Limb malformations in a 1982 Museum collection of *Pleurodeles waltl* larvae.

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Limb malformations in amphibians are well-known occurrences, especially among Caudata (Johnson et al., 2003; Laurentino *et al*., 2016). However, most of the reported cases are of single observations with a malformation prevalence in a population, rarely exceeding 2% (Ouellet *et al*. 1997, Vandenlangenberg *et al*. 2003, Martínez-Silvestre et al., 2014; Mester *et al*. 2015; Laurentino *et al*., 2016). The majority of reported cases show that the number and proportion of limbs and digits (ectromelia – absence or unproportioned limbs; ectrodactyly – absence of digits; polymely – excess limbs; and polydactyly – excess digits) are the most common type of malformations (Ouellet, 2000; Laurentino *et al*., 2016).

Several deformities have been registered in sharp-ribbed newts (*Pleurodeles waltl* Michaelles, 1830) such as: bifurcated limbs (Martins d'Alte, 1941), polymely, polydactyly, brachydactyly (short digital bones) (Héron-Royer, 1884; Lyedig, 1879; Zamora-Camacho, 2016, 2020), limb synostosis (two or more bones fused together) (Sanchiz & Pérez, 1974), ectrodactyly, ectromelia (Lauthier, 1971, Zaamora-Camacho, 2020), polyphalangy (presence of extra digital bones) (Torres & Hidalgo, 2016), syndactyly (fused digits) and ectrodactyly (Zamora-Camacho, 2020).

Here we report the case for the collection of the Sea Museum - King D. Carlos (Cascais, Portugal) that holds 32 specimens of *Pleurodeles waltl* larvae conserved in 4% formaldehyde. We found that 26 out of these 32 specimens (over 80% of the collection) had limb malformations (Table 1, Figure 1). All individuals were captured on the 30th of March of 1982 at Quinta do Marquês de Angeja in Alcabideche, Cascais (38º 73' 85'' 13 N, 9º 41' 24'' 20 W). Since 1982 the main water source has been diverted, and water quantity and quality are frequently compromised. It is doubtful that a relic population still persists.

The most common malformation was brachydactyly (Figure 1A) with over 65% of the affected individuals and over 50% of the collection having at least one malformation of this kind, followed by Amelia (Figure 1C) (absence of limbs) and Polydactyly (Figure 1E G) on over 15% of the individuals that had any malformation. Our findings support the idea that brachydactyly is one of the most common cases for skeletal malformations in urodeles (Diego-Rasilla *et al*., 2007; Williams *et al*., 2008). However, we also found syndactyly (Figure 1F G) and polymelia (Figure 1H) that are among the most uncommon cases of malformations (Escoriza & García-Cardenete, 2005; Diego-Rasilla *et al*., 2007; Williams *et al*., 2008; Hinckley *et al*., 2015).

Like most of the reported cases the reason(s) for the limb malformations could not be accessed (Johnson et al., 1999; Blaustein & Johnson, 2003). Nonetheless the most commonly advanced reasons for limb deformities are related with the loss of limbs, extremely common during their larval development due to predation and even cannibalism - and to the regeneration process, that does not always occur with full success (Sessions & Ruth, 1990, Ballengée & Sessions, 2009; Bowerman *et al*., 2010, Thompson *et al*., 2014). However, there are other environmental and anthropogenic factors that are known to induce morphological malformations in amphibian populations such as parasites (Johnson *et al*., 2003), pollution (Taylor *et al*., 2005), UV radiation (Blaustein *et al*., 1997), temperature (Dournon *et al*., 1998) or the interaction among factors (Ouellet, 2000; Johnson *et al*., 2006 Laurentino *et al*., 2016). A better knowledge on cases like this can be very important for amphibian conservation as the majority of reasons associated with malformations are also associated with mortality events (Blaustein *et al*., 1994, 1997; Paull et al., 2012; Hayden *et al*., 2015).

Although we could not point out a reason for these malformations, registers like this increase the knowledge on amphibian malformations, and might contribute for future

studies as a historical record for the presence of a population with a high prevalence of deformities. This is especially relevant when considering the rate amphibian populations are decreasing worldwide and in biodiversity hotspots in particular such as the case for the sharp-ribbed newt in the Iberian Peninsula.

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Legends of Tables and Figures

Table 1: Deformities found in the collection of *Pleurodeles waltl* present at Sea Museu – King D. Carlos in Cascais per limb and in the total of individuals.

Figure 1: Malformations found in the collection of *Pleurodeles waltl* present at Sea Museu – King D. Carlos in Cascais, Portugal. A – Brachydactyly (short digits); B – Micromelia (short limb); C – Amelia (absent limb); D – Oligodactyly (absent digits); E – Polydactyly (excess digits); F – Syndactyly (fused digits); G – Polysyndactyly (excess digits fused together); H – Polymelia (excess limbs); I – Joint malformations; J – Other digits malformations.

Caption to Figures

Malformations	Limb				Individuals
	Forelimbs		Hindlimbs		
	Right	Left	Right	Left	
Brachydactyly	10	7	3	0	17
Micromelia	0	0	1	2	2
Amelia	1	1	1	1	4
Oligodactyly	0	0	0	1	1
Polydactyly	2	1	1	0	4
Syndactyly	0	1	0	0	1
Polysyndactyly	0	0	1	0	1
Polymelia	1	0	0	0	1
Joint malformations	1	1	0	0	2
Other digits malformations	3	1	0	0	4
Total	18	12	7	4	26

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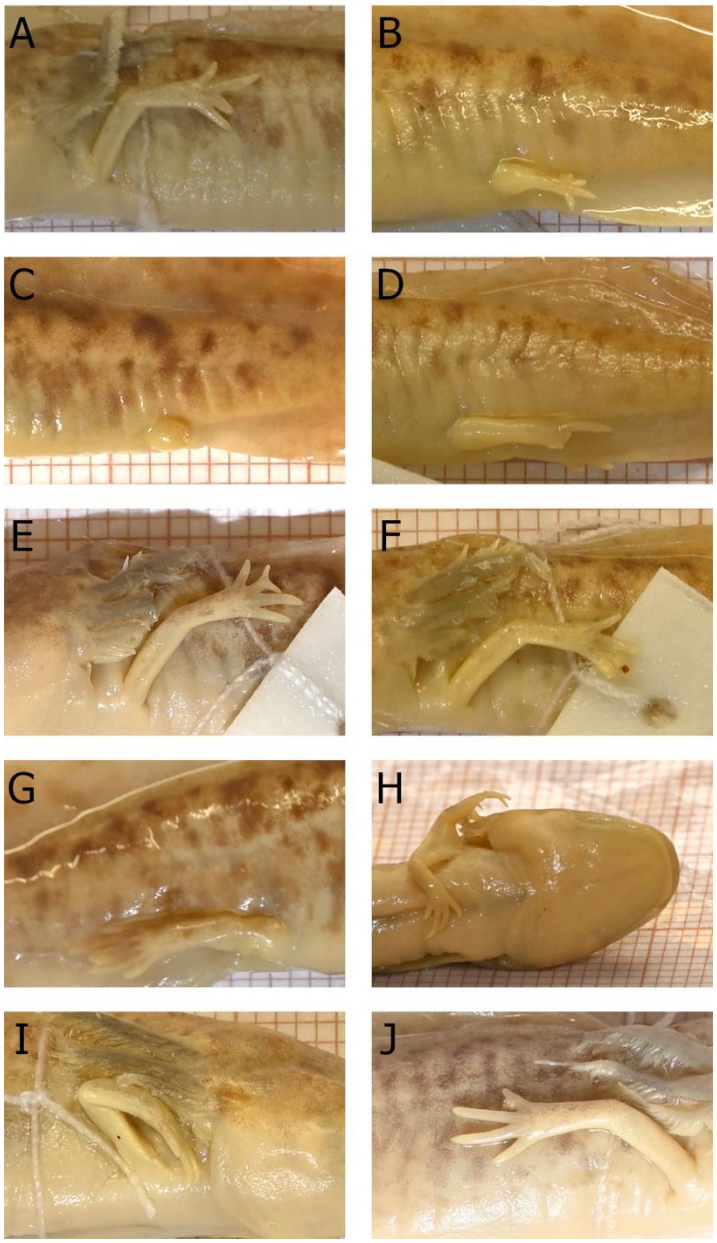

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