

Skeletal Morphogenesis of the Vertebral Column of the Miniature Hylid Frog *Acris crepitans*, With Comments on Anomalies

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ABSTRACT Although the vertebral columns of anurans have received much study in the last 150 years, few detailed descriptions exist of the skeletal morphogenesis of this anatomical unit. Herein, the ontogeny of the vertebral skeleton of the hylid frog *Acris crepitans* is described based on cleared and double-stained specimens, radiographs, and 3D reconstructions generated from synchrotron microCT scans. The adult axial formula is 1-7-1-1, and the vertebral centra are epichordal and procoelous. The neural arches are nonimbricate, and there is a medial articulation between the laminae of Presacrals I and II. Free ribs are absent. The sacral diapophyses are uniform in width or slightly expanded distally. The urostyle is slender, round in cross section, and about equal in length to the presacral region. Presacral vertebrae are the first to form, developing in a cephalic-to-caudal sequence. However, development and growth are decoupled and growth is fastest initially in the posterior presacrals and sacrum. In addition, there is a time lag between the formation of the presacral/sacral region and the postsacral region. More than 8.5% of the specimens examined have vertebral anomalies, and about 50% display small variants from the typical vertebral column morphology. However, these malformations do not seem to have been so severe as to have affected survival. *J. Morphol.* 270:52–69, 2009. © 2008 Wiley-Liss, Inc.

KEY WORDS: Anura; Hylidae; *Acris*; vertebral column; skeleton; morphogenesis; anomalies

The anuran vertebral column has attracted the interest of morphologists for more than 150 years. One of the most important contributions to our understanding of the anuran vertebral column is that of Nicholls (1916). His views on the structure of the column (particularly on the morphogenesis of the vertebral centra), and its significance as a basis for classification, generated great interest and stimulated research for decades (e.g., Noble, 1922, 1931; Mookerjee, 1931; Mookerjee and Das, 1939; Ritland, 1955; Griffiths, 1963; Kluge and Farris, 1969; Trueb, 1973). Other aspects of the vertebral column have also been investigated. For example, in leptodactylid frogs Lynch (1969, 1971) defined three types of cervical cotyles, which have been applied extensively to other anurans (e.g., Lynch, 1973; Trueb, 1973; Sanchiz, 1984; Cannatella, 1985; Clarke, 1988; Báez and Basso, 1996).

Noble (1931), Tihen (1960), Lynch (1973), Cannatella (1985), and Cannatella and Trueb (1988a), among others, discussed the fusion of the atlas and second vertebra. The length, orientation, and shape of the transverse processes, and the presence of free ribs in larvae and/or adults have also received wide attention (e.g., Zweifel, 1956; Kluge and Farris, 1969; Lynch, 1971; Cannatella and Trueb, 1988a,b; Clarke, 1988; Duellman and Trueb, 1994; Báez and Basso, 1996; da Silva, 1998; Maglia, 1998; Blanco and Sanchiz, 2000; Pramuk, 2002, 2006; Fabrezi, 2006). In addition, the fusion between the sacrum and urostyle has been regarded as a diagnostic feature of some anurans (Nicholls, 1916; Noble, 1922; Tihen, 1960; Trueb, 1971; Estes and Reig, 1973; Lynch, 1973; Cannatella, 1985; Cannatella and Trueb, 1988a,b; Báez and Trueb, 1997; Báez and Pugener, 2003; Frost et al., 2006).

A survey of the literature reveals that deformities among natural frog populations have been documented since the late 1800s. Interestingly, early reports were concerned almost exclusively with the vertebral column. Among the first contributions are publications by Howes (1886, 1893) and Adolphi (1892, 1895), describing vertebral fusions in pelobatids, bufonids, and ranids. Taylor (1942), Schiromany (1950), Tihen (1959), Holman (1963), Lynch (1965), Madej (1965), and Sanchiz and Perez (1974) described anuran sacral fusions and other vertebral column malformations in a variety of taxa. A more extensive article is that of Trueb (1977), who described intrapopulation variations in the osteology of *Hyla lanciformis*. Her findings suggest that, unlike the skull and limbs,

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the vertebral column seems to be a highly variable architectural unit. Moreover, Trueb (1977) recommended that morphological descriptions and discussions of variability and anomalies should be assessed in light of ontogenetic development.

Acris crepitans, commonly known as Northern Cricket Frogs, are small anurans residing in streams, rivers, and wetlands of the eastern half of the United States and northeastern Mexico (Duellman, 2001; McCallum and Trauth, 2003; Gray et al., 2005a). Despite being members of the tree frog family Hylidae, *A. crepitans* are not arboreal, but rather, are semiaquatic. These frogs have received much attention owing to reports of population declines and malformations (e.g., Greenwell et al., 1996; Brodman and Kilmurry, 1998; Hemesath, 1998; Moriarty, 1998; Hammerson and Livo, 1999; Gray, 2000; Lipps, 2000; Johnson et al., 2001; McCallum and Trauth, 2003; Gray and Brown, 2005; Gray et al., 2005b; and Irwin, 2005, to name a few). Yet, only recently has the adult skeletal anatomy of *A. crepitans* been thoroughly studied (Maglia et al., 2007). Maglia et al. (2007) reported that these miniature anurans exhibit several novel morphologies and a large number of osteological abnormalities, several of which occur in the vertebral column. The skeletal and larval development of *A. crepitans* has yet to be investigated. Herein, we describe the skeletal ontogeny of the vertebral column of *A. crepitans* to understand further the malformations described by Maglia et al. (2007). We compare the normal vertebral development to that of other frogs (and in particular hylids), and we discuss malformations observed in the context of vertebral anomalies known from other species.

MATERIALS AND METHODS

We examined the vertebral columns of 49 premetamorphic and 80 postmetamorphic *A. crepitans* [Duméril and Bibron (1841) (Table 1)]. Specimens from 14 other hylid species were also examined (Table 2). A developmental series of 38 specimens representing Gosner (1960) Stages 32 through adult were staged, measured, eviscerated, and cleared and double-stained for cartilage and bone following methods adapted from Taylor and van Dyke (1985). Additional postmetamorphic and adult specimens were examined using X-rays. The radiographs were taken at The University of Kansas Natural History Museum and Biodiversity Research Center using a Picker Hot Shot TFI 805D radiographic system operating at 30 kV, with exposure times of 90 s.

Measurements of snout–vent length (SVL) were taken using electronic digital calipers, accurate to 0.03 mm, prior to clearing and staining or image processing. Angle of orientation of the transverse processes and sacral diapophyses were measured following the methods of Trueb (1977). When referring to transverse processes/diapophyses, we consider the length to be the medial–lateral extension and the width to be the anterior–posterior extension. Specimens were considered to be adults if they were 20 mm in SVL (the size at sexual maturity reported by Hulse et al., 2001) or larger. Cleared and stained specimens and radiographs were examined with the aid of an Olympus

SZX12 stereo microscope equipped with a camera lucida and a 5-megapixel digital camera.

A three-dimensional reconstruction of the vertebral column of an adult *A. crepitans* was generated using data obtained via synchrotron microCT at the beamline 2-BM of the Advanced Photon Source of the Argonne National Laboratory with the following settings: energy = 13.3 kiloelectron volts (keV); lens = 1.25× objective; and time = 470 ms. (For a detailed explanation of the 2-BM fast microtomography system, please see de Carlo et al., 2006.) A series of 900 slice images was used to generate the reconstruction with the aid of the ImageJ and 3D Doctor© software packages. An interactive version of the 3D image is available for examination in the MorphologyNet web-based library of anatomical reconstructions (www.morphologynet.org; Leopold et al., 2005), under number MN 008. Specimens used in this study are deposited in the herpetological collections of the Natural History Museum and Biodiversity Research Center at The University of Kansas (KU). Thirteen specimens (KU 303230-42) were examined previously in the postmetamorphic osteology study of Maglia et al. (2007).

Vertebrae are designated via Roman numerals in the order they first appear in ontogeny, starting with the first morphological discrete vertebra visible, Presacral I, or the atlas. Vertebra IX is generally referred to as the sacrum. Postsacral vertebrae—when distinguishable as separate elements prior to fusing to form the urostyle—are designated in an anterior-to-posterior sequence using Arabic numerals. The neural arches of all presacral vertebrae, except the atlas, and the sacrum bear laterally oriented processes known as diapophyses, or transverse processes. Here, the term transverse process is used in reference to the processes of the presacral vertebrae, and the term diapophysis is restricted to the processes of the sacrum.

The term coccyx has been used extensively as a synonym of urostyle (e.g., Ritland, 1955; Lynch, 1973; Trueb, 1973; Cannatella, 1985; Cannatella and Trueb, 1988a,b; Clarke, 1988; Wiens, 1989; Duellman and Trueb, 1994; Báez and Basso, 1996; Wild, 1997; Zug et al., 2001; Frost et al., 2006). Coccyx is defined as a small, bony element at the base of the vertebral column, consisting of several fused, rudimentary caudal vertebrae (Pugener, 2002). A coccyx is present in the tail-less apes (Kent, 1987) and in anurans (Pugener, 2002). A comparable osseous structure called pygostyle also occurs in birds (Baumel, 1979). The coccyx of anurans fuses synostotically to an unsegmented ventral structure, the hypochord, to form the urostyle (Mookerjee, 1931; Griffiths, 1963; Branham and List, 1979; Maglia and Pugener, 1998; Pugener, 2002; Ročková and Roček, 2005). Therefore, the coccyx and urostyle are only partially homologous structures, and the use of these terms as synonyms should be avoided.

RESULTS

The notochord and vertebral column are the main longitudinal structural elements of the body that provide support for the head and viscera. The notochord is a rod of fibrous connective tissue surrounding a core of fluid-filled cells that lies dorsal to the digestive system and directly ventral to the spinal cord. The notochord is present during early development in all anurans, but gives way to the vertebral column after metamorphosis.

The vertebral column is a metameric, semiflexible, arched bar located in the dorsal part of the trunk, and is formed by a series of bony vertebrae. The vertebral column provides suspension for the appendicular skeleton and protection for the spinal nerve cord. In anurans, the vertebral column is divided into three regions, namely, presacral, sacral,

TABLE 1. *Acris crepitans* specimens examined

Catalogue no.	Stage	SVL (mm)	Preparation	Locality	Collection date
KU 307866	Gosner L 32	9.43	C&S	Cherokee/KS	July 1977
KU 307867	Gosner L 32	10.03	C&S	Cherokee/KS	July 1977
KU 307868	Gosner 33	9.14	C&S	Cherokee/KS	July 1977
KU 307869	Gosner 33	10.15	C&S	Cherokee/KS	July 1977
KU 307870	Gosner 33	10.48	C&S	Cherokee/KS	July 1977
KU 307871	Gosner 33	10.69	C&S	Cherokee/KS	July 1977
KU 307876	Gosner 33	12.48	C&S	Crawford/KS	July 1975
KU 307872	Gosner L 33	10.83	C&S	Cherokee/KS	July 1977
KU 307873	Gosner L 33	11.27	C&S	Cherokee/KS	July 1977
KU 307877	Gosner E 34	11.62	C&S	Crawford/KS	July 1975
KU 307896	Gosner E 34	13.14	C&S	Pottawatomie/KS	July 1977
KU 307878	Gosner L 34	11.61	C&S	Crawford/KS	July 1975
KU 307879	Gosner 35	13.01	C&S	Crawford/KS	July 1975
KU 307913	Gosner 36	11.11	C&S	Cherokee/KS	July 1977
KU 307880	Gosner 36	13.37	C&S	Crawford/KS	July 1975
KU 307914	Gosner 36	13.91	C&S	Cherokee/KS	July 1977
KU 307881	Gosner E 37	13.28	C&S	Crawford/KS	July 1975
KU 307882	Gosner 37	13.40	C&S	Crawford/KS	July 1975
KU 307883	Gosner L 37	13.81	C&S	Crawford/KS	July 1975
KU 307884	Gosner 38	14.10	C&S	Crawford/KS	July 1975
KU 307885	Gosner 38	14.52	C&S	Crawford/KS	July 1975
KU 307874	Gosner 38	13.31	C&S	Cherokee/KS	July 1977
KU 307898	Gosner L 38	10.91	C&S	Pottawatomie/KS	July 1974
KU 307886	Gosner L 38	13.89	C&S	Crawford/KS	July 1975
KU 307887	Gosner 39	14.48	C&S	Crawford/KS	July 1975
KU 307888	Gosner 39	15.84	C&S	Crawford/KS	July 1975
KU 307897	Gosner E 40	13.92	C&S	Pottawatomie/KS	July 1977
KU 307889	Gosner 40	15.38	C&S	Crawford/KS	July 1975
KU 307890	Gosner 40	14.51	C&S	Crawford/KS	July 1975
KU 307891	Gosner 40	16.77	C&S	Crawford/KS	July 1975
KU 307892	Gosner 41	14.89	C&S	Crawford/KS	July 1975
KU 307875	Gosner 41	16.03	C&S	Cherokee/KS	July 1977
KU 307893	Gosner 41	18.15	C&S	Crawford/KS	July 1975
KU 307900	Gosner 41	14.04	C&S	Bourbon/KS	July 1975
KU 307903	Gosner 42	13.26	C&S	Bourbon/KS	July 1977
KU 307994	Gosner 42	12.75	C&S	Crawford/KS	July 1975
KU 307995	Gosner 42	13.17	C&S	Crawford/KS	July 1975
KU 307902	Gosner L 42	13.73	C&S	Douglas/KS	June 1967
KU 307903	Gosner 43	11.23	C&S	Wabaunsee/KS	July 1975
KU 307901	Gosner 43	12.39	C&S	Bourbon/KS	July 1975
KU 307904	Gosner E 44	11.49	C&S	Cherokee/KS	August 1975
KU 307905	Gosner 44	11.69	C&S	Chase/KS	July 1975
KU 307906	Gosner 44	12.22	C&S	Chase/KS	July 1975
KU 307909	Gosner 44	12.53	C&S	Chase/KS	July 1975
KU 307907	Gosner E 45	14.03	C&S	Chase/KS	July 1975
KU 307908	Gosner 45	13.02	C&S	Chase/KS	July 1975
KU 307910	Gosner 45	12.79	C&S	Chase/KS	July 1975
KU 307899	Gosner 46	12.27	C&S	Pottawatomie/KS	July 1974
KU 307911	Gosner 46	13.36	C&S	Douglas/KS	July 1975
KU 307930	Juvenile	17.31	C&S	Maries/MO	June 2005
KU 307931	Juvenile	19.24	C&S	Maries/MO	June 2005
KU 307932	Juvenile	19.54	C&S	Maries/MO	June 2005
KU 307933	Juvenile	16.11	C&S	Maries/MO	June 2005
KU 307934	Juvenile	17.95	C&S	Maries/MO	June 2005
KU 307935	Juvenile	18.19	C&S	Maries/MO	June 2005
KU 307936	Juvenile	13.11	C&S	Maries/MO	June 2005
KU 307937	Juvenile	12.73	C&S	Maries/MO	June 2005
KU 307938	Juvenile	13.24	C&S	Maries/MO	June 2005
KU 307939	Juvenile	12.50	C&S	Maries/MO	June 2005
KU 307940	Juvenile	15.55	C&S	Maries/MO	June 2005
KU 307941	Juvenile	13.32	C&S	Maries/MO	June 2005
KU 307942	Juvenile	17.71	C&S	Maries/MO	June 2005
KU 307943	Juvenile	19.38	C&S	Maries/MO	June 2005
KU 303233	Juvenile	19.97	C&S	Phelps/MO	April 2003
KU 303236	Juvenile	16.98	C&S	Phelps/MO	April 2003
KU 303241	Juvenile	19.08	C&S	Phelps/MO	April 2003
KU 303231	Juvenile	18.45	C&S	Phelps/MO	April 2003
KU 158223	Juvenile	18.24	X-ray	Cherokee/KS	October 1974
KU 170957	Juvenile	14.49	X-ray	Crawford/KS	July 1976

TABLE 1. (Continued)

Catalogue no.	Stage	SVL (mm)	Preparation	Locality	Collection date
KU 13534	Juvenile	19.19	X-ray	Ottawa/OK	April 1929
KU 13537	Juvenile	18.79	X-ray	Ottawa/OK	April 1929
KU 303230	Adult	21.22	C&S	Phelps/MO	April 2003
KU 303232	Adult	20.68	C&S	Phelps/MO	April 2003
KU 303234	Adult	22.19	C&S	Phelps/MO	April 2003
KU 303235	Adult	22.43	C&S	Phelps/MO	April 2003
KU 303237	Adult	24.12	C&S	Phelps/MO	April 2003
KU 303238	Adult	20.41	C&S	Phelps/MO	April 2003
KU 303239	Adult	25.19	C&S	Phelps/MO	April 2003
KU 303240	Adult	21.50	C&S	Phelps/MO	April 2003
KU 303242	Adult	21.00	C&S	Phelps/MO	April 2003
KU 307944	Adult	25.53	C&S	Maries/MO	June 2005
KU 307945	Adult	25.36	C&S	Maries/MO	June 2005
KU 307946	Adult	23.19	C&S	Maries/MO	June 2005
KU 307947	Adult	27.37	C&S	Maries/MO	June 2005
KU 307948	Adult	20.95	C&S	Maries/MO	June 2005
KU 307949	Adult	21.51	C&S	Maries/MO	June 2005
KU 307950	Adult	20.90	C&S	Maries/MO	June 2005
KU 307951	Adult	21.69	C&S	Maries/MO	June 2005
KU 307952	Adult	20.58	C&S	Maries/MO	June 2005
KU 307953	Adult	20.89	C&S	Maries/MO	June 2005
KU 307954	Adult	22.41	C&S	Maries/MO	June 2005
KU 307955	Adult	21.28	C&S	Maries/MO	June 2005
MN 008	Adult	—	CT scan	Maries/MO	June 2005
KU 84689	Adult	25.62	X-ray	Jasper/MO	May 1963
KU 84690	Adult	24.51	X-ray	Jasper/MO	May 1963
KU 84691	Adult	27.93	X-ray	Jasper/MO	May 1963
KU 158091	Adult	23.56	X-ray	Crawford/KS	March 1974
KU 158092	Adult	21.69	X-ray	Crawford/KS	March 1974
KU 158093	Adult	21.40	X-ray	Cherokee/KS	March 1974
KU 158095	Adult	20.89	X-ray	Linn/KS	March 1974
KU 158096	Adult	23.55	X-ray	Linn/KS	March 1974
KU 158246	Adult	24.21	X-ray	Crawford/KS	May 1975
KU 158247	Adult	27.06	X-ray	Crawford/KS	May 1975
KU 158248	Adult	31.05	X-ray	Crawford/KS	May 1975
KU 158249	Adult	25.62	X-ray	Crawford/KS	May 1975
KU 158250	Adult	23.04	X-ray	Crawford/KS	May 1975
KU 158251	Adult	26.17	X-ray	Crawford/KS	May 1975
KU 158252	Adult	27.89	X-ray	Crawford/KS	May 1975
KU 170956	Adult	30.72	X-ray	Crawford/KS	July 1976
KU 170958	Adult	28.72	X-ray	Crawford/KS	July 1976
KU 174837	Adult	30.51	X-ray	Labette/KS	April 1976
KU 38912	Adult	25.78	X-ray	Jasper/MO	April 1955
KU 13526	Adult	21.26	X-ray	Ottawa/OK	April 1929
KU 13527	Adult	26.04	X-ray	Ottawa/OK	April 1929
KU 158111	Adult	28.42	X-ray	Douglas/KS	April 1974
KU 158112	Adult	25.55	X-ray	Douglas/KS	April 1974
KU 158132	Adult	24.53	X-ray	Douglas/KS	April 1974
KU 158176	Adult	25.49	X-ray	Pottawatomie/KS	July 1974
KU 158119	Adult	21.23	X-ray	Douglas/KS	October 1974
KU 158206	Adult	22.24	X-ray	Douglas/KS	October 1974
KU 158216	Adult	25.51	X-ray	Douglas/KS	October 1974
KU 170825	Adult	30.42	X-ray	Pottawatomie/KS	June 1975
KU 170834	Adult	26.61	X-ray	Pottawatomie/KS	June 1975
KU 170915	Adult	29.68	X-ray	Pottawatomie/KS	June 1976
KU 170921	Adult	25.21	X-ray	Douglas/KS	June 1976
KU 170941	Adult	26.05	X-ray	Douglas/KS	June 1976
KU 187701	Adult	30.05	X-ray	Pottawatomie/KS	June 1977
KU 187703	Adult	29.33	X-ray	Pottawatomie/KS	June 1977
KU 294953	Adult	25.58	X-ray	Douglas/KS	May 1974

C&S, clear and stained; E, early; KS, Kansas; KU, Natural History Museum and Biodiversity Research Center, The University of Kansas, USA; L, late; MO, Missouri; OK, Oklahoma; SVL, snout-vent length. Locality information includes county and state.

and postsacral. In *A. crepitans*, the presacral region consists of eight discrete vertebrae; the sacral region is composed of a single vertebra, and the postsacral region is formed by the urostyle.

A typical anuran presacral vertebra consists of two essential parts, a dorsal neural arch and a ventral centrum (see Fig. 1). The neural arch is formed by a pair of pedicels—which form first dur-

TABLE 2. *Hylid specimens examined*

Species name	Catalogue no.	Stage
<i>Hyla alboguttata</i>	KU 104418	Adult
<i>H. alboguttata</i>	KU 104419	Adult
<i>H. armata</i>	KU 163334	Adult
<i>H. lanciformis</i>	KU 205977	Gosner 26
<i>H. lanciformis</i>	KU205982	Gosner 33
<i>H. lanciformis</i>	KU 205984	Gosner 37
<i>H. lanciformis</i>	KU 205965	Gosner 38
<i>H. lanciformis</i>	KU 205967	Gosner 39
<i>H. lanciformis</i>	KU 205969	Gosner 41
<i>H. lanciformis</i>	KU 205971	Gosner 42
<i>H. lanciformis</i>	KU 205973	Larva
<i>H. lanciformis</i>	KU 99301	Juvenile
<i>H. lanciformis</i>	KU 124175	Juvenile
<i>H. lanciformis</i>	KU 126411	Juvenile
<i>H. lanciformis</i>	KU 205960	Juvenile
<i>H. lanciformis</i>	KU 205961	Juvenile
<i>H. lanciformis</i>	KU 205962	Juvenile
<i>H. lanciformis</i>	KU 205963	Juvenile
<i>H. lanciformis</i>	KU 205964	Juvenile
<i>H. lanciformis</i>	KU 104910	Adult
<i>H. lanciformis</i>	KU 104913	Adult
<i>H. lanciformis</i>	KU 104914	Adult
<i>H. lanciformis</i>	KU 99300	Adult
<i>H. lanciformis</i>	KU 99299	Adult
<i>H. minuscula</i>	KU 127820	Adult
<i>H. minuscula</i>	KU 127821	Adult
<i>H. minuta</i>	KU 122915	Adult
<i>H. minuta</i>	KU 182928	Adult
<i>Osteopilus septentrionalis</i>	KU 84661	Adult
<i>Pachymedusa dacnicolor</i>	KU 78454	Adult
<i>Trachycephalus coriacea</i>	KU 207658	Adult
<i>Phyllomedusa lemur</i>	KU 31707	Adult
<i>P. lemur</i>	KU 68627	Adult
<i>P. lemur</i>	KU 68628	Adult
<i>P. lemur</i>	KU 101815	Adult
<i>Plectrohyla glandulosa</i>	KU 209699	Adult
<i>P. matudai</i>	KU 59948	Adult
<i>Pseudacris crucifer</i>	KU 84668	Adult
<i>P. crucifer</i>	KU 207433	Adult
<i>P. ocularis</i>	KU 291049	Gosner 46
<i>P. ocularis</i>	KU 291052	Adult
<i>P. triseriata</i>	KU 201017	Gosner 46
<i>P. triseriata</i>	KU 291021	Gosner 46
<i>P. triseriata</i>	KU 98318	Adult
<i>P. triseriata</i>	KU 207491	Adult
<i>P. triseriata</i>	KU 207492	Adult

U, Natural History Museum and Biodiversity Research Center, The University of Kansas, USA.

ing development—and a pair of laminae. The anterior and posterior ends of each neural arch pedicel are indented to form anterior and posterior intervertebral notches; thus, when two vertebrae articulate, the anterior notch of one vertebra and the posterior notch of the other create a bilateral intervertebral space. These spaces accommodate the spinal nerves as they emerge from the spinal canal. The neural arch laminae cover the spinal cord dorsally, thereby enclosing the neural canal. The lateral margin of each lamina rests dorsal to the superior end of the pedicel, and the medial end fuses to the medial end of the opposite lamina. Usually, in the area of contact of both laminae, a neural spine develops.

Each vertebra possesses six apophyses, four zygapophyses and two transverse processes. The zygapophyses consist of paired processes at the cephalic (=prezygapophyses) and caudal (=postzygapophyses) ends of the neural arch. The articular facets of the prezygapophyses of one vertebra face dorsomedially and articulate with the lateroventrally facing facets of the postzygapophyses of the preceding vertebra. Zygapophyses function as interlocking structures between adjacent vertebrae, and limit dorsoventral flexion and lateral movement of the column in the trunk region. Transverse processes project on each side from the point where the lamina joins the pedicel. The processes of Presacrals II–IV are longer than those of the last four presacral vertebrae, and provide surfaces for the attachment of the muscles that originate mainly from the head, the scapula, and the suprascapula. The transverse processes of Presacrals V–VIII are subequal in length and serve mainly for the attachment of muscles from the caudal region of the vertebral column.

The vertebral bodies, or centra, functionally replace the notochord. In *A. crepitans*, the vertebral centra are procoelous and epichordal. Procoelous defines a centrum that is concave at the anterior end. A procoelous centrum bears a condyle on the posterior end for the articulation with the posterior adjacent vertebral centrum, forming a ball-and-socket joint that allows extensive motion in most directions. Epichordal defines a centrum that originates from ossification of the dorsal portion of the perichordal tube, as opposed to ossification of the entire perichordal tube (=perichordal condition).

The atlas and sacral vertebra are similar, for the most part, to a typical presacral vertebra. The

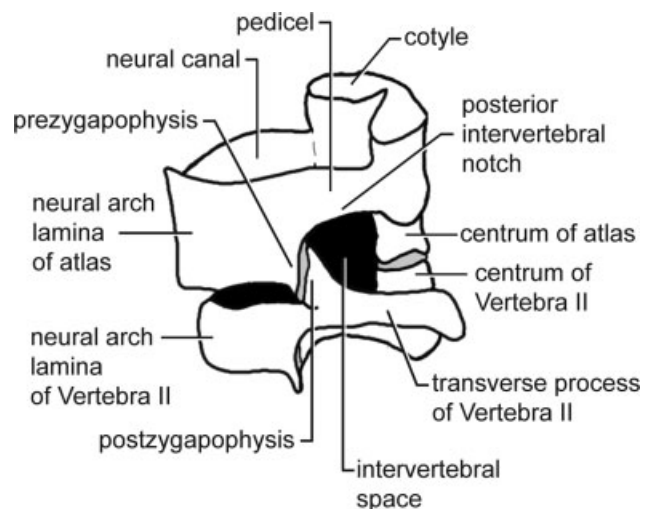


Fig. 1. Schematic representation of Presacral Vertebrae I and II of anurans, lateral view. Gray denotes cartilage; white denotes bone.

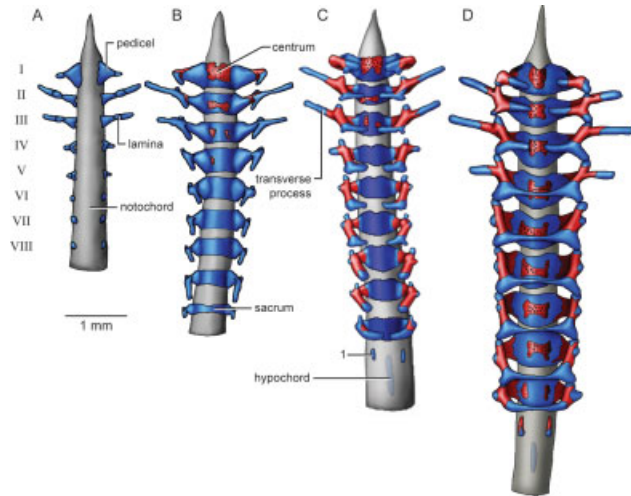


Fig. 2. Ontogeny of the vertebral column of *Acris crepitans*, dorsal views. (A) Late Gosner Stage 32 (KU 307866), (B) Late Gosner Stage 34 (KU 307878), (C) Gosner Stage 36 (KU 307914), (D) Gosner Stage 37 (307882). Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; 1, Postsacral Vertebra 1.

atlas is distinguished by lacking transverse processes and prezygapophyses, and possessing anterior, cervical cotyles for the articulation with the occipital condyles. The sacral vertebra has sacral diapophyses (i.e., transverse processes modified for the support of the pelvic girdle), two posterior condyles (only one in some species) for the articulation with the urostyle, and lacks postzygapophyses. Posterior to the sacral vertebra, anurans possess a single, rod-like caudal element, the urostyle, which lies within a compartment formed by the elongated, anteriorly oriented ilia (=one of the three pairs of elements that constitute the anuran pelvic girdle). The function of the urostyle is to provide an intrapelvic mechanism for transmitting hindlimb generated forces to the axial skeleton (Shubin and Jenkins, 1995).

Premetamorphic Morphogenesis

At Gosner Stage 32, the earliest stage examined, the notochord is well developed and tapered at both ends; from slightly posterior to the level of the anterior margin of the otic capsules, at the base of the chondrocranium, the notochord extends to the tip of the tail. At this stage, the perichordal tube around the notochord seems to be made entirely of fibrous connective tissue. The components of the vertebral column include seven (e.g., KU 307867) or eight (e.g., KU 307866) pairs of neural arch pedicels that develop dorsolaterally to the notochord (Fig. 2A). The base of each atlas pedicel is about three times as wide as the pedicel of the other vertebrae. The pedicels of the first five pairs of vertebrae possess small oval neural arch

laminae that are restricted to their dorsal margins. Presacrals II and III also have incipient, anteriorly oriented transverse processes with round distal termini. The transverse processes of Presacral II are about one-third longer than those of Presacral III. The extent of development of the presacral vertebrae of some Stage-33 specimens (e.g., KU 307868) is similar to that of Stage 32. In most individuals, the sacrum is represented by a pair of short, laterally oriented, cone-shaped pedicels posterior to Presacral VIII, and the perichordal tube is chondrified dorsally in the area between the neural arch pedicels of the presacral vertebrae, forming rudimentary cartilaginous centra. By late Stage 33, the neural arch laminae of all vertebrae, including the sacrum, are present and slightly expanded posteromedially.

By Gosner Stage 34, osteogenesis has begun (Figs. 2B, 3A). The first indication of ossification is present in one early Stage-34 specimen (KU 307877), in which a pair of minute bony centers is visible on the cartilaginous centrum of the atlas, near the bases of the neural arch pedicels. By late Stage 34, these centers of ossification have fused medially. The cartilaginous precursor of Presacral II also has a rudimentary centrum consisting of two centers of ossification that are fused at the midline. In KU 307878, in addition to the coalesced central ossifications of Presacrals I and II, the developing centra of Presacrals III and IV also possess paired centers of ossification. In all specimens, ossification also occurs in the dorsal half of the pedicels of the atlas and in the pedicels of Postsacral II underneath their transverse processes. All presacrals, except the atlas and the sacrum, possess small prezygapophyses. By Gosner Stage 35, the neural arch laminae of the first four

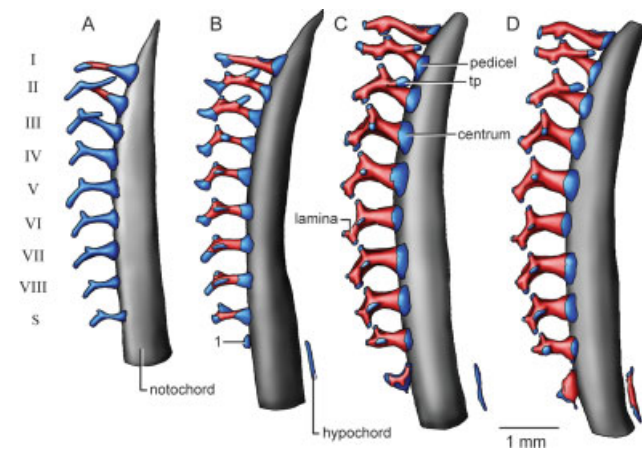


Fig. 3. Ontogeny of the vertebral column of *Acris crepitans*, lateral views. (A) Late Gosner Stage 34 (KU 307878), (B) Gosner Stage 36 (KU 307914), (C) Late Gosner Stage 38 (KU 307886), (D) Gosner Stage 40 (KU 307890). Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; 1, Postsacral Vertebra 1; S, sacrum; tp, transverse process.

presacrals are expanded medially; growth of the laminae is more significant in Presacrals II–IV than in the atlas. The transverse processes of Presacral II remain about one-third longer than those of Presacral III.

Stage 36 is characterized by the development of Postsacral 1 and the hypochord, and by a significant increase in the amount of ossification of the neural arches (Figs. 2C, 3B). Postsacral 1 is present in all Stage-36 specimens examined, and emerges as small, paired neural arch pedicels posterior to the sacrum. The hypochord is present only in some of the specimens examined (e.g., KU 307914), and is first seen as a thin sliver of cartilage about one-third longer than the length of the atlantal centrum. The hypochord originates parallel to, and beneath, the notochord, although not in direct contact with it. The anterior end of the hypochord lies at the level of the neural arches of Postsacral 1. The neural arches of all presacral vertebrae and the sacrum are ossified. Ossification extends from the upper portion of each neural arch pedicel to the lateral aspect of the lamina, being more widespread in the atlas and less extensive in the sacrum. Each atlantal neural arch pedicel has a thin, but well-developed, cartilaginous cervical cotyle that occupies slightly less than half of the anterior margin of the pedicel. Presacrals I–VI bear small postzygapophyses, although they still do not articulate with the prezygapophyses. The transverse processes of Presacrals II and III are about equal in length, and their proximal portions are ossified. In addition, in some specimens (e.g., KU 307880, KU 307913) Presacrals IV–VI have small transverse processes, whereas in other specimens (e.g., KU 186096) the transverse processes are present in all presacral vertebrae. In all cases, the newly developed transverse processes are knob-like in dorsal view and dorsoventrally elongate in lateral view; the transverse processes of Presacral IV are slightly more developed than those of the posterior vertebrae. The anterior and posterior margins of the cartilaginous vertebral centra are clearly concave and convex, respectively. The degree of ossification of the centra varies among specimens; in some, such as in KU 307913, the centra are ossified in all presacral vertebrae and the sacrum, whereas in others (e.g., KU 307914) only the centra of the first three presacral vertebrae are ossified.

By Stage 37, the neural arch laminae of Presacral IV–sacrum fuse at the midline (Fig. 2D). The transverse processes of Presacral III have lengthened, and are about one-third longer than the transverse processes of Presacral II. The transverse processes of Presacral IV also are longer—slightly shorter than that of the transverse processes of Presacral II—and their proximal ends are ossified. The transverse processes of Presacrals V–VIII remain small and knob-like, and ossification

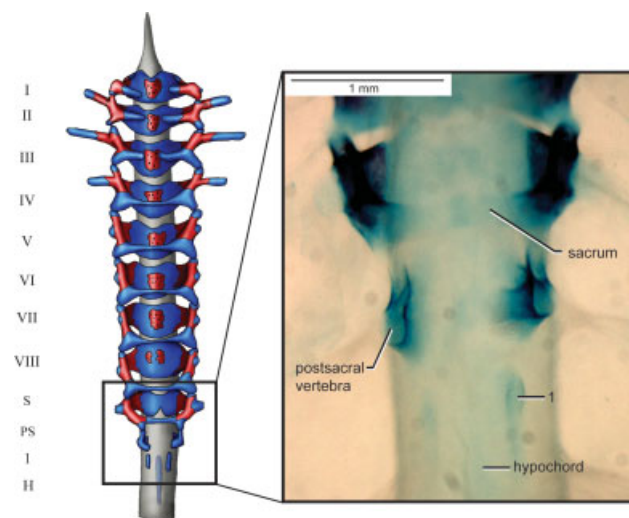


Fig. 4. Dorsal view of the vertebral column of late Gosner Stage 37 (KU 307883) *Acris crepitans* and (inset) close-up photograph of the sacral and postsacral regions. The specimen exhibits a small vertebra posterior to the sacrum that is morphologically similar to the presacral vertebrae. It bears a pair of well-developed neural arch pedicels and a pair of prezygapophyses that articulate with a pair of postzygapophyses present in the sacrum. Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; 1, Postsacral Vertebra 1; H, hypochord; PS, anomalous postsacral vertebra; S, sacrum.

has invaded the bases of the transverse processes of Presacrals II–IV. The sacrum bears a pair of small, round diapophyses. In KU 307882, the neural arches of Postsacral 1 show ossification, whereas in KU 307881, they are completely cartilaginous. The hypochord is small—about the same length as the centrum of the atlas—and located posterior to the pedicels of Postsacral 1 in KU 307882; the hypochord is absent in KU 307881. In KU 307883, a late Gosner Stage-37 specimen, there is a small vertebra posterior to the sacrum that is morphologically similar to the presacral vertebrae. This postsacral vertebra bears a chondrified centrum, a pair of well-developed neural arches, a medially fused neural arch lamina, and a pair of prezygapophyses that articulate with a pair of postzygapophyses present on the sacrum (see Fig. 4). Posterior to this vertebra, there is a pair of neural arch pedicels similar to those of Postsacral 1 of other specimens. The hypochord is about two-thirds longer than the centrum of the atlas, and its anterior end lies at the level of the anterior margin of the neural arch pedicels of the second postsacral vertebra.

By Stage 38, bony plates are developing at the anterolateral corners of the neural arch lamina of Presacrals III–VIII and the anterior and posterior edges of the neural arch pedicels of most vertebrae (Fig. 3C). The ossified contralateral halves of the neural arch laminae of all vertebrae, except Postsacral 1, are joined at the midline by a strip of car-

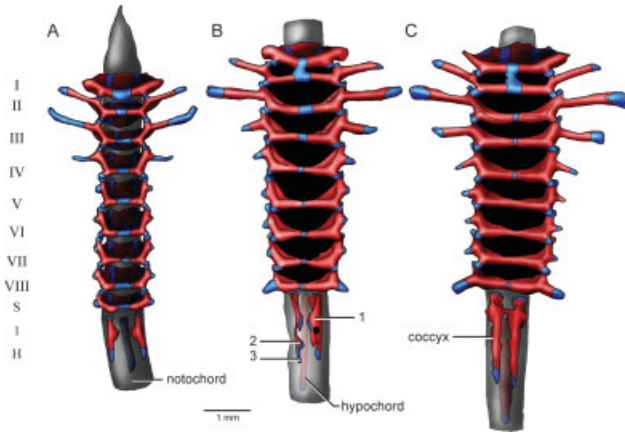


Fig. 5. Ontogeny of the vertebral column of *Acris crepitans*, dorsal views. (A) Early Gosner Stage 40 (KU 307897), (B) Gosner Stage 41 (KU 307892), (C) Late Gosner Stage 42 (KU 307902). Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; 1, Postsacral Vertebra 1; 2, Postsacral Vertebra 2; 3, Postsacral Vertebra 3; H, hypochord; S, sacrum.

tilage. The prezygapophyses and postzygapophyses are cartilaginous, but articulate with each other, thereby providing more stability to the developing vertebral column. The distal ends of the transverse processes of Presacrals II and III are slightly expanded distally. In some Stage-38 and in all Stage-39 specimens, the hypochord has begun to ossify at the midline. The right and left counterparts of Postsacral 1 remain dorsally and ventrally separated, but each side has an anterior cartilaginous cotyle. In one late Gosner Stage-38 specimen (KU 307886), the central portion of the neural arch lamina of Presacral IV has failed to form.

In tadpoles of Gosner Stage 40, all the main components of the vertebral column are present (Figs. 5A, 3D). The neural arch pedicels of Postsacral 1 are expanded at their posterior end, indicating the emergence of the neural arch pedicels of Postsacral 2. The posteroventral margins of the neural arch pedicels of the sacrum and adjacent areas of the sacral centrum exhibit newly developed cartilaginous condyles. At Stage 41, both halves of Postsacral 2 are well developed (Fig. 5B). The boundary between the neural arch pedicels of Postsacral 1 and 2 is indicated by the presence of a large foramen. Additional cartilaginous growth of the posterior margin of Postsacral 2 suggests the development of a third pair of postsacral neural arch pedicels. Thus, by this stage, the right and left counterparts of a coccyx are present. The hypochord is completely ossified, except for its cartilaginous posterior terminus, and is ~50% the length of the presacral portion of the vertebral column. The neural arch pedicels of all vertebrae are completely ossified. The transverse processes of Presacral III are larger than in previous stages, and thus, are the most robust processes of the pre-

sacral vertebrae. The transverse processes of Presacral V are about half the length of the transverse processes of Presacral IV, are posterolaterally oriented, and their proximal ends are ossified. The sacral diapophyses are narrower at their distal termini than at their bases, and are about one-third the length of the transverse processes of Presacral III. The midsection of the posterior margin of the neural arch lamina of the atlas is fused to the central portion of the anterior margin of Presacral II. In one Stage-41 specimen (KU 307875), there is a postzygapophysis on the right side of the sacrum and a prezygapophysis on the corresponding right half of the coccyx; articulation does not occur.

The most significant feature of Gosner Stage 42 is the onset of regression of the notochord. This process is evident particularly at the posterior end of the notochord, which exhibits a large number of small creases and grooves. In addition, the moniliiform shape of the notochord, caused by the pressure exerted by the developing vertebrae on the perichordal tube and notochord, is noticeable (Fig. 6A). The degree of development of the vertebral column, in contrast, is similar to that of Stage-41 larvae (Fig. 5C). The cartilaginous end of each transverse process of Presacral III has a small expansion at the posterolateral corner. The sacral diapophyses are uniform in width, and ~1.5 times the width at the proximal end and 50% the length of the transverse processes of Presacral III. Both halves of Postsacral 3 are relatively well developed, and the boundary between the neural-arch pedicels of Postsacral 2 and 3 is indicated by the presence of a small foramen. The hypochord is about 60% the length of the presacral portion of the vertebral column, and its anterior terminus

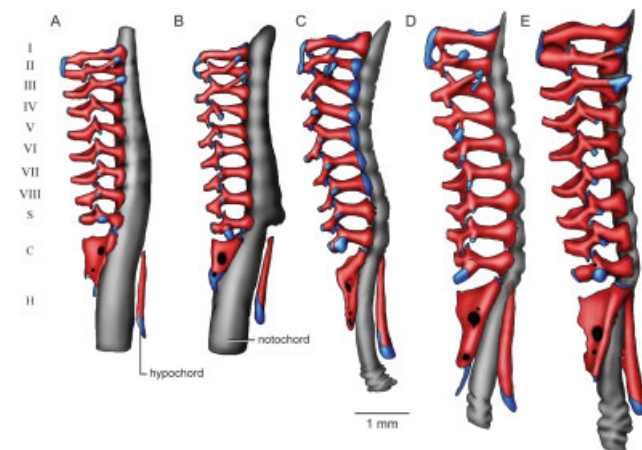


Fig. 6. Ontogeny of the vertebral column of *Acris crepitans*, lateral views. (A) Late Gosner Stage 42 (KU 307902), (B) Gosner Stage 43 (KU 307901), (C) Early Gosner Stage 44 (KU 307904), (D) Gosner Stage 44 (KU 307906), (E) Early Gosner Stage 45 (KU 307907). Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; C, coccyx; H, hypochord; S, sacrum.

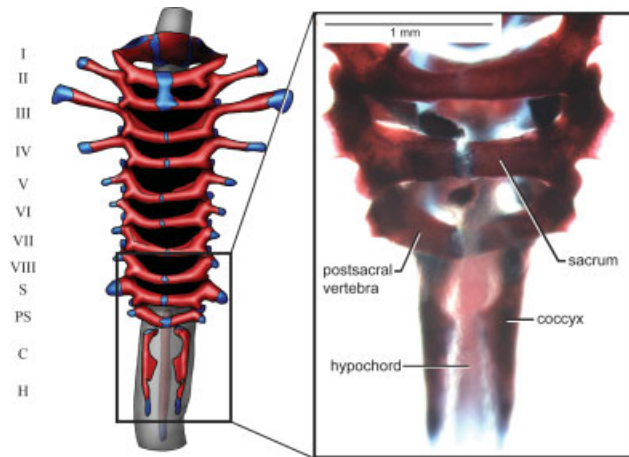


Fig. 7. Dorsal view of the vertebral column of Gosner Stage 42 *Acris crepitans* (KU 307903) and (inset) close-up photograph of the sacral and postsacral regions. The specimen exhibits a vertebral element posterior to the sacrum that bears a pair of well-developed neural arches, a medially fused neural arch lamina, a pair of prezygapophyses that articulate with the sacral postzygapophyses, a pair of posteriorly directed transverse processes, and an ossified centrum. Anteriorly, it articulates with the sacral condyles and posteriorly with the coccygeal cotyles. Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; C, coccyx; H, hypochord; PS, anomalous postsacral vertebra; S, sacrum.

lies at the level of the neural arch pedicels of Postsacral 1. In KU 307903, there is a vertebra posterior to the sacrum that, although small, has a morphology closely resembling that of the presacral vertebrae (see Fig. 7). This postsacral vertebra bears a pair of well-developed neural arches, a medially fused neural arch lamina, a pair of prezygapophyses that articulate with a pair of postzygapophyses present on the sacrum, a pair of posteriorly directed transverse processes, and an ossified centrum. The anterior margin of the centrum has a pair of cotyles that articulate with the condyles of the sacral centrum, and the posterior margin bears a pair of condyles that articulate with the coccygeal cotyles. Posterior to this vertebra, the contralateral halves of the coccyx resemble those of other Stage-41 and 42 specimens (i.e., in being formed by the fusion of three pairs of neural arch pedicels). The hypochord is about 60% the length of the presacral portion of the vertebral column and its anterior terminus lies at the level of the centrum of the first postsacral vertebra, immediately posterior to the sacrum.

Degeneration of the notochord continues during Stages 43 and 44, particularly at the level of the anterior end of the hypochord, which has migrated dorsally to constrict the notochord (Fig. 6B–D). The moniliform shape of the notochord becomes more conspicuous. The distal ends of the transverse processes of Presacral III continue to expand posteriorly, and the distal ends of the transverse

processes of Presacral IV show signs of posterior enlargement in some Stage-43 and all Stage-44 specimens. The transverse processes of the last three presacral vertebrae are ossified at their base, but the distal ends remain cartilaginous and round. These processes have grown laterally to a length almost equal to that of the transverse processes of Presacral V, which in turn are about one-third shorter than the transverse processes of Presacral IV. At Stage 43, the transverse processes of Presacral VI and VIII are oriented posterolaterally and those of Presacral VII are oriented laterally. Although the transverse processes of Presacrals VI and VII maintain the same orientation, by Stage 44, those of Presacral VIII are perpendicular to the longitudinal axis of the vertebral column.

At Gosner Stage 45, the anterior end of the hypochord joins the anterior end of the coccyx (Fig. 6E). The notochord is considerably shortened at both ends, and the moniliform shape is most pronounced. At the end of metamorphosis (Gosner Stage 46), the notochord is eroded completely and the anterior half of the coccyx is fused to the underlying hypochord, thereby forming the urostyle (Figs. 8A, 9A). The urostyle is more than 60% the length of the presacral portion of the vertebral column. The transverse processes of Presacrals V–VIII are about equal in length to the transverse processes of Presacrals II and IV, and their cartilaginous distal ends are acuminate. In addition, the transverse processes of Presacral VIII are oriented anterolaterally and are curved. The sacral diapophyses are about 60% the length of the transverse processes of Presacral III, and a cartilaginous sesamoid element is present lateral to the distal terminus of each diapophysis.

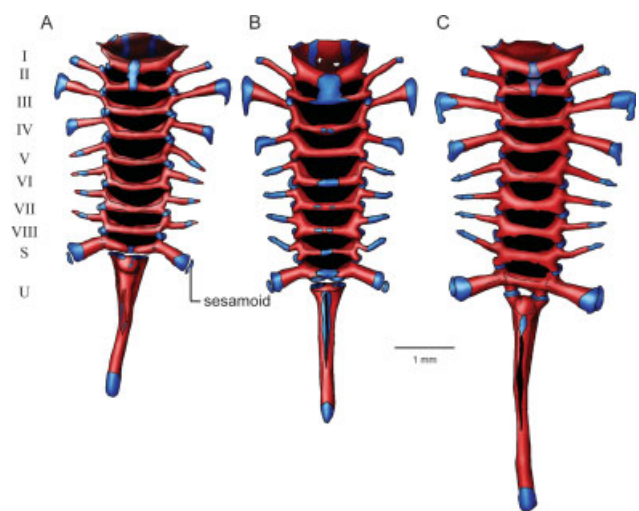


Fig. 8. Ontogeny of the vertebral column of *Acris crepitans*, dorsal views. (A) Gosner Stage 46 (KU 307911), (B) 13.24-mm SVL juvenile (KU 307938), (C) 17.95-mm SVL juvenile (KU 307934). Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; S, sacrum.

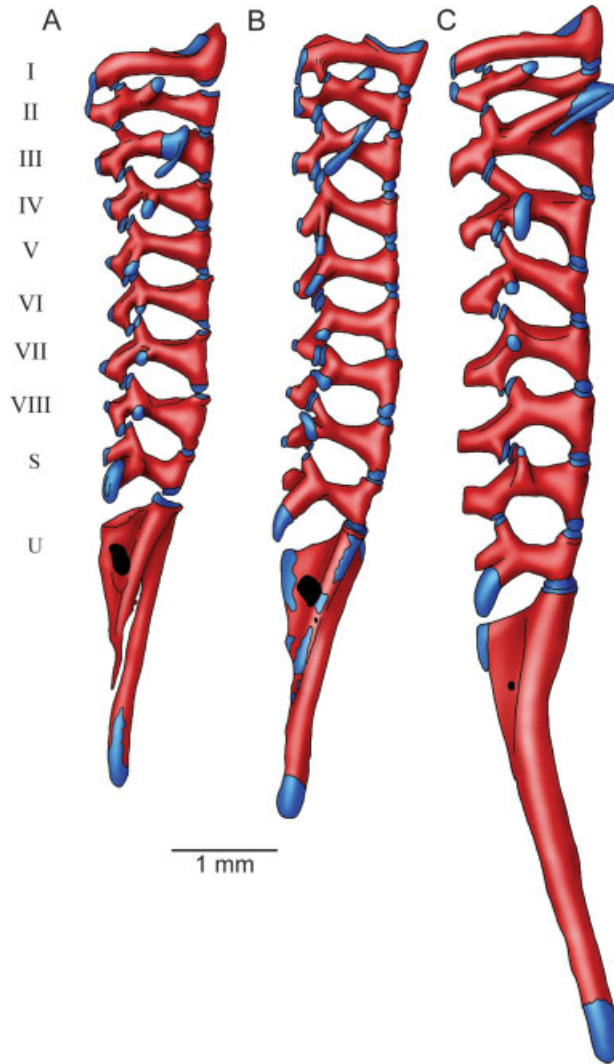


Fig. 9. Ontogeny of the vertebral column of *Acris crepitans*, lateral views. (A) Gosner Stage 46 (KU 307911), (B) 13.24-mm SVL juvenile (KU 307938), (C) 17.95-mm SVL juvenile (KU 307934). Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; S, sacrum; U, urostyle.

Postmetamorphic Morphogenesis

In young postmetamorphic specimens of about 13-mm SVL, the face or articular facet of each prezygapophysis and postzygapophysis is cartilaginous, whereas the opposite side of the face or counterface is ossified (Figs. 8B, 9B). By this stage, the distal ends of the transverse processes of the last four presacral vertebrae have developed small tear-shaped expansions. The area of contact between the neural arch pedicels and the vertebral centra is cartilaginous. The coccyx is completely fused to the hypochord, but a suture on either side is clearly discernible. The combined neural arches of the postsacral vertebrae form a prominent, longitudinal ridge on the anterodorsal aspect of the urostyle; the dorsal ends of the neural arches,

however, are not fused at the midline. A pair of large spinal nerve foramina pierces the lateral sides of the urostyle at the base of the longitudinal ridge, close to the anterior margin of the urostyle. The second pair of spinal nerve foramina is no longer present.

The configuration of the vertebral column in juveniles of ~18-mm SVL (Figs. 8C, 9C) is similar to that of the adult (see Fig. 10). The cervical cotyles are Type IB of Pugener (2002). They are widely separated, with the intercotylar distance being greater than the length of the long axis of the oval articular facet (see Fig. 11). The articular facets of the cervical cotyles are ossified in all specimens larger than 19-mm SVL and the prezygapophyses and postzygapophyses are ossified in all specimens larger than 20-mm SVL, except KU 307944. The centrum of the atlas is shorter than the centra of the posterior vertebrae. Anterior to the centrum, the intercotylar space is covered by a thin lamina with a slightly convex anterior margin. The neural arch laminae of all vertebrae, except Presacrals I and II, are completely ossified. The laminae of Presacrals I and II, in contrast, are cartilaginous at the midline, although some mineral deposits are visible; they remain cartilaginous even in the largest adult specimen examined, although they do become heavily mineralized. The synchondrotic fusion between both vertebrae, however, is replaced by an articular union. The neural canal is round in cross section.

None of the presacral vertebrae is fused and they are all about equal in overall size. The width of the vertebral centra, in contrast, decreases

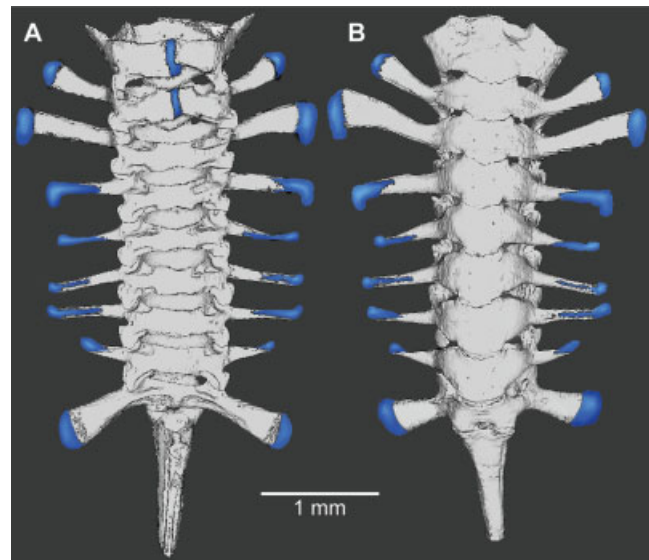


Fig. 10. Three-dimensional visualization of the vertebral column of the adult *Acris crepitans* (MN 008). (A) Dorsal view, (B) Ventral view. Blue denotes cartilage; gray denotes bone. The posterior half of the urostyle is not depicted.

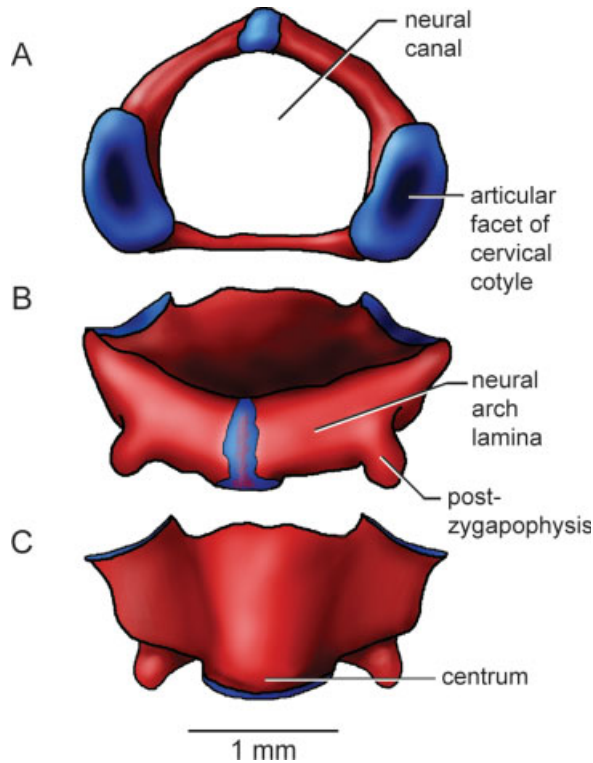


Fig. 11. Atlas of the adult *Acris crepitans* (KU 307950). (A) Anterior view, (B) Dorsal view, (C) Ventral view.

gradually toward the fourth or fifth vertebra (depending on the specimen), and increases slightly toward the posterior end of the presacral region. The neural arches are nonimbricate (non-overlapping), even in the largest specimens examined; therefore, relatively large portions of the spinal cord are exposed dorsally between the vertebrae. Presacral vertebrae are slightly larger toward the caudal end of the presacral region. Neither the neural arches nor the vertebral centra of any of the vertebrae are ornamented; neural spines are absent. In one specimen (KU 303239), the neural arches of Presacral III are not fused at the midline. Normally, the anterior, concave articular facets of the vertebral centra ossify prior to the posterior, convex facets. However, a few specimens in our sample do not follow the pattern. For example, in KU 307947 (27.37-mm SVL), both ends of the vertebral centra are cartilaginous, whereas in KU 307945 and 307944 (25.36- and 25.53-mm SVL, respectively), the anterior end is ossified and the posterior one is cartilaginous. And in KU 303237 and 303239 (24.12- and 25.19-mm SVL, respectively), both the anterior and posterior ends are ossified. In KU 307937, the centra between Presacrals VI and VII and between Presacral VII and the sacrum have half procoelous and half opisthocelous articulations (see Fig. 12). Vertebral centra with half procoelous and half opisthocelous

articulations are also present in KU 307939 between Presacral VIII and the sacrum, and in KU 303236 between Presacrals IV and V. In the case of the first two specimens, these anomalous formations do not seem to have affected the overall symmetry of the vertebral column, whereas in the case of KU 303236, the malformation seems to have caused an asymmetry of the centra of all the posterior presacral vertebrae and the sacrum. In KU 303237, there is a minor asymmetry of the centra of the last three presacral vertebrae and the sacrum.

The transverse processes of Presacrals II–IV are slender, but slightly more robust than those of the last presacrals. The transverse processes of the last four presacral vertebrae are equal in length to the transverse processes of Presacral IV, and the sacral diapophyses are about 90% the length of the transverse processes of Presacral III. Thus, the vertebral column profile in decreasing order is: III > IV \approx V \approx VI \approx VII \approx VIII \approx sacrum > II. The transverse processes of the third vertebra are positioned more ventrally than those of the rest of the vertebrae. The typical orientation of the transverse processes is: anterolateral for Presacrals II, III, VII, and VIII (about 70°, 80°, 80°, and 75° to the perpendicular axis of the vertebral column, respectively), posterolateral for Presacrals IV and V (about 100° and 110° to the perpendicular axis of the vertebral column, respectively), and perpendicular to the axis of the vertebral column for Presacral VI. In a few specimens, however, the orientation of the transverse processes of the last three presacral vertebrae may differ from the norm. The transverse processes of Presacral VI may have a posterolateral orientation of up to 105°; those of Presacral VII may be perpendicular to the axis of the vertebral column, and those of Presacral VIII may vary between 65° and 80°. The curvature of

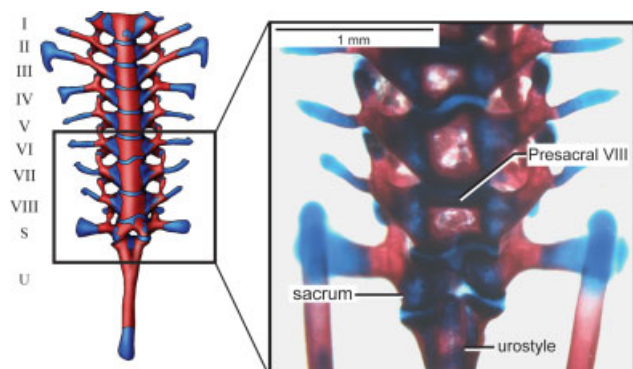


Fig. 12. Ventral view of the vertebral column of 12.73-mm SVL juvenile *Acris crepitans* (KU 307937) and (inset) close-up photograph of the postsacral region. The vertebral centra between Presacrals VI and VII and between Presacral VII and the sacrum exhibit half procoelous and half opisthocelous articulations. Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; S, sacrum; U, urostyle.

the transverse processes of Presacral VIII that is characteristic of juvenile specimens disappears in the adult. Uncinate processes are absent in all specimens examined. The distal ends of all transverse processes and diapophyses are cartilaginous; occasionally the transverse processes of Presacral II may be calcified. In several specimens (KU 307949, 84689, 84691, 158251, 170834, 170915, and 170941), the distal ends of the transverse processes of Presacral IV exhibit an expansion similar to that of the transverse processes of Presacral III; in addition, they are almost completely ossified.

The condyles of the sacrum and cotyles of the urostyle are ossified in specimens of about 25-mm SVL or larger. A straight margin or a small notch usually separates the cotyles of the urostyle, but in several specimens (KU 158112, 170834, 187701, 303231, 303232, 303235, and 307955) there is a pointed intercotylar process. In KU 303238, the centrum and postzygapophyses of Presacral VIII are fused to the centrum and prezygapophyses of the sacrum, respectively, and the intercotylar process of the urostyle is fused to the posteroventral margin of the corpus of the sacral vertebra (see Fig. 13).

The osseous portion of each sacral diapophysis has uniform width in about 50% of the specimens, whereas in other specimens, the distal end can be as much as one-third wider than the proximal end. In all specimens, the cartilaginous portion is slightly expanded. In most adults, the leading edge of the sacral diapophyses is directed posteriorly, with the angle to the longitudinal axis of the vertebral column being between 95° (57% of the specimens) and 110° (41% of the specimens). However, in a few individuals (e.g., KU 84691) the

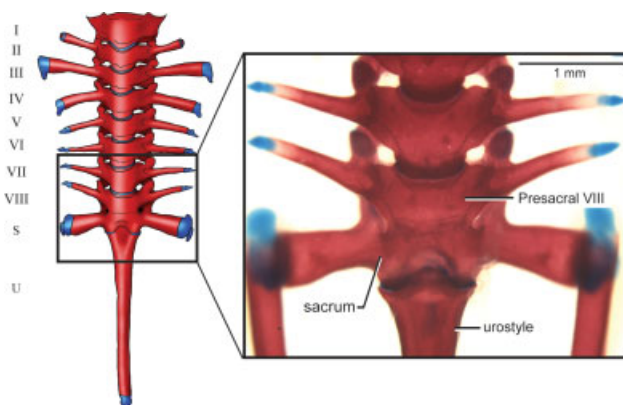


Fig. 13. Ventral view of the vertebral column of a 20.41-mm SVL adult *Acris crepitans* (KU 303238) and (inset) close-up photograph of the sacral region. The centrum and postzygapophyses of Presacral VIII are fused to the centrum and prezygapophyses of the sacrum, respectively, and the intercotylar process of the urostyle is fused to the posteroventral margin of the corpus of the sacral vertebra. I–VIII, Presacral Vertebrae I–VIII; S, sacrum; U, urostyle.

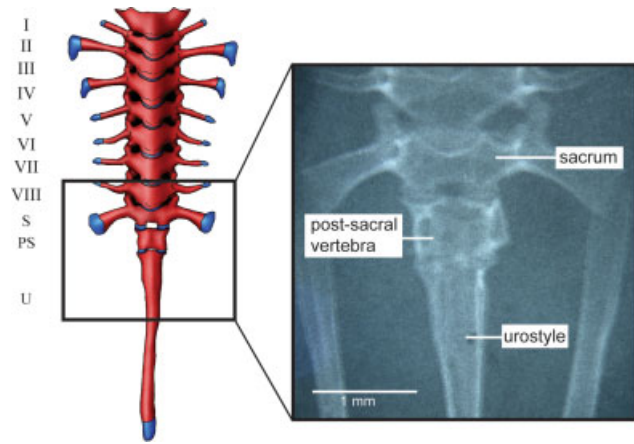


Fig. 14. Ventral view of the vertebral column of a 21.23-mm SVL adult *Acris crepitans* (KU 158199) and (inset) close-up radiograph of the sacrum and postsacral region. This specimen exhibits a vertebral element posterior to the sacrum that has a configuration closely resembling the morphology of the presacral vertebrae. Blue denotes cartilage; red denotes bone. I–VIII, Presacral Vertebrae I–VIII; PS, anomalous postsacral vertebra; S, sacrum; U, urostyle.

anterior margin of the diapophyses is 120° to the longitudinal axis of the vertebral column. The urostyle is slender, round in cross section, and its length is approximately equal to the length of the presacral portion of the vertebral column. The anterior end of the urostyle is slightly wider than the posterior cartilaginous end. The urostyle ridge is highest at its most anterior end, where it is flat and its dorsal margins are covered by a small oval cartilage that becomes heavily mineralized in the largest specimens. In lateral view, the anterior margin of the ridge is perpendicular to the longitudinal axis of the urostyle. Minute spinal nerve foramina pierce the urostyle. In KU 158199, there is a distinct vertebral element posterior to the sacrum and anterior to the urostyle (see Fig. 14); the anterior margin of the centrum has a pair of cotyles that articulate with the condyles of the sacral centrum and the posterior margin bears a pair of condyles that articulate with the cotyles of the urostyle. Posterior to this vertebra, the urostyle is slightly shorter ($\approx 85\%$ the length of the presacral region of the vertebral column), but morphologically similar to the urostyle of other adult specimens.

DISCUSSION

The skeletal morphology of the vertebral column of *A. crepitans* is typical of that of a hylid frog. All hylids have a 1-7-1-1 axial formula (i.e., an atlas, seven discrete postatlantal vertebrae, a sacrum formed by a single vertebra, and a rod-like urostyle). The number of vertebrae is not reduced via fusion as it is in some brachycephalids, bufonids,

dendrobatids, pipids, and microhylids (McDiarmid, 1969; Pugener, 2002). Free ribs are absent; the vertebral centra are procoelous, and a medial articulation of the neural arch laminae of Presacral I and II is present in most hylids (also observed by Gaudin, 1969). In addition, the neural arches of all vertebrae are nonimbricate, although Lynch (1973) indicated that the neural arches of phyllomedusines are imbricate.

The vertebral column of *A. crepitans* differs from those of other hylids with regard to the presence/absence of neural spines and the degree of distal expansion of the sacral diapophyses. Neural spines are absent in *A. crepitans*, but are present in other hylids, such as *Hyla*, *Plectrohyla*, *Pseudacris*, and *Trachycephalus*. Also, the sacral diapophyses of *A. crepitans* are cylindrical in cross section and uniform in width or slightly expanded distally, whereas those of other hylids, such as *Hyla*, *Pachymedusa*, *Phyllomedusa*, and *Pseudacris*, are expanded distally to at least two to three times the width of the base of the diapophysis. Gaudin (1969) stated that in most hylids, except *Acris*, the transverse processes of Presacral II are longer than the transverse processes of the last four presacral vertebrae; we were unable to corroborate these findings. A feature that seems to be unique to *A. crepitans* is the presence of small cartilaginous, tear-shaped expansions at the distal end of the transverse processes of the posterior four presacral vertebrae. Ephemeral cartilaginous vestiges of ribs, such as those found in association with the anterior presacrals of *H. arborea* larvae (Blanco and Sanchiz, 2000), were not detected.

The presacral region of the vertebral column is the first to appear during development. Vertebrae form in a cephalic-to-caudal sequence (Maglia and Pugener, 1998; Sheil, 1999; Pugener, 2002; Maglia, 2003; Handrigan et al., 2007) emulating the appearance of somites during somitogenesis, which also proceeds in time and space in an anterior-to-posterior order (Baker et al., 2003; Takahashi, 2005). Vertebral development and growth, however, seem to be decoupled. In other words, development occurs in an anterior-to-posterior gradient but the rate of growth (at least initially) is most rapid in the posterior presacrals. In *A. crepitans*, vertebral development begins before Gosner Stage 32 and proceeds in an anterior-to-posterior gradient. By Stages 36 and 37, there is an acceleration of the growth rate of Presacrals IV–VIII and the sacrum, as compared to the anterior three presacral vertebrae. By Stage 40, the growth rate of the anterior presacrals and the rest of the vertebrae is equal, and by Stage 42, all of the vertebral components of both regions are formed. The postsacral region, on the contrary, begins to develop around Gosner Stage 36, and is not formed completely until Stage 46. After metamorphosis, however, growth rates are similar in all regions of the verte-

bral column. Growth extends into adulthood, and continues throughout life.

The epichordal vertebral centra of *A. crepitans* originate from chondrification and subsequent ossification of the dorsal portion of the perichordal tube, between the bases of the neural arch pedicels. In the adult, this mode of development results in a centrum that is greatly depressed in cross section. Similar developmental modes are found in other hylids, such as *Hyla lanciformis* (Pugener, 2002) and *Phyllomedusa vaillanti* (Sheil and Alamillo, 2005). Epichordal centra form in slightly different ways in other anurans. For example, in the amphignathodontid *Gastrotheca riobambae*, the centra form by chondrification and subsequent ossification of the dorsal half of the perichordal tube (Pugener, 2002). In the adult, this results in centra that are oval instead of depressed in cross section. Mookerjee (1931) and Kluge and Farris (1969) stated that the centrum of the atlas of all anurans arises by ossification of the entire perichordal tube. In *A. crepitans*, however, the atlantal centrum clearly is epichordal. The atlantal centra of *H. lanciformis* and *P. vaillanti* are also epichordal.

In adult *A. crepitans*, as well as in most other anurans, the transverse processes associated with Presacral II usually are the shortest, whereas the processes of Presacral III tend to be the longest, and bear large, cartilaginous distal ends. Exceptions include pipids (Cannatella and Trueb, 1988a,b; Pugener, 2002), the hylid *Sphaenorhynchus* (da Silva, 1998), and the ceratophryid *Ceratophrys* (Wild, 1997), in which the transverse processes of Presacral IV tend to be the longest and bear the largest cartilaginous ends. In young *Acris* larvae (Gosner Stage 36 and younger), the transverse processes of Presacral II are the longest. Only after Stage 37 do the transverse processes of Presacral III grow to be longer than those of Presacral II. A similar developmental configuration was reported by Sheil (1999) for *Pyxicephalus adspersus*.

Emerson (1982) identified the lengths of the transverse processes of the posterior presacrals as functionally important to the iliosacral articulation (discussed below). She (Emerson, 1982) suggested that, in general, an increase in sacral distal expansion is correlated with a decrease in the relative length of the transverse processes of the posterior presacral vertebrae. Species with expanded sacral diapophyses are characterized by relatively short posterior presacral transverse processes, thereby preventing physical interference with the antero-posterior sliding of the ilia. Taxa with this morphology include bombinatorids, pipids, most hylids, pelobatids, and rhinophrynids, among others (Pugener, 2002). In contrast, species with cylindrical sacral diapophyses, such as *A. crepitans*, have relatively longer transverse processes

because there is no movement of the pelvic girdle in the horizontal plane, and thus, physical interference between the transverse processes and ilia does not present a problem. Other anurans showing this morphology include *Ascaphus*, *Ceratophrys*, dendrobatids, and some leptodactylids, among others (Pugener, 2002). In *Acris*, the ultimate length of the posterior transverse processes is attained at metamorphosis. In Gosner Stage-42 larvae and younger, the transverse processes of the last presacrals are cartilaginous and knob-like; lateral growth begins at Stage 43. A similar growth pattern occurs in *Bombina orientalis* (Maglia and Pugener, 1998), *Discoglossus sardus* (Pugener and Maglia, 1997), and *Pyxicephalus adspersus* (Haas, 1999; Sheil, 1999).

The orientation of the transverse processes has been used as a source of phylogenetic information (e.g., Cannatella, 1985; Báez and Basso, 1996; da Silva, 1998; Gao and Wang, 2001; Pramuk, 2002, 2006; Fabrezi, 2006). In *A. crepitans*, the orientations of the transverse processes of Presacrals II–IV are maintained throughout ontogeny. In contrast, the transverse processes of Presacral VI are posterolaterally oriented until Gosner Stage 46, at which time they become laterally orientated, and those of the last two presacrals are posterolaterally oriented until Stage 43, when they begin to move toward their characteristic adult orientation, which does not appear until after metamorphosis. In a few adult specimens, however, the orientation of the transverse processes of the last presacrals varies from the norm. These ontogenetic and intrapopulational variations clearly suggest that in the case of *A. crepitans*, the orientation of the transverse processes, and in particular that of the last presacrals, should be used cautiously as a phylogenetic character. According to Ritland (1955), similar ontogenetic changes in the orientation of the transverse processes also occur in *Ascaphus truei*, but according to Pugener (2002) there is no visible variation in adult individuals. On the contrary, in *Leiopelma* the orientation of the transverse processes of all presacrals (except the second) appears to be highly variable among adults (Pugener, 2002).

A. crepitans are strong jumpers and, when approached by a potential predator, make several quick zig-zag leaps, each often a meter or more in length (Zug, 1985; Gray et al., 2005a; pers. obs.). This behavior is reflected in the morphology of the sacral diapophyses, and is consistent with Emerson's (1982) Type-IIB iliosacral articulation. This kind of articulation maximizes vertical rotation and restricts movements in the horizontal plane, and is typically found in anurans that are accomplished, long-distance leapers (Emerson, 1982). It is worth noting that although there was a slight expansion of the distal ends of the sacral diapophyses of about half of the *Acris* examined, this does

not indicate variation in the type of iliosacral articulation. *Ascaphus truei* also has a Type-IIB iliosacral articulation, despite the fact that the sacral diapophyses are distally expanded (Emerson, 1982). However, instances of distal diapophyses expansion in *A. crepitans* could represent the remnants of a plesiomorphic morphology. Other hylids (e.g., *Hyla*, *Pachymedusa*, *Phyllomedusa*) have well-developed, distally expanded diapophyses that are characteristic of Emerson's (1982) Type-I iliosacral articulation. This type of articulation maximizes anteroposterior sliding movements of the pelvic girdle in the horizontal plane and minimizes lateral and dorsoventral rotation, and is better suited for the climbing (Emerson, 1982) that is characteristic of "typical" tree frogs.

In *A. crepitans*, the posterior end of the sacral centrum bears two condyles; thus, the articulation between the sacrum and urostyle is synovial and bicondylar. All other hylids have a bicondylar sacrum-urostyle articulation (Gaudin, 1969; Duellman and Trueb, 1994), but the posterior end of the sacral centrum varies among other anurans. In *Ascaphus*, *Leiopelma*, and *Notobatrachus* the posterior end of the sacrum is concave, and it is separated from the anterior end of the urostyle by a continuous intervertebral cartilage (Stephenson, 1952, 1960; Ritland, 1955; Cannatella, 1985; Báez and Basso, 1996). In some megophryids (e.g., *Leptotalax gracilis*, *Megophrys montana*, some *M. monticola*, and *Scutigera mammatus*), the posterior end of the centrum is concave, and it is separated from the urostyle by a free intervertebral body; Lynch (1973) described this condition as monocondylar. In some bufonids, some megophryids, all pipids, pelobatids, and scaphiropodids, the sacrum and urostyle are fused (Lynch, 1973; Cannatella, 1985; Cannatella and Trueb, 1988a,b; Wiens, 1989; Báez and Trueb, 1997; Maglia, 1998; Pugener, 2002; Banbury and Maglia, 2006; Frost et al., 2006). And in bombinatorids, the articulation between the sacrum and urostyle is synovial and monocondylar (Maglia and Pugener, 1998). Jenkins and Shubin (1998) interpreted the nature of the joint between the sacrum and urostyle in a functional context, and showed that sacrourostylic mobility is critical to launching a jump. They (Jenkins and Shubin, 1998) concluded that a bicondylar articulation constitutes the best joint for saltatorial locomotion because it restricts movement to a hinge-like flexion/extension, and that other kinds of joints are found in species in which jumping is not the main mode of locomotion.

Several features of the vertebral column of *A. crepitans* are highly variable, including: the distal expansion of the transverse processes of Presacral IV; the orientation of the transverse processes of the posterior presacrals; the angle of the leading edges of the sacral diapophyses; the distal expansion of the sacral diapophyses; and the configura-

tion of the intercotylar space of the urostyle. Interestingly, two of these structures that are highly variable in *A. crepitans* (i.e., the angle between the anterior edge of the sacral diapophyses and the longitudinal axis of the vertebral column and the orientation of the transverse processes of Presacral VI) showed the least amount of variation in Trueb's (1977) study of *Hyla lanciformis*. In addition, 11 of the *A. crepitans* we examined have some vertebral anomalies. Many genetic and environmental factors can affect somite formation (Baker et al., 2003), producing different defects in vertebrae (Pourquié and Kusumi, 2001; Turnpenny et al., 2003; Kusumi and Turnpenny, 2007). The nature and extent of the malformations depend on the stage of somitogenesis in which the perturbation occurs (Rivard et al., 1979), and may consist of vertebral fusion or failure of vertebral formation, among others (Pourquié and Kusumi, 2001; Dunwoodie et al., 2002; Kusumi and Turnpenny, 2007). Three *Acris* specimens examined display barely noticeable anomalies, including neural arch laminae that did not develop incomplete midline ossification, and minor vertebral asymmetries. The remaining eight specimens, on the other hand, show a host of more significant malformations, as discussed below.

Three *A. crepitans* specimens exhibit a malformation that, to our knowledge, has not been reported for any other anuran species. The malformation consists of vertebral centra with half procoelous (the typical condition) and half opisthocoeleous articulations. Mookerjee (1931) and Griffiths (1963) proposed that the kind of articulation of the centrum is determined by the nature of the intervertebral cartilage and its association to the centrum. In *A. crepitans*, however, there is no evidence of intervertebral cartilages, and the developing centra present a procoelous morphology when they first form. This developmental pattern could be explained in one of two ways. Either the intervertebral cartilage fuses to the centrum early in development or the intervertebral cartilage is absent and the articular condyle is generated from the centrum. Considering our limited understanding of the development of the articular condyle on a normal vertebral centrum of *A. crepitans*, we are hesitant to suggest a scenario for the origin of the articular anomalies.

In one adult *A. crepitans*, the centrum and postzygapophyses of Presacral VIII are fused to the centrum and prezygapophyses of the sacrum, respectively, and the intercotylar process of the urostyle is fused to the posteroventral margin of the corpus of the sacral vertebra. In some anurans (e.g., the bufonids *Dendrophryniscus brevipollicatus*, *Nectophryne afra*, and *Osornophryne guacamayo* and all species of the pipid *Hymenochirus*), the last presacral vertebra, the sacrum, and the urostyle are fused to form a compound structure

called the synsacrum (Pugener, 2002). In mature individuals of these species, sutures delineating the limits between the incorporated elements are not visible. However, the occurrence of a synsacrum usually can be recognized by the presence of: 1) more than one pair of spinal nerve foramina piercing the ventrolateral sides of the sacral structure; 2) sacral diapophyses with broad proximal ends (because they originate from two pairs of modified, subequal transverse processes); and 3) a pair of bony plates (=webbings) connecting the posteromedial margins of the diapophyses with the lateral margins of the anterior portion of the urostyle. The fusions observed in *Acris* clearly do not represent a synsacrum. Rather, they are likely the consequence of hyperossification or a developmental anomaly, and almost certainly had an adverse effect on midtrunk flexion and jumping ability. Similar anomalous fusions have been reported for a wide range of anurans, including alytids (Sanchiz and Perez, 1974), bombinatorids (Madej, 1965), bufonids (Taylor, 1942; Holman, 1963; Lynch, 1973; Pugener, 2002), dendrobatids (Holman, 1963), hylids, leptodactylids, microhylids (Pugener, 2002), pelobatids (Holman, 1963; Kluge, 1966), pipids (Pugener, 2002), and ranids (Holman, 1963).

Although the sacrum typically lacks postzygapophyses, and the urostyle is devoid of prezygapophyses, one *Acris* tadpole has a postzygapophysis on the right side of the sacrum and a prezygapophysis on the corresponding right half of the coccyx. These zygapophyses, however, do not articulate with one another. A similar malformation was reported in *Ascaphus truei* and *Megophrys nasuta* (Pugener, 2002). Three *Acris* examined have a discrete vertebral element posterior to the sacrum. In two of the specimens, the postsacral vertebra articulates with the urostyle posteriorly; the third specimen is a young larva in which the caudal neural arches and hypochord are barely developed. The presence of a postsacral vertebra is not uncommon in anurans. It has been observed in *Bombina bombina* and *B. variegata* (Madej, 1965), *B. orientalis* (pers. obs.), *Notobatrachus degiustoi* (Báez and Basso, 1996), *Microhyla ornata* (Pugener, 2002), and *Rana pipiens* (Holman, 1963). Moreover, in the fossil †*Eopelobates anthracinus* there are two discrete postsacral vertebrae anterior to the urostyle (Špinar, 1972). In addition, Stephenson (1952) reported that in *Leiopelma* (characterized by the presence of nine presacral vertebrae), the sacral diapophyses form occasionally on the ninth vertebra, in which case the tenth vertebra articulates with the urostyle (instead of being fused to it).

†*Triadobatrachus massinoti*, a salientian from the Early Triassic of Madagascar that has been interpreted as the sister group of anurans, has six postsacral vertebrae (Griffiths, 1963; Estes and

Reig, 1973; Rage and Roček, 1989). In this taxon, the first caudal vertebra has a pair of short transverse processes, whereas the posterior vertebrae are ring-like and become sequentially smaller posteriorly. Salamanders also possess caudal vertebrae, although the number is highly variable (Wake, 1966), and may consist of 20 to more than 100 (Duellman and Trueb, 1994). These vertebrae exhibit a gradual reduction in the sizes of their transverse processes and zygapophyses, but such structures are never entirely absent. Supernumerary caudal vertebrae also occur in the larvae of some megophryid anurans (Griffiths, 1963; Haas et al., 2006; Handrigan and Wassersug, 2007; Handrigan et al., 2007). The normal occurrence of postsacral vertebrae in the tadpoles of these frogs constitutes an adaptation for their riparian lifestyle, and the vertebral elements disappear during metamorphosis through apoptosis, leaving only the urostyle in the adult (Handrigan et al., 2007). The presence of caudal vertebrae in megophryids seems to be a homoplastic reversal to a more ancestral condition (Handrigan et al., 2007). The occasional formation of a postsacral vertebra in *A. crepitans* and other anurans could suggest retention of the genetic and developmental mechanisms needed to form vertebral elements posterior to the sacrum (as it does in megophryids) or it might represent the anomalous formation of an extra somite. During somitogenesis, certain molecular or environmental factors may induce the formation of additional somites (Veini and Bellairs, 1986; Roy et al., 1999; Baker et al., 2003). Under these circumstances, the embryo has an extra vertebral precursor, while the "normal" somites and vertebral precursors would resemble those of typical embryos. Interestingly, in frogs with a vertebra posterior to the sacrum the morphology of the urostyle does not seem to differ from the configuration typically found in normal members of the species.

The preceding description and discussion demonstrate that the vertebral column is highly variable in *A. crepitans*. More than 8.5% of the specimens examined have vertebral anomalies (11 of a total sample of 129 specimens), and about 50% display small, normal variants from the typical morphology. High malformation and variation rates are not exclusive to *Acris*. Even a cursory perusal of the literature (see introductory comments) is sufficient to indicate that vertebral deformities are frequent among anurans. Moreover, the percentages of vertebral anomalies observed in *A. crepitans* concur with those reported for other species. For example, Madej (1965) observed a 10% rate (of 1,368 specimens) of vertebral column anomalies in *Bombina*, and Trueb (1977) found 9.4% of 53 specimens with vertebral malformations and over 50% deviating from the typical morphology in *Hyla*. Most vertebral column malformations, however, do

not seem to be severe enough to affect survival. Moreover, it seems that the vertebral column is highly plastic in its development, and that frogs are able to survive a wide range of vertebral variability and anomaly.

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