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Macroscopic morphology of the male genitalia of *Caenolestes caniventer* (Caenolestidae: Paucituberculata)

Morfología macroscópica del genital masculino de *Caenolestes caniventer* (Caenolestidae: Paucituberculata)

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The anatomy of the penis in the Paucituberculata has received little attention. The only treatise addressing the genitalia and prostate in this group of marsupials comes from a century ago. The present work describes the macroscopic morphology of the male genitalia of the gray-bellied shrew opossum, *Caenolestes caniventer*, a structure that can be useful in the taxonomic, reproductive, and evolutionary fields. The genitals were removed (n = 2) through a basal incision during the taxidermy of specimens and preserved in a 90 % alcohol solution. In the laboratory, genitals were prepared following eversion protocols and stained to highlight the structures. The penis and its structures were described. The penis has a long-wide glans with two distinctive prongs at distal end. A medium spermatic groove extends to the prongs. The prongs are 1.6 times longer than the glans, relatively cylindrical and striated, progressively tapering to a hook-shaped distal tip. Prong length and penile spines may be key to reproductive efficiency. We hypothesize that the family Caenolestidae generally displays similar penis shapes, with differences between species. These results are useful in taxonomic, reproductive, and evolutionary fields.

Key words: Genitals; glans; penile spines; phallus; spermatic sulcus.

La anatomía del pene en paucituberculados ha merecido poca atención. El único tratado que aborda la genitalia y próstata en este grupo de marsupiales proviene de hace un siglo. El presente trabajo describe la morfología macroscópica de la genitalia masculina del ratón marsupial de vientre gris, *Caenolestes caniventer*, estructura que puede ser de utilidad en el campo taxonómico, reproductivo y evolutivo. Los genitales fueron removidos (n = 2) con una incisión basal durante la taxidermia de los especímenes y conservados en una solución de alcohol al 90 %. En el laboratorio los genitales fueron preparados siguiendo protocolos de eversión y tinturado los genitales para resaltar las estructuras. Se describió el genital y sus estructuras. El pene presenta un glande largo y ancho, con dos distintivos apéndices ubicados en su extremo. Un surco medio espermático se extiende hasta los apéndices. Los apéndices son 1.6 veces más largos que la longitud del glande, de forma relativamente cilíndrica y estriada, estrechándose progresivamente hasta terminar en una punta distal con aspecto de garfio. El largo de los apéndices y las protuberancias en forma de espina podrían ser clave para la eficiencia reproductiva. Hipotetizamos que la familia Caenolestidae presenta de manera general formas similares del pene, pero con diferencias entre especies. Los resultados son de utilidad en el campo taxonómico, reproductivo y evolutivo.

Palabras clave: Falo; genitales; glande; surco espermático; tubérculos espinosos.

Knowledge of penile morphology has been moderately documented, mainly for medium-sized and large mammals (Mollineau *et al.* 2006; Amezcua *et al.* 2009; Carneiro *et al.* 2010; Rocha *et al.* 2017) and, to a lesser extent, for small mammals, such as marsupials (Didelphidae) and rodents (Cricetidae and Muridae; Hooper and Musser 1964; Voss and Linzey 1981; Nogueira 1988; Martinelli and Nogueira 1997; Nogueira *et al.* 2004). For the Order Paucituberculata, a group of marsupials endemic to the New World (Goin *et al.* 2009; Ojala-Barbour *et al.* 2013; Patterson 2016; Voss and Jansa 2021), knowledge of this trait is scarce, as well as its anatomical, morphological and functional adaptations. The only work dealing with the genitalia and prostate in Paucituberculata comes from *Caenolestes fuliginosus*, studied by Osgood (1921) a century ago. Rodger (1982) and Woolley (1987) provided an anatomical approach to the seminiferous tubes, epididymis, *rete testis*, and efferent and deferens ducts of this species.

This work describes the macroscopic morphology of the penis of *Caenolestes caniventer* (Figure 1), a species restricted to the western Andes of Ecuador and northwestern Perú (Patterson 2016) and classified by IUCN as Nearly Threatened (Solari and Martínez-Cerón 2015). This is the first work that fully illustrates the gross morphology

Figure 1. Adult male specimen of *Caenolestes caniventer* (MECN 4821). Chillacocha, Cordillera de Chila, Provincia de El Oro, Ecuador. Photograph: J. Brito.

of a male genital of Paucituberculata, which can be useful in taxonomic, reproductive, and evolutionary fields.

Two adult specimens of *C. caniventer* were captured in Chilla (3° 30' 9.35" S, 79° 37' 32.49" W, 3,338 m), a locality in the Provincia de El Oro, Ecuador, using Sherman traps (7.5 × 9 × 27 cm; H. B. Sherman Traps, Tallahassee, Florida) baited with a mixture of oat and coconut oil. Specimens were handled following the guidelines of Sikes *et al.* (2016). The voucher specimens (catalog numbers: MECN 4821 and MECN 4841, head-body length (total) 125 mm and 120 mm, respectively) are deposited in the mastozoological collection of the Instituto Nacional de Biodiversidad (MECN), Quito, Ecuador.

The genitals were removed through a basal incision during the taxidermy and preserved in a 90 % alcohol solution. The penises were prepared based on reptile hemipenis eversion protocols (Pesantes 1994; Betancourt *et al.* 2018), modified according to the anatomical structures of specimens. The genitals were immersed in a sodium dodecyl sulfate solution for 24 hr. Petroleum jelly was melted with a laboratory burner, and red vegetable dye was added for staining. Once confirmed that organ tissues were soft and flexible, organs were slowly inflated with the stained petroleum jelly using 2 ml insulin syringes, mainly for the glans prongs. The procedure was repeated until the organs reached their maximum expansion. The description of the penis and its structures is based on direct observations of the sexual organs, following the considerations of Osgood (1921).

The measurements used (with their respective abbreviations) to describe each anatomical structure and its proportions include phallus length (PL), measured from the base to the tip where it splits into prongs; glans width (GW), measured horizontally along the widest segment of the glans; length of the right glans prong (LRP), measured from the insertion base at the outer edge of the phallus to the tip; length of the left glans prong (LLP); length of the distal right glans prong capsule (LRC), measured from the base of the capsule to the distal region of the capsule; length of the distal left glans prong capsule (LLC); basal width of the right glans prong (BWRP), measured at the widest base of each glans prong; basal width of the left glans prong (BWLP); distal basal width of the right glans prong (DWRP), measured at the distal base of the glans; distal basal width of the left glans prong (DWLP). All measurements were taken with a Buffalo Tools digital calibrator to the nearest 0.1 mm.

The phallus is longer than wide, with two distinctive prongs at the glans tip (Figure 2A). The phallus has an average length of 17.27 mm, with an average glans width of 6.8 mm (Table 1). The glans prongs emerge from the lateral tips of the penis and are 1.6-fold longer than the phallus length. They are relatively cylindrical and striated, progressively tapering to a hook-shaped distal tip (Figure 2A, 2B, 2D, 2E).

The average glans prong length is 27.72 mm, with an average basal width of 5.52 mm, tapering to 3.21 mm at the base of the distal prong tip. The sagittal glans surface exhibits a middle spermatic groove (Figure 2C) that stretches along the ¾ parts of the prongs; the distal quarter is formed by a sub-distal capsule that wraps around the middle of the distal tip, conspicuously showing the spermatic groove (Figure 2E). Both the capsule and the distal tip are covered by penile spines (Figure 2F).

Male genitalia of the genus *Caenolestes* was partially studied by Osgood (1921) based on *C. fuliginosus*, who most probably did not evert the genitals to fully visualize the two glans prongs. The anatomy of the male genitalia of *Caenolestes* displays a unique structure, such as the presence of a spermatic sulcus and a bifurcated glans, a condition that has also been reported in didelphid marsupials, birds, and reptiles (Tyndale-Biscoe and Renfree 1987; Ricaurte-Galindo 2006; Passos and Lynch 2010).

Differences in the shape and proportions of male genitalia in some mammal groups are related to a certain extent to body proportions and evolutionary processes (Amezcua *et al.* 2009). However, the large dimensions of the glans prongs, the filiform structure of the penis, the hook-shaped glans prongs tips, and the presence of penile spines in *C. caniventer* may suggest that these are adaptations to deposit the sperm as close uterus as possible. This has also

Table 1. Morphometric measurements (mm), $\overline{X}$ mean and ± standard deviation (SD) of the male genital of 2 adult specimens of *Caenolestes caniventer*. Abbreviations of measurements are described in the text.

Measurements	MECN 4821	MECN 4841	$\overline{X}$	± SD
PL	15.14	19.39	17.27	3.01
GW	6.76	6.83	6.80	0.05
LRP	28.66	26.63	27.65	1.44
LLP	28.86	26.58	27.72	1.61
LLC	4.92	6.06	5.49	0.81
LRC	4.95	6.08	5.52	0.80
BWRP	3.04	3.37	3.21	0.23
BWLP	3.37	3.34	3.36	0.02
DWRP	1.14	1.16	1.15	0.01
DWLP	1.12	1.26	1.19	0.10

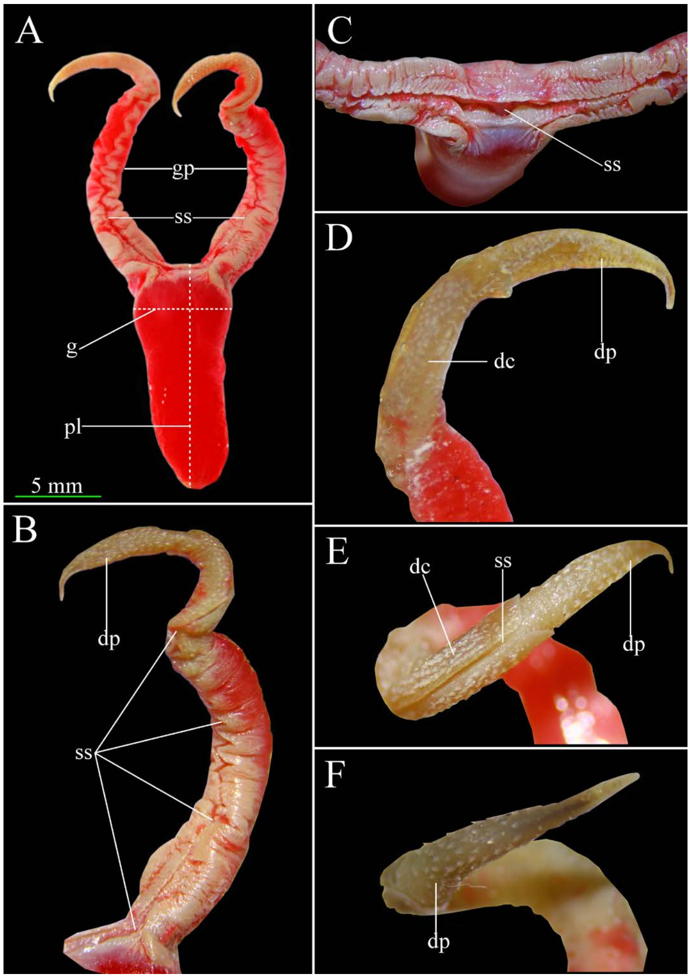

Figure 2. Male genital of an adult specimen of *Caenolestes caniventer* (MECN 4841). A) Ventral view of the genital: (pl) phallus length; (g) glans; (gp) glans prongs; (ss) spermatic sulcus. B) Enlargement of the left glans prong, showing the spermatic sulcus (ss) and distal prong tip (dp). C) Anterior view of the glans at the bifurcation of the prongs. D) Detail of (dp) and the subdistal capsule (dc). E) Detail of the (dc), showing the (ss). F) Detail of (dp). Note in E and F the penile spines around the (dc) and (dp). Scale = 5 mm. Photographs: M. Yánez-Muñoz.

been observed in some marine mammals and ungulates with long and filiform penises (Bonet *et al.* 2013; Brennan 2016; Orbach *et al.* 2017) and in reptile species that have deeply bifurcated hemipenises with hooks and spines, such in *Thamnophis radix* (King *et al.* 2009).

Penile spines are also associated with sensory functions during the coitus, promoting stimulation between the male organs and the vaginal wall (McLean *et al.* 2011; Orbach *et al.* 2017). In rodents and primates, these spines appear to play a crucial role. Dixson (1986) showed as the experimental removal of these structures resulted in a lesser stimulus, with males requiring a longer time for penetration and ejaculation. Mammals with spineless penises have prolonged copulations so that the sensory stimulation lengthens, and the presence of penile spines no longer seems essential (McLean *et al.* 2011). The presence of these structures in the glans prongs of *C. caniventer* suggests that the copulation is probably short-lasting, and according to this reproductive behavior, the penile spines increase the stimulation and the probability of successful deposit sperm in the female genital tract.

The coevolution of male and female genitalia plays a central role in the shape of the penis (Brennan *et al.* 2007; Masly 2012; Orbach *et al.* 2017). The morphology of the penis must match the female anatomy (following the key-lock hypothesis) of the species to avoid intromission into females of other species; in turn, the female must display reproductive characters unique to its species (Hershkovitz 1966; Brennan *et al.* 2007; Masly 2012; Brennan and Prum 2015). These interspecific morphological variations have been reported for multiple small mammal species (Hooper and Musser 1964; Hershkovitz 1966; Langguth and Neto 1993). Therefore, we hypothesized that the Caenolestidae species would display similar genital morphology and structures, albeit with interspecific differences. However, only the comparison between genitalia of different species would allow determine if there is a significant anatomical variation and its intrinsic value in the taxonomy.

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