

Re-evaluating the morphological evidence for the re-evolution of lost mandibular teeth in frogs

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Dollo's law of irreversibility states that once a complex structure is lost, it cannot be regained in the same form. Several putative exceptions to Dollo's law have been identified using phylogenetic comparative methods, but the anatomy and development of these traits are often poorly understood. *Gastrotheca guentheri* is renowned as the only frog with teeth on the lower jaw. Mandibular teeth were lost in the ancestor of frogs more than 200 million years ago and subsequently regained in *G. guentheri*. Little is known about the teeth in this species despite being a frequent example of trait "re-evolution," leaving open the possibility that it may have mandibular pseudoteeth. We assessed the dental anatomy of *G. guentheri* using micro-computed tomography and histology and confirmed the longstanding assumption that true mandibular teeth are present. Remarkably, the mandibular teeth of *G. guentheri* are nearly identical in gross morphology and development to upper jaw teeth in closely related species. The developmental genetics of tooth formation are unknown in this possibly extinct species. Our results suggest that an ancestral odontogenic pathway has been conserved but suppressed in the lower jaw since the origin of frogs, providing a possible mechanism underlying the re-evolution of lost mandibular teeth.

KEY WORDS: Anura, dentition, Dollo's law, Hemiphractidae, trait reversal.

Dollo's law of irreversibility proposes that complex structures that are lost over evolutionary time cannot be regained in the same form (Gould 1970). Recent studies exploring trait evolution in a phylogenetic context, however, suggest that many complex traits have been lost and then regained, including wings in stick insects (Whiting et al. 2003), limbs in lizards (Brandley et al. 2008), aquatic larvae in salamanders (Chippindale et al. 2004), and teeth on the lower jaw in frogs (Wiens et al. 2011). In most cases, the evidence for these putative exceptions to Dollo's law derives from reconstructing trait evolution on phylogenies. Further evaluating the plausibility of these exceptions requires a more thorough understanding of the anatomy, development, and genetics of these traits (Kerney et al. 2011; Sadier et al. 2021).

Teeth originated in stem gnathostomes more than 400 million years ago (Rücklin et al. 2012) and have been broadly maintained across vertebrates. Within modern amphibians

(Lissamphibia), all salamanders and caecilians maintain teeth on the upper and lower jaws, but nearly all frogs lack dentition on the lower jaw (i.e., mandible) and variably possess teeth on the upper jaw and palate (Paluh et al. 2021). Of the more than 7000 species of frogs, only one species, the marsupial frog *Gastrotheca guentheri* (Boulenger 1882; Hemiphractidae), possesses teeth on the dentary bone of the lower jaw. Boulenger (1882) recognized the distinctiveness of these mandibular teeth when describing *G. guentheri* and thus placed the species in a monotypic family (Amphignathodontidae) and genus (*Amphignathodon*; from the Greek: amphí—on both sides, gnathos—jaw, and odon—tooth). Noble (1922) illustrated the toothed mandible of *Amphignathodon*, described the mandibular teeth as being similar to the maxillary teeth, and noted that the mandibular teeth contained a pulp cavity, dentin, and enamel, but presented no histological evidence to support this. He further recognized that the species

was “simply a specialized *Gastrotheca*,” which was later confirmed with immunological and molecular data (Duellman et al. 1988; Wiens et al. 2007). Laurent (1983) suggested that *G. guentheri* re-evolved mandibular teeth due to the lifting of a “very old inhibition,” such as a suppressor regulatory gene that disappeared, and that the developmental and genetic basis for teeth on the lower jaw had not been lost in the ancestors of these frogs. Recently, Wiens (2011) used ancestral state reconstruction methods to demonstrate that mandibular teeth were lost in the ancestor of crown-group frogs more than 200 million years ago and subsequently regained in *G. guentheri* during the Miocene.

The “re-evolution” of mandibular teeth in frogs may be unlikely given the exceptionally long period of time between their loss and subsequent re-acquisition (>200 million years). Only two brief anatomical descriptions (Boulenger 1882; Noble 1922), one illustration (Noble 1922), and one photograph (Berkovitz and Shellis 2016) of the teeth of *G. guentheri* have been published. There remains a need to verify that the mandibular dentition of *G. guentheri* is composed of true teeth. Alternatively, these structures may be bony odontoid serrations (i.e., pseudoteeth) as seen in some other anurans, such as the mandibular odontoids found in *Cornufer guentheri* and species of *Hemiphractus* (Shaw 1989; Fabrezi and Emerson 2003; Paluh et al. 2021), which would indicate that they do not represent a “re-evolved” trait. If true mandibular teeth are present, they may be degraded or simplified, such as through the loss of enamel or a bicuspid shape, due to deterioration of the odontogenic pathway. If the mandibular teeth of *G. guentheri* are identical to those on the upper jaw, these results may suggest the ancestral tooth development program has been highly conserved, but long suppressed, on the lower jaw of frogs. *Gastrotheca guentheri* was always a rare species in the cloud forests of Colombia and Ecuador, and a living specimen has not been observed since 1996 (De la Riva et al. 2020). Museum specimens are thus our only resource to investigate this putative violation of Dollo’s law. Here, we re-evaluate the dental anatomy of *G. guentheri* using both high-resolution micro-computed tomography scanning and histology.

Methods

SAMPLING

Females in the genus *Gastrotheca* brood developing embryos in a pouch on their back (Duellman 2015). Some species, including *G. guentheri*, have direct development (i.e., no free-living and feeding larval stage; Gomez-Mestre et al. 2012) and young emerge from the pouch as miniature versions of the adult. We examined six postnatal specimens of *G. guentheri* that vary in ontogenetic stage: a neonate froglet (KU:KUH:200260, 17.1 mm snout-vent length [SVL]), two juveniles (KU:KUH:178464, 30.9 mm SVL; KU:KUH:221634, 47.6 mm SVL), and three adults

(KU:KUH:195628, 62.4 mm SVL; KU:KUH:164226, 71.5 mm SVL; KU:KUH:221635, 78.9 mm SVL). The largest reported body size for the species is 82.00 mm SVL (Duellman 2015). One adult (KU:KUH:221635) was a brooding female with late-stage embryos in its dorsal pouch, which allowed us to assess embryological anatomy of individuals with an approximate SVL of 13 mm. We compared the jaw and tooth morphology of *G. guentheri* to 19 additional specimens of other *Gastrotheca* (Table S1; four juveniles and 15 adults), representing 14 species and each of the eight recognized subgenera (Duellman 2015; Duellman and Cannatella 2018; but see Echevarría et al. 2021). Specimens for this study are from the herpetological collections of the University of Kansas Biodiversity Institute (KU) and Florida Museum of Natural History (UF).

MICROCT

To examine the dental ontogeny of *G. guentheri*, we generated high-resolution X-ray computed tomography (microCT) scans of the entire body, as well as for just the head at higher resolution, for each specimen using a GE v|tome|x M 240 at the University of Florida’s Nanoscale Research Facility. A-180 kV X-ray tube and diamond-tungsten target were used for all scans. We adjusted the voltage (between 70 and 90 kV) and current (between 100 and 200 μ A) to maximize absorption range for each specimen, with final voxel resolutions ranging from 7 to 46 μ m. The raw X-ray data were processed using the GE datos|x software to produce tomogram and volume files. The volume files were imported into VG StudioMax (Volume Graphics, version 3.4), and the skull and lower jaw were segmented using the region-growing and smoothing tools. Linear measurements of the skull, jaws, and teeth were recorded using the polyline length tool in VG StudioMax. One late-stage embryo was removed from the pouch of the brooding female (KU:KUH:221635) and scanned at 4.9 μ m to identify the state of developing teeth. We additionally CT-scanned a dissected segment of the upper jaw (maxilla and premaxilla) and lower jaw (dentary) of one juvenile specimen of *G. guentheri* (KU:KUH:221634) at 1.6 μ m to examine the jaw and dental anatomy at high resolution prior to histological sectioning. Tooth counts (number of attached functional teeth and number of total tooth loci) were recorded for each paired dentigerous element (left and right maxilla, premaxilla, vomer, dentary) in the series of *G. guentheri* and in the additional 19 specimens of other *Gastrotheca* species for the right side of the skull only. All resulting CT data are available via MorphoSource (see Table S1).

HISTOLOGY

We dissected upper and lower jaw tissue from a juvenile specimen of *G. guentheri* (KU:KUH:221634) to evaluate the cellular structure and tissue composition of the upper jaw, lower jaw, and corresponding dentition. For comparison, we sampled two other

species of *Gastrotheca* (*Gastrotheca peruana*, UF:Herp:65788; *Gastrotheca riobambae*, UF:Herp:98222) and a representative from the Hylidae (*Osteopilus septentrionalis*, UF:Herp:171513), a family that is closely related to the Hemiphractidae (Feng et al. 2017; Streicher et al. 2018). We removed one late-stage embryo from the dorsal pouch of a brooding female (KU:KUH: 221635); there is no staging table for direct-developing hemiphractid frogs, but the staging table developed for *Eleutherodactylus* by Townsend and Stewart (1985) suggests that this embryo is at a stage just before hatching (Townsend-Stewart [TS] 14 or 15). The embryo and the dissected jaw tissues from *G. guentheri* and the comparative species were decalcified in EDTA for 48 h and 4–6 days, respectively, and processed for paraffin sectioning using a standard protocol. The embedded paraffin blocks were sectioned to 8–11 μm on a Leica RM2145 microtome and stained using a standard Hematoxylin and Eosin (H&E) Alcian blue protocol (Johanson et al. 2019). Slides were imaged on a Leica DM2500 LED compound microscope. We additionally stained the *G. guentheri* jaw sections using a fluorescent DAPI (diamidino-2-phenylindole) stain to visualize cell nuclei and assess gross cell morphology.

Results

DENTAL ONTOGENY IN *G. guentheri*

Pedicellate, bicuspid teeth are present on the maxilla, premaxilla, vomer, and dentary in all six postnasal *G. guentheri* specimens examined (Fig. 1). Tooth size and shape vary little across the four dentigerous elements within each specimen, indicating a homodont condition. Pedicel height (range: 0.3–1.2 mm), pedicel width (range: 0.1–0.4 mm), and crown height (range: 0.1–0.4 mm) increase with body size (Table S2; Fig. S1). Tooth number varies considerably across the four dentigerous elements and through ontogeny (Fig. 1; Table S2). The vomer has the fewest functional teeth (range: 0–5) and tooth loci (range: 3–7) and the maxilla has the most (functional teeth range: 11–40; tooth loci range: 28–53). The smallest postnatal individual examined has seven to 10 functional teeth and 21 tooth loci on each dentary, whereas the three adults have 27–30 functional teeth and 40–49 tooth loci on each dentary. The relative increase in tooth loci and functional teeth is similar on the maxilla and dentary through ontogeny (Fig. S2). The dentition in *G. guentheri* first develops as an alternating series in which each functional tooth on the premaxilla, maxilla, dentary, and vomer is separated by a locus undergoing replacement (either empty, undergoing resorption, or developing the next generation tooth; Fig. 1). In the three adult specimens examined, this alternating sequence is broken on the maxilla and dentary where several functional teeth are adjacent to one another (a similar pattern has been shown in the closely related *Hemiphractus*; Shaw 1989). On all ele-

ments, the teeth are replaced using a one-for-one tooth replacement system, which is typical of amphibians (Davit-Béal et al. 2007). The late-stage embryos within the pouch of the brooding female (KU:KUH:221635) do not have observable teeth, although many skeletal elements are undergoing ossification, including those of the axial skeleton (vertebral column, urostyle), appendicular skeleton (ilium, femur, tibiofibula, humerus), and skull (basicranium, maxilla, premaxilla). The lower jaw is not observable in the microCT data and thus presumably not yet ossified.

Gastrotheca TOOTH COUNT VARIATION

The number of functional teeth and tooth loci generally increases with body size across all species of *Gastrotheca* examined and for all four dentigerous elements (Fig. 2; Table S1). The numbers of functional teeth and tooth loci on the premaxilla, maxilla, and vomer in the *G. guentheri* series are within the range observed across other species of *Gastrotheca*. However, *G. guentheri* often has fewer teeth and tooth loci than congeners at a similar body size. For example, an adult *Gastrotheca weinlandii*, which is the sister taxon to *G. guentheri* (Wiens et al. 2011), has 15 more tooth loci on the right maxilla and two more tooth loci on the right premaxilla and right vomer than a similarly sized *G. guentheri*. Dentary teeth are present only in *G. guentheri*.

HISTOLOGY

True teeth were confirmed present in the upper and lower jaws of *G. guentheri* through standard histology stained with H&E and Alcian blue (Figs. 3 and 4). A successional dental lamina is present on both the upper and lower jaws of *G. guentheri*, suggesting a similar method of tooth addition and replacement. The dental lamina is a band of dental epithelium continuous with the oral epithelium. It invaginates into the underlying dental mesenchyme and continuously develops new teeth in polyphyodont vertebrates (Fraser et al. 2020). This essential tissue for tooth replacement appears to play an important and conserved role in both tooth development and replacement in the dentary teeth of *G. guentheri*. Replacement teeth at various stages of development (from tooth buds to ankylosed functional teeth) are in close association with the dental lamina and are composed of a pulp cavity, odontoblasts, dentin, ameloblasts, enamel organ, and a thin layer of enamel. Similar dental tissues were observed in the replacement teeth on the upper jaw in *Osteopilus septentrionalis*, *G. peruana*, and *G. riobambae* (Fig. 4). In the four species examined, the tissue composition and progression of tooth replacement on the upper jaw resembles previously published dental descriptions in toothed frogs (*Rana pipiens*, Gillette 1955; *Hyla cinerea*, Goin and Hester 1961; *Xenopus laevis*, Shaw 1979; *Hemiphractus proboscideus*, Shaw 1989). The dental lamina of the upper jaw extends on the lingual side of each functional tooth, from which the

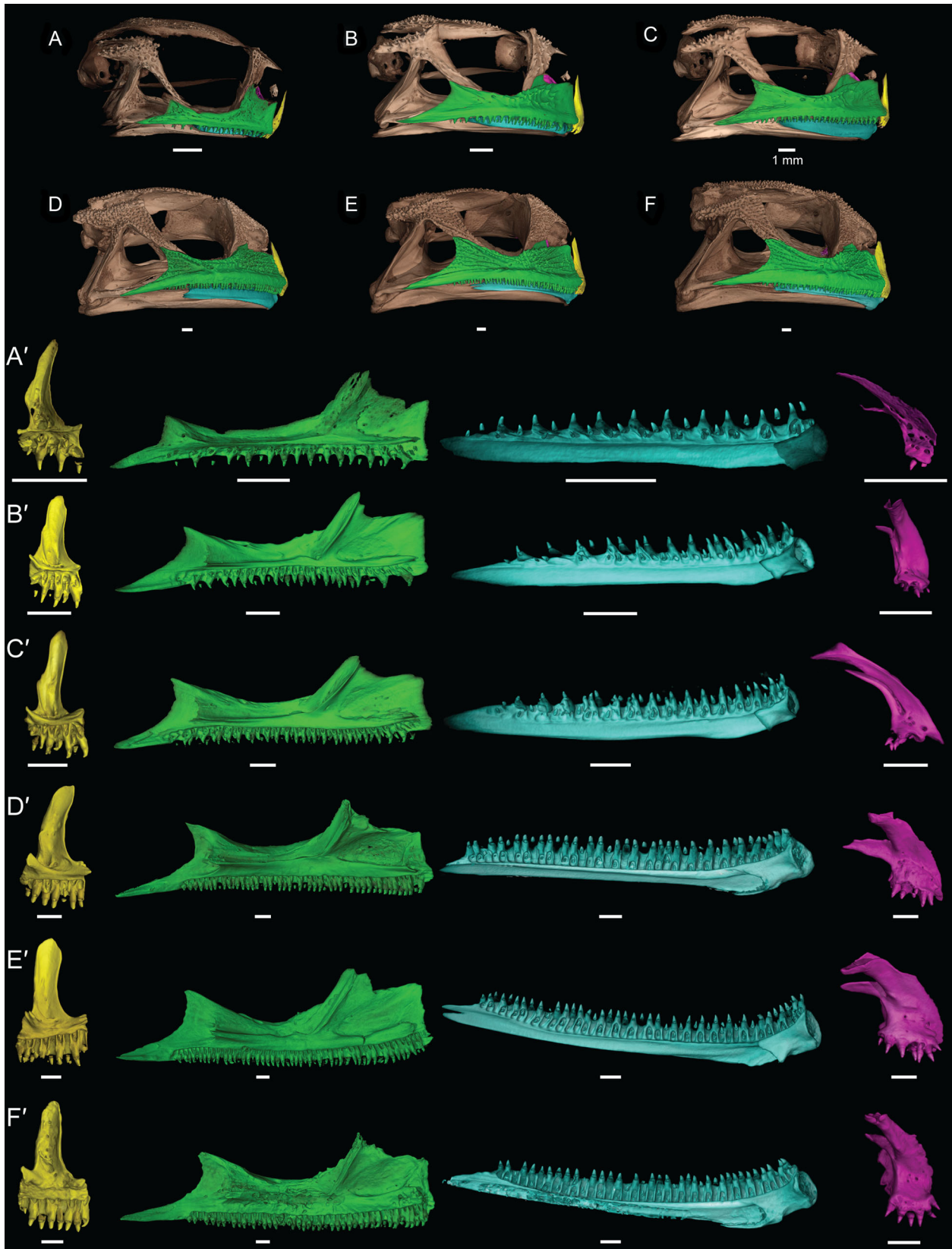


Figure 1. Dental ontogeny of *Gastrotheca guentheri*. (A) 17.1 mm snout-vent length (SVL) neonate, KU:KUH:200260; (B) 30.9 mm SVL juvenile, KU:KUH:178464; (C) 47.6 mm SVL juvenile, KU:KUH:221634; (D) 62.4 mm SVL adult, KU:KUH:195628; (E) 71.5 mm SVL adult, KU:KUH:164226; and (F) 78.9 mm SVL adult, KU:KUH:221635. Skulls in lateral view: dentigerous cranial elements are colored. Isolated premaxilla (yellow), maxilla (green), dentary (blue), and vomer (purple) in lingual views. Scale bars = 1 mm.

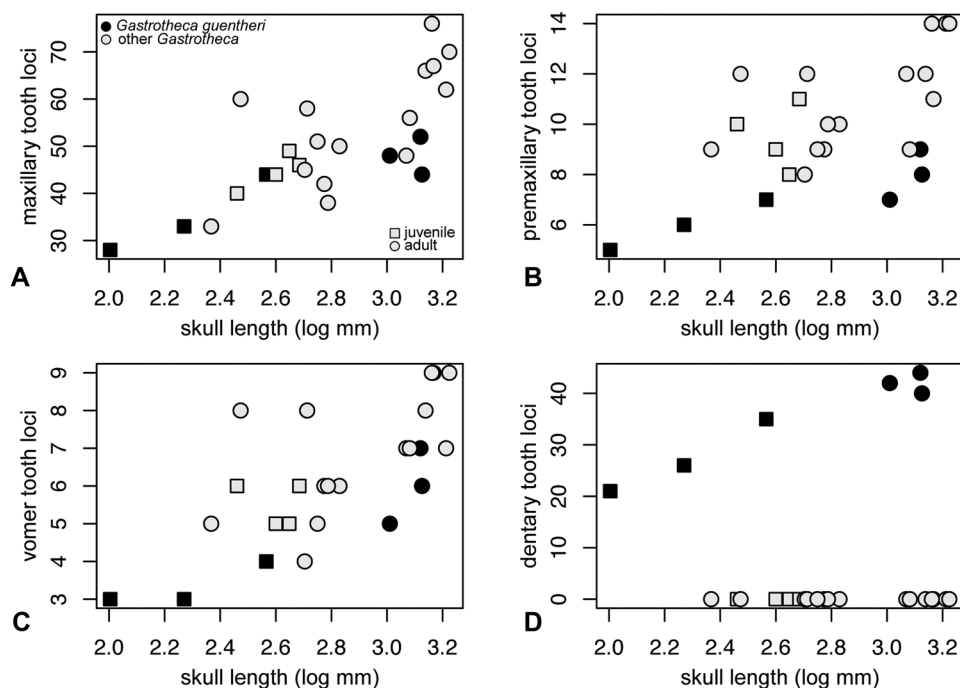


Figure 2. Relationship between tooth loci number and log transformed skull length on the (A) maxilla, (B) premaxilla, (C) vomer, and (D) dentary across the *Gastrotheca guentheri* ontogenetic series (black points) and other *Gastrotheca* species (gray points). Tooth loci counts were recorded from the right side of each specimen. Specimen point labels are provided in Figure S3 and corresponding data are provided in Table S1.

next tooth will develop. The dental lamina of the lower jaw in *G. guentheri* also extends on the lingual side of each functional tooth and is similar to the lower jaw dental morphology seen in salamanders (Davít-Béal et al. 2007). There is no evidence of a dental lamina or dentition on the lower jaw in *Osteopilus*, *G. peruana*, or *G. riobambae* (Fig. 4).

Although no teeth are visible in the microCT reconstruction of the late-stage *G. guentheri* embryo, histological sections confirm that odontogenesis has initiated on both the upper and lower jaws with the formation of the primary dental lamina that gives rise to the first-generation teeth (Fig. 5). The dental lamina on the upper jaw is proliferating into a cup shape at its extremity and is therefore at a more advanced developmental stage than the dental lamina of the lower jaw. Due to tissue shrinkage that likely resulted from poor initial preservation of the developing embryos in the dorsal pouch, the oral epithelium and dental lamina have detached from the underlying mesenchyme (see Fig. 5).

Discussion

Perhaps our most remarkable finding is that the teeth on the lower jaw in *G. guentheri* are nearly identical in gross morphology, histology, and development to the teeth on the upper jaw and palate. Our microCT and histological results confirm the longstanding

assumption that *G. guentheri* has true teeth on the lower jaw. Bicuspid, pedicellate teeth are present on the upper jaw (premaxilla and maxilla), palate (vomer), and lower jaw (dentary) throughout postnatal ontogeny, and the tissue composition of the dentition, from dental lamina to erupted teeth, is similar on both jaws. Tooth size and shape are similar across the tooth-bearing bones within each *G. guentheri* specimen, but tooth size increases with body size. The numbers of functional teeth and tooth loci increase through ontogeny on all four dentigerous elements. This is generally consistent with the limited data for dental ontogeny available from other anuran species (Smirnov and Vasil'eva 1995; Davít-Béal et al. 2007). *Gastrotheca guentheri* has relatively fewer teeth and tooth loci on the premaxilla, maxilla, and vomer than several other species of *Gastrotheca* that attain a similar body size (Fig. 2). One explanation for this pattern is that a consequence of re-evolving mandibular teeth might be a reduction in tooth number on other dentigerous elements due to the energetic costs of continuous dental development and replacement on an additional skeletal element. Alternatively, tooth development in *G. guentheri* may be delayed (see below), and this slowed development could be responsible for the reduced tooth count. Other likely consequences of re-evolving mandibular teeth warrant further investigation, such as modifications to the blood supply and innervation of the lower jaw and modifications to tongue morphology.

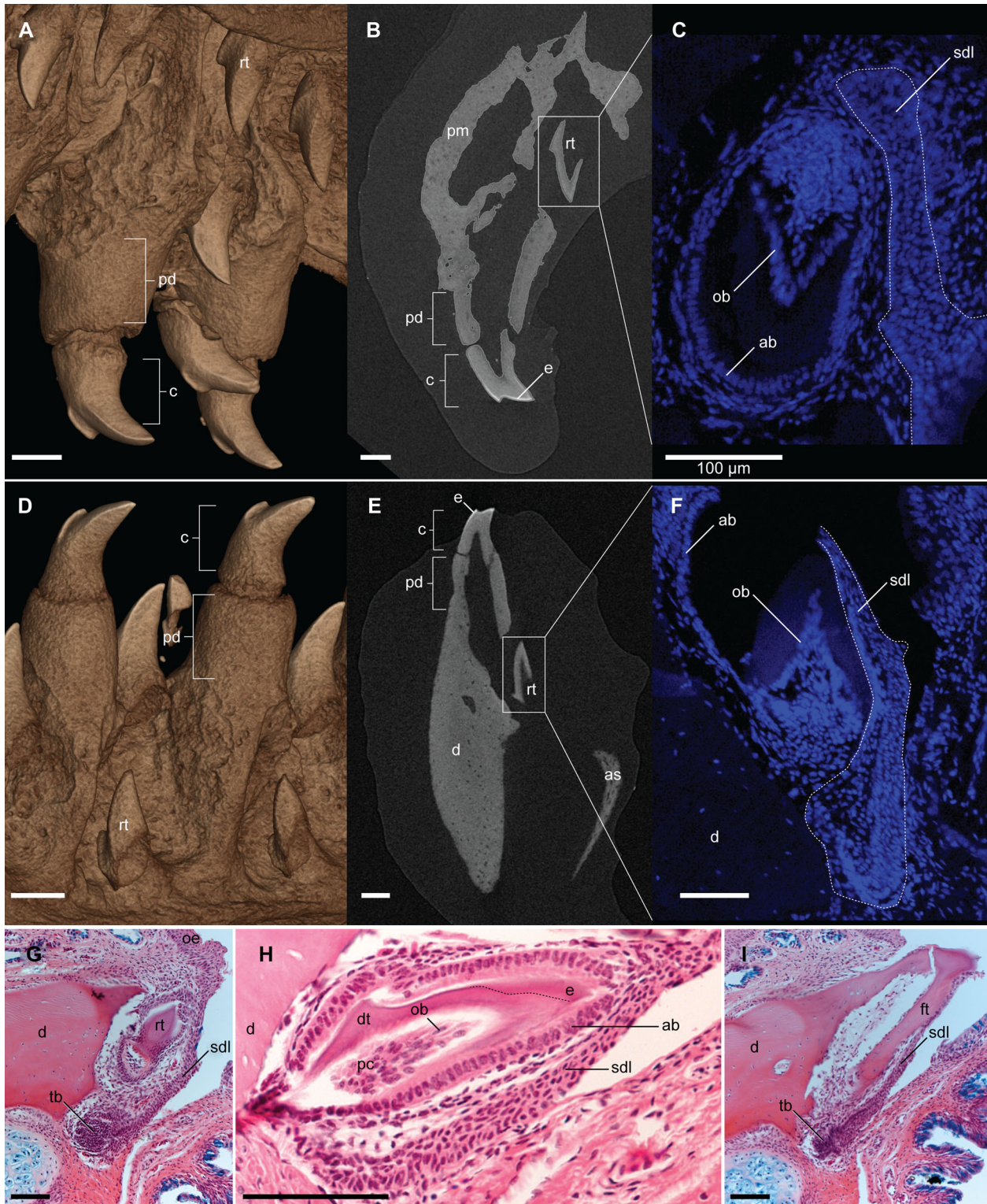


Figure 3. High-resolution dental anatomy of *Gastrotheca guentheri* (KU:KUH:221634) from the upper jaw (A–C) and lower jaw (D–F and G–I) based on microCT volume segmentation (A and D), microCT tomograms (B and E), DAPI stained sections (C and F), and H&E-stained sections (G–I). Abbreviations: ab, ameloblasts; as, angulosplenic; c, crown; d, dentary; dt, dentin; e, enamel; ft, functional tooth; ob, odontoblasts; oe, oral epithelium; pc, pulp cavity; pd, pedicel; pm, premaxilla; rt, replacement tooth; sdl, successional dental lamina; tb, tooth bud. Scale bars = 100 μm .

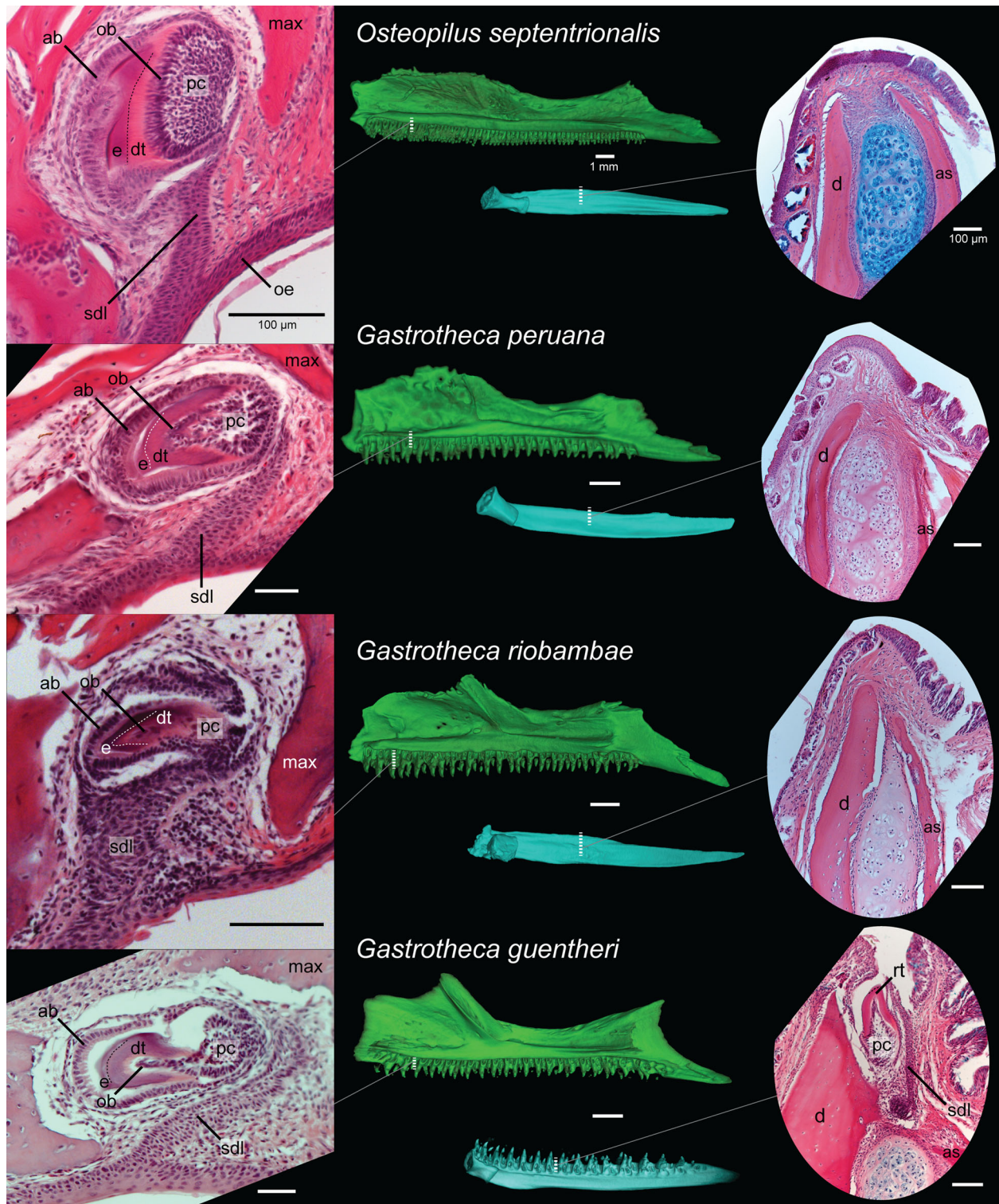


Figure 4. Jaw anatomy of *Osteopilus septentrionalis* (microCT of UF:Herp:63656, histology of UF:Herp:171513), *Gastrotheca peruana* (microCT of UF:Herp:65783, histology of UF:Herp:65788), *G. riobambae* (both UF:Herp:98222), and *G. guentheri* (both KU:KUH:221634) based on microCT volume segmentation (center; green = maxilla, blue = dentary; anterior is to the left) and H&E-stained sections of the upper jaw (left) and lower jaw (right). Upper jaw teeth are present in all species and mandibular teeth are present only in *G. guentheri*. Abbreviations: ab, ameloblasts; as, angulosplenic; d, dentary; dt, dentin; e, enamel; ob, odontoblasts; oe, oral epithelium; m, maxilla; pc, pulp cavity; rt, replacement tooth; sdl, successional dental lamina.

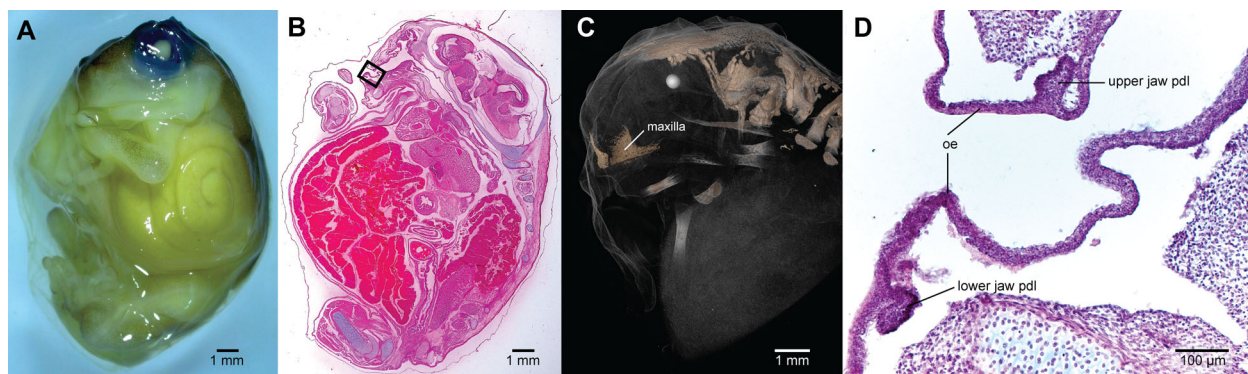


Figure 5. Anatomy of a late-stage *Gastrotheca guentheri* embryo (A) removed from the pouch of a brooding female (KU:KUH:221635) examined using histology (B) and microCT (C). (D) Inset of mouth opening outlined in panel B. No teeth are visible in the microCT reconstruction of the embryo, but histological sections show the initiation of tooth development on the upper and lower jaws with the formation of the primary dental lamina. Abbreviations: oe, oral epithelium; pdl, primary dental lamina.

The initiation of odontogenesis typically occurs during early larval or embryonic development (Lainoff et al. 2015), including in salamanders and caecilians (Davit-Béal et al. 2007). In frogs that have a biphasic life history, the onset of tooth development is delayed and typically occurs during metamorphosis (Gosner Stages [GS] 40–46; Smirnov and Vasil'eva 1995, Davit-Béal et al. 2007) or during the mid-larval phase in *Xenopus laevis* (Niewkoop-Faber Stage 53–55/GS 31–35; Shaw 1979, Davit-Béal et al. 2007). Tooth formation likely occurs ontogenetically late in frogs because the anuran mouth undergoes dramatic restructuring during metamorphosis (McDiarmid and Altig 1999), transitioning from an herbivorous tadpole with a keratinized beak and pseudoteeth (i.e., keratodonts or labial teeth) to an insectivorous frog with true teeth. Tooth development has not been directly studied in any frogs that have direct development, but the evolution of this life history mode may provide an opportunity to repattern the jaw and alter the timing of tooth germ initiation. No erupted teeth are present in hatchlings of *Eleutherodactylus jasperi* (Eleutherodactylidae; Wake 1978) or *Pseudophilautus silus* (Rhacophoridae; Kerney et al. 2007), although tooth buds are present and undergoing mineralization on the upper jaw of *E. jasperi* up to 4 days prehatching (Wake 1978). Maxillary teeth are visible in *Haddadus binotatus* (Craugastoridae) in the final stage (TS 15) prior to hatching (Vera Candiotti et al. 2020), but it is difficult to interpret from the images of cleared-and-stained specimens whether these teeth are erupted and functional. Cumulatively, these observations suggest that odontogenesis does not shift to earlier embryonic stages in direct-developing frogs. The embryo of *G. guentheri* that we examined is likely near the final stage of development prior to hatching (TS 14 or 15) because of the presence of fully developed limbs, toepads, and dorsal pigmentation. If true, tooth development may be further delayed in *G. guentheri* in comparison to what little is known in other direct-developing lineages. The earliest stages of odontogenesis (for-

mation of the primary dental lamina; no mineralization present) may begin shortly before emerging from the maternal pouch. The stage of tooth development between the upper and lower jaws is slightly offset with the dental lamina of the upper jaw being more advanced, forming into a cup shape at its extremity (Davit-Béal et al. 2007).

Little is known of the ecology of *G. guentheri*. The stomach contents of one specimen (KU:KUH:164226) revealed that this species is capable of consuming large prey relative to its body size, suggesting that mandibular dentition may play some role in prey capture (Paluh et al. 2019). The species has also been reported to consume small vertebrates, including frogs and lizards (Arteaga et al. 2013; De la Riva et al. 2020). The shape, hyperossification, and articulation of the skull in *G. guentheri* are similar to other carnivorous frog species that specialize on eating large vertebrate prey, such as *Ceratophrys*, *Pyxicephalus*, *Cornufer guentheri*, and *Hemiphraactus* (Paluh et al. 2020). These frogs are typically sit-and-wait predators that independently evolved bony odontoid fangs and serrations on the lower jaw that are thought to improve prey capture but are not true teeth (Fabrezi and Emerson 2003). Wiens (2011) speculated that the repeated evolution of odontoid fangs in these anurans suggests that selection can favor tooth-like structures on the mandible, but true teeth have not evolved in any of these taxa due to an unspecified developmental constraint. The longstanding question of how *G. guentheri* regained mandibular dentition after more than 200 million years of absence in frogs and their nearest relatives (Boulenger 1882; Noble 1922; Laurent 1983; Wiens et al. 2011) remains challenging to investigate using developmental genetic techniques due to the lack of living specimens and tissue resources for this species.

Recent work has documented that teeth have been completely lost more than 20 times in frogs, which is a higher occurrence of edentulism than in any other major vertebrate lineage

(Paluh et al. 2021). The analyses of Paluh et al. (2021) also indicated as many as six reversals from a complete edentulous to a toothed state and at least one reversal from toothless vomers to toothed vomers in *Hylambates* (formerly *Phlyctimantis*) *leonardi* (Hyperoliidae). Five of the six inferred reversals from toothless to toothed jaws occurred in the Microhylidae. These results suggest that the regain of mandibular teeth in *G. guentheri* is only one of several dental reversals in frogs, although still the only reversal documented on the dentary. The remaining inferred reversals require further anatomical and developmental investigation to confirm that these all represent true teeth and not, for example, small bony serrations that lack the tissue composition of true dentition (Shaw et al. 1989).

Several studies have used comparative methods to identify traits that seem to reject Dollo's law (Whiting et al. 2003; Brandle et al. 2008; Wiens et al. 2011), but few of these phylogenetic patterns have been further scrutinized using integrative approaches to assess the developmental and genetic mechanisms responsible for the evolutionary regain of these traits (Collin and Miglietta 2008). The lack of developmental studies has hindered the interpretation of "re-evolved" traits, especially because many of these structures may only be absent in adult life stages (Sadier et al. 2021). If lost structures are developmentally transient, their gene regulatory networks and developmental pathways are likely retained and provide a mechanism for recovering lost phenotypes (Collin and Miglietta 2008; Kerney et al. 2011; Smith-Paredes et al. 2021). Similarly, Wake et al. (2011) hypothesized that the re-evolution of serially repeated structures in different positions within an organism may be more likely to occur through the retention of ancestral developmental pathways. We hypothesize that a suppressed tooth developmental program may be maintained in the lower jaw of anurans and is disrupted by a conserved mechanism that originated in the ancestor of all frogs. *Triadobatrachus*—the oldest known "stem frog" that lived during the Early Triassic—already lacked teeth on the lower jaw (Ascarunz et al. 2016), and the absence of mandibular dentition is considered a synapomorphy of Salientia (Milner 1988). Presumably this suppression was somehow removed to generate true teeth on the lower jaw in *G. guentheri*. If true, signaling molecules and transient rudimentary structures, such as early thickenings of the oral epithelium that typically gives rise to tooth buds, might be seen before the abortion of tooth development in the mandible of frogs.

The loss of mandibular teeth in anurans may be due to the loss of a single signal that orchestrates odontogenesis and thus arrests tooth formation early in development. This would then be comparable to the loss of odontogenic *Bmp4* expression in birds (Chen et al. 2000) or termination of *Msx2* expression in turtles (Tokita et al. 2013). Alternatively, Wiens (2011) suggested that because most frog species maintain teeth on the premaxilla, max-

illa, and vomer, *G. guentheri* may have re-evolved mandibular teeth by using the developmental machinery used in the formation of upper jaw teeth. If true, the entire odontogenic pathway may have decayed in the lower jaw of all other frogs and thus there may be no early signs of tooth development. Regional tooth loss among different regions of the skull has repeatedly occurred in vertebrates, including in fishes (Aigler et al. 2014), amphibians (Paluh et al. 2021), squamates (Voris 1966), and birds (Brocklehurst and Field 2021). The gene regulatory network that controls tooth initiation, development, and differentiation is likely modular (Stock 2001; Sadier et al. 2020), and this organization may be responsible for shaping this pattern of heterogeneous tooth loss across decoupled jaw regions. Odontogenesis is poorly understood in amphibians, especially when compared to our understanding of tooth development in fishes and amniotes (Fraser et al. 2004; Tucker and Sharpe 2004; Thiery et al. 2017). It is not yet known if all the genes critical for tooth formation in fishes and amniotes are also expressed during the morphogenesis of teeth in amphibians (but see Soukup et al. 2021). Investigating the developmental genetics of tooth formation in the jaws of frogs may provide insights into whether a transient tooth signaling program is present in the lower jaw, providing the possible mechanism underlying the re-evolution of lost mandibular teeth in *G. guentheri*.

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AUTHOR CONTRIBUTIONS

DJP curated the data, performed visualization, and wrote the original draft. DJP, GJF, and DCB conceptualized the idea of the study. DJP and DCB acquired funding. GJF provided resources. All authors performed investigation and reviewed and edited the manuscript.

DATA ARCHIVING

Computed tomography data have been deposited in MorphoSource (see Table S1). All measurement and tooth count data are available in Tables S1 and S2.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

LITERATURE CITED

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Supporting Information

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Figure S1. Ontogenetic variation in pedicel height (left), crown height (center), and pedicel width (right) on the maxilla (row A), premaxilla (row B), vomer (row C), and dentary (row D) in *Gastrotheca guentheri*.

Figure S2. Increase in tooth loci and functional tooth number on the maxilla and dentary (right and left side of each specimen) in *Gastrotheca guentheri* through ontogeny.

Figure S3. Relationship between tooth loci number and log transformed skull length (Fig. 2) with specimen point labels. Corresponding data are provided in Table S1.

Table S1. Species and specimens examined in this study with associated measurement and tooth count (functional teeth and total tooth loci) data.

Table S2. Linear measurement (in mm) and tooth count (functional teeth and total tooth loci) data for *Gastrotheca guentheri* specimens examined.