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Morphology of the Blood Sinus in the Expanded Toe Tips of the Arboreal Salamander, *Aneides lugubris* (Plethodontidae)

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The lungless climbing salamander *Aneides lugubris* (family Plethodontidae) respires through cutaneous and buccal respiration rather than via lungs. A preliminary study from 1899 noted that there were two blood sinuses in the expanded toe tips of *A. lugubris* and hypothesized they facilitated respiration. Our goal is to histologically confirm the presence of these blood sinuses. Our work sets the stage for future work on the biomechanics and physiological function of toe tips in these climbing and lungless salamanders.

ARBOREALITY in plethodontid salamanders has evolved at least five times (Baken and Adams, 2019) and is common; approximately 50% of tropical species and 35% of temperate species are obligately or facultatively arboreal (McEntire, 2016). While the focus has been specifically on arboreality (McEntire, 2016; Baken and Adams, 2019), the distinction between arboreality and climbing in general (over rocks or on rock walls for example) has not been explored in detail. Climbing is an ability needed for arboreality, but of course is used in many other contexts as well. While there is a consistent body shape and foot shape among the arboreal species studied, these morphospaces are also shared by non-arboreal species (Baken and Adams, 2019). There was convergence, however, in foot shape between arboreal and cave-dwelling species, suggesting perhaps a common "climbing" morphology (Baken and Adams, 2019). Body size and foot shape are correlated with the ability of a salamander to attach to the substrate, an important skill for climbers (O'Donnell and Deban, 2020). In frogs, expanded toes tips have evolved independently across several lineages and are considered an adaptation for arboreality (Green, 1979). And while foot contact area and centroid size are correlated with clinging ability (Baken and O'Donnell, 2021), there are relatively few studies specifically on the dynamics of expanded toe tips in salamanders (but see Brown et al., 2025).

In an early description of expanded toe tips in the plethodontid *Aneides lugubris*, Ritter and Miller (1899) suggested that expanded toe tips were an adaptation for climbing as well as serving as an "external gill" (p. 695) for respiration. Based on dissections, Ritter and Miller (1899) noted two highly vascularized blood sinuses on either side of the terminal phalanx. More recently, the sinuses in the expanded toe tips of the climbing salamanders *Aneides aeneus* (Diefenbacher, 2008) and *A. vagrans* (Brown et al., 2025) have been described. Diefenbacher (2008) hypothesized that the expanded toe tips of *A. aeneus* enhance climbing ability. This hypothesis is supported by recent data from the Wandering Salamander, *A. vagrans*, that showed blood perfusing and leaving the sinus at different stages of locomotion (Brown et al., 2025).

Our objective is to confirm and histologically describe the blood sinuses in the expanded toe tips of *A. lugubris*, as first described by Ritter and Miller (1899).

MATERIALS AND METHODS

The third digit on the forelimb or hindlimb from sexually mature male ($n = 6$) and female ($n = 4$) *A. lugubris* (California Department of Fish and Game permits 4023 and 4755; all methods were approved by Gonzaga University's IACUC committee) were dissected and decalcified (Presnell and Schreibman, 1997). Using standard histological procedures (Presnell and Schreibman, 1997), tissue samples were embedded in paraffin, sectioned at 10 μm (Leica Jung Biocut 2035), and mounted onto microscope slides. Four toes were cut horizontally, one was cut sagittally, and the remaining five were cut transversely. Slides were stained with hematoxylin and eosin or hematoxylin and eosin-phloxine for general morphology, Verhoeff's elastic/Masson's trichrome (O'Connor and Valle, 1982) to identify elastic tissue surrounding the sinus and blood vessels, Giemsa stain to identify blood cells, or the Quad stain (Floyd, 1990; Staub and Paladin, 1997) to identify skin glands. Verhoeff's elastic/Masson's trichrome stain distinguishes different tissue types: bones stain red, muscle and collagen stain blue/green, muscle stains pink, and elastic tissue stains dark brown or black. With the Giemsa stain, nuclei are dark blue and erythrocytes are pink. The Quad stain (Floyd, 1990; Staub and Paladin, 1997) was used to differentiate mucous glands (Alcian blue positive) from granular glands (Schiff reaction positive or naphthol yellow positive).

Sections were analyzed with a Leica DME light microscope and photographed with a microscope-mounted Canon EOS Rebel 5 camera.

RESULTS

All toe tips examined had a terminal blood sinus (Fig. 1). Horizontal (Fig. 1A, B) and sagittal (Fig. 1C) sections provide a general overview of the toe-tip morphology with the blood sinus filling the distal region of the toe tip, ventral to the distalmost phalanx. The distal phalanx slightly curves dorsally to ventrally at the distal end of the toe tip (Figs. 1, 2). The sinus at the tip appears to be a collection of vessels and interstitial tissue forming a spongiform mass, as indicated in Figure 2A–B. The empty spaces of the sinus as seen in Figure 1 are most likely artifacts of preservation and processing. The poor nuclear staining in Figure 2 is most likely due to over-decalcification (Presnell and Schreibman, 1997).

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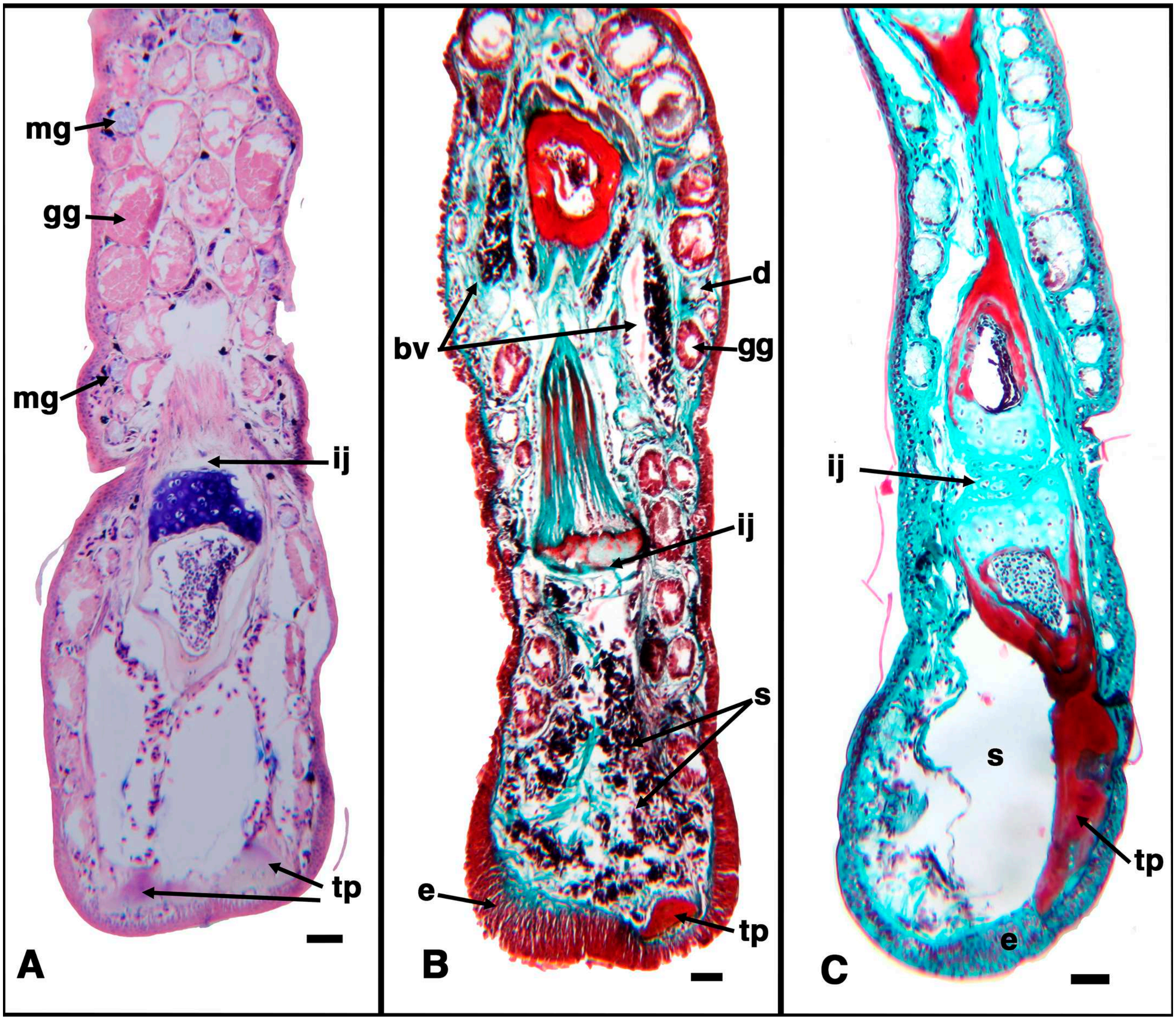

Fig. 1. Toe sections of *Aneides lugubris*. These 10 μm horizontal (A, B) and sagittal (C) sections show the distal two phalangeal regions of the toe. Note the large blood sinus at the toe tip. (A) Because of the curvature of the toe, the distal region is cut more ventrally than the proximal region, which shows many glands on the dorsal surface (Giemsa stain). Note the large blood vessels (bv) in (B) running laterally along the toe (Verhoeff's elastic/Masson's trichrome stain). (C) This sagittal section shows a different perspective of the toe tip. This section is more lateral than at the midline (note the penultimate phalanx is not cut through the midline; (Verhoeff's elastic/Masson's trichrome stain). bv: blood vessel; d: dermis; e: epidermis; gg: granular gland; ij: interphalangeal joint; mg: mucous gland, s: blood sinus; tp: terminal phalanx. Scale bars are 100 μm.

The cross sections of the toe tip (Fig. 2A–D) illustrate how the sinus changes distally to proximally along the phalange. The large interconnected sinus, seemingly composed of interconnecting compartments distally (Fig. 2A, B), is divided more proximally by connective tissue into two lateral sinuses. A large process extends from the ventral side of the distalmost phalanx, to which digital muscles attach (Fig. 2C, D; Wake, 1963).

Mucous and granular glands are on the lateral and dorsal surfaces of the toes but not on the ventral surface. Because of the curvature in the toe in Figure 1A, the sectioned plane along the penultimate phalanx is more dorsal than at the toe tip, and consequently shows many granular and mucous glands.

The figure in Ritter and Miller (1899) shows bilateral blood vessels widening at the distal end of the toe, extending to the toe's tip, and then doubling back, running proximally up the toe. We did not find evidence of that vessel arrangement. Rather, the vessels meet at a common sinus compartment at the toe tip. How blood returns from the toe needs more careful analysis. Large vessels are present adjacent to the distalmost interphalangeal joint (Fig. 1D).

DISCUSSION

We confirm the presence of a blood sinus, as first noted by Ritter and Miller (1899), in the expanded toe tip of *A. lugubris*. The sinus lies ventral to the terminal phalange that is recurved

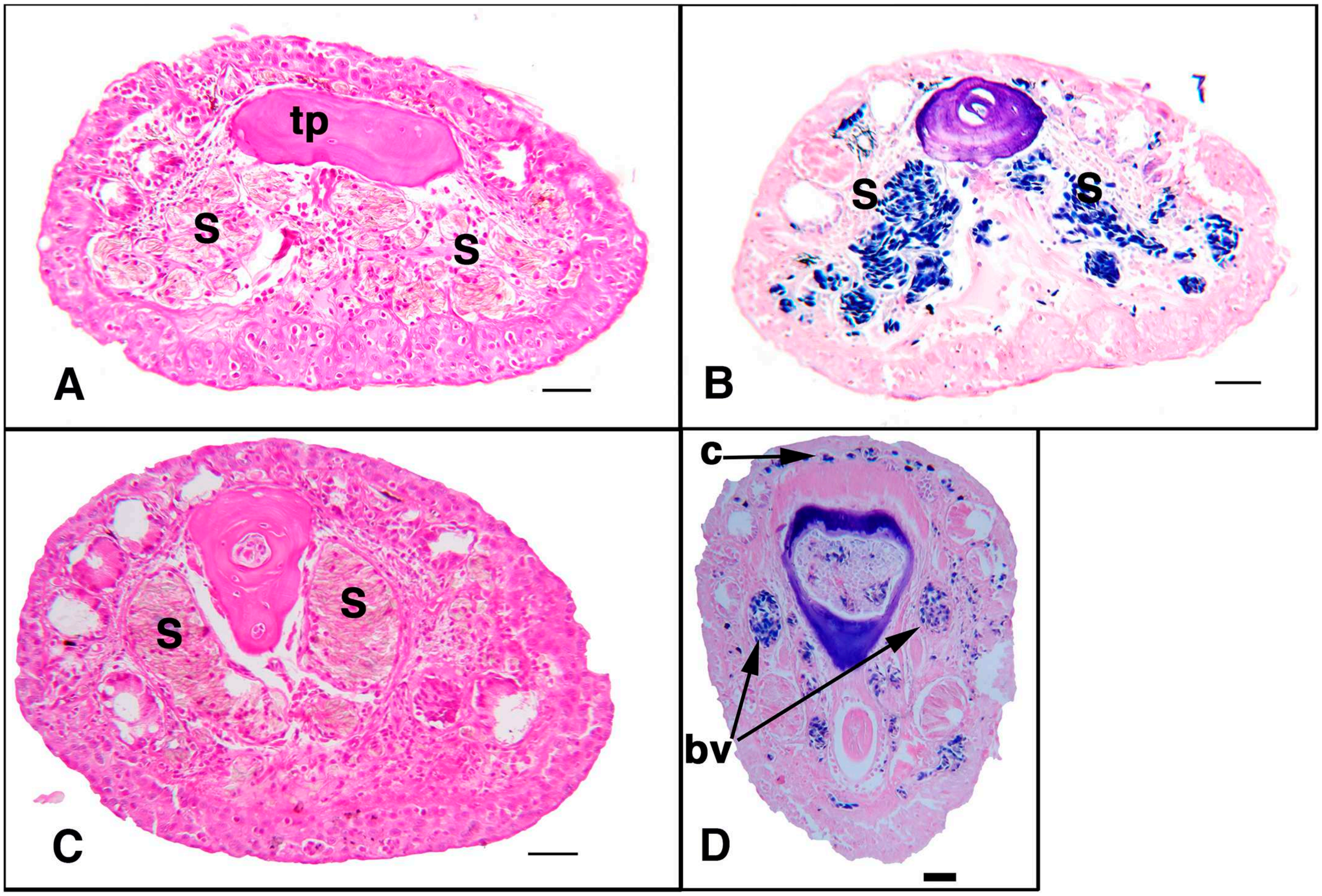

Fig. 2. Cross sections of the toe tip of *Aneides lugubris*. These 10 μm transverse sections show sections from distal (A) to proximal (D) along the distalmost phalanx of the toe. Note the large blood sinus at the toe tip (A) and that the sinus is separated into two chambers more proximally (B–C). The sinus chambers, just distal to the distalmost interphalangeal joint, are reduced to two vessels (D). Sections in (A) and (C) are stained with hematoxylin and eosin-phloxine; sections in (B) and (D) are stained with Giemsa. bv: blood vessel; c: capillaries; s: blood sinus; tp: terminal phalanx. Scale bars are 100 μm.

and bifid distally (Wake, 1963; Wake et al., 1983). This sinus has been observed in other species as well, for example, in *A. aeneus* (Diefenbacher, 2008) and *A. vagrans* (Brown et al., 2025), both climbing salamanders. More interestingly perhaps, is the presence of a terminal blood sinus in two species of *Desmognathus*, *Desmognathus monticola* and *Desmognathus fuscus* (as seen in figure 2 of Caldwell and Trauth, 1979). These species are not considered arboreal or climbing salamanders, but perhaps moving along rocky substrates semi-aquatically constitutes a type of climbing behavior. Alternatively, the blood sinus may be a common or even ancestral feature of plethodontid toe tips. *Plethodon patraeus*, similar to the climbing species of *Aneides*, has a recurved terminal phalanx and more blunt and expanded terminal phalanges compared to other members of its *Plethodon glutinosus* species group (Wynn et al., 1988). We predict that it too has a terminal blood sinus. Along these lines, analyses of the toe tips across a wide array of plethodontids, climbing and non-climbing, would address the question of whether the sinus is ancestral or has specializations in climbing taxa.

In the distalmost region of the toe tip, the sinus is one main compartment and more proximally is separated into two compartments (Fig. 2), an arrangement noted in *A. vagrans* as well (Brown et al., 2025). It appears that the sinus compartments are reduced to large vessels at the most distal interphalangeal joint (Figs. 1B, 2D). Future work similar to the high-speed video studies of Brown et al. (2025) will help determine patterns of blood flow through the vessels and sinus compartments of the toe.

Two different types of glands are present in the toe tip, both granular and mucous (Fig. 1). Examination of the role of mucous glands among plethodontids, and of different types of mucus, on clinging and attachment ability across substrates will be informative, as Baken and O'Donnell (2021) suggest. The distribution of mucous glands in the foot will prove interesting as well. Some climbing species of *Bolitoglossa* have mucous glands on the soles of their feet (Green and Alberch, 1981). While we observed no mucous glands on the ventral surface of the toes in *A. lugubris*, the presence of mucous glands on the ventral surface of the foot pad itself has not yet been determined. The function of granular glands in the context of climbing is a promising area of research as well. Comparative studies that combine morphology, gland distribution, and gland composition, as Langowski et al. (2019) analyzed in tree frogs, would be valuable.

Recent studies have examined the clinging performance and attachment characteristics of plethodontids by analyzing contact area of the salamander, behavior, posture, as well as centroid size (O'Donnell and Deban, 2020; Baken and O'Donnell, 2021). The structure of the toe tip epithelium is another variable to consider as well (Li et al., 2022). Examining variation in the digital sinus and toe shape across species should help disentangle the contributions of these different variables to locomotor ability. As Baken and O'Donnell (2021) point out, the relationship between clinging performance and climbing ability is another worthwhile area of study.

Ritter and Miller (1899) hypothesized that the blood sinuses in the expanded toe tips may be adaptations for respiration and for climbing. Noble (1925) argues that the sinuses are not used for respiration, based on the number and distribution of capillaries in terrestrial and aquatic species. Interestingly, in *A. lugubris,* the dorsal surface of the toe tip is covered with capillaries (Fig. 2D), which could effectively increase the surface area for respiration. Other support for this hypothesis is based on bullfrog research that shows that the more capillaries are perfused with blood, the more carbon dioxide is eliminated (Burggren and Moalli, 1984). Of course, the capillaries are different from the sinus, but in any event, examining the structure and biomechanics of the toe tips, including the sinus, associated vessels, and skin glands, will be valuable in determining the interplay among morphology, blood flow, and locomotory style across different clades of salamanders.

DATA ACCESSIBILITY

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AI STATEMENT

The authors declare that no AI-assisted technologies were used in the design and generation of this article and its figures.

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