



# Vertebral anomalies in a natural population of *Taricha granulosa* (Caudata: Salamandridae)

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## Abstract

Developmental plasticity, a common pattern in lissamphibian evolution, results in numerous alternative morphologies among species and also within populations. In the present study, a natural population of the salamander *Taricha granulosa* (Salamandridae) was examined to detect variation in the vertebral count and to identify potential deformities of their vertebral column. The number of trunk vertebrae varied between 11 and 13 and we recorded 58 individuals with 69 anomalous vertebral elements. These anomalies range from congenital malformations (block vertebrae, unilateral bars, hemivertebrae), extra ossifications in the haemal region, to posttraumatic pathologies. Most osseous pathologies were encountered in the caudal region of the axial skeleton. Our data suggest a high frequency of vertebral malformations in salamanders; however, the identification of the exact causes remains challenging.

**Keywords** Lissamphibians · Vertebral column · Development · Congenital malformations · Tail duplication

## Introduction

The vertebral column forms the longitudinal axis of the body and is composed of serially repeated vertebrae. In salamanders, the vertebral column consists of five regions (cervical – trunk – sacral – caudal-sacral – caudal) and the vertebrae may vary in size and shape within these regions (Wake 1966; Wake and Dresner 1967). A typical vertebra of the trunk region, for example, consists of a cylindrical vertebral body (or centrum) and a neural arch, which surrounds and protects the spinal cord dorsally (Fig. 1a). The neural arch has several individual processes that provide articular support and attachments for ligaments and muscles. All caudal vertebrae possess an additional haemal arch that extends ventrally around the caudal artery and vein.

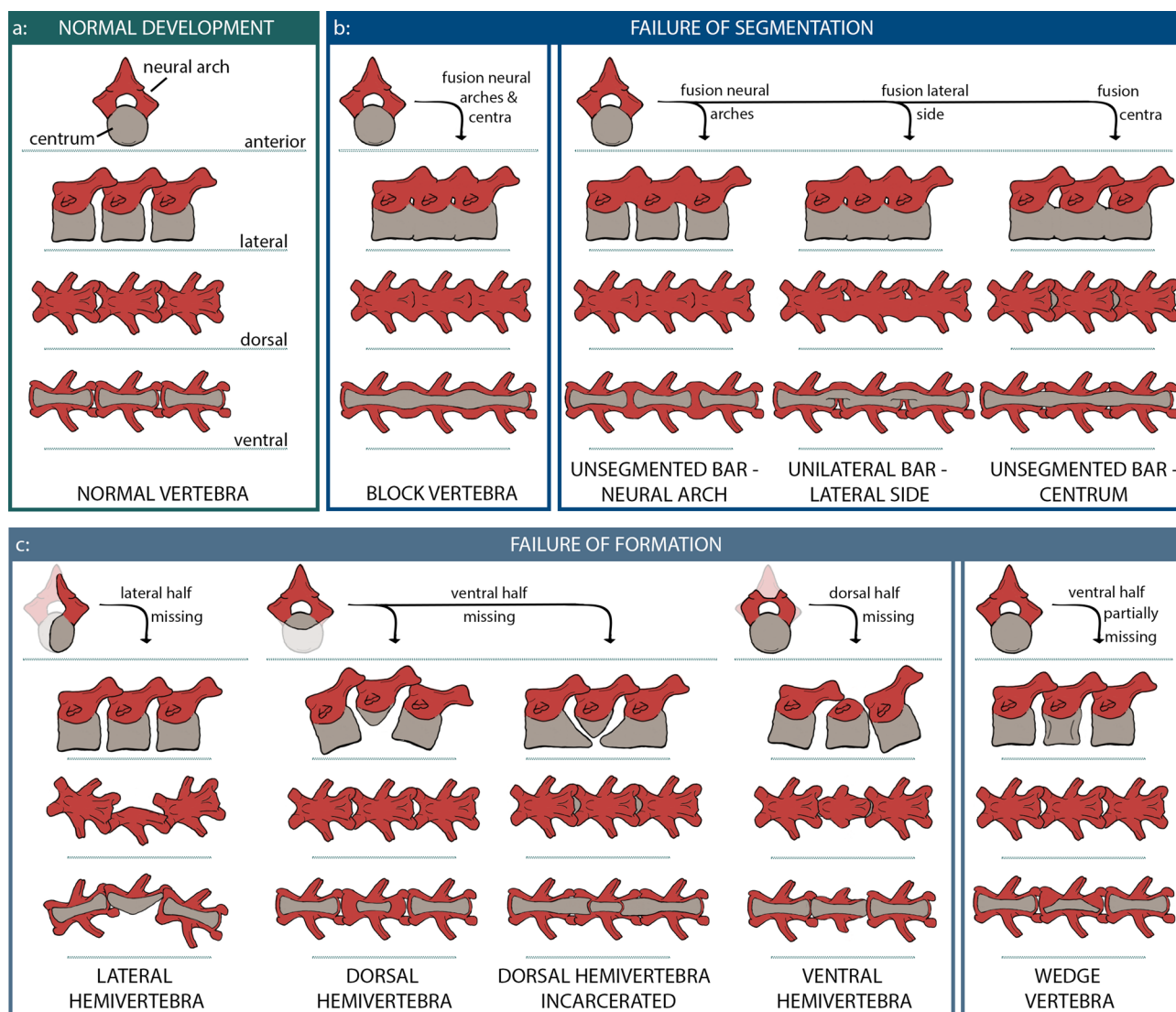
Congenital malformations within the axial skeleton of vertebrates are common and encompass deformities of the

vertebral elements, changes in the number of vertebral elements, and can include axial distortion (McMaster 1998; Erol et al. 2002, 2004; Kaplan et al. 2005; Witzmann et al. 2021). Congenital vertebral malformations are a byproduct of anomalous vertebral development in the embryo, specifically during somitogenesis, in which serially repeating blocks of cells, the somites, are formed bilaterally along the neural tube (Pourquié and Kusumi 2001; Erol et al. 2002). In the literature, congenital vertebral malformations have been classified into three categories: (1) failure of segmentation, (2) failure of formation, and (3) neural tube defects (Nasca et al. 1975; McMaster and Singh 1999; Jaskwlich et al. 2000; Kaplan et al. 2005). The first classification category (“failure of segmentation”) occur when two adjacent somites fail to divide properly during development, which results in two or more vertebrae that are fused together (Erol et al. 2002; Kaplan et al. 2005). Such a congenital block vertebra is characterized by a complete or bilateral failure of segmentation of both neural arch and vertebral centrum (Fig. 1b). A partial or unilateral failure of segmentation leads to a unilateral bar whereby the fusion can either involve the neural arches, the vertebral centra or solely one lateral half of the vertebrae (Fig. 1b) (Gutierrez-Quintana et al. 2014). The second classification category (“failure of formation”) consists of vertebral elements, in which a portion of the vertebra completely fails to form (Kaplan et al. 2005; Dias 2007).

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**Fig. 1** Examples of congenital vertebral malformations. **a** Normally developed vertebrae in anterior, lateral, dorsal, and ventral view. **b** Failure of segmentation which results in a congenital block ver-

tebra or unsegmented bar. **c** Failure of formation which results in a hemivertebra or wedge vertebra. Red = neural arch; grey = centrum

Thereby, any region of the vertebra may be affected: dorsal (i.e. neural arch), ventral (i.e. vertebral centrum), lateral (i.e. right or left side of the vertebra) (Fig. 1c). Hemivertebrae are most common and characterized by the lack of formation of one half of the vertebral element and they can be fully segmented (normal disc space anteriorly and posteriorly), semi-segmented (fusion with either anterior or posterior vertebra), incarcerated (both anterior and posterior vertebra conform in shape) or non-segmented (fusion with anterior and posterior vertebra) (Witzmann et al. 2008; Johal et al. 2016). Other types of vertebral malformations attributed to perturbed formation include wedge vertebrae (partial failure of formation of one side; Fig. 1c) and butterfly vertebrae (failure of fusion of the lateral halves of the centrum due to

persistent notochordal tissue) (Jaskwlich et al. 2000; Hopkins and Jh 2015; Chaturvedi et al. 2018; Katsuura and Kim 2019). Frequently, combined deformities are observed (e.g. a unilateral bar with hemivertebra) and the defects of formation and segmentation can result in simple to complex structural and neurologic disorders (Kaplan et al. 2005). The third classification category (“neural tube defects”) comprises congenital malformations, in which the neural tube fails to completely close during embryonic development (Moore and Persaud 1998; Greene and Copp 2014; Kaplan et al. 2005). Different variants exist, ranging from a mild form in which the spinal cord and surrounding tissue do not protrude (spina bifida occulta) to a more severe form with exposed and damaged spinal nerves (myeloschisis). The

specific causes are still unknown, but different risk factors such as folate deficiency, obesity, medications, and poorly controlled diabetes have been documented for humans and other mammals.

The majority of reports of congenital vertebral malformation are based on clinical studies of humans (among many others: McMaster 2001; Erol et al. 2002, 2004; Green and Copp 2014; Katsura and Kim 2019) and domestic mammals (Wong et al. 2005; Westworth and Sturges 2010; Schlensker and Dislt 2013; Gutierrez-Quintana et al. 2014). Developmental anomalies such as supernumerary limbs, the absence of limbs (i.e. amely), and increased numbers of fingers and toes (i.e. polydactyly) are relatively common in lissamphibians and have been reported in the scientific literature for more than 200 years (among many others: De Superville 1740; Meteyer 2000; Ouellet 2000; Johnson et al. 2002; Blaustein and Johnson 2003; Lunde and Johnson 2012; Rothschild et al. 2012; Silva-Soares and Mônico 2017). Reports on developmental deformities of the spine, however, are less frequent as they can only be diagnosed via radiographic examination, dissection, histological sections or different clearing and staining techniques (Adolphi 1893, 1895, 1898; Trueb 1977; Martinez et al. 1992; Alvarez et al. 1992, 1995; Gamble et al. 2005; Maglia et al. 2007; Park et al. 2010; Pugener and Maglia 2009; Perpiñán et al. 2010; Buckley et al. 2013; Liu et al. 2016; Danto et al. 2020).

In this study, we describe different types of vertebral pathologies found in a natural population of *Taricha granulosa*, including block vertebrae, unilateral bars and hemivertebrae.

## Materials and methods

In 1991, more than 500 individuals of *Taricha granulosa* (Caudata: Salamandridae) were collected in Marin County, California (Shubin et al. 1995). All specimens belong to a single population, which died in a mass kill (sudden and complete freeze of a small, shallow pond). At that time, following the protocol of Hanken and Wassersug (1981), the material was cleared and double stained to visualize the chondrification and ossification of the skeleton. The material is catalogued in the collection of the Museum of Vertebrate Zoology at UC Berkeley. In the present study, we examined the vertebral columns of 459 specimens. All specimens are considered to be postmetamorphic (determined by the distinct morphology of the hyobranchium, after Rose (2003)), and can be divided into 422 male and 37 female specimens. Salamandridae is a large family of small to medium sized salamanders that are distributed across Europe, North America, and East Asia (Duellman and Trueb 1994). The life cycle of *Taricha granulosa* can be described as follows: the fully aquatic larvae metamorphose into terrestrial juveniles

and adults. The adults migrate seasonally between terrestrial and aquatic habitats, whereby the specimens undergo substantial morphological changes during the breeding season (AmphibiaWeb, February 24th, 2020). The material was examined with a Zeiss Stemi 1000 Stereo Microscope using ordinary transmitted light. Images were taken with a Plugable USB2-MICRO-250X Digital Microscope.

## Results

In all 459 specimens, the vertebral column was examined and both vertebral number and shape of the individual vertebrae were documented. In three specimens, the specific number of vertebral elements could not be determined, as the atlas was missing. In the remaining 456 specimens, the vertebral column (neural arches, vertebral centra, and haemal arches) was fully ossified from the cranial to the caudal end. We did not observe variation in the number of cervical vertebrae, as *Taricha granulosa*, is characterized by a single vertebra in this region. The number of trunk vertebra was either eleven (in one female and six males), twelve (in 35 females and 398 males), or 13 (in one female and 15 males). The sacral region consisted of a single vertebra in all 459 individuals.

From a total of 459 specimens, 58 individuals (i.e. 12.64%) had osseous pathologies along the vertebral column. Most individuals ( $n = 50$ ) exhibited a single pathology, but six specimens had two pathologies, one specimen had three pathologies, and one specimen had four. In total, 69 anomalous vertebral elements were documented (Table 1). The majority of anomalous vertebrae were located in the postsacral region (57 individuals) and both males (53 individuals) and females (5 individuals) exhibited vertebral deformities.

### Failure of segmentation

We identified 26 individuals of *Taricha granulosa* with 29 defects of segmentation (Table 1). In the majority of these specimens, the fusion is complete involving the neural arches, centra, and haemal arches thereby creating a congenital block vertebra. The abnormal vertebrae are elongated along the anterior–posterior axis, but their heights do not deviate from the normal. The articulation to the adjacent vertebrae is intact, and the zygapophyses are well formed (Fig. 2a). Block vertebrae of the trunk and anterior caudal region are characterized by the possession of two, well-developed transverse processes (Fig. 2b). However, because the size of the transverse processes gradually diminishes along the tail, this feature becomes less visible in block vertebrae located in the posterior region of the tail.

**Table 1** List of specimens with anomalous vertebral elements

Collection number	Sex	Vertebral count				In total	Observed deformity	Vertebra affected	Type of pathology
		Cerv	Trun	Sacr	Caud				
MVZ 216158	Female	1	12	1	32	<b>46</b>	Reduced neural arch	#30 (caudal vertebra)	Ventral hemivertebra
MVZ 216160	Female	1	12	1	45	<b>59</b>	Extra-ossification in the haemal region	#35 (caudal vertebra)	Supernumerary bone
MVZ 216161	Female	1	12	1	48	<b>62</b>	Extra-ossification in the haemal region	#42 (caudal vertebra)	Supernumerary bone
MVZ 216164	Female	1	12	1	43	<b>57</b>	Fusion of two vertebrae	#43 (caudal vertebra)	Block vertebra
MVZ 216175	Female	1	12	1	49	<b>63</b>	Reduced neural arch	#35 (caudal vertebra)	Ventral hemivertebra
MVZ 216195	Male	1	12	1	46	<b>60</b>	Fusion of lateral side of two vertebrae	#40 (caudal vertebra)	Unilateral bar—lateral side
MVZ 216202	Male	1	12	1	42	<b>56</b>	Extra-ossification in the haemal region	#43 (caudal vertebra)	Supernumerary bone
MVZ 216221	Male	1	12	1	43	<b>57</b>	Reduced lateral side of vertebra	#47 (caudal vertebra)	Lateral hemivertebra
MVZ 216243	Male	1	12	1	41	<b>55</b>	Fusion of haemal arches of two vertebrae	#35 (caudal vertebra)	Unsegmented bar—ventral side
MVZ 216244	Male	1	12	1	46	<b>60</b>	Fusion of two vertebrae fusion and large swelling of bone	#41 (caudal vertebra) #25—#26 (caudal vertebra)	Block vertebra Posttraumatic pathology
MVZ 216247	Male	1	12	1	40	<b>54</b>	Reduced haemal arch Abnormal shape and large swelling of bone	#45 (caudal vertebra) #36 (caudal vertebra)	Dorsal hemivertebra Posttraumatic pathology
MVZ 216256	Male	1	12	1	40	<b>54</b>	Fusion of two vertebrae	#28 (caudal vertebra)	Block vertebra
MVZ 216258	Male	1	12	1	46	<b>60</b>	Reduced neural arch	#39 (caudal vertebra)	Ventral hemivertebra
MVZ 216262	Male	1	12	1	43	<b>57</b>	Reduced haemal arch	#34 (caudal vertebra)	Dorsal hemivertebra
MVZ 216268	Male	1	12	1	37	<b>51</b>	Fusion of two vertebrae	#49 (caudal vertebra)	Block vertebra
MVZ 216288	Male	1	12	1	42	<b>56</b>	Abnormal shape and large swelling of bone	#37 (caudal vertebra)	Posttraumatic pathology
MVZ 216292	Male	1	12	1	40	<b>54</b>	Fusion of two vertebrae	#33 (caudal vertebra)	Block vertebra
MVZ 216295	Male	1	12	1	38	<b>52</b>	Reduced haemal arch	#38 (caudal vertebra)	Dorsal hemivertebra
MVZ 216297	Male	1	12	1	41	<b>44</b>	Fusion of two vertebrae	#51 (caudal vertebra)	Block vertebra
MVZ 216299	Male	1	12	1	44	<b>58</b>	Fusion of two vertebrae Reduced neural arch	#36 (caudal vertebra) #37 (caudal vertebra)	Block vertebra Ventral hemivertebra
MVZ 216302	Male	1	12	1	40	<b>54</b>	Fusion of two vertebrae	#45 (caudal vertebra)	Block vertebra
MVZ 216312	Male	1	12	1	44	<b>58</b>	Extra-ossification in the haemal region	#54 (caudal vertebra)	Supernumerary bone
MVZ 216324	Male	1	12	1	39	<b>53</b>	Fusion of two vertebrae	#42 (caudal vertebra)	Block vertebra
MVZ 216335	Male	1	12	1	44	<b>58</b>	Reduced haemal arch	#30 (caudal vertebra)	Dorsal hemivertebra
MVZ 216339	Male	1	12	1	42	<b>56</b>	Fusion of two vertebrae Reduced haemal arch	#31 (caudal vertebra) #33 (caudal vertebra)	Block vertebra Dorsal hemivertebra
MVZ 216348	Male	1	12	1	37	<b>51</b>	Fusion of two vertebrae	#20 (caudal vertebra)	Block vertebra
MVZ 216355	Male	1	12	1	43	<b>57</b>	Fusion of two vertebrae	#44 (caudal vertebra)	Block vertebra
MVZ 216358	Male	1	12	1	45	<b>59</b>	Fusion of haemal arches of two vertebrae	#42 (caudal vertebra)	Unsegmented bar—ventral side
MVZ 216359	Male	1	12	1	43	<b>57</b>	Reduced neural arch Fusion of two vertebrae	#37 (caudal vertebra) #54 (caudal vertebra)	Ventral hemivertebra Block vertebra
MVZ 216361	Male	1	12	1	43	<b>57</b>	Fusion of two vertebrae	#52 (caudal vertebra)	Block vertebra
MVZ 216367	Male	1	12	1	40	<b>54</b>	Fusion of two vertebrae	#24 (caudal vertebra)	Block vertebra
MVZ 216370	Male	1	12	1	36	<b>50</b>	Fusion of two vertebrae Reduced haemal arch Reduced neural arch	#26 (caudal vertebra) #27 (caudal vertebra) #32 (caudal vertebra)	Block vertebra Dorsal hemivertebra Ventral hemivertebra

**Table 1** (continued)

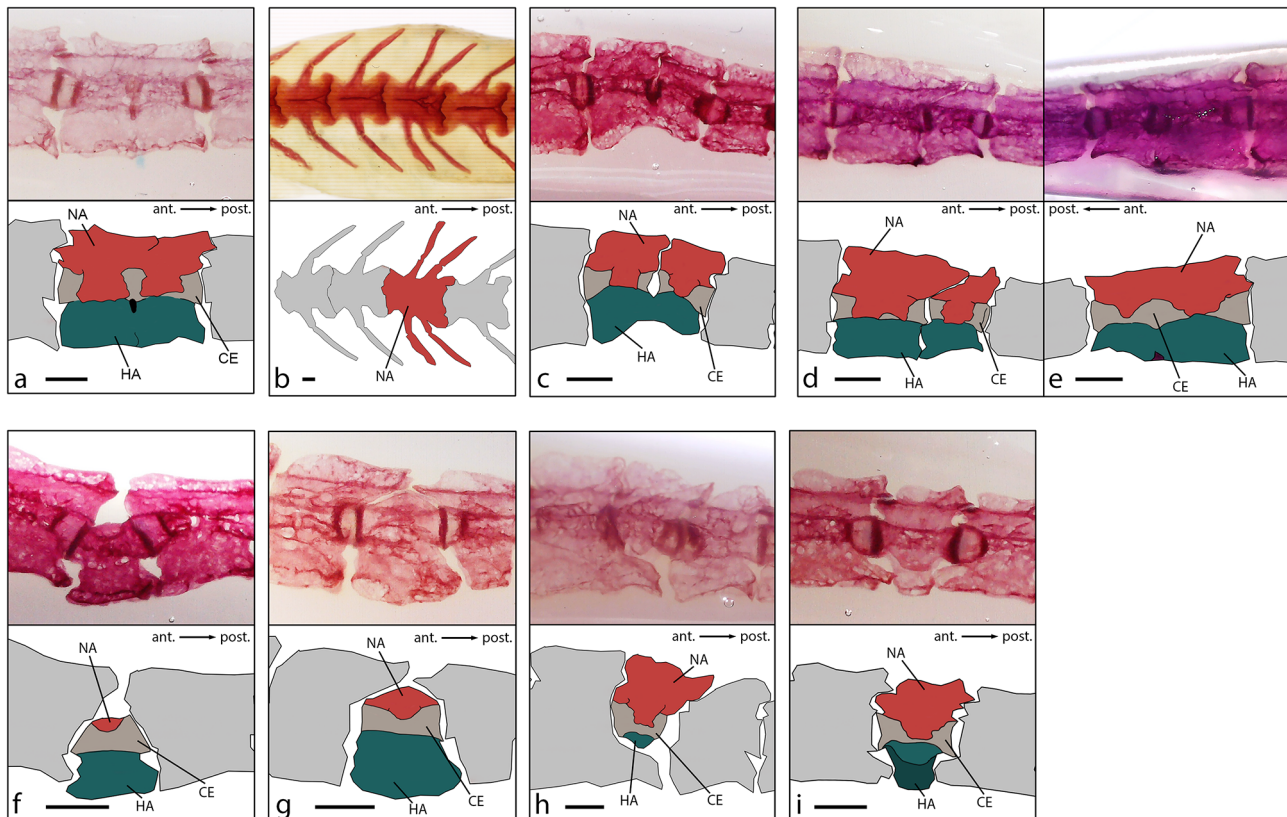
Collection number	Sex	Vertebral count				In total	Observed deformity	Vertebra affected	Type of pathology
		Cerv	Trun	Sacr	Caud				
MVZ 216371	Male	1	12	1	42	<b>56</b>	Reduced haemal arch	#39 (caudal vertebra)	Dorsal hemivertebra
MVZ 216388	Male	1	12	1	44	<b>58</b>	Reduced neural arch	#44 (caudal vertebra)	Ventral hemivertebra
MVZ 216391	Male	1	12	1	47	<b>61</b>	Reduced neural arch	#43 (caudal vertebra)	Ventral hemivertebra
MVZ 216401	Male	1	12	1	46	<b>60</b>	Reduced neural arch	#43 (caudal vertebra)	Ventral hemivertebra
MVZ 216424	Male	1	12	1	41	<b>55</b>	Reduced haemal arch	#30 (caudal vertebra)	Dorsal hemivertebra
MVZ 216428	Male	1	12	1	46	<b>60</b>	Reduced neural arch	#43 (caudal vertebra)	Ventral hemivertebra
MVZ 216466	Male	1	12	1	40	<b>54</b>	Reduced neural arch	#45 (caudal vertebra)	Ventral hemivertebra
MVZ 216468	Male	1	13	1	34	<b>49</b>	Enlarged haemal arch	#32 (caudal vertebra)	Posttraumatic pathology
MVZ 216472	Male	1	12	1	40	<b>54</b>	Reduced neural arch	#47 (caudal vertebra)	Ventral hemivertebra
MVZ 216480	Male	1	12	1	45	<b>59</b>	Fusion of two vertebrae	#31 (caudal vertebra)	Block vertebra
MVZ 216489	Male	1	12	1	35	<b>49</b>	Fusion and large swelling of bone	#37 (caudal vertebra)	Posttraumatic pathology
MVZ 216490	Male	1	12	1	49	<b>63</b>	Reduced haemal arch	#44 (caudal vertebra)	Dorsal hemivertebra
MVZ 216500	Male	1	12	1	44	<b>58</b>	Reduced neural arch	#46 (caudal vertebra)	Ventral hemivertebra
MVZ 216511	Male	1	12	1	38	<b>52</b>	Extra ossification in the haemal region	#21 (caudal vertebra)	Supernumerary bone
MVZ 216514	Male	1	12	1	38	<b>52</b>	Fusion of two vertebrae	#31 (caudal vertebra)	Block vertebra
MVZ 216526	Male	1	12	1	44	<b>58</b>	Fusion of two vertebrae	#15 (caudal vertebra)	Block vertebra
MVZ 216529	Male	1	12	1	41	<b>55</b>	Reduced neural arch	#38 (caudal vertebra)	Ventral hemivertebra
MVZ 216530	Male	1	12	1	41	<b>55</b>	Reduced haemal arch	#27 (caudal vertebra)	Dorsal hemivertebra
MVZ 216555	Male	1	12	1	39	<b>53</b>	Fusion of two vertebrae	#42 (caudal vertebra)	Block vertebra
MVZ 216558	Male	1	12	1	43	<b>57</b>	Fusion of two vertebrae	#30 (caudal vertebra)	Block vertebra
MVZ 216561	Male	1	12	1	42	<b>56</b>	Reduced haemal arch	#26 (caudal vertebra)	Dorsal hemivertebra
MVZ 216565	Male	1	12	1	42	<b>56</b>	Reduced neural arch	#35 (caudal vertebra)	Ventral hemivertebra
MVZ 216565	Male	1	12	1	43	<b>57</b>	fusion of three vertebrae	#28 (caudal vertebra)	Unsegmented bar—dorsal side
MVZ 216571	Male	1	11	1	40	<b>53</b>	Fusion of two vertebrae	#31 (caudal vertebra)	Block vertebra
MVZ 216572	Male	1	12	1	42	<b>56</b>	Reduced haemal arch	#40 (caudal vertebra)	Dorsal hemivertebra
MVZ 216579	Male	1	12	1	41	<b>55</b>	Fusion of two vertebrae	#42 (caudal vertebra)	Block vertebra
MVZ 216579	Male	1	12	1	41	<b>55</b>	Fusion of two vertebrae	#09 (trunk vertebra)	Block vertebra
MVZ 216579	Male	1	12	1	41	<b>55</b>	Reduced neural arch	#47 (caudal vertebra)	Ventral hemivertebra
MVZ 216613	Male	1	12	1	42	<b>56</b>	Fusion of neural arches of two vertebrae	#32 (caudal vertebra)	Unsegmented bar—dorsal side
MVZ 216613	Male	1	12	1	42	<b>56</b>	Reduced neural arch	#33 (caudal vertebra)	Ventral hemivertebra

Abbreviations: *caud* caudal, *cerv* cervical, *sacr* sacral, *trun* trunk

A small number of specimens exhibited unilateral unsegmented bars, e.g. the fusion of the vertebrae is incomplete and only includes a specific region of the vertebral element. Similar to the congenital block vertebrae, the partially unsegmented bars are elongated along the anterior–posterior axis compared to normally developed vertebrae. Different morphological variants were observed: in two specimens, solely the haemal arches were fused (Fig. 2c), in one specimen, the lateral side of the abnormal vertebra was fused (Fig. 2d and e), and in two specimens, the neural arches and centra were fused, but not the haemal arches.

### Failure of formation

We identified 29 individuals of *Taricha granulosa* with 30 defects of formation (Table 1). Numerous specimens are characterized by abnormal development of the neural arches (ventral hemivertebra). We observed a wide range of morphological variants. However, in all specimens, the malformed vertebra is fully segmented. In some specimens, the neural arch is poorly developed and the specific vertebra consists mainly of the vertebral centrum and haemal arch (Fig. 2f). Frequently, the preceding and succeeding neural arches conform in shape and incarcerate the anomalous structure (Fig. 2g). In other specimens, the neural arch is



**Fig. 2** Examples of defects of segmentation and defects of formation along the vertebral column of *Taricha granulosa*. **a** Congenital block vertebra in the caudal region of MVZ 216292, in lateral view. **b** Congenital block vertebra in the trunk region of MVZ 216571, in lateral view. **c** Unsegmented bar in the caudal region of MVZ 216358, in lateral view. **d** and **e** Unilateral bar in the caudal region of MVZ 216195, from the left and right lateral view, respectively. **f** Ventral

hemivertebra in the caudal region of MVZ 216391, in lateral view. **g** Ventral hemivertebra in the caudal region of MVZ 216428, in lateral view. **h** Dorsal hemivertebra in the caudal region of 216262, in lateral view. **i** Dorsal hemivertebra in the caudal region of 216295, in lateral view. Scale bars equal 1 mm. Abbreviations: *ant* anterior, *CE* centrum, *HA* haemal arch, *NA* neural arch, *post* posterior

incompletely ossified but still characterized by its paired, contralateral halves. The neural spine is absent or very low and pre- and postzygapophyses are often missing. The remaining specimens display a partial failure of development of the neural arches, meaning that one lateral half of the neural arch is poorly differentiated and reduced in size.

Abnormal development of the haemal arches was observed in twelve individuals (dorsal hemivertebra). Thereby, the morphological variation is large and ranges from completely absent haemal arches (Fig. 2h) up to low elements, which are still characterized by their paired haemal spines. In some specimens, the abnormal growth is asymmetric, meaning that one lateral haemal spine is more strongly reduced in size and underdeveloped (Fig. 2i). Frequently, the dorsal hemivertebrae are incarcerated, i.e. both the preceding and succeeding haemal arches conform in shape. Only in a single specimen is the deformed vertebra not separated from both its preceding and succeeding vertebrae (non-segmented hemivertebra).

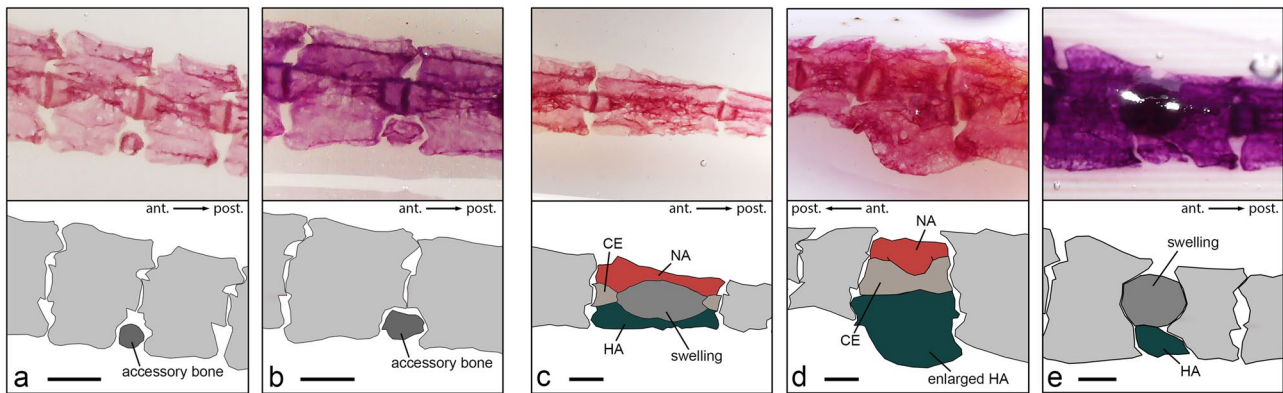
A single individual was characterized by a lateral hemivertebra. The right side of the vertebra is poorly developed and missing. The element is enclosed by the neural and haemal arches of the preceding vertebra.

### Supernumerary bone

Five specimens are characterized by an extra ossification in the haemal region. The additional bony elements are round, small, fully segmented and located between two haemal arches (Fig. 3a, b). The preceding and succeeding haemal arches conform in shape and are slightly smaller in size compared to normally developed haemal arches.

### Posttraumatic pathologies

In five individuals, the external morphology of the abnormal vertebrae differs compared to the aforementioned congenital deformities. Two individuals display fused vertebral elements, which exhibit a protuberant mass of abnormal



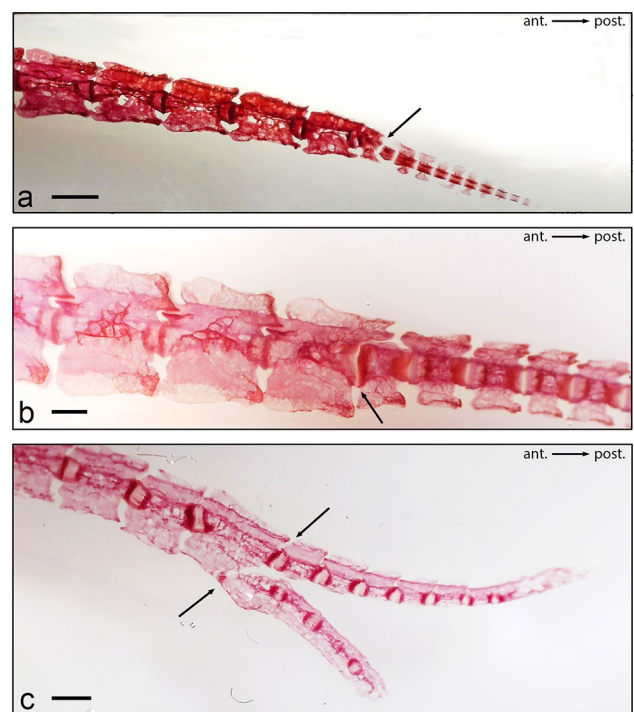
**Fig. 3** Supernumerary bones and posttraumatic pathologies along the vertebral column of *Taricha granulosa*. **a** Accessory bone in the caudal region of MVZ 216161, in lateral view. **b** Accessory bone in the caudal region of MVZ 216202, in lateral view. **c** Elongated and swollen caudal vertebra of MVZ 216489, in lateral view. **d** Enlarged

haemal arch in the caudal region of MVZ 216468, in lateral view. **e** Deformed and swollen caudal vertebra of MVZ 216288, in lateral view. Scale bars equal 1 mm. Abbreviations: *ant* anterior, *CE* centrum, *HA* haemal arch, *NA* neural arch, *post* posterior

bone growth on their lateral sides (Fig. 3c). The elements are elongated and both the neural and haemal arches are fully fused. The height of the anomalous vertebrae does not deviate from the normal and the articulation to the adjacent vertebrae is intact. In another specimen, the vertebra is fully segmented and composed of a neural arch, centrum and haemal arch. The haemal arch, however, has an abnormally large size and extends far ventrally (Fig. 3d). The remaining two specimens are characterized by an unusual shape of the vertebrae and by a large swelling of bone on the centrum (e.g. MVZ 216288, Fig. 3e) and the neural arch (e.g. MVZ 216247), respectively.

### Tail regeneration

In the present study, we identified 62 specimens with regenerated tails (i.e. 13.51%). However, we assume that some regenerated individuals remain undetected as the regenerative capacity is high in salamanders and that some regenerated tails can no longer be distinguished from a normally developed tail. In all 62 specimens, the regenerated tail consists of ossified vertebrae whereby we observed a reverse order of development: first formation of centra followed by neural and haemal arches. Furthermore, the height and length of regenerating vertebrae are distinctly smaller compared to normally developing tails (Fig. 4a). The degree of ossification of the regenerated tail varies greatly among the individuals: in some specimens, regeneration is limited to the posterior end of the tail and solely the vertebral centra are ossified. In others, the regenerated tail is longer and composed of numerous ossified vertebrae, which consist of vertebral centra, neural arches, and haemal arches. We further observed that the vertebral element located anterior to the site of injury often has an anomalous external morphology



**Fig. 4** Regenerated tails. **a** Regenerated tail of MVZ 216391, in lateral view. **b** Regenerated tail of MVZ 216261, in lateral view. **c** Duplicated tail in MVZ 216569, in lateral view. Arrows indicate the shift from normal to regenerated tail. Scale bars equal 1 mm

(Fig. 4b). The vertebrae are elongated along their anterior–posterior axis, the neural arches and haemal arches are deformed and may extend posteriorly. Additionally, we found a single specimen bearing a tail duplication (Fig. 4c). The site of injury is located at the posterior end of the tail (i.e. 49<sup>th</sup> vertebra) and exhibits a cleavage into a ventral and

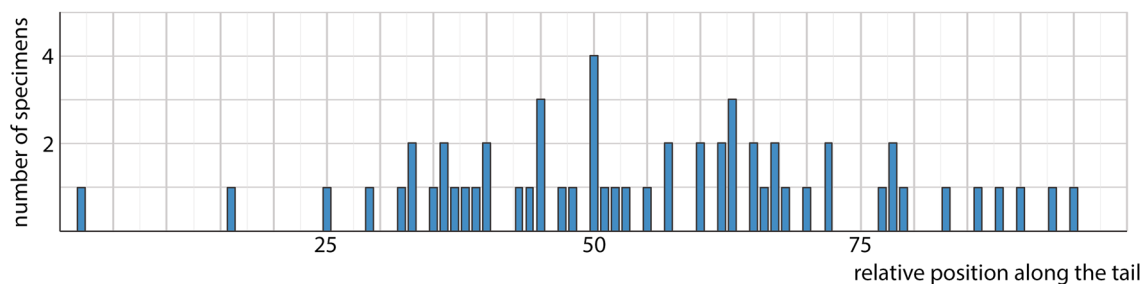
dorsal tail tip. The vertebrae in both regenerated tail tips are distinctly smaller than the regular caudal vertebrae.

## Discussion

This study represents one of the first attempts to investigate a natural salamander population in terms of development, intraspecific variation and congenital deformities of the vertebral column. Within the population of *Taricha granulosa*, we found variation in the number of trunk vertebrae. The level of variation seems to be low, and in the majority of the specimens (95%) the number of trunk vertebrae was twelve, followed by 13 trunk vertebrae in 3.5% of the specimens, and eleven trunk vertebrae in 1.5% of the specimens. A similar range of variation (two or fewer vertebrae) has been observed in different salamander taxa, and the range of variation seems to be larger in species with higher modal numbers of trunk vertebrae (Peabody and Brodie 1975; Jockusch 1997; Litvinchuk and Borkin 2003; Litvinchuk et al. 2005; Lanza et al. 2006; Slijepčević et al. 2015; Govedarica et al. 2017). In salamanders, the count of vertebral elements may also display variation between different populations (“geographic variation”), among species (“interspecific variation”), between sexes (“sexual dimorphism”) and between families (Highton 1960; Jockusch 1997; Arntzen and Wallis 1999; Litvinchuk and Borkin 2003; Lanza et al. 2006; Arntzen et al. 2015). For instance, in different salamander taxa, the number of trunk vertebrae varies significantly from 11 (Salamandridae) to 64 (Amphiumidae) (Duellman and Trueb 1994; Litvinchuk and Borkin 2003). These observed variations have been explained by environmental (e.g. temperature, annual precipitation), genetic and developmental factors (Highton 1960; Jockusch 1997; Arntzen et al. 2015). It should be mentioned that a regional variation in centrum morphology has been discussed for *Taricha granulosa* by Worthington and Wake (1972). They assumed that an increased length of some vertebrae compensates for a reduction in vertebral number. Axial developmental plasticity (which is related to a high level of morphological

variability) has not only been observed in salamanders but also in other taxa, including fishes and avian and non-avian reptiles (Lindell 1994; Morin-Kensicki et al. 2002; Head and Polly 2007; Ward and Brainerd 2007; Müller et al. 2010). In most mammals, however, the precaudal vertebral number is highly conserved and under a high degree of constraint (Narita and Kuratani 2005). Interestingly, the number of the single cervical and sacral vertebra, however, does not vary at all which indicates a strong developmental constraint in this region of the vertebral column. This agrees with a hypothesis of Geoffroy St. Hilaire (1832): organs or skeletal structures, which consist of several homologous elements placed in linear series are subject to a higher degree of variation than organs or skeletal structures with a smaller number of elements (see also Schultz and Straus 1945; Woolfenden 1961).

The clearing and staining method involving Alcian blue and Alizarin red for cartilage and bone allows the visualization of malformations or posttraumatic pathologies (e.g. injuries, infections) in the vertebral column of salamanders. In total, 55 individuals of *Taricha granulosa* display 59 congenital malformations along the vertebral column (failure of segmentation and failure of formation). Most of these malformations were encountered in the caudal region of the axial skeleton, whereas they occur almost equally in the proximal and distal parts of the tail (Fig. 5). Only a single malformation was located in the presacral region of the axial skeleton. In humans, vertebral deformities are often associated with simple to complex structural and neurologic disorders and the frequency of presacral congenital vertebral malformations is estimated at around 1 per 1000 births or lower (Wynne-Davies 1975; Detrait et al. 2005; Goldstein et al. 2005; Kaplan et al. 2005; Giampietro et al. 2013; Katsura and Kim 2019). In the same way, the frequency of presacral congenital vertebral malformations in *Taricha granulosa* is estimated at around 2 specimens per 1000. However, if caudal deformities are added to the calculation, the frequency of congenital malformations increases to around 120 specimens per 1000 (excluding posttraumatic pathologies). The data obtained in this study indicate a higher frequency of



**Fig. 5** Histogram of the relative position of every single congenital malformation (failure of segmentation and failure of formation) along the caudal region of the vertebral column



vertebral congenital deformities in lissamphibians than previously documented in the literature. A reason why these deformities often remain undetected is that many anomalies cause minimal external deformity and therefore might go unrecognized. The development of osseous pathologies in salamanders (and lissamphibians) is complex and linked to genetic, physiological, environmental, nutritional, and parasitological factors (Blaustein and Johnson 2003 and references therein). Still, the identification of the exact causes is often difficult, and their relationship is poorly understood. One potential explanation for the high frequency of salamanders with vertebral deformities in this population could be linked to the above-mentioned developmental plasticity of the vertebral column in lissamphibians. However, it is also possible that skeletal deformities in the *Taricha* population studied here are related to environmental causes (UV-B radiation, chemical pollution, environmental degradation). Additional studies on other salamander populations will be needed to understand if the high frequency of abnormalities observed here is a characteristic of salamanders or this specific population.

A potential association between an abnormal number of trunk vertebrae and the occurrence of a congenital malformation along the vertebral column remains inconclusive. Of the 58 individuals with anomalous vertebral elements, only two specimens (MVZ 216468 and MVZ 216571) display a differing number of trunk vertebrae (see Table 1): the first specimen (MVZ 216468) is characterized by 13 trunk vertebrae. However, the osseous pathology in the caudal region consists of a posttraumatic pathology that does not result from a disruption in early development. In the second specimen (MVZ 216571), the lower number of 11 trunk vertebrae results from a failure of segmentation, meaning two vertebral elements are fused to a congenital block vertebra. Interestingly, this is also the only individual, in which an anomalous vertebral element has been identified in the trunk region. It raises the question if changes in the number of trunk vertebrae in salamanders are potentially associated with segmentation and formation defects in this vertebral region—a pattern that is well described in mammals (Ten Broek et al. 2012). Nevertheless, the data suggest no association between an abnormal number of trunk vertebrae and the occurrence of congenital malformations in the caudal region.

It remains to be determined why the caudal region is more susceptible to anomalies than others. Fowler (1970), for instance, suggested that the more posterior somites are more sensitive to environmental changes and that the development of the axial skeleton consists of highly sensitive periods, which alternate with less sensitive ones. Galis et al. (2006), on the other hand, proposed a strong natural selection against changes in the anterior region of the vertebral column as these changes are associated with major abnormalities and a dramatically reduced fitness. Development in

the posterior region of the vertebral column, however, is less vulnerable which results in a lower evolutionary constraint of this region. The study of Vaglia et al. (2012) showed that the tail of salamanders is a region of ongoing development and growth which suggest that the tail is evolutionary the most plastic portion of the vertebral column. Another aspect that should be considered is a possible correlation between vertebral malformations and tail regeneration—questioning if deformities located in the tail are associated with the underlying regenerative processes. Tail regeneration exists in all salamander groups, independent of whether the species can autotomize their tails or not, and the regenerated tail is fully functional and composed of calcified or ossified vertebral elements and associated musculature (Holtzer et al. 1955; Holtzer 1956; Wake and Dresner 1967; Mufti and Simpson 1972; Dinsmore 1995; Vaglia et al. 1997; Babcock and Blais 2001; Mchedlishvili et al. 2012). In the present study, we found no indication of a correlation, as only two specimens (MVZ 216268 and MVZ 216295) had congenital malformations in a regenerated tail. However, as mentioned above, some regenerated individuals most possibly remain undetected as the tail is already fully developed and morphologically not distinguishable from a normally developed tail. Recently, congenital malformations (hemi-, wedge- and block vertebrae) have also been described in fossil lissamphibians (Skutschas et al. 2018) and among extinct temnospondyls, the presumed stem group of lissamphibians (Witzmann 2007; Witzmann et al. 2014). Given that the caudal region in early tetrapods is often damaged or poorly preserved, it could be possible that the frequency of congenital malformations may be higher in the fossil record than documented until now.

Inspection of the external morphology of a small number of individuals reveals vertebral deformities that do not result from a disruption in early development. Parasite-induced malformations are common in natural amphibian populations and normally encompass limb abnormalities and cysts (Johnson et al. 1999, 2002; Blaustein and Johnson 2003). Pathologies of the vertebral column resulting from parasitic infection, however, have only rarely been reported in salamanders to date (Gamble et al. 2005; Perpiñán et al. 2010; Danto et al. 2020). In the present study, two deformed vertebrae (MVZ 216288 and MVZ 216247) could possibly have resulted from parasitic infection. The vertebrae are characterized by a large lump of bone on their lateral side. In two other pathological specimens, multiple vertebrae are completely fused to one another and the lateral sides are characterized by a protuberant mass of abnormal bone growth. The abnormal bone surface and structure are irregular and rough. The specific nature of these malformed vertebrae remains undefinable, and we can only speculate about the aetiology: the abnormal bone mass could be caused for instance by a bone infection or disease.

## Conclusions

The vertebral column is composed of serially repeating chondrified and ossified vertebrae that surround the spinal cord and the notochord. We reported numerous vertebral deformities in a natural population of the salamander *Taricha granulosa*. The deformations include congenital malformations (block vertebrae, unilateral bars, hemivertebrae), supernumerary bones, posttraumatic pathologies, and the majority of these are located in the caudal region. The data in this study indicate a high frequency of vertebral congenital deformities in salamanders, however, the identification of the exact causes remains challenging. They could be related to a high developmental plasticity of the vertebral column, anthropogenic and natural factors, or tail regeneration. Further studies on other salamander populations are needed to increase the available comparative data on salamander vertebral development and vertebral congenital malformations.

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**Data availability statement** The data that support the findings of this study are available from the corresponding author upon reasonable request.

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