A Description of the Skin Glands and Cloacal Morphology of the Plethodontid Salamander *Karsenia koreana*

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The skin glands and cloacal morphology of the Korean crevice salamander, *Karsenia koreana*, were similar to those of other plethodontids. The skin contained mucous, granular, and modified granular glands in varying frequencies and sizes. Males had sexually dimorphic glands in the skin of the chin (mental glands) and the dorsal tail base (caudal courtship glands). On the ventral surface of the tail base, modified granular glands were sexually dimorphic in size, with males larger than females. The cloacal glands in males, as in other plethodontids, consisted of four eosinophilic gland clusters (dorsal pelvic glands, lateral pelvic glands, caudal pelvic glands, and vent glands) and three basophilic glands (anterior ventral glands, posterior ventral glands, and Kingsbury’s glands). In females, the only cloacal gland was the spermatheca, which, as in other plethodontids, was a compound tubulo-alveolar gland in the roof of the cloaca.

**MATERIALS AND METHODS**

Tissue was excised from the cloaca, mentum, dorsal tail base, and ventral tail base (just posterior to the cloacal opening) from three adult male and three adult female *Karsenia koreana* (personal collection of D. R. Vieites).

Specimens were fixed in 10% formalin except for one initially preserved in ethanol. Tissues were dehydrated in a graded series of ethanol, cleared in Citrisolv (Fisher Scientific) or toluene, embedded in paraffin, sectioned at 10–12 µm with Jung Biocut 2035 or RMC XL microtomes, and processed using standard histological procedures (Presnell and Schrieberman, 1997). Sections were stained with either hematoxylin and eosin or the quin stain (Floyd, 1990; described by Staub and Paladin, 1997). The quin stain includes Alcian blue (pH 2.5; identifies mostly carboxylated glycosaminoglycans), cresyl violet (counterstain used to identify DNA), naphthol yellow (identifies protein), and the periodic acid–Schiff (PAS) reaction (identifies neutral carbohydrates by a magenta color). For some runs, methyl green was used as a counterstain instead of cresyl violet. Control slides (dimedone or no periodic acid) were used in each run to confirm PAS reactions.

Gland height was measured using an ocular micrometer in a Leica DME light microscope. One to eight glands were measured per individual in sections with the gland duct. Measurements were analyzed using JMP software and Microsoft Excel to test for differences between males and females and between different gland locations and to calculate gland size.

**RESULTS**

**Skin glands.**—The skin of *Karsenia koreana* contained mucous, granular, and modified granular glands, as well as glands that comprised male secondary sexual characters (Figs. 1, 2). Mucous glands were identified by their flocculent secretion and positive reaction to Alcian blue, although staining intensity with Alcian blue varied. Typical granular glands contained a grainy secretion and stained positively for protein (yellow with naphthol yellow). The cytoplasm was syncytial with oval or flattened nuclei scattered around the gland periphery. Modified granular glands also possessed a grainy secretion and reacted positively with PAS, which...
resulted in a magenta colored product. The lumina of modified granular glands was generally full of secretory product (Fig. 1B). The nuclei of modified granular glands were rounder and more numerous than those in typical granular glands. The cytoplasm was syncytial as in typical granular glands. Transmission electron microscopy studies showed that mucous glands and male sexually dimorphic skin glands (mental glands, caudal courtship glands) were not syncytial but had individual cells delineated by plasma membranes (Sever and Siegel, 2015; Sever, 2016).

The tail base region, both dorsally and ventrally, showed gland sexual dimorphism. On the dorsal tail base surface in males, PAS+ caudal courtship glands ($\bar{x} = 0.12 \pm 0.015$) were scattered among larger, typical granular glands (Fig. 1A). The histology of the caudal courtship glands and associated
An overview of male cloacal surface (male pelvic glands, caudal pelvic glands, and vent glands) and eosinophilic gland clusters (dorsal pelvic glands, lateral glands, as in other plethodontids, consisted of four anatomy was pieced together by examining cloacal larger (P. cinereus consisted of four glands were sexually dimorphic in size, with male glands mm), but because of sample size (Table 1), gland size was not modified granular glands that did not appear significantly different in size from male caudal courtship glands ( dome). The only cloacal gland in female Karsenia koreana was the spermatheca, which, as in other plethodontids, was a compound tubulo-alveolar gland in the roof of the cloaca (Sever, 1994). The common tube that opened into the roof of the short cloacal tube was composed of stratified epithelium and passed caudally to the middle of the cloacal chamber, where it branched into narrow neck tubules (Fig. 4B) that expand into distal bulbs (Fig. 5). Six tubules were noted in the most complete specimen (Fig. 4A, B). Otherwise, the cloaca anatomy of K. koreana was quite unremarkable, and the pseudostratified epithelium lining the cloacal tube and dorsal portions of the anterior cloacal chamber was gradually replaced by the stratified epithelium until the entire posterior end of the cavity was lined with epithelium (Fig. 4C, D). As in males, ciliated epithelium was lacking in the female cloaca.

The spermatheca in one specimen (Figs. 4, 5A) was inactive and lacked sperm, but another specimen possessed copious amounts of sperm in the spermatheca (Fig. 5B). In the inactive condition, the lumen was narrow, the apical cytoplasm was basophilic, and the nuclei were bunched together. In the active condition, the simple columnar nature of the epithelium was apparent, and the apical cytoplasm was eosinophilic. Heads of some sperm were closely associated with the apical cytoplasm. In the active form, some distortion of tissue was apparent, as a strange vacuolated space separated the tubules from their surrounding fibrous sheath (Fig. 5B).

### Table 1. Mean (±SD) gland size (maximum diameter) for different gland types in males and females. * indicates significant difference between males and females. n refers to number of individuals, and dm refers to diameter. Males were mean 48.8 mm (SD±0.06) SVL, and females were mean 49.3 mm (SD±0.87) SVL.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Type</th>
<th>n</th>
<th>( \bar{x} ) (mm)</th>
<th>SD (±)</th>
<th>Range (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Dorsal granular</td>
<td>3</td>
<td>0.082</td>
<td>0.011</td>
<td>0.04–0.11</td>
</tr>
<tr>
<td>Female</td>
<td>Dorsal granular</td>
<td>3</td>
<td>0.079</td>
<td>0.024</td>
<td>0.05–0.14</td>
</tr>
<tr>
<td>Male</td>
<td>Ventral granular</td>
<td>3</td>
<td>0.21</td>
<td>0.029</td>
<td>0.12–0.28</td>
</tr>
<tr>
<td>Female</td>
<td>Ventral granular</td>
<td>3</td>
<td>0.26</td>
<td>0.092</td>
<td>0.15–0.23</td>
</tr>
<tr>
<td>Male</td>
<td>Caudal courtship</td>
<td>3</td>
<td>0.19</td>
<td>0.013</td>
<td>0.07–0.08</td>
</tr>
<tr>
<td>Female</td>
<td>Caudal courtship</td>
<td>3</td>
<td>0.20</td>
<td>0.074</td>
<td>0.11–0.29</td>
</tr>
<tr>
<td>Male</td>
<td>Dorsal modified</td>
<td>3</td>
<td>0.12</td>
<td>0.015</td>
<td>0.06–0.16</td>
</tr>
<tr>
<td>Female</td>
<td>Dorsal modified</td>
<td>1</td>
<td>0.11</td>
<td></td>
<td>0.09–0.12</td>
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<tr>
<td>Male</td>
<td>Ventral modified*</td>
<td>3</td>
<td>0.19</td>
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<tr>
<td>Female</td>
<td>Ventral modified*</td>
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<td>0.14</td>
<td>0.012</td>
<td>0.10–0.18</td>
</tr>
<tr>
<td>Male</td>
<td>PAS+ on mentum*</td>
<td>3</td>
<td>0.15</td>
<td>0.015</td>
<td>0.09–0.19</td>
</tr>
<tr>
<td>Female</td>
<td>PAS+ on mentum*</td>
<td>3</td>
<td>0.083</td>
<td>0.002</td>
<td>0.06–0.12</td>
</tr>
</tbody>
</table>
Fig. 3. Transverse sections through cloacal fragments of male Karsenia koreana from anterior (A) to posterior (F; hematoxylin-eosin). (A) First specimen, probably cloacal tube. (B) Same specimen, anterior cloacal chamber. (C–F) Second specimen, sections proceeding caudally through the cloacal chamber. Ad, anterior depression; Av, anterior ventral glands; Cc, cloacal chamber; Cp, caudal pelvic glands; Dp, dorsal pelvic glands; Kg, Kingsbury’s glands; Lp, lateral pelvic glands; Lr, lateral recess; Pv, posterior ventral glands; Sp, sperm; Vg, vent glands.
Fig. 4. Transverse sections through cloacal fragments of a female *Karsenia koreana*, from anterior (A) to posterior (D; hematoxylin-eosin). Note that a dorsal portion of the right cloacal sheath is detached from the remaining tissue. (A) Cloacal tube. (B–D) Sections proceeding caudally through the cloacal chamber. Cc, cloacal chamber; Cot, common tube; Ct, cloacal tube; St, spermathecal tubules.
DISCUSSION

The glands found in the integument of *Karsenia koreana* are similar to the mucous, granular, modified granular, and sexually dimorphic glands that have been found in other plethodontid species (Delfino et al., 1986; Sever, 1989, 2003; Staub and Paladin, 1997; Hecker et al., 2003; Mary and Trauth, 2006; Largen and Woodley, 2008). The variable intensity of Alcian blue staining of mucous glands may indicate the presence of some neutral mucous, as found in *Ensatina* (Fontana et al., 2006).

As expected, males have well-developed mental gland tubules in the dermis of the lower jaw (Fig. 2). Sever (1976) induced development of mental glands in female *Eurycea quadridigitata* by injection of testosterone. He concluded that the induced mental glands are derived from mucous glands since only mucous glands were present in this region in control females. Modified granular glands are present in the female mentum of *Karsenia koreana* and are smaller and less frequent than mental glands in the male. This is the first report of glands in the mentum of females that stain PAS-like the male mental gland, but with much less intensity. Often, the female mentum is not examined; thus, whether the presence of modified granular glands in the mentum of *K. koreana* is unusual requires additional histological work on other plethodontids. The function of these glands in females is unknown. Testosterone is important in the development of mental glands in males (Sever and Staub, 2010), and the hormones involved in modifying granular glands in the mentum of females would be interesting to reveal.

During the plethodontid courtship behavior known as the tail-straddling walk, the female swings her chin across the dorsal tail base of the male (Houck and Arnold, 2003), and the caudal courtship glands are thought to facilitate successful courtship via pheromone release (Houck and Sever, 1994). Most of the plethodontids that have been examined for caudal courtship glands have them, e.g., *Desmognathus* (Noble, 1931), *Eurycea nana*, *Eurycea neotenes* (Sever, 1985), the *Eurycea bislineata* complex (Sever, 1989), *Eurycea lucifuga* (Hamlett et al., 1998), *Plethodon albagula*, *P. ouachitae*, *D. brimleyorum* (Mary and Trauth, 2006), and *Plethodon cinereus* (Sever and Siegel, 2015). *Typhlomolge rathbuni*, a paedomorphic plethodontid, does not have either mental or caudal courtship glands (Sever, 1985). The glands on the dorsal tail surface of *Plethodon shermani* (caudal to the tail base region and extending the length of the tail) are only slightly PAS positive (Largen and Woodley, 2008), rather than strongly PAS positive, as for typical caudal courtship glands. These glands empty after handling, suggesting they are used for defense rather than during courtship (Largen and Woodley, 2008).

Interestingly, *Plethodon shermani* has modified granular glands (which stain as courtship glands) in the post-cloacal ventral tail region, as do the species *P. cinereus* (Hecker et al., 2003) and *Aneides lugubris* (Staub and Paladin, 1997), suggesting the ventral area is important in scent-marking the substrate (Largen and Woodley, 2008). From a time-course histological study with *P. cinereus*, these glands are...
known to empty during scent-marking (Simons and Felgenhauer, 1992; Simons et al., 1999). In some studies and species, these glands stain positively with PAS (Staub and Paladin, 1997; Hecker et al., 2003) and others are PAS negative (Simons and Felgenhauer, 1992). A comprehensive review of non-mental glands involved in pheromone production, whether for courtship or for other social interactions, is sorely needed. Isolating and identifying pheromones secreted from caudal courtship glands will make a valuable contribution.

Male cloacal anatomy is similar to that of many other plethodontids (Sever, 1994). The most remarkable features are the lateral recesses in the anterior cloacal chamber. Males of some other plethodontids, especially in Desmognathus and Chiroteptorotriton, possess lateral recesses, but these are situated dorsally, rather than just inside the cloacal orifice (Sever, 1994). The lateral recesses of Karsenia koreana do, however, resemble the anatomy of such structures in some male ambystomatids (Sever, 1981, 1992; Licht and Sever, 1991).

In female Karsenia koreana, the absence of cloacal glands other than spermathecae is characteristic of a number of plethodontines, including species of Aneides and many species of Plethodon (Sever, 1994). Female cloacal anatomy of K. koreana, like male cloacal anatomy, is very generalized and in no way remarkable among plethodontids.

In summary, K. koreana has typical plethodontid skin and cloacal morphology with the exception of the presence of modified granular glands in the mentum of females. Future work on these glands promises to deepen our understanding of the structure, function, and behavior of the glandular salamander.

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LITERATURE CITED


