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Sperm Morphology in the Malagasy Rodents (Muroidea: Nesomyinae)

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ABSTRACT The morphology of the spermatozoon of representative species of the subfamily Nesomyinae (Muroidea: Nesomyidae), a monophyletic group of rodents endemic to Madagascar, was examined by light and electron microscopy to determine the sperm head shape and tail length across the species. Marked interspecific differences were found to occur in both the form of the sperm head and length of the tail. The species that possess a sperm head with an apical hook, which largely contains acrosomal material, generally displayed longer sperm tails, and a species with a spatulate sperm head had the shortest tail. The association between sperm head shape and tail length mirrors that previously found in Eurasian and Australasian murine rodents. Thus, the repeated association between sperm head shape and tail length across these groups of muroid rodents clearly indicates a functional relationship between these two features. A comparison of sperm morphology of the nesomyines to that of related muroid rodents on the mainland of Africa suggests that the possession of an apical hook is the ancestral condition. J. Morphol. 271:1493–1500, 2010.

KEY WORDS: sperm morphology; rodents; Nesomyinae; Madagascar

INTRODUCTION

Mammalian spermatozoa are remarkably diverse in form and size across species with members of the Rodentia exhibiting the greatest interspecific variability for any taxon of mammals. Why has such a diversity of sperm forms evolved, and what are the selective forces that have brought about such differences in morphology? Although early interpretations suggested nonadaptive changes to explain interspecific differences in sperm morphology (Friend, 1936), the repeated associations between certain character states across the various murine rodent lineages suggests that such features may be functionally correlated and have evolved due to either natural and/or sexual selection (reviews: Roldan et al., 1992; Gage, 1998; Breed, 2002; Pitnick et al., 2009).

In rodents, the greatest variation of sperm form occurs within the Muridae, a large radiation indigenous to the Old World (about 730 species representing 150 genera, Musser and Carleton, 2005). Their sperm head shapes vary from those having one to three rostrally projecting hooks consisting of extensions of the nucleus, acrosome, and cytoskeleton, to spatulate or pear-shaped sperm heads with less extensive sperm head cytoskeletal material (Friend, 1936; Bishop and Austin, 1957; Bishop and Walton, 1960; Breed, 2002, 2004). The sperm tail similarly shows marked species differences in length, ranging in murine rodents from around 56–170 μm (Cummins and Woodall, 1985; Breed and Taylor, 2000). Recent studies of sperm morphology in various Eurasian and Indoaustralian representatives of the subfamily Murinae have revealed a consistent association between sperm head shape and tail length, which is longer in species that possess one or more apical hooks on the sperm head (Breed and Musser, 1991; Roldan et al., 1992; Breed and Taylor, 2000; Breed, 2002, 2004; Immler et al., 2007; review: Pitnick et al., 2009). The constancy of this association implies that selection has influenced sperm tail length in relation to head shape (Gage, 1998), although this association was not found to occur in gerbils (Muridae: Gerbillinae), the second largest clade

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within the Muridae, although only a few taxa were investigated (Breed, 2005).

To determine whether this relationship between shape of the sperm head and tail length extends to other rodent groups, we have examined sperm morphology in species of the subfamily Nesomyinae (Nesomyidae), a clade of muroid rodents that comprises a small, but diverse, subfamily endemic to the island of Madagascar. From a common ancestor that is suggested as having arrived in the early Miocene 24–20 million years ago (Jansa and Carleton, 2003; Poux et al., 2005; Ali and Huber, 2010), the radiation of extant Nesomyinae includes 27 species in nine genera (Goodman et al., 2008, 2009). Nesomyine rodents are related to a few small groups of sub-Saharan African muroid rodents all of which are currently recognized as subfamilies within the family Nesomyidae (Musser and Carleton, 2005) with the closest living relatives probably being members of the subfamily Cricetomyinae and/or Mystromyinae (Jansa et al., 1999). This, and other broader molecular studies, have indicated that the Nesomyidae arose from a common ancestor of the Families Cricetidae and Muridae, the most species-rich family-level clades of Muroidea (Michaux et al., 2001; Jansa and Weksler, 2004; Steppan et al., 2004; Jansa et al., 2009).

Although relatively few in number, the genera of Malagasy rodents are strikingly differentiated in external morphology and their natural history. They range from saltatorial, gerbil-like animals (*Macrotarsomys*), to arboreal forms with long tufted tails (*Eliurus*), to short-tailed, vole-like species (*Brachyuromys*). Little has been published on the sperm morphology of members of this group apart from brief mention of sperm structure of *Nesomys audeberti* (Bedford, 2004) and three species of *Eliurus* (Breed, 2005). In the present study, we expand the survey of sperm morphology of Nesomyinae to include five genera and 12 species. We show that there is a correlation between sperm head shape and tail length and we briefly consider some possible functional implications of these morphological findings.

MATERIALS AND METHODS

The spermatozoa all came from adult animals collected in the wild with the specimens being housed in either the Field Museum of Natural History, Chicago (FMNH) or the United States National Museum of Natural History, Washington, DC (USNM). For FMNH specimens, the epididymides were removed in the field, fixed in either 10% buffered formaldehyde or 3% glutaraldehyde, and shipped to Cornell Medical College, New York, whereas the whole bodies of the USNM animals were fixed in the field and then sent to the museum where cauda epididymides were subsequently dissected. We include the following taxa and cataloged specimens in this study together with the locations of collection of the individuals.

Brachytarsomys villosa: Province de Mahajanga, 13.5 km SW Befingotra (FMNH 167469).

Brachyuromys betsileoensis: Province de Fianarantsoa, 3 km NNW Vohiparara (USNM 449218, 449223, 449224).

Eliurus antsingy: Province de Toliara, 3.5 km E Bekopaka (FMNH 172721).

E. grandidieri: Province de Mahajanga, 13.0 km SW Befingotra (FMNH 167464).

Eliurus majori: Province de Mahajanga, 13.0 km SW Befingotra (FMNH 167453):

E. minor: Province de Fianarantsoa, Vatoharanana, 4 km SW Ranomafana (ville) (FMNH 170815, 170817).

E. myoxinus: Province de Toliara, Forêt d'Analavelona, 16.5 km NW d'Andranoheza (FMNH 169750, 169754).

E. tanala: Province de Fianarantsoa, Vatoharanana, 4 km SW Ranomafana (ville) (FMNH 170824); Forêt de Vinantelo, 15.5 km SE Vohitrafeno (FMNH 170840).

E. webbi: Province de Fianarantsoa, 2 km NE Andrambovato (USNM 449267); 9 km ESE Kianjavato (USNM 449265).

Macrotarsomys bastardi: Province de Mahajanga, Ampijoroa (USNM 341822).

N. audeberti: Province de Fianarantsoa, Ambodiamontana, Ranomafana (USNM 44895); Vatoharonoma 4 km SW Ranomafana (ville) (FMNH 170846).

N. rufus: Province de Fianarantsoa, Ambodiamontana about 7 km. west of Ranomafara (USNM 448965); 3 km NNW Vohiparara (USNM 449235, USNM 449238, USNM 449239); and Vatoharanana 4 km SW Ranomafana (ville) (FMNH 170852).

All dissected materials were subsequently transferred to The University of Adelaide, South Australia, where sperm were prepared for both light and/or electron microscopy. The cauda epididymides were removed, cleaned, and sperm were extruded into neutral buffered formalin. For light microscopy, small drops of sperm suspensions were placed on microscope slides and studied by phase contrast and/or Nomarski differential interference microscopy. The morphology of the sperm head was determined for 10–30 spermatozoa from each individual, and mean lengths were recorded. To determine nuclear shape, a few drops of 4′-6′-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma, St Louis, MO) were added to the slide and the resultant images viewed by ultraviolet fluorescent microscopy.

For transmission electron microscopy (TEM), small pieces of cauda epididymides, and occasional adjacent vasa deferentia, that had been fixed in formaldehyde were placed in 3% glutaraldehyde/3% paraformaldehyde made up in 0.1 mol l^{-1} phosphate buffer to which 2.5% polyvinylpyrrolidone had been added. These were postfixed in 1% osmium tetroxide, dehydrated in a graded series of alcohols, and embedded in TAAB epoxy resin. Thick plastic sections were cut, stained with toluidine blue in borate buffer, and then, where appropriate, ultrathin sections cut. These were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined at 80 kV with a Philips TEM 100.

For scanning electron microscopy (SEM), the fixed spermatozoa were placed in 0.1 mol l^{-1} phosphate buffer on polylysine-coated coverslips, dehydrated by passing the coverslips through a graded series of acetones, critical point dried, coated with 10 nm of platinum and 5 nm of carbon, and observed in a Philips SEM.

Measurements on the sperm were made with a Nomarski light microscope using a five megapixel Nikon digital camera and analyzed with elements BR 3.1. Sperm measurements include head length from its base to the apical tip, breadth at its maximum width, total tail length from the connecting piece to the tip of the end piece, and midpiece length from the neck to the point where the sperm tail diameter decreased, which was often the site of a cytoplasmic droplet. To assess possible correlations between sperm heads with apical hooks and longer tails, and sperm heads lacking hooks and a shorter tail, a linear mixed effects model was used, and calculations were performed using SAS Version 9.2 (SAS Institute, Cary, NC). One way analyses of variance and bivariate regressions were implemented using Systat for windows, Version 10.2 (Systat Software, Chicago, IL, 2002).

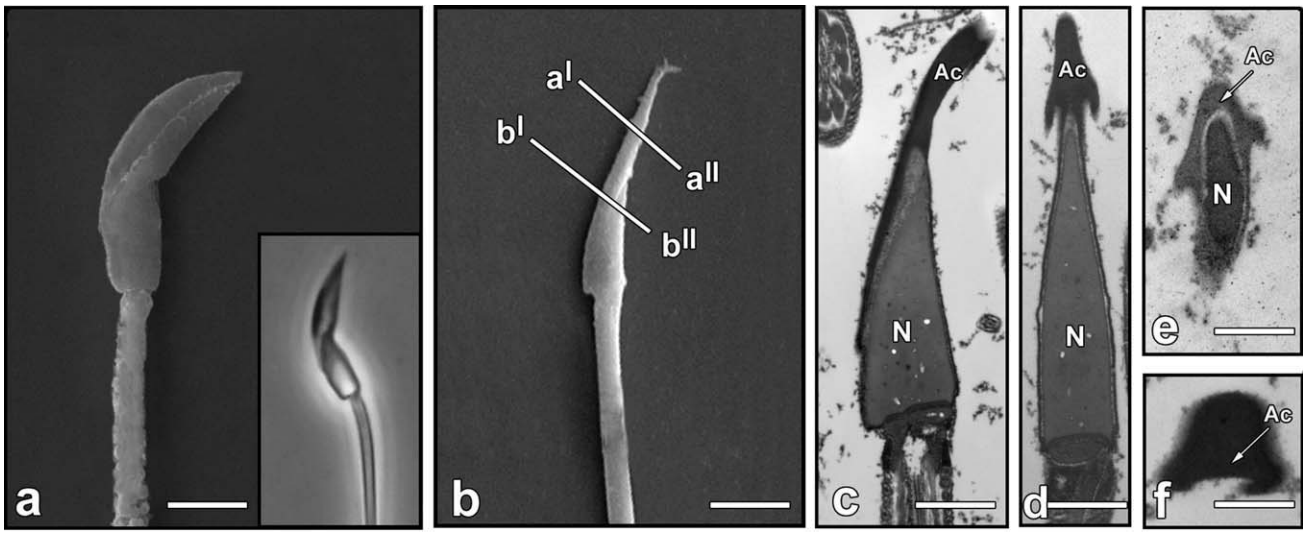

Fig. 1. Scanning electron micrographs (**a**, **b**), phase contrast (a, inset), and TEMs (**c**–**f**) of sperm heads *Brachytarsomys villosa* (a), and *Brachyuromys betsileoensis* (b–f) showing presence of an apical hook and lateral extensions of the acrosome in sperm of the latter species; approximate sections e and f shown in b′–b" and a′–a" (b). Scale bars: a = 3.4 μm; b = 3.6 μm; c = 1.4 μm; d = 1.1 μm; e = 0.5 μm; f = 0.5 μm.

RESULTS

Marked differences in the shape of the nucleus and acrosome of the sperm head and in midpiece and total length of the sperm tail were observed to occur across the different species of Malagasy rodents.

Sperm Head Shape

In the single specimen of *Brachytarsomys villosa*, a large, sickle-shaped, sperm head was evident, the apical region of which forms an optuse angle of about 140° to the rest of the sperm head (Fig. 1a). This apical region is largely composed of acrosomal material, which is highly asymmetrical (Fig. 1a, inset), whereas the tail attaches to the midbasal region.

The sperm head of *Brachyuromys betsileoensis* is also long and narrow (Fig. 1b) with its apical region being flexed to a variable degree. Transmission electron micrographs showed that it was bilaterally flattened (Fig. 1c,d), and that the acrosome, which has small lateral flanges (Fig. 1c–e), extends beyond the tip of the nucleus and becomes triangular in transverse section with a rounded apex (Fig. 1f).

Considerable interspecific differences in sperm head shape were encountered among the seven species of *Eliurus* (Fig. 2, Table 1). In *E. majori*, the sperm head is bilaterally flattened, and has a distinctive apical hook about 3.5-μm long that is set at right angles to the rest of the head (Fig. 2a, inset). Transmission electron micrographs revealed that the bilaterally flattened nucleus extends into the base of the hook (Fig. 2b) with the rostral portion of the hook consisting of a triangular-shaped acrosomal extension (Fig. 2c). The sperm tail attaches medially to the base of the head. In all other species of *Eliurus* examined, the spermatozoa lacks an apical hook, and the midpiece of the tail attaches to the head in an offset often lateral position (Fig. 2d–g). The sperm head is generally similar in size and shape in the four species, *E. grandidieri* (Fig. 2d), *E. minor* (Fig. 2f), *E. webbi* (Fig. 2e), and *E. tanala* (Fig. 2g). It is broad and subcircular to kidney shaped in form, with a rounded anterior region composed of an acrosome that thickens apically (Fig. 2h,i). Phase contrast microscopy confirmed that the acrosome forms a thickened cap, or dome, over the apical and upper convex surface of the nucleus (Fig. 2f,g, inset), and that the tail attaches to the lower concave region of the sperm head. Somewhat different sperm head shape occurs in *E. antsingy*, where it is more paddle shaped (Fig. 2j), and in *E. myoxinus*, where it is narrower and more ovate with near midbasal attachment of the tail (Table 1, Fig. 2k,l).

The sperm head of *Macrotarsomys bastardi* lacks an apical hook, is spatulate in shape, and is relatively small, being about 5-μm long and 3-μm wide (Fig. 3a). The midpiece of the sperm tail attaches medially to the base of the head with the form of the nucleus reflecting the overall head shape and capped by a thin, symmetrical acrosome (Fig. 3b).

In both species of *Nesomys*, the sperm head is narrow and thin and tapers distally to form a distinctive apical hook with an angle of about 130° to the rest of the sperm head (Fig. 3c, Table 1). In both species, the midpiece of the tail attaches to

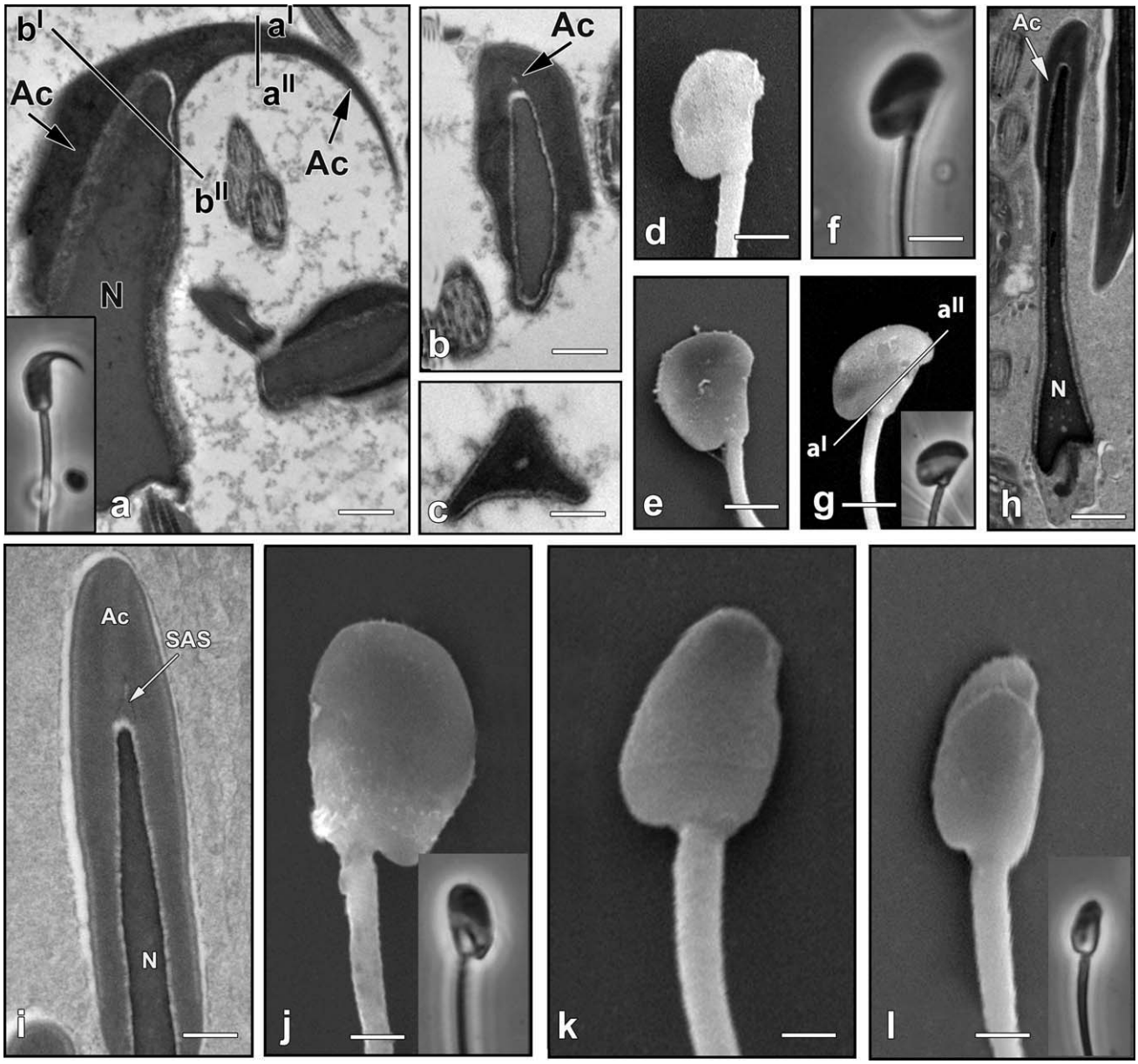

Fig. 2. Sperm of *Eliurus* species showing marked differences in head morphology across species. Scanning electron micrographs (d, e, g, j–l), phase contrast LMs (f and insets in a, g, j, i) and TEMs (a–c, h, i). Sperm heads are of *Eliurus majori* (a–c); *E. grandidieri* (d, h, i), *E. minor* (f), *E. webbi* (e), *E. tanala* (g), *E. antsingy* (j), and *E. myoxinus* (k, l). N, nucleus; Ac, acrosome; sections b′–b" and a′–a" in a shown in b and c, and g a′-a" shown in h. Scale bars: a = 0.7 μm; b = 0.4 μm; c = 0.2 μm; d, i = 2.4 μm; e = 2.8 μm; f = 5.0 μm; g = 4.0 μm; h = 0.6 μm; i = 1.1 μm; j = 1.4 μm; k = 0.9 μm; l = 1.3 μm.

the lower concave surface of the head. In *N. audeberti*, the apical hook is 6.8–7.5 μm in length, DAPI stained heads showing that the nucleus only extends into the base of the hook (Fig. 3c, inset on right), whereas TEM and phase contrast microscopy demonstrated that the hook was composed almost entirely of acrosomal material distally (Fig. 3c, inset on left); TEM also delineated an apical extension of the subacrosomal space (Fig. 3d,e). The sperm head morphology of *N. rufus* is similar to that of *N. audeberti* (cf. Fig. 3c vs. 3f); the nucleus is capped by an acrosome that extends apically and narrows to a tapering triangular structure beyond the tip of the bilaterally flattened nucleus (Fig. 3g–j).

Sperm Tail Length

Length of the sperm tail varies from about 60 μm in *Macrotarsomys bastardi* to well over 200 μm in *Brachytarsomys betsileoensis* (Table 1). Proportional development of the tail and other sperm

TABLE 1. Sperm head and tail lengths and widths (±SD) of Nesoymine rodents

Species	Specimens	Sperm head length (μm)	Sperm head width (μm)	Apical hook	Midpiece length (μm)	Total tail length (μm)
Brachytarsomys villosa	FMNH 167469	10.2 ± 0.2	6.5 ± 0.2	Present	59 ± 22	124 ± 1
Brachyuromys betsileoensis	USNM 449224, USNM 449218	9.9 ± 0.7, 9.9 ± 0.8	2.7 ± 0.5, 2.7 ± 0.5	Present	74 ± 6, –	243 ± 14, 212 ± 18
Eliurus antsingy	FMNH 172721	7.5 ± 0.4	5.1 ± 0.5	Absent	14 ± 1.5	66 ± 2.0
E. grandidieri	FMNH 167464	7.7 ± 0.5	5.1 ± 0.6	Absent	15 ± 1	80 ± 2.8
E. majori	FMNH 167453	6.6 ± 0.2	3.3 ± 0.2	Present	23 ± 1	125 ± 3
E. minor	FMNH 170817, FMNH 170815	8.5 ± 0.3	6.0 ± 0.4	Absent	15 ± 0.6, 14 ± 0.2	97 ± 1.8
E. myoxinus	FMNH 169750, FMNH 169754	6.4 ± 0.3, 5.3 ± 0.2	3.4 ± 0.2, 3.2 ± 0.3	Absent	16 ± 0.5, 16 ± 0.5	76 ± 0, 73 ± 1.4
E. tanala	FMNH 170824, FMNH 170840	7.0 ± 0.2, 5.8 ± 0.2	5.6 ± 0.2, 5.1 ± 0.1	Absent	16 ± 0.4, 16 ± 1.0	77 ± 1.4, 80 ± 1.3
E. webbi	USNM 449267, USNM 449265	7.5 ± 0.4, 7.6 ± 0.5	6.3 ± 0.2, 5.6 ± 0.6	Absent	15 ± 0.5, 14 ± 1.1	78 ± 1.2, 72 ± 3.2
Macrotarsomys bastardi	USNM 341822	5.0 ± 0.3	3.0	Absent	40 ± 6	59 ± 22
Nesomys audeberti	FMNH 170846, USNM 448559	10.9 ± 1.1, 11.7 ± 0.9	3.9 ± 0.7, 3.2 ± 0.2	Present	44 ± 1, 42 ± 1	159 ± 0, 152 ± 5
N. rufus	FMNH 170852, USNM 448965	13.3 ± 1.8, 11.7 ± 0.8	3.2 ± 0.8, 3.5 ± 0.4	Present	48 ± 3, 46 ± 3	192 ± 3.8, 183 ± 8.1

dimensions are strongly associated with the general shape of the sperm head, in particular, the occurrence of a well-formed apical hook. Entered as a categorical effect in one-way analyses of variance, the presence/absence of an apical hook accounted for substantial variation recorded for all four sperm measurements (F values = 9.05–52.04, $P \leq$ 0.05–0.0001). Thus, the lengths of the sperm head and tail are highly positively correlated, and those species with an apical hook have a markedly longer sperm head (Fig. 4, top). Similarly, length of the midpiece strongly correlates with overall length of the sperm tail, and those species with an apical hook have longer tails (Fig. 4, bottom). A linear mixed effects model fitted to the latter data (including a random animal effect to adjust for measurement interdependence from the same animal) confirmed that both midpiece length and total sperm tail length are significantly longer in species with a sperm head apical hook (mean midpiece length 45.7 μm vs. 15.1 μm, $P \leq$ 0.0001; mean sperm tail length 182.8 μm vs. 73.7 μm, $P \leq$ 0.0001). *Brachytarsomys villosa* and *Macrotarsomys bastardi* possess very long midpieces relative to overall length of the tails, although sperm of the former possesses an apical hook whereas the latter does not. Species whose sperm have an apical hook have not only longer tails but also tend to have sickle-shaped, narrower heads that contain a rostral extension of acrosomal material.

Noteworthy in both bivariate plots is the outlier position of *Eliurus majori* (Fig. 2a), whose sperm head has an apical hook in contrast to other members of this genus. This intrageneric variation suggests that such proportional relationships vis-à-vis an apical hook are not an inherent consequence of smaller body size. *E. majori* is a moderately large species within the genus, matched or exceeded only by *E. antsingy* and *E. tanala*, and being of larger body mass than *E. grandidieri*, *E. minor*, *E. myoxinus*, and *E. webbi*.

DISCUSSION

Clearly considerable differences in sperm morphology have evolved within the Nesomyinae, which is a small, evolutionarily old and geographically isolated, lineage of muroid rodents compared with the highly diverse, broadly distributed, and speciose rodent families Cricetidae and Muridae (Musser and Carleton, 2005). Current phylogenetic evidence supports the monophyly of the Nesomyinae and suggests that they are an insular remnant of a formerly abundant clade Nesomyidae that diversified within sub-Saharan Africa and is currently represented by other surviving relicts classified within the subfamilies Cricetomyinae, Dendromurinae, and Mystromyinae (Carleton and Musser, 1984; Michaux et al., 2001; Jansa and Weksler, 2004; Steppan et al., 2004; Jansa et al., 2009). These

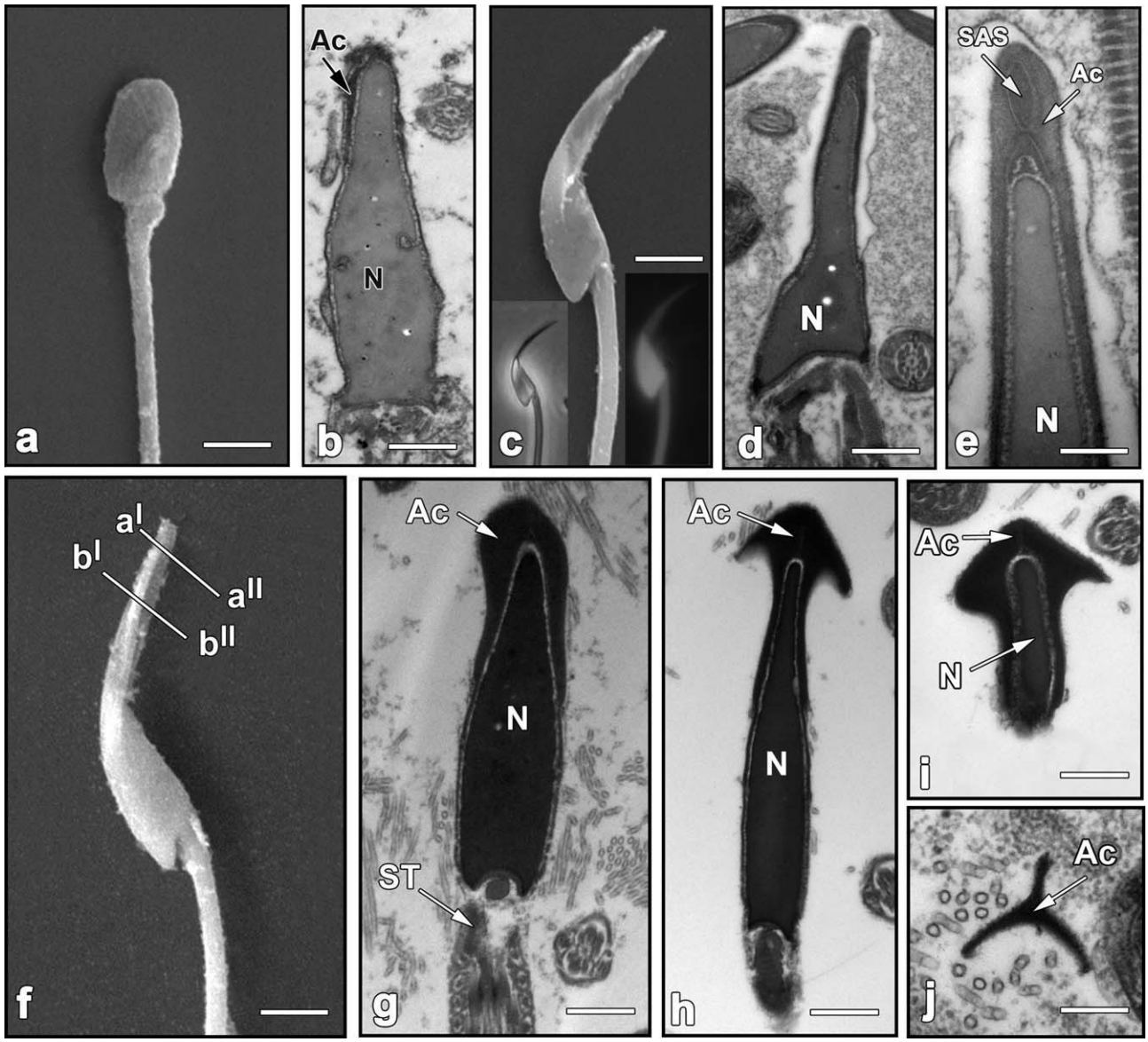

Fig. 3. Scanning electron micrographs (**a**, **c**, **f**), phase contrast (inset c on left), and fluorescent image after staining with DAPI (inset c on right), and TEMs (**b**, **d**, **e**) and (**g**–**j**) of sperm images of *Macrotarsomys bastardi* (a, b), *Nesomys audeberti* (c–e), and *N. rufus* (f–j) showing presence of apical hook in sperm head of both *Nesomys* species. Scale bars: a = 2.3 μm; b = 1.0 μm; c = 2.4 μm; d = 0.8 μm; e = 0.3 μm; f = 1.9 μm; g = 0.8 μm; h = 0.9 μm; i, j = 0.8 μm.

mainland African groups provide an outgroup comparison for interpreting the evolution of spermatozoal characters amongst the Malagasy taxa.

The sperm of species of Cricetomyinae (*Cricetomys gambianus* and *Saccostomus campestris*), Mystromyinae (*Mystromys albicaudatus*), and most Dendromurinae display a long decurved apical hook of acrosomal material and a tail length of between 130 and 150 μm (Breed, 1995, 2005). Among the Nesomyinae we examined, similar long sperm tails and sperm heads with an apical hook are evident in *E. majori*, as well as in species in the genera *Brachyuromys*, *Nesomys*, and *Brachytarsomys*. *Brachyuromys betsileoensis* has a sperm tail longer than that recorded for any species of murine rodent. The similarity of sperm form in these species to that in the nesomyid rodents on the African mainland suggests that they have retained an ancestral condition of sperm morphology within the Nesomyinae. Other species of *Eliurus* and *M. bastardi* thus are likely to have evolved a more recently derived sperm form characterized by a short and wide sperm head together with an absence of an apical hook and shorter sperm tail.

In murine rodents, the apical hook of the sperm head comprises of an extension of the nucleus, acrosome, and perforatorium (Friend, 1936; Lalli and Clermont, 1981; Oko and Clermont, 1988; Breed,

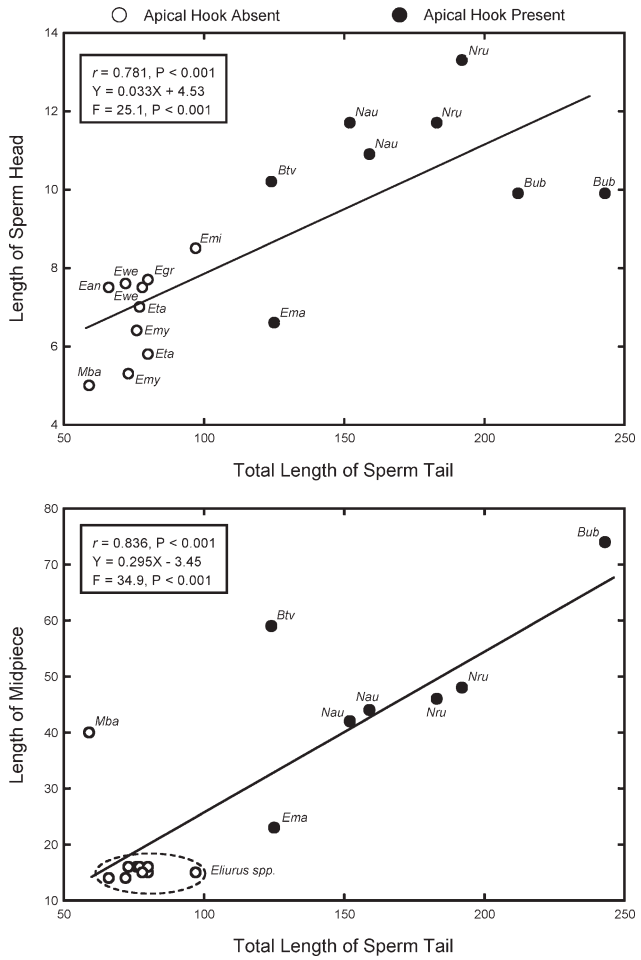

Fig. 4. Scatter plots of selected dimensions of nesomyine spermatozoa in relation to the occurrence of an apical hook (see Table 1). Inset boxes include the correlation coefficient (Pearson's *r*), line regression statistics, and attained probabilities for the variable relationship plotted. Btv, *Brachytarsomys villosa*; Bub, *Brachyuromys betsileonensis*; Ean, *Eliurus antsingy*; Egr, *E. grandidieri*; Ema, *E. majori*; Emi, *E. minor*; Emy, *E. myoxinus*; Eta, *E. tanala*; Ewe, *E. webbi*; Mba, *Macrotarsomys bastardi*; Nau, *Nesomys audeberti*; Nru, *N. rufus*.

2004), whereas in Cricetomyinae and *Mystromys*, the apical hook, as in the Cricetidae, consists largely of acrosomal material with the nucleus terminating near its base (Yanagimachi and Noda, 1970; Breed, 2005). A similar substructure of the apical hook of largely acrosomal material occurs in the Malagasy rodents with its composition much like that of members of the Cricetomyinae, albeit the hook is not so strongly caudally reflected.

At a lower taxonomic level, some of the variation in sperm morphology supports current understanding about kinship among nesomyine genera and species, whilst others do not. *Brachyuromys* and *Nesomys* emerge as sister taxa in gene-sequencing investigations (Jansa et al., 1999, 2009; Jansa and Carleton, 2003), with the species in both genera retaining a narrow sperm head and a pronounced apical hook and long sperm tail. *E. majori*, the only species with an apical hook on the sperm head among the seven *Eliurus* examined, retains several plesiomorphic traits and is thought to represent an older clade within the genus (Carleton, 1994). Thus, together with its relatively longer sperm tail, retention of an apical hook on the sperm head of this species is consistent with an earlier cladistic differentiation and with its placement in its own species group (Carleton and Goodman, 2007). The uniform lack of an apical hook and shorter sperm tails found in the other six species of *Eliurus* do not support other specific groupings as proposed by Carleton and Goodman and as revealed in gene-sequencing studies (Jansa et al., 1999). Furthermore, the lack of an apical hook and the presence of a short sperm tail in these *Eliurus* species and in *M. bastardi*, are likely to reflect parallel evolution rather than a close phylogenetic relationship. The general morphology of these two genera is strikingly divergent (Carleton, 2003; Carleton and Goodman, 2003) as is the detailed anatomy of the sperm head shape as shown in the present study. The applicability of sperm morphology to other questions of intergeneric relationships must await examination of the remaining nesomyine genera *Gymnuromys*, *Hypogeomys*, *Monticolomys*, and *Voalavo*.

What is the functional significance, if any, of the differences in the sperm head morphology and tail length of these rodents? The study by Humphries et al. (2008) indicates that sperm length per se does not correlate with sperm velocity although in a wide ranging study of mammals, Gage (1998) demonstrated an association between sperm head shape and tail length. Recently, Immler et al. (2007) showed that, in murine rodents, both the length of the apical hook and the extent of its curvature associates with relative testis mass, whereas investigations of sperm morphology and behavior in the wood mouse, *Apodemus sylvaticus*, have shown that the long, caudally reflected, apical hook facilitates sperm grouping behavior with these groups moving faster than individual sperm (Moore et al., 2002). The generality of this latter phenomenon within the Muroidea remains unknown, but Fisher and Hoekstra (2010) have reported similar sperm behavior in a species of North American *Peromyscus* (Cricetidae: Neotominae). Whether such behavior also occurs in the nesomyine rodent sperm remains to be determined; within these species there are clearly interspecies differences in length and orientation of the sperm head apical hook whereas in others, e.g., *M. bastardi* and most species of *Eliurus*, an apical hook is completely lacking. It would be interesting indeed to determine whether, in these nesomyine rodents, the presence/absence of an apical hook on the sperm head, together with its length and orientation, associate with sperm grouping behavior, testis mass, as well as the intensity of intermale sperm competition. At the present time, however, too little is known

about the reproductive anatomy and breeding system of these native Malagasy rodents to determine whether such associations exist.

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