

## Developmental anomalies in the smooth snake, *Coronella austriaca* Laurenti, 1768 (Squamata, Colubridae) from Poland

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## Abstract

We present four cases of rare developmental anomalies in the smooth snake *Coronella austriaca* Laurenti, 1768 from western Poland. These include brachycephaly, lordosis, a supernumerary row of 'ventral' scales, and the third reported case of dicephalism in snakes from Poland. All the cases are supported by X-ray radiography. One of the possible explanations for these anomalies is a low genetic variation in populations from western Poland.

## Key Words

brachycephaly, dicephalism, malformations, radiography, reptiles

### Introduction

Animal anomalies are relatively commonly reported (e.g. Rothschild et al. 2012). Individuals with anomalies are usually much more likely to die earlier than healthy ones, hence our knowledge on the frequency of anomalies in wild populations is very limited. This includes snakes, which are commonly held in captivity, and most of the data on snake anomalies come from captive animals (e.g. Mulder 1995; Wallach 2007). However, a high frequency of pathologies may suggest significant populational disturbances, such as low genetic diversity or inbreeding depression (e.g. Olsson et al. 1996); therefore, data on developmental anomalies in wild animals are needed.

The smooth snake, *Coronella austriaca* Laurenti, 1768, is a relatively small, non-venomous and viviparous

colubrid snake. It is distributed throughout nearly the whole of Europe and western Asia (Speybroeck et al. 2016). Despite its wide range, it is considered a rare species in many areas and is, therefore, strictly protected in many European countries, including Poland (e.g. Kolanek et al. 2017; Profus et al. 2018). In addition to its rarity, it is also very secretive and its biology is less known compared to more conspicuous European snakes. This also includes anomalies and pathologies (Jablonski and Mikulíček 2015). Below, we describe some basic reproductive parameters with a particular focus on developmental anomalies of neonatal and juvenile smooth snakes that were recently observed in Poland. Our additional goal was to assess the utility of two various sets of veterinary digital radiography tools for studying the congenital anomalies in smooth snakes.

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#### Methods

The study was conducted in 2012–2014 at three sites in western Poland. The Szczecin site (53°24'N, 14°41'E, Fig. 1) is an uncultivated area covered by grass, well-exposed and sunny (a half-open glade). It is located on the edge of a mixed forest, close to an active railway, and is covered by self-seedings and saplings of the Scots pine (*Pinus sylvestris*). The Rybocice site (52°17'N, 14°38'E, Fig. 1) was a long-used, illegal landfill. Occasionally, the contents of backyard cesspits were released into the area. The site is sunny, covered by grass, self-seedings and saplings of the Scots pine, false acacia (*Robinia pseudoacacia*) and hawthorns (*Crataegus*). The Świętoszów site (51°28'N, 15°23'E, Fig. 1) is an unused railway that includes embankments bordering pine monocultures and heaths.

We have observed eleven gravid females and one juvenile. All of them were captured and temporarily kept in terrariums resembling their natural habitat. After giving birth, females and neonates were released at the site where they were captured. The only exceptions were the individuals exhibiting anomalies, which were kept until their natural death, and then deposited at the Faculty of Biological Sciences (University of Zielona Góra, Poland).

To obtain the images, we used two radiographic sets. The first one was an indirect radiography set Agfa CR-30X with the image plate Agfa HealthCare CR MD4.0T General Set. The X-ray generator used was the Gierth HF 200 A Plus generator with a system of manual and automatic exposure parameters and the voltage between 30 and 100 kV (hereinafter "CR set"). The second set was the Midmark Digital Dental X-ray with the Progeny Preva Plus generator for intraoral exposures. The high-resolution generator provided a focal spot of 0.4 mm increasing the resolution, and allowed for automatic and manual regulation of exposure parameters between 60, 65 and 70 kV, and the exposure time between 0.02 and 2 seconds. A direct radiography detector was attached to this set (hereinafter "DR set").

#### Results

The number of viable offspring varied between three and eight. In five of the clutches, we observed unfertilised eggs and/or dead embryos. Three neonates exhibited congenital morphological anomalies which are described in detail below. The snout-vent length (SVL) of the neonates was between 12.2 and 15.4 mm and their mass between 1.7 and 3.8 g. The basic parameters of the neonates are summarised in Tables 1, 2 and Suppl. material 1: Table S1.

The CR set did not allow obtaining high quality radiographs due to their significant overexposure. The obtained images were highly fogged and the anatomical details were not discernible. The DR set with automatic exposure parameters provided slightly fogged images which were also of insufficient quality. Manual adjustment allowed to



Figure 1. Location of the study sites.

obtain high quality radiographs, suitable for anatomical descriptions.

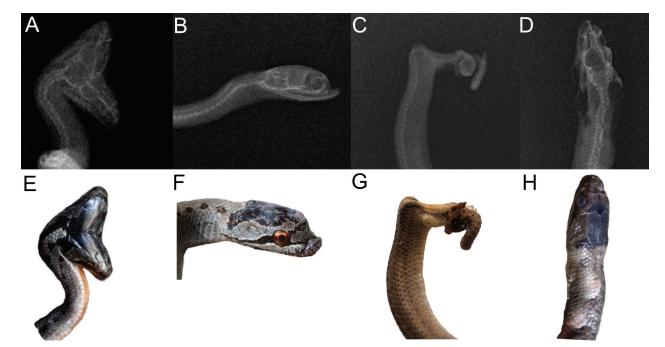
The first specimen (no. 76c) exhibited a duplicated head, with the split extending to posterior parts of the head (Table 1, Fig. 2A, E). X-ray scanning indicated that the parietal lobe was duplicated but the basioccipital bone was single and the bifurcation did not include the vertebral column. Thus, the anomaly could be classified as craniodichotomy. The snout-vent length (SVL) was almost the same from the left snout to the vent (12.2 mm) as from the right snout to the vent (12.5 mm). However, the right head was more asymmetrical. On the left head, the number of supralabial scales was six, on both left and right side, but on the right head, there were seven supralabial scales (including two small, anomalous scales) on the left side and six on the right side. On the left head, there were six sublabial scales on both sides, on the right head, however, there were seven sublabial scales on the right side and six sublabial scales on the left side. Apart from the heads, the specimen showed no other anomalies. It was born alive but died about 30 minutes after parturition. It was one of four siblings and the only one showing a developmental anomaly.

The second specimen (no. 79e) showed a very short upper jaw and an approximately normal-sized mandible (Table 1, Fig. 2B, F). X-ray scanning indicated an almost complete absence of bones in the pre-orbital region of the skull. Thus, the snake could be considered brachycephalic. SVL was 14.9 mm. Apart from the abnormal snout, the specimen showed no other anomalies. It was born alive but died on the next day after parturition. It was one of six siblings and the only one showing a developmental anomaly.

The third specimen (no. 112g) exhibited a strongly ventrally-bent tail which could probably be regarded as a case of lordosis (Table 1, Fig. 2C, G). Caudally to that, there was a large mass of anomalous tissues. Because it was present in a neonatal individual, it most proba-

Locality	Female	Female SVL/	Female mass	Date of	No. of offspring (with anomalies)	Offspring SVL [cm]	
	ID	TAL [cm]	before/after	parturition		Mean ± SD	Min-Max
			parturition [g]				
Świętoszów	68	47.9/9.6	69.9/49.7	10.08.2012	6 (0)	$13.1 \pm 0.2$	12.9–13.4
	72	51.5/9.7	69.6/41.5	3.08.2012	7 (0)	$13.9 \pm 0.1$	13.8-14.1
	74	53.2/10.1	75.4/49.9	9.08.2012	7 (0)	$13.4 \pm 0.2$	13.0-13.6
	75	55.9/9.6	77.2/45.1	17.08.2012	5+3 dead embryos (0)	$13.6 \pm 0.1$	13.4-13.7
	76	50.8/9.7	54.5/38.9	10.08.2012	3+1 unfertilised egg (1)	$12.9\pm0.6$	12.2-13.3
	77	53.2/9.5	92.7/61.2	15.08.2012	8 (0)	$14.2 \pm 0.2$	13.9-14.5
	97	50.7/9.2	96.8/69.1	22.08.2013	5 (0)	$15.0 \pm 0.3$	14.6-15.4
	112	47.9/10.3	79.0/47.9	13.08.2014	7 + 2 dead embryos (1)	$13.8 \pm 0.2$	13.5-14.2
Szczecin	79	50.4/10.5	79.3/44.9	27.08.2012	6(1)	$15.0 \pm 0.0$	14.9-15.0
	90	57.8/9.7	108.9/66.2	3.09.2012	6 + 2 unfertilised eggs (0)	$14.7 \pm 0.4$	14.1-15.2
Rybocice	110	51.8/11.1	74.5/?	5.08.2014	3 + 1 dead embryo + 3 unfertilised eggs	$15.1 \pm 0.2$	14.9–15.3

Table 1. Description of the collected females and their offspring. SVL – snout-vent length, TAL – tail length.



**Figure 2.** X-ray (A–D), and photography (E–H) of specimens with developmental anomalies: A, E. Two-headed snake; B, F. Brachycephalic snake; C, G. A snake with lordosis; D, H. A snake with a supernumerary row of 'ventral' scales.

bly represented a congenital malformation. SVL was 13.6 mm. Apart from the abnormalities in the tail, the specimen showed no other anomalies. It was one of nine siblings – one of them was stillborn and the other (the one described here) showed a developmental anomaly.

The fourth specimen (no. 3) was born in the current year and lived independently. It exhibited several scale anomalies posterior to the head (Table 1, Fig. 2D, H). Most interestingly, a supernumerary row of 'ventral' scales developed on the lateral side of the specimen and a patch of similar scales was present on the dorsum (Fig. 2H). The normally-developed, 'genuine' ventral scales were present along the entire length of the venter between the gulars and the anal scale. Additionally, several dorsal scales located immediately posterior to the head were enlarged or abnormally shaped. It was a juvenile individual (SVL - 17.4 mm). This individual died three days after it was discovered. The death of

this snake was probably caused by a damage to the neck region. Apparently, shortly before death, it was in good condition, as evidenced by three prey items (two juvenile slow worms, *Anguis fragilis* Linnaeus, 1758, and one unidentified small mammal) preserved in its stomach.

#### Discussion

The obtained data on the number of offspring in the clutch, as well as on the body size and mass, are consistent with the available information about developmental parameters of smooth snakes from western Poland (Najbar 2006) and Poland in general (Juszczyk 1974).

Acquisition of radiographs was difficult due to early ontogenetic age of the studied specimens resulting in their small size and low mineralization of the skeleton. However, the X-ray lamps used in the stomatological set

ID	Locality	Date of birth/	Anomaly
		Date of death	
76c	Świętoszów	13.08.2012/	dicephalism
		13.08.2012	
79e	Szczecin	27.08.2012/	brachycephaly
		28.08.2012	
112g	Świętoszów	13.08.2014/	bone outgrowth in the tail; lordosis
e	C C	14.08.2014	
3	Szczecin	22.09.2012*/	supernumerary row of
		25/26.09.2012	'ventral' scales

**Table 2.** General information about specimens with developmental anomalies.

\* – date of collection.

are of low voltage and their proximity to the tissue allows obtaining a collimated radiation dose with a high transfer coefficient. In combination with high-resolution detectors, this allowed to obtain high quality radiographs, suitable for descriptions of congenital anomalies.

Dicephalism is a taxonomically widespread anomaly in reptiles, recorded in extinct choristoderes (Buffetaut et al. 2007), turtles (Sönmez et al. 2017), lizards (Spadola and Insacco 2009) and especially snakes (Wallach 2007, 2018). However, the existing body of information is based predominantly on records from captive animals (Wallach 2007). Although the number of records in wild populations is increasing, this mostly concerns North and South America (e.g. Albuquerque et al. 2010, 2013; Esqueda et al. 2016; Hileman et al. 2017). Records from Europe come especially from the Balkans (Jovanović 2011; Pezdirc et al. 2013; Gvozdenović and Čavor 2015; Gvozdenović et al. 2021), while from Poland they are almost completely missing. To our knowledge, the only dicephalic snakes (one adder, Vipera berus (Linnaeus, 1758), and one grass snake, Natrix natrix (Linnaeus, 1758)) recorded from Poland so far, have been mentioned only in the popular press (Polsat News 2019). Thus, this is the third documented case of dicephalism in snakes from Poland, the first one in the endangered in Poland smooth snake, and the twelfth one in the whole species (Wallach 2018). Additionally, both two-headed snakes from Poland described previously exhibited prodichotomy (i.e. their heads were completely separated and had two short necks), while the smooth snake described above had an incompletely divided head, i.e. it was craniodichotomous. Prodichotomy is the most common type of axial bifurcation, accounting for about 59% of all cases. Craniodichotomy is almost two times rarer (29.9% of all cases) (Wallach 2018).

Brachycephaly seems to be even rarer than axial bifurcation in snakes. Rothschild et al. (2012) list several examples of this anomaly in lizards, turtles and crocodylians but only two in snakes – one in the grass snake, *Natrix natrix*, and one in the garter snake, *Thamnophis sirtalis* (Linnaeus, 1758). A similar pathology was observed by Mulder (1995) in a captive individual of the Ottoman viper, *Montivipera xanthina* (Gray, 1849). Brachygnathia is more common in snakes but the specimen described above had a mandible of a normal length.

Congenital spine anomalies, such as scoliosis, lordosis or kyphosis, are not uncommon in snakes. They can be caused by vertebral malformations such as hemivertebra. However, it is unclear from the X-ray images whether this is the case in the specimen described above. Lordosis is relatively rare in two viviparous Neotropical viperid snakes, Crotalus durissus Linnaeus, 1758, and Bothrops jararaca (Wied-Neuwied, 1824) in which it occurs with 11.1% and 12.6% frequency, respectively (Sant'Anna et al. 2013). If such anomalies concern the caudal parts of the spine (as is the case here), they do not necessarily cause death of the animal. However, the specimen described above had also an anomalous swollen tissue in the most caudal part of the vertebral column. Probably, it was caused by bone outgrowth, but its actiology is unclear.

The ventral scales in snakes are developmentally tightly associated with vertebrae, so that the number of ventral scales closely corresponds with the number of vertebrae (e.g. Alexander and Gans 1966). However, the X-rays did not show any evident anomalies of the vertebral column. It is thus difficult to identify the factor responsible for such a strong asymmetry of the ventral scales. The ventral scales play an important role in the locomotion of snakes (e.g. Bury et al. 2019), and anomalies of these scales, such as their asymmetry, affect locomotor performance (Löwenborg and Hagman 2017). The effect of such anomalies is probably the greatest in terrestrial snakes (such as *C. austriaca*), because the ventral scales are relatively the widest in these snakes and are in an almost constant contact with the substrate (Bury et al. 2019). However, the preserved stomach contents (see above) indicate that the hunting abilities of this specimen were not handicapped by the strong asymmetry of the ventral scales. Ventral scale anomalies are known in snakes (e.g. Löwenborg and Hagman 2017) and occur frequently (in ca. 39% of individuals) in smooth snakes in the studied area (Najbar 2006) but we are unaware of other cases of such 'misplaced ventral' scales.

The exact aetiology of a given anomaly is usually difficult to determine. Numerous potential causes were suggested to explain, for example, polycephalism in snakes. Among them were incomplete division of a single embryo, partial fusion of two embryos, abnormally low or high temperatures during incubation or gestation, regeneration after an embryonic lesion, anoxia during embryogenesis, toxic effects of metabolic secretion, inbreeding depression, hybridisation, environmental pollution or radiation (e.g. Wallach 2007). While establishing the cause of the anomalies described above seems to be impossible, it is worth noting that the specimens described above belonged to populations where the inbreeding coefficient  $(F_{IS})$  was statistically significant (Sztencel-Jabłonka et al. 2015). Moreover, it is well known that in highly inbred or bottlenecked populations, the incidence of different developmental anomalies and dead neonates is high (e.g. Olsson et al. 1996; Gautschi et al. 2002).

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## Supplementary material 1

#### Table S1

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Data type: morphological

Explanation note: Morphometric data of neonatal smooth snakes.

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