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GROSS MORPHOLOGY OF TEETH ON THE PREMAXILLAE OF STREAMSIDE SALAMANDERS (*AMBYSTOMA BARBOURI*) AND SMALL-MOUTHED SALAMANDERS (*AMBYSTOMA TEXANUM*) FROM MIDDLE TENNESSEE

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Abstract.— Until 1989, the Streamside Salamander (*Ambystoma barbouri*) was considered conspecific with the Small-mouthed Salamander (*Ambystoma texanum*). Although they have distinct natural histories, particularly reproductive behaviors, individuals of these two species are nearly indistinguishable from each other. The similarity in appearance typically is not an issue because the two species are largely allopatric and geography can be used to determine which species is present. However, several narrow zones of contact (i.e. parapatry) have been reported from Kentucky, Indiana, and Ohio, and identifying an individual to species in these zones requires an examination of the dentition in postmetamorphic individuals. We used scanning electron microscopy to examine the gross morphology of teeth from adult Streamside Salamanders and adult Smallmouth Salamanders from middle Tennessee. Our observations of tooth morphology do not differ from those of these two sibling-species from other regions of their range. The lingual cusps of teeth on the upper jaw (premaxillae and maxillae) of Streamside Salamanders are short and rounded; whereas, cusps of these teeth in Small-mouthed Salamanders are long and narrow. Tooth morphology can be used to identify postmetamorphic individuals of each of these species from middle Tennessee.

Key Words.— Central Basin, Eastern Highland Rim, labial cusps, lingual cusps, sibling species, teeth, tooth morphology

The Streamside Salamander (*Ambystoma barbouri*) and the Small-mouthed Salamander (*Ambystoma texanum*) are a pair of cryptic species that inhabit middle Tennessee (Fig. 1; Redmond and Scott 1996, Scott et al. 1997). No external feature can be used to reliably identify salamanders of either of these two species (Krause and Petranka 1989). However, the dentition is different between the two species, and tooth structure has been used to identify species of individuals collected in zones of contact (Kraus and Petranka 1989) and in newly discovered populations (Scott et al. 1997).

Little is known about the variation in tooth structure of either Streamside Salamanders or Small-mouthed Salamanders. Kraus and Petranka (1989) describe the dentition of both species, but they did not examine specimens of either species from middle Tennessee. Their descriptions of the dentition of the Small-mouthed Salamander are based on specimens they obtained throughout the range of the species, and they describe distinct variation in morphology of the teeth from western and eastern populations. Beneski and Larsen (1989) describe the morphology of the teeth of the Small-mouthed Salamander, but their publication precedes the recognition of the Streamside Salamander as a distinct species, and they do not indicate the collection locality of the

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FIG 1. (A) Streamside Salamander (*Ambystoma barbouri*) from Wilson County, Tennessee. (B) Small-mouth Salamander (*Ambystoma texanum*) from Coffee County, Tennessee. (Photographs by Brian T. Miller).

specimens they use in their study. Gregory et al. (2016) describe tooth morphology of both species, but also do not indicate collection locality of their specimens. Thus, variation in morphology of the teeth occurs among populations of Small-mouthed Salamanders, but too little information exists to determine if similar variation occurs among populations of Streamside Salamanders.

The Streamside Salamander is known from fewer than fifty sites in middle Tennessee, all restricted to the Central Basin Physiographic region (Niemiller et al. 2006; Anderson et al. 2015, Lockwood et al. 2016). In contrast, the Small-mouthed Salamander is widespread in west Tennessee, but has a more limited distribution in middle Tennessee, where it is known primarily from a few locations in the Barrens of the Western Highland Rim physiographic region (Redmond and Scott 1996) and even populations from the Barrens of the Eastern Highland Rim (Miller et al. 2005). No aspect of the dentition has been described for either of these two species in middle Tennessee. The objective of this study is to use scanning electron microscopy to describe and document the morphology of teeth on the premaxillary bone of Streamside Salamanders and Small-mouthed Salamanders from middle Tennessee.

METHODS AND MATERIALS

We collected sexually mature Streamside Salamanders during January 1997 as they migrated to a small, ephemeral pond in the vicinity of Sinking Pond in Arnold Air Force Base in northern Coffee County, Tennessee. We also collected sexually mature salamanders as road kill in southern Rutherford County, Tennessee during breeding migrations of winter and spring of 2002. To prepare for SEM, we fixed specimens in 10% buffered formalin and preserved in 70% ETOH. We macerated three heads of preserved specimens of each species in a 4% KOH solution. We washed resulting skulls or disarticulated bones with distilled water for at least 24 h to remove the KOH. We then dehydrated the bones via a graded series of ethanol rinses (70%–95%–100%). Following dehydration, we air dried bones, mounted them on aluminum stubs, and sputter-coated specimens of each stub with about 30 nm of gold in a Hummer 6.2 sputter coater (Anatech USA, Union City, California, USA). We utilized a Hitachi S-3400N scanning electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan) to examine preparations at 20 KV.

RESULTS

Gross morphology of teeth on the premaxillary bone was similar in these two species (Figs. 2, 3). In each species, two rows of functional teeth were attached to the premaxillae, and the teeth on this jaw bone were pedicellate and bicuspid (Figs. 2, 3). The labial cusp was spade-like in each species; whereas, the lingual cusp was either short, blunt, and rounded (Streamside Salamander), or long, tapered and pointed (Small-mouthed Salamander; Fig. 3). Furthermore, in each species the outer surfaces of the cusps (labial and lingual) had an intricate network of ridges (Fig. 3).

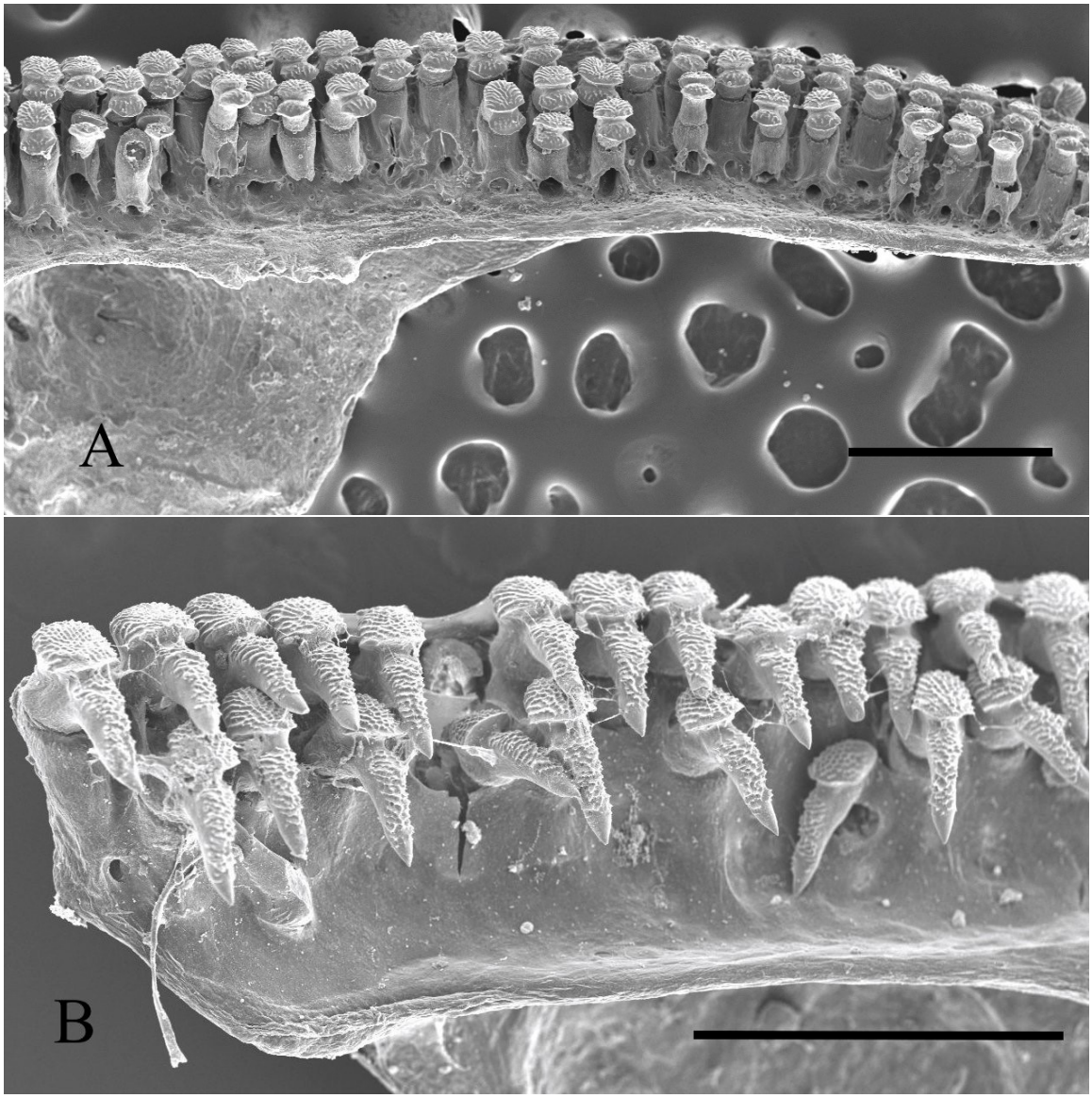

FIG. 2. (A) Teeth on the premaxilla of a Streamside Salamander (*Ambystoma barbouri*) from Rutherford County, Tennessee. (B) Teeth on the premaxilla of a Small-mouthed Salamander (*Ambystoma texanum)*, from Coffee County, Tennessee. The scale bar in the lower right of each photograph is 500 µ. (Photographs by Joyce L. Miller)

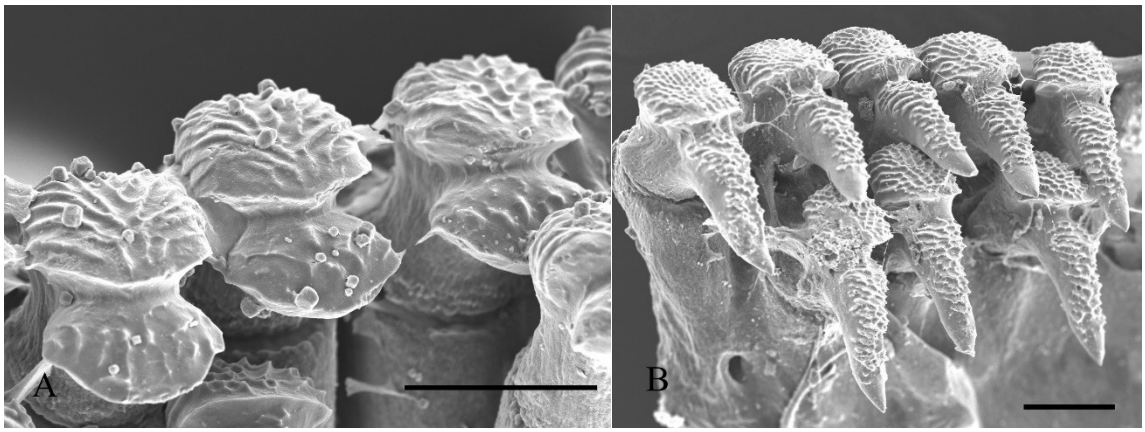

FIG. 3. (A) Teeth on the premaxilla of a Streamside Salamander (*Ambystoma barbouri*) from Rutherford County, Tennessee. (B) Teeth on the premaxilla of a Small-mouthed Salamander (*Ambystoma texanum*) and from Coffee County, Tennessee. The scale bar in the lower right of each photograph is 100 µ. (Photographs by Joyce L. Miller).

DISCUSSION

Teeth of the premaxillary bone of Streamside Salamanders and Small-mouthed Salamanders from middle Tennessee are similar to the few descriptions provided for these species from other regions (Beneski and Larsen 1989; Kraus and Petranka 1989; Gregory et al. 2016). At least in central Kentucky, the lingual cusps of teeth on the upper jaw of Streamside Salamanders are short and rounded; whereas, these cusps are long and narrow in eastern populations of the Small-mouthed Salamander (Kraus and Petranka 1989). The presence of multiple rows of functional teeth on the jaws of these two species and a few other ambystomatids has long been noted and is unusual (Beneski and Larsen 1989; Kraus and Petranka 1989); however, the functional significance, if any, is unknown. Furthermore, the functional significance of the peculiar ridges on the outer surfaces of the cusp is unknown (Beneski and Larsen 1989; Gregory et al. 2016).

Although the Streamside Salamander has been known to occur in Tennessee for approximately 20 years (Scott et al. 1997), relatively little information has been published on either the ecology or morphology of the species in the state. Regester and Miller (2000), Niemiller et al. (2009), and Mattison and Miller (2011) report on aspects of reproduction in the species, and Anderson and Miller (2011) report on iron deposition in the teeth of larvae. Most other published information is concerned with distribution of the species in the Central Basin, and most of these reports are based on discovery of either egg masses or adults (Niemiller et al. 2006; Niemiller et al. 2011; Anderson et al. 2014; Lockwood et al. 2016). Certainly, the typical reproductive behavior of Streamside Salamanders laying eggs on the undersurface of rock differs from that of the Smallmouth Salamander, which will usually lay eggs on in small clusters attached to vegetation in ponds (Kraus and Petranka 1989; Petranka 1998). However, Small-mouth Salamanders occasionally breed in ditches and streams, and Streamside Salamanders also occasionally breed in ponds (Petranka 1998). Thus, location of breeding site and manner of egg deposition is not necessarily a definitive means of identifying species. We suggest that the teeth of adult salamanders also be examined to ensure proper identification of this cryptic pair of sibling species.

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