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Limb malformation and tail bifurcation in sand lizards (*Lacerta agilis*) and common lizards (*Zootoca vivipara*) from Poland

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The sand lizard *Lacerta agilis* Linnaeus, 1758 and the common lizard *Zootoca vivipara* (Lichtenstein, 1823) are medium-sized lacertid lizards with wide Palaearctic distributions. Both are fairly common species in many parts of their range and are commonly studied, yet reports of their developmental anomalies are relatively scarce. In this article we describe six cases of limb and tail malformations of these two lizard species.

On the 7th June 2017 near the forest edge surrounded by numerous fish ponds in the vicinity of Ruda Milicka (51.5320°N, 17.3289°E) we observed an adult, probably gravid, female sand lizard (ca. 6 centimetres in snout-vent length). This site is located in the Barycz Valley Natura 2000 protected area. The lizard had severely malformed right hindlimb. It was not differentiated into stylopodium, zeugopodium and autopodium, being a short, rod-like appendage (Fig. 1A, 1B). Its scalation was more similar to that of toes but it did not end with a claw. The appendage was stiff and was used for body support during locomotion, which suggests that it was supported by bone or cartilage. Despite the anomaly, the observed individual was in good condition and the malformation did not hinder its mobility. Four other sand lizards were observed on that day and none of them exhibited any anomalies. The malformed lizard was observed again on 5th July 2017.

Similar limb malformations have been described in several lacertids and skinks by Marcucci (1930) and recently in two *Podarcis* species from the Mediterranean (Cortada et al., 2017; Gkourtsouli-Antoniadou et al., 2017). Cortada et al. (2017) suggested that the tail-like leg in a male *P. lilfordi* was a result of an incomplete limb regeneration. In experimental conditions the limb regeneration in lizards is to some extent possible, especially regarding stylo- and zeugopodium (Alibardi, 2017a). Indeed, such incomplete regeneration and further somatic growth may explain the presence of tail-like legs in lizards (Alibardi, 2017b). However, we did not find any scarring or other pieces of evidence pointing to the traumatic injury of the sand lizard right hindlimb. Moreover, its scalation is different from that of the possibly regenerated *P. lilfordi* tail-like limb (Cortada et al., 2017), being more similar to the tail-like limb recorded in *P. erhardii* (Gkourtsouli-Antoniadou et al., 2017). The latter, however, had well developed thigh, from which the malformed appendage grown out, while in the sand lizard the whole leg was malformed.

Another case of a sand lizard with limb malformation was observed on the 11th July 2016 on a meadow near an oxbow lake of the Odra River in the vicinity of Milsko (51.9440°N, 15.7741°E). It was a juvenile sand lizard with missing right hindfoot. A short, stout appendage grown out of its ankle, slightly resembling a very short tail (Fig. 1D, 1E). This individual did not show any other anomalies and the limb malformation did not apparently reduce its mobility.

Ectomely is defined as an absence of at least some parts of the limb, especially the most distal ones. It includes amely (absence of whole limbs), hemimely (defective limbs, especially their distal parts) and meromely (absence of digits) (Rothschild et al., 2012). Thus, both these sand lizards could be classified as both hemimelic and meromelic. While the etiology of these anomalies is unknown, generally they are regarded as having environmental origin (Rothschild et al., 2012). For example, Raynaud (1990) found that 1β-arabinofuranosylcytosine (Ara-C) may cause numerous anomalies in developing lizard embryos, including ectromely (incomplete limb with missing

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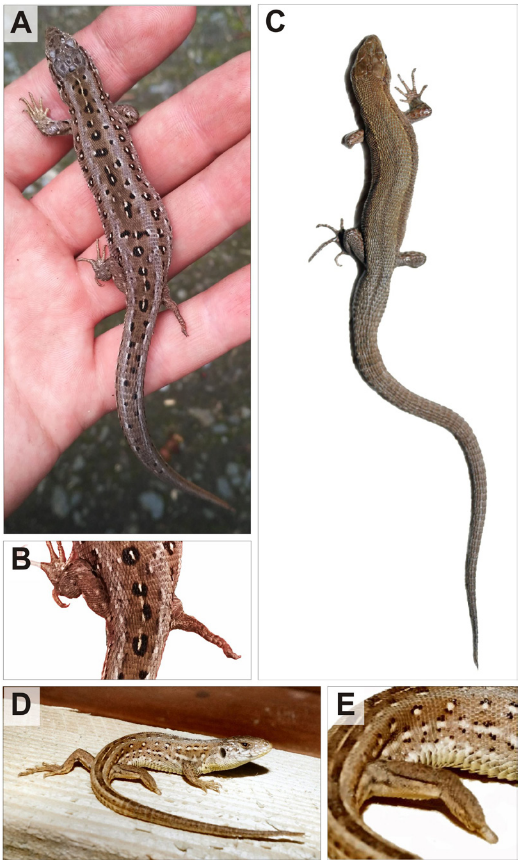

Figure 1. A) Sand lizard (*Lacerta agilis*) female with malformed leg. B) Close-up of the malformed leg. C) Common lizard (*Zootoca vivipara*) male with missing distal parts of the left forelimb and the right hindlimb. D) Juvenile sand lizard with malformed right hindlimb. E) Close-up of the malformed limb. Photos by K. Kolenda (A, B) and M. Wieczorek (C, D, E).

proximal portion) or ectrodactyly (absence of at least one digit) (Rothschild et al., 2012). While we cannot be certain about the cause of these anomalies, the partial regeneration seems a possible explanation, particularly if the appendage contained cartilage rather than bones (Alibardi, 2017b). Sand lizards with such malformed appendages have not been hitherto reported from Poland.

An adult male of the common lizard was observed on 27th May 2016 on a meadow near an oxbow lake of the Odra River in the vicinity of Milsko (51.9437°N, 15.7738°E). It was the only individual observed. Its left forelimb and right hindlimb were truncated at elbow and knee, respectively (Fig. 1C). Again, this lizard could be regarded as both hemimelic and meromelic. It is unclear whether this condition was caused by predation attempts or developmental anomaly. However, no scarring was observed. It seemed that the limb malformations did not decrease the lizard's mobility. This is another piece of evidence that even severe limb anomalies do not necessarily affect lizard mobility (e.g. Gleed-Owen, 2012). There are only a few published records of lizards with missing digits or parts of limbs from Poland (Szwajkowski, 2006) but it is also unclear whether they represent signs of predation attempts or much rarer developmental anomalies.

Compared to limbs, lizard tails have much greater regeneration potential (Alibardi, 2017b). Tail autotomy (tail-shedding) is a major anti-predator strategy in at least 13 family-level lizard clades (Bateman and Fleming, 2009). It is known from at least 150 million years ago (Delfino and Sánchez-Villagra, 2010). The tail breaks along a fracture plane and the vertebrae of the shed tail are then replaced by a cartilaginous rod (Bateman and Fleming, 2009). Incomplete autotomy or injury of the tail can stimulate regeneration of an additional tail, resulting in presence of two or more tails. There are numerous reports of bifurcation (e.g. Dudek and Ekner-Grzyb, 2014) or trifurcation (e.g. Koleska and Jablonski, 2015; Passos et al., 2016) of tails in lizards. In some cases, even six regenerated tails may be present in a single individual (Pelegrin and Leão, 2016).

Despite being widespread, cases of tail regeneration anomalies are very rare on a populational scale (e.g. Dudek and Ekner-Grzyb, 2014) and the knowledge on their frequency is still scarce. Here, we report on two observations of a tail bifurcation in sand lizards and one in common lizard. First was made on 8th May 2012 on densely vegetated meadow (abandoned building site) in the vicinity of farmlands in Zielona Góra, Ochla district (51.8697°W, 15.4555°E). The sand lizard was an adult male in green, breeding colouration. The regenerated tail was longer than the rest of the original tail, because the latter was autotomised and only partially regenerated. Its colour and scalation were as in normally developed tail (Fig. 2A, 2B). About nine other sand lizards were observed at the site, none of which exhibited tail anomalies. Second observation was made on 28th May 2016 in Żary, in an abandoned quarry covered by meadows and several small ponds (51.6294°W, 15.0855°E). Twelve sand lizards were observed, one of which (a juvenile individual) had the tail bifid at the tip. The bifurcation was only shallow and the supernumerary tail did not differ from the basic one in

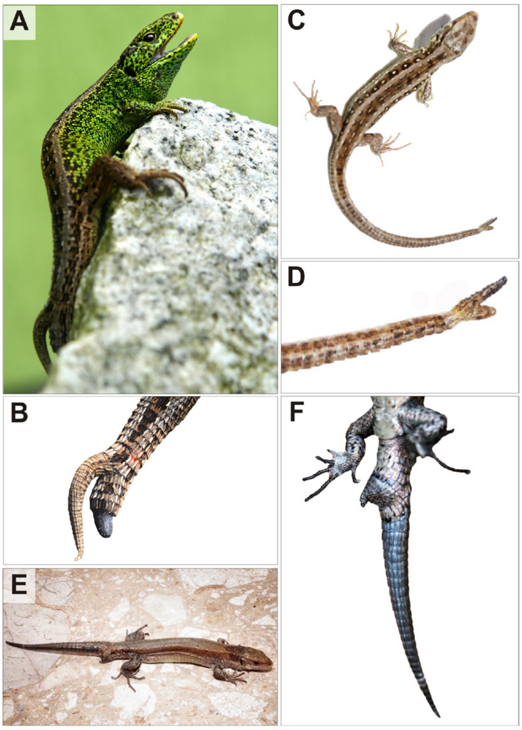

Figure 2. A) Sand lizard (*Lacerta agilis*) male with a double tail. B) Close-up of the bifurcated tail. C) Juvenile sand lizard showing a tail bifurcation. D) Close-up of the bifid tail. E) Common lizard (*Zootoca vivipara*) female with a double tail. F) Close-up of the supernumerary tail. Photos by A. Najbar (A, B) and M. Wieczorek (C, D, E, F).

colouration, being slightly shorter and stouter (Fig. 2C, 2D). Hitherto, tail anomalies in sand lizard from Poland were described only by Juszczyk (1987) and Dudek and Ekner-Grzyb (2014). Juszczyk (1987) mentioned several cases of sand lizards having two, three, four or even five regenerated tails but gave no details on specific cases, remarked only that such anomalies are very rare. Thus, the lizards described above are the only documented records of a tail bifurcation in sand lizards from Poland, after that described by Dudek and Ekner-Grzyb (2014).

A single common lizard was observed on 8th September 2017, again in the vicinity of Milsko (51.9437°N, 15.7738°E). It was a single female individual during skin-shedding. The tail was autotomised near its base and completely regenerated. A supernumerary tail was growing out of the tail base more anteriorly than the fracture plane. It was very stout, especially at its base and its colouration was more similar to the regenerated rather than the original tail (Fig. 2E, 2F). Tail autotomy and regeneration are quite widespread in common lizard, being noted in about 38% of individuals (Juszczyk, 1987). However, anomalies during tail regeneration are rarer than in sand lizards, as only one case was hitherto reported from Poland (Dudek and Ekner-Grzyb, 2014).

Both the limb malformations and tail bifurcations are very rare on the populational scale. Between 28th March and 3rd October 2016, 102 sand lizards were observed in Żary, and between 12th April and 11th September 2017 – 106 individuals. Only one of them showed a bifid tail. Thus, the frequency of the tail bifurcation in this population equals ca. 0.5%. In 2016 and 2017 in Milsko about 18 common lizards were observed, two of which showed anomalies (one, bifurcated tail and the other, limb malformation), which equals about 11%. Further studies could indicate whether there are indeed differences in the frequency of developmental anomalies, or such a disproportion reflects only a sampling bias.

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