Submission date: 9 September 2010

First record of bicephaly in *Lissotriton boscai* (Amphibia, Caudata, Salamandridae)

F.A. Fernández-Álvarez[1], E. Recuero[1], I. Martínez-Solano[2], D. Buckley[1,*]

[1]*Museo Nacional de Ciencias Naturales, C.S.I.C., c/ José Gutiérrez Abascal, 2. 28006 Madrid, Spain.*

[2] *Instituto de Investigación en Recursos Cinegéticos, C.S.I.C.-U.C.L.M.-J.C.C.M., Ronda de Toledo, s/n, 13005 Ciudad Real Spain*

[*]corresponding author: email: dbuckley@mncn.csic.es

Running title: Bicephaly in *Lissotriton boscai*

Abstract:

Teratologies are frequent among vertebrates, but with differing prevalence among groups. For instance, cases of bicephaly are extremely scarce in amphibians, in contrast with other groups, like reptiles. Here we report the first case of bicephaly in *Lissotriton boscai*. The anomaly is a consequence of the duplication of the skeleton axis and the subsequent development of most of the cephalic structures in each axis, with heads fused at the level of the cranial post-otic structures. Despite its young age and small size, the larva presents an advanced stage of development. The low frequency of cases of bicephaly among amphibians can be a consequence of high mortality rates in early stages, but it could also reflect differences in the developmental properties between vertebrate lineages.

Developmental abnormalities are frequently reported both among vertebrates born in captivity and those from natural conditions. Teratological individuals are common in mammals (e.g. Wu 2002) and reptiles, for instance (e.g. lizards [Pleticha 1968, Spadola & Insacco 2009], turtles [e.g. Vanni & Nistri 1987, Diong et al. 2003], and occurring very frequently in snakes [Heasman 1933, Belluomini 1959, Da Cunha 1968, De Lema 1982, 1994, Khaire & Khaire 1984, Mitchell & Fieg 1996, Oros et al. 1997, Maryan 2001, Hoser & Gibbons 2003, de Albuquerque et al. 2010]).

Some cases of developmental abnormalities are also frequent in amphibians, especially polymelia (presence of supernumerary limbs) and polydactyly (presence of supernumerary digits) (Sealander 1944, Johnson et al. 2001, Recuero-Gil & Campos-

Asenjo 2003). Reports of bicephaly in amphibians are, however, more scarce. To our knowledge, bicephaly has only been documented in three anuran larvae (Loyed 1897, Lebedinsky 1921, Dragoiu & Busnitza 1927) and in three urodele larvae of species included in the family Salamandridae (Pereira & Rocha 2004; Velo-Antón et al. 2007). In this note, we describe a new case of bicephaly in a larva of the Iberian endemic Bosca's newt, *Lissotriton boscai*, a species also included in Salamandridae.

A sample of water, vegetation, and aquatic invertebrates was taken from a pond in Somao (Asturias, Spain, 43º 31' 37.81"N, 6º 07' 07.96" W, 254 masl) in March 2000. The teratological individual of *L. boscai* described in this note (Figs. 1 and 2) was accidentally removed from the pond at the egg stage and was raised in captivity, mimicking natural conditions, until it died a few days later. The individual presented two fully formed and opened (but non-functional) mouths. Teeth were clearly visible in both mouths. Two eyes were present on each head. The anterior-most ones (right eye of the left head, and left eye of the right head on dorsal view, Fig. 1) were well developed and seemed fully functional. The two other eyes were reduced in size. This reduction seemed related to the abnormal location of gills. The left gills of the left head and the right ones in the right head were normally located, but the pairs of gills in the area where the two heads fused were misplaced and have developed dorsally, posterior to the eyes (Fig 1). All the gills were very reduced, corresponding to the size of gills in normal pre-metamorphic *L. boscai* larvae. Two skeleton axes could be clearly perceived running posterior to each head and fusing posterior to the pectoral girdle. Unlike some rare examples of craniofacial duplications (diprosopus) (Wu et al. 2002, Velo-Antón et al. 2007), this case of bicephaly implies the early embryonic duplication of the skeleton axis and the development of (most) of the cephalic structures in each axis. The two axes, however, did not give rise to two semi-independent bodies (e.g. de Albuquerque et

al. 2010), but the two bodies remained fused at the level of the cranial post-otic structures. The teratological individual presented two hind- and two fore-limbs, fully formed and with well-developed digits (pre-metamorphic stage). The dorsal fin was still visible, but very reduced in size. The anomalous larva, with morphological characteristics of pre-metamorphic stage, was unable to feed normally and died after a few days, with a size of 16mm. Size at hatching of normal *L. boscai* larvae ranges between 8 and 10mm; they normally grow to 30-35mm prior to metamorphosis (Montori & Herrero 2004).

The scarcity of examples of bicephalous amphibians is in striking contrast with data gathered for other vertebrate groups. So far, for instance, more than 1000 cases of bicephaly have been reported in snakes (see de Albuquerque et al. 2010, and references therein). Several factors may account for this difference. First, bicephalic salamanders may be as frequent as in other groups, but harder to detect. Amphibian metamorphosis is a rather challenging process from a developmental, morphological, and physiological point of view; a challenging process that is seldom overcome by teratological bicephalous larvae. In amphibians, thus, we would expect to find this kind of abnormalities at the larval stage and in very few, if any, adults. Given that aquatic larval stages are short transient phases, and that life expectancy of teratological larvae would be further reduced especially due to difficulties to feed and the increased chances of predation, the probability of observing cases of bicephaly in amphibians will be necessarily lower than in other groups. Second, the difference in the rate of occurrence of the abnormalities between groups may be real, reflecting differences in the developmental properties of the various vertebrate lineages. Accounting for and reporting such differences in the rate and kind of specific abnormalities is not, thus, trivial. The comparative analysis of the type, frequency, and anatomical characteristics

(skeletal elements and tissues involved) of the teratological individuals among lineages would shed some light on the specific ecological and/or developmental processes responsible for the differential occurrence of abnormalities among groups.

Acknowledgements:

The work of F. A. Fernández-Álvarez is funded by a C.S.I.C. “Iniciación a la Investigación” fellowship. I. Martínez-Solano is a "Ramón y Cajal" postdoctoral fellow funded by the Universidad de Castilla la Mancha and the Spanish Ministerio de Ciencia e Innovación (MICINN), and D. Buckley is a “JAE-DOC” postdoctoral fellow funded by the C.S.I.C. Partial funds were provided by a grant from the MICINN (Ref: CGL2008-04271-C02/01/BOS, PI: IMS).

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Figure 1. Dorsal view of the bicephalic *L. boscai* larva. Scale bar = 0,5mm © F. A. Fernández-Álvarez.

Figure 2. Ventral view of bicephalic larva in *L. boscai*. Scale bar = 0,5mm © F. A. Fernández-Álvarez.