Herpetological Review, 2010, 41(2), 157–159.

The Hemipenis of *Trimorphodon quadruplex*

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Scott and McDiarmid (1984:2) stated that "Most early attempts to clarify the relationships of *Trimorphodon* have been misled by an inadequate description of the hemipenis…" Recently, the *Trimorphodon biscutatus* complex has received some significant attention both in molecular and morphological analyses (Devitt 2006; Devitt et al. 2008) and much of its evolutionary history is becoming better understood. However, these recent studies have not included hemipenial morphology and earlier papers appear to provide inaccurate descriptions (e.g., Klauber 1940; Scott and McDiarmid 1984; Smith 1941). Cope (1895, 1900) dissected the tails of male *Trimorphodon* and noted that their hemipenes possessed calyces. This was later rejected by Scott and McDiarmid (1984) and although Savage (2002) stated that *Trimorphodon* possess calyces, he did not provide a reference or evidence for his statement. In this study we dissect, illustrate, and describe the hemipenis of one of the species in the *Trimorphodon biscutatus* complex, *T. quadruplex*.

Methods

A partially everted left hemipenis from a large adult-male specimen (SVL 1075 mm; tail length 226 mm; 89 divided subcaudals) of *Trimorphodon quadruplex* (UTA R-44978) was measured and then removed at the base. We followed the hemipenial preparation procedures of Myers and Cadle (2003) and Zaher and Prudente (2003) with the addition of blue petroleum jelly which was inserted into the base of the hemipenis resulting in full eversion of the hemipenis. The latter method is further described and illustrated in Smith and Ferrari-Castro (2008). Finally, we examined the retracted left hemipenis of USNM 32274—*in situ*, as originally studied and illustrated by E. D. Cope in 1895 and 1900. The description follows the terminology of Dowling and Savage (1960), Myers and Campbell (1981), and Zaher (1999), as adopted by Savage (2002).

Hemipenis Description

Trimorphodon quadruplex (UTA R-44978); Figs. 1, 2

The specimen comes from the town of Santa Cruz, Guanacaste, Costa Rica, and was collected by M. Sasa in 1997. The everted left hemipenis extends approximately 55 mm in length and is 12.5 mm at its maximum width. The pedicel of the hemipenis is long and narrow, extending approximately 20 mm, and its distal half is covered in minute spines. The spinous truncus occupies approximately 10 mm of the hemipenis. This region possesses about 87 hooks (noticeably large spines), ranging from about 1.0 to 1.6 mm in length. When fully everted the spines start at the beginning of the 8th subcaudal and end at the 13th subcaudal. The calyculate apical region extends to the 21st subcaudal. The single semicentripetal *sulcus spermaticus* extends to the tip of the organ. Following the spines is the spinous and papillate calyx region, extending for about 25 mm and includes 20 rows of calyces on the sulcate side. The calyces bear on their edges minute papilla, distally, or spines, proximally. A unique feature of the hemipenis is the enlargement of the two most proximal calyces on the asulcate side, over part of the truncus and resembling naked or striated pockets. The first and largest calyx extends about 4 mm. At the apex of the hemipenis the final 2–3 rows of ornamentation consist of numerous, small papillae.

Trimorphodon quadruplex (USNM 32274); Fig. 1

The specimen comes from San Juán, Nicaragua, and was collected by M. Oviedo in 1997. The SVL could not be taken of this specimen but the tail has a length of 173 mm and has 91 subcaudals, excluding the tip. The left hemipenis was examined uneverted, as illustrated by Cope (1895 and 1900). This hemipenis extends in length to the 25th subcaudal, the first minute spines appear at the level of subcaudal 8, and the first hooks at the level of subcaudal 14.

Discussion

The only illustration of *Trimorphodon* hemipenes we are aware of was presented twice by Cope (1895: plate 30, figure 7; 1900: plate 28, figure 7). Cope (1900, p. 1101) mentioned examining only four specimens of *T. biscutatus* (then including *T. quadruplex*), so his illustration was thought to come from one of them. Cope

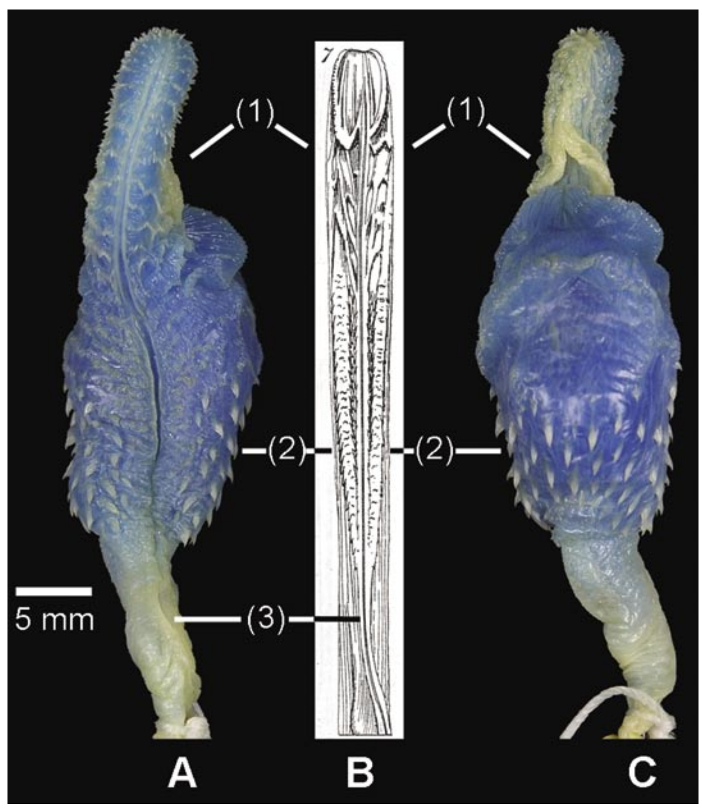

Fig. 1. Hemipenis of *Trimorphodon quadruplex*. Sulcate (A) and asulcate (C) view of the left hemipenis of UTA R-44978, compared to Cope's (1900: plate 28, image 7) *in situ* illustration of the left organ of USNM 32274 (B), showing calyces (1), spines (2), and sulcus spermaticus (3).

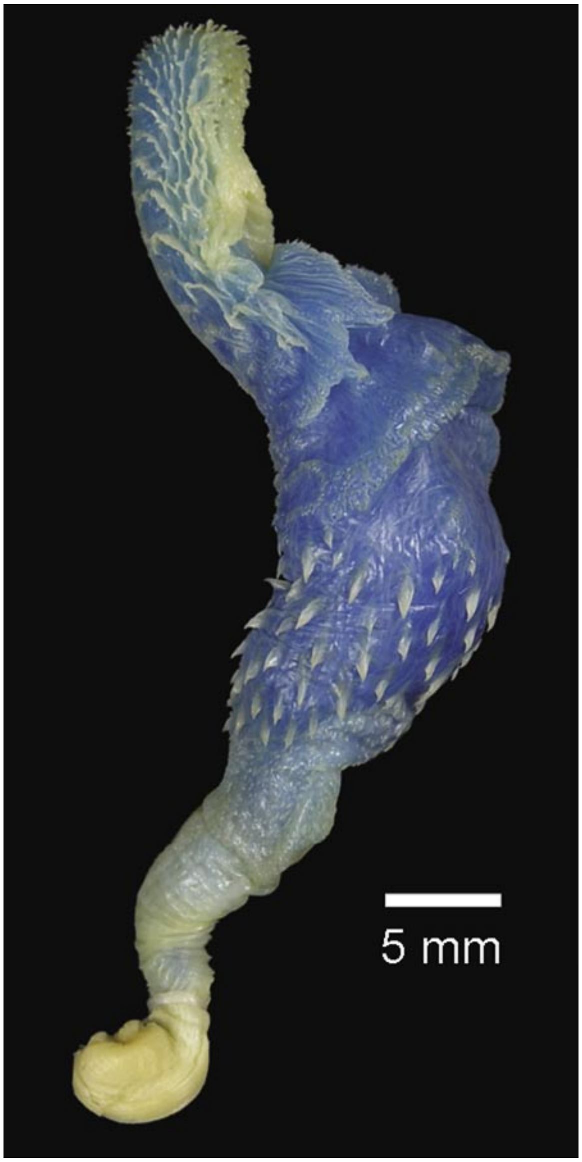

Fig. 2. Side view of the hemipenis of *Trimorphodon quadruplex* (UTA R-44978), showing the protruding lower calyces and curvature of the hemipenis.

(1861) mentioned a specimen of *T. biscutatus* (= *T. quadruplex*) from Realejo Nicaragua (USNM 5569) and in 1870 (1869) described *T. major* (shortly synonymized by him as *T. biscutatus*, Cope 1876 [1875], p. 131) from Tehuantepec, Oaxaca, based on three specimens (two specimens mentioned by him but three cotypes, USNM 30427–9, are listed by Cochran 1961). However, our examination determined that the hemipenes of none of these specimens were examined by Cope. The illustrated hemipenis belongs to the left hemipenis of a *T. quadruplex* (as currently recognized by Devitt et al. 2008), USNM 32274, a paratype from San Juan, Nicaragua (Smith 1941), which was returned by Cope's estate after his death. Our figure 1 includes an image of Cope's illustration for direct comparison of the retracted and the everted hemipenes. The existence of calyces is quite obvious on the fully everted hempenis (Figs. 1, 2), and as mentioned by Cope (1900), these structures are very unusual.

Although beyond the scope of this study, we examined specimens of *T. biscutatus* (UTA R-51832, from Carretera El Camaron-Mitla Km 106 on Mex 190, Oaxaca) and *T. paucimaculatus* (UTA R-52654, from Arroyo Colorado, Municipio La Huerta, Jalisco) and found these same features (e.g., unusual and extended proximal calyces) on their everted hemipenes. A thorough analysis of the hemipenis of the other species of *Trimorphodon* (*biscutatus*, *lambda*, *lyrophanes*, *paucimaculatus*, *tau*, *vilkinsonii*) is needed to add morphological support to the currently recognized species. King et al. (2009) found that reproductive behavior may correlate with hemipenis morphology. An investigation of the reproductive behavior causing this odd morphological evolution would be interesting.

Acknowledgments.—We thank T. Hartsell, K. Tighe, R. Wilson, A. Wynn, and R. McDiarmid (USNM), and C. J. Franklin and J. A. Campbell (UTA) for providing space at their institution and allowing us to examine and dissect hemipenes from *Trimorphodon* specimens under their care. This paper is based in part upon work supported by the National Science Foundation under grant no. DEB-0613802 to Jonathan A. Campbell and DEB-0416160 to ENS; and a grant from the Instituto Bioclon, Mexico, to ENS. Necessary permits for Mexican specimens were issued by SEDUE (now SEMARNAP).

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TECHNIQUES

Herpetological Review, 2010, 41(2), 159–162.

Effectiveness of Using Burlap Bands to Sample Arboreal Green Salamander Populations in the Blue Ridge Mountains of Georgia and North Carolina

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Green Salamanders, *Aneides aeneus*, occur as two distinct populations in the eastern United States. The more extensive population is located throughout the Cumberland and Allegheny Mountain regions from Pennsylvania to Alabama and northeastern Mississippi. The smaller disjunct population is located in the Blue Ridge Mountains of Georgia, South Carolina, and North Carolina (Petranka 1998). A study conducted in the Blue Ridge Escarpment of western North Carolina, northwestern South Carolina, and northeastern Georgia documented a 98% decline in some populations of Green Salamanders since 1970 (Corser 2001). The U.S. Fish and Wildlife Service currently recognizes *A. aeneus* as a species of concern. The Blue Ridge Escarpment population is listed as rare in Georgia, endangered in North Carolina, and a species of special concern in South Carolina. Corser (2001) attributed declines to climate change, loss and alteration of habitat, a chytrid fungal pathogen, and exploitation through collection by researchers. The issue of forest ecosystem alteration and its importance in understanding Green Salamander arboreality and species declines have recently garnered attention (Pauley and Watson 2005; Waldron and Humphries 2005).

Green Salamanders are generally considered crevice-dwelling species associated with rock outcrops, and only "weakly" arboreal (Pauley and Watson 2005). Green Salamander morphology is consistent with rock-crevice habits, particularly their dorso-ventrally compressed bodies with elongate limbs and toes with expanded square toe tips (Petranka 1998). Most associations with Green Salamanders and non-rock outcrop habitats were reports of large numbers of individuals under the bark of dead and fallen trees, particularly large American Chestnut (Barbour 1949). Contradicting the perception of Green Salamanders as weakly arboreal, Waldron and Humphries (2005) recently documented large numbers of Green Salamanders using arboreal habitats in some Blue Ridge Escarpment populations. Using day and night searches with flashlights, they recorded 345 Green Salamander observations between April and October, of which 43% occurred in trees, 41% in rock outcrops, and only 15% on or in logs. They also reported arboreal nesting. Additionally, Wilson (2003) documented Green Salamanders in woody and arboreal habitats at sites throughout the Blue Ridge Mountains. These studies raise questions about the importance of arboreal habits to Green Salamander populations and highlight the potential need to include methods for searching arboreal habitats as part of Green Salamander monitoring.

Although the technique used by Waldron and Humphries (2005) was successful at discovering many salamanders using arboreal habitats, it was very time consuming (more than 210 h over a 3.5 yr period) and difficult to standardize. Survey success varied widely based on weather conditions and other variables. Further, the effectiveness of the technique is likely to vary greatly among observers who differ in ability to spot salamanders high in trees. A standardized and less labor-intensive technique for studying arboreal habits of Green Salamanders would be useful.

Artificial cover is widely utilized as a technique for standardized monitoring of terrestrial salamanders (Houze and Chandler 2002; Monti et al. 2000). Artificial cover can provide the necessary microclimates to attract salamanders during diurnal retreat, making detection of species less dependent upon the immediate climate. Artificial cover generally involves the application of boards or other materials to the forest floor, or litter bags or baskets along stream banks (Monti et al. 2000). We are not aware of any standardized artificial cover technique for capturing salamanders in arboreal habitats. However, burlap fabric attached to trees (known as burlap bands) has been used to sample arboreal invertebrates and reptiles (Campbell and Sloan 1977; Duguay et al. 2000; Horn and Hanula 2006; Reardon 1976). In addition to attracting invertebrates, these artificial shelters are sometimes occupied by salamanders, including Red-backed Salamanders (*Plethodon cinereus*) and Northern Two-lined Salamanders (*Eurycea bislineata*) (J. Waldron, pers. comm.). Attaching artificial cover to trees at distance and heights determined within the predicted range of Green Salamanders may provide a reliable technique for capturing Green Salamanders occupying arboreal habitats. In 2005, we deployed burlap bands on trees at four study sites in Georgia and North Carolina in order to determine whether Green Salamanders will occupy burlap bands as an artificial cover type.

Methods.—The populations of Green Salamanders surveyed were located in the Nantahala National Forest in Jackson County, North Carolina, and Chattahoochee National Forest in Rabun