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Short Communication

Morphological abnormalities in a population of *Pleurodeles waltl* (Caudata: Salamandridae) from southwestern Spain

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Palavras-chave: bifurcação da cauda, braquidactilia, braquimelia, hipodactilia, membranas cutâneas, polidactilia, sindactilia, tritão-de-costelas-salientes, urodelos.

Prevalence of morphological abnormalities usually is less than 5% in most amphibian populations (Ouellet *et al.* 1997, Vandenlangenberg *et al.* 2003, Mester *et al.* 2015). Morphology is closely related to whole-organism performance in amphibians (Zamora-Camacho 2018, Zamora-Camacho and Aragón 2019a), and thus, is under strong selection (Watkins 1996). Therefore, this low frequency likely reflects the negative effects of abnormalities on whole-organism performance (Zamora-Camacho and Aragón 2019b). However, amphibian populations are experiencing an alarming increase in morphological abnormality rates worldwide (Lanoo 2008, Johnson and Bowerman 2010, Laurentino *et al.* 2016). Amongst these, the most common are limb malformations, such as misshapen or fused limbs, and missing, or presence of extra limbs and toes (Harris *et al.* 2008, Johnson and Bowerman 2010, Reeves *et al.* 2013).

Herein, I report six cases of morphological abnormalities recorded in a sample of 172 Iberian ribbed newts (*Pleurodeles waltl* Michahelles, 1830) from southwestern Spain. This species occurs across the central and southern Iberian Peninsula, as well as northwestern Africa (Salvador 2015). It is the largest salamandrid across its range; adults often exceed 30 cm in total length, roughly half of which corresponds to the tail (Salvador 2015). Normal newts have two forelimbs with four digits and two slightly larger hind limbs with five digits (Salvador 2015). The species is fairly aquatic, mainly from the autumn through the spring, when it inhabits several types of bodies of water—mainly medium-to-large temporary ponds (Salvador 2015). However, *P. waltl* typically aestivates during the summer, and can be found wandering on land on rainy nights.

Sampling was conducted in Pinares de Cartaya (37°19' N, 70°11' W, 60 m a.s.l.), an

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11,000-ha *Pinus pinea* L. grove with an undergrowth dominated by *Cistus ladanifer* L., *Pistacia lentiscus* L., and *Rosmarinus officinalis* L. scrub. Although it is unclear whether this formation is natural or human-introduced, there is evidence of its predominant role in the area from at least the last 4000 years; so, it is widely considered as a natural landscape (Martínez and Montero 2004). Between 2016 and 2018, the area was sampled 21 times for adult *Pleurodeles waltl*. Sampling was conducted on rainy autumn and winter nights, when the salamanders were intercepted during their terrestrial activity. A total of 172 individuals (93 females and 79 males) were captured. I checked morphology of each newt in search of morphological abnormalities. Newts were released after toe-clipping as a part of a long-term research project. Toe-clipping is a common marking and tissue-sampling technique with little effect on urodele behavior, growth, and survival (Ott and Scott 1999, Kinkead *et al.* 2006, Pfleeger *et al.* 2016). Therefore, recaptures were identified and not counted more than once.

In total, 3.49% of the sample (six individuals) had abnormalities of some type; the proportion was greater in females (4.30%, four individuals) than in males (1.53%, two individuals). Specifically, the abnormal individuals encountered were one female with polydactyly, syndactyly, and/or brachydactyly in both forelimbs and in one hindlimb (described in Zamora-Camacho 2016), along with the individuals shown in Figure 1.

In this sample, brachydactyly was the most frequent abnormality (1.74% of individuals in the sample), followed by syndactyly (1.16%), hypodactyly, polydactyly, brachymelia, interdigital skin webbing, and bifurcated tail (0.58%). These findings match previous ones that the prevalence of morphological abnormalities in amphibian populations is usually around or below 3% (Gillilland *et al.* 2001, Mester *et al.* 2015, Laurentino *et al.* 2016, Zambrano-Fernández *et al.* 2020); however, the frequency can be higher in some local populations (Worthington 1974,

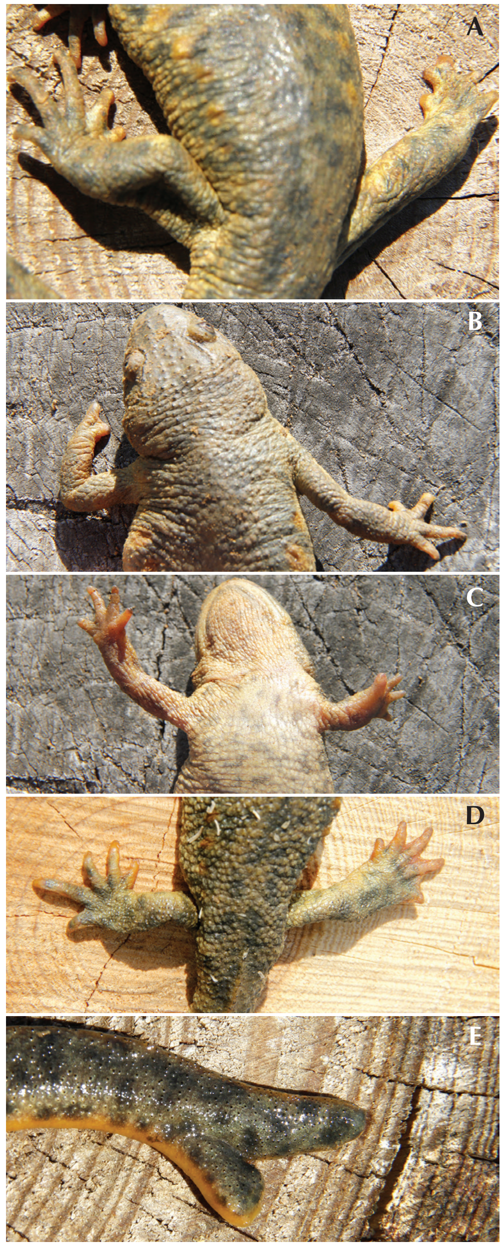

Figure 1. Morphological abnormalities found in *Pleurodeles waltl*. (**A**) A female with brachydactyly and syndactyly in right hindlimb, (**B**) a male with hypodactyly in left forelimb, (**C**) a female with brachymelia in left forelimb, (**D**) a female with brachydactyly and interdigital skin webbing in right hind limb, and (**E**) a male with bifurcate tail.

Hanken 1983, Zamora-Camacho and Medina-Gálvez 2019). In an isolated population of *Pleurodeles waltl* in southern Spain, three of eighteen individuals (16.7%) were polyphalangic (Torres and Hidalgo 2016).

These results are consistent with previous reports that brachydactyly is amongst the most frequent, and hypodactyly amongst the least frequent, morphological abnormalities in amphibians (Stocum 2000, Williams *et al.* 2008). However, the prevalence of syndactyly was higher, and that of polydactyly was lower, in this sample than in other cases reported (Stocum 2000, Williams *et al.* 2008). Three individuals had multiple abnormalities, which is considered to be rare (Stocum 2000, Williams *et al.* 2008). However, taken together, these limb abnormalities are relatively common in urodeles (Johnson *et al.* 2003, Martínez-Silvestre *et al.* 2014, Laurentino 2016). Skin webbing (not necessarily interdigital) is relatively common in anurans (Gardiner and Hoppe 1999, Meteyer *et al.* 2000, Johnson *et al.* 2001a, 2001b, Ankley *et al.* 2004), but not many such cases are known in urodeles (Meyer-Rochow and Asashima 1988, Johnson *et al.* 2006, D'Amen *et al.* 2008, Laurentino *et al.* 2016). Likewise, tail bifurcation is uncommon in wild adult urodeles (reviewed in Henle *et al.* 2012).

The potential causes of these abnormalities are unclear. In many cases, habitat pollution has been reported as a trigger of morphological abnormalities in amphibians (Álvarez *et al.* 1995, Taylor *et al.* 2005, D'Amen *et al.* 2008). However, the individuals described here were sampled in a well-preserved natural habitat; thus, pollution does not seem plausible. Excess ultraviolet radiation (Pahkala *et al.* 2001) and anomalous temperatures (Worthington 1974) during embryonic development also can provoke morphological abnormalities in amphibians. But again, this was a low-elevation, covered-canopy forest, which makes both phenomena unlikely. Parasite infections also can produce abnormalities (Johnson *et al.* 2003), sometimes synergistically with predation events (Johnson *et al.* 2006). Also, failed predator attacks often result in lost limbs and/or tails that, in this (Elewa *et al.* 2017) and other urodeles (reviewed in Nye *et al.* 2003), can be regenerated. An aberrant regeneration may lead to a morphological anomaly (Young 1977). For example, skin webbing may appear in newts (*Notophthalmus viridescens*) that have been forced experimentally to regenerate a limb several times (Dearlove and Dresden 1976). Finally, a genetic origin of these abnormalities cannot be dismissed (Droin and Fischberg 1980).

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