Coexistence and intermediate morphological forms between *Vipera aspis* and *V. latastei* in the intensive agriculture fields of north-western Iberian System

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Resumen: Se presenta la distribución de *Vipera aspis* y *V. latastei* en la zona de contacto de Oja-Tirón (noroeste del Sistema Ibérico) caracterizada por un paisaje predominantemente agrícola intensivo. *Vipera aspis* ha sido detectada en un total de 106 celdas UTM 1x1 km, mientras que *V. latastei* en 57. Las dos especies se han detectado en simpatría en 15 celdas UTM 1x1 km y en sintopía en cuatro de ellas. De los 549 ejemplares capturados y encontrados atropellados se han detectado 46 individuos con características morfológicas intermedias entre las dos especies, incluyendo un ejemplar con coloración gris uniforme (concolor), que constituye el primer registro de una víbora mediterránea occidental con esta coloración en la península ibérica. De manera similar a lo que ocurre en la zona del Alto curso del río Ebro, los ejemplares con características intermedias pueden ser resultado de un proceso de hibridación entre las dos especies de víboras.

European vipers (genus *Vipera*) display parapatric distributions that reflect contrasting climatic niches and past evolutionary trajectories (Saint-Girons, 1980). In regions of climatic transition, species distributions can meet in contact, i.e. contact zones, where, at local scale, species frequently show variable levels of habitat segregation, in accordance to ecological and evolutionary differentiation (Luiselli, 2006; Martínez-Freiria *et al.*, 2008; Tarroso *et al.*, 2014; Mebert *et al.*, 2015; Guiller *et al.*, 2017).

The mechanisms allowing species coexistence among European vipers have been intensively studied in distinct contact zones, including: 1) *Vipera aspis* and *V. berus* in the Atlantic Loire region of north-western France (Saint-Girons, 1975; Naulleau, 1986; Guiller *et al.*, 2017), in the Massif Central in central France (Duguy & Saint-Girons, 1978), in the Bernoises Pre-Alps of Switzerland (Monney, 1996) and in the Alpine region of Lombardy in northern Italy (Scali *et al.*, 2011); 2) *V. aspis* and *V. ursinii* in the Abruzzo mountains of central Italy (Luiselli *et al.*, 2007); 3) *V. latastei* and *V. seoanei* in the Peneda-Gerês Mountains of north-western Portugal (Brito & Crespo, 2002); 4) *V. aspis*, *V. berus* and *V. ammodytes* in the Julian Alps in north-western Slovenia (Mebert *et al.*, 2015, 2017); and 5) *V. aspis*, *V. latastei* and *V. seoanei* in the High course of the Ebro river in northern Spain (Martínez-Freiría *et al.*, 2006, 2008, 2009, 2010; Tarroso *et al.*, 2014).

Recent insights into the contact zones of north-western Slovenia (*V. aspis*, *V. berus* and *V. ammodytes*; Mebert *et al.*, 2015) and north-western France (*V. aspis* and *V. berus*; Guiller *et al.*, 2017) revealed syntopic specimens and punctual hybridization,

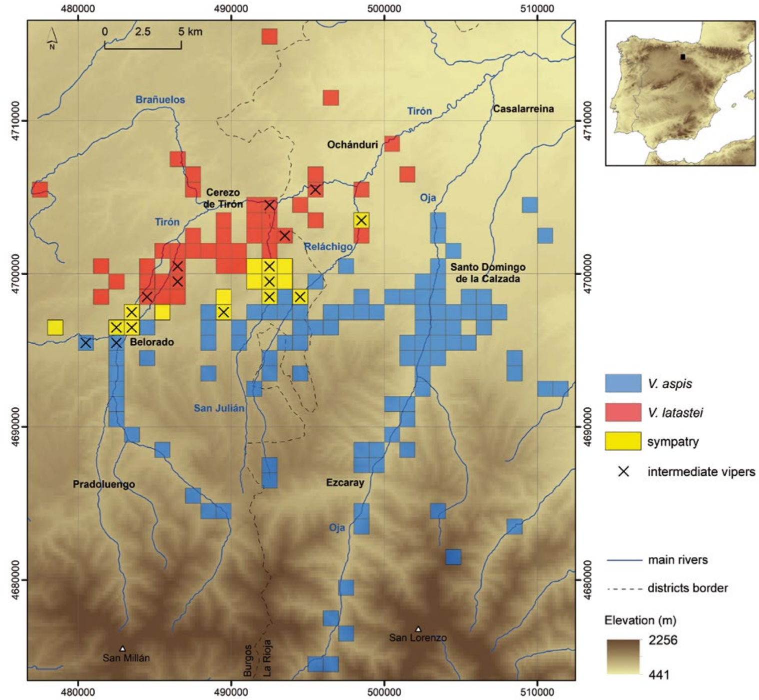

Figure 1: Distribution of *V. aspis*, *V. latastei* and vipers with intermediate traits between *V. aspis* and *V. latastei* (i.e. intermediate vipers) in the Oja-Tirón contact zone, based on 1x1 km UTM squares (ETRS1989). Location of the study area is showed (top right).

Figura 1: Distribución de *V. aspis*, *V. latastei* y víboras con características intermedias entre *V. aspis* y *V. latastei* en la zona de contacto de Oja-Tirón, basada en celdas UTM 1x1 km (ETRS1989). Se muestra la localización del área de estudio (arriba a la derecha).

respectively; however, extensive syntopy and hybridization has been only documented among the sibling species *V. aspis* and *V. latastei* in northern Spain (Martínez-Freiría *et al.*, 2006; Tarroso *et al.*, 2014).

The Western Mediterranean vipers, *V. aspis* and *V. latastei*, are closely related species that exhibit several contact zones across their distribution areas (Martínez-Freiría, 2014; Martínez-Freiría *et al.*, 2014). Sympatry between these species has been suggested in six areas of the Iberian Peninsula: four in the southern slopes of the Pyrenees, one in the Iberian System, and one in the High course of the Ebro River (Martínez-Freiría, 2014; Martínez-Freiría *et al.*, 2014). In the

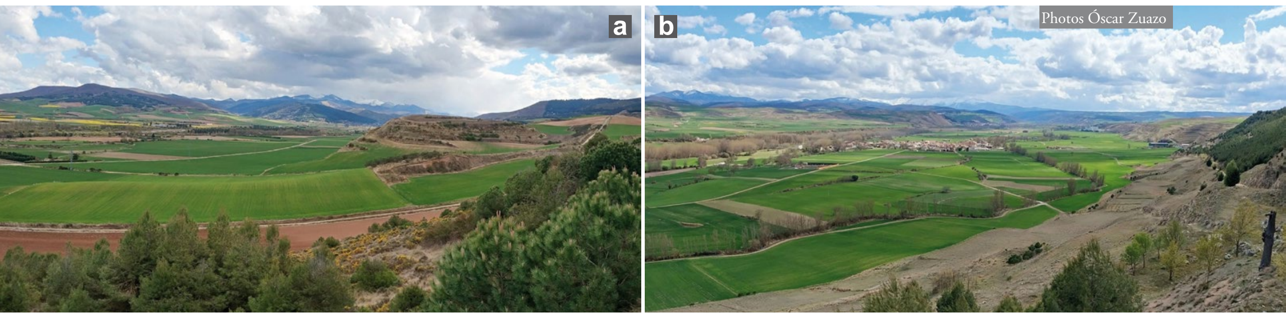

Figure 2: Pictures of the landscapes in Oja (a) and Tirón (b) river valleys, where *V. aspis* and *V. latastei* occur, respectively. Pictures were taken from north to south. Intensive cereal cultures are in the second plane, while Demanda Mountains in the background.

Figura 2: Imágenes de los paisajes de los valles del río Oja (a) y Tirón (b), donde se encuentran *V. aspis* y *V. latastei*, respectivamente. Las imágenes fueron sacadas desde el norte en dirección sur. Los campos de cultivo se encuentran en segundo plano, mientras que la Sierra de la Demanda en el fondo.

High Ebro, sympatry and syntopy were reported in a relatively large area of 8 km^2, where about 20% of captured specimens showed intermediate morphology between *V. aspis* and *V. latastei*, and were mostly confirmed as hybrids between these species by genetic studies (Martínez-Freiría *et al.*, 2006; Tarroso *et al.*, 2014). This hybrid zone is coincident with a steep ecotone between the Atlantic and Mediterranean bioclimatic provinces, with hybrids being found in areas of suboptimal habitat for parental taxa (Tarroso *et al.*, 2014). Aside from this well-studied contact zone, little is known about the remaining contact zones between *V. aspis* and *V. latastei* (e.g. Ferrer *et al.*, 2018). In this note, we report sympatry, syntopy and the existence of intermediate morphological forms between the two Iberian vipers, *V. aspis* and *V. latastei*, in the north-western slopes of the Iberian System in north-central Spain.

The study area is located north to the Demanda Mountains (Iberian System) and south to La Bureba region (centroid: 30T 495000; 4695000; ETRS1989 datum), between Burgos and La Rioja provinces (Figure 1). It is shaped by the valleys of two main rivers, the Tirón and the Oja, tributaries of the Ebro River. Unlike the High Ebro contact zone, which is embedded in a vast natural landscape (Martínez-Freiría *et al.*, 2006), the study area is located in a zone of substantial anthropogenic disturbance, consisting mostly of intensive culture fields of cereals. Altitude ranges from 480 to 2271 masl. The area matches with the transition between Euro-Siberian and Mediterranean regions, resulting in a Mediterranean-Atlantic climatic character (Sillero *et al.*, 2009). The climate is characterised by low levels of precipitation, with annual rainfall ranging from 604-1016 mm/year, and low annual temperature, ranging from 4.1 to 12.5º C (Hijmans *et al.*, 2005).

The study area was surveyed by foot and car (to find road-killed vipers), along a four-years period (March 2015 - November 2018). Alive specimens were captured, measured and photographed, and their geographic position (UTM coordinates, ETRS1989 datum) recorded with a Global Positioning System. Road-killed specimens were collected and preserved in ethanol for further analyses. Specimens' classification as *V. aspis*, *V. latastei* or intermediate forms was done by a set of diagnostic morphological traits (see Table 1) following the criteria used to identify species in the High Ebro contact zone (see Martínez-Freiría *et al.*, 2006, 2009). Specimens were classified as intermediate forms when displaying intermediate morphological traits between each species or a combination of traits which are considered diagnostic for each species.

Table 1: Diagnostic morphological traits used to differentiate specimens of *V. aspis* from specimens of *V. latastei* (from Martínez-Freiría *et al.*, 2006).
Tabla 1: Rasgos morfológicos diagnósticos usados para diferenciar ejemplares de *V. aspis* y *V. latastei* (de Martínez-Freiría *et al.*, 2006).

Trait	*V. aspis*	*V. latastei*
Snout	Slightly upwards	Snout upwards, forming an appendix
Number of apical scales	2 - 3	3 - 9
Shape of the dorsal draw	Alternated cross bands with a thin vertebral line (type 0) or narrow angular zigzag (type 1)	Wide zigzag (type 2) or rounded-rhomboidal marks running together to form a wavy or zigzag stripe (type 3)
Number of dorsal marks	45 - 78	33 - 57

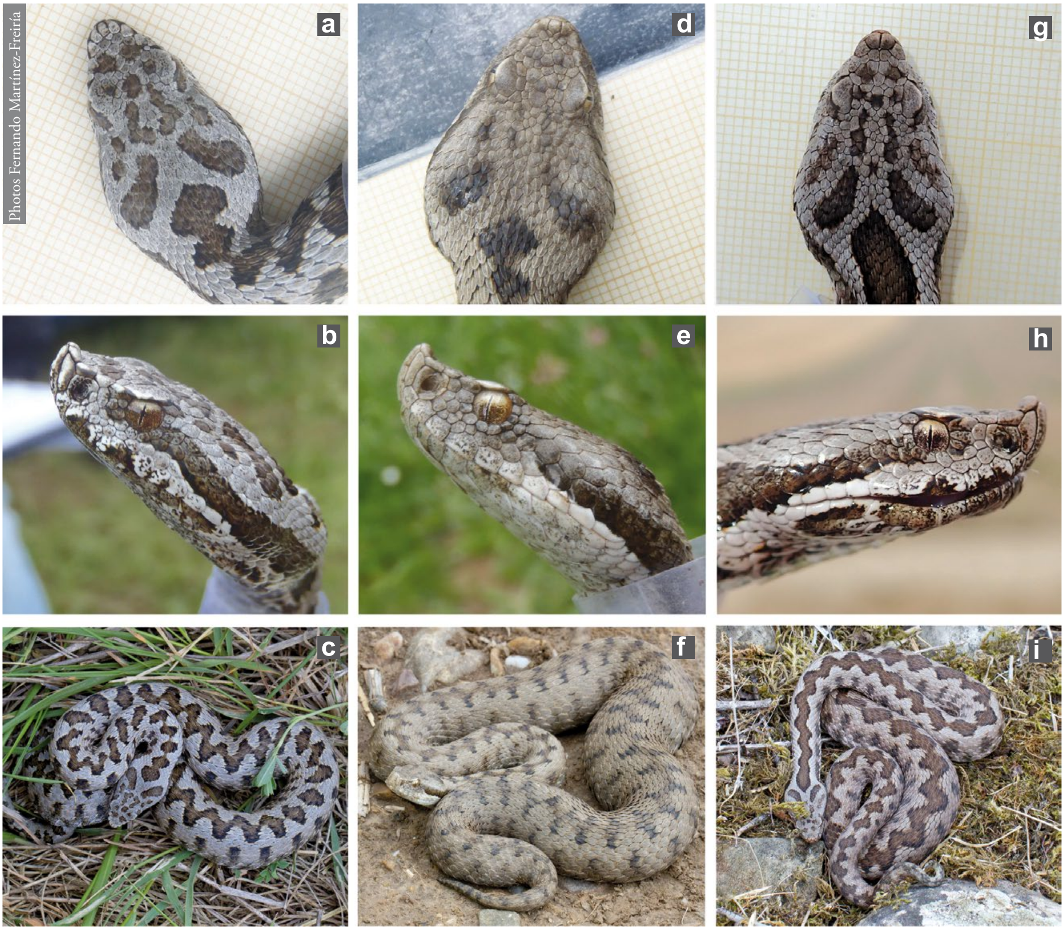

Figure 3: Pictures of three individuals classified as intermediate vipers (i.e. vipers with intermediate traits between *V. aspis* and *V. latastei*). a), b) and c), correspond to a male with three apical scales, snout slightly upwards, 49 dorsal marks and dorsal draw type 1/2. d), e) and f) correspond to a female with five apical scales, snout upwards, 46 dorsal marks and dorsal draw type 1. g), h) and i) correspond to a male with two apical scales, snout upwards, 42 dorsal marks and dorsal draw type 3.

Figura 3: Imágenes de tres individuos clasificados como víboras intermedias (es decir, víboras con rasgos intermedios entre *V. aspis* y *V. latastei*). a), b) y c) corresponden a un macho con tres escamas apicales, hocico levemente hacia arriba, 49 marcas dorsales y diseño dorsal de tipo 1/2. d), e) y f) corresponden a una hembra con cinco escamas apicales, hocico hacia arriba, 46 marcas dorsales y diseño dorsal de tipo 1. g), h) e i) corresponden a un macho con dos escamas apicales, hocico hacia arriba, 42 marcas dorsales y diseño dorsal de tipo 3.

A total of 549 specimens were collected (501 alive, 48 road-killed), 320 classified as *V. aspis* (133 females, 154 males) and 186 as *V. latastei* (82 females, 80 males). *V. aspis* was distributed throughout the southern zone of the study area, while *V. latastei* occurred in the northern zone (Figure 1). Both species were mainly found along hedges connecting extensive cereal fields (Figure 2), but *V. aspis* tended to be present in habitats that were more humid and less exposed to solar radiation than *V. latastei*. Both species showed a general allopatric distribution with sympatry in the central zone of the study area (Figure 1). *V. aspis* and *V. latastei* were detected in 106 and 57 1x1 km UTM squares respectively, and were found in sympatry in 15 squares. Syntopic specimens of both species were found in four of these sympatric squares.

A total of 46 specimens (19 females, 24 males) with intermediate traits were observed in 17 1x1 km UTM squares, mostly in sympatric areas (eight squares in sympatry) or adjacent squares (Figure 1). These specimens presented two to five apical scales, the shape of the dorsal draw including type 0, type 1, type 2 and mixtures of types 0/2, 1/2 (the most common dorsal pattern; 30% of specimens), and 2/3, and 33 to 65 dorsal marks (Figure 3). One adult male (470 mm of body size) captured on March 2017 within a sympatric square (surroundings of Redecilla del Camino, Burgos; 30T VM99), presented three apical scales and a greyish uniform colouration without dorsal zigzag pattern (i.e. concolor colouration; see Figure 4).

Figure 4: Picture of three males captured on March 2017 in the surroundings of Redecilla del Camino (Burgos). From left to right, *V. aspis* (male with three apical scales and dorsal draw type 1), *V. latastei* (male with five apical scales and dorsal draw type 2) and the concolor *Vipera* sp.

Figura 4: Imagen de tres machos capturados en marzo de 2017 en los alrededores de Redecilla del Camino (Burgos). De izquierda a derecha, *V. aspis* (macho con tres escamas apicales y diseño dorsal de tipo 1), *V. latastei* (macho con cinco escamas apicales y diseño dorsal de tipo 2) y el ejemplar concolor.

In the absence of complete reproductive isolation between these species and with the existence of ecological conditions that allow sympatry, *V. aspis* and *V. latastei* may be hybridizing at this contact zone; being intermediate vipers likely hybrids between both species (Martínez-Freiría *et al.*, 2006, 2009; Tarroso *et al.*, 2014). Landscape transformation can facilitate species interactions by reducing habitat heterogeneity and limiting natural resources, but it can also lead to fragmentation of suitable habitats of sympatric species by reducing landscape interconnectivity between them. Comparative studies addressing gene flow dynamics, morphological variability, and habitat selection between species in both contact zones are needed to understand the mechanisms allowing coexistence of these viper species, as well as the role of landscape transformation in species distribution.

The spatial distribution of sympatric areas and hybrids in the Oja-Tirón contact zone are quite dissimilar to the patterns reported for the High Ebro, where a single continuous sympatric area was found in the confluence of Sedanillo and Rudrón rivers and intermediate vipers were all located inside this area (Martínez-Freiría *et al.*, 2006). Landscape configuration of Oja-Tirón is probably the key to understand such spatial pattern: the occurrence of south-north oriented rivers from the Demanda Mountains to the lower Tirón Valley (Tirón, Retorto, San Julián and Reláchigo; Figure 1) likely produces a patchy distribution of suitable conditions allowing coexistence of both species, and consequently hybridization. Dispersal of putative hybrids northwards through river valleys would explain their occurrence outside sympatric areas within the area of *V. latastei*. Nevertheless, *V. aspis* could have passed unnoticed in the sampling campaigns developed in these areas and future prospections could provide northwards records for this species. Further studies addressing spatial ecological patterns such as home range or dispersal of parental species and putative hybrids are essential to understand the ecological dynamics of viper species at Oja-Tirón contact zone.

The finding of the concolor viper constitutes the first record of a Western Mediterranean viper with this colouration in the Iberian Peninsula. We base this statement in the revision of more than 700 specimens of *V. aspis* and 1100 of *V. latastei* from all the Iberian Peninsula, including specimens from fieldwork and collections in European museums and universities. Concolor individuals of *V. aspis* are known in montane populations of the French and Italian Alps (Mebert *et al.*, 2011; Tessa, 2016). Some populations present more than 50% of individuals with this coloration, which has been referred as result of local adaptive pressures (Dubey *et al.*, 2015). However, we suggest that this specimen is the result of hybridization between *V. aspis* and *V. latastei*. The number of apical scales can be attributed to both species, while the snout height and other traits such as the high division of the dorsal cephalic shields suggests *V. aspis* and *V. latastei*, respectively (see Martínez-Freiría, 2014; Martínez-Freiría *et al.*, 2014). Hybridization is known to promote colour polymorphism in other groups of animals (e.g. Medina *et al.*, 2013) and it is expectable in this contact zone.

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