZP3 that possesses sperm receptor activity', *Developmental Biology,* vol. 154, pp. 309-317.

Roth, TL, Weiss, RB, Buff, JL, Bush, LM, Wildt, DE & Bush, M 1998, 'Heterologous *in vitro* fertilization and sperm capacitation in an endangered African antelope, the Scimitar-Horned Oryx (*Oryx dammah*)', *Biology of Reproduction,* vol. 58, pp. 475-482.

Schmell, E & Gulyas, BJ 1980, 'Mammalian sperm-egg recognition and binding *in vitro*. I. Specificity of sperm interactions with live and fixed eggs in homologous and heterologous inseminations of hamster, mouse and guinea pig oocytes', *Biology of Reproduction,* vol. 23, pp. 1075-1085.

Scobie, G, Kerr, LE, MacDuff, P & Aitken, RJ 1999, 'Cloning, sequencing and site of origin of the rat sperm receptor protein, ZP3', *Zygote,* vol. 7, pp. 27-35.

Shur, BD 1998, 'Is sperm galactosytransferase a signaling subunit of a multimeric gamete receptor?' *Biochemical and Biophysical Research Communications,* vol. 250, pp. 537-543.

Shur, BD, Evans, S & Lu, Q 1998, 'Cell surface galactosyltransferase: current issues', *Glycoconjugate Journal,* vol. 15, pp. 537-548.

Sinowatz, F, Kolle, S & Topfer-Petersen, E 2001, 'Biosynthesis and expression of zona pellucida glycoproteins in mammals', *Cells Tissues Organs,* vol. 168, pp. 24-35.

Sinowatz, F, Wessa, E, Neumuller, C & Palma, G 2003, 'On the species-specificity of sperm binding and sperm penetration of the zona pellucida', *Reproduction in Domestic Animals,* vol. 38, pp. 141-146.

Slavik, T, Pavlok, A & Fulka, J 1990, 'Penetration of intact bovine ova with ram sperm *in vitro*', *Molecular Reproduction and Development,* vol. 25, pp. 345-347.

Steppan, SJ, Adkins, RM & Anderson, J 2004, 'Phylogeny and divergence-date estimates of rapid radiations in murioid rodents based on multiple nuclear genes', *Systematic Biology,* vol. 53, no. 4, pp. 533-553.

Steppan, SJ, Adkins, RM, Spinks, PQ & Hale, C 2005, 'Multigene phylogeny of the Old World mice, Murinae, reveals distinct geographic lineages and the declining utility of mitochondrial genes compared to nuclear genes', *Molecular Phylogenetics and Evolution,* vol. 37, pp. 370-388.

Strahan, R 2002, *The Mammals of Australia,* 2nd edn, Australian Museum, Reed New Holland, Sydney.

Swann, CA, Hope, RM & Breed, WG 2002, 'cDNA nucleotide sequence encoding the ZPC protein of Australian hydromyine rodents: a novel sequence of the putative sperm-combining site within the family Muridae', *Zygote,* vol. 10, pp. 291-299.

Swanson, WJ, Yang, Z, Wolfner, MF & Aquadro, CF 2001, 'Positive Darwinian selection drives the evolution of several female reproductive proteins in mammals', *Proceedings of the National Academy of Sciences USA,* vol. 98, pp. 2509-2514.

Swanson, WJ & Vacquier, VD 2002, 'The rapid evolution of reproduction proteins', *Nature Reviews Genetics,* vol. 3, no. 2, pp. 137-144.

Swanson, WJ, Nielsen, R & Yang, Q 2003, 'Pervasive adaptive evolution in mammalian fertilization proteins.' *Molecular Biology and Evolution,* vol. 20, no. 1, pp. 18-20.

Swenson, CE & Dunbar, BS 1982, 'Specificity of sperm-zona interaction', *Journal of Experimental Zoology,* vol. 219, pp. 97-104.

Talbot, P, Shur, BD & Myles, DG 2003, 'Cell adhesion and fertilization: steps in oocyte transport, sperm-zona pellucida interactions, and sperm-egg fusion', *Biology of Reproduction,* vol. 68, pp. 1-9.

Thaler, CD & Cardullo, RA 1996, 'The initial molecular interaction between mouse sperm and the zona pellucida is a complex binding event', *Journal of Biological Chemistry,* vol. 271, no. 38, pp. 23289-23297.

Thompson, JD, Higgins, DG & Gibson, TJ 1994, 'CLUSTAL-W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice', *Nucleic Acids Research,* vol. 22, pp. 4673-4680.

Topper, EK, Kruijt, L, Mann, CK, Topfer-Petersen, E & Woelders, H 1997, 'Identification of bovine zona pellucida glycoproteins.' *Molecular Reproduction and Development,* vol. 46, pp. 344-350.

Tulsiani, DRP, Nagdas, SK, Cornwall, GA & Orgebin-Crist, M-C 1992, 'Evidence for the presence of high-mannose/hybrid oligosaccharide chain(s) on the mouse ZP2 and ZP3', *Biology of Reproduction.,* vol. 46, pp. 93-100.

Turner, LM & Hoekstra, HE 2006, 'Adaptive evolution of fertilization proteins within a genus: variation in ZP2 and ZP3 in deer mice (*Peromyscus*)', *Molecular Biology and Evolution,* vol. 23, no. 9, pp. 1656-1669.

Vacquier, VD 1998, 'Evolution of gamete recognition proteins', *Science,* vol. 281, pp. 1995-1998.

Vandevoort, CA, Tollner, TL & Overstreet, JW 1992, 'Sperm-zona pellucida interaction in Cynomologus and Rhesus Macaques', *Journal of Andrology,* vol. 13, pp. 428-432.

Van Duin, M, Polman, JEM, Verkoelen, CCEH, Bunschoten, H, Meyerink, JH, Olijve, W & Aitken, RJ 1992, 'Cloning and characterization of the human sperm receptor ligand ZP3: evidence for a second polymorphic allele with a different frequency in the Caucasian and Japanese populations', *Genomics,* vol. 14, pp. 1064-1070.

Wassarman, PM 1988, 'Zona pellucida glycoproteins', *Annual Review of Biochemistry,* vol. 57, pp. 415-442.

Wassarman, PM & Litscher, ES 1995, 'Sperm-egg recognition mechanisms in mammals', *Current Topics in Developmental Biology,* vol. 30, pp. 1-19.

Wassarman, PM, Liu, C & Litscher, E 1996, 'Constructing the mammalian egg zona pellucida: some new pieces of an old puzzle', *Journal of Cell Science.,* vol. 109, pp. 2001-2004.

Wassarman, PM 1999a, 'Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis and fusion', *Cell,* vol. 96, pp. 175-183.

Wassarman, PM 1999b, 'Zona pellucida glycoprotein mZP3: a versatile player during mammalian fertilization', *Journal of Reproduction and Fertility,* vol. 116, pp. 211-216.

Wassarman, PM, Chen, J, Cohen, N, Litscher, E, Liu, C, Qi, H & Williams, Z 1999, 'Structure and function of the mammalian egg zona pellucida', *Journal of Experimental Zoology Part B Molecular and Developmental Biology,* vol. 285, pp. 251-258.

Wassarman, PM & Litscher, ES 2001, 'Towards the molecular basis of sperm and egg interaction during mammalian fertilization', *Cells Tissues Organs,* vol. 168, pp. 36-45.

Wassarman, PM, Jovine, L & Litscher, E 2001, 'A profile of fertilization in mammals', *Nature Cell Biology*, vol. 3, pp. E59-E64.

Wassarman, PM 2002, 'Sperm receptors and fertilization in mammals', *The Mt Sinai Journal of Medicine,* vol. 69, no. 3, pp. 148-155.

Wassarman, PM, Jovine, L & Litscher, ES 2004a, 'Mouse zona pellucida genes and glycoproteins', *Cytogenetic and Genome Research,* vol. 105, pp. 228-234.

Wassarman, PM, Jovine, L, Litscher, E, Qi, H & Williams, Z 2004b, 'Egg-sperm interactions at fertilization in mammals', *European Journal of Obstetrics, Gynecology and Reproductive Biology,* vol. 115S, pp. S57-S60.

Wassarman, PM 2005, 'Contribution of mouse egg zona pellucida glycoproteins to gamete recognition during fertilization', *Journal of Cellular Physiology,* vol. 204, pp. 388-391.

Wassarman, PM, Jovine, L, Qi, H, Williams, Z, Darie, C & Litscher, E 2005, 'Recent aspect of mammalian fertilization research', *Molecular and Cellular Endocrinology,* vol. 234, pp. 95-103.

Watts, CHS & Aslin, HJ 1981, *The Rodents of Australia,* Angus and Robertson, Sydney.

Watts, CHS, Baverstock, PR, Birrell, J & Kreig, M 1992, 'Phylogeny of the Australian rodents (Muridae): a molecular approach using microcomplement fixation of albumin', *Australian Journal of Zoology,* vol. 40, pp. 81-90.

Watts, CHS & Baverstock, PR 1994a, 'Evolution in some South-east Asian Murinae (Rodentia), as assessed by microcomplement fixation of albumin, and their relationship to Australian murines', *Australian Journal of Zoology,* vol. 42, pp. 711-722.

Watts, CHS & Baverstock, PR 1994b, 'Evolution in New Guinea Muridae (Rodentia) assessed by microcomplement fixation of albumin', *Australian Journal of Zoology,* vol. 42, pp. 295-306.

Watts, CHS & Baverstock, PR 1995, 'Evolution in the murinae (Rodentia) assessed by microcomplement fixation of albumin', *Australian Journal of Zoology,* vol. 43, pp. 105-118.

Williams, Z & Wassarman, PM 2001, 'Secretion of mouse ZP3, the sperm receptor, requires cleavage of its polypeptide at a consensus furin cleavage-site', *Biochemistry,* vol. 40, pp. 929-937.

Williams, Z, Litscher, E & Wassarman, PM 2003, 'Conversion of Ser to Thr residues at the sperm combining-site of mZP3 does not affect sperm receptor activity', *Biochemical and Biophysical Research Communications,* vol. 301, pp. 813-818.

Williams, Z, Litscher, E, Jovine, L & Wassarman, PM 2006, 'Polypeptide encoded by mouse *Zp*3 exon-7 is necessary and sufficient for binding of mouse sperm *in vitro*', *Journal of Cellular Physiology,* vol. 207, pp. 30-39.

Wong, WSW, Yang, Z, Goldman, N & Nielsen, R 2004, 'Accuracy and power of statistical methods for detecting adaptive evolution in protein coding sequences and for identifying positively selected sites', *Genetics,* vol. 168, pp. 1041-1051.

Wyckoff, GJ, Wang, W & Wu, C-I 2000, 'Rapid evolution of male reproductive genes in the descent of man', *Nature,* vol. 403, pp. 304-309.

Yanagimachi, R 1972, 'Penetration of guinea-pig spermatozoa into hamster eggs *in vitro*', *Journal of Reproduction and Fertility,* vol. 28, pp. 477-480.

Yanagimachi, R, Yanagimachi, H & Rogers, BJ 1976, 'The use of zona-free animal ova as a test-system for the assessment of the fertilizing capacity of human spermatozoa', *Biology of Reproduction,* vol. 15, pp. 471-476.

Yanagimachi, R 1978, 'Sperm-egg association in mammals', *Current Topics in Developmental Biology,* vol. 12, pp. 83-105.

Yanagimachi, R 1994, 'Mammalian Fertilization', in E Knobil & JD Neill (eds), *The Physiology of Reproduction*, 2nd edn, Raven Press, New York, pp. 189-317.

Yang, Z, Kumar, S & Nei, M 1995, 'A new method of inference of ancestral nucleotide and amino acid sequences', *Genetics,* vol. 141, pp. 1641-1650.

Yang, Z 1997, 'PAML: a program package for phylogenetic analysis by maximum likelihood', *Computer Applications in the Biosciences,* vol. 13, pp. 555-556.

Yang, Z 1998, 'Likelihood ratio tests for detecting positive selection and application to primate lysozyme evolution', *Molecular Biology and Evolution,* vol. 15, no. 5, pp. 568-573.

Yang, Z & Nielsen, R 2000, 'Estimating synonymous and nonsynonymous substitution rates under realistic evolutionary models', *Molecular Biology and Evolution,* vol. 17, no. 1, pp. 32-43.

Yang, Z, Wong, WSW & Nielsen, R 2005, 'Bayes empirical bayes inference of amino acid sites under positive selection', *Molecular Biology and Evolution,* vol. 22, no. 4, pp. 1107-1118.

Yurewicz, EC, Sacco, AG, Gupta, SK, Xu, N & Gage, DA 1998, 'Hetero-oligomerization-dependent binding of pig oocyte zona pellucida glycoproteins ZPB and ZPC to boar sperm membrane vesicles', *Journal of Biological Chemistry,* vol. 273, no. 12, pp. 7488-7494.

Publications

Image on reverse: Australian Old Endemic rodent, *Leggadina forresti*.
Modified image from the private collection of Assoc. Prof. Bill Breed

Swann, C.A., Hope, R.M. and Breed, W.G. (2002) cDNA nucleotide sequence encoding the ZPC protein of Australian hydromyine rodents: a novel sequence of the putative sperm-combining site within the family Muridae
Zygote, v. 10 (4) pp. 291-299, November 2002

http://dx.doi.org/10.1017/S0967199402004021

Swann, C.A., Cooper, S.J.B. and Breed, W.G. (2007) Molecular evolution of the carboxy terminal region of the zona pellucida 3 glycoprotein in murine rodents. *Reproduction, v. 133 (4) pp. 697-708, April 2007*

ABSTRACTS

Forty-ninth AGM

The Australian Mammal Society

7-9 July 2003

Sydney University

Spoken papers pages 1-37
* Asterisks indicate the person delivering the paper

Poster papers pages 39-49

SPEIGHT, N., Leigh, C., and Breed, W.*
Department of Anatomical Sciences, University of Adelaide SA 5005

FEMALE REPRODUCTIVE TRACT MORPHOLOGY OF THE HOPPING MOUSE (*NOTOMYS ALEXIS)* AND ITS INFLUENCE ON SPERM TRANSPORT

In most murine rodents the cervix is a large fibrous structure through which sperm transport is facilitated by males depositing a large vaginal plug. However, in most hopping mice, including *Notomys alexis,* the cervix is small, whereas males have minute seminal vesicles and coagulating glands and deposit a minute plug.

The following questions on hopping mice were addressed: (i) What is the difference between the vaginal and cervical luminal diameter?, (ii) What is the sperm distribution shortly after mating?, and (iii) Can artificial insemination (AI) be performed per vaginam? For (i) a mould of the reproductive tract was obtained using resin, for (ii) female hopping mice were euthanased between 10 min and 4 h pc, and sperm distribution determined, and for (iii) primed females were inseminated with cauda sperm and prostatic secretions per vaginam and resultant sperm distribution determined.

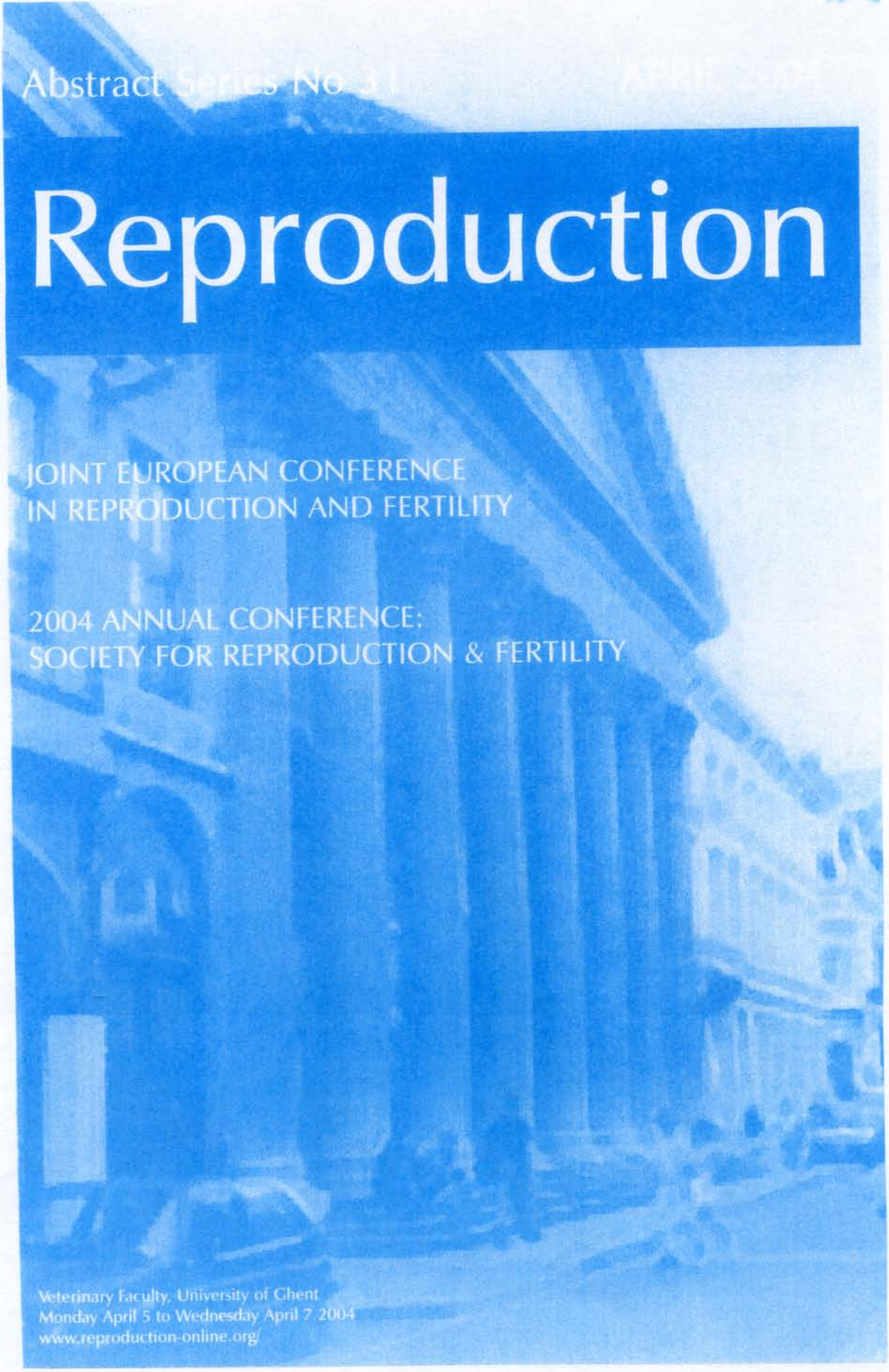
The resin casts showed that in hopping mice the size of vaginal and cervical canals is similar. Histology of the reproductive tract of recently mated females showed 10% of the sperm population reached the upper uterus by 10 min pc, whereas about 45% were still present in the vagina 4 h pc. AI resulted in only 2/7 females having tubal sperm.

These findings on female hopping mice indicate that the cervical lumen is not markedly reduced compared to that of the vagina, invaginal insemination occurs, and rapid sperm transport to the uterotubal junction results. Attempts to develop AI per vaginam were, however, largely unsuccessful perhaps due to sperm transport being facilitated by female tract contractions that were not mimicked during AI.

SWANN[1*], C.A.; Cooper[2], S.; Hope[3], R.M. and Breed[1], W.G.
[1] Department of Anatomical Sciences, University of Adelaide University, Australia
[2] Evolutionary Biology Unit, South Australia Museum
[3] University of Adelaide

EVOLUTION OF ZONA PELLUCIDA C GLYCOPROTEIN IN AUSTRALASIAN NATIVE RODENTS

To successfully fertilise an egg the spermatozoon must first traverse the female reproductive tract, pass through the cumulus matrix surrounding the oocyte, bind to and penetrate the egg coat, the zona pellucida (ZP), and fuse with the oolemma. The ZP is comprised of 3 to 5 glycoproteins and it is to these glycoconjugates that the sperm binds. In the laboratory mouse, the primary sperm-ZP binding site is the O-linked oligosaccharides attached to serine residues within exon 7 of zona pellucida C (ZPC) glycoprotein. Recently, it has been claimed that this region has undergone rapid evolution due to positive Darwinian selection. Here the nucleotide sequence of exon 7 of ZPC of Australasian native rodents is determined to ascertain whether there is any evidence of rapid evolution. Primers, designed from *Notomys alexis* cDNA, were used to PCR amplify and sequence a region from exon 6 through to exon 7 of ZPC (~350 nt) in a range of Australasian native rodents. Evidence of positive selection was determined using the codon sequences and the software program PAML. Amino acid residues within exon 7 of ZPC show a remarkable degree of conservation among all hydromyine species sampled. A number of codons are specific to this group, including two serine residues (potential glycosylation sites) not present in any other group of mammals, including *Mus* species. These results do not support evidence for positive Darwinian selection at the putative sperm-combining site in this group of rodents, suggesting that other factors are likely to be involved in reproductive isolation of these species.



Abstract Series No 31 April 2004

Reproduction

JOINT EUROPEAN CONFERENCE IN REPRODUCTION AND FERTILITY

2004 ANNUAL CONFERENCE: SOCIETY FOR REPRODUCTION & FERTILITY

Veterinary Faculty, University of Ghent
Monday April 5 to Wednesday April 7 2004
www.reproduction-online.org/

Results and Discussion: Active immunization of 10 µg and 30 µg of M1 antigen respectively showed a significant decrease in number of implantation sites ($P < 0.05$). Histological analyses on testes of immunized males clearly showed a sign of aspermatogenesis particularly at 30µg of M1 antigen injections. These results suggest a possible basis for the development of M1 antigen as a potential contraceptive antigen. (This study was supported by project grants from IRPA 09-0202-0060 and TORAY foundation).

O28. Oocyte-cumulus communication: Impact of gap junctional communication and GDF-9 on peroxiredoxin 6 expression

G Leyens [1], B Knoops [2] & I Donnay [1]

[1]Université Catholique de Louvain, Institut des Sciences de la Vie, Unité des Sciences Vétérinaires, Louvain-la-Neuve, Belgium; [2]Université Catholique de Louvain, Institut des Sciences de la Vie, Unité de Biologie Animale, Louvain-la-Neuve, Belgium.

Introduction: Peroxiredoxins (PRDX1 to PRDX6) form a new family of peroxidases involved in cell signalling and antioxidant protection. We showed earlier that bovine oocytes and cumulus cells present an up-regulation of PRDX6 transcripts and protein after *in vitro* maturation. Our objective was to study the effect of cumulus-oocyte communication and GDF-9 on the expression of PRDX6.

Methods: Experiment 1: Clumps of cumulus cells were matured in presence or absence of denuded oocytes, or as cumulus-oocyte complexes. Clumps of cumulus were obtained by aspiration through a 75 µm pipette. These samples were matured *in vitro* for 24 hours in TCM-199 supplemented with EGF. Experiment 2: Clumps of cumulus cells were matured in TCM-199 supplemented with medium conditioned either by mouse recombinant GDF-9 transfected cells, or by mock transfected cells. In the two experiments, PRDX6 expression was studied using semi-quantitative RT-PCR with normalisation to histone H2a expression.

Results and Discussion: An up-regulation of PRDX6 was observed in oocytes only when they were matured as cumulus-oocyte complexes. On the contrary, cumulus-oocyte junctions were not needed for the up-regulation in cumulus cells, even though the presence of oocytes was required. It therefore seems that cumulus cells regulate PRDX6 expression in oocytes through cell junctions, whereas oocytes regulate PRDX6 expression in cumulus cells via paracrine signalling. Next, we tested whether GDF-9 could be the paracrine factor. Our results showed that GDF-9 increased PRDX6 expression by two folds in cumulus cells cultured alone ($p<0.001$ at 100 ng/ml GDF-9 versus control). Together, our results demonstrated that interaction between oocytes and cumulus cells are necessary for the regulation of PRDX6 expression in both cell types. GDF-9 might be the oocyte factor responsible for the increase of PRDX6 expression in cumulus cells. (Gregory Leyens is a Research Fellow of the Fonds National de la Recherche Scientifique, Belgium.)

O29. Novel functional characterization of expression of prostaglandin $F_{2\alpha}$ synthase (PGFS) and microsomal prostaglandin E_2 synthase (mPGES) in porcine oestrous cycle endometrium and in early pregnancy

A Waclawik [1], NA Rahman [2], A Rivero-Muller [2], LJS Brokken [2], K Watanabe [3], A Blitek [1] & AJ Ziecik [1]

[1] Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland; [2] Department of Physiology, University of Turku, Turku, Finland; [3] Division of Applied Life Sciences, University of East Asia, Yamaguchi, Japan.

Introduction: Prostaglandins produced in the uterine endometrium and the $PGF_{2\alpha}/PGE_2$ ratio play important role in the regulation of the oestrous cycle and establishment of pregnancy. PGFS and mPGES are the two distinct downstream catalyzing enzymes, which regulate the production of both prostaglandins. We hereby investigated the functional appearance of the PGFS and mPGES expression along with the porcine oestrous cycle and early pregnancy.

Methods: Endometrium samples were analyzed from cyclic (n=27) and pregnant gilts (n=7). PGFS expression was examined by RT-PCR, quantitative RT-PCR and Western blot, whereas mPGES expression was investigated by RT-PCR and quantitative RT-PCR.

Results and Discussion: A 978-bp RT-PCR product which corresponds to the positive control (lung) mRNA, was identified both in endometrium collected from the oestrous cycle (4-21 days) and pregnant (15-25 days) gilts. PGFS mRNA expression levels (arbitrary units) were highest on days 12-13 and 14-15 of the oestrous cycle (mean+/-SEM, 0.037+/-0.0005, 0.031+/-0.006; $p<0.05$ and $p<0.01$, compared to midluteal and follicular phase), medium during midluteal 6-9 days (0.013+/-0.002; $p<0.05$ and $p<0.01$, compared to 12-13, 13-14 days and follicular phase), and lowest during follicular phase (0.004+/-0.001; $p<0.01$, compared to both 12-13, 14-15 days and midluteal days) and high in 15-, but low in the 20-25 days of pregnancy ($p<0.05$, compared to all groups). A 37-38 kDa protein band of PGFS was significantly increased on days 13-14 of the oestrous cycle and also showed similar mRNA expression patterns in pregnancy, compared to follicular phase. A 333-bp RT-PCR product of mPGES was detected in endometrium and mPGES mRNA quantitation demonstrated no significant upregulation across the oestrous cycle comparing to PGFS but similar increase on 15-25 of the pregnancy was observed. Taken together, this is a novel report characterizing the functional expression and changes of PGFS and mPGES in porcine endometrium during the oestrous cycle and early pregnancy.

O30. Role for prostaglandins in the autocrine/paracrine regulation of 11B-hydroxysteroid dehydrogenase (11BHSD) activity in human granulosa-lutein (hGL) cells

KC Jonas [1], C Chandras [1], DRE Abayasekara [2] & AE Michael [1, 2]

[1]Department of Biochemistry & Molecular Biology, Royal Free Campus, University College London, London, UK; [2]Reproduction & Development Group, Department of Veterinary Basic Sciences, Royal Veterinary College, London, UK.

Introduction: In the ovary, cortisol is inactivated by isoforms of 11BHSD. In placenta and uterus, glucocorticoid metabolism by 11BHSD can be increased by prostaglandins (PGs). Since PGs are paracrine regulators of ovarian function, the objective of this study was to establish whether endogenous PGs can regulate the activity and expression of 11BHSD in hGL cells.

Methods: hGL cells were isolated from follicular aspirates of women undergoing assisted conception (n=6). After isolation on 60% Percoll, cells were cultured in serum-supplemented medium. On day 3 of culture, cells were transferred to serum-free medium and treated for 4h or 24h with the preferential PGH synthase inhibitor meclofenamic acid (MA: 0, 0.01, 0.1, 1, 10, 100uM). 11BHSD activities were assessed by radiometric conversion assays using either 100nM 3H-cortisol or 3H-cortisone to assess 11B-dehydrogenase (11B-DH) or 11-ketosteroid reductase (11-KSR) activities, respectively. Steroids were extracted, resolved by TLC, and quantified using a radiochromatogramme scanner. Parallel experiments were performed using 0uM and 100uM MA and Western blots conducted to determine effects of MA on 11BHSD protein expression (n=3).

Results and Discussion: Suppression of local PG synthesis in hGL cells with MA resulted in concentration-dependent decreases in both 11B-DH and 11-KSR activities. Over 4h, 100uM MA decreased 11B-DH and 11-KSR activities by 31.9+/- 4.8% ($p<0.05$) and 50.6+/- 4.9% ($p<0.01$) respectively. Although treatment with MA for 24h had no further effect on 11B-DH activity (25.9+/-4.8% inhibition, $p<0.01$), the effects on 11-KSR activity was more marked: maximal suppression of 11-KSR activity was achieved at 0.1uM MA (56.2 +/- 9.3% decrease, $p<0.01$). Since 100uM MA had no effect on 11BHSD protein expression ($p>0.05$) over both 4h and 24h, we conclude that endogenous PGs are necessary to maintain 11BHSD activities within hGL cells and are implicated in the post-translational regulation of 11BHSD within the human ovary. (Supported by MRC studentship G69/1756)

O31. Glycoconjugates of the zona pellucida of murid rodents: Is there evidence for species-specificity?

CA Swann, CM Leigh & WG Breed

Department of Anatomical Sciences, University of Adelaide, Adelaide, Australia.

Introduction: Oligosaccharides account for approximately half the zona pellucida (ZP) mass. They are important for structural support and provide adhesive ligands for sperm receptors that may bind species-selectively. Lectins, sugar-binding proteins, may be used to probe sugar composition. If the sperm-adhesion property of the ZP is species-selective then it may be that the ZP of closely related species have different sugar compositions. To test

this, we compare lectin-binding patterns, and hence sugar composition, of ZP from three murid rodent species: *Mus musculus* (laboratory mouse) and two Australian hydromyine species, *Notomys alexis* (spinifex hopping mouse) and *Pseudomys australis* (plains mouse).
Methods: Ovaries from young *M. musculus*, *N. alexis* and *P. australis* were fixed for 24h in Rossman's Fluid, washed in 95% alcohol, routinely processed and embedded in paraffin wax. 7 um sections were incubated with various FITC-labelled lectins and viewed by epifluorescent and phase contrast microscopy. Intensity of the lectin staining of the ZP around oocytes of antral follicles was determined both qualitatively and quantitatively with digital image analysis.
Results and Discussion: The ZP of all three species stained with lectins specific for N-acetylglucosamine (DSA, LEA, S-WGA and WGA), B-gal(1-4)N-acetylglucosamine (ECA) and B-gal(1-3)N-acetylgalactosamine (PNA), albeit at varying intensities; however, there was no staining with lectins specific for a-mannose (ConA), a-glucose (ConA), or a-fucose (UEA-I). The ZP of *M. musculus*, but not those of *N. alexis* or *P. australis*, stained with DBA and SBA, indicating presence of a-N-acetylgalactosamines. These results suggest that the ZP of the hydromyine rodents have more similar sugar composition to each other than either does to that of the more distantly related *M. musculus*. They do not support the hypothesis that closely related species have different ZP sugar composition; the findings thus question species differences in oligosaccharides and hence perhaps species-specificity of zona-sperm adhesion.

O32. Ploidy and development of artificial mouse oocytes and zygotes

B Heindryckx, S Lierman, J Van der Elst & M Dhont

Infertility Centre, Ghent University Hospital, Ghent, Belgium.

Introduction: For patients without gametes, there are currently no treatment options leading to genetically own children. These sterile patients have to rely on sperm or oocyte donation. Artificial production of gametes through haploidization may offer an alternative strategy. The artificial creation of a gamete involves the transfer of a diploid somatic nucleus into an enucleated oocyte followed by induction of polar body extrusion to reduce artificially the diploid chromosome number to haploid status. The aim of this study was to evaluate the efficiency of producing artificial oocytes and zygotes with correct ploidy. We also analysed the developmental capacity of the artificial zygotes and artificial oocytes fertilised by IVF or ICSI.
Methods: Somatic cumulus cell nuclei were injected into non-enucleated oocytes to produce artificial zygotes and into enucleated mature mouse oocytes to produce artificial oocytes. The expected ploidy of artificial zygotes and oocytes is 40 and 20 chromosomes, respectively. Further we verified whether different time intervals (3, 5, 8h) between injection and activation influenced the number of artificial oocytes and zygotes showing correct chromosome number. Finally fertilisation and developmental potential was investigated.
Results and Discussion: The expected chromosome numbers were found in 12% of artificial zygotes and 15% of artificial oocytes. Varying the time interval between injection of the somatic nucleus and activation (3, 5, 8hrs) tended to increase the efficiency up to 18% and 23%, respectively in the 5h time interval. Fertilization rate was 90% for artificial zygotes, 37% for artificial oocytes after IVF and 53% after ICSI. Blastocyst formation rates were 14, 8 and 9%, respectively. Ploidy analysis shows that the efficiency of obtaining artificial zygotes and oocytes with correct ploidy was low and that developmental potential was severely hampered. These observations question the possibility of obtaining chromosomally normal embryos from artificial oocytes or zygotes.

O33. PKA localises to mitochondria in mouse oocytes

RJ Webb & J Carroll

Department of Physiology, University College London, London, UK.

Introduction: It has recently been shown that both isoforms of the regulatory subunit of Protein Kinase A (PKA) RI and RII are present in mouse oocytes. PKA-RII is known to bind to A Kinase Anchoring Proteins (AKAPs) that are present on many intracellular structures, including the endoplasmic reticulum, mitochondria, golgi and the cytoskeleton. In oocytes the RII subunit shows a punctate distribution in the cytoplasm with an aggregation around the germinal vesicle (GV). The aim of this study is to identify the structure which binds the RII subunit in mouse oocytes.
Methods: Oocytes were obtained from PMSG primed and 14 day old MF1 mice. mRNA for the catalytic and regulatory subunits of PKA, fused with eCFP and YFP respectively, was microinjected and left for 2 hours to express prior to imaging. Mitochondria were labelled by incubation in tetramethylrhodamine ethyl (TMRE; 100 nm, 10 min). The cells were then scanned using a confocal microscope fitted with a metahead to allow the separation of the 2 closely emitting fluorophores YFP and TMRE.
Results and Discussion: In GV stage oocytes both PKA and mitochondria show a punctate distribution in the cytoplasm with perinuclear localisation around the GV, overlay of these two signals demonstrated that PKA was co-localised with the mitochondria (n=8). However, in meiotically incompetent oocytes obtained from pre-antral follicles, different patterns of distribution for PKA and mitochondria were observed. PKA was diffuse throughout the cytoplasm (n=7), while the mitochondria showed a punctate pattern throughout the oocyte. These results demonstrate a structure for localised PKA signalling is formed during the acquisition of meiotic competence and that this is localised to the mitochondria. (This study was supported by a project grant from the Wellcome Trust.)

O34. The effects of caffeine on MPF and MAPK kinase activities and parthenogenetic development of ageing ovine oocytes

JH Lee & KHS Campbell

Division of Animal Physiology, School of Biosciences, The University of Nottingham, Sutton Bonington Campus, Loughborough, UK.

Introduction: Maturation-promoting factor (MPF) and mitogen-activated protein kinase (MAPK) are key regulators of both meiotic and mitotic cycles. MII oocytes contain high levels of both kinases, however, these activities decline with age. Caffeine (an inhibitor of Myt1/Wee1 activity can increase MPF and MAPK activities in ovine oocytes (Lee and Campbell, 2004 Reprod Fertil Dev), however the effects of caffeine treatment on the activation and developmental potential of ovine oocytes is unknown. The aims of this study were to examine the effects of ageing and caffeine treatment on MPF and MAPK activities, activation rates and development.
Methods: Ovine oocytes were matured in vitro. Control and enucleated (16-18h post onset of maturation (hpm)) oocytes were cultured until 24 hpm and then treated with caffeine 10mM for 6 hr. 10 oocytes were sampled and analysed for MPF and MAPK as previously described (Ye JP et al.,2003 Reprod.125, 645-656). Oocytes were activated in medium containing 5ug/ml A23187, and then cultured in SOF, 7.5ug/ml cytochalasin B, with or without 10ug/ml of cycloheximide (CHXM) for 5 hrs. Cleavage was assessed on day 2 and development to blastocyst on day 7. Statistical analysis was performed using the Chi-square test.
Results and Discussion: Both kinases reached maximum activities 24 hpm and then decreased. Enucleation did not affect activities but caffeine treatment significantly increased both. The decline of MPF and MAPK on activation was not affected by caffeine treatment. A significant difference was observed in activation rates between A23187 alone or A23187 + CHXM in 24hpm oocytes (23.7% vs 83.6%) and 30hpm caffeine treated oocytes (42.8% vs 88.5%), but not in 30hpm control oocytes (92.2% vs 94.2%). Developmental to blastocyst was higher in 30hpm oocytes activated with A23187 + CHXM (30.2%) than 24hpm oocytes (22.2%), and in 30hpm caffeine treated oocytes a significant difference in development was observed (3.1% vs 30.2%).

O35. Connexin 43 as a parameter of cumulus functionality in meiotic maturation of bovine oocytes in vitro

M Tománek [1] & M Machatková [2]

[1]Department of Biology of Reproduction, Research Institute of Animal Production, 104 01 Prague - Uhrineves, Czech Republic; [2]Veterinary Research Institute, Brno, Czech Republic

Introduction: It was shown previously that mechanical disruption of cumulus-oocyte gap junctional communication by removal of cumulus cells before maturation did not prevent oocytes from resuming the meiotic maturation in vitro but resulted in suppressed developmental competence of oocytes and decreased number of blastocysts. The current study was aimed a) to study the effect of cdk kinases inhibitors roscovitine and butyrolactone-I octane identified in estrus and post estrus. The compounds, 9-octa decenoic acid and 4-methyl phenol are repeatedly identified only in the estrus urine sample. The present study concludes that the excretion of volatile compounds in the urine of female buffaloes differs from one phase to other, and findings provide additional support to the possibility of identifying the estrus phase of buffalo by detecting urinary pheromonal compounds.

P12. Expression of Nrip1 during development of the male reproductive tract of mice

VJ Pocock, R Hudson-Davies & SR Milligan

Centre for Reproduction, Endocrinology & Diabetes, King's College, London UK.

Introduction: The transcriptional activity of nuclear receptors is mediated by cofactor proteins (coactivators or corepressors) that are implicated in chromatin remodelling and the recruitment of the transcription machinery. Adult male mice devoid of *Nrip1* gene (encoding a nuclear receptor corepressor) have a high percentage of 'hairpin-looped' epididymal sperm. In the adult mouse, Nrip1 is present in Leydig cells, some seminiferous tubules, the efferent ducts and parts of the epididymis suggesting that *Nrip1* plays a role in both endocrine and spermatogenic functions in the adult male. The present study focussed on determining the pattern of *Nrip11* expression during early development in the testis and male tract.
Methods: *Nrip1* expression can be identified in *Nrip1* -- mice in which *Nrip1* has been replaced by a lacZ-neo fusion gene expressing beta-galactosidase. Wildtype, heterozygote and knockout male mice were killed at 2-day intervals from the day of birth to postnatal day 20 and the testes and reproductive tract were stained using X gal.
Results and Discussion: There was no endogenous beta-galactosidase activity in the testis or efferent ducts of wildtype animals at any age, but became evident in the vas deferens and cauda epididymis by day 12 and in the corpus epididymis by day 14. Endogenous activity was present in the initial segment of the caput epididymis by day16 and in the rest of the epididymis by day 20. In heterozygote and *Nrip1* -- mice, beta-galactosidase activity (reflecting *Nrip1* expression) was evident from day 0 and persisted until beyond Day 20 in the interstitial tissue and was present throughout the seminiferous tubules from day 12. There was also strong expression throughout development in the efferent ducts and along the whole length of the epididymis. These results are consistent with a regulatory role of *Nrip1* in nuclear receptor mediated responses of the male reproductive tract development.

P13. RNAi evidence that GDF9 mediates oocyte regulation of cumulus cell expansion in mice

L Gui & IM Joyce

School of Biology, University of Leeds, Leeds, UK.

Introduction: In mice, growth differentiation factor-9 (GDF9) is an oocyte-specific secreted protein that plays an essential role in early follicular development. However, the role of GDF9 at later stages of follicle development is uncertain. In this study, RNAi was used to knockdown GDF9 levels in oocytes in order to investigate the possible role of this protein in mediating oocyte regulation of cumulus expansion.
Methods: Fully-grown oocytes from mice maintained in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986 were injected with either GDF9 dsRNA; BMP15 (a closely related gene also expressed by oocytes) dsRNA or injection buffer alone and cultured for 24 h. To determine the efficacy of the RNAi procedure, oocytes were then used for measurement of GDF9 and BMP15 mRNA levels using real-time RT-PCR, and for measurement of GDF9 protein levels using Western blotting and immunofluorescence. To investigate the role of GDF9 in cumulus expansion, 24 h after injection oocytes in the three treatment groups were cocultured with oocytectomised cumulus cell complexes in the presence of 0.5 IU / ml rFSH for a further 24 h.
Results and Discussion: GDF9 dsRNA but not BMP15 dsRNA or injection buffer knocked down GDF9 mRNA levels in oocytes within 24 h of injection. Similarly, GDF9 protein levels were lower in the GDF9 dsRNA-injected oocytes. During a further 24 h culture period, oocytes injected with either BMP15 dsRNA or injection buffer alone, but not oocytes injected with GDF9 dsRNA, enabled FSH-stimulated cumulus cell expansion. This study strongly supports the idea that GDF9, but not BMP15, is a key mediator of oocyte-enabled cumulus cell expansion in mice. (This work was supported by project grants from the Royal Society and the BBSRC)

P14. Evolution of the putative sperm adhesion region of zona pellucida C glycoprotein in murid rodents: Is there evidence for species-selectivity?

CA Swann [1], S Cooper [2], RM Hope [1] & WG Breed [1]

[1]Department of Anatomical Sciences, University of Adelaide, Adelaide, Australia; [2]Evolutionary Biology Unit, South Australian Museum, Adelaide, Australia.

Introduction: The mammalian egg coat, the zona pellucida (ZP), is an extracellular matrix of 3 to 5 glycoproteins. Studies on the laboratory mouse suggest that ZPC O-linked oligosaccharides attached to the serine/threonine residues of exon 7 provide adhesive ligands for sperm receptors. Furthermore, exon 7 amino acid residues may be under positive Darwinian selection. Changes to the amino acid sequence within this region may alter glycosylation, and hence sugars available for sperm adhesion. If this adhesion is species-selective then the amino acid sequence of closely related species might differ. To test this we determined the amino acid sequence of the putative sperm adhesion region of ZPC from more than 40 species of Australian hydromyine rodents.
Methods: DNA was extracted using either the salt extraction method or PureGene DNA Isolation Kit (Gentra, Minneapolis, MN). Primers, designed from *Notomys alexis* cDNA, were used to PCR amplify, and sequence, the ZPC region of exon 6 through to exon 7, the sequences visually aligned, then compared.
Results and Discussion: Within the sperm adhesion region of exon 7 (Cys-328 to Gln-343), more than three-quarters of species show 100% amino acid identity. Unlike *Mus* and *Rattus*, all have an additional serine residue, at position 336 and all, except two *Melomys* species, have a second serine residue at position 341. Five species of pebble-mound mice have a serine to proline substitution at position 334 and two other species of *Pseudomys* have a serine to leucine substitution at position 331. This study shows that, in the Australian hydromyine rodent radiation, there was full conservation of the putative sperm adhesion region between closely-related species; it argues against this region, in at least this group of rodents, being under positive Darwinian selection.

P15. Centrosome functions in first cell cycle organisation of horse oocytes following parthenogenesis and nuclear transfer

Xihe Li & WR Allen

Department of Clinical Veterinary Medicine Equine Fertility Unit, University of Cambridge, Newmarket, UK.

Introduction: In animal cells, the microtubules of the cytoskeleton typically associate with the centrosome to assemble the complex microtubule-organizing centre (MTOC). Some animal cells lack centrosomes entirely yet they are still capable of forming complex microtubular structures such as the mitotic spindle. In this study, we monitored the functions of the centrosome on first cell cycle organisation in the horse oocytes following parthenogenesis and nuclear transfer.
Methods: MII oocytes generated by in vitro culture system were subjected to parthenogenetic stimulation or nuclear transfer. The oocytes were cultured for 12-14 h after each treatment and then fixed in a glycerol-based microtubule-stabilising solution followed by 2.5% paraformaldehyde in PBS (Simerly and Schatten, 1993). First cell cycle organisation was detected by indirect immuno-fluorescent staining, utilising a mouse anti-alpha-tubulin antibody which stained the microtubules green and a rabbit anti-gamma-tubulin antibody which stained the centrosome red. The stained oocytes were mounted under a coverslip in anti-fade mounting medium containing TOT3 (blue chromatin), and examined by confocal microscopy.
Results and Discussion: In parthenogenetically activated oocytes centrosomes were not found at opposite poles of the microtubular spindles at the MII stage, or after their further development following chromosomes separation. However, red staining gamma-tubulin did remain associated with the microtubules. On the other hand, 2-4 red-stained centrosome-like structures were observed in oocytes reconstructed by nuclear transfer, and