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Phallic morphology of six species of Soricid shrew

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Abstract: The features of phallic morphology are powerful tools in understanding the phylogenetics of relationships in many mammalian species, especially sibling species. However, the traits of phallic morphology within the Soricidae remain poorly known. In this study, phallic morphological features from thirty-one penis specimens representing six species within the Soricidae were investigated and sketched. The results showed that the baculum of these penis specimens was absent as shown by examination of tissue sections. The urethra was dorsal to the corpus cavernosum which partially surrounds it, and no single corpus spongiosum urethra was present. The spermaducts were surrounded by the cavernous body of penis, and two branches of the spermaducts were conjoined in the distal part of the penes in these six species. A lingulate structure might be regarded as a unique feature within *Blarinella quadraticauda*, which was treated as a taxonomic criterion to identify this species. Within *Sorex* (*Sorex bedfordiae*, *S. cylindricauda*, *S. excelsus*), the boundary line of the glans penes was hardly observed. In contrast, the boundary line of glans penes was observed significantly within *Blarinella* (*B. quadraticauda*), *Anourosorex* (*Anourosorex squamipes*) and *Crocidura* (*Crocidura attenuata*).
Key words: Penis; Phallic morphology; Soricidae

鼩鼱科内6个种的阴茎形态学

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摘要：阴茎形态学特征是研究哺乳动物亲缘关系的有力工具，尤其是在近缘种之间。本试验选取鼩鼱科内的黑齿鼩鼱（*Blarinella quadraticauda*）、小纹背鼩鼱（*Sorex bedfordiae*）、大纹背鼩鼱（*S. cylindricauda*）、云南鼩鼱（*S. excelsus*）、四川短尾鼩（*Anourosorex squamipes*）和灰麝鼩（*Crocidura attenuata*）6个种共31号阴茎标本观察其阴茎形态学特征。研究结果表明：这6个种的阴茎均缺乏阴茎骨；在6个种的阴茎横切面上，尿道靠近阴茎腹侧面为阴茎海绵体包裹，没有单独的尿道海绵体存在，输精管由阴茎海绵体包裹，在阴茎末端形成两条输精管侧支；黑齿鼩鼱的阴茎具有独特的舌状结构，可作为鉴定黑齿鼩鼱的一个重要特征，鼩鼱属内的小纹背鼩鼱、大纹背鼩鼱和云南鼩鼱的阴茎头界限很难观察到，而黑齿鼩鼱、四川短尾鼩和灰麝鼩的阴茎头则具有明显的界限。
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1 Introduction

The baculum is known to occur in seven orders of Mammalia: Insectivora, Chiroptera, Primates, Rodentia, Carnivora, Pinnipedia, and Cetacea. Phallic morphology has been widely used as an important taxonomic character among related species of small mammals. The baculum is also possibly a much more conservative structure from the evolutionary standpoint than other structures which must keep up in their adaptions with changing environment (Burt, 1936). The characteristics of the glans penis have been proven useful in understanding phylogenetic relationships in many mammalian groups. For instance, the topography of the glans and the gross anatomy of other segments of the penis have been used to determine phylogenetic position within Rodentia and Chiroptera (Hamilton, 1946; Burt, 1960). Besides, phallic morphology has also been used as evidence to describe and support some new species. In 2007, a new vole species *Proedromys liangshanensis* was reported by Liu *et al.* (2007). The glans penis and bacular morphology were treated among its distinguishing features.

However, Little is know about phallic morphology in the Order Insectivora, especially for the Family Soricidae. This subject has been treated in only a few studies (Butler, 1979; Heaney, 1994; Ruedi, 1996). In China, we could not find any published research about phallic morphology in insectivores that

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was used taxonomically. Within Soricidae, some species are difficult to identify due to their similar appearance such as in body length and pelage. In 2008, we investigated the phallic morphology of six species within Soricidae (*Sorex bedfordiae*, *S. cylindricauda*, *S. excelsus*, *Blarinella quadraticauda*, *Crocidura attenuata* and *Anourosorex squamipes*) in order to find their unique features. In this report we elaborate the phallic morphology of these six species with particular emphasis on distinguishing these species from each other. This is the first description of the phallic morphologies of these six species.

2 Material and Method

Penes were removed from 31 specimens representing six species (*Sorex bedfordiae*, *S. cylindricauda* *S. excelsus*, *Blarinella quadraticauda*, *Crocidura attenuata* and *Anourosorex squamipes*), preserved in 100% ethanol or 10% buffered formalin. These specimens were collected from many localities (Appendix) and were archived in the collections of the Sichuan Academy of Forestry, Sichuan Province, China.

Firstly, penes were placed in clean water for several minutes, and examined using a dissecting microscope. External features of penes were sketched using an ocular micrometer accurate to the nearest 0.1 millimeter(mm). Morphological parameters included length of glans, width of glans, and length of urethral orifice. Secondly, phalli used in this study were cleared and stained following Hooper(1958) and Lidicker(1968). Then, penes tissue were thin-sectioned to allow us to observe the interior structure of these penes clearly. Anatomical terminology using in all descriptions follows Hooper(1958) and Carleton(1977).

3 Results

3.1 *Sorex bedfordiae*

The penis is small, rod-shaped, slender, smooth and with a conspicuous ventral-curvature. It is about six times longer than wide (Table 1). Ventrally, the urethral orifice is slightly recurved, the whole shape appears like an arc, and it is vertical on the shaft of the penis (Fig. 1, A1). Laterally, the distal region is deeply bent inward, the size of the proximal region also swollen, and the maximum width of the proximal region is similar to the maximum width of distal section (Fig. 1). A protuberance at the proximal region is about 3 mm in length (Fig. 1, A3). Two spermducts and the urethra converge in the distal region approach to the urethral orifice of the glans penis (Fig. 1, B).

Table 1 Measurements (in mm) of the glans penis in six species of Soricidae

	S. bedfordiae	*S. cylindricauda*	*S. excelsus*	*B. quadraticauda*	*C. attenuata*	*A. squamipes*
n	9	3	3	3	4	9
LDT	10.00(9.00 – 11.00)	10.00(9.00 – 11.00)	5.00(4.50 – 6.00)	2.60(2.50 – 2.70)	6.50(5.00 – 7.00)	6.20(4.50 – 7.50)
LG	–	–	–	2.50(2.40 – 2.60)	1.00(0.90 – 1.10)	2.00(1.90 – 2.10)
WG	1.60(1.30 – 2.00)	1.80(1.50 – 2.00)	1.44(1.20 – 1.50)	1.90(1.80 – 2.00)	1.34(1.00 – 1.40)	1.40(1.20 – 2.00)
PHH	1.40(0.90 – 1.70)	1.60(1.50 – 1.70)	0.95(0.80 – 1.00)	1.30(1.20 – 1.40)	1.12(1.00 – 1.40)	1.50(1.00 – 2.50)
LDT/WG	6.25	5.55	3.47	1.36	4.85	4.42

LDT (Length of distal tract): Distance on the ventral face of the glans from its base, to its greatest inner corner of the penis. LG: Length of glans. WG (Diameter of glans): Greatest width of the glans. PHH (Pallus height): Greatest height of the glans. LDT/WG: Length of distal tract/Width of glans. The glans is not observed, and the length of glans is not measured.

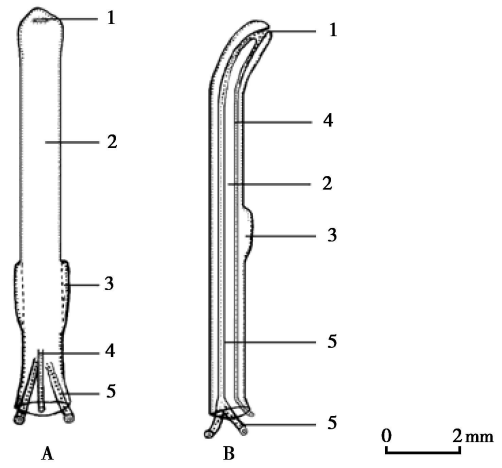

Fig. 1 Penis morphology of *S. bedfordiae*. A. Ventral view, B. Lateral view. 1. Urethral orifice; 2. Glans; 3. Protuberance; 4. Urethra; 5. Spermaduct.

3.2 *Sorex cylindricauda*

The penis is relatively small, rod-shaped, slender, and with a conspicuous ventral-curvature. It is about five times longer than wide (Table 1). Ventrally, the surface of the glans penis curved inward, it appears like a curved finger tip. The urethral orifice appears like a V-shaped funnel, and it is located in the front of glans penis. Laterally, the distal region is deeply bent inward, the size of the proximal region also swollen, and the maximum width of the proximal region is similar to the maximum width of distal section. A protuberance at the proximal region is about 4 mm long (Fig. 2).

3.3 *Sorex excelsus*

Ventrally, the glans penis is flat with a slight curve about 2.0 mm from the apex (Fig. 3). The urethral orifice is situated in the tip of the glans penis. The shape of urethral orifice is abnormal, but the orifice

is located at the tip of glans. When removing the outer crater of the penis body, three branches including one distal branch and two lateral branches are found in the distal part of the glans penis.

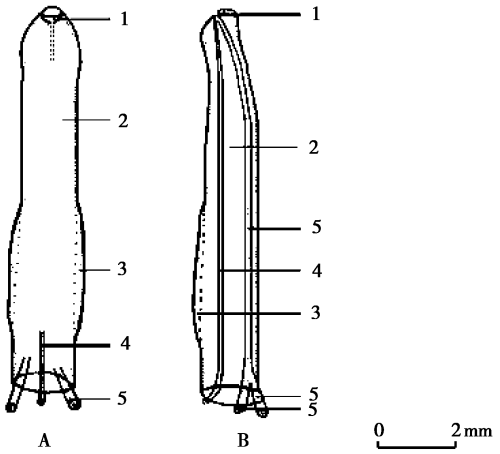

Fig. 2 Penis of *S. cylindricauda*. A. Ventral view; B. Lateral view. 1. Urethral orifice; 2. Penis; 3. Protuberance; 4. Urethra; 5. Spermaduct.

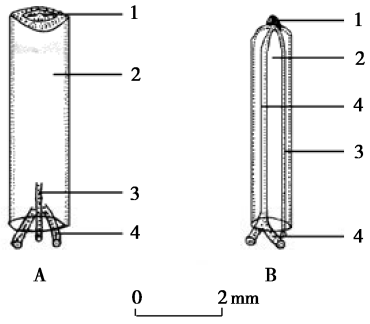

Fig. 3 Penis morphology of *S. excelsus*. A. Ventral view; B. Lateral view. 1. Urethral orifice; 2. Glans; 3. Urethra; 4. Spermaduct.

3.4 *Blarinella quadraticauda*

The glans penis of *Blarinella quadraticauda* is substantial, medium in size, oval-shaped, thick and short, and approximately 1.3 times longer than wide (Table 1). Ventrally, the proximal section of the glans is narrow and small. A piece of tissue covering the surface of the glans (see Fig. 4, A2) appears linguoid and is here referred to as the lingulate structure. Most of the surface of glans penis is occupied by the lingulate structure, which can be lightly flipped over with forceps. The maximum length of the lingulate structure is about 2.0 mm, and the maximum width is about 1.5 mm. The urethral orifice is located on the midventral surface of the glans penis and is about 0.8 mm in diameter (Fig. 4, A1). Laterally, the shaft of the penis was substantially curved (Fig. 4B).

3.5 *Crocidura attenuata*

The penis has a distinct balanus which averages 1.0 mm in length. The surface of the glan penis is rugose (Fig. 5, A). The surrounding tissue of the urethral orifice is rugose, and the width of the urethral orifice is approximately 0.5 mm. The surface of the glans penis is covered with small, irregular spines that are sharp at the tip (Fig. 5, C). The spines are distributed irregularly and sparsely.

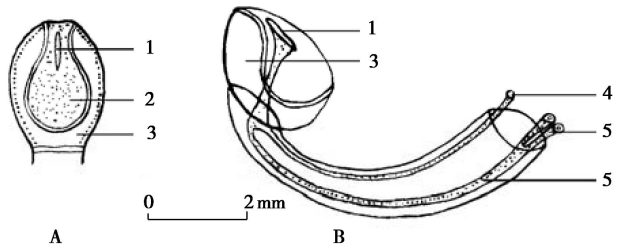

Fig. 4 Penis morphology of *B. quadraticauda*. A. Ventral view, B. Lateral view. 1. Urethral orifice; 2. Lingulate structure; 3. Glans; 4. Urethra; 5. Spermaduct.

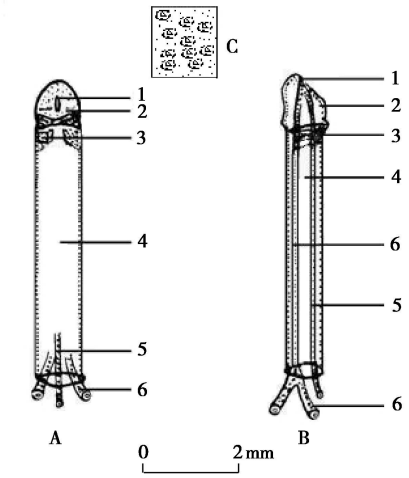

Fig. 5 Penis of *C. attenuata*. A. Ventral view; B. Lateral view; C. Epidermal spines (magnified 10). 1. Urethral orifice; 2. Glans; 3. Epidermal spines; 4. Penis; 5. Urethra; 6. Spermaduct.

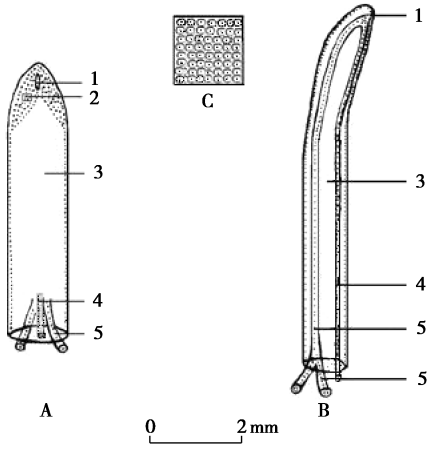

Fig. 6 Penis morphology of *A. squamipes*. A. Ventral view; B. Lateral view; C. Epidermal spines (magnified × 10). 1: Urethral orifice; 2: Epidermal spines; 3: Glans; 4: Spermaduct; 5: Urethra; 6. Cavernous body of penis.

3.6 *Anourosorex squamipes*

The penis of *Anourosorex squamipes* is cylindrical and is about 3 – 5 times longer than wide (Table 1). The glans is about 2 mm in length (Fig. 6). Ventrally, the bending urethral orifice located in the midline of the penis in the distal region, which is about 0.5 mm in length (Fig. 6, B). The body size of the penis is symmetrical. At the distal end of the glans body there is a broad V-shaped region, which is densely covered with directed spines which are short and blunt and distributed symmetrically on both the dorsal and ventral surfaces (Fig. 6, C).

3.7 Penes tissue slice

Penes tissue slice were taken in these 6 species. In accordance with tissue procedure, we sketched several common features of the transection in these penes in (Fig. 7). One section was located in the terminal of the penes (Fig. 7, A), and the othe section located in the middle of these penes (Fig. 7, B). The urethra was adjacent to both the corpus cavernosum penis and the ventral surface of the penis, whereas two seminal ducts were near to the dorsal wall of the penis and were surrounded with cavernous body of penis. The aperture of the spermaduct was larger than the urethral canal, and the wall of tube was also thicker too. The two spermaducts and urethra were joined in the sulcus urethralis which appears in the end of the glans. There are two parallel spermaducts in the inner structure. No baculum is found in these species.

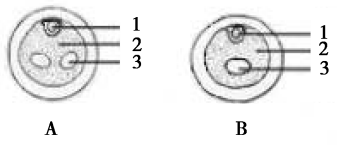

Fig. 7 Gross morphology of transection in 6 six species. A. section 1 (from the root of the penis body); B. section 2 (from the middle of the penis body). 1. Urethra; 2. Corpus cavernosum penis; 3. Spermduct

4 Discussion

Several studies have investigated the baculum in mammals (Yang, 1988; Liu, 2000; Yu, 2007). These authors claimed that most species in the order Insectivora had a baculum; but no one had studied the penes of specimens of insectivores. However, our results indicated that the baculum is evidently absent within the Soricidae, these results are consistent with Pucek (1964) and Atalar and Ceribasi (2006).

According to Cao *et al.* (1995), the histological structure of the Insectivora can be distinguished from that of species in the Rodentia. Their results indicated that spermaducts were situated out of corpus cavernosum penis, and the urethra was surrounded by corpus spongiosum urethra. However, our results showed that the spermaducts were within the cavernous body of penis in the six species of shrew, the urethra was over the corpus cavernosum penis and no single corpus spongiosum urethra alone.

As viewed from the penis specimens of these selected Chinese Soricidae, each penis showed its own unique features species level. However, phallic morphology was extremely stable within eath species. Among these six species, *Blarinella quadraticauda* has a special lingulate structure that distinctly differs from the rest species. We suggested that the lingulate structure can be treated as a taxonomic criterion. Similarly, among extraspecies, phallic morphology also indicated some stable characteristics. Within *Sorex* (*S. bedfordiae*, *S. cylindricauda*, *S. excelsus*), the penes are small, slender and smooth. Length of the distal tract (LDT) of *S. bedfordiae* and *S. cylindricauda* are equivalent. Diameter of the glans (WG) of *S. cylindricauda* is sightly wideer than the WG of *S. bedfordi*ae, However, phallic morphology is similar between *S. cylindricauda* and *S. bedford* iae., and this finding indicates that these two species may be closely related. On the contrary, phallic morphology showed significant differences among genera. The boundary of the glans penes could hardly observed within *Sorex* (*S. bedfordiae*, *S. cylindricauda*, *S. excelsus*), while the boundary of glans penes was significant within *Blarinella* (*B. quadraticauda*), *Anourosorex* (*Anourosorex squamipes*) and *Crocidura* (*Crocidura attenuata*). Thus, these results can be regarded as an important feature in *Sorex*. Among the different genera, phallic morphology showed some stable and variable features. Epidermal spines were found in *Anourosorex* (*A. Squamipes*) and *Crocidura* (*C. attenuata*), but the region of distribution and shape showed significant differences.

In conclusions, phallic morphology revealed there are many distinct intrageneric and intraspecific differences. These results lay the foundation for further studies in determining phylogenetic relationships within Insectivora.

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Appendix I

Appendix of all specimens examined

Species	Field Number	Sample Number	Locality
Blarinella quadraticauda	ELSA02001	SAF06In001	Erlangshan, Tianquan County
B. quadraticauda	ELSA01004	SAF06In002	Erlangshan, Tianquan County
B. quadraticauda	ELS01014	SAF06In003	Erlangshan, Tianquan County
Sorex bedfordiae	ELSB05	SAF06In004	Erlangshan, Tianquan County
S. bedfordiae	ELSC01004	SAF06In005	Erlangshan, Tianquan County
S. bedfordiae	ELSB5	SAF06In006	Erlangshan, Tianquan County
S. bedfordiae	WL060603	SAF06In007	Wanglang, Pingwu County
S. bedfordiae	WL0694123	SAF06In008	Wanglang, Pingwu County
S. bedfordiae	MGDW505	SAF06In009	Lanlong, Meigu County
S. bedfordiae	MGDW507	SAF06In010	Lanlong, Meigu County
S. bedfordiae	MGLL75120103	SAF07In001	Lanlong, Meigu County
S. bedfordiae	MGLL75190102	SAF07In002	Lanlong, Meigu County
Anourosorex squamipes	JJAA197	SAF08In001	Jiajinshan, Baoxing County
A. squamipes	JJSA339	SAF08In002	Jiajinshan, Baoxing County
A. squamipes	ELSB0205	SAF06In011	Erlangshan, Tianquan County
A. squamipes	ELSA01007	SAF06In012	Erlangshan, Tianquan County
A. squamipes	ELSA02003	SAF06In013	Erlangshan, Tianquan County
A. squamipes	ELSA02006	SAF06In014	Erlangshan, Tianquan County
A. squamipes	ELSA01006	SAF06In015	Erlangshan, Tianquan County
A. squamipes	ELSA02001	SAF06In016	Erlangshan, Tianquan County
A. squamipes	ELSA01005	SAF06In017	Erlangshan, Tianquan County
Sorex excelsus	JZ01004	SAF02In001	Changhai, Jiuzhaigou County
S. excelsus	LT10001	SAF04In001	Zaisang, Litang County
S. excelsus	LT0402	SAF04In002	Zaisang, Litang County
Sorex cylindricauda	JJSA461	SAF98In001	Jiajinshan, Baoxing County
S. cylindricauda	JJSB106	SAF98In002	Jiajinshan, Baoxing County.
S. cylindricauda	ELSA0202	SAF98In003	Erlangshan, Tianquan County.
Crocidura attenuata	QC690301	SAF06In018	Labahe, Tianquan County
C. attenuata	LBH11004	SAF02In002	Labahe, Tianquan County
C. attenuata	KX1001	SAF07In003	Kaixian County, Chongqing
C. attenuata	BQ001	SAF97In001	Kaixian County, Chongqing