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Sulawesi and Philippine Rodents (Muridae): A Survey of Spermatozoal Morphology and Its Significance for Phylogenetic Inference

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ABSTRACT

Variation in spermatozoal morphology among 26 species of murine rodents native to Sulawesi and two indigenous to the Philippines was surveyed with the light microscope. Spermatozoa from most of the species were composed of an asymmetrical head, falciform or scythelike in shape, and a tail attached to the ventrocaudal surface of the head. Similarity in traits reflecting size and shape of the sperm head, size of the apical hook, and length of the tail defined distinct clusters of species in genera defined by other characters: *Rattus*, *Paruromys*, *Margaretamys*, *Haeromys*, *Tateomys*,

Melasmothrix, *Crunomys*, and *Chrotomys*. In contrast, significant variation in sperm morphology existed among species within *Bunomys*, *Taeromys*, and *Maxomys*. Spermatozoa from *Eropeplus canus* and *Lenomys meyeri* had a symmetrical club-shaped head with a very short tail attached to the midbasal part of the head, a configuration unlike that of any of the other species surveyed. The significance of the variation for estimating phylogenetic relationships among the species, and between them and other Indo-Australian groups, is discussed.

INTRODUCTION

In this paper we document results of our survey of variation in the external morphology of spermatozoa, using light microscopy, from species native to Sulawesi and the Philippine Islands. Our initial intent was to sam-

ple only the Sulawesi fauna, but we had material from two important Philippine species at hand and included them for comparison. Spermatozoal traits provide a set of morphological data that might offer an in-

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dependent assessment of the pattern of phylogenetic relationships among these endemic species, and between them and faunas from the New Guinea–Australia region to the east and Southeast Asia to the west.

Spermatozoal morphology, viewed at the resolution levels of both light and scanning electron microscopy, has been a significant source of data for inferring phylogenetic relationships among some groups of native Indo-Australian murids. There is, for example, appreciable variation in sperm morphology among native Australian murids. Limits of that variation define two primary groups. One consists of the species that are currently placed in *Rattus*, which share a spermatozoal conformation consisting of an elongate falciform head with a prominent apical hook and a tail attached to the ventrocaudal margin of the head. The species in *Hydromys*, *Xeromys*, *Conilurus*, *Leggadina*, *Leporillus*, *Mastacomys*, *Mesembriomys*, *Notomys*, *Pseudomys*, *Zyzomys*, *Melomys*, and *Uromys*, however, form an assemblage in which most are defined by a sperm head with two ventral hooks in addition to the apical one. This design suggests that these species are part of an evolutionary radiation different from the one out of which the species of *Rattus* evolved (Breed and Sarafis, 1979; Breed, 1983, 1984).

Outside Australia, only the native murids found on the Malay Peninsula have been surveyed for variation in spermatozoal morphology (Breed and Yong, 1986). Most of these species possess spermatozoa that are either closely similar to that of *Rattus* or a modification derived from that configuration. A sperm head with two ventral hooks in addition to the apical hook is not represented among the Malaysian samples and appears to be unique to the Australian (Breed and Sarafis, 1979) and New Guinea non-*Rattus* fauna (Breed, in prep.).

What does the interspecific variation in external spermatozoal morphology tell us about the phylogenetic alliances of the endemic Sulawesi murid fauna and that small part of the indigenous Philippine fauna that we sampled? We explore this question in the pages to come by first describing the sperm morphology of the species and then discussing the significance of the traits for phylogenetic

inference. The variation and conclusions we record here will be tested with results from phylogenetic analyses of the Sulawesi murids using other sets of characters (morphological and biochemical) when they become available (Musser, in prep.).

SPECIMENS AND TECHNIQUES

Twenty-eight species—two that are indigenous to the Philippine Islands, the rest native to Sulawesi—were surveyed. The specimens from which samples of cauda epididymal sperm were obtained are listed below. All are stored in either the Department of Mammalogy at the American Museum of Natural History, New York (AMNH), or the Division of Mammals at the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM).

SULAWESI (localities in the central region)

Rattus hoffmanni: AMNH 223755

Rattus facetus: AMNH 224287

Bunomys chrysocomus: AMNH 224694 and 224707

Bunomys andrewsi: AMNH 225661

Bunomys penitus: AMNH 223806, 223818, 225263, and 225266

Bunomys sp. A: AMNH 225028

Lenomys meyeri: AMNH 224317

Eropeplus canus: AMNH 223553

Paruromys dominator: AMNH 224828, 225699, 225729, and 225731

Taeromys hamatus: AMNH 223461

Taeromys callitrichus: AMNH 224637

Taeromys celebensis: AMNH 225672

Taeromys arcuatus: AMNH 223561

Echiothrix leucura: AMNH 225684 and 226815

Haeromys minahassae: AMNH 226050

Haeromys sp.: AMNH 223958

Margaretamys elegans: AMNH 223681 and 223691

Margaretamys parvus: AMNH 225066 and 225067

Margaretamys beccarii: AMNH 225667

Maxomys hellwaldii: AMNH 225925 and 226015

Maxomys musschenbroekii: AMNH 225021

Maxomys sp. B: AMNH 224861

Tateomys rhinogradoides: AMNH 223966

Tateomys macrocercus: AMNH 225077

Melasmothrix naso: AMNH 225099 and 225111

Crunomys celebensis: AMNH 224316

PHILIPPINES (Luzon)

Rattus everetti: USNM 399568

Chrotomys mindorensis: AMNH 256202

The animals were preserved in the field by injecting their body cavities and large muscle masses with formalin and then submersing them in a formalin solution for several days until tissues were fixed. Back at the American Museum, all rats were soaked in changes of water for several days, then stored in a solution of 70 percent ethanol. Testes and epididymides were dissected out, packaged, and mailed by air to the Department of Anatomy & Histology at the University of Adelaide where Breed processed them.

A small piece of cauda was extracted from each specimen and macerated in a watch glass that contained 10 percent buffered formalin. Drops of the solution with cellular material were placed on microscope slides. After a coverslip was placed on each slide and the sample ringed with DePeX, the material between coverslip and slide was observed under Nomarski differential and phase-contrast optics.

Photomicrographs were taken of intact spermatozoa. Approximate length and breadth of each sperm head, together with the hook (if present), were determined for several spermatozoa when the material was examined with $\times 100$ objective. Approximate length of the sperm tail was also measured. Because most of the tails were not positioned in a straight line and the terminal piece was missing from some, the maximum length was assumed to be the most accurate measurement and those values are listed in table 1. Total length of the sperm head was taken as the straight-line distance from the caudal extremity of the postacrosomal region to the top of the convex surface of the curvature of the head. Where the curvature of the hook was obtuse, no accurate length could be determined and no measurement was recorded. Breadth of the sperm head was measured at the site of the connecting piece for spermatozoa with a hook, or the maximum

distance between the dorsal and ventral surfaces for spermatozoa without hooks. Length of the hook was taken as the straight-line distance from the inner concave surface of the sperm head to the apical tip of the spermatozoon. Measurements are in micrometers (μm).

Terminology we use for describing orientation of the sperm head and morphology of the head and tail follows that defined by Breed (1983, 1984). The *dorsal* portion of the head, for example, is "the convex surface of the hooked sperm" (Breed, 1983: 613), and the *ventral* portion is the concave surface of the hooked sperm head. Acrosome, postacrosomal region, sperm hook, and connecting piece of the sperm tail—terms we use here—are also defined and illustrated in Breed's reports cited above.

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INTERSPECIFIC VARIATION IN SPERM MORPHOLOGY

The accounts that follow contain descriptions of the overall shape of the sperm head together with length of the sperm tail, as seen with a light microscope. Heads and portions of tails are illustrated in figures 1 and 2; measurements are listed in table 1. Descriptions are arranged in three groups that correspond to the provisional generic assemblages discussed by Musser (1981b: 165–167): Group III consists of *Rattus*; Group II contains genera with *Rattus*-like cranial and dental deri-

TABLE 1
Approximate Measurements (μm) of Spermatozoal Heads and Tails in Samples of Native Murid Rodents from Sulawesi and Luzon (Philippines)

Species	Head			Tail
	length	breadth	length of apical hook	length
<i>Rattus hoffmanni</i>	12	2	6	170
<i>Rattus facetus</i>	12	2	5	155
<i>Rattus everetti</i>	11–12	2	6	160
<i>Bunomys chrysocomus</i>	10–11	2	7	160
<i>Bunomys andrewsi</i>	8	2.5	7	155
<i>Bunomys</i> sp. A	9	2.5	3.5	135
<i>Bunomys penitus</i>	10	2.5	5	135
<i>Lenomys meyeri</i>	6.3	3.5	—	60
<i>Eropeplus canus</i>	6	5	—	80
<i>Paruromys dominator</i>	11	6.5	3	130
<i>Taeromys callitrichus</i>	9	4	3	110
<i>Taeromys arcuatus</i>	11	3	6	165
<i>Taeromys hamatus</i>	7	4	—	100
<i>Taeromys celebensis</i>	7	3	—	60
<i>Echiothrix leucura</i>	15	2	4	180
<i>Haeromys minahassae</i>	9.5	4	4	115
<i>Haeromys</i> sp.	10	5	4	110
<i>Margaretamys elegans</i>	15	3	11	168
<i>Margaretamys parvus</i>	14	3	8	175
<i>Margaretamys beccarii</i>	15	2.5	8	160
<i>Maxomys hellwaldii</i>	—	2	—	245
<i>Maxomys</i> sp. B	—	2.5	8	195
<i>Maxomys musschenbroekii</i>	12	4	4.5	130
<i>Tateomys rhinogradoides</i>	9.5	2	5	120
<i>Tateomys macrocercus</i>	10.5	2	5	125
<i>Melasmothrix naso</i>	9	3	5	105
<i>Crunomys celebensis</i>	8	4	2	52
<i>Chrotomys mindorensis</i>	8.5	2.4	4	135

vations; and Group I is formed of genera usually considered an old endemic faunal stratum. Within the latter is a subgroup native to the New Guinea and Australian region as well as the Philippine Islands, and another subgroup in which the phylogenetic affinities of the genera are with the fauna of islands on the Sunda Shelf and mainland Southeast Asia.

Group III: *Rattus* is the only genus in this category (Musser, 1981b).

Shape of the sperm head is similar in the Sulawesi *Rattus hoffmanni* and *R. facetus* as well as the Philippine *R. everetti*. Asymmetrical and falciform in outline (fig. 1A and B), the sperm head of each species is 11.0–12.0 μm long and 2.0 μm wide; length of the apical

hook is 5.0–6.0 μm . The connecting piece of the sperm tail is attached to the ventrocaudal surface of the head; length of the tail ranges from 155 to 170 μm (table 1).

Group II: Musser (1981b) placed *Bunomys*, *Eropeplus*, *Lenomys*, *Paruromys*, and *Taeromys* in this assemblage.

Spermatozoal morphology is variable among the four species of *Bunomys*. Shape of the sperm head and length of the tail in samples from *B. chrysocomus* (fig. 1C) and *B. andrewsi* (fig. 1D) are similar to spermatozoa in the species of *Rattus*; the sperm head of *B. andrewsi*, however, is shorter and slightly wider ($8.0 \times 2.5 \mu\text{m}$) than that of the *Rattus* species ($11.0\text{--}12.0 \times 2.0 \mu\text{m}$) and shorter than

those of any other species of *Bunomys* (9.0–11.0 μm). In samples from *Bunomys* sp. A (fig. 1E) and *B. penitus* (fig. 1F), the sperm head is slightly broader than that of *B. chrysocomus*, and has a shorter hook (3.5–5.0 μm versus 7.0 μm) and tail (135 μm versus 155–160 μm) than that found in the other two species of *Bunomys* that were investigated.

Gross morphology of spermatozoa from samples of *Lenomys meyeri* and *Eropeplus canus* (fig. 1H–J) is very different from that seen in species of either *Rattus* or *Bunomys* or any of the other genera we examined. The sperm head lacks a hook (present in species of other genera), the connecting piece of the tail inserts at the midbasal region of the head (insertion is at the ventrocaudal surface in other species), and the tail is short (60–80 μm). In the sample from *Lenomys* (fig. 1H), the symmetrical sperm head has a transverse ridge—perhaps at the posterior border of the acrosome—and a domelike anterior apical tip.

Two morphological forms of spermatozoa appear to be present in samples of *Eropeplus*. One type (fig. 1I—a fragment of the other form is also shown) has a broad face (up to 5.0 μm wide), and the connecting piece of the tail inserts off-center at the base of the sperm head. The other form (fig. 1J—a piece of the other morph can be seen in the bottom half of the figure) has a narrower face (up to 2.0 μm) and the connecting piece of the tail inserts at the midbasal portion of the sperm head. In both images, the sperm head narrows toward the connecting piece of the tail. These two morphs could be alterations of a single morphological type, one that was slightly bilaterally flattened and differed in its appearance depending on the orientation of the spermatozoa on the microscope slide. Alternatively, the morphologies may represent two forms of spermatozoa within the same individual.

The sperm head in the sample of *Paruromys dominator* (fig. 1G) contrasts strikingly with the spermatozoal morphology characteristic of species in *Rattus*, *Bunomys*, *Lenomys*, and *Eropeplus*. The asymmetrical sperm head of *P. dominator* has a very broad lateral face (6.5 μm), broader than any sperm from species of either *Rattus* or *Bunomys*, for example (table 1). The apical hook is short (3.0 μm) and blunt, and the moderately long tail

(130 μm) inserts on the lower ventral surface of the sperm head.

Differences in shape of sperm heads and length of sperm tails among the species of *Taeromys* are impressive. Samples from *T. callitrichus* (fig. 1L) and *T. arcuatus* (fig. 2B) contain spermatozoa with a fairly typical sperm head and apical hook, along with a tail (table 1) connected to the ventrocaudal portion of the head. Except for their broader sperm head (3.0–4.0 μm), as well as the short apical hook (3.0 μm) and sperm tail (110 μm) of *T. callitrichus* and *T. arcuatus*, general conformation of the spermatozoa resembles the sperm morphology we see in species of *Rattus* and *Bunomys*. In contrast, gross morphology of spermatozoa from *T. hamatus* (fig. 1K) is unlike these other two species of *Taeromys* in that the sperm head has an extremely attenuated and blunt apical hook as well as a short tail (100 versus 110 and 165 μm).

The most divergent morphology observed among the samples of *Taeromys* is seen in spermatozoa from *T. celebensis* (fig. 2A). In this species, the paddle-shaped sperm head is elongate and symmetrical, without an apical hook. A very short sperm tail (60 μm), the shortest we recorded among the species of *Taeromys*, is inserted, by a connecting piece, to the midbasal region of the head. This configuration, so peculiar among species of *Taeromys*, somewhat resembles the distinctive shape of sperm head, place of tail insertion, and relative length of sperm tail which are characteristic of the samples from *Lenomys* and *Eropeplus* (fig. 1H–J; table 1).

Group I: In this cluster, Musser (1981b) listed *Echiothrix*, *Haeromys*, *Margaretamys*, *Maxomys*, *Melasmothrix*, and *Tateomys* as members of an “Asian midland subgroup”; *Crunomys* and *Chrotomys* were included within a “New Guinea–Australian–Philippine subgroup.”

All the species sampled share a similar sperm morphology, at least at the resolution of our observations. All spermatozoa have asymmetrical heads and apical hooks of variable lengths. The sperm tail is generally long (table 1) and invariably inserted on the ventrocaudal surface of the sperm head.

In samples from *Echiothrix leucura* (fig. 2C), the sperm head is long (15.0 μm) and sickle-shaped, with a short apical hook (4 μm).

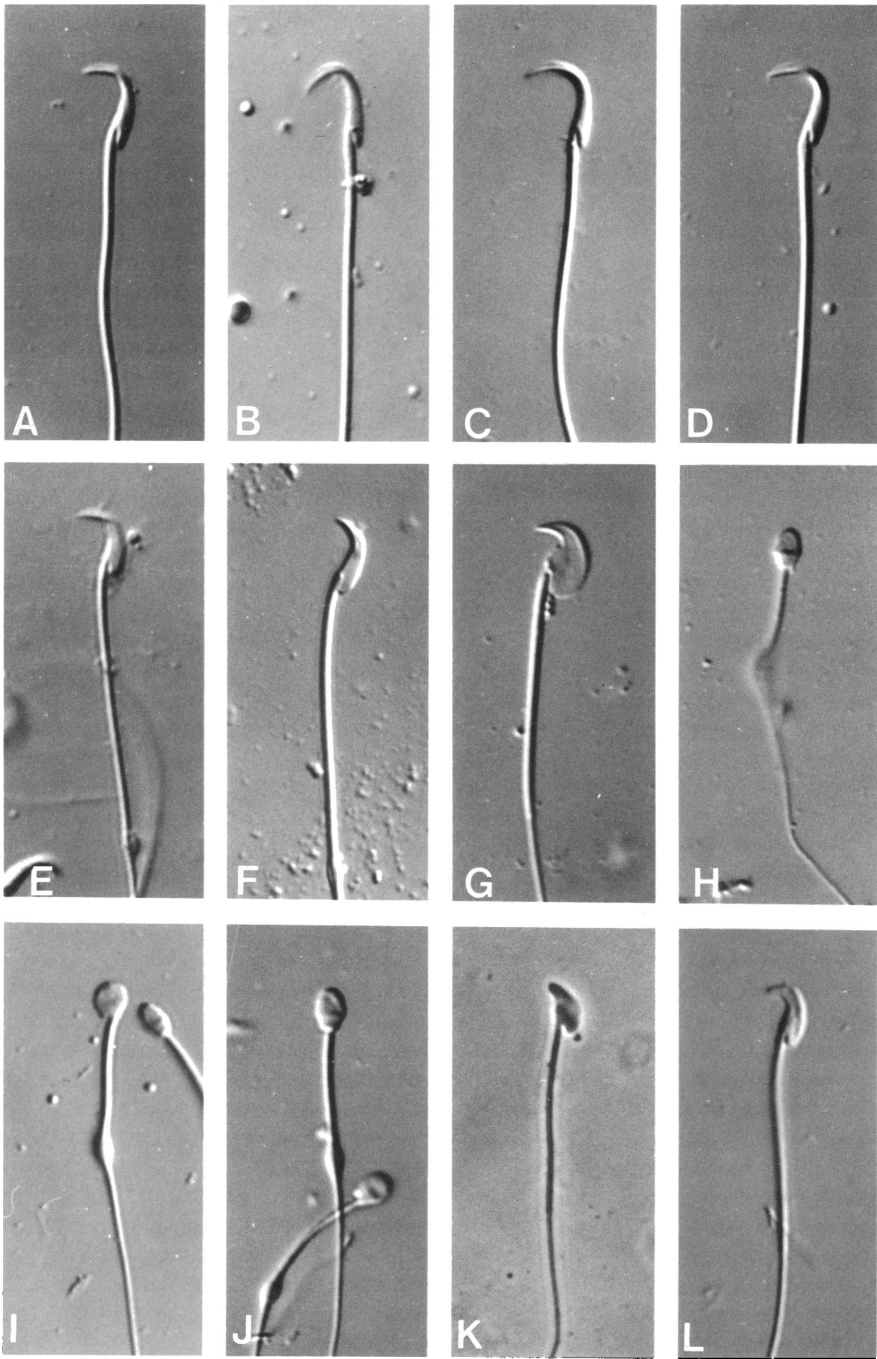


Fig. 1. Micrographs (Nomarski optics, A–J and L; phase-contrast optics, K) of spermatozoa from murid rodents native to Sulawesi. $\times 820$. **A**, *Rattus everetti*; **B**, *Rattus facetus*; **C**, *Bunomys chrysocomus*; **D**, *Bunomys andrewsi*; **E**, *Bunomys* sp. A; **F**, *Bunomys penitus*; **G**, *Paruromys dominator*; **H**, *Lenomys meyeri*; **I**, **J**, *Eropeplus canus*; **K**, *Taeromys hamatus*; **L**, *Taeromys callitrichus*.

The tail is long (180 μm). General shape of the sperm head (but not its size or the relatively small hook) as well as the tail proportion resemble the conformation in samples from *Rattus* (fig. 1A, B).

Shapes and relative sizes of sperm elements are similar in samples from the two kinds of *Haeromys* (fig. 2D, E). Spermatozoal features of both species include a head with a broad lateral face (4.0–5.0 μm), a short apical hook (4.0 μm), and a moderately short tail (110–115 μm). A small ventral spike is also evident just above the connecting piece of the sperm tail.

All three species of *Margaretamys*—*elegans* (fig. 2F), *parvus* (fig. 2G), and *beccarii* (fig. 2H)—share a similar sperm morphology. The sperm heads are long (14.0–15.0 μm), narrow (2.5–3.0 μm), and sickle-shaped, with long apical hooks (8.0–11.0 μm). Sperm tails are long (160–175 μm).

Spermatozoa from the species of *Maxomys* that we sampled are dissimilar in gross morphology. The large, sickle-shaped sperm head (we could not obtain an accurate measurement of its length) of *M. hellwaldii* (fig. 2I) is distinctive among the species of *Maxomys*, although its overall conformation resembles sperm heads from species of *Margaretamys* (fig. 2F–H). Spermatozoa from *M. hellwaldii* also have the longest sperm tails (245 μm) of any murid rodent for which observations have been published. A sperm head of somewhat similar shape is characteristic of *Maxomys* sp. B (fig. 2K), and the sperm tail is also long (195 μm). The sperm head in the sample from *M. musschenbroekii* (fig. 2J) is shorter and wider (12.0 by 4.0 μm) than those from the other species of *Maxomys*, the apical hook is shorter (4.5 versus 8.0 μm), as is the sperm tail (130 versus 195 and 245 μm).

Of the three genera of small-bodied shrew rats, *Tateomys rhinogradoides* (fig. 2L) and *T. macrocercus* (fig. 2M) are very similar in gross morphology of their spermatozoa. The sperm heads are sickle-shaped, similar in conformation as well as size to those from samples of *Rattus* (fig. 1A, B; table 1). Sperm tails are moderately long (120–125 μm). Spermatozoa from *Melasmothrix naso* (fig. 2N) differ from those of the other two species by having a slightly wider sperm head (3.0 versus 2.0 μm) in which the caudal region is

tapered distally (apparently a unique trait), and a shorter tail (105 versus 120–125 μm).

Crunomys celebensis (fig. 2O) has distinctive spermatozoa. The sperm head has a broad (4.0 μm) lateral face, and a very short (2.0 μm) and truncated apical hook. The sperm tail is very short (52 μm), the shortest of any species studied (table 1).

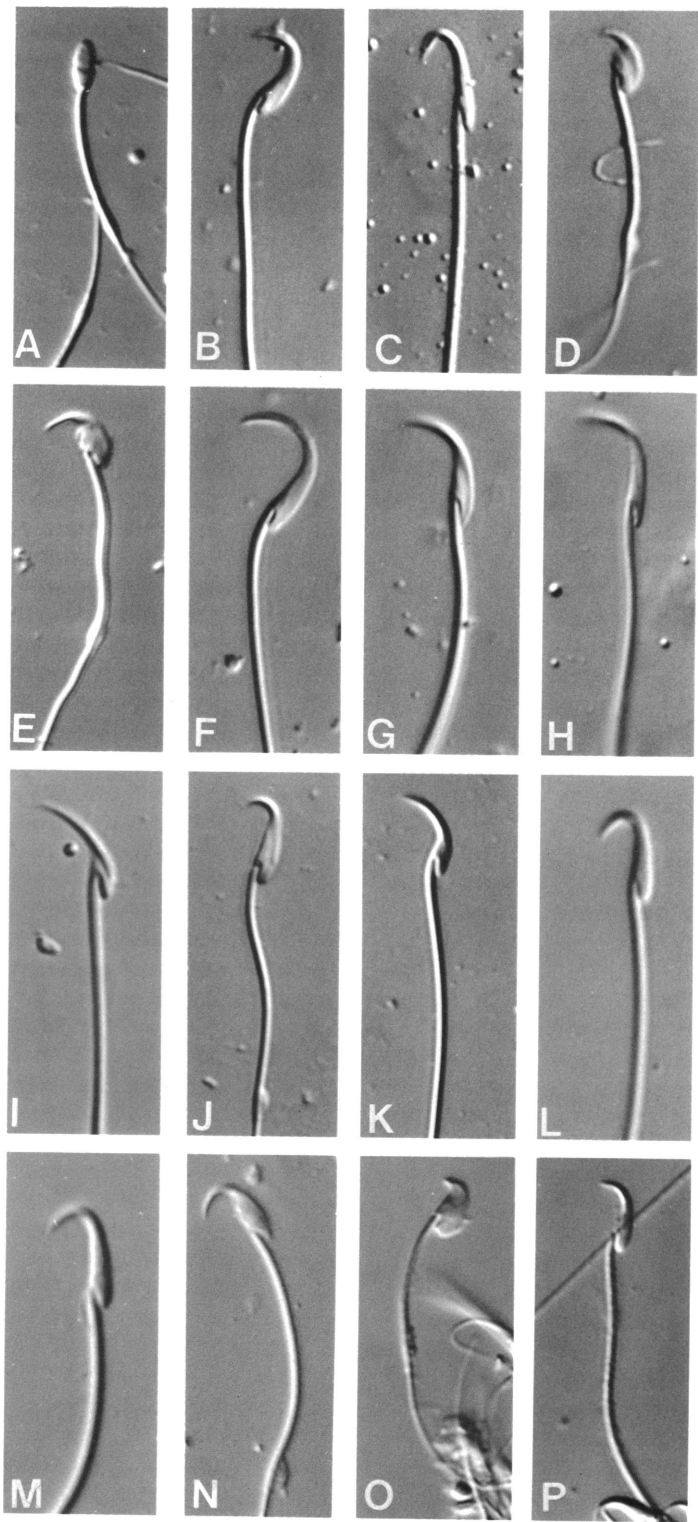
Gross morphology of spermatozoa from the sample of *Chrotomys mindorensis* (fig. 2P), the large-bodied Philippine shrew rat, is undistinguished. Although smaller (8.5 μm long), the sperm head is shaped somewhat like that in species of *Rattus* (fig. 1A, B) and *Tateomys* (fig. 2L, M). The sperm tail (135 μm long) is shorter than those characteristic of *Rattus* and other species but longer than in the samples of *Tateomys* (table 1).

SIGNIFICANCE

CHARACTER POLARITY

Morphology of spermatozoa provides a set of data that might be useful in inferring phylogenetic relationships among the species of endemic murids we sampled. In this context, we assume certain spermatozoal features to be primitive, others to be derived. The assumption is based on surveys of groups of Rodentia other than the genera we studied as well as other orders of mammals. We are also sensitive to the high degree of homoplasy in morphological characters within the Muroidea (Carleton and Musser, 1984) and realize that some of the presumed derived spermatozoal features shared among species may represent convergence. We are also aware that many other species of native Asian and African murines have yet to be surveyed for spermatozoal characters. Results from any future surveys of those geographic groups may alter our perception of primitive and derived traits of the sperm we observed at the gross morphological level.

An apical hook that includes a large extension of the subacrosomal space in the form of a perforatorium on the sperm head of many murines has not been recorded in spermatozoa of other mammalian groups. The morphology is probably derived within the Rodentia and evolved early in the history of the Murinae because it is characteristic of sperm in most murines from Australia (Breed and



Sarafis, 1979; Breed, 1983, 1984), the Malay Peninsula (Breed and Yong, 1986), and those sampled from Africa (Bishop and Austin, 1957; Gordon and Watson, 1986; Breed et al., 1988); it was presumably present in a common ancestor of all these lineages. The absence of apical hooks on the sperm heads of a few Australian species (Breed, 1983) as well as at least one species from Eurasia (Friend, 1936), one from Malaysia (Breed and Yong, 1986), and one from Africa (Gordon and Watson, 1986; Breed et al., 1988), is likely to be a more recently derived state. The presence of two extra ventral hooks on the sperm head of most of the species in the non-*Rattus* genera native to Australia (Breed, 1983, 1984; Flaherty and Breed, 1983, 1987) is also probably a new specialization. We assume that a sperm head with an apical hook represents the primitive condition within the Murinae; additional hooks, or their absence, are derived traits.

SPECIES CLUSTERS

Based on the spermatozoal features we have seen, the species surveyed fall into two primary groups. One contains *Lenomys meyeri* and *Eropeplus canus*. The sperm heads of both species are symmetrical and do not have apical hooks or any evidence of them. The sperm tails are short and are either attached to the midbasal regions of the head (*Lenomys* and one morphological form in *Eropeplus*) or off-center at the base of each sperm head (the other form observed in samples of *Eropeplus*). Morphology of the spermatozoa is more like that of other mammalian groups (such as members of Chiroptera, primates, and Carnivora, for example). None of the spermatozoa in samples from the few other species of murines which have sperm heads without apical hooks appear to be similar to those of *Lenomys* and *Eropeplus*. Among other Sulawesian endemics, the closest con-

formation to sperm from these two genera is seen in *Taeromys celebensis*. The sperm head of this species is elongate, symmetrical, and without an apical hook. Its tail is short and is attached to the midbasal part of the sperm head.

Musser's (1981b: 167) inclusion of *Lenomys* and *Eropeplus* within a *Rattus*-like cluster of genera is certainly not supported by spermatozoal morphology. Sperm from each is derived, within our definition of polarities, and these derived traits are shared by both genera. The two also share a similar cranial conformation—composed mostly of derived features (Musser, 1981b)—and pelage (Musser, personal obs.); molar occlusal patterns, however, are dissimilar. Shared distinctive spermatozoal traits as well as cranial and pelage features suggest that *Lenomys* and *Eropeplus* are phylogenetically more closely related to each other than to the other murines native to Sulawesi. This estimate of affinities is the hypothesis to test with other kinds of data.

Past studies of their relationships have focused on molar cusp patterns. Tate (1936), for example, placed *Lenomys* in the Phloeomyinae (genera with “complexly folded molars”) and *Eropeplus* in Murinae (genera having basically *Rattus*-like molars). Later, Ellerman (1941) allocated both genera to the Murinae but included *Lenomys* as part of a group “retaining the posterointernal cusp and having no extreme specialization of molars, i.e., those in which no reduction of the main cusps has started” and relegated *Eropeplus* to a cluster of genera “without the posterointernal cusp of the upper molars, and without other specializations, containing the majority of the subfamily, and centering round the genus *Rattus*.”

Misonne (1969), in a report on evolutionary trends in African and Asian murids, placed *Lenomys* and *Eropeplus* in a “*Lenothrix-Parapodemus* Division,” a major group

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Fig. 2. Micrographs (Nomarski optics) of spermatozoa from murid rodents native to Sulawesi and the Philippines. $\times 820$. A, *Taeromys celebensis*; B, *Taeromys arcuatus*; C, *Echiothrix leucura*; D, *Haeromys minahassae*; E, *Haeromys* sp.; F, *Margaretamys elegans*; G, *Margaretamys parvus*; H, *Margaretamys beccarii*; I, *Maxomys hellwaldii*; J, *Maxomys musschenbroekii*; K, *Maxomys* sp. B; L, *Tateomys rhinogradoides*; M, *Tateomys macrocercus*; N, *Melasmothrix naso*; O, *Crunomys celebensis*; P, *Chrotomys mindorensis*.

in which he included "the fossil and recent genera with both archaic and advanced molar characters." In the context of Misonne's work, his arrangement simply indicated that the two genera were relict in some way, arboreal to a degree, isolated on mountains or islands, and adapted to tropical forest. But spermatozoal characteristics of *Lenomys* and *Eropeplus* are unlike those from any of the other genera that have been surveyed and which could be similarly characterized: *Chiropodomys* and *Lenothrix* from the Malay Peninsula, for example (Breed and Yong, 1986). Of the genera in the *Lenothrix-Parapodemus* Division, the closest in sperm morphology to *Lenomys* and *Eropeplus* is *Pithecheir* from the Malay Peninsula—another relict in which the sperm head lacks an apical hook (Breed and Yong, 1986).

Our second major group that is defined by spermatozoal traits contains all the other species surveyed here. Basic to nearly all of them is an asymmetrical falciform or scythe-shaped sperm head with a prominent apical hook, and a tail which inserts, by a connecting piece, on the ventrocaudal surface of the sperm head. Sperm from one, or perhaps two, species of *Taeromys* are the only exception to this design.

By this clustering we do not mean to imply that all the species with such a shape are more closely related to one another than they are to *Lenomys meyeri* and *Eropeplus canus*. We simply mean to indicate that they all share a general morphology which we assume to be primitive, and which contrasts with the presumed derived spermatozoal configurations found in samples of *Lenomys* and *Eropeplus*.

There is sufficient interspecific variation in shape of sperm head and length of tail within the second major group to allow us to comment on how the species may be related among themselves, and to others outside of Sulawesi and the Philippines. It is clear that spermatozoal traits we noted cannot be used to sort the species into the three discrete groups that were suggested by Musser (1981b). It is also evident that the variation in sperm morphology among the species from Sulawesi and the two from Luzon is not expansive enough to embrace the distinctive shapes peculiar to the native Australian genera (ex-

cluding *Rattus*). Most of the Australian species have a sperm head with two extra ventral hooks in addition to the dorsal apical hook (Breed and Sarafis, 1979; Breed, 1983, 1984). None of the species we studied are characterized by this distinctive, and likely highly derived, configuration. Results from our observations do not support a view that the native Sulawesi murines and at least two of the Philippine species are part of the same radiation which resulted in the old Australian endemics (*Hydromys* and *Xeromys*; *Conilurus*, *Leggadina*, *Leporillus*, *Mastacomys*, *Mesembriomys*, *Notomys*, *Pseudomys*, and *Zyzomys*; *Melomys* and *Uromys*). If they are part of the same radiation, then the characters uniting the groups of Australian endemics (hydromyines, conilurines, and uromyines) evolved after these lineages diverged from the ancestral stock out of which the Sulawesi and Philippine species evolved. That the apomorphic double ventral hooks evolved prior to separation of the genera within Australia is suggested by the presence of such hooks on the sperm head of most of the species in all three of the Australian groups (Breed, 1984).

Sperm morphology is similar among the three species of *Rattus* we surveyed and also closely resembles that of other species now placed in that genus, whether they be native to the Malay Peninsula (Breed and Yong, 1986), Australia (Breed and Sarafis, 1979), or elsewhere (*R. rattus* and *R. norvegicus*; see Retzius, 1909; Friend, 1936).

Among the Sulawesi species we surveyed, spermatozoa from *Bunomys* resemble sperm from species of *Rattus* more closely than the sperm from any of the other genera. There are differences, however, not only between the two genera but among the species of *Bunomys*. Sperm morphology characterizing *B. chrysocomus* and *B. andrewsi* resembles that of *Rattus*, but *Bunomys* sp. A and *B. penitus* are less similar in that they have a wider sperm head, shorter apical hook, and shorter sperm tail. This corresponds with variation seen in the skull, dentition, and external traits of the various species of *Bunomys*. In those features, *B. chrysocomus* and *B. andrewsi* resemble each other; *Bunomys* sp. A and *B. penitus* are each distinctive, quite

different in morphology from the first two, and probably more closely related (Musser and Newcomb, 1983; Musser, in prep.).

The species *dominator* has long been associated with the genus *Rattus* (Tate, 1936; Ellerman, 1941), but its distinctive cranial characters finally stimulated Ellerman (in Laurie and Hill, 1954) to place it in his new subgenus *Paruromys*, which Musser and Newcomb (1983) regarded as a distinct genus, one that is defined by a diagnostic suite of cranial, dental, and external traits. Sperm morphology clearly supports the removal of *dominator* from the embrace of *Rattus*. Its sperm head with a broad lateral face, short apical hook, and short tail is unlike the sperm morphology characteristic of *Rattus*. Superficially (except for its shorter hook), it resembles sperm heads seen in samples from *Mus*, *Chiropodomys*, and the species of *Maxomys* from the Malay Peninsula (Breed and Yong, 1986). Musser and Newcomb (1983) indicated there might be a link between *Paruromys* and *Taeromys*, but sperm morphology does not support that tentative suggestion.

Marked interspecific differences in sperm morphology are evident among the species of *Taeromys* that we surveyed. Except for its broader sperm head, the spermatozoal configuration of *T. arcuatus* somewhat resembles that of *Rattus*. The sperm of *T. callitrichus* is basically similar in shape to that of *T. arcuatus*, but it has a much shorter apical hook and sperm tail. The sperm of *Taeromys hamatus* is distinctive with its short and wide head, blunt apical hook, and short tail. And the sperm morphology of *T. celebensis* is the most divergent among the four species: an elongate sperm head without an apical hook and a midbasal (as contrasted with ventrocaudal) insertion of a very short sperm tail.

The variation in the apical portion of the sperm head seems to represent a transformation species. It extends from a head with a prominent apical hook (*arcuatus* and *callitrichus*) to an intermediate stage where the hook is reduced to a short and blunt projection (*hamatus*), and ends with a paddle-shaped head lacking an apical hook (*celebensis*). Assuming the four species are part of the same monophyletic group, and given the polarity in sperm morphology that is our work-

ing hypothesis, it seems likely that the sperm shape seen in *T. arcuatus* and *T. callitrichus* is primitive, and the shortening or elimination of the apical hook so characteristic of *T. hamatus* and *T. celebensis* is a more recently derived configuration.

As in *Bunomys*, the diversity in spermatozoal morphology within *Taeromys* reflects the variation in cranial, dental, and external characters among the species. *Taeromys callitrichus* and *T. celebensis* represent the opposite ends of the range of morphologies within the genus (compare the crania and molar rows illustrated in Musser and Newcomb, 1983: 488–493). *T. arcuatus* is more like *T. callitrichus*, and *T. hamatus* has its own distinctive traits and is no more closely related to *T. callitrichus* or *T. arcuatus* than to *T. celebensis* (Musser, in prep.).

All species of *Taeromys* were for a long time included within *Rattus* (see the history in Musser, 1970, and Musser and Newcomb, 1983), and *callitrichus* and *celebensis* were closely linked to *infraluteus* and *muelleri*, two species that had been placed in *Rattus* but are now part of *Sundamys* (Musser and Newcomb, 1983). Sperm morphology does not tie *Taeromys* closely to *Rattus*, and demonstrates no strong link between *Taeromys* and *Sundamys*. Configuration of sperm head and relative length of sperm tail in samples of *S. muelleri*, for example (*S. infraluteus* and *S. maxi* have not been sampled), are very similar to sperm of *Rattus* (Breed and Yong, 1986), but not to species of *Taeromys*.

A broad sperm head, short apical hook, and short sperm tail are traits uniting the two species of *Haeromys*. A ventral spike is also present in both species, which, among Sulawesi murines appears—at this level of resolution—to be unique to *Haeromys*. The spermatozoal configuration and relative sizes of its elements resemble sperm of *Chiropodomys gliroides* from the Malay Peninsula (see fig. 2 in Breed and Yong, 1986). This resemblance is important if the sperm traits of both genera (a broad sperm head, short apical hook, and short tail) are shared, derived features. The similarity would reinforce the weak affinity between *Haeromys* and *Chiropodomys* that was speculated by Musser and Newcomb (1983). If the characteristics are

primitive, the resemblance indicates only that the two sets of species share primitive spermatozoal traits. Then sperm morphology could be added to all the primitive cranial and dental traits (Musser and Newcomb, 1983), as well as chromosomal features (Musser, 1990), that distinguish *Haeromys* from all other Sundaic and Sulawesi murines (Musser and Newcomb, 1983). Unfortunately, we cannot assign a primitive-derived polarity to either a sperm shape like that in *Rattus* (long and narrow sperm head, long apical hook, and moderately long tail) or to something similar to the shape characteristic of *Haeromys*.

Long and narrow sperm heads, long apical hooks, and moderately long tails unite the species of *Margaretamys*, a group also defined by shared cranial, dental, and external traits (Musser, 1981a). Although somewhat similar to the sperm of *Rattus* in general shape, the very large sperm heads and apical hooks of *Margaretamys* clearly set it apart from *Rattus* or any of the other Sulawesi endemics except for some *Maxomys*.

Except for the longer sperm tails, the spermatozoal configuration seen in *Maxomys hellwaldii* and *Maxomys* sp. B resembles sperm shapes of the three species of *Margaretamys*. These two species of *Maxomys* are unlike their counterparts on the Malay Peninsula (*M. surifer*, *M. whiteheadi*, and *M. inas*) which have shorter and broader sperm heads and shorter tails (Breed and Yong, 1986). The other *Maxomys* species from Sulawesi that we examined, *M. musschenbroekii*, has spermatozoa that in shape and size resemble those in samples from the Malayan species.

The three kinds of Sulawesi *Maxomys* also fall into two groups that are defined by traits of the skull, molars, hind feet, and fur. *Maxomys hellwaldii* and *Maxomys* sp. B cluster and stand apart from *M. musschenbroekii* (Musser, in prep.). For example, cusp t4 (the posterolingual cusp on the second cusp row of each first and second upper molar) is partially or completely divided, a derived trait unique to *M. hellwaldii* and *Maxomys* sp. B; comparable cusps in *M. musschenbroekii* are undivided.

The taxa now included in *Maxomys* were once placed in the genus *Rattus* (Ellerman,

1941; Laurie and Hill, 1954). Geographic range of the group encompasses parts of Indochina, islands on the Sunda Shelf, and Sulawesi; and the species reflect diverse cranial, dental, and chromosomal morphologies (Musser et al., 1979; Musser and Newcomb, 1983). Variation in sperm morphology is no exception.

The phylogenetic affinities of *Echiothrix leucura* are enigmatic. The genus was once closely associated with the Philippine shrew rat *Rhynchomys*, has been thought to be a specialized derivative of the *Rattus xanthurus* group (which contains *R. facetus*), was considered to be so morphologically aberrant that it had no close relatives, or was placed with *Crunomys* in a group near or transitional to *Hydromys* and its relatives (see the history recounted in Musser, 1969; Misonne, 1969). Sperm morphology cannot identify *Echiothrix*'s closest relatives. Shape and size of the various spermatozoal elements of this shrew rat resemble the sperm configuration in species of *Margaretamys* and some *Maxomys* (*M. hellwaldii* and *Maxomys* sp. B), although the apical hook is shorter. And apart from size, the general sperm shape also resembles that characteristic of *Rattus*.

In addition to being placed in the same group as *Echiothrix*, the Philippine *Crunomys* has at one time or another been allied to the Philippine shrew rats *Chrotomys* and *Celaenomys*, and included in a group which also contained *Rattus* (Ellerman, 1941; Misonne, 1969; see the historical summary in Musser, 1982). Information about sperm morphology in the Philippine species of *Crunomys* is unavailable, but spermatozoa in the Sulawesi form have a conformation and size unlike those of any species of either *Echiothrix*, *Chrotomys*, or *Rattus*. Except that the sperm does not look like that from species of *Lenomys* and *Eropeplus*, and does not have ventral hooks as do most of the non-*Rattus* Australian endemics, sperm morphology is not especially useful in identifying the closest relative of the Sulawesi *Crunomys*.

Each of the three species of small-bodied Sulawesi shrew rats is characterized by a suite of diagnostic traits that relates it more closely to the other two species than to any other murine rodent native to Sulawesi (Musser, 1982). Sperm morphology is also similar

among the three. Sperm from *Tateomys rhinogradoides* and *T. macrocercus* are nearly identical in conformation and size. Spermatozoa from *Melasmothrix naso* differ slightly in shape and size. Based only on spermatozoal characters, the species of *Tateomys* could be grouped with some species of *Bunomys* or even with *Chrotomys mindorensis*; and except for length of the sperm tail, their sperm morphology somewhat resembles that of *Rattus*. These alliances are not reflected in any other set of morphologies studied, and likely indicate the retention of similar primitive spermatozoal traits.

Spermatozoa of the Philippine shrew rat *Chrotomys mindorensis* resemble those of *Tateomys* in shape and size, and the shape is even reminiscent of the *Rattus* sperm. Among the species we surveyed, sperm morphology of the shrew rat is not a helpful source of data for estimating its phylogenetic links. Clearly, however, the sperm head of *Chrotomys*, with its simple and likely primitive conformation, is unlike those of *Hydromys* and *Xeromys*, with their specialized ventral hooks (see plate 1 in Breed, 1984). No evidence from sperm morphology supports the grouping of *Chrotomys* with *Hydromys* and its relatives as Missone (1969) has suggested.

CONCLUSION

External morphology of spermatozoa—as seen with the light microscope—does vary among the Sulawesi and Philippine species we sampled. The significance of the distribution of sperm traits for inferring phylogenetic relationships is also variable. If our assumptions about the spermatozoal configurations which represent primitive and derived states are correct, then *Eropeplus canus* and *Lenomys meyeri* share a derived set of features that distinguishes them from any of the other species. This pairing is consistent with the distribution of certain cranial and external attributes. Sperm shape, size, and proportions appear to provide significant phylogenetic information for these two species.

All the other species we record here have sperm with a different shape, a conformation defined by traits we assume to be primitive among murines. That this design is found in

the Philippine *Chrotomys* is significant because it is unlike the highly derived sperm heads of *Hydromys* from the region of New Guinea and Australia. Specialized dental traits have been used to cluster these two genera within the same taxonomic category. Here again, external sperm morphology seems to contain significant phylogenetic data.

Because we are ignorant about the primitive versus derived polarities of the sperm traits associated with the remainder of the species, the significance of their morphology in the context of our inquiry is reduced to that of clustering by morphological similarity, which may or may not reflect phylogenetic ties. In some cases it clearly does: a similar conformation is shared by the species of *Rattus*; a common shape unites the three *Margaretamys*; the two species of *Haeromys* share a similar spermatozoal external morphology; a certain sperm shape and size are typical of both kinds of *Tateomys*; the spermatozoa of *Melasmothrix* resemble those of *Tateomys* but are still distinct; and the respective spermatozoa of *Crunomys* and *Paruromys* are distinct from those of other species. Although we can use spermatozoal traits to cluster the species within each of these genera we cannot estimate the pattern of their phylogenetic relationships to one another.

Among the remainder of genera, sperm morphology is ambiguous in its ability to determine species similarity, let alone resolve phylogenetic links. There is considerable variation in the configuration of spermatozoa within *Bunomys*, *Taeromys*, and *Maxomys*. Spermatozoal features by themselves do not support the assumption that each of these genera consists of a monophyletic group of species.

The range of shapes that characterizes *Taeromys* is especially impressive. At one end is the general configuration: a falciform sperm head with a prominent apical hook and a long tail inserting on the ventrocaudal surface of the head (*T. callitrichus*, for example). The other extreme is represented by a paddle-shaped sperm head with a short tail inserting at its midbasal portion (*T. celebensis*), a shape which more closely resembles that of sperm from *Eropeplus* and *Lenomys* than those from the other species of *Taeromys*. The range in sperm head shapes recalls that documented

for the Australian *Pseudomys* in which most species have an asymmetrical and falciform-shaped head with a large apical hook (as well as two ventral hooks, unlike those in *Taeromys*), a few have lost the hooks, and one closely resembles the configuration recorded for *Eropeplus*, *Lenomys*, and *Taeromys celebensis* (Breed, 1983).

We suggest that these ambiguities might be resolved by using three approaches. One would be to determine the functional significance of the various sperm morphologies we have described, a difficult inquiry. A second would be to survey ultrastructure of the spermatozoa at the level of resolution provided by scanning and transmission electron microscopy. Greater resolution might demonstrate additional traits and allow us to better evaluate whether the similarities among sperm morphologies we record here really represent homologous states. The third would be to estimate phylogenetic relationships among the Sulawesi and Philippine species and between them and groups from nearby regions (especially Southeast Asia) using other sets of data—morphological (external features, skeleton, reproductive tract, and other organ systems) and biochemical—and see how information derived from the study of spermatozoal morphology fits those results.

One result of our survey is unambiguous. In their spermatozoal morphology, the endemic Sulawesi murids and the two species of Philippine rats are clearly allied to faunas native to islands on the Sunda Shelf and mainland of Southeast Asia, and not to the indigenous, non-*Rattus*, Australian genera.

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