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A unique case of limb abnormalities of a lizard (Reptilia, Lacertidae): Growth and development

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Abstract

Limb abnormalities are one of the most common deformities of vertebrates. They can be caused by both external and internal reasons. Limb abnormalities of amniotes are a fairly rare phenomenon, and mass limb abnormalities have not been found in amniote populations. Isolated cases of skeletal abnormalities are described mainly externally, without detailing the structure of the skeleton. This article presents descriptions of three abnormal specimens of hybrids of subspecies of *Lacerta agilis* (*L. a. boemica* × *L. a. exigua*). Two of them have oligodactyly of the right fore-limb. The third specimen demonstrates a unique combination of oligodactyly of the right and polydactyly of the left forelimbs.

KEYWORDS

forelimb, *Lacerta agilis*, oligodactyly, polydactyly

1 | INTRODUCTION

Limb abnormalities are one of the most common deformities of vertebrates. They can be caused by both external and internal reasons. Among them are mutations, injuries, parasites, thermal shock, various infections, environmental pollution with chemicals, and so on (e.g., Henle et al., 2017; Kovalenko & Kovalenko, 1996; Rothschild et al., 2012; Svinin et al., 2020). Limb abnormalities are most common in anamniotes, among which amphibians occupy a leading position. Limb abnormalities, like other abnormalities found in natural amphibian populations, are often mass and are often used as an indicator of environmental pollution (Henle et al., 2017). Limb abnormalities of amniotes are a fairly rare phenomenon and mass limb abnormalities have not been found in amniote populations. Isolated cases of skeletal abnormalities are described mainly externally, without detailing the structure of the skeleton (Bauer et al., 2009; Decemson et al., 2021; Vyas, 2018). This article presents descriptions of three abnormal specimens of hybrids of subspecies of *Lacerta agilis* Linnaeus, 1758. Two of them have oligodactyly of the right fore-limb. The third specimen demonstrates a unique combination of oligodactyly of the right and polydactyly of the left forelimbs.

2 | MATERIALS AND METHODS

In August 2021, to conduct experiments on the hybridization of subspecies of the sand lizard in laboratory conditions, we captured adult male *Lacerta agilis boemica* Suchow, 1929 (Russia, Stavropol Krai, Kirovsky Municipal District, near the village of Maryinskaya, 43.8725 N 43.4295 E) and an adult female *L. a. exigua* Eichwald, 1831 (Russia, Stavropol Krai, Predgorny Municipal District, near the village of Verkhnetambukansky, 43.9854 N 43.3314 E).

On 1 February 2022, they were brought out of hibernation; mating took place from February 11 to 12; on March 25, the female laid seven eggs, which were placed in the Lucky Reptile incubator “Egg-O-Bator” (Germany). The size and weight of the eggs corresponded to the parameters known for the species: length 12.09–13.74 mm ($X \pm Sx = 13.06 \pm 0.55$), width 9.45–10.28 mm ($X \pm Sx = 9.81 \pm 0.34$), weight 0.63–0.71 g ($X \pm Sx = 0.67 \pm 0.02$). Incubation took place at a temperature of 27–28°C. One of the eggs did not

TABLE 1 Hatching sizes of *Lacerta agilis* hybrid specimens (*L. a. boemica* × *L. a. exigua*).

No. ZISP	Body length (mm)	Tail length (mm)	Weight (g)	Anomaly
32563	30	47	0.75	Anomaly of the forelimb girdle, monodactyly of the right forelimb
32564	30	41	0.63	Anomaly of the axial skeleton, forelimb girdle, monodactyly of the right, polydactyly of the left forelimb
32565	32	43	0.74	Anomaly of the forelimb girdle, bidactyly of the right forelimb
32866	30	46	0.71	-
32867	30	40	0.71	-
32868	30	45	0.74	-

develop and was removed. By April 22, the eggs had the following dimensions: length 17.28–18.61 mm ($X \pm Sx = 17.69 \pm 0.48$), width 14.45–16.07 mm ($X \pm Sx = 15.17 \pm 0.58$), weight 1.93–2.33 g ($X \pm Sx = 2.14 \pm 0.15$).

On 29 April 2023, six specimens hatched, three of which had abnormalities in the development of the forelimbs. All specimens had normal size and weight for the species (Table 1). After 6 months, they were transferred to the collection of the Zoological Institute of the Russian Academy of Sciences (ZISP 32563–32565, 32866—33868).

For a detailed study of the skeletal structure of these specimens, a Neoscan N80 tomograph (CCP "Taxon" ZISP) was used. For comparison, we chose descriptions of the normal morphology of the limbs of lacertid lizards (Baranov et al., 1976; Kotok, 1993). The finger numbers were determined by the phalangeal formula and/or by their location relative to the elements of the zygopodium and basipodium.

3 | RESULTS

Below we provide a description of the morphology of the limb skeleton of specimens with anomalies.

ZISP 32565, female (Figures 1a and 2).

The pectoral girdle is asymmetrical. The right glenoid cavity is half the size of normal. It is located slightly further to the left. The right lateral outgrowth of the presternum is displaced cranially, and the right clavicle is displaced caudally.

In the right forelimb, the medial part of the proximal epiphysis of the humerus is underdeveloped. The right forearm consists of the ulna. The wrist consists of ulnare, intermedium, pisiforme, carpalia distalia III, reduced compared to the norm, and two fused carpalia distalia IV and V. The duality of this element is visible from the inside of the palm.

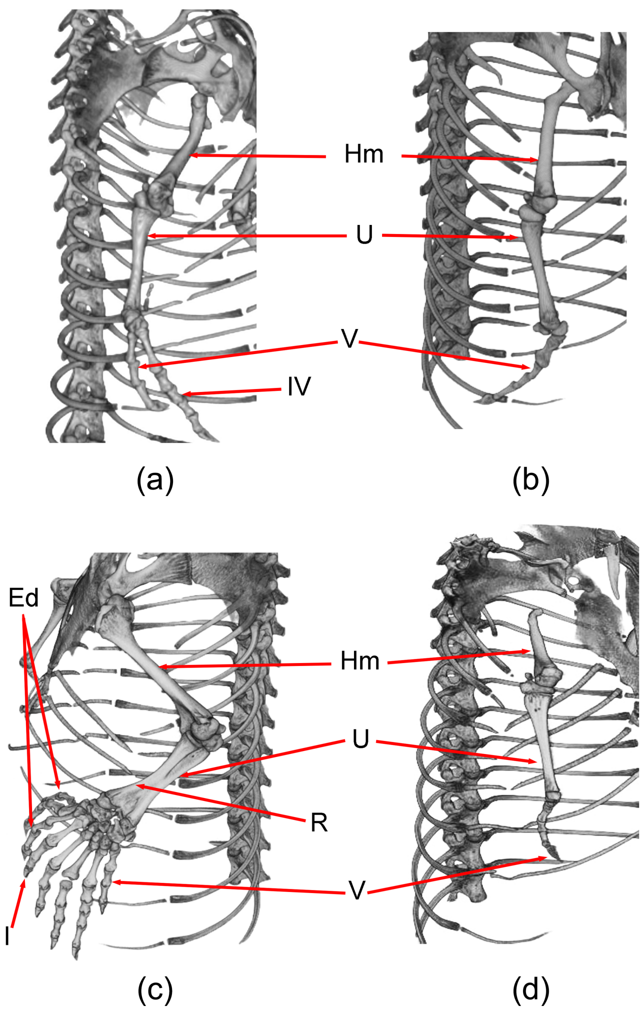

FIGURE 1 Computed microtomography of the abnormal forelimbs of *Lacerta agilis*: (a) (ZISP 32565), (b) (ZISP 32563)—oligodactyly of the right forelimbs; (c) polydactyly of the left forelimb, (d) oligodactyly of the right limb (ZISP 32564). Hm, humerus; U, ulna; R, radius; Ed, additional fingers; roman numerals (I, IV, V), finger numbers.

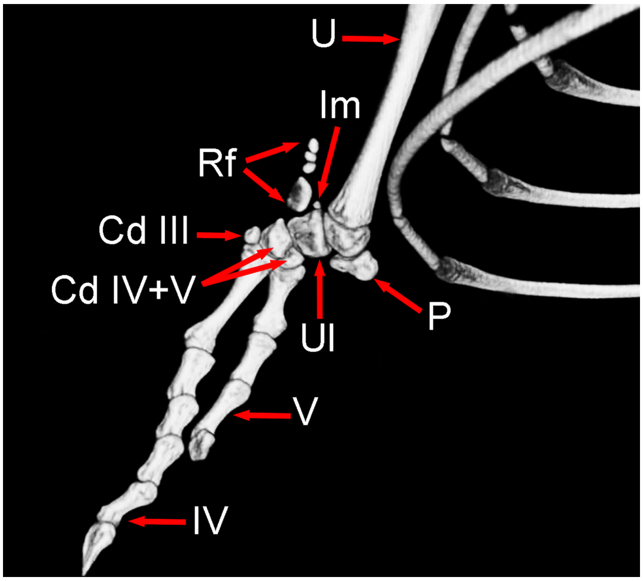

FIGURE 2 Computed microtomography of the abnormal right forelimb of *Lacerta agilis* (ZISP 32565). Cd III, carpalia distalia III; Cd IV + V, fused carpalia distalia IV and V; Im, intermedium; P, pisiforme; Rf, reduced finger; St, sternum; U, ulna; Ul, ulnare; roman numerals (IV, V), finger numbers.

Two fingers consist of five and three phalanges. Judging by the phalangeal formula, these are fingers IV and V. Tomography revealed four more small bones lined up in one line. They may represent another reduced finger. The proximal bone is elongated, similar to the metacarpal. There are three small globular bones, possibly phalanges. The beam of this "finger" is directed proximally, along the ulna (Figure 2). Externally, on a live specimen, it was invisible.

ZISP 32563, male (Figure 1b).

The pectoral girdle is asymmetrical. All its elements on the right side, including the glenoid cavity, lie further away than the corresponding elements on the left side. The right coracoid is ossified to a lesser extent than the left one. A large foramen remains in its center. The right glenoid cavity is practically not pronounced. The epiphysis of the humerus does not enter it. The humerus is adjacent to the pectoral girdle by the proximal part of the diaphysis. The shoulder joint is not formed.

The right forelimb consists of a humerus, an ulna, and a hand with a single finger. The medial part of the proximal epiphysis is underdeveloped in the humerus. The ulnar sesamoid is four times smaller than normal.

The wrist consists of three elements: carpalia distalia, ulnare, and intermedium. The single metacarpal bone is shortened and thickened compared to the normal condition. There is a small ridge on the distal part of the element. The only, probably, third finger consists of four phalanges.

The left forelimb is formed normally, with the exception of the intermedium: it is rounded, not elongated, compared to the normal condition.

ZISP 32564, female (Figure 1c,d).

The pectoral girdle is asymmetrical. The craniolateral end of the right half of the sternum is underdeveloped. The episternum is also underdeveloped. It is represented by a triangular element, the vertex of which is directed caudomedially. The element itself is slightly shifted to the left of the middle line. The right shoulder joint is not formed. The scapula, coracoid, and clavicle are much smaller than the normal ones, and their shape has changed compared to the normal. There is no boundary between the scapula and the supra-scapular cartilage. The left shoulder joint, scapula, and coracoid are formed normally. The clavicle is reduced to a small triangular element.

In the right forelimb, the proximal epiphysis of the humerus is underdeveloped. The bone is thinning from the center proximally. The forearm consists of the ulna. The ulnar sesamoid is four times smaller than normal. The wrist is represented by a single bone, probably carpalia distalia V. The single finger (presumably V) consists of three phalanges.

In the left forelimb the humerus is of normal shape and size. The forearm is represented by two bones: an almost completely fused radius and ulna. There is a small gap only between the proximal thirds of the diaphysis. On the lateral surface of the forearm, the contours of two bones are visible. Complete fusion is observed on the medial side of the forearm.

The hand exhibits preaxial polydactyly and includes eight fingers. The phalanx formula is 3:4:3:3:3:4:5:3. The wrist consists of ulnare, radiale, intermedium, centrale, pesiforme, carpalia distalia I–VI. The intermedium has an unusual, elongated shape. Palmar sesamoids are represented by three elements, one of which is much smaller than the others.

The metacarpus consists of seven bones. The first bone is wider and shorter than the normal elements. There is a foramen at its distal end, indicating that it is the result of the fusion of two elements. Two fingers are attached to it.

4 | DISCUSSION

The variability of the number of fingers among the limb abnormalities of terrestrial vertebrates is the most common disorder. These are oligodactyly (fingers less than 5) and polydactyly (fingers more than 5). Polydactyly, as a variant of the norm, is much less common than oligodactyly and is characteristic only of the early amphibians

(Coates & Clarck, 1990; Lebedev & Coates, 1995) and, as a secondarily acquired trait, for secondary aquatic reptiles—Ichthyosauria, Plesiosauria (Sander, 2000). Some variants of oligodactyly have been evolutionarily entrenched: as the norm in a large number of both extinct and extant tetrapods (Alberch & Gale, 1985; Gans, 1975; Kruzhkova, 2005; Lande, 1978). For example, in the modern amphibian fauna, the four-toed forelimb is the most common variant of the normal structure (see Kruzhkova, 2005). However, representatives of the families Amphiumidae, Proteidae, and Serenidae show more serious changes in the number of fingers—up to three, two, and even one finger (Means, 1996).

In reptiles, weakly expressed oligodactyly is typical as a norm for crocodiles (four fingers on the hind limbs) and turtles (four fingers on the forelimbs). However, the greatest variety of finger reduction options is observed in representatives of the order Squamata, for example, in the families Cordilidae and Scincidae (Essex, 1927; Leonard, 1979; Siebenrock, 1895; Whiting et al., 2004). Oligodactyly is also present as a variant of the norm in all birds and a number of mammals, for example, in ungulates and rodents (Kingsley, 1925; Klimov, 1927; Lewis, 1964). Heterochrony is considered to be the most likely mechanism of such evolutionary transformations. In this case, pedomorphosis, in which a new morphological variant of the limb structure occurs by inhibiting the development of this structure at earlier stages than in the ancestral form (Hinchliffe & Vorobyeva, 1999; Shapiro, 2002; Werneburg & Sánchez-Villagra, 2015). In some forms, we can observe regressive changes directly in ontogenesis. Thus, in *Anguis fragilis* Linnaeus, 1758 (Anguidae), the elements of the basipodium are first laid down in accordance with the usual sequence for a five-toed limb, and then dissolve (Raynaud, 1985; Skawinski et al., 2021); the same pattern is observed in snakes (Guerra-Fuentes et al., 2023).

Judging by the phalangeal formula and by the position relative to the elements of the basi- and zeigopodium, the abnormal lizards described in this study have fingers No. IV and/or No. V. Thus, the rays that are the last to appear in the ontogenesis of amniotes are absent (Vorobyeva, 1999). This feature of the structure is characteristic of the variants that appeared as a result of the heterochrony of limb development. However, in the abnormal specimens described here, the reduction affects both the distal and two proximal parts. The proximal epiphysis is underdeveloped at the shoulder. Moreover, the more the hand is reduced, the more the shoulder is reduced. Also, all specimens lack a radius in the forearm. The ulna is developed almost normally. In oligodactyly, which occurs as a norm, even with its strongest manifestation, for example, in *Bachia trisanale* (Cope, 1868) (Gymnophthalmidae), the stylopodium and zeigopodium are always normally developed, although they may be reduced in size compared with the corresponding parts of the five-toed limbs of related groups (Shapiro, 2002). The listed structural features indicate that these deformities cannot be the result of heterochrony.

The morphology of the abnormal limbs of two lizards (ZISP 32563, 32565) corresponds to radial oligodactyly, which is part of several syndromes (Bergman et al., 2020). The third specimen (ZISP 32564) demonstrates a combination of radial oligodactyly and radial polydactyly. Polydactyly is in most cases the result of mutations (Umair et al., 2018). But the combination of these two anomalies is unique. We have not been able to find similar descriptions in either biological or medical literature.

The pectoral girdle in all abnormal specimens is asymmetrical, although all its elements are present. On the side of the reduced limb, they are usually less developed than the corresponding elements of the opposite side. It is known that skeletal growth is stimulated, among other things, by muscle load (Schoenau & Fricke, 2008; Szulc et al., 2005). During their lifetime, lizards moved their abnormal limbs in a limited way; hence, the pectoral muscles practically did not work and did not give the necessary load to the elements of the girdle itself, which affected the definitive structure of the latter.

Poorly developed glenoid cavity or even its complete absence on the side of the abnormal limb is also quite understandable. It is well known that for the formation of a movable joint, contact of two elements is necessary, as well as their movement relative to each other (Hall, 1963; Lamb et al., 2003; Pitsillides, 2006). Obviously, the incorrect relative position of the girdle and shoulder, as well as the limited movement of the abnormal limb, has become an obstacle to the normal development of the shoulder joint.

The reason for the appearance of the abnormalities described here in *L. agilis* remains unknown. We can exclude the injuries, because the animals have already hatched with these deformities. Note that on 8 June 2022, nine eggs incubated under the same conditions were received from the same pair. No developmental abnormalities were found in this clutch. Egg incubation conditions can also be excluded, because at the same time in the same incubator and under the same conditions there was a clutch of six eggs from another pair of sand lizards (male *L. a. exigua* and female *L. a. boemica*) and no abnormalities were found in the hatched specimens.

It can also be assumed that hybridization is an additional risk factor for the appearance of anomalies. Increased mortality and increased frequency of deformities of various organ systems are quite common during hybridization. They have been observed in bony fish and in representatives of all modern classes of tetrapods (Serebrovsky, 1935; Slijepcevic et al., 2015), including representatives of the tribe *Lacertini* (Danielyan, 1987; Rykena, 1996).

The experimental protocols were approved by the Bioethics Commission of the Zoological Institute of the Russian Academy of Sciences (conclusion No. 1-3/15-06-2021).

AUTHOR CONTRIBUTIONS

Igor V. Doronin: Conceptualization; investigation; methodology; validation; data curation; supervision; project administration; formal analysis; writing – original draft; visualization. **Marina A. Doronina:** Conceptualization; methodology; resources. **Yulia I. Tsuryumova:** Conceptualization; investigation; methodology; validation; formal analysis; resources.

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