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DNA barcoding confirms the presence of *Hydria cervinalis* (Scopoli, 1763) in the Iberian Peninsula (Lepidoptera: Geometridae: Larentiinae)

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The genus *Hydria* Hübner, 1822 is distributed across the Palearctic and Nearctic regions, and includes more than 50 species in South America. In Europe, this genus includes five species subdivided into three species-groups, the *undulata*, the *cervinalis* and the *montivagata* species group (Hausmann & Viidalepp 2012). Of these five species, three have been reported from the Iberian Peninsula, *Hydria undulata* (Linnaeus 1758), *H. gudarica* (Dufay 1983), and *H. montivagata andalusica* (Ribbe 1912) (Redondo *et al.* 2009; Hausmann & Viidalepp 2012). *Hydria cervinalis* (Scopoli, 1763) is widely distributed from England to western European Russia to the east and from southern Fennoscandia, to the Alps and Carpathians Mountains in the south. This distribution matches a Euro-Caucasian pattern (Hausmann & Viidalepp 2012). *H. cervinalis* was cited from Eastern Pyrenees (Mazel & Peslier 1997), in the southern Iberian System based on one female from Uña (Cuenca) (Dominguez 1991, unpublished) and from Albarracín (Teruel) (Zerny 1927). However, according to Redondo *et al.* (2009), all these records must be referred to *H. gudarica*. Recently, *H. cervinalis* has been cited by Hausmann and Viidalepp (2012) in the Iberian Peninsula based on our data.

In this paper, we use morphological and DNA barcoding data to confirm the presence of *Hydria cervinalis* in the Iberian Peninsula. Based on this evidence, one female specimen was preliminarily classified as unidentified and, thereafter, female genital morphology and DNA barcoding were integrated to identify it as *Hydria cervinalis*.

A single adult female of *Hydria cervinalis* was collected in Valle del Lago-Pola de Somiedo, Asturias, Spain (Lat/Lon: 43.063, -6.1785; Elevation; 1295 m; Date: 22-V-2011; Juan José Guerrero leg.). The specimen is deposited in the collection of the Department of Zoology and Physical Anthropology of University of Murcia (Spain).—The female was examined externally in order to evaluate possible differences in its colouration and wing shape, and it was dissected using standard procedures (Hausmann 2001) with minor modifications. The female genitalia was compared with drawings and photographs provided by Leraut (2009), by the Lepidoptera Dissection Group (2013) and by Hausmann and Viidalepp (2012). Barcoding was carried out by removing two legs for DNA extraction. This sample was submitted to the Canadian Centre for DNA Barcoding (CCDB) at the Biodiversity Institute of Ontario, University of Guelph and it was processed following standard protocols. Voucher data, image, sequence, and trace files are publicly available on the Barcode of Life Database (BOLD) (Ratnasingham and Hebert 2007). Specimen sequence was compared to a reference barcode database of Lepidoptera barcodes using the identification engine (BOLD-ID) and identified as *H. cervinalis*, a Eurasian species not known to occur in Iberian Peninsula.

The Iberian specimen showed morphological traits typical of European individuals of *H. cervinalis*, although we found slight differences in the sclerotized appendix of the genitalia, which is sharper than that of European individuals, and also in the small patch at the base of the appendix, which showed few spinules (Figure 1). The COI sequence of the sampled specimen had 658 nucleotide bp. Intraspecific genetic distance of a total of 25 specimens of *H. cervinalis* from seven European countries averaged 0.80% with a maximum value of 2.63% (referring to a diverging specimen from northern Italy). The Iberian specimen from Somiedo showed sequence identity with 10 other specimens from Austria, Finland, France, Germany and Netherlands. Integrating the evidence from COI mitochondrial DNA sequences, and adult morphology, we conclude that the *H. cervinalis* specimen collected in the UNESCO Biosphere Reserve of the Natural Park of Somiedo (Asturias) is genetically identical with specimens from western, central and northern Europe based on mitochondrial data, although the characteristic female genital morphology seems to present slight differences which could indicate a relict population in the Northern Iberian Peninsula. We emphasize here the importance of combining traditional morphological analysis and ecological traits with the additional dataset of DNA sequences for taxonomic groups whose identification is particularly difficult and mainly based on differences in the genitalia.