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Source: Mammal Study, 46(4) : 269-278

Published By: Mammal Society of Japan

URL: <https://doi.org/10.3106/ms2020-0096>

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Comparative morphology of the male genitalia of Japanese Muroidea species

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Abstract. We examined the morphology of the male genitalia of six Muridae and five Cricetidae in the Muroidea focusing on the medial and lateral bacular mounds, as well as their ossification patterns to discuss the diversity and the movement mechanism of the trident structure. All examined species possessed a medial bacular mound and two lateral bacular mounds, which collectively formed a trident structure. In the Muridae species, the medial bacular mound was ossified or consisted of cartilage, while the lateral bacular mounds were composed of soft tissue. By contrast, both the medial and lateral bacular mounds were ossified in the Cricetidae species. Among the Muridae species, the medial bacular mound was well developed, and the lateral bacular mounds were small in *Mus* and *Micromys* species while the medial bacular mound was highly developed, and the lateral bacular mounds were developed in *Apodemus speciosus*. Different combinations of developmental characteristics of the medial and lateral bacular mounds produced variation in the glans penis morphology. Histological examination of *A. speciosus* and *Craseomys rufocanus* suggested that the movement of the lateral bacular mounds was driven by blood flowing into the cavernous space, and the movement increases the cross-sectional area of the glans penis.

Key words: baculum, Cricetidae, Muridae, reproductive strategy, trident structure.

Diverse morphologies found in mammals are related primarily to feeding, defense, or reproductive strategies (Feldhamer et al. 2020). The diversity of morphology of the glans penis in male genitalia might reflect adaptation to different reproductive strategies or sexual selection. Individual intraspecific variation can then lead to enhanced reproductive success in the specific males with the most adaptive morphology. Therefore, the morphology of male genitalia has been compared between species with respect to differences in mating systems (Eberhard 1985). The surface of the glans penis of mammals directly interacts with the female's vaginal epithelium, and is characterized by hooks, spines, papilla, lappets, domes, and flaps in many taxa (Orr and Brennan 2016). Many species of the mammalian orders Rodentia, Chiroptera, Carnivora, and Primates and a few species of Eulipotyphla, Afrosoricida, and Lagomorpha have a bone, namely the baculum (*os penis*) inside the glans penis, but others do not (Schultz et al. 2016). Schultz et al. (2016) suggested

that the baculum was gained a minimum of nine times and lost a minimum of ten times during mammalian evolution concurrently with changes in reproductive strategies. The baculum acts as a supporting structure for the glans penis, and its morphology exhibits considerable diversity among various groups of mammals (Tokuda 1941; Hamilton 1946; Burt 1960). Therefore, the morphological study of both the glans penis and baculum is expected to increase our understanding of the diversity in the mating systems of mammals.

There are many studies describing the baculum as a taxonomic trait, but only a few studies which focused on the baculum length and width and testis size (Birkhead 2000; Larivière and Ferguson 2002; Stockley et al. 2013). Even fewer studies exist on the functional morphology of the glans penis and baculum (Hart and Melese-D'Hospital 1983; Kelly 2000). The baculum is generally spoon-shaped at the proximal part and rod-shaped, tapering toward the distal part, where diverse morphological char-

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acteristics have been reported among several taxa (Burt 1960; Hooper and Hart 1962). Among rodents, the ground squirrel (Sciuridae: Xerinae) has an asymmetric spine-like structure in the distal part of the baculum, while pocket gophers (Geomysidae) and kangaroo mice (Heteromyidae: Dipodomysinae) have an elongated, generally rod-shaped baculum. These characteristics are representative of the diverse forms of the baculum stalk. On the other hand, the baculum of the Muroidea is unique in that it consists of a stalk and distal segments composed of bone and cartilage, which are separate from the stalk (Burt 1960). With regard to the presence of distal segments, the glans penis of the Muroidea also has features such as spines on the glans, a medial bacular mound (papilla centralis), lateral bacular mounds (papilla lateralis or lateral bacular process), a urethral process (papilla lingualis), a dorsal papilla (papilla dorsalis), and rim papillae (Tokuda 1934; Hooper and Hart 1962; Yang and Fang 1988).

In Cricetidae and Muridae species in the Muroidea, bacular mounds at the distal part of the glans penis include two lateral bacular mounds and a medial bacular mound (hereinafter referred to collectively as a trident structure) (Hooper and Hart 1962). The medial bacular mound has a medial distal segment that consists of bone or cartilage and the lateral bacular mounds have lateral distal segments that consist of bone or cartilage (Kaneko 1985; Hooper and Hart 1962; Yang and Fang 1988). In Arvicolinae species of the Cricetidae, the degree of ossification in the trident structure varies among species, e.g., only the medial distal segment is ossified and the lateral distal segments are not ossified in *Microtus pinetorum* and *M. ochrogaster*, while both the medial and lateral distal segments are ossified in *M. pennsylvanicus* and *M. oregoni* (Hooper and Hart 1962). On the other hand, several species of *Apodemus* and *Rattus* in the Muridae are known to also carry a trident structure, and no ossification of lateral distal segments has been reported in the Muridae (Yang and Fang 1988).

In this study, we conducted a comparative study of 11 Japanese species of the Muridae and Cricetidae to elucidate the diversity in the male genitalia of the Muroidea and, in particular, to understand the diversity of the trident structure. We also performed a histological comparison of the penile structures of Muridae (*Apodemus speciosus*) and Cricetidae (*Craxomys rufocanus*), and discuss the movement mechanism and function of the trident structure.

Materials and methods

In total, 22 specimens of 11 Muridae and Cricetidae species from Japan were examined to observe the external, internal, and histological features of the glans penis. The external and internal morphologies of the glans penis were analyzed in 14 of the specimens. The specimens were deposited at the Kyoto University Museum, Kyoto University (KUZ, Kyoto, Japan) and Takashi O. Yato private collection (TOY, Japan). Murinae species in the Muridae included the large Japanese field mouse *A. speciosus* ($n = 3$, TOY-074 from Hokkaido prefecture, TOY-151 from Miyakejima Island, and TOY-214 Miyagi prefecture), the small Japanese field mouse *A. argenteus* ($n = 2$, TOY-040 from Kyoto prefecture and TOY-215 from Miyagi prefecture), the house mouse *Mus musculus* ($n = 1$, TOY-224 from Tottori prefecture), the Ryukyu mouse *M. caroli* ($n = 1$, KUZ M2272 from Okinawa prefecture), the harvest mouse *Micromys minutus* ($n = 1$, KUZ M12843 from Mie prefecture), and the brown rat *Rattus norvegicus* ($n = 1$, TOY-309 from Hokkaido prefecture). Arvicolinae species in the Cricetidae included the Japanese grass vole *Alexandromys montebelli* ($n = 1$, KUZ M12810 from Nara prefecture), the northern red-backed vole *Myodes rutilus* ($n = 1$, TOY-292 from Hokkaido prefecture), the gray red-backed vole *C. rufocanus* ($n = 1$, TOY-121 from Hokkaido prefecture), Smith's red-backed vole *C. smithii* ($n = 1$, KUZ M13298 from Kyoto prefecture), and Anderson's red-backed vole *C. andersoni* ($n = 1$, KUZ M11486 from Nagano prefecture). All specimens were adults and in a reproductive state based on body weight and testis size, except for *R. norvegicus*, which was in the juvenile state because we were unable to capture an adult individual. For histological analyses, an additional four specimens of *A. speciosus* (body weights 11.4, 20.0, 31.6, and 34.2 g; TOY-320 from Hokkaido prefecture, and TOY-271, 260, and 274 from Yamanashi prefecture) and four specimens of *C. rufocanus* (18.4, 18.4, 30.4, and 37.8 g; TOY-294, 298, 288, and 289 from Hokkaido prefecture) were examined. These two species are known to mature sexually at 30 g or more, and we used individuals of various body weights to evaluate developmental changes from juvenile to adult stages (Abe 1968; Murakami 1974). Specimens were collected in the field and stored at Kyoto University Museum, Kyoto University. The species taxonomies and scientific names followed Denys et al. (2017) and Pardiñas et al. (2017). Terminology used for the glans penis and baculum followed Hooper and Hart (1962), Tokuda (1934),

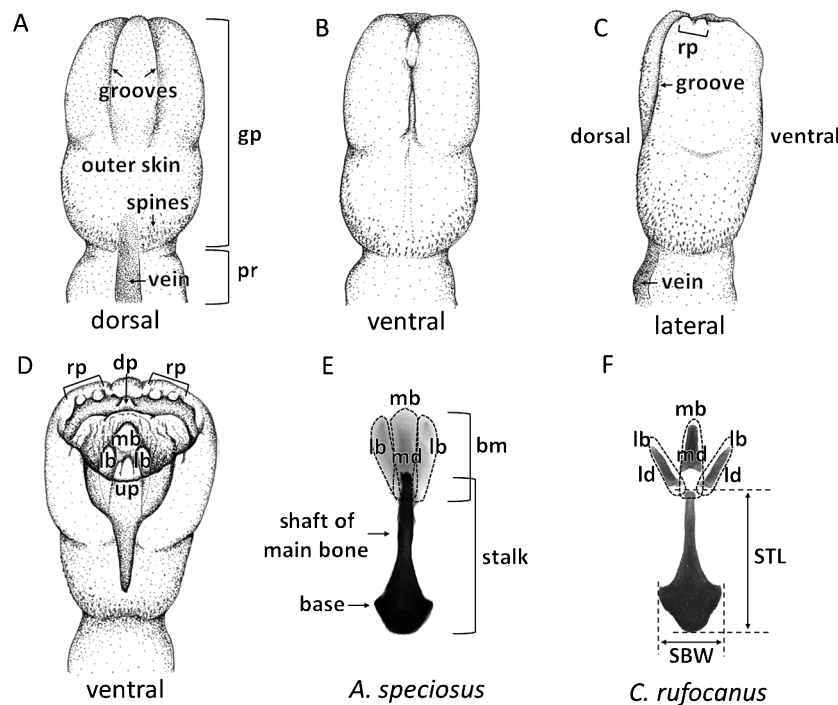


Fig. 1. Illustrations of external dorsal (A), ventral (B), and lateral (C) views of the glans penis of *Apodemus speciosus*; internal ventral view of the glans penis of *A. speciosus* (D); and baculum and bacular mounds of *A. speciosus* (E) and *Craseomys rufocanus* (F). gp, glans penis; pr, prepuce; rp, rim papillae; dp, dorsal papilla or papilla lingualis; mb, medial bacular mound or papilla centralis; lb, lateral bacular mounds or papilla lateral; up, urethral process; bm, bacular mounds or trident structure; md, medial distal segment; ld, lateral distal segments; STL, stalk length; SBW, stalk base width.

Kaneko (1985), and Stockley et al. (2013) as shown in Fig. 1.

Glandes penium were cut at the basal part for external and internal observation, fixed with 70% ethanol or 10% formalin, and stored in 70% ethanol. The dorsal, ventral, and lateral sides of the external glandes penium were observed and photographed with a stereomicroscope. Then, the glandes penium were cut open along the mid-line of the ventral side to observe the internal structure with a stereomicroscope. Following observation of external and internal features of the glandes penium, the samples were transferred to 2% KOH overnight, followed by transfer to Alizarin Red S (Wako Pure Chemical Industries, Ltd., Osaka, Japan) overnight for staining. The samples were then stored in glycerin. The soft tissue was removed except for lateral bacular mounds. The stained samples were observed and photographed with a stereomicroscope and a digital microscope system (VHX-S50 observation system, Keyence Corporation, Osaka, Japan). Stalk length (STL) and stalk base width (SBW) were measured digitally in mm using Adobe Photoshop CS software (Fig. 1C).

Glandess penium used for histological examination

were fixed in formalin and transferred to 70% ethanol for storage. Glandess penium were removed from the body to the proximal junction, decalcified with Morse solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan), dehydrated in a graded ethanol series (70% to 100%), clarified in xylene, embedded in paraffin, and serially sectioned (transversely) into 10- μ m sections with a microtome (Microm HM 335 E, Microm International GmbH, Walldorf, Germany) to clarify the structure of the tissue. The tissue sections were stained with hematoxylin and eosin, and observed and photographed with a microscope (BX50, Olympus, Tokyo, Japan). We adopted the terminology defined by Kelly (2000) to identify the cavernous tissues (corpus cavernosum and corpus spongiosum).

Results

Morphologies of Murinae species

The features of the glans penis and baculum are summarized in Table 1. All examined Murinae species (*A. speciosus*, *A. argenteus*, *M. musculus*, *M. caroli*, *M. minutus*, and *R. norvegicus*) had a medial bacular mound

Table 1. Measurements (mm) and features of the glans penis and baculum in six Murinae species and five Arvicolinae species

Species name	Measurements (mm)		Medial bacular mounds		Lateral bacular mounds		Dorsal papilla	Urethral process	
	Stalk length (STL)	Stalk base width (SBW)	Tissue	State	Tissue	State			
Muridae: Murinae									
<i>Apodemus speciosus</i>	4.29, 4.61, 3.31	1.41, 1.57, 1.30	cartilage	developed	soft tissue	developed	present	trident	
<i>Apodemus argenteus</i>	2.33, 2.34	1.25, 0.98	cartilage	developed	soft tissue	developed	present	trident	
<i>Mus musculus</i>	2.74	0.94	cartilage	highly developed	soft tissue	only trace	absent	bifurcate	
<i>Mus caroli</i>	2.46	0.82	cartilage	highly developed	soft tissue	only trace	absent	bifurcate	
<i>Micromys minutus</i>	2.52	0.51	cartilage	highly developed	soft tissue	only trace	present	bifurcate	
<i>Rattus norvegicus</i>	2.72	0.64	cartilage	developed	soft tissue	developed	absent	bifurcate	
Cricetidae: Arvicolinae									
<i>Alexandromys montebelli</i>	2.60	1.46	bone	highly developed	bone	highly developed	present	trident; with small central one	
<i>Myodes rutilus</i>	2.50	1.23	bone	highly developed	bone	highly developed	present	trident; with small central one	
<i>Craseomys rufocanus</i>	2.77	1.25	bone	highly developed	bone	highly developed	present	trident; with small central one	
<i>Craseomys smithii</i>	2.72	1.49	bone	highly developed	bone	highly developed	present	trident; with small central one	
<i>Craseomys andersoni</i>	2.79	1.65	bone	highly developed	bone	highly developed	present	trident; with small central one	

and lateral bacular mounds that together formed a trident structure (bacular mounds) (Fig. 2A–F). In the Murinae species, the stalk was stained by Alizarin Red S, while the medial and lateral bacular mounds were not stained.

Apodemus speciosus: The medial and lateral bacular mounds were developed and protruded slightly outside of the outer skin of the glans penis (Fig. 2A). A dorsal papilla was present, and the tip of the urethral process was divided into three forks (Table 1); the medial fork was larger than the outer two forks. Two grooves were present on the dorsal part of the outer skin (Fig. 1A). The medial distal segment of the cartilage was rod-like and tapered slightly toward the distal part.

Apodemus argenteus: The medial and lateral bacular mounds were developed but protruded only slightly from the outer skin of the glans penis (Fig. 2B). A dorsal papilla was present, and the tip of the urethral process was divided into three forks (Table 1); the medial fork was as large as the outer two forks. No groove was present on the dorsal part of the outer skin. The stalk of the baculum was wider than in *A. speciosus* of a similar body size. The medial distal segment was thin and short, and its junction with the stalk was rounded.

Mus musculus: The medial bacular mounds were developed, whereas the lateral bacular mounds were

buried in the surrounding tissue and only the tips were exposed (Fig. 2C). The tip of the medial bacular mound was divided into two small parts and the tip of the urethral process was divided into two parts (Table 1). One shallow groove was present on the ventral surface of the outer skin. The stalk was thin at the tip and widened gradually as it approached the base, and the medial distal segment was short, even though the medial bacular mound protruded to the outside.

Mus caroli: The medial bacular mounds were developed, whereas the lateral bacular mounds were buried in the surrounding tissue and only the tips were exposed (Fig. 2D). The tip of the medial bacular mound was divided into two small parts and the tip of the urethral process was divided into two parts (Table 1). There were two pairs large rim papillae and some small rim papillae on the outer crater. One shallow groove was present on the ventral surface of the outer skin. The stalk was thin at the tip and widened gradually as it approached the base.

Micromys minutus: The medial bacular mounds were well developed, whereas the lateral bacular mounds were buried in the surrounding tissue and only the tips were exposed (Fig. 2E). The tip of the medial bacular mound was not divided and the tip of the urethral process was divided into two parts (Table 1). The ventral side of the

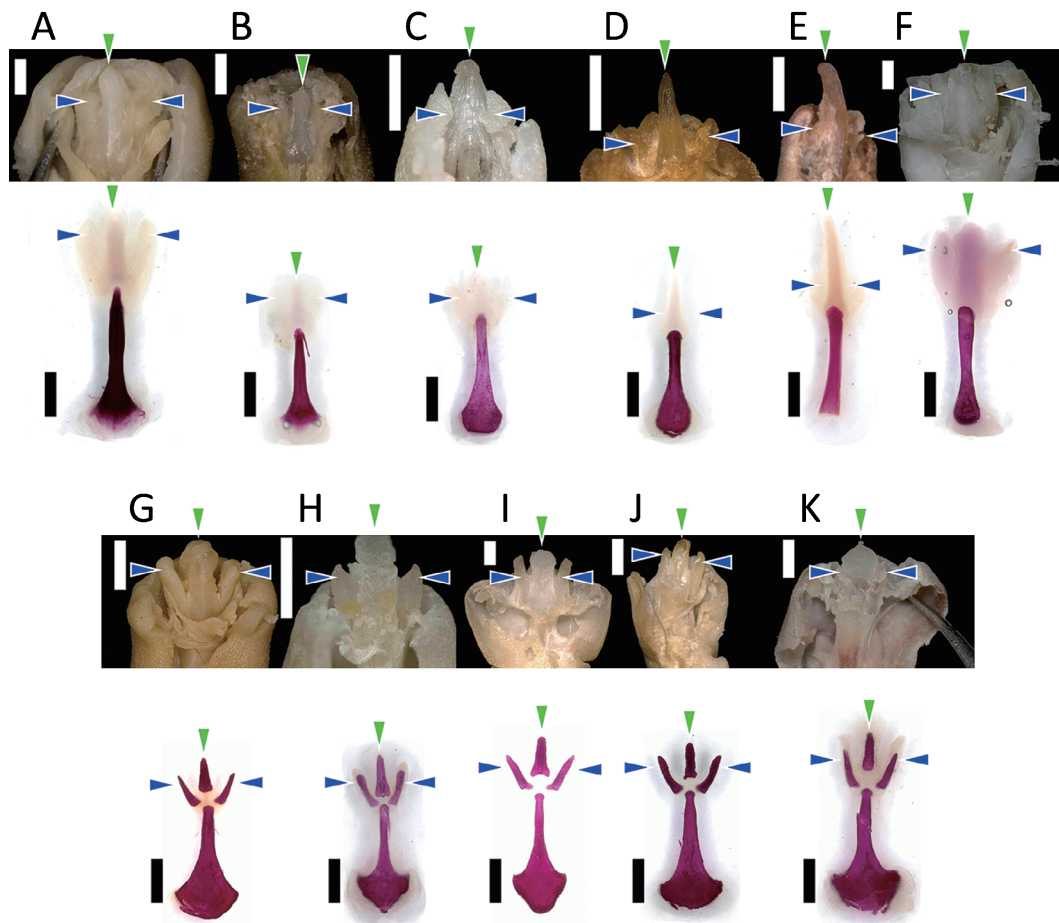


Fig. 2. Views of the internal features of the distal end of the glans penis (upper row) and ventral views of the stalk and bacular mound (lower row) of Murinae (A–F) and Arvicolinae (G–K) species. (A) *Apodemus speciosus* (TOY-214), (B) *A. argenteus* (TOY-215), (C) *Mus musculus* (TOY-224), (D) *M. caroli* (M2272), (E) *Micromys minutus* (M12843), (F) *Rattus norvegicus* (TOY-309), (G) *Alexandromys montebelli* (M12810), (H) *Myodes rutilus* (TOY-292), (I) *Craseomys rufocanus* (TOY-121), (J) *C. smithii* (M13298), and (K) *C. andersoni* (M11486). Blue and green arrows represent lateral and medial bacular mounds, respectively. The base of the *M. minutus* stalk was broken (E). Scale bars represent 1 mm.

outer skin had one deep groove. The base of the stalk was broken, so we do not know the details of its morphology, but it exhibited little widening at the base. The medial distal segment was exceptionally long, in contrast to those of *M. musculus* and *M. caroli*.

Rattus norvegicus (juvenile): The tip of the urethral process was divided into two parts and the presence of a dorsal papilla was not confirmed (Table 1). Most of the trident structure was encased inside the outer skin of the glans penis and was not prominent (Fig. 2F). There was no groove in the center of the ventral surface, but a pair of shallow grooves was present on the lateral side. The base of the stalk was not very wide; the medial distal segment was deep and thick.

Morphologies of Arvicolinae

All Arvicolinae species (*A. montebelli*, *M. rutilus*, *C.*

rufocanus, *C. andersoni*, and *C. smithii*) had a developed medial bacular mound and lateral bacular mounds that formed a trident structure (Fig. 2G–K). In the Arvicolinae species, the stalk, as well as the medial and lateral distal segments of the bacular mounds, were stained by Alizarin Red S. The medial bacular mound and stalk were not connected by bone, but by cartilage. Only the proximal part of the medial and lateral bacular mounds was associated with the surrounding tissue; the other parts were independent and outward protrusion was confirmed. The urethral process was divided into three parts consisting of a small medial part and two lateral parts, and each part had smaller projections (Table 1). There was a groove on the ventral surface of the outer skin that was identified as common to all five species. Stalks also had similar shapes in the five Arvicolinae species, and the width of the base of the stalk was wider than in the Murinae spe-

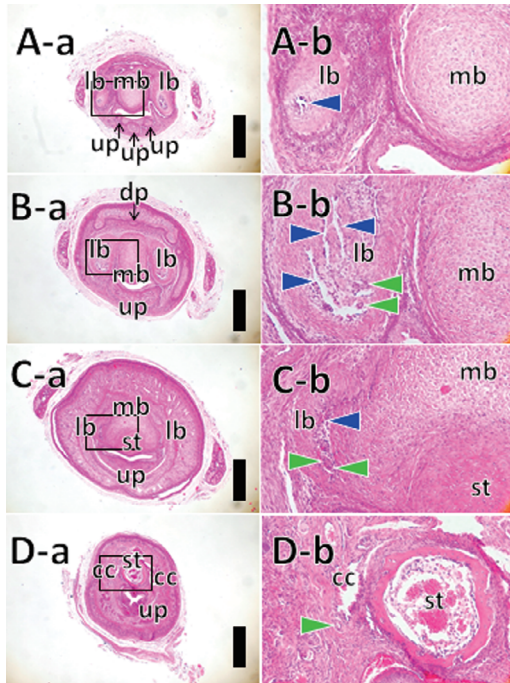


Fig. 3. Penis sections of *Apodemus speciosus*. Photographs of whole penis sections (A-a, B-a, C-a, and D-a), and enlarged views of the regions in the rectangles in A-a, B-a, C-a, and D-a (A-b, B-b, C-b, and D-b). mb, medial bacular mounds consisting of fibrocartilage; lb, lateral bacular mounds; up, urethral process; dp, dorsal papilla; cc, corpus cavernosum; st, stalk. Blue and green arrowheads indicate cavernous spaces and veins, respectively. Scale bars represent 0.5 mm. Diagram of the locations of four *A. speciosus* penis sections (lower panel). A, distal part of the lateral bacular mounds; B, central part of the lateral bacular mounds; C, region connecting the medial and lateral bacular mounds and the stalk; and D, central part of the stalk.

cies (ranges of SBW/STL: 0.24–0.48 in Murinae and 0.45–0.59 in Arvicolinae). *Myodes rutilus* had a specific morphology of the lateral distal segments in their angle and shallower tip as seen from the lateral view, distinct from other Arvicolinae species (Fig. 2H).

Analysis of *A. speciosus* tissue sections

Medial and lateral bacular mounds were typically

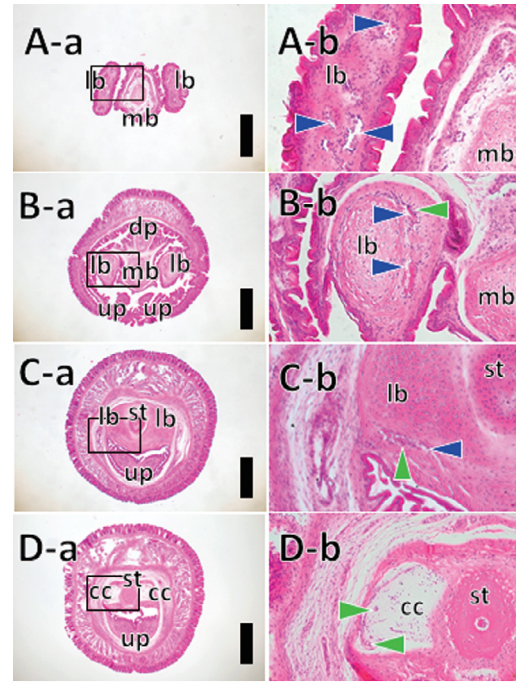


Fig. 4. Penis sections of *Craseomys rufocanus*. Photographs of whole penis sections (A-a, B-a, C-a, and D-a), and enlarged views of the regions in the rectangles in A-a, B-a, C-a, and D-a (A-b, B-b, C-b, and D-b). mb, medial bacular mounds consisting of fibrocartilage; lb, lateral bacular mounds; up, urethral process; dp, dorsal papilla; cc, corpus cavernosum; st, stalk. Blue and green arrowheads indicate cavernous spaces and veins, respectively. Scale bars represent 0.5 mm. Diagram of the locations of four *C. rufocanus* penis sections (lower panel). A, distal part of the lateral bacular mounds; B, central part of the lateral bacular mounds; C, region connecting the medial and lateral bacular mounds and the stalk; and D, central part of the stalk.

found in sexually mature adults as well as in juveniles. The medial distal segment of the medial bacular mound (mb) was composed of fibrocartilage (Fig. 3A-a, B-a). Lateral bacular mounds (lb) were composed of corpus spongiosum, with cavernous spaces in the middle of the distal part of the lateral bacular mounds (Fig. 3A-b). The more proximal part of the lateral bacular mounds was occupied by cavernous spaces throughout (Fig. 3B-b).

The development of cavernous spaces in the lateral bacular mounds was also observed in juveniles. The lateral bacular mounds had multiple vessels along the cavernous spaces (Fig. 3C-b). The lateral bacular mounds did not have skeletal muscle tissue (Fig. 3A-b, B-b). The corpus cavernosum (cc) along the stalk (st) was found to have blood vessels extending from the lateral bacular mounds (Fig. 3D-b). The urethral process (up) and part of the dorsal papilla (dp) which composed of corpus spongiosum also had cavernous spaces (Fig. 3A-a, B-a). By contrast, the medial bacular mound did not have cavernous spaces (Fig. 3A-a, B-a). The medial and lateral bacular mounds were connected to each other on the dorsal plane, and the back was also connected to other surrounding tissues (Fig. 3A-a, B-a). There were epidermis continuous with the outer skin of the glans penis and distal tip outside of the medial and lateral bacular mounds. The lateral bacular mounds, the urethral process, and the dorsal papilla, were mostly composed of corpus spongiosum, and were surrounded with other tissues; thus, those corpora spongiosa were not connected to each other and existed independently. The stalk was mostly composed of lamellar bone and proximal end of stalk was composed of hyaline cartilage.

Analysis of C. rufocanus tissue sections

Medial and lateral bacular mounds were typically found in the sexually mature adults as well as the juveniles. The medial and lateral distal segments of the medial bacular mound (mb) and lateral bacular mounds (lb) were composed of bones (Fig. 4A-a, B-a). The lateral bacular mounds consisted of bone on the outside and soft tissue consisting of cavernous space in both the distal region (Fig. 4A-b) and inside (Fig. 4B-b). Blood vessels were observed in soft tissue (Fig. 4B-b, C-b) and skeletal muscle tissue was not present. The corpus cavernosum (cc) along the stalk (st) contained blood vessels extending from the lateral bacular mounds (Fig. 4D-b). The urethral process (up) and the dorsal papilla (dp) also contained cavernous spaces (Fig. 4B-a, C-a). The dorsal papilla was large and similar in size to cross sections of the medial or lateral bacular mounds (Fig. 4B-a). By contrast, the medial bacular mound did not contain cavernous spaces (Fig. 4A-a, B-a). The lateral bacular mounds, the urethral process, and the dorsal papilla, were mostly composed of corpus spongiosum, and were surrounded with other tissues; thus, those corpora spongiosa were not connected to each other and existed independently.

Discussion

Differences in the baculum and glans penis among species

This is the first study that compares both the external and internal morphologies of the male genitalia of Japanese Murinae and Arvicolinae. Medial and lateral bacular mounds were identified in all of the species examined in seven genera of both subfamilies, indicating the common presence of a trident structure (Fig. 2). In *M. caroli*, medial bacular mounds were present as a protrusion from the foreskin (Tokuda 1934), while the presence of lateral bacular mounds was confirmed for the first time in this study. The medial bacular mound protruded from the glans penis and was highly developed while the lateral bacular mounds were degenerate in *M. musculus*, *M. caroli*, and *M. minutus* (Fig. 2). Yang and Fang (1988) reported the presence of lateral bacular mounds in *M. musculus* and *M. minutus*, but it is not clear from their figures whether the medial bacular mound is projecting. Rodriguez et al. (2011) reported the presence of MCC (MUMP corpus cavernosum) in the glans penis in laboratory mice, and this was thought to correspond to the lateral bacular mounds in the present study.

In this study, all Murinae species had medial distal segments that consisted of cartilage and lateral bacular mounds that consisted of soft tissue occupied by the corpus spongiosum (Fig. 2A–F). In the Murinae, two types of medial distal segments have been reported: those were ossified and cartilaginous segments (Yang and Fang 1988). The medial distal segment has been reported to ossify in some rat species including *R. norvegicus* and two *Niviventer* species (Yang and Fang 1988; Yonezawa et al. 2011). In laboratory rats, the medial distal segment does not ossify in young individuals up to day 40, but ossifies later as they grow (Yonezawa et al. 2011). The specimen of *R. norvegicus* used in this study was still juvenile (BW, 25.5 g), and the medial distal segment may not have ossified yet and was still present as cartilage. Therefore, it is suggested that the medial distal segment does not ossify in small Murinae, but is thought to ossify concurrently with growth in large Murinae such as *Rattus* and *Niviventer*. Yang and Fang (1988) described cartilage inside the lateral bacular mounds, but histological studies of rats (Murakami 1987; Kelly 2000) and the present study suggest that the lateral bacular mounds do not consist of cartilage, but instead are soft tissue consisting of corpus spongiosum.

Large interspecific differences were found in the medial and lateral bacular mounds of the glans penis in Murinae,

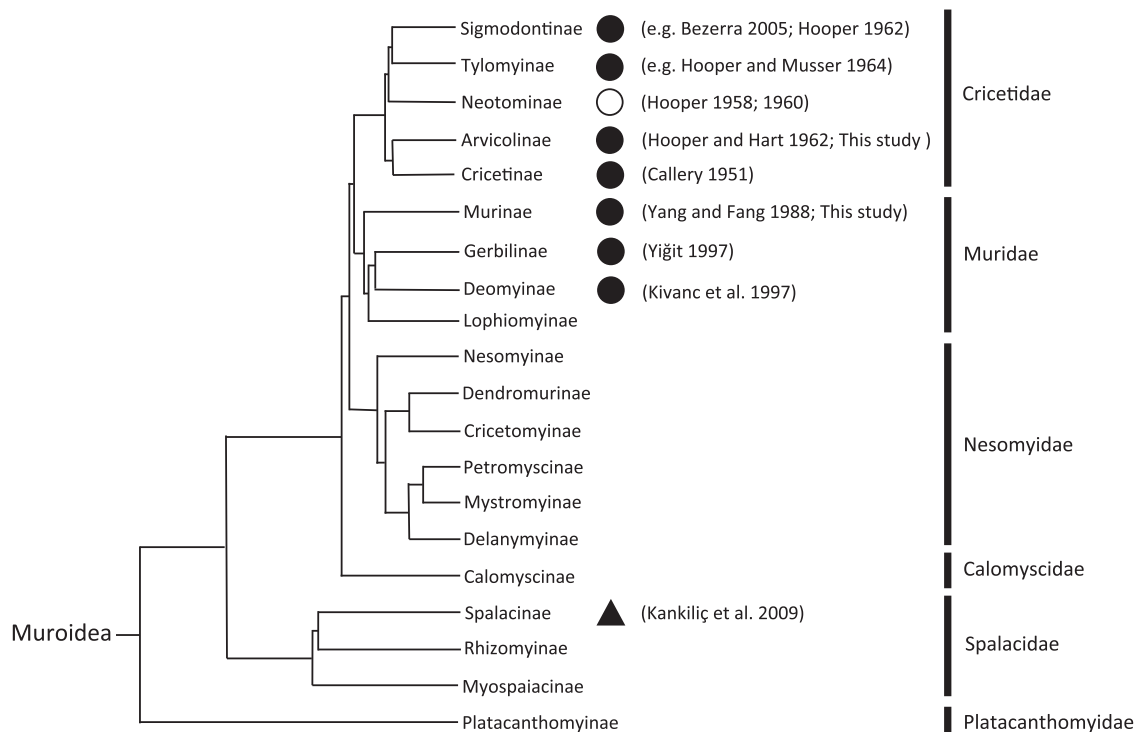


Fig. 5. Muroidea subfamilies with confirmed trident structures indicated on the molecular phylogenetic tree of Steppan and Schenk (2017). Black circles indicate subfamilies in which the trident structure was confirmed. White circle indicates the one subfamily in which the trident structure was not confirmed. Black triangle indicates a subfamily where dorsal lobe and lateral lobes that are suspected to be homologous to the medial bacular mound and the lateral bacular mounds of the trident structure were confirmed. There have been no studies on the internal penis structures of the remaining taxa.

but differences were also found in other areas. The glans penis of the genus *Apodemus* is distinguished by the presence of a trigeminal urethral process in contrast with bifurcated processes in other genera; the tip of the medial distal segment of genus *Mus* was bifurcated and the tips of other genera were not bifurcated; the glans penis of genus *Micromys* differed in that the medial distal segment was exceptionally long; and the glans penis of genus *Rattus* also resembled *Apodemus* in its juveniles but was distinguishable by bifurcation of the urethral process and the absence of dorsal papilla (Fig. 2 and Table 1). Consistent with Tokuda (1934), who advocated the importance of penile morphology as a diagnostic taxonomic character, the penile morphology of each species was distinct and diverse, possibly related to and dependent on genus-specific reproductive strategies.

On the other hand, both the medial and lateral distal segments were ossified in all five species of Arvicolinae examined in this study (Fig. 2G–K). In Arvicolinae, various types of ossification have been reported: distal segments entirely ossified and others entirely cartilaginous, an ossified medial distal segment and lateral

bacular mounds that are soft tissue, an ossified medial distal segment and lateral distal segments that are cartilaginous, and a cartilaginous medial distal segment and lateral bacular mounds that are soft tissue (Hooper and Hart 1962). In addition, the taper of lateral distal segments of *M. rutilus* differed from that of the other species so that the distal part of the lateral distal segments appeared wider in this study (Fig. 2H).

Medial and lateral bacular mounds that form a trident structure have been identified in many subfamilies of Muridae and Cricetidae including Deomyinae (Kivanc et al. 1997), Gerbilinae (Yiğit et al. 1997), and Murinae (Yang and Fang 1988; this study) in the Muridae; and Sigmodontinae (Hooper 1962; Hooper and Musser 1964; Bezerra 2005), Arvicolinae (Hooper and Hart 1962; this study), Tylomyinae (Hooper 1960; Hooper and Musser 1964), and Cricetinae (Callery 1951) in the Cricetidae (Fig. 5). By contrast, no trident structure has been reported in Neotominae (Hooper 1958; Hooper 1960) in the Cricetidae. The presence or absence of a trident structure in Lophiomyinae of the Muridae is unknown due to a lack of study. In reference to phylo-

genetic relationship of Muroidea (Steppan and Schenk 2017), trident structure is suggested to have evolved before the split between Muridae and Cricetidae with secondary loss in Neotominae of Cricetidae (Fig. 5). Dorsal lobe and lateral lobes have been identified in Spalacinae of the Spalacidae (Kankiliç et al. 2009). If these structures in Spalacinae are homologous to medial and lateral bacular mounds in Muridae and Cricetidae, acquisition of the trident structure is suggested to have occurred in earlier stage of Muroidea diversification. Presence or absence of the trident structures in species of Nesomyidae, Calomyscidae, Platacanthomyidae, and Spalacidae should be examined to reconstruct the evolution of the trident structure beyond the stalk in the Muroidea. Further study using bone staining and tissue sections is required to cover Muroidea species.

Model of the movement mechanism of the lateral bacular mounds

Kelly (2000) proposed that the baculum increases the overall flexural stiffness of the penis during copulation by transferring bending and compressive forces from the distal end of the glans to the tensile wall of the corpus cavernosum. In this study, the presence of cavernous spaces and blood vessels were confirmed in the lateral bacular mounds in both *A. speciosus* and *C. rufocanus*. Blood flowing through the vessels of the corpus cavernosum is suggested to enter the vessels of the lateral bacular mounds and accumulate in the cavernous space of the corpus spongiosum, and induce erection of the glans penis. Since no muscle tissue was found in the corpus spongiosum of lateral bacular mounds or corpus cavernosum, muscle tissue may not be involved in the mobility of lateral bacular mounds. In *A. speciosus*, the lateral bacular mounds have no ossified lateral distal segment, and therefore, almost all the internal space was occupied by the corpus spongiosum, and proximal parts of the lateral bacular mounds were attached to the distal part of the stalk (Fig. 3). In *C. rufocanus*, lateral bacular mounds had lateral distal segments and therefore less cavernous space (Fig. 4). Inflow of blood into the lateral bacular mounds is considered to increase the volume of the lateral bacular mounds in *A. speciosus* and *C. rufocanus*, thereby moving the lateral bacular mounds laterally using the junction of the proximal parts of the lateral bacular mounds and the stalk as a fulcrum (Fig. 6). This blood flow mechanism is thought to contribute to an efficient increase in the cross-sectional area of the glans penis. Kelly (2000) and Rodriguez et al. (2011)

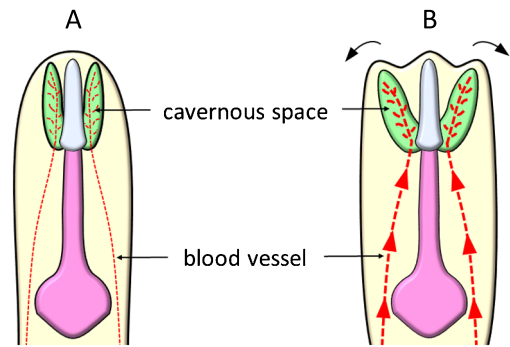


Fig. 6. Model of the movement mechanism of the lateral bacular mounds in non-erection (A) and erection (B) states. The cavernous space serves as a pool of blood in the corpus cavernosum in the lateral bacular mounds. Lateral bacular mounds expand driven with blood flow in the cavernous space and move laterally using the junction of the proximal parts of the lateral bacular mounds and the stalk as a fulcrum.

have suggested that corpus spongiosum of lateral bacular mounds were involved in penis expansion and hardening during copulation. Additionally, the present study clarified the specific movement mechanism for the first time. In laboratory rats, it has been reported that contraction of ischiocavernosus muscles attached to the proximal part of the pelvis and the corpus spongiosum causes blood flow into the corpus spongiosum, resulting in expansion of the cross-sectional area of the glans penis (Hart and Melese-D'Hospital 1983). Ischiocavernosus muscles contacting the pelvis may be involved in initiating blood flow in the corpus cavernosum connected to the cavernous space of corpus spongiosum.

The size of the dorsal papilla differed significantly between *A. speciosus* and *C. rufocanus* (Figs. 3B-a, 4B-a). Although the dorsal papilla of *A. speciosus* was very small, the dorsal papilla of *C. rufocanus* was as large as the lateral bacular mounds and was almost fully occupied by cavernous space. Therefore, in *C. rufocanus*, it is thought that blood flowing into the dorsal papilla also contributes to the increase in the cross-sectional area of the glans penis, in addition to the lateral bacular mounds. These findings suggest the importance of blood flow into the corpus spongiosum for expansion and erection of the glans penis in Muroidea species and the differences between families in the structures involved in blood flow: lateral bacular mounds in Muridae (*A. speciosus* in this study), and lateral bacular mounds together with the dorsal papilla in Cricetidae (*C. rufocanus* in this study). Differences in the way the glans penis expands are suggested to be related to the pattern of ossification of the trident structure.

Acknowledgments: We thank Tsutomu Hikida, Taku Okamoto, Kanto Nishikawa, laboratory members, and others for valuable comments on early versions of the manuscript and/or help with fieldwork for collecting samples. We also thank Takushi Kishida for instruction on tissue section analyses. This research was supported, in part, by JSPS KAKENHI Grant JP20J20295 and JP18H03602 and the Cooperation Research Program of Wildlife Research Center, Kyoto University.

References

- Abe, H. 1968. Growth and development in two forms of *Clethrionomys*. 1. External characters, body weight, sexual maturity and behavior. Bulletin of Hokkaido Forest Experimental Station 6: 69–89 (in Japanese with English abstract).
- Bezerra, A. M. R. 2005. Phallic morphology of *Kunsia tomentosus* (Rodentia: Sigmodontinae). Mastozoologia Neotropical 12: 227–232.
- Birkhead, T. 2000. Promiscuity: An Evolutionary History of Sperm Competition. Faber and Faber, London, 272 pp.
- Burt, W. H. 1960. Bacula of North American mammals. Miscellaneous Publications of the Museum of Zoology, University of Michigan 113: 1–75.
- Callery, R. 1951. Development of the *os genitale* in the golden hamster, *Mesocricetus (Cricetus) auratus*. Journal of Mammalogy 32: 204–207.
- Denys, C., Taylor, P. J. and Aplin, K. P. 2017. Family Muridae (true mice and rats, gerbils and relatives). In (Wilson, D. E., Lacher, T. E. and Mittermeier, R. A., eds.) Handbook of the Mammals of the World. Vol. 7. Rodents II, pp. 536–886. Lynx Edicions, Barcelona.
- Eberhard, W. G. 1985. Sexual Selection and Animal Genitalia. Harvard University Press, Cambridge and London, 244 pp.
- Feldhamer, G. A., Merritt, J. F., Krajewski, C., Rachlow, J. L. and Stewart, K. M. 2020. Mammalogy: Adaptation, Diversity, Ecology, Fifth edition. Johns Hopkins University Press, Baltimore, 728 pp.
- Hamilton, W. J. Jr. 1946. A study of the baculum in some North American Microtinae. Journal of Mammalogy 27: 378–387.
- Hart, B. L. and Melese-D'Hospital, P. Y. 1983. Penile mechanisms and the role of the striated penile muscles in penile reflexes. Physiology and Behavior 31: 807–813.
- Hooper, T. E. 1958. The male phallus in mice of the genus *Peromyscus*. Miscellaneous Publications Museum of Zoology, University of Michigan 105: 5–24.
- Hooper, T. E. 1960. The glans penis in *Neotoma* (Rodentia) and allied genera. Occasional Papers, Museum of Zoology, University of Michigan 618: 1–21.
- Hooper, T. E. 1962. The glans in *Sigmodon*, *Sigmomys*, and *Reithrodon* (Rodentia, Cricetinae). Occasional Papers, Museum of Zoology, University of Michigan 625: 1–11.
- Hooper, T. E. and Hart, S. B. 1962. A synopsis of recent North American Microtine Rodents. Miscellaneous Publications, Museum of Zoology, University of Michigan 120: 5–68.
- Hooper, T. E. and Musser, G. G. 1964. The glans penis in neotropical Cricetines (Family Muridae) with comments on classification of Muroid Rodents. Miscellaneous Publications, Museum of Zoology, University of Michigan 123: 1–57.
- Kaneko, Y. 1985. Examinations of diagnostic characters (mammary and bacula) between *Eothenomys smithi* and *E. kageus*. The Journal of the Mammalogical Society of Japan 10: 221–229 (in Japanese with English abstract).
- Kelly, D. A. 2000. Anatomy of the baculum–corpus cavernosum interface in the Norway rat (*Rattus norvegicus*), and implications for force transfer during copulation. Journal of Morphology 244: 69–77.
- Kankiliç, T., Çolak, E. and Kankiliç, T. 2009. Macro-anatomical and karyological features of two blind mole rat subspecies (Rodentia: Spalacidae) from Turkey. Anatomia Histologia Embryologia 38: 145–153.
- Kıvanç, E., Sözen, M., Çolak, E. and Yiğit, N. 1997. Karyological and phallic aspects of the spiny mouse, *Acomys cilicicus* Spitzenberger, 1978 (Rodentia: Muridae) in Turkey. Turkish Journal of Zoology 21: 167–169.
- Larivière, S. and Ferguson, S. H. 2002. On the evolution of the mammalian baculum: vaginal friction, prolonged intromission or induced ovulation? Mammal Review 32: 283–294.
- Murakami, O. 1974. Growth and development of the Japanese wood mouse (*Apodemus speciosus*). I. The breeding season in the field. Japanese Journal of Ecology 24: 194–206 (in Japanese with English abstract).
- Murakami, R. 1987. A histological study of the development of the penis of wild-type and androgen-insensitive mice. Journal of Anatomy 153: 223–231.
- Orr, T. J. and Brennan, P. L. R. 2016. All features great and small—the potential roles of the baculum and penile spines in mammals. Integrative & Comparative Biology 56: 635–643.
- Pardiñas, U. F. J., Myers, P., León-Paniagua, L., Ordóñez Garza, N., Cook, J., Kryštufek, B., Haslauer, R., Bradley, R., Shenbrot, G. and Patton, J. 2017. Family Cricetidae (true hamsters, voles, lemmings and new world rats and mice). In (Wilson, D. E., Lacher, T. E. and Mittermeier, R. A., eds.) Handbook of the Mammals of the World. Vol. 7. Rodents II, pp. 204–279. Lynx Edicions, Barcelona.
- Rodríguez Jr, E., Weiss, D. A., Yang, J. H., Menshenina, J., Ferretti, M., Cunha, T. J., Barcellos, D., Chan, L. Y., Risbridger, G., Cunha, G. R., et al. 2011. New insights on the morphology of adult mouse penis. Biology of Reproduction 85: 1216–1221.
- Schultz, N. G., Lough-Stevens, M., Abreu, E., Orr, T. and Dean, M. D. 2016. The baculum was gained and lost multiple times during mammalian evolution. Integrative and Comparative Biology 56: 644–656.
- Steppan, S. J. and Schenk, J. J. 2017. Muroid rodent phylogenetics: 900-species tree reveals increasing diversification rates. PLOS ONE 12: e0183070. DOI: 10.1371/journal.pone.0183070.
- Stockley, P., Ramm, S. A., Sherborne, A. L., Thom, M. D., Paterson, S. and Hurst, J. L. 2013. Baculum morphology predicts reproductive success of male house mice under sexual selection. BMC Biology 11: 66. DOI: 10.1186/1741-7007-11-66.
- Tokuda, M. 1934. Kleine Bestimmungstabelle der Muridae Nippons nach dem Charakter des Penis. Botany and Zoology 2: 1995–2008 (in Japanese with German summary).
- Tokuda, M. 1941. A revised monograph of the Japanese and Manchou-Korean Muridae. Transactions of the Biogeographical Society of Japan 4: 1–155.
- Yang, A. P. and Fang, L. X. 1988. Phallic morphology of 13 species of the family Muridae from China, with comments on its taxonomic significance. Acta Theriologica Sinica 8: 275–278 (in Chinese with English summary).
- Yiğit, N., Çolak, E., Kıvanç, E. and Sözen, M. 1997. Gerbil record from Turkey: *Gerbillus (Hendecapleura) dasyurus* (Wagner, 1842) (Rodentia: Gerbillinae). Israel Journal of Zoology 43: 13–18.
- Yonezawa, T., Higashi, M., Yoshioka, K. and Mutoh, K. 2011. Distribution of aromatase and sex steroid receptors in the baculum during the rat life cycle: effects of estrogen during the early development of the baculum. Biology of Reproduction 85: 105–112.

Received 26 September 2020. Accepted 4 March 2021.

Published online 17 June 2021.

Editor was Daisuke Koyabu.