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Reproductive Biology of the Mice and Rats (Family Muridae) in New Guinea—Diversity and Evolution

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Abstract. In New Guinea there are around 100 species of native rodents in the family Muridae that are members of two tribes, the Hydromyini and Rattini, and five divisions—the Hydromys, Uromys, Mallomys, Pogonomys, and Rattus divisions. Here we review their basic reproductive biology so far as it can be determined from the material available. We find that females of most species in the Hydromys and Uromys divisions have 4 nipples, most species in the Pogonomys division have 6, whereas in the Mallomys division nipple number across species ranges from 2 to 6, and in the Rattus division from 4 up to 12. The number of fetuses observed in pregnant individuals in species of all of the hydromyine divisions was generally between 1 and 3 but in three species in the Rattus division up to 6, or even occasionally more, occurs. In males, the relative testes mass (RTM) of most species in the Hydromys, Uromys, Mallomys, and Rattus divisions was usually between 1 and 3% of body mass, whereas in the Pogonomys division it varied markedly from only around 0.4% in *Hyomys goliath* up to 5% in two species of *Pogonomys*. The spermatozoa of species in the Hydromys and Uromys divisions, like in the Australian species of these divisions, contained a head with an apical hook together and two ventral processes, whereas in the Pogonomys and Mallomys divisions marked interspecific differences occurred with some having a sperm head with an apical hook and ventral processes but in others there were no ventral processes but a long apical hook. Sperm tail length of most species was generally between 90 and 130 µm but *Chiruromys* and *Xenuromys* had sperm tail lengths of 150–153 µm. Male accessory sex glands were generally similar across the species except for that of the preputial glands which appeared to be absent in species of *Pogonomys* and *Chiruromys* but very large in *Hyomys*. The findings of large relative testes mass in *Pogonomys* and long sperm tails in *Chiruromys* and *Xenuromys* suggest selection for high levels of intermale sperm competition and hence multimale breeding systems in these species, whereas the variation in preputial gland size suggest interspecific differences in social organization.

Introduction

Australia, New Guinea and its adjacent land-bridge islands, Sahul, have a unique diversity of mammals with the original mammalian fauna being composed of marsupials and monotremes. However various groups of eutherian mammals also occur with murid rodents first arriving around 6 million years ago in the late Miocene or early Pliocene. Whereas there are, in Australia, around 60 species of native rodents in the family Muridae that make up around 20% of the current extant mammalian fauna, the number of species of mice and rats in New Guinea is still not known in detail

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although there appears to be at least 100 species (Tate, 1951; Menzies & Dennis, 1979; Flannery, 1995a, 1995b; Musser & Carleton, 2005; Aplin, 2006; Musser *et al*., 2008; Musser & Lunde, 2009; Helgen, 2005a, 2005b; Helgen & Helgen, 2009; Helgen *et al*., 2010; Aplin & Ford, 2013; Rowe *et al*., 2008, 2019; Roycroft *et al*., 2020) with only a few species occurring in both Australia and New Guinea.

Diversity of Australian rodents

In Australia, there are two major groups of murid rodents—the "Old Endemics", tribe Hydromyini, and the more recently arrived genus *Rattus*, classified in the tribe Rattini. The Hydromyini are composed of around 10 genera most of which are classified in the Pseudomys and Conilurus "divisions" (use of divisions as a semi-formal taxonomic grouping follows Musser & Carleton, 2005; Aplin & Helgen, 2010; Rowe *et al*., 2019). They vary markedly in body mass from just a few grams in the Delicate Mouse, *Pseudomys delicatulus*, up to nearly 1 kg in the case of the Water Rat, *Hydromys chrysogaster*, and the Giant White-tailed Rat, *Uromys caudimaculatus*. These species are present in a variety of habitats (see Watts & Aslin, 1981; Van Dyck & Strahan, 2008; Breed & Ford, 2007) although there are only a few species that occur in the rainforest environment with many species having adapted for living in semiarid or arid regions, with the most extreme arid-adapted species being members of the genus *Notomys,* or hopping mice. These old endemic hydromyine rodents include omnivores, herbivores, folivores, frugivores, and even two aquatic carnivores as in the case of the Water Rat (*H. chrysogaster*) and Water Mouse (*Xeromys myoides*) (Watts & Aslin, 1981; Breed & Ford, 2007; Van Dyck & Strahan, 2008; Aplin & Ford, 2013). Studies on the reproductive biology of females of these species have shown that, compared to many other murids, they have comparatively long pregnancies with females of nearly all species having only 4 nipples, which somewhat limits the number of pups that can be raised at any one time. By contrast, males vary greatly in their relative testes mass and thus in the numbers of sperm produced; a finding that suggests a considerable diversity across the species in the intensity of intermale sperm competition and hence breeding system (Kenagy & Trombulak, 1986; Breed, 1997b; Breed & Taylor, 2000). This is also suggested by the marked interspecific differences in quantity and quality of sperm produced (Breed, 1997a; McLennan *et al*., 2017).

In contrast to the "Old Endemics" the members of the genus *Rattus* include seven species which show much less diversity in body form (Rowe *et al*., 2011) though they occur in a variety of very different habitats. Females have much shorter gestation lengths than do those of the hydromyine rodents with considerable diversity in litter size, with species living in grasslands and deserts having the highest ovulation rates and potential litter sizes (see Taylor & Horner, 1973; Breed, 1978; Yom-Tov, 1985; Taylor *et al*., 1990; Breed & Ford, 2007; Geffen *et al.*, 2011).

Diversity of New Guinea rodents

In previous studies of rodent diversity in New Guinea Lidicker (1968) and Lidicker & Brylski (1987) suggested that there were five major tribes. These were the Hydromyini with species in the genera *Hydromys, Leptomys, Paraleptomys, Mayermys, Neohydromys, Pseudohydromys,* and *Xeromys*; the Uromyini, with species in the genera *Uromys, Melomys, Coccymys, Pogonomelomys,* and *Xenuromys*; the Anisomyini which included the "residue" of the old Papuan group in the genera *Anisomys, Hyomys, Lorentzimys, Mallomys, Pogonomys, Macruromys,* and *Chiruromys*; the Conilurini with just two, largely Australian, genera, represented by *Conilurus penicillatus* and *Pseudomys delicatulus*; and the Rattini, with species of *Rattus.* Subsequently Watts & Baverstock (1994) questioned the monophyly of the hydromyin and uromyin clades and included within their Hydromyini the various genera that had previously been placed in both of these groups. These authors suggested that "further clarification" (p. 303) of the position of *Lorentzimys, Coccymys,* and some *Melomys* species also needed to be investigated and they suggested that *Mallomys* "may be misplaced" (p. 303). In more recent decades, New Guinea murines have been subject to considerable clarifying taxonomic review and revision (e.g., Flannery, 1995a, 1995b; Menzies, 1996; Musser & Carleton, 2005; Helgen, 2005a, 2005b; Musser *et al*., 2008; Helgen & Helgen, 2009; Musser & Lunde, 2009; Helgen *et al*., 2010), and murine tribes and divisions, including those of the New Guinea species, have been investigated extensively using molecular phylogenetic methods (e.g., see Lecompte *et al*., 2008; Smissen & Rowe, 2018; Rowe *et al*., 2019; Roycroft *et al*., 2020). In the current study, we follow the systematic arrangement as detailed by Roycroft *et al*. (2020) which includes the following five divisions and two tribes in New Guinea and adjacent islands.

1. **Hydromys division (tribe Hydromyini)** with the genera *Hydromys*, *Parahydromys*, *Baiyankamys, Crossomys, Xeromys, Leptomys, Paraleptomys, Microhydromys, Pseudohydromys,* and *Mirzamys*. Helgen (2005a, 2005b), Helgen & Helgen (2009), Helgen *et al*. (2010), and Musser *et al*. (2008) have recently expanded this division to include a number of new species; additionally Helgen (2005b) showed that *Baiyankamys* deserves generic recognition, and Helgen & Helgen (2009) described a new genus, *Mirzamys*.

2. **Uromys division (tribe Hydromyini)** with the genera *Uromys, Melomys, Paramelomys, Protochromys,* and *Solomys*.

3. **Pogonomys division (tribe Hydromyini)** with the genera *Pogonomys, Hyomys, Chiruromys, Anisomys*, and *Lorentzimys*.

4. **Mallomys division (tribe Hydromyini)** which includes species in the genera *Mallomys, Coccymys, Abeomelomys, Pogonomelomys,* and *Mammelomys*. Musser & Lunde (2009) revised the genus *Coccymys* and recognized an additional genus, *Brassomys*, which likely also belongs in this division.

5. **Rattus division (tribe Rattini)** with the various species of *Rattus.*

Aims of current study

In the current study an overview of the female and male reproductive biology of the rodents from New Guinea is presented. Since many of the species are poorly known, and only a very few have been bred in captivity, knowledge of their reproductive biology is, by necessity, very limited. For females some indication of reproductive potential can be determined from the number of nipples, and the number of fetuses found in utero of pregnant specimens, and these data are given here where known. For males an indication of intensity of intermale sperm competition, and hence the breeding system, can be inferred from their relative testes mass (RTM) (Harcourt *et al*., 1981; Kenagy & Trombulak, 1986; Parker, 1993, 2016; Birkhead & Møller, 1998; Gómez Montoto *et al*., 2011), as well as from some aspects of their sperm head morphology and sperm tail length (see Gomendio & Roldan, 1991; Immler *et al*., 2007; Pitnick *et al*., 2009; Tourmente *et al*., 2011; Simmons & Fitzpatrick, 2012; Šandera *et al*., 2013; Van der Horst & Maree, 2014; McLennan *et al*., 2017; Pahl *et al*., 2018; Peirce *et al*., 2018). Thus the testes weight and body mass together with the testis organization and sperm morphology will be given here where known. Because the size and abundance of the various male accessory sex organs may also reflect the intensity of postcopulatory sexual selection (Ramm *et al*., 2005) and/or social organization (Bronson & Caroom, 1971; Brown & Williams, 1972; Zhang *et al.,* 2008), these morphological traits are also summarized. Similarities and differences in the reproductive biology of the various species across the five divisions will be discussed and where the data suggest a potential breeding system it will be indicated.

Materials and methods

Specimens discussed here are vouchered in the mammalogical collections of the Australian Museum, Sydney (AM M.); the Australian National Wildlife Collection, CSIRO, Canberra (ANWC M); the Bernice P. Bishop Museum, Honolulu (BBM-NG); the Queensland Museum, Brisbane (QM JM); and the South Australian Museum (SAM M). Additional, non-vouchered specimens of *Hydromys chrysogaster* were wild caught in South Australia (see Leigh & Breed, 2020).

Most of the material used in this study are specimens that had been fixed in formalin, most of which had their body cavity opened, with the consequent fixation of the reproductive organs. For examination the gastrointestinal tract was displaced to one side so that the reproductive tract could be visualized. If the individual was female the uterus was inspected for indication of the presence of fetuses and, when present, the number of swellings recorded.

In scrotal males, one or both testes and epididymides were removed, and testis weight of the individual determined. When one testis was obtained its weight was doubled to give the approximate total testis mass for the individual. To gain some insight into sperm production, histology of the testis was carried out on some individuals and, for this, small pieces of tissue were transferred to 0.1 M buffered formaldehyde/glutaraldehyde, dehydrated by passing the tissues through a series of alcohols and then embedding in epoxy resin. Sections were cut, at 0.5 to 1 μm thickness, with an ultramicrotome and stained with toluidine blue in 0.5% sodium tetraborate. Some indication of the sperm production within the testes was then determined by ascertaining the relative proportion of seminiferous tubules to interstitial tissue in the testes cross sections by bright field light microscopy linked to an image analysis system.

From most of the males, spermatozoa were extruded from the cauda epididymides into 10% buffered formaldehyde and then observed by phase contrast or Nomarski optics light microscopy. If the sperm head had an apical hook and/or ventral processes, the latter of which is a characteristic feature of most species of Australian hydromyine rodents, their approximate lengths were determined by measuring the distance from the base of the apical hook, and when present, the base of the ventral processes, to the tips of these processes as previously indicated in McLennan *et al*. (2017) and Pahl *et al*. (2018). The maximum length of the sperm tail was also recorded. Care was taken to only measure intact spermatozoa. Some spermatozoa were stained with 4^1-6^1-diamidino-2-phenylindole dihydrochloride (DAPI), Sigma, for fluorescence microscopy using a UV filter performed to determine the shape of the sperm nucleus. Scanning electron microscopy of cauda epididymal spermatozoa from most species was also undertaken (Breed & Leigh, 2009). For this, sperm were attached to polylysine coated coverslips, dehydrated by passing the coverslips through a graded series of acetones, critical point dried, and coated with 10 nm of carbon and 5nm of platinum. They were subsequently viewed at 20 kV with a Philips XL20 SEM.

Male accessory sex glands from some individuals, including seminal vesicles, coagulating glands, ventral and dorsal prostates, and preputial glands, if present, were also dissected and camera lucida drawings of the accessory sex glands were made, indicating the relationships between the glands from anterior, posterior, and lateral perspectives. These drawings were then used to obtain measurements, when possible, of the maximum length and width of the seminal vesicles, coagulating glands, ventral prostates and preputial glands (Linzey & Layne, 1969). Since the glands occur in pairs, measurements of each gland were obtained separately from anterior, posterior and/or lateral perspective and the values averaged to give a single measurement of the length and maximum width of the glands of each individual. For the seminal vesicles, measurements of length were taken from the attachment of the gland to the rest of the male reproductive tract, along the midline longitudinal axis, to its tip, following the gland's curvature. The width was taken as the average maximum width of the proximal, uncurved segment of the gland. For the coagulating glands and ventral prostates, the greatest length from the base to the tip, and width across the widest part of the gland, were recorded. Similarly, the size of preputial glands, when present, was also documented.

Male reproductive tracts used came from the following specimens:

Hydromys division

Hydromys chrysogaster (Hc38, Hc40, Hc41, Hc42, Hc61, Hc58, Hc100) (field numbers from Leigh & Breed, 2020)
Leptomys elegans (AM M.18618)
Leptomys ernstmayri (AM M.14862)
Parahydromys asper (AM M.17319)
Paraleptomys rufilatus (BBM-NG 104629)
Pseudohydromys pumehanae (AM M.14827, AM M.15324)
Xeromys myoides (ANWC M10844)

Uromys division

Melomys leucogaster (AM M.14655)
Melomys lutillus (AM M.16396, AM M.18597, ANWC M29326, ANWC M29330, ANWC

M29331)
Melomys rufescens (AM M.13485, AM M.15177, AM M.19052, AM M.19053, AM M.21678)
Paramelomys lorentzii (AM M.32089, ANWC M35536)
Paramelomys mollis (ANWC M35526, ANWC M35509)
Paramelomys platyops (AM M.14671, AM M.14828, AM M.16168, AM M.18625, AM M.21682)
Paramelomys rubex (AM M.16252, AM M.24994)
Protochromys fellowsi (ANWC M10141)
Uromys caudimaculatus (AM M.16697, ANWC M24354, ANWC M29303, QM JM2041)
Uromys anak (AM M.16695)

Pogonomys division

Anisomys imitator (AM M.13770)
Chiruromys forbesi (AM M.19956, AM M.29318)
Chiruromys lamia (AM M.28325)
Chiruromys vates (AM M.14658, AM M.17172, AM M.18590, AM M.18594)
Hyomys goliath (AM M.18452, AM M.18487)
Lorentzimys nouhuysi (AM M.13778, ANWC M35465, ANWC M35850)
Pogonomys championi (AM M.13463, AM M.13502, AM M.13719, AM M.17721)
Pogonomys loriae (AM M.13792, AM M.13828, AM M.15119, AM M.15126, AM M.15127, ANWC M35897, ANWC M35898)
Pogonomys macrourus (AM M.13802, AM M.15137, AM M.15149, AM M.24974, AM M.30295,)
Pogonomys sylvestris (ANWC M25472, AM M.2832, ANWC M29428, ANWC M29430, ANWC M29432)

Mallomys division

Abeomelomys sevia (AM M.13465)
Mallomys aroaensis (AM M.17362)
Mammelomys rattoides (ANWC M35847, BBM-NG 22308)
Mammelomys lanosus (BBM-NG 100148)
Xenuromys barbatus (AM M.17363, AM M.17703)
Coccymys shawmayeri (BBM-NG 100673)

Rattus division

Rattus leucopus (AM M.13934, AM M.14686, ANWC M35541, ANWC M35545, QM JM2388, QM JM974)
Rattus niobe (AM M.14710, AM M.14714, AM M.21689, ANWC M35545, ANWC M35541)
Rattus praetor (ANWC M35881, ANWC M35885)
Rattus sordidus (ANWC M29343, ANWC M29354, QM JM1392)
Rattus steini (AM M.14651, AM M.14880, ANWC M35887, ANWC M14880)
Rattus verecundus (ANWC M14832, SAM M15124)

Results

Female reproductive biology

The number of nipples recorded in the literature for each species is summarized in Table 1. The data show that there are considerable interspecific differences across the divisions in the number of nipples present (see Fig. 1).

Nearly all females of species in the Hydromys and Uromys divisions have 4 nipples, all of which were inguinally located, with the one exception being *Pseudohydromys patriciae*, which had only 2 inguinal nipples.

All species in the Pogonomys division have 6 nipples, with a pair of pectoral nipples in addition to the 2 pairs of inguinal nipples, except the species of *Hyomys* and *Macruromys*, which have 4, with 2 pairs of inguinal nipples.

The Mallomys division shows the greatest variation in nipple number. Species of *Mammelomys* have only 2 nipples. *Abeomelomys* and *Pogonomelomys* have 4. Species of *Mallomys*, *Coccymys,* and *Xenuromys* have 6, with a pair of pectoral nipples in addition to the 2 pairs of inguinal nipples.

Species in the Rattus division (genus *Rattus*), also showed marked interspecific differences in nipple number which ranged from 4 in *Rattus vandeuseni*, to 6 in *R. leucopus, R. niobe, Rattus omichlodes,* and *R. verecundus*, 8 in most species of New Guinea *Rattus*, and up to 12, with 3 pairs of pectoral as well as 3 pairs of inguinal, nipples in *R. sordidus* (see Table 1, Fig. 1). In *R. steini* and *R. jobiensis* the number reported varies from 6 to 8 (Flannery, 1995a, 1995b).

Is this difference in nipple number reflected in the number of fetuses in the uteri of pregnant individuals of these species? The results show that most pregnant individual members of the Hydromys and Uromys divisions had only 1 or 2 fetuses although in *Melomys lutillus* and *M. rufescens,* as well as *H. chrysogaster,* and the two *Uromys* species, 3 or even occasionally 4, occurred (see Table 1, Fig. 2). In the Pogonomys division, amongst the species with 6 nipples, individuals of 3 species of *Pogonomys* and one species of *Chiruromys* species had up to 3 fetuses with a few others having just 1 or 2, whereas only one fetus was present in the pregnant *Mallomys* and *Hyomys* individuals in spite of having 6 nipples. Similarly in the pregnant *Mammelomys rattoides,* which has 2 nipples, only a single fetus occurred. *Rattus* species with 6 or 8 nipples had an average of 2 to 4 fetuses, whereas in *R. sordidus* up to 9 fetuses have been recorded as being present although only 2 were present in the pregnant individual that we dissected (see Table 1, Fig. 2).

Male reproductive biology

Relative testes mass (RTM) in members of the Hydromys and Uromys divisions was generally similar across species and ranged from 0.8 to 2.9% of body mass (see Table 2). In the Pogonomys division a considerably greater range of RTM occurred across the species (see Fig. 3, Table 2), with small-bodied species like *Pogonomys macrourus* and *P. championi* tending to have a very large relative testes mass around 5% of body mass. By contrast, in a sexually mature *Hyomys goliath* specimen the RTM was just 0.4%. In the Mallomys division RTM ranged from 0.8 to 2.4%, and in the Rattus division from 1.1 to 3.0% (see Table 2, Fig. 3).

The percentage of the testis occupied by the sperm producing seminiferous tubules varied from 71–76% in *Hydromys chryogaster* and *Mallomys aroaensis* to over

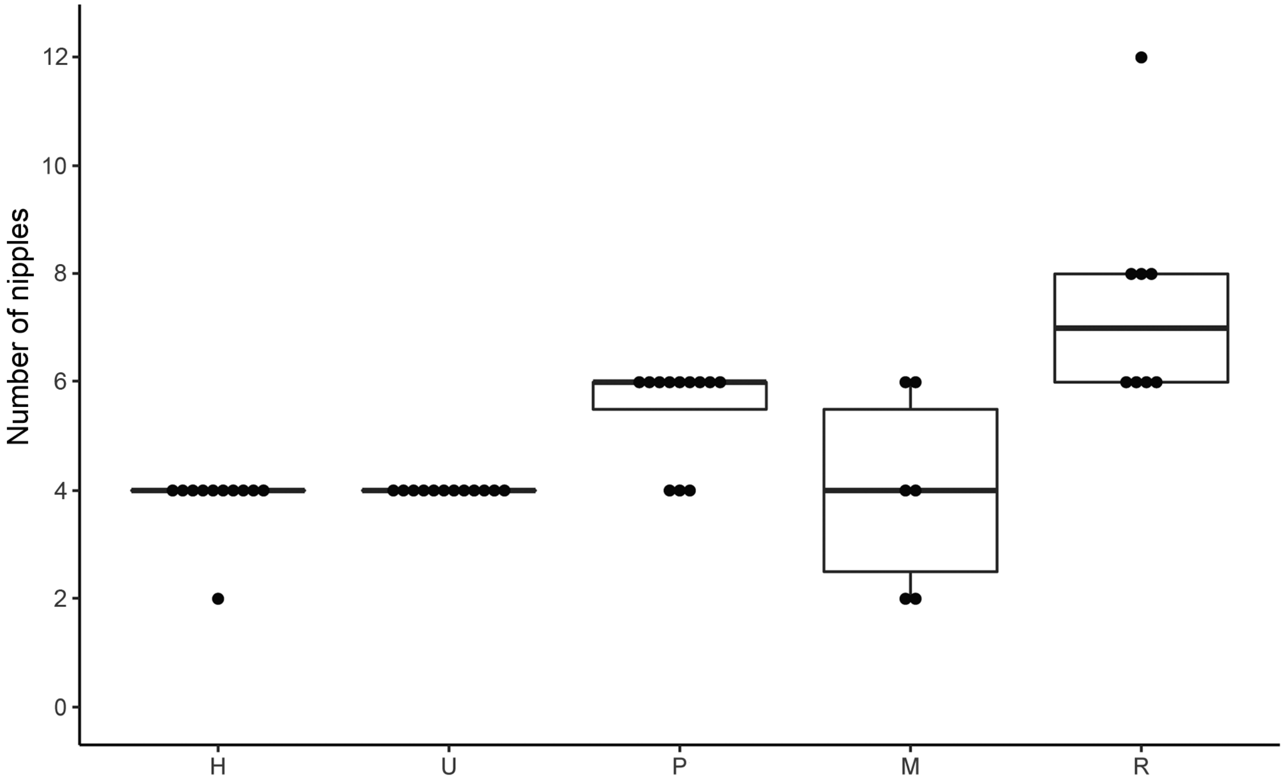

Figure 1. Boxplot showing nipple numbers for species in the divisions (H) Hydromys, (U) Uromys, (P) Pogonomys, (M) Mallomys, and (R) Rattus.

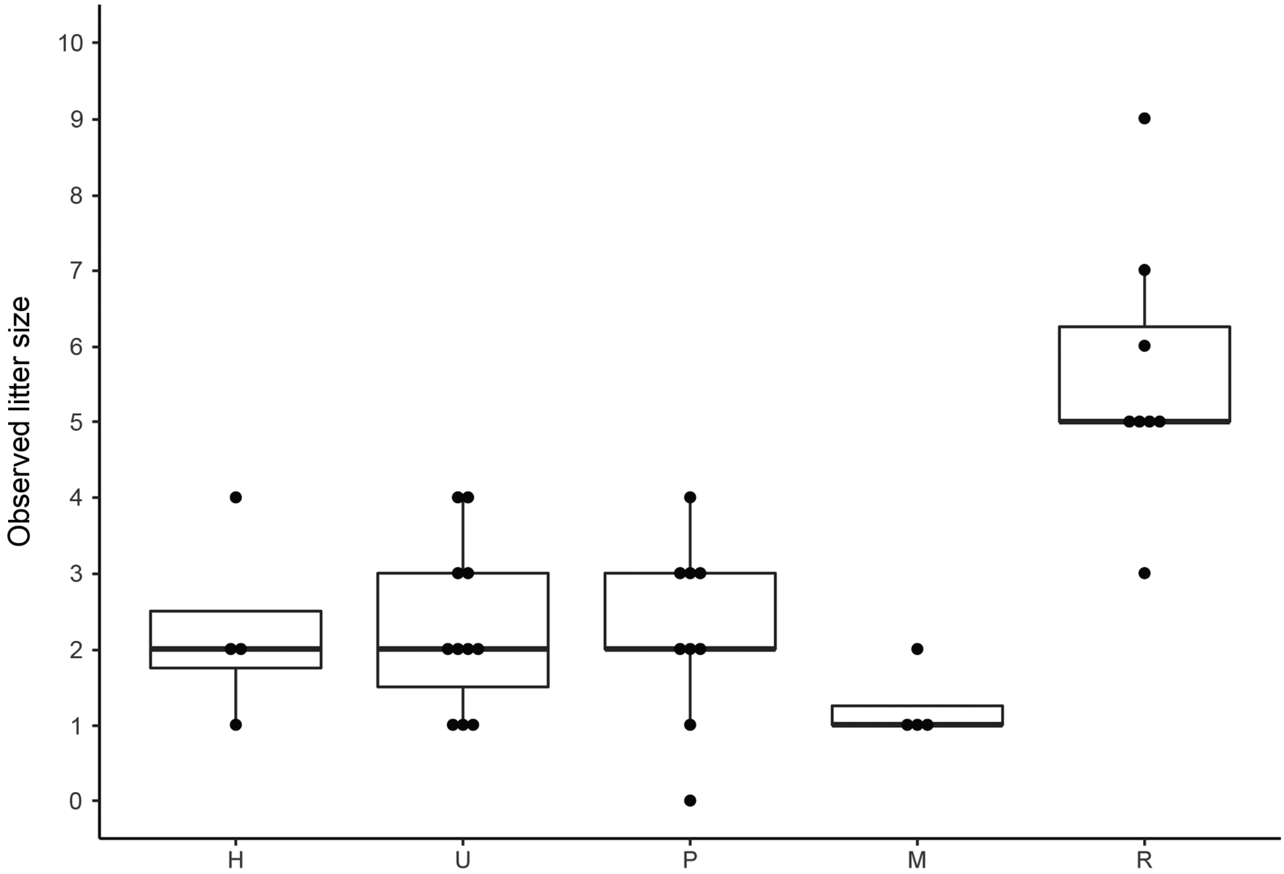

Figure 2. Boxplot showing maximum numbers of fetuses across the species in the divisions (H) Hydromys, (U) Uromys, (P) Pogonomys, (M) Mallomys, and (R) Rattus.

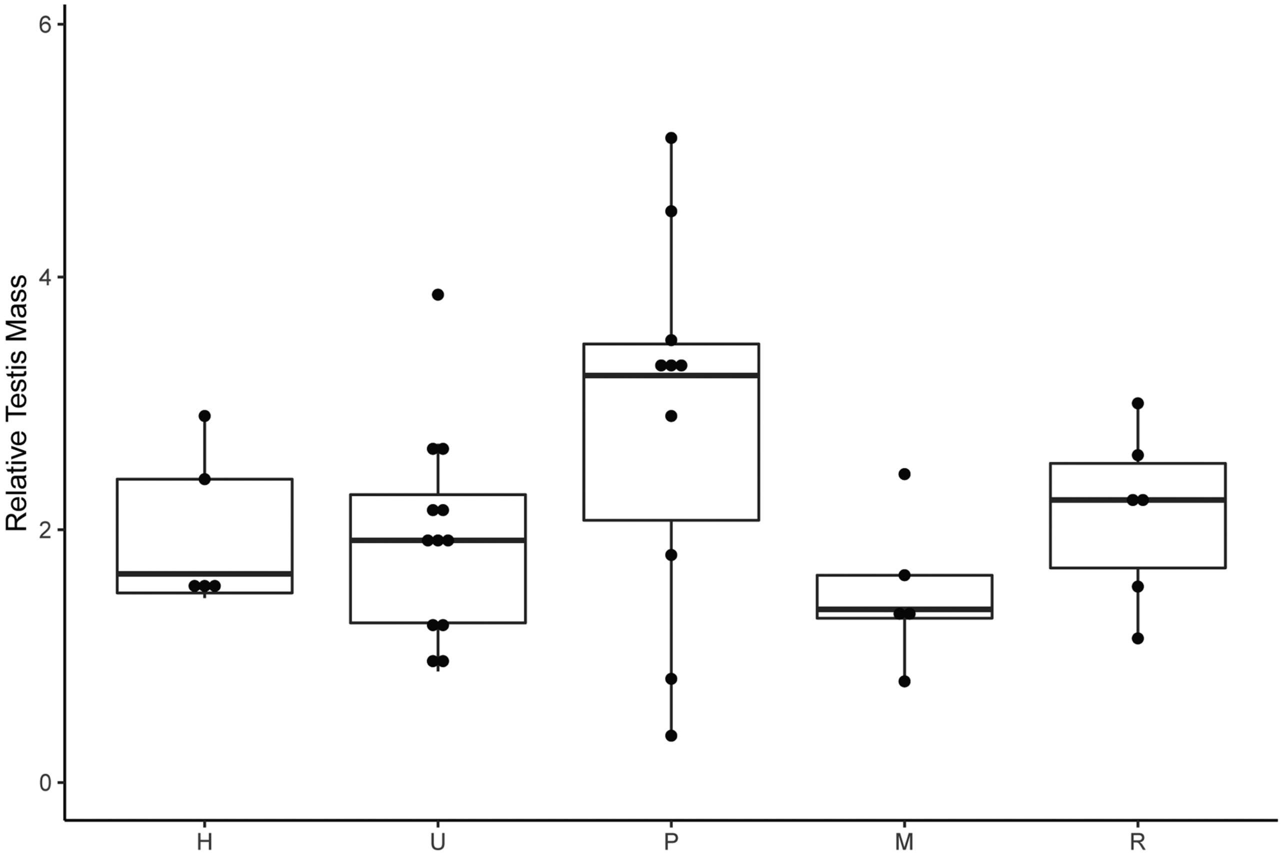

Figure 3. Boxplot of relative testes mass (g) for species in the divisions (H) Hydromys, (U) Uromys, (P) Pogonomys, (M) Mallomys, and (R) Rattus.

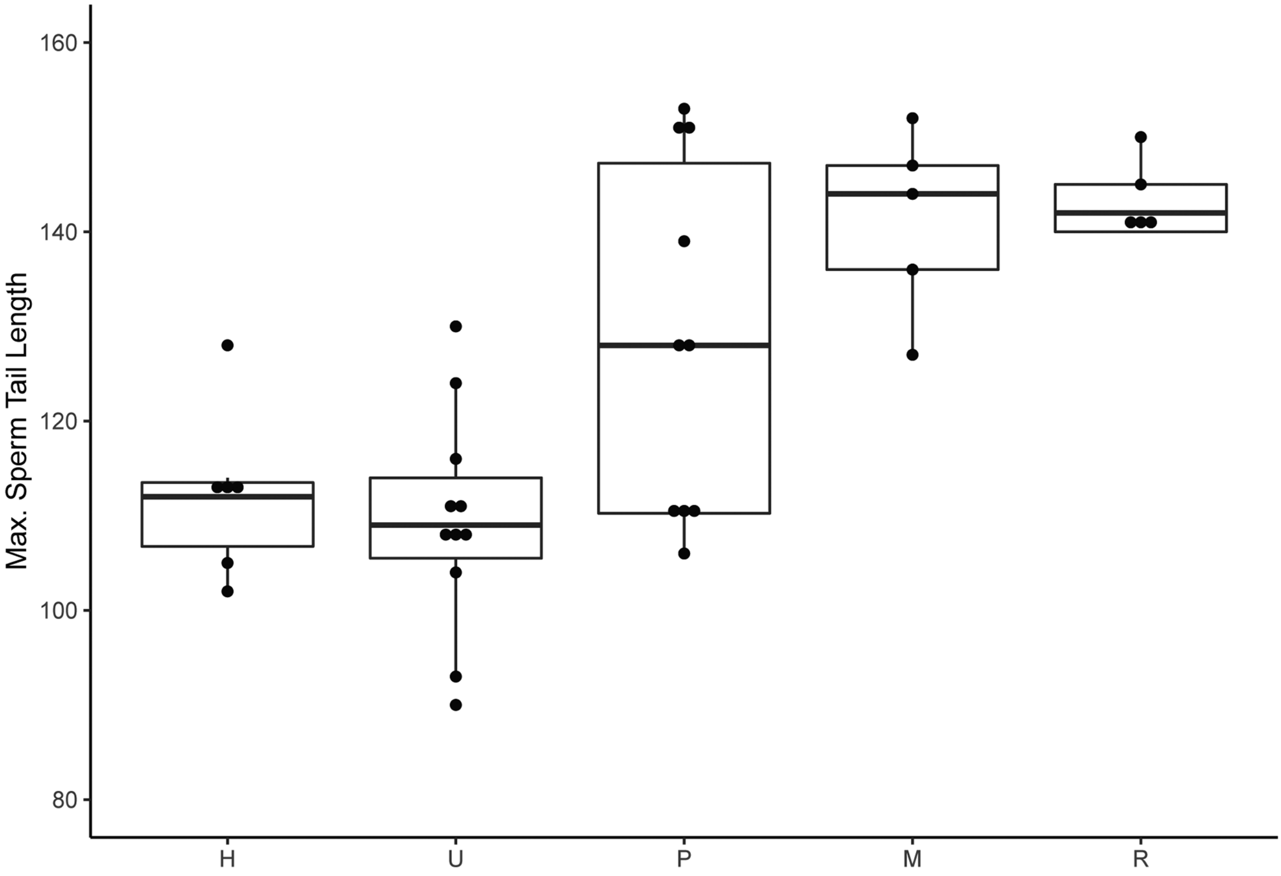

Figure 4. Boxplot of maximum sperm tail length (µm) for species in the divisions (H) Hydromys, (U) Uromys, (P) Pogonomys, (M) Mallomys, and (R) Rattus.

Table 1. Data on female reproductive morphology of New Guinea rodents. Average and range of body mass, number of nipples (with number of pairs of pectoral and inguinal nipples given in brackets), and female fetal number where known (as quoted in the literature). Data for number of fetuses of dissected specimens at the Australian National Wildlife Collection, Canberra (ANWC) and the South Australian Museum, given with specimen numbers (e.g., 2F- = 2 fetuses).

species	tribe	division	body mass (g)	number of nipples	female fetal number
Hydromys chrysogaster	Hydromyini	Hydromys	700 ± 14	4 (0+2)	up to 4, 2F-M35849
Parahydromys asper	Hydromyini	Hydromys	540	4 (0+2)	2
Crossomys moncktoni	Hydromyini	Hydromys	165	4 (0+2)	—
Microhydromys spp.	Hydromyini	Hydromys	—	4 (0+2)	—
Baiyankamys shawmayeri	Hydromyini	Hydromys	78 ± 7.4	4 (0+2)	—
Leptomys erstmayeri	Hydromyini	Hydromys	42	4 (0+2)	1
Leptomys elegans	Hydromyini	Hydromys	80, 66–90	4 (0+2)	av. 1.75, 1
Pseudohydromys pumahanae	Hydromyini	Hydromys	17 ± 3	4 (0+2)	—
Pseudohydromys patriciae	Hydromyini	Hydromys	22	2 (0+1)	—
Mirzamys louiseae	Hydromyini	Hydromys	—	4 (0+2)	—
Xeromys myoides	Hydromyini	Hydromys	42	4 (0+2)	—
Melomys leucogaster	Hydromyini	Uromys	65–156	4 (0+2)	2
Melomys rufescens	Hydromyini	Uromys	c. 60, 54 ± 6	4 (0+2)	av. 2, (1–4)
Melomys lutillus	Hydromyini	Uromys	30–40	4 (0+2)	av. 2.2, 3F-M29329
Protochromys fellowsi	Hydromyini	Uromys	—	4 (0+2)	—
Paramelomys moncktoni	Hydromyini	Uromys	c. 100	4 (0+2)	2
Paramelomys mollis	Hydromyini	Uromys	85, 72–97	4 (0+2)	1
Paramelomys lorentzii	Hydromyini	Uromys	130–150	4 (0+2)	1, 1F-M35529
Paramelomys platyops	Hydromyini	Uromys	65–80	4 (0+2)	1
Paramelomys rubex	Hydromyini	Uromys	c. 48, 52 ± 6	4 (0+2)	2, 2F-M15119
Paramelomys levipes	Hydromyini	Uromys	100–130	4 (0+2)	1–2
Uromys caudimaculatus	Hydromyini	Uromys	450–695	4 (0+2)	av. 2.5
Uromys anak	Hydromyini	Uromys	450	4 (0+2)	4
Chiruromys vates	Hydromyini	Pogonomys	23–68	6 (1+2)	1F-M15108, 2F-M15109
Chiruromys forbesi	Hydromyini	Pogonomys	—	6 (1+2)	1–3, 1F-M2815
Chiruromys lamia	Hydromyini	Pogonomys	40–48	6 (1+2)	—
Hyomys goliath	Hydromyini	Pogonomys	882	4 (0+2)	1
Pogonomys loriae	Hydromyini	Pogonomys	70–128	6 (1+2)	2–3
Pogonomys macrourus	Hydromyini	Pogonomys	35–56	6 (1+2)	1–3
Pogonomys championi	Hydromyini	Pogonomys	41–59	6 (1+2)	2
Pogonomys sylvestris	Hydromyini	Pogonomys	38 ± 5	6 (1+2)	2–3
Macruromys elegans	Hydromyini	Pogonomys	—	4 (0+2)	—
Macruromys major	Hydromyini	Pogonomys	350	4 (0+2)	—
Anisomys imitator	Hydromyini	Pogonomys	500–525	6 (1+2)	—
Lorentzimys nouhuysi	Hydromyini	Pogonomys	12–22	6 (1+2)	2
Xenuromys barbatus	Hydromyini	Mallomys	1000	6 (1+2)	—
Abeomelomys sevia	Hydromyini	Mallomys	52, 60–66	4 (0+2)	1
Mallomys rothschildi	Hydromyini	Mallomys	925–1500	6 (1+2)	1
Mammelomys lanosus	Hydromyini	Mallomys	112	2 (0+1)	—
Mammelomys rattoides	Hydromyini	Mallomys	172–236	2 (0+1)	1F-M35841
Pogonomelomys mayeri	Hydromyini	Mallomys	110	4 (0+2)	2
Coccymys shawmayeri	Hydromyini	Mallomys	26	6 (1+2)	—
Rattus niobe	Rattini	Rattus	36–50	6 (1+2)	av. 2 (1–3)
Rattus verecundus	Rattini	Rattus	55 ± 6	6 (1+2)	av. 2.8 (1–5)
Rattus steini	Rattini	Rattus	110–220	8 (2+2) & 6 (1+2)	av. 3.4 (2–5)
Rattus leucopus	Rattini	Rattus	175–315	6 (1+2)	av. 3.5 (2–5), 4F-M29321
Rattus novaeguinae	Rattini	Rattus	129	8 (2+2)	av. 5 (4–6)
Rattus sordidus	Rattini	Rattus	c. 100	12 (3+3)	av. 6 (2-9), 2F-M29339
Rattus jobiensis	Rattini	Rattus	—	8 (2+2) & 6 (1+2)	—
Rattus vandeuseni	Rattini	Rattus	—	4 (0+2)	—

Data sourced from: Dwyer, 1975; Flannery, 1995a, 1995b; Helgen & Helgen, 2009; Helgen, 2005a, 2005b; Helgen, 2007; Helgen *et al.*, 2010; Jackson & Woolley, 1993; McPhee, 1988; Menzies & Dennis, 1979; Menzies, 1996; Musser & Lunde, 2009; Musser *et al.*, 2008; Olson, 1982; Taylor & Horner, 1973; Taylor *et al.*, 1990; and Woolley & Furby, 1996.

90% in several species with small body mass (*Paramelomys platyops, P. rubex, Chiruromys vates, C. lamia,* and *Lorentzimys nouhuysi*), all having at least 92% of the relative area of the testis occupied by seminiferous tubules (Table 2).

The morphology of the spermatozoon also differed markedly across the species in the various divisions (Figs 5–8). Nevertheless, in members of the Hydromys division it was very similar across the species with the sperm head having both an apical hook and two ventral processes extending from its upper concave surface which were a little longer in *Leptomys* and *Paraleptomys* (Fig. 5a, Table 2) with the length of the sperm tail ranging from 102–128 µm (see Fig. 4, Table 2). In members of the Uromys division, similar sperm morphology with a sperm head having an apical hook and two ventral processes also invariably occurred with the length of the apical hook and ventral processes generally

Table 2. Data on male reproductive morphology of New Guinea rodents. Abbreviations: *H*—tribe Hydromyini or Hydromys division, *M*—Mallomys division, *P*—Pogonomys division, *R*—tribe Rattini or Rattus division, *U*—Uromys division (see Roycroft *et al*., 2020). Data of *Hydromys chrysogaster, Xeromys myoides*, and *Uromys caudimaculatus* were from Australian specimens.

species	tribe	division	no. males sampled	male body mass (g), mean ± SD	mean (± SD) testis mass (mg)	mean RTM	% seminiferous tubules in testes sections	sperm head apical hook length µm	sperm head ventral process length µm	maximum sperm tail length µm	mean accessory sex glands, length×width (mm)		
											seminal vesicles	coagulating glands	ventral prostates
Hydromys chrysogaster	H	H	4	804 ± 53	13780 ± 2320	1.7	71.2	2	2	112	—	—	—
Parahydromys asper	H	H	1	490	7152	1.46	—	2.5–3	2.5–3	112	32.7×6.9	12.5×4.0	7.4×6.5
Leptomys spp.	H	H	2	61 ± 12	1432 ± 271	2.4	—	5	4	128	19.7×4.6	7.9×3.0	5.9×4.0
Paraleptomys rufilatus	H	H	1	54	440	0.81	—	5	4	105	—	—	—
Pseudohydromys pumehanae	H	H	2	19 ± 2	539 ± 142	2.9	—	2.5	2.5	114	24.0×4.8	5.7×2.5	5.5×2.7
Xeromys myoides	H	H	1	34	568	1.65	89.6	3.5	2.5	102	—	—	—
Melomys leucogaster	H	U	1	104	1102	1.04	87	3	3	104	—	—	—
Melomys rufescens	H	U	5	71 ± 15	1341 ± 521	1.74	—	3	2.5	112	34.2×7.6	14.3×5.0	10.6×5.1
Melomys lutillus	H	U	5	40 ± 14	806 ± 131	2.09	—	2.5	2.5	109	25.1×5.2	9.5×3.6	7.4×5.5
Paramelomys mollis	H	U	2	118 ± 42	2386 ± 48	2.17	—	3	3	107	—	—	—
Paramelomys lorentzii	H	U	2	129 ± 9.2	2476 ± 746	1.92	—	4	2.5	130	35.5×10.4	15.7×5.9	7.9×4.3
Paramelomys platyops	H	U	5	90 ± 14	1670 ± 447	1.93	92.4	3	3	110	33.2×6.2	15.1×5.7	8.0×4.1
Paramelomys rubex	H	U	2	80 ± 28	1847 ± 35	2.59	94.4	2.5	2.5	108	29.3×6.1	12.0×5.7	9.2×4.4
Protochromys fellowsi	H	U	1	132	1600	1.21	—	3	2.5	—	—	—	—
Uromys caudimaculatus	H	U	4	825 ± 162	7440 ± 1445	0.93	90	5	5	90	56.8×9.1	23.0×6.9	14.3×5.9
Uromys anak	H	U	1	879	11592	1.31	—	3	2.5	93	76.4×13.0	31.1×8.3	13.7×7.6
Chiruromys vates	H	P	4	45 ± 3.5	1557 ± 187	3.63	93.8	3	2.5	152	29.0×5.3	12.6×5.0	6.9×4.6
Chiruromys forbesi	H	P	2	112 ± 13	2082 ± 391	1.8	—	2.5	2.5	150	34.5×5.9	15.1×5.4	6.0×3.4
Chiruromys lamia	H	P	1	120	2292	1.91	93.1	2.5	2.5	153	24.0×4.1	10.4×3.6	5.3×3.2
Hyomys goliath	H	P	2	1000 ± 10	3751 ± 1091	0.38	86.4	5.5	0	128	66.6×9.6	20.9×5.6	20.7×8.5
Pogonomys macrourus	H	P	5	49 ± 8.4	2462 ± 356	5.14	88.7	3	3	110	32.5×7.5	10.6×3.2	7.7×4.5
Pogonomys championi	H	P	2	53 ± 1.4	2097 ± 117	5.16	—	3	3	106	28.7×6.9	11.3×4.2	6.2×3.4
Pogonomys sylvestris	H	P	4	50 ± 7.4	1556 ± 239	3.12	85.3	3	3	111	23.0×4.5	9.0×2.9	4.5×2.5
Pogonomys loriae	H	P	6	104.8 ± 11.4	3483 ± 657	3.31	85.8	2	2	110	30.7×6.8	11.4×3.8	8.0×4.6
Anisomys imitator	H	P	1	508	4167	0.82	—	5	0	139	—	—	—
Lorentzimys nouhuysi	H	P	3	17 ± 1.3	403 ± 82	2.35	92.8	5	0	128	13.6×2.9	7.2×2.5	3.4×2.4
Abeomelomys sevia	H	M	1	52	854	1.64	—	7	0	144	—	—	—
Mallomys aroaensis	H	M	1	1087	14350	1.37	76.7	6	4	147	60.5×14.0	17.6×4.3	15.3×8.2
Mammelomys lanosus	H	M	1	123	3004	2.44	83.6	6	0	136	—	—	—
Mammelomys rattoides	H	M	2	216 ± 1.4	1621 ± 30	0.8	—	6	0	127	24.5×4.7	7.6×3.2	9.1×5.5
Xenuromys barbatus	H	M	2	1000 ± 141	18941 ± 14	1.8	—	7	0	152	43.8×10.2	10.5×6.0	10.4×5.7
Coccymys shawmayeri	H	M	1	—	—	—	—	5	0	124	—	—	—
Rattus niobe	R	R	5	47 ± 6	1450 ± 698	3	—	6	0	140	25.8×4.9	10.6×4.0	8.9×4.8
Rattus verecundus	R	R	1	100	1984	1.98		6	0	145	25×4.3	10.7×3.8	7.4×0
Rattus steini	R	R	3	88 ± 22	1045 ± 334	1.33	—	6	0	150	—	—	—
Rattus leucopus	R	R	4	146 ± 51	3289 ± 922	2.26	—	6	0	140	—	—	—
Rattus sordidus	R	R	3	162 ± 12	2170 ± 1204	1.8	—	6	0	142	—	—	—
Rattus praetor	R	R	2	193 ± 7	3094 ± 1436	1.55	—	—	—	—	—	—	—

being 2.5–3.5 µm (Figs 5b, 6b–d; Table 2) although a slightly longer apical hook and ventral processes of 5 µm was present in *Uromys caudimaculatus* (Fig. 6e) whereas the sperm tail ranged from 90–130 µm (see Table 2).

In members of the Pogonomys division there were, by contrast, marked interspecific differences in sperm head morphology as well as in sperm tail length. All species of *Pogonomys* and *Chiruromys* had, like the species in the Hydromys and Uromys divisions, a sperm head with two ventral processes as well as an apical hook (Figs 6f, 7a–d) with the overall shape of the sperm head being longer and narrower in *Chiruromys vates* than the others (Fig. 7a). Other species in this division such as *Hyomys goliath* (Fig. 7e), *Anisomys imitator* (Fig. 5f), and *Lorentizimys nouhuysi* (Fig. 8a), had a sperm head that totally lacked the ventral processes although, in *H. goliath,* a short ventral spike extending from the lower ventral surface was evident (see Fig. 7e). The length of the sperm tail across these species was generally 106–130 µm but in the three *Chiruromys* species a longer sperm tail of 150 µm to 153 µm was present (Fig. 3, Table 2).

In the Mallomys division neither the sperm head of *Mammelomys* (Fig. 8b–c) nor those of *Xenuromys barbatus* (Fig. 8e), *Coccymys shawmayeri* (Fig. 8d), or *Abeomelomys sevia* (Fig. 5e) had ventral processes but the apical hook was generally longer than in species in the other divisions with the longest apical hook occurring in *X. barbatus* (Fig. 8e). In *Mallomys aroaensis* a uniquely shaped sperm head with a deep invagination at the site of sperm tail attachment occurred with the ventral extensions extending more caudally than in the other species (see Fig. 7f). The length of the sperm tails ranged from 124 µm in *Coccymys* up to 152 µm in *Xenuromys* (Fig. 4).

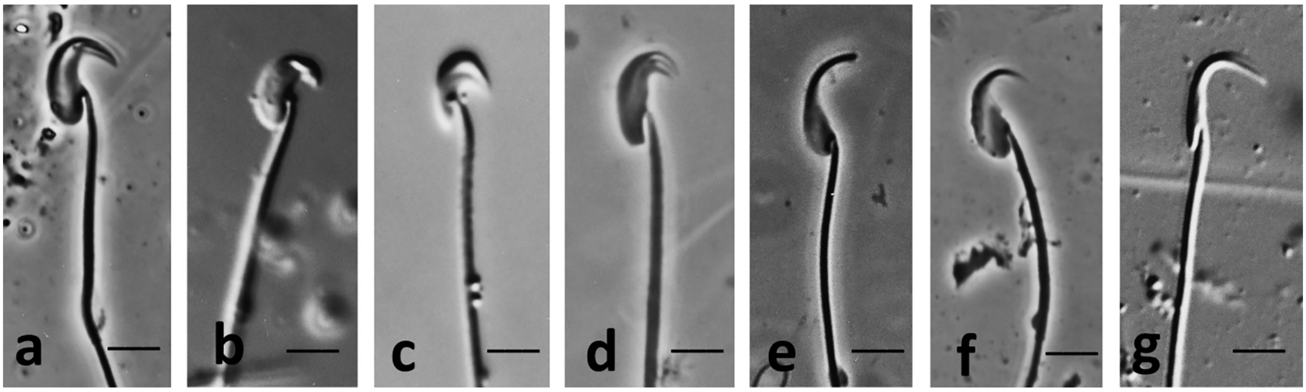

Figure 5. Light micrographs of spermatozoa from (*a*) *Leptomys elegans,* (*b*) *Melomys rufescens,* (*c*) *Pogonomys championi,* (*d*) *Pogonomys loriae,* (*e*) *Abeomelomys sevia,* (*f*) *Anisomys imitator,* and (*g*) *Rattus steini.* Scale bars a = 4 µm, b–g = 5 µm.

All members of the Rattus division had very similar sperm head morphology with a single, comparatively long, apical hook of about 6 µm (Fig. 8f) into which the nucleus extended whereas the length of the sperm tails ranged from 140–150 µm (see Fig. 4, Table 2).

The accessory sex glands (Table 2) invariably included large saccular seminal vesicles together with coagulating glands lying along the inner curvature of the seminal vesicles with the distal region of the coagulating gland being embraced by a subterminal flexure of the seminal vesicle (Fig. 9a–b). However, uniquely, the seminal vesicles of *Hyomys goliath* appeared less saccular and the coagulating gland relatively short compared to those of the other species (Fig. 9c). Modestly developed ventral and dorsal prostates, ampullary glands, and bulbourethral glands appeared to be universally present (Fig. 9b, Table 2) and showed relatively little interspecific variability.

The accessory gland that lies either side of the glans penis, the preputial gland, showed marked interspecific differences across the species. In some, for example, all four species of *Pogonomys,* two species of *Chiruromys,* and *Melomys lutillus,* it appeared to be very small or even absent, with species of the Hydromys division as well as *Paramelomys rubex* and *Paramelomys platyops* having small preputial glands. By contrast in *Hyomys goliath* the preputial gland was large and conspicuous (Fig. 9c).

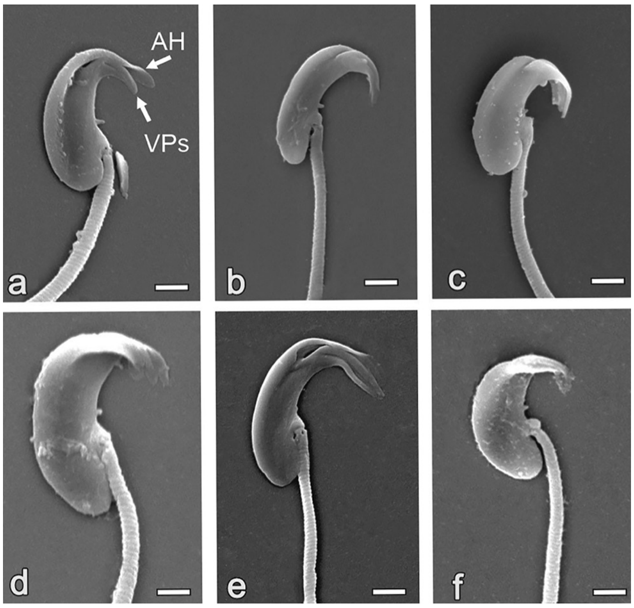

Figure 6. Scanning electron micrographs of spermatozoa from (*a*) *Pseudohydromys pumehanae,* (*b*) *Melomys lutillus,* (*c*) *Paramelomys platyops,* (*d*) *Uromys anak,* (*e*) *Uromys caudimaculatus,* and (*f*) *Chiruromys lamia*. AH = apical hook and VPs = ventral processes of sperm head. Scale bars a = 2 µm, b = 1.3 µm, c = 2 µm, d = 1 µm, e and f = 2 µm.

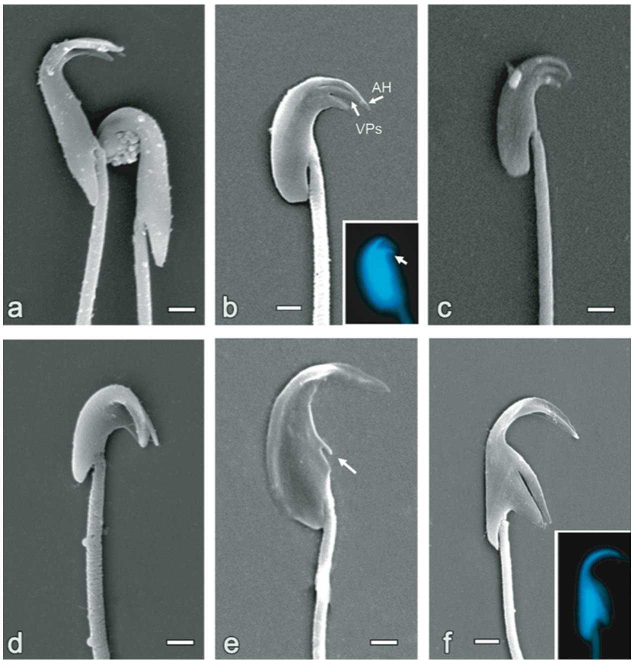

Figure 7. Scanning electron micrographs of spermatozoa from (*a*) *Chiruromys vates,* (*b*) *Pogonomys loriae* with inset a fluorescent LM stained with DAPI showing nucleus, (*c*) *Pogonomys macrourus,* (*d*) *Pogonomys sylvestris,* (*e*) *Hyomys goliath* with arrow indicating ventral spike on sperm head, and (*f*) *Mallomys aroaensis* inset fluorescent LM stained with DAPI showing nucleus. AH = apical hook and VPs = ventral processes of sperm head. Scale bars a–d = 0.7 µm, e and f = 1.4 µm.

Discussion

This comparative investigation shows considerable interspecific differences in both female and male reproductive anatomy across the various species of mice and rats from New Guinea with findings from a few species suggesting a polyandrous or promiscuous breeding system.

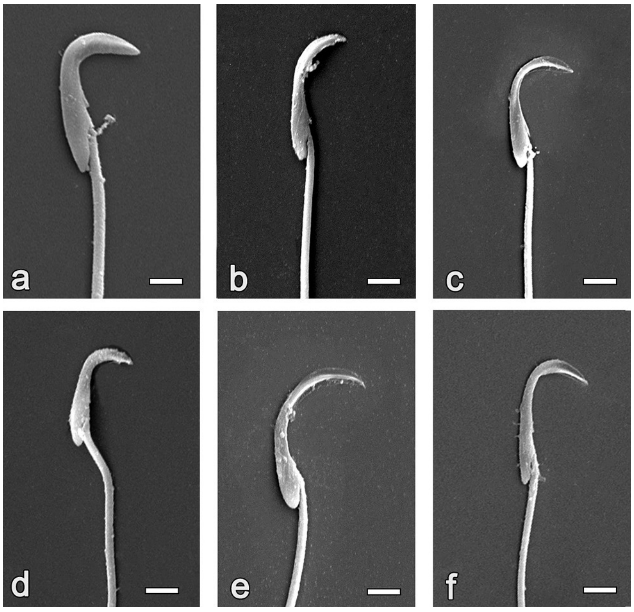

Figure 8. Scanning electron micrographs of spermatozoa from (*a*) *Lorentzimys nouhuysi,* (*b*) *Mammelomys lanosus,* (*c*) *Mammelomys rattoides,* (*d*) *Coccymys shawmayeri,* (*e*) *Xeneromys barbatus,* and (*f*) *Rattus niobe.* Scale bars a = 1.5 µm, b–f = 2.5 µm.

New Guinea clearly has a very diverse murid rodent fauna. The current study on female reproductive biology suggests, in general, similarity across the species in the Hydromys and Uromys divisions to those of the Australian members of these groups (e.g., Breed & Ford, 2007; Van Dyck & Strahan, 2008; Geffin *et al*., 2011), generally involving 4 nipples and a low number of fetuses in pregnant females.

Members of the Pogonomys and Mallomys divisions, by contrast, showed greater interspecific diversity in the nipple number which ranged from 6 in most species of the Pogonomys division to just 2 in *Mammelomys*, and with *Mallomys aroaensis* occasionally having 8 (Helgen, 2007a). Although individuals of several dissected animals of the species with 6 nipples had three fetuses, there was in general a low number of fetuses present in the pregnant females in the Pogonomys division even though most species had 2 additional nipples compared to the species in the Hydromyine and Uromyine divisions. These findings support the conclusion that, in general, the hydromyine rodents have a low fecundity in spite of the presence of 6 nipples in a number of species.

By contrast in the genus *Rattus* nipple number varied from 4 in *R. vandeuseni* up to 12 in *R. sordidus* (see also Taylor & Horner, 1973; Breed, 1978; Taylor *et al*., 1990) indicating a high potential litter size in the latter species. There is a suggestion in the literature that females in some populations of *R. steini* and *R. jobiensis* have 6 nipples whereas others have 8 (Taylor *et al*., 1982, 1990; Flannery, 1995a, 1995b), a finding that suggests the possibility of a species complex (Helgen, 2007b; Robins *et al*., 2014; Rowe *et al*., 2020). That individuals of *Rattus* species with numerous nipples can sometimes have a large number of fetuses at the one time is indicated by the findings in *R. sordidus* where up to 9 fetuses have been recorded, although the litter size of *R. niobe* and *R. verecundus* appears to be no greater than that of many of the hydromyine rodents. The high number of fetuses in *R. sordidus*, which is a species that also occurs in northern Australia, is similar to that of two closely related Australian *Rattus* species *R. colletti* and *R. villosissimus*, which live in grassland or desert habitats (Taylor & Horner, 1973; Yom-Tov, 1985; Breed & Ford, 2007; Van Dyck & Strahan, 2008) where rapid population increase during times of abundant resources sometimes occurs. By contrast the rainforest species *R. leucopus* has a lower number of nipples and lower number of fetuses (Dwyer, 1975; Taylor *et al*., 1990; Geffen *et al*., 2011). Clearly there is greater interspecific variability in ovulation rate, nipple number, and potential litter sizes across *Rattus* species than there is between species in the hydromyine tribe.

When it comes to interspecies differences in relative testes mass (RTM) it was found that none of the New Guinea species had such small relative testes mass as occurs in some of the semiarid and arid adapted Australian hydromyine rodents in the genera *Notomys* and *Pseudomys* (Breed, 1997b; Breed & Taylor, 2000; Bauer & Breed, 2008). Nevertheless, some differences between species in RTM are indicated for the rodents from New Guinea in the 40–100 g body weight range with two *Pogonomys* species, *P. macrourus* and *P. sylvestris*, having larger RTM than any of the species in the Hydromys or Uromys divisions of similar body mass. Evidence from observations of individuals of these species in the natural environment suggests that *Pogonomys* and *Chiruromys* may exhibit colonial or group-living behaviour, in either holes in trees or burrows underground (Dennis & Menzies, 1979; Flannery & Seri, 1990; Flannery, 1988, 1995a). The high RTM in these *Pogonomys* species, as well as in *C. vates,* suggest that considerable intermale sperm competition may at times take place with these species having potentially a polyandrous or promiscuous mating system.

Interspecies differences in the organization of the testis may also occur (e.g., Lüpold *et al.*, 2009; Ramm & Schärer, 2014; Peirce *et al*., 2018) and in the New Guinea species those with relatively small body mass, unlike the two species with large body mass such as *Mallomys* and *Hyomys* species, had at least 90% of the testes composed of seminiferous tubules. A finding that suggests greater numbers of sperm producing capacity of the testes are produced in these species.

So what is the significance of the interspecific differences in sperm morphology that have been found to occur? Previous studies on sperm morphology of Australian murids have shown clear differences between hydromyine rodents and *Rattus* species. Whereas all *Rattus* species have a sperm head with a single long apical hook and long tail, in most members of the Uromys and Hydromys divisions, as well as in most species in the Pseudomys and Conilurus divisions, a more complex sperm head with 2 ventral processes, in addition to the apical hook, occurs, with their angle, together with sperm tail length, correlating with relative testes mass (McLennan *et al*., 2017). Amongst the *Rattus* species from New Guinea the sperm also have a single apical hook like those of the Australian *Rattus*, whereas in species of the Uromys and Hydromys divisions the sperm head morphology is more complex and contains, in addition, two ventral processes similar to those of the closely related Australian species in the same divisions (Breed, 1984; Breed & Aplin, 1994; Breed & Leigh, 2010). Studies on spermatozoa of an Australian *Pseudomys* species have shown that the two ventral processes contain cytoskeletal proteins (Flaherty *et al*., 1983; Breed

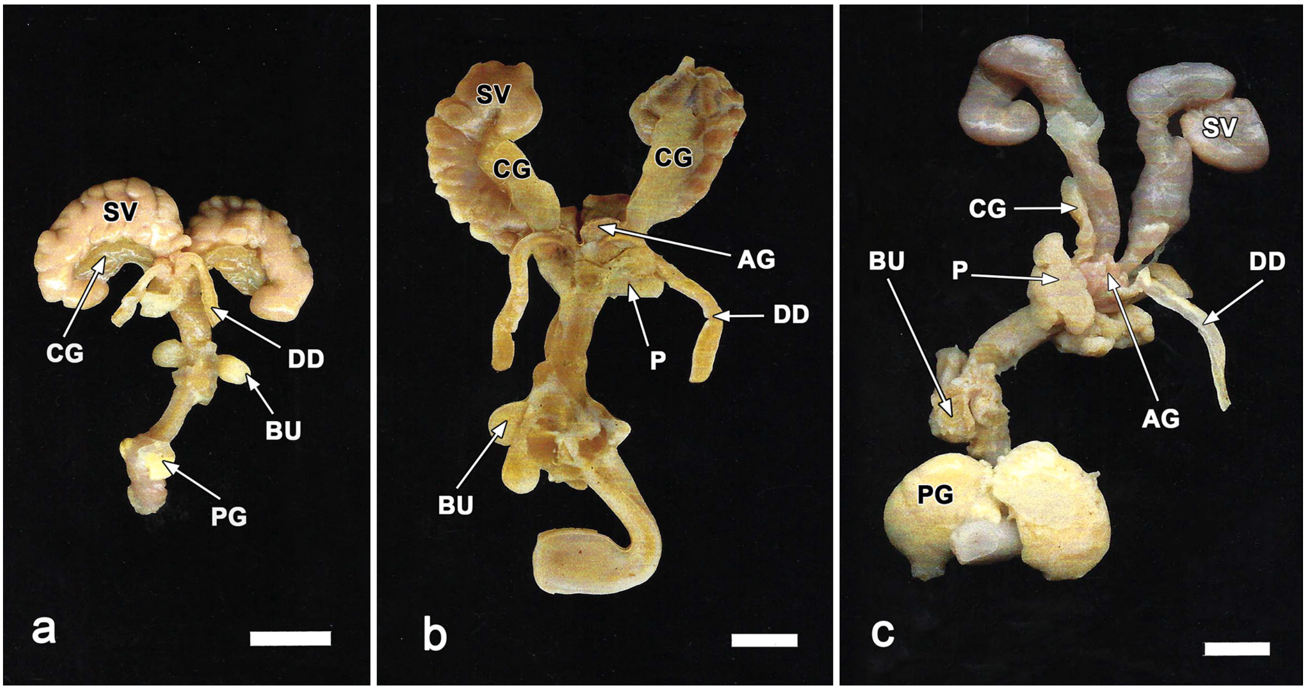

Figure 9. Dissected male reproductive accessory sex glands from (*a*) *Paramelomys rubex,* (*b*) *Uromys caudimaculatus,* and (*c*) *Hyomys goliath.* Note large sacculated seminal vesicles in (*a*) and (*b*) with coagulating glands on their inner curvatures; *H. goliath* has rather different gross morphology of seminal vesicles and coagulating glands and strikingly large preputial glands (PG). SV = seminal vesicles, CG = coagulating glands, P = prostate glands, AG = ampullary glands, BU = bulbourethral glands, PG = preputial glands, and DD = ductus deferens. Scale bars a–c = 10 mm.

et al., 2009) with studies on spermatozoa of an Australian *Pseudomys* species having shown that these processes aid in the binding of the sperm head to the extracellular coat that surrounds the egg as well as to egg coat penetration at the time of fertilization (Breed, 1997a; Drew *et al*., 2014).

In members of the Pogonomys and Mallomys divisions the current findings clearly indicate marked differences in sperm morphology across the species albeit that there is generally similar sperm morphology between species within the same genus. In species of *Pogonomys* and *Chiruromys* the sperm head contains, like those in the Hydromys and Uromys divisions, two ventral processes extending from the upper concave surface with the three species of *Chiruromys* having somewhat longer sperm tails; a finding that supports the view, together with the fact that *C. vates* has a more streamlined sperm head (see Fig. 7a), that these species also have high levels of intermale sperm competition and multimale mating systems. In *Mallomys* a very different sperm head shape was found to occur with a more caudal orientation of the two ventral projections, with the divergent nature of these sperm suggesting the possibility of independent evolution of these structures, and perhaps a different function, from those of the species in the other genera.

Other species in the Pogonomys and Mallomys divisions in the genera *Abeomelomys, Anisomys, Xenuromys,* and *Mammelomys*, as well as that of *Lorentzimys,* have a sperm head in which the ventral processes are entirely absent but the apical process is generally longer than that of most other hydromyine rodents. The presence of a single apical process is similar to that of sperm morphology of various other genera of murid rodents that occur in Southeast Asia and the Philippines (Breed & Yong, 1986; Breed & Musser, 1991; Pahl *et al*., 2018; Breed *et al*., 2019) including species that are members of the Chrotomys division in the hydromyine tribe as well as members of the Maxomys, Dacnomys, and Bunomys divisions of the Rattini (Rowe *et al*., 2019).

The morphology of most of the accessory sex glands did not show marked differences across the species. In murids a full complement of accessory sex glands is considered to be the ancestral condition, with the seminal vesicles usually being the largest accessory sex gland (Linzey & Layne, 1969; Voss & Linzey, 1981). Its secretions, together with those from the coagulating glands, form a large intravaginal copulatory plug at time of mating, the main function of which may be a chastity enforcing device to protect the male's investment (Voss, 1979; Mangels *et al*., 2016), and/or to facilitate sperm migration through the highly fibrous cervix into the uterus (Carballada & Esponda, 1992). In general, regardless of tribe or division, there was a similar complement of seminal vesicles, coagulating glands, and ventral and dorsal prostates albeit that in the one individual *Hyomys goliath* investigated divergent morphology occurred.

Unlike the other male accessory sex glands the size of the preputial glands differed markedly across the species with this gland being absent in some members of the Pogonomys division, whereas by contrast prominent preputial glands were evident in the male *Hyomys goliath.* The function of these glands may be to secrete pheromones that elicit sexual attraction in females, aggressive behaviour in male conspecifics, and/or possibly induce various other reproductive behaviours (Bronson & Caroom, 1971; Brown & Williams, 1972; Orsulak & Gawienowski, 1972; Zhang *et al*., 2008; Rowe *et al*., 2020) with interspecific differences in preputial glands size suggesting differences in social organization across the species, although more data from *H. goliath* are needed to ascertain if the highly derived gland found in the one individual observed in this study is a characteristic feature of this species.

In conclusion this study indicates that, within the New Guinea Pogonomys and Mallomys divisions, marked interspecies differences have evolved in several aspects of both female and male reproductive anatomy. In the female there are species differences in number of nipples although the number of fetuses in the hydromyine rodents was generally low. In males interspecific differences in both relative testes mass and in sperm morphology were evident. The full functional significance of these differences has yet to be determined but the findings of large RTM in *Pogonomys* and the long sperm tails in *Chiruromys,* suggest that these species have high levels of intermale sperm competition and hence multimale breeding systems.

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