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Anomalies in the vertebral column and ilio-sacral articulation of some anuran amphibians

Alexander Haas[1], Sophie Schwippert[1], Sebastian Büsse[2], Thomas Kleinteich[3], André Beerlink[4], Jörg U. Hammel[5], Stephanie Köhnk[1], Stanislav Gorb[2] & Karolin Engelkes[1]

1) Centrum für Naturkunde, Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany
2) Functional Morphology and Biomechanics, Institute of Zoology, University of Kiel, Am Botanischen Garten 1–9, 24118 Kiel, Germany
3) TPW Prüfzentrum GmbH, Xantener Str. 6, 41460 Neuss, Germany
4) YXLON International GmbH, Essener Bogen 15, 22419 Hamburg
5) Institute of Materials Research, Helmholtz-Zentrum Geesthacht, Max-Planck-Str. 1, 21502 Geesthacht, Germany

Corresponding author: Alexander Haas, e-mail: alexander.haas@uni-hamburg.de

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Abstract. We present accounts of vertebral anomalies in 17 individuals representing 13 species of anuran amphibians. These cases were detected while perusing a larger survey on the skeleton of frogs, for which μCT scans of a broad range of species were collected and evaluated. Our data and reports from the literature suggest that malformations, asymmetries, and irregularities, if present, appear to be particularly prevalent in the posterior region of the axial skeleton in frogs. Anomalies at the trunk-tail boundary, i.e., at the sacrum and neighbouring segments, were relatively common. Malformations at the trunk-tail boundary often include sacralization of pre- and postsacral elements with asymmetrically or symmetrically developed diapophyses, fusion with the posteriormost presacral vertebra, occurrence of postsacral vertebrae, unusual transverse processes at the proximal end of the urostyle, formation of additional zygapophyses, or fusion of elements that normally articulate. Vertebral fusion in the anterior vertebral column (Presacral Vertebrae I+II) has been reported both in evolutionary context and in cases of individual developmental anomalies. Malformations in the middle section of the vertebral column, such as the case of *Epidalea calamita* reported herein, are rare.

Key words. Amphibia, Anura, axial skeleton, pelvis, sacrum, malformation.

Introduction

The anuran body plan is unique among vertebrates making members of the monophylum Anura easily recognizable: apart from losing the tail, the truncation of the vertebral column, the reduction in the number of vertebrae, the elongation of the ilia, expanded sacral diapophyses with ventral articulation to the ilium, and the formation of the urostyle are the most obvious synapomorphies in early frog evolution (Shubin & Jenkins 1995, Pugener & Maglia 2009a). Frogs are the tetrapod group with the fewest vertebrae. Species of the Ascaphidae and Leiopelmatidae possess nine presacral vertebrae, all other anurans have a vertebral count of eight or less (Duellman & Trueb 1994). There is only moderate differentiation into functional groups in the presacral vertebral column, for example, in the size of vertebral transverse processes at the level of the suprascapula. Presacral Vertebra I, or Presacral I for short, is the only neck vertebra and stands out by typically lacking transverse processes (Gaupp 1896). A single sacral vertebra establishes the articulation between the vertebral column and the pelvis. The sacral vertebra is pivotal for the function of the sacro-urostylic complex (Emerson 1982). The tail vertebral column is highly reduced in all extant species; only vestiges of the tail vertebrae contribute to the formation of the urostyle that is otherwise an evolutionary novelty (Roček & Roček 2005, Pugener & Maglia 2009a).

The anatomical details and the shape of the ilio-sacral joint vary among the frog lineages and are strongly correlated with a species' specialization in locomotor mode, such as jumping versus swimming or burrowing, as well as habitat choice (arboreal, fossorial, aquatic, terrestrial) (Emerson 1979, Jorgensen & Reilly 2013). For example, the very wide sacral diapophyses of the sacral vertebra in pipids allow for a forward sliding of the pelvis (Videler & Jorna 1985, Cundall et al. 2017), whereas the almost cylindrical diapophyses in many ranid frogs provide the necessary articular freedom for dorsoventral extension at the initial jumping phase (Emerson 1979, 1982, Jenkins & Shubin 1998).

Considering the crucial functional role of the vertebral column, ilio-sacral articulation, and sacro-urostylic complex, one would expect that mutational malformations of the skeleton were strongly selected against in evolution and should be rare in nature (Zamora-Camacho & Aragón 2019). Congenital skeletal anomalies, however, can have many different causes other than genetic mutations. Particularly in organisms that complete a substantial part of their skeletal development during larval life and metamorphosis, when they can be exposed to teratogens in the environment. Recently, Ouellet (2000) and Henle & Dubois (2017) have assessed amphibian anomalies in general. These hitherto most extensive compilations and evaluations of published reports cover anomalies in colour pattern and morphology, and review hypotheses about causes of such pathologies. Specific skeletal abnormalities, however, have been covered only marginally apart from external features of general limb or body deformations. Henle & Dubois (2017) provided a glossary, specific cases, and general methodological recommendations.

In a different project on skeletal features of the shoulder girdle (Engelkes et al. 2020), we had access to and examined micro-computed tomography (µCT) scans of a broad taxonomic sample of anuran amphibians. During the examination we recognized several individuals with vertebral and sacral malformations or anomalies compared to conspecifics or congeners. Various categories of vertebral anomalies have been described before (Madej 1965). We classify vertebral malformations herein, first, as any asymmetry that was so substantial that it could be detected visually even without further measurement, such as much inflated or present processes on only one side. In specimens that could be considered normal, in contrast, asymmetries were not perceivable. A second category of anomalies does not involve asymmetry but the fusion of consecutive vertebral structures. Third, supernumerary elements can be present. Sometimes a combination of these major types of malformations can occur in the same specimen. In the following we describe the osteological malformations of the vertebral column and ilio-sacral articulation present in specimens examined. Providing a comprehensive compilation on the topic is beyond the scope of this work, but by reporting the phenomenology of some new cases of axial skeleton anomalies we hope to contribute to the knowledge of anomalies in anurans in general.

Materials and methods

For a study on the shoulder girdles in anuran amphibians (Engelkes et al. 2020), our group assembled a data set of 125 µCT scans of 74 frog species that could be assessed for anomalies of the axial skeleton (Supplementary document S1). Among these, 28 datasets were downloaded from MorphoSource (Duke University) online 3D-database (Supplementary document S1).

Most of the µCT data were generated on either a Bruker Corp. Skyscan 1172, a YXLON FF20 CT or FF35 CT, a GE Sensing & Inspection Technologies GmbH Phoenix Nanotom S or M, or a GE Sensing & Inspection Technologies GmbH Phoenix v|tome|x L 450. Depending on the size of the specimen, the scanning parameters were set appropriately (Supplementary document S1). Volumes were reconstructed from X-ray projections using the software delivered with the respective scanner. The resulting voxel size ranged from 11.87–106.01 µm, depending on the specimen size and CT system used.

Amira 6.0.1. software (Thermo Fisher Scientific) was used to visualize and inspect µCT volumes. For each available µCT image stack an Isosurface of the calcified tissues (i.e., bones and calcified cartilage) was generated for inspection. If the specimen showed anomalies in the vertebral column or the ilio-sacral articulation, relevant skeletal structures were segmented with Amira's Segmentation Editor (Brush and Magic Wand tools). As this study focuses on the vertebral column and sacral structures, only affected skeletal elements were accurately separated during segmentation; cruder segmentation methods were applied in other areas of the skeleton. Because cartilage does not visualize reliably in µCT datasets of plain specimens and because it does not contribute additional aspects to the phenomena treated herein, cartilage tissue was generally neglected for this study; only calcified cartilage was occasionally segmented and visualized in skeletal parts that were unaffected by malformations if its grey values fell within the range of bone grey values. Segmented skeletons were exported with the mulitExport macro (Engelkes et al. 2018) as polymesh surfaces (.obj format) and, if necessary, further processed in MeshLab 1.3.3 (Cignoni et al. 2008); specifically, Quadric Edge Collapse Decimation and Taubin Smooth procedures were applied in MeshLab to reduce the number of polygons and to smooth the surfaces while still preserving the original surface as well as possible. Next, the surfaces were imported into various versions of Modo (7–13; The Foundry Visionmongers Ltd.) for subtle further smoothing (if necessary), topology hole filling, assigning colours (malformations red; corresponding structures without indication of malformation green, all other skeletal parts grey), and final rendering (Dome Light and Directional Light). Colour plates were arranged using Graphic 3.1 (Picta Inc.) software.

Abbreviations: AMNH, American Museum of Natural History; CAS, California Academy of Sciences; CM, Carnegie Museum of Natural History; UF, Florida Museum of Natural History; USNM, Smithsonian, National Museum of Natural History; ZMH, Zoological Museum Hamburg; ZSM, Zoologische Staatssammlung München; µCT, micro-computed tomography.

Results

The comparison of *Barbourula busuangensis* CAS-SUA 21240 (Fig. 1A) and *B. busuangensis* CAS-SUA 21247 (Fig. 1B; Table 1) in our study revealed severe malformations in CAS-SUA 21247, i.e., it showed: Presacral I and

Table 1. Specimens with anomalies in their axial skeleton reported herein. CAS, California Academy of Sciences. FMNH, Field Museum of Natural History. UF, Florida Museum of Natural History. USNM, National Museum of Natural History. ZMH, Zoologisches Museum, Hamburg. ZSM, Zoologische Staatssammlung München. See Supplementary Information Table 1 for a list of all specimens examined.

Species	Museum collection number	Anomaly
Barbourula busuangensis Taylor & Noble, 1924	CAS-SUA 21247	Fusion Presacrals I+II and Presacrals VII+VIII; supernumerary transverse processes at urostyle base.
Bombina bombina (Linnaeus, 1761)	ZMH A09674	Supernumerary tenth vertebra with broad left diapophysis; unequal diapophyses at sacral vertebra.
Bombina bombina (Linnaeus, 1761)	ZMH A05619	Wedge vertebra following Presacral VI.
Bombina bombina (Linnaeus, 1761)	ZMH A05617	Sacralization of Presacral VIII with left broad diapophysis; sacral vertebra(?) fused to urostyle and forming a broad diapophysis only on right side.
Bombina orientalis (Boulenger, 1890)	ZMH A14350	Fusion Presacrals I+II.
Bombina variegata (Linnaeus, 1758)	ZMH A11873	Unclear segmental pattern; wedge vertebra following Presacral IV. Sacral vertebra fused to urostyle and possibly representing segment IX or X; presacral element forming unusually large and flat transverse processes (sacralization).
Crossodactylus caramaschii Bastos & Pombal, 1995	USNM 318234	Right side sacralization of Presacral VIII; reduced right diapophysis in sacral vertebra (IX).
Discoglossus montalentii Lanza, Nascetti, Capula & Bullini, 1984	ZSM 1300/2006	Sacralization of Presacral VIII, i.e. extended transverse processes.
Ecnomiohyla miliaria (Cope, 1886)	UF Herp 137208	Failed formation of right diapophysis in sacral vertebra; supernumerary tenth vertebra with right side sacralization.
Eleutherodactylus coqui Thomas, 1966	ZMH A10152	Malformed , laterally curved urostyle.
Epidalea calamita (Laurenti, 1768)	ZMH A06868	Fusion Presacrals V+VI.
Microhyla nepenthicola Das & Haas 2010	ZMH A11645	Reduced vertebral count, seven presacrals; fusion Presacral I+II is norm for the species, but Presacral VII (=eighth vert. segment) fused with sacral vertebra and developing unilateral transverse process.
Microhyla nepenthicola Das & Haas 2010	ZMH A11639	Supernumerary postsacral vertebral element with distinct neural arch but synostosis with the sacral vertebra at centrum and (possibly) zygapophyses.
Pelobates fuscus (Laurenti, 1768)	UF Herp 36935	Bifurcated left transverse process of Presacral IV.
Pelobates fuscus (Laurenti, 1768)	ZMH A07151	Atlas with transverse processes that articulate with transverse processes of Presacral II.
Pleurodema bibroni Tschudi, 1838	FMNH 132507	Supernumerary ascending neural arch and transverse process on the right side at Presacral VIII; fused.
Pseudacris triseriata (Wied-Neuwied, 1838)	CAS 188145	Reduced left diapophysis in Presacral IX; Presacral IX fused to a subsequent, supernumerary sacralized vertebra (right diapophysis broad, left slender); the latter fused to urostyle.

II were fused into one skeletal element (synostosis, block vertebra). A second synostosis in the same specimen comprised Presacral VII and VIII. Supernumerary transverse processes were present at the base of the urostyle (two pairs instead of one).

Our 23 specimens of *Bombina* included normally developed specimens (such as *Bombina bombina* ZMH A05110; Fig. 2A) that possessed eight clearly articulating presacral vertebrae, symmetrical, broadly expanded sacral diapophyses, and a urostyle that had developed a pair of symmetrical processes near its base. Within the specimens examined for the genus, we found three with anomalies in their vertebral columns. *Bombina bombina* ZMH A09674 (Fig. 2A) and *B. bombina* ZMH A05617 (Fig. 2C), *B. bombina* ZMH A05619, *B. orientalis* ZMH A14350, and *B. variegata* ZMH A11873 Fig. 2C) differed in the patterns observed.

In *Bombina bombina* ZMH A09674, the count of presacral vertebrae was normal (eight) and the presacral vertebral column was symmetrical. However, the neural arch of Presacral I was incompletely ossified mid-dorsally; possibly the ossification process had not been finished in this 25 mm snout–vent-length specimen. The sacral region possessed a duplication of the sacral vertebra. The total count of vertebrae was, thus, ten in this specimen. The posterior sacral vertebra formed a sacral diapophysis asymmetrically on the left side. On the right side, there was a slender transverse process, instead of a dilated sacral diapophysis, in this supernumerary vertebra. The anterior sacral vertebra formed two sacral diapophyses, the right one was larger and longer than the left one. All three diapophyses of the two vertebrae contributed to the ilio-sacral articulation. Altogether each of the diapophyses appeared relatively smaller than the diapophyses in *B. bombina* ZMH A05110 that showed no obvious signs of malformations.

In *Bombina bombina* ZMH A05617 the count of presacral vertebrae was seven and two sacral vertebrae were present, suggesting that the eighth presacral vertebra had developed into a sacral vertebra. Both sacral vertebrae in this specimen bore broad diapophyses laterally, to the left in the anterior and to the right in the posterior sacral vertebra, both articulated with the respective ilium. In *B. bombina* ZMH A05619 the ilio-sacral joint was very similar to the one in ZMH A05617.

Bombina variegata ZMH A11873 (Fig. 2D) presents with multiple interesting malformations: The total count of vertebral elements (urostyle excluded) was ten; however, more than one interpretation is possible (Fig. 2D). Beginning with unequivocal position IV, two vertebral "elements" follow (V? + VI?) of which V? does not encircle the neural canal but is wedged between the anterior IV and posterior VI elements (wedge vertebra) and restricted to the right side of the body. The last vertebra without contact to the ilium formed unusually long and flat transverse processes, appearing intermediate between normal transverse processes of the vertebra anterior to it and the diapophyses of the vertebra posterior to it. The latter established the ilio-sacral articulation and is confluent with the urostyle posteriorly. Note also the thickened end of the urostyle and the relatively short and curved ilia in this specimen. In *B. bombina* ZMH A05619, Presacrals VI and VII were similarly malformed as Presacrals IV and V? in *B. variegata* ZMH A11873. We further observed the fusion of Presacrals I+II in *B. orientalis* ZMH A14350 similar to the occurrence in *Barbourula busuangensis* CAS-SUA 21247.

Comparing two specimens of *Discoglossus montalentii*, ZSM 1299/2006 showed no obvious anomalies in the vertebral column and ilio-sacral joint. In contrast, we detected sacralization of Presacral VIII in specimen ZSM 1300/2006 (Figs 3A–B). The sacralization in specimen ZSM 1300/2006 was characterized by longer, thicker, and more transversely oriented processes, reaching the ilium on the right and almost reaching the ilium on the left side (Fig. 3B).

The comparison of two individuals from two *Crossodactylus* species (Figs 3A–B) revealed an ilio-sacral deformity in *C. caramaschii* USNM 318234. We consider the perfectly symmetrical formation of Presacral VIII and the sacral vertebra in *C. trachysomus* (CM Herp 2662, Fig. 3D) the normal vertebral development. In *C. caramaschii* USNM 318234, however, Presacral VIII was sacralized on the right side: A broad transverse process, reminiscent of a proper sacral diapophysis, was formed and articulated with the ilium. On the left side of the same vertebral body, a normal cylindrical, thin transverse process with anterolateral orientation was formed. The proper sacral vertebra (ninth vertebra) featured a relatively normal diapophysis on the left side. On its right side, however, a vestigial diapophysis, thin and short in dimensions and deflected posteriorly, was present.

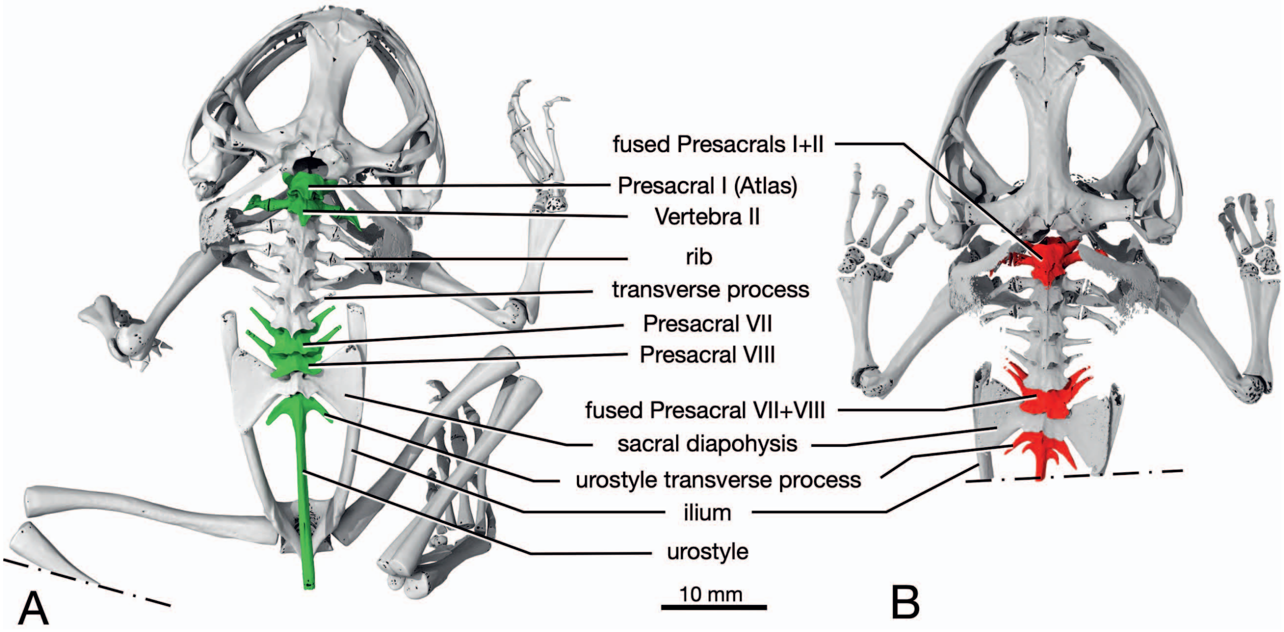

Figure 1. Comparison of the skeleton in two specimens of *Barbourula busuangensis*. A) CAS-SUA 21240, specimen with normal vertebral column development; B) CAS-SUA 21247 showing fusions between Presacral I (atlas) and II, as well as Presacrals VII and VIII. Furthermore, the urostyle of CAS-SUA 21247 possesses four lateral processes. Polymesh surfaces derived from computed tomography scans, rendered in dorsal views. Dashed lines indicate stop levels of scans from which the polymesh was derived; colours highlight corresponding skeletal parts, green: normal bone formation, red: anomalous bone elements.

Another category of deformities was detected in *Epidalea calamita* ZMH A06868 (Fig. 3E). This specimen presented with Presacrals V and VI that appeared compressed in the antero-posterior dimension, relative to the sizes of neighbouring vertebrae. Furthermore, the two elements were fused at the level of the neural arches, zygapophyses and centra. In neighbouring vertebrae, these structures were clearly separate between successive structures so that we can exclude CT artefacts.

In *Pleurodema*, we had no access to normally developed individuals but asymmetric structures in an otherwise bilaterally symmetrical organism allowed us to locate deformities in Presacral VIII and the urostyle of *P. bibroni* FMNH 132507 (Fig. 3F). This specimen showed a supernumerary vertebral element, specifically a supernumerary ascending neural arch and transverse process on the right side of the vertebral column only. We detected a bulge at the anterior base of the posterior process, reminiscent of a zygapophysis. An intervertebral foramen was present between the two right neural arches of Presacral VIII but dorsal and ventral to this foramen the neural arches, centra and zygapophyses were ankylosed. Despite the considerable deviations for the body plan on the right side, the left part of Presacral VIII did not exhibit apparent deformities. The sacral vertebra appeared unaffected by the malformation of the preceding vertebra. The urostyle, however, appeared anomalous as it possessed a small lateral process at its base on the left side.

The genus *Pseudacris* was represented in our study by a seemingly normally, symmetrically developed specimen of *P. streckeri* (AMNH A184936; Fig. 4A). A specimen of *P. triseriata* (CAS 188145), however, showed clear malformations in the sacral vertebra and diapophyses (Fig. 4B). The malformation of the latter was characterized by a very asymmetrical development of the sacral diapophysis of the actual sacral vertebra (Presacral IX). The left diapophysis was replaced by a process that was identical in shape and orientation with the transverse process of the preceding vertebra; this process did not articulate with the ilium. What seemed to be the anterior end of the urostyle, however, showed sacralization and formed a left-side process that had the length, orientation and shape of a sacral diapophysis, and articulated with the ilium. On the right side of the same element, yet another process was formed and reached toward the ilium. It was more cylindrical and less blade-like flat than the sacral diapophysis of the preceding sacral vertebra. Furthermore, in this aberrant specimen, the left ilium was shorter than the right ilium, corresponding to the respective, broad diapophyseal process. A similar but mirrored arrangement was discovered in *Ecnomiohyla milia-*

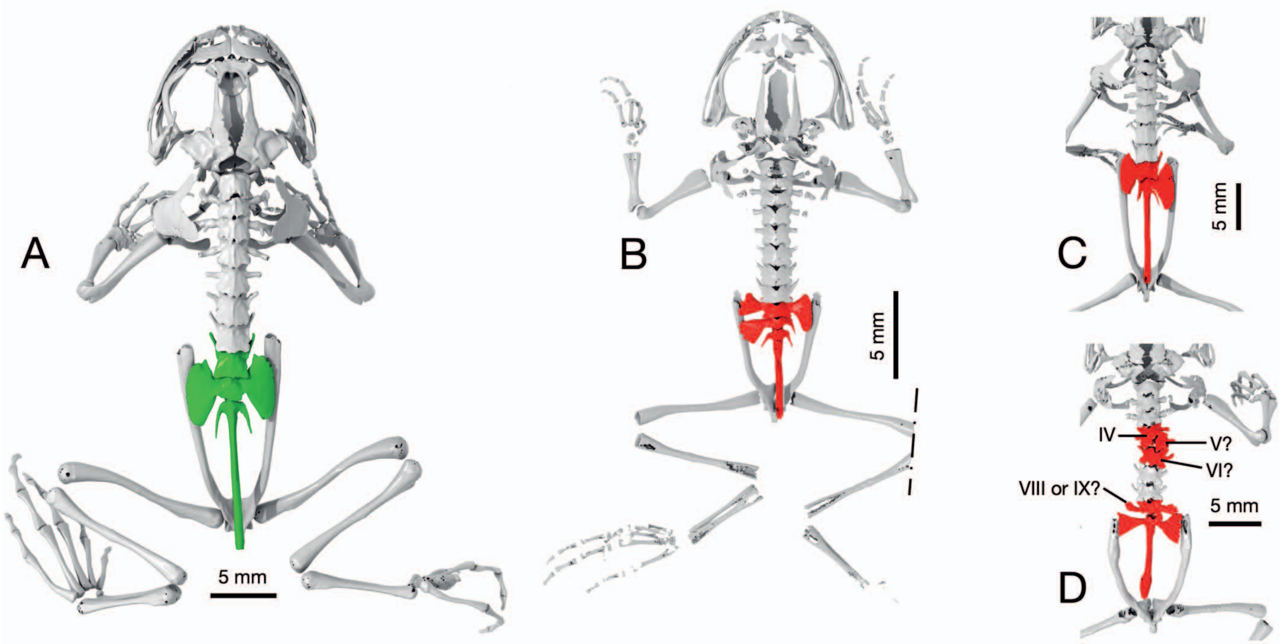

Figure 2. Vertebral column morphology in *Bombina*. A) *B. bombina* ZMH A05110, green colour highlights the normally developed Presacral VIII, the sacral vertebra with sacral diapophyses, and the urostyle. B) *B. bombina* ZMH A09674, vertebral column with malformations (red) in the sacral region: presence of two sacral vertebra (IX, X) with a total of three articulating diapophyses. C) *B. bombina* ZMH A05617, vertebral column with malformation (red) in the sacral region. The articulation with the pelvic ilium is established by Presacral VIII on the left side and a structure on the right side that may include the proper sacral vertebra but is confluent with the urostyle. D) *B. variegata* ZMH A11873, vertebral column with several malformations in the vertebral column; unclear segmental pattern in mid vertebral column. The sacral articulation is formed between the ilium and an element that bears sacral diapophyses but is confluent with the urostyle posteriorly. The vertebra anterior to that element forms transverse processes that are flat and expanded, reminiscent of sacral diapophyses. Polymesh surfaces derived from computed tomography scans, rendered in dorsal views; dashed lines indicate stop level of original scan in B, C and D cropped from original rendered images.

ria UF Herp 137208 (Fig. 4C), in which the sacral vertebra (IV) had diapophyseal contact with the ilium only on the left side and a tenth vertebral element was formed and articulated via a diapophysis on the right side with the ilium; elements were not fused with each other and the urostyle.

A more subtle sacral malformation was detected in the genus *Microhyla*. There were eight presacrals in *M. pulchra* USNM 278542 and no obvious anomalies. In all four *M. nepenthicola* examined, however, Presacrals I+II were indistinguishably fused and the resultant fusion element possessed transverse processes. Thus the presacral count was seven in ZMH A11639, A11647 (Fig. 4D), and A11650, respectively; we consider this the species' norm. In *M. nepenthicola* ZMH A11645 (Fig. 4E), however, there were only six free presacrals. A left side transverse process was present anterior to the sacral vertebra. Our interpretation is that the last presacral was partially and asymmetrically developed and fused to the sacral vertebra. Furthermore, in *M. nepenthicola* A11639 a supernumerary vertebra was formed posterior to the sacral vertebra. The data suggested that this element and the preceding sacral vertebra were synostosed at their vertebral bodies and possibly at the zygapophyses, however, their neural arches were clearly distinct (not depicted herein).

Two specimens of *Pelobates fuscus* showed some noteworthy differences (Fig. 5). *Pelobates fuscus* ZMH A07151 possessed transverse processes at the atlas (Presacral I) that articulated with Presacral II; whereas *P. fuscus* UF Herp 36935 had a normal atlas, without transverse processes. The latter specimen, however, formed a bifurcating transverse process at Presacral IV, whereas the former seemed normal in that respect (no bifurcation).

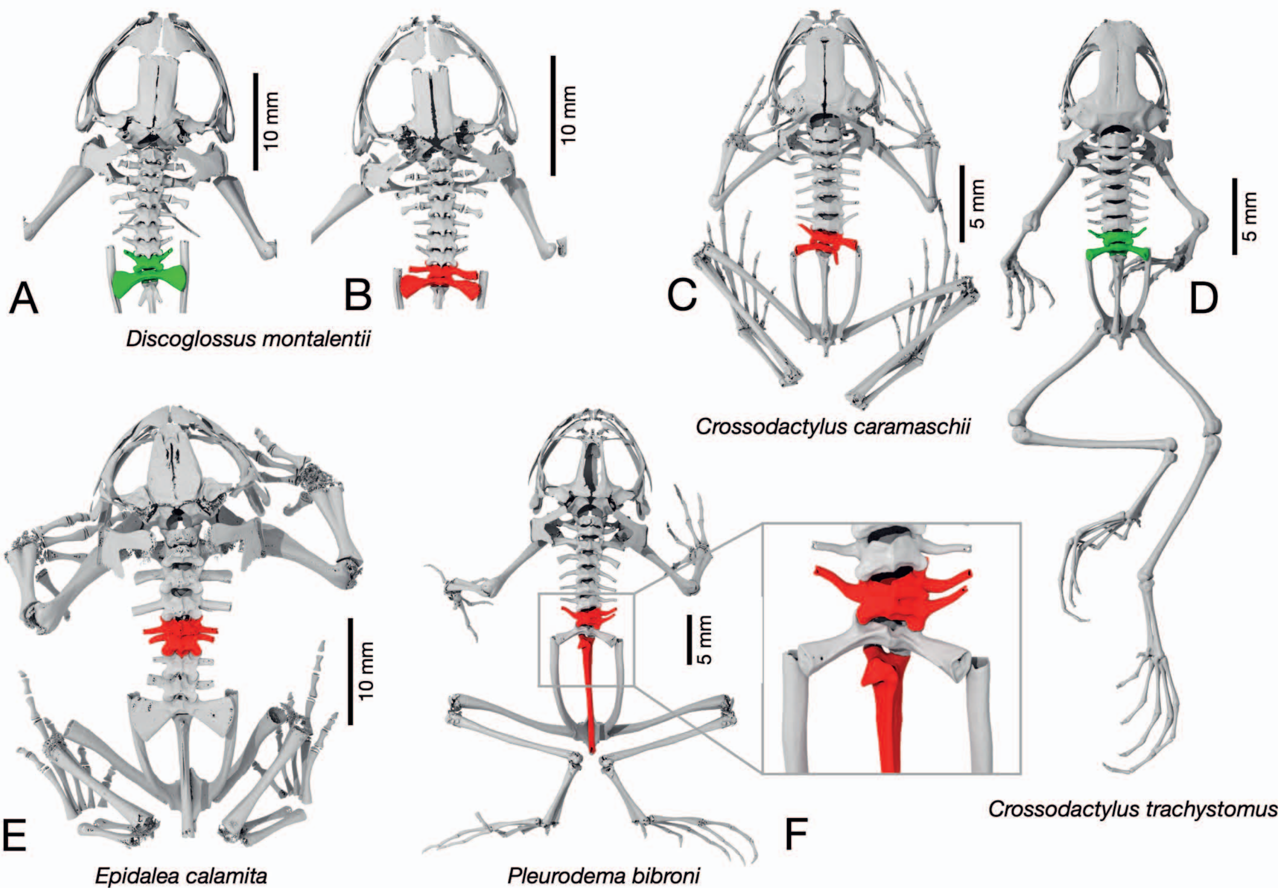

Figure 3. A–B) Comparison between an apparently normal vertebral element (green) in *Discoglossus montalentii* ZSM 1299/2006 (A) and specimen ZSM 1300/2006 (B) of the same species with ilio-sacral anomaly that features expanded transverse processes in Presacral VIII (red) and slightly narrow sacral diapophyses (red). C–D) Intrageneric comparison of two *Crossodactylus* species. C) *C. caramaschii* USNM 318234 presenting an ilio-sacral anomaly (red) in which Presacral VIII forms a dilated transverse process that articulates with the ilium on the right side, whereas the sacral vertebra proper has a vestigial right diapophysis and only articulates with the ilium by its left diapophysis. D) *C. trachystomus* CM Herp 2662, Presacral VII and sacral vertebra (green) without malformations. E) *Epidalea calamita* ZMH A06868. In this specimen, Presacrals V and VI (red) possess anomalous features: both elements seem compressed in the anteroposterior axis and are ancylosed. F) *Pleurodema bibroni* FMNH 132507. The specimen has malformations in Presacral VIII as well as the urostyle (red). Presacral VIII seems serially duplicated but exclusively on the right side. All images are dorsal views of specimens reconstructed from µCT data sets and rendered as polymesh surface. A and B cropped.

Finally, *Eleutherodactylus coqui* ZMH A10152, a F1-generation captive-bred specimen, among two specimens of that species examined, showed a strongly curved urostyle (not depicted herein).

Discussion

Any classification of aberrant, abnormal, deformed or malformed anatomical structures requires the "norm" as reference. A multifaceted discussion of this topic has been provided by Kovalenko & Kruzhkova (2013a,b,c) in a detailed study on *Bufo bufo*. In the current study, the number of "normal" specimens for intraspecific reference in a particular species was small (often n=1), yet the number of available CT scans across major clades of the Anura was large (125 specimens). Knowledge of the morphology of species that were closely related to species in our sample, and literature accounts, were considered sufficient to detect and describe the cases presented herein. We did not address anomalies of the vertebral column with statistical methods at the population level but rather qualitatively at a higher level of abstraction, that is, the bilateral symmetry and segmentation of the amphibian body plan in general. Thus, we focus on presenting observations that clearly and unambiguously break the patterns of either symmetry (substantial asymmetry) or seriality (disturbed segmentation pattern) in the vertebral column and ilio-sacral complex. Asymmetries, such as unilateral sacralization of vertebrae (for example, Peloso 2016), are easily and unam-

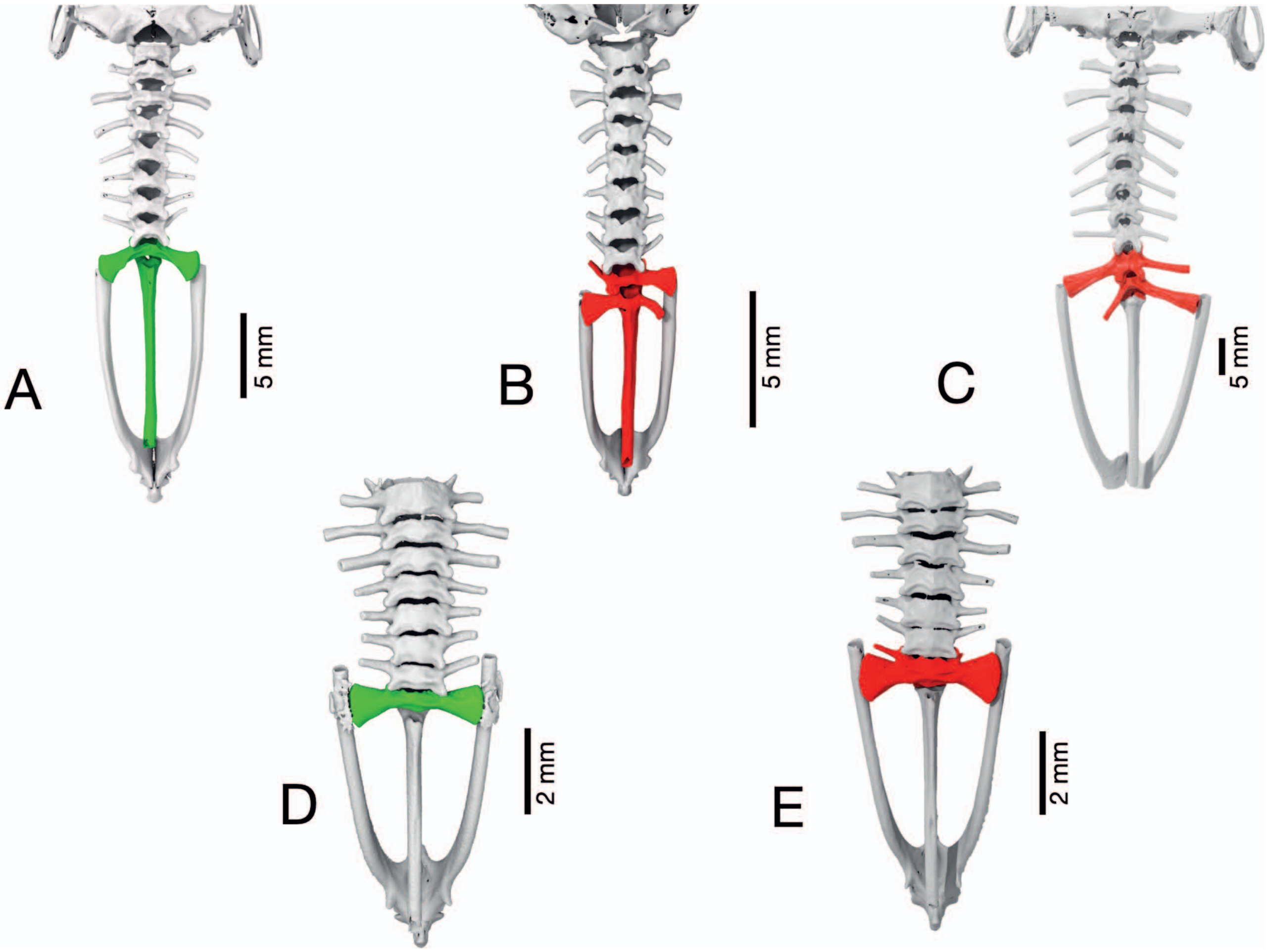

Figure 4. A–B) Two species of the genus *Pseudacris*: A) *Pseudacris streckeri* AMNH A184936 (cropped). The symmetrical presacral and sacral vertebrae indicate a normal state, whereas in B) *Pseudacris triseriata* CAS 188145 (cropped) shows a strongly malformed sacral vertebra and anterior urostyle. C) *Ecnomiohyla miliaria* UF Herp 137208 (cropped) unilaterally sacralized Presacral VIII and incompletely formed left sacral diapophysis. D–E) Comparisons between two specimens of *Microhyla nepenthicola*: D) Vertebral column and pelvis of *M. nepenthicola* ZMH A11647 without obvious anomalies; E) Vertebral column and pelvis of *M. nepenthicola* ZMH A11645 with a left side transverse process at the anterior face of the sacral vertebra and vertebral count is only six. The fusion of Presacral I+II and a total of seven vertebrae was found in four specimens of this species and is considered normal for this miniature species. All specimen representations are dorsal views of specimens, images rendered from polymesh surfaces derived from computed tomography datasets; cropped from original data to vertebral column and pelvic regions.

biguously recognized as anomalies. In cases of symmetrical or serial anomalies (vertebra number), more caution is in place. The data presented may be considered preliminary until larger series are examined. Consultation of the literature on the morphology of a species or its close relatives is essential to assess the normal condition.

The symmetry, serial patterns, and development of the vertebral column and ilio-sacral complex in anurans have been well-documented in a broad range of studies that can serve as references for the norm of the anuran body plan (e.g., Gaupp 1896, Lynch 1973, Wiens 1989, Wild 1997, Maglia & Pugener 1998, Haas 1999, Trueb et al. 2000, Sheil & Alamillo 2005, Banbury & Maglia 2006, Handrigan & Wassersug 2007, Pugener & Maglia 2009a,b, Hoyos et al. 2012, Kovalenko & Kruzhkova 2013b, Vera & Ponssa 2013, Biton et al. 2016, Soliz & Ponssa 2016, Senevirathne et al. 2020). Although Shearman & Maglia (2014) concluded that the available accounts on anuran skeletal morphology cover only a small fraction of the known number of species (Frost 2020), many of the anuran subclades are actually covered by reports of exemplar species. Backed by this background knowledge, misidentification and confusion of a norm character in the vertebral column for an anomaly, or vice versa, is unlikely in extant frogs (but see Maglia & Pugener (1998) below). Caution, however, is advised in general in applying axial skeleton features derived from single specimens in classification (Trueb 1977). Abnormal symmetrical features in single anuran fossils may overlap phenomenologically with normal morphologies during the processes of skeletal modification in early frog evolution, for example in the reduction of the number of vertebral elements in the trunk and the tail (Estes & Reig 1973, Lynch 1973, Handrigan & Wassersug 2007).

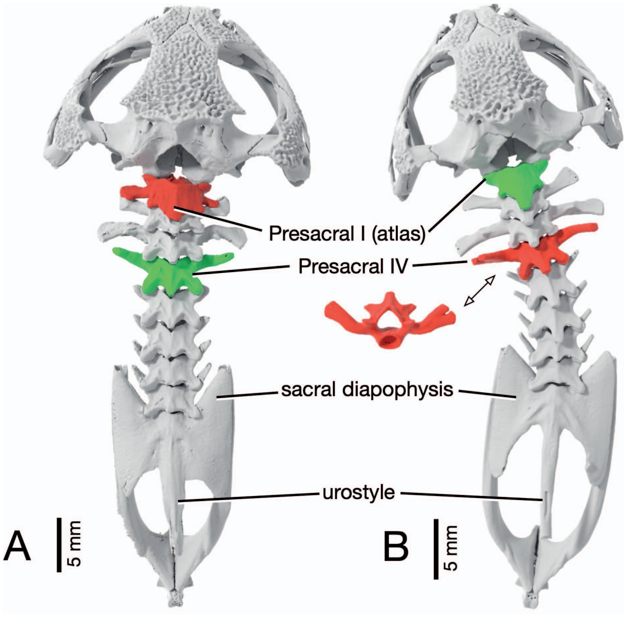

Figure 5. Two specimens of *Pelobates fuscus*: A) The atlas of *Pelobates fuscus* ZMH A07151 developed transverse processes that articulate with Presacral II; B) *Pelobates fuscus* UF Herp 36935 possesses an atlas without transverse processes and Presacral IV forms a bifurcated left transverse process. Dorsal views of partial renditions (skull, vertebral column, pelvis); Presacral VI in B separated and shown in anterior view; polymesh surfaces derived from computed tomography datasets; anomalous elements red, normal elements green.

Unfortunately, the prevalence of axial skeleton anomalies in natural populations has not received much attention in the literature. Madej (1965) and Kovalenko & Kruzhkova (2013b) to our knowledge provided the most comprehensive assessment of intraspecific variations and anomalies in anuran species. Madej (1965) examined 555 skeletons of *Bombina bombina* and 813 skeletons of *B. variegata*, whereas Kovalenko & Kruzhkova (2013b) assessed anomalies of the vertebral column and ilio-sacral joint in 1633 lab-reared offspring of one mating pair of *Bufo bufo*. Both studies categorized the observed phenomena and presented quantitative data on the intraspecific prevalence in the respective sample examined. The anomalies we could detect rather haphazardly in our study with taxa across the anuran order all were subsets of anomaly categories described in *Bombina* and *Bufo* by these authors; new categories were not discovered.

The scans downloaded from MorphoSource and our own CT-scanning of individuals across the Anura uncovered 17 individuals (13%) with axial anomalies (Table 1) in a total sample of 125 examined specimens belonging to 73 species. Madej (1965) detected an overall rate of anomalies of 10% in a large sample of *Bombina*. Meza-Joya et al. (2013) reported 22% individuals with anomalies in the vertebral column in a captivity-reared sample of 58 embryos and 96 postembryonic individuals of *Eleutherodactylus johnstonei*, a species with direct development. This is, to our knowledge, the highest known percentage. The axial malformations they observed included fusion of Presacrals I+II, fusion of Presacral VIII to the sacral vertebra (IX), development of postzygapophyses at the sacral vertebra, and extra postsacral vertebral elements (half or full).

Dasgupta & Grewal (1970) described vertebral fusion in *Euphlyctis cyanophlyctis* and found 8–12% of the population affected. Most cases of fusion occurred between Presacrals I and II, as well as VIII and IX; fewer incidences were observed in the middle section of the vertebral column. Pugener & Maglia (2009b) found that more than 8.5% (11 out of 129 *Acris creptitans* specimens) of the specimens in their study had vertebral anomalies, and that approximately 50% showed minor variation from the typical morphology, that, however, were not classified as anomalies.

In the larger scale studies, intraspecific variation of vertebral number was detected as a relatively rare event: 3.06% and 2.51% in *Bombina* and *Bufo bufo*, respectively (Madej 1965, Kovalenko & Kruzhkova 2013b). Ritland (1955) noted less than 3% unilateral sacralization of Presacral IX in *Ascaphus truei*, a species with ten vertebrae. Malformations of variable degree in the coccygeal part of the urostyle, its lateral processes and zygapophyses, however, were common (more than a third of specimens). In her study on 58 specimens of *Boana lanciformis* (then *Hyla lanciformis*), Trueb (1977) reported vertebral anomalies in 9.4% of the adult specimens. Greer & Byrne (2007) observed one case (2.4%) of sacralization of the posterior presacral vertebra in their sample of *Litoria aurea*. Sacralization of the presacral vertebra is commonly described in reports we found and has been reported early on (Morgan 1886); our findings are in support of this general statement (Figs 2–4).

Anomalies at the sacrum and its neighbouring segments have been described frequently (for example, Ritland 1955, Madej 1965, Kovalenko & Danilevskaya 1994, Kovalenko 1995, Pugener & Maglia 2009b, Kovalenko & Kruzhkova 2013b; Figs. 2–4); anomalies in the anterior part of the vertebral column, in contrast, appear to be less frequent. The most anterior anomalies observed in our sample involved the atlas: Presacrals I+II were fused (synostosis) in *Barbourula busuangensis* CAS-SUA 21247 (Fig. 1A) and in *Bombina orientalis* ZMH A14350, while the atlas of *Pelobates fuscus* ZMH A07151 developed transverse processes (Fig. 5A). The normal vertebral characteristics in the two known species of *Barbourula* (*B. busuangensis* and *B. kalimantanensis*) have been described in Clarke (1987). Interestingly, along with the anterior synostosis, *B. busuangensis* CAS-SUA 21247 had additional malformations in the posterior vertebrae and the urostyle. Multiple malformations in one individual have been reported previously (for example, Madej 1965, Trueb 1977, Maglia & Pugener 1998). In the study on *Bombina* (Madej 1965), a fusion of Presacrals I+II was not explicitly illustrated. Only the total number of vertebrae (including the sacral vertebra) was reported to vary from eight to ten. We, however, observed fusion of Presacrals I+II in *B. orientalis* ZMH A14350. Interestingly, Maglia & Pugener (1998) in their work on the skeletal development and adult osteology of *B. orientalis*, depicted the axial skeleton in the adult KU 129703. The drawing shows the specimen with fusions of Presacrals I+II as well as VII+VIII, and an asymmetric development of the basal lateral processes of the urostyle. This description is also remarkably congruent to the condition in *Barbourula busuangensis* CAS-SUA 21247 examined herein, that belongs to the same anuran family (Bombinatoridae). Although KU 129703 (in Maglia & Pugener 1998) clearly was aberrant from the normal development of the axial skeleton in *Bombina* (Madej 1965), its unusual condition was not further expanded on in that paper. Anomalous fusions of Presacrals I+II have been reported, among others, in some individuals of *Denrophryniscus brevipollicatus* (Izecksohn 1971), *Bufotes viridis* (Adolphi 1892; then *Bufo variabilis*), and *Pelobates fuscus* (Adolphi 1895).

The reduction in number of vertebrae has played a major role in frog evolution (Lynch 1973), particularly the evolution of the jumping locomotor mode. The stem-group fossil *Triadobatrachus massinoti* possessed more presacral (trunk) vertebrae and shorter ilia (Rage & Roček 1989, Roček & Rage 2000, Ascarrunz et al. 2016) than any crown group frogs (Trueb 1973, Haas 2003, Frost et al. 2006, Handrigan & Wassersug 2007). A reduction of the number of trunk vertebrae, and the concomitant (or possibly subsequent) lengthening of the ilia and reduction of tail vertebra, has been a major process in the evolution of modern frogs and their jumping ability (Gans & Parsons 1966, Shubin & Jenkins 1995, Jenkins & Shubin 1998, Přikryl et al. 2009, Sigurdsen et al. 2012, Ascarrunz et al. 2016). The most recent common ancestor of frogs has been hypothesized to have possessed nine presacral vertebrae (Ford & Cannatella 1993). Among extant species nine presacrals are only present in the Leiopelmatidae and Ascaphidae, followed by a reduction to eight or less in other extant groups (Haas 2003, Frost et al. 2006). Further reductions in vertebral number have been reported in a diversity of frog groups, among others in some species of the Brachycephalidae, Calyptocephalellidae, Ceratophryidae, Craugastoridae, Dendrobatoidea, Pipidae, Bufonidae, Ptychadenidae, or Myobatrachidae (Stannius 1856, Izecksohn 1971, Trueb 1973, Cannatella & Trueb 1988, Baez & Pugener 2003, Guayasamin 2004, Frost et al. 2006, Muzzopappa et al. 2015).

Reductions of vertebrae in taxa in crown-group anurans have evolved in different ways. For example, the evolution of seven presacral vertebrae in *Pipa* has been explained by fusion of anterior vertebrae (Trueb et al. 2000) whereas the integration of the most posterior presacral vertebra into the sacrum has been suggested to have resulted in seven presacral vertebrae in the closely related *Hymenochirus* and *Pseudhymenochirus* (Cannatella & Trueb 1988) or the bufonid *Dendrophryniscus* (Izecksohn 1971). For one of the smallest frogs in the world, *Microhyla nepenthicola*, we report here the complete fusion of atlas and second vertebra into one element as the species' norm character. One specimen (ZMH A11645, Fig. 4E) had the last presacral incompletely developed and fused to the sacral vertebra as an anomaly, resulting in only six articulating presacral elements. In a sample of four specimens, two possessed axial anomalies in this highly miniaturized species. The hypothesis that miniaturization might be linked to vertebral count reduction and developmental instability deserves future attention. Adolphi (1892) already concluded that events that we classify as anomalies in some species may correspond to the norm character in other taxa acquired in the course of evolution (see also Izecksohn 1971).

The anuran urostyle has been studied thoroughly and its development has been clarified in various taxa (e.g., Hodler 1949, Branham & List 1979, Wiens 1989, Močkova & Roček 2005, Handrigan & Wassersug 2007, Pugener & Maglia 2009a, Kruzhkova & Kovalenko 2010; Meza-Joya et al. 2013, Muzzopappa et al. 2015, Senevirathne et al. 2020). Variation or externally induced disturbances (Madej 1965) in the developmental program of the ontogenetically and evolutionarily crucial posterior trunk boundary (Handrigan & Wassersug 2007) have been hypothesized to be associated with anomalies in that region that occurred in the single digit range percentage (Kovalenko & Kruzhkova 2013b). A noteworthy case of perfectly normal development of multiple postsacral vertebrae, however, has been reported in megophryid tadpoles (Haas et al.

2006, Handrigan et al. 2007). Megophryid tadpoles possess up to 30 ossified centra in pre-metamorphic stages.

Axial skeleton anomalies can be induced by external agents (see review in Henle et al. 2017). Pesticides have been shown to cause vertebral scoliosis possibly by influence on the vitamin D regulated pathways (Alvarez et al. 1995). Yet, the genetic backgrounds and developmental mechanisms behind vertebral anomalies remain largely unresolved in frogs (Handrigan & Wassersug 2007). It is intriguing to ask, if vertebral developmental instability is linked to phenotypic plasticity in other traits and thereby (indirectly) positively selected for in certain environments and times (DeWitt et al. 1998). In *Euphlyctis cyanophlyctis,* one dominant gene has been linked to vertebral fusion and a higher temperature tolerance during development as potential selective advantage for heterozygosity (Dasgupta & Grewal 1968, 1970). The anterior shift of *Hox10* gene expression domain defining the trunk-tail boundary (sacrum) (Handrigan & Wassersug 2007) could play a role in both the evolutionary reduction of presacral vertebrae in some taxa and the occurrence of anomalies in the posterior axial region in other taxa if that boundary is perturbed. The surprisingly high number of anomalies in our survey and the data from the literature suggests that vertebral anomalies are not rare in anurans. This could suggest that they may have little effect on performance and are not heavily selected against.

Trunk-tail boundary pertubations probably played a role in fossil taxa as well and anomalies have been reported from ancient amphibians (Witzmann 2007, Witzmann et al. 2013). Vertebral anomalies have been well known from other vertebrate groups, such as salamanders (e.g., Pogoda & Kupfer 2019; Danto et al. 2020), humans (for example, Beals et al. 1993) or fishes (Jawad 2017). The phenomenologies may be similar across vertebrate groups. Wedge vertebrae that develop on one side of the spine only, as observed in *Bombina variegata* ZMH A11873, and sacralization of the presacral vertebra(e) are relatively common in humans (Werenskiold 1937, Beals et al. 1993, McMaster 1998).

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Supplementary data

The following data are available online:

Supplementary document 1. List of specimens / datasets examined for this study.