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First record of *Trocheta subviridis* Dutrochet, 1817 (Arhynchobdellida, Erpobdellidae) from the north-eastern Iberian Peninsula (Navarra, Spain)

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ABSTRACT

First record of *Trocheta subviridis* Dutrochet, 1817 (Arhynchobdellida, Erpobdellidae) from the north-eastern Iberian Peninsula (Navarra, Spain)

We reported the first record of the rare species *Trocheta subviridis* Dutrochet, 1817 (Arhynchobdellida, Erpobdellidae) from north-eastern Spain (Suspiro stream at Señorio de Bértiz Natural Park, Navarra, Spain). The species was previously recorded in Spain by Cordero del Campillo (1980) and García Más and Jiménez (1981). However, the latter authors admitted (Garcia Más & Jiménez, 1984) that both citations could be considered erroneous due to a misinterpretation by Cordero del Campillo of Blanchard (1893) (Jueg, 2008). Thus, considering the limited and uncertain information about *T. subviridis* in Spain, we can argue that our specimen is the first valid record reported for the species in the Iberian Peninsula.

Key words: Hirudinea, leech, Suspiro stream, Natural Park Señorio de Bertiz

RESUMEN

Primer registro de la especie* Trocheta subviridis *Dutrochet, 1817 (Arhynchobdellida, Erpobdellidae) desde el noreste de la Peninsula Iberica

En esta publicación reportamos el primer registro de la rara especie Trocheta subviridis *Dutrochet, 1817 (Arhynchobdellida, Erpobdellidae) en el noreste de España (arroyo Suspiro en el Parque Natural del Señorío de Bértiz, Navarra, España). La especie fue registrada previamente en España por Cordero del Campillo (1980) y García-Más y Jiménez (1981). Sin embargo, estos últimos autores admitieron (García Más y Jiménez, 1984) que ambas citas podrían considerarse erróneas debido a una mala interpretación de Blanchard (1893) por parte de Cordero del Campillo (Jueg, 2008). Por lo tanto, teniendo en cuenta la limitada e incierta información sobre* T. subviridis *en España, podemos argumentar que nuestro ejemplar es el primer registro válido citado para esta especie en la Península Ibérica.*

Palabras clave: *Hirudinea, sanguijuela, regata Suspiro, Parque Natural del Señorio de Bertiz*

INTRODUCTION

Morphological identification of leeches (Hirudinea) is often extremely challenging, resulting in researchers' reluctance to study the taxonomy and biodiversity of these interesting animals. The subclass Hirudinea constitutes a monophyletic group with ca. 680 described species worldwide (Sket & Trontelj, 2007). These invertebrates are voracious predators (Miler et al., 2019) or semiaquatic bloodsucking ectoparasitic (Borda & Siddall, 2004) widespread in different habitats on all continents, except Antarctica (Sket & Trontelj, 2007). In Europe, *Dina* Blanchard, 1892 and *Trocheta* Dutrochet, 1817 are genera with the great species richness, and both are belonging to Erpobdellidae family. Erpobdellids are typical predators of freshwater habitats, and it is generally possible to find them hiding under flat stones in the riverbed or slithering around the riparian zone. Distinguishing the genus *Dina* from *Trocheta* is tricky since only one morphological character is helpful for that scope: annulation pattern (Trontelj & Sket, 2000). Other morphological characters as body size, colour, and shape are strongly biased by the fixation method. Thus, these anatomical characteristics are often inappropriate for an accurate morphological classification when the animal is died (Trontelj & Sket, 2000). Furthermore, the lack of definite morphological differences among different leech species has led to incorrect identification over the years within this family (Khomenko et al., 2020). An example of taxonomic mislabelling for the genus *Trocheta*, is the species *Trocheta subviridis* Dutrochet, 1817. This large and uncommon leech inhabits freshwater environments, preferably with some organic contamination (Hartley, 1962). In Spain, the first record of *T. subviridis* was published by Cordero del Campillo (1980) and later reviewed by García Más and Jiménez (1981). Notably, a few years after their first publication, the same authors admitted the incorrectness of the record García Más and Jiménez (1984) due to a Cordero del Campillo's misinterpretation of the dichotomous key of Blanchard (1893) (Jueg, 2008). The actual distribution of *T. subviridis* is reported in Fauna Europaea (De Jong et al., 2014), including countries as France, Ireland, Italy, Luxembourg, Romania, Ukraine, former Yugoslavia and Spain (erroneous record). Recently, this species has also been reported from Germany (Roos, 2019). Taking all mentioned facts dealing with incorrect identification, the previously reported presence of *T. subviridis* in the Iberian Peninsula could be easily questioned (Jueg, 2008). In the pre-

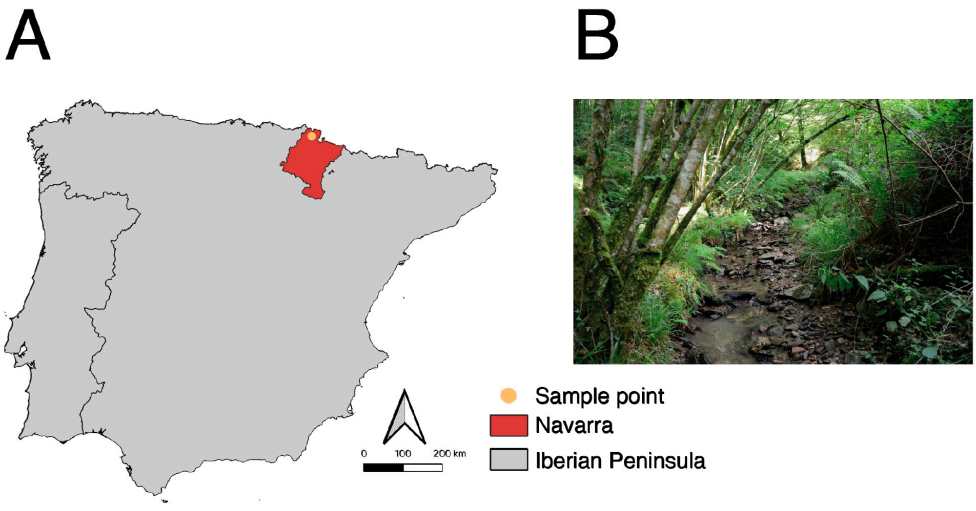

Figure 1. Sampling site (A) and habitat (B) of the new records of *T. subviridis*. (Photo: J. Oscoz). *Sitio de muestreo y hábitat del nuevo record de* T. subviridis. *(Foto: J. Oscoz).*

sent note, we provide the first valid record of *T. subviridis* for north-eastern Spain as well as for the whole Iberian Peninsula. We also provided a detailed morphological identification of our specimen and a summary of the main biological and physiochemical environmental characters of the site where the sample has been collected.

MATERIALS AND METHODS

Material

1 specimen; leg.: Javier Oscoz; det.: C. Grosser. The leech was detected in the upper stretch of the Suspiro stream (Navarra, Spain; coordinates EPSG: 4326 WGS 84 -1° 37' 50.7"; 43° 9' 40.2", 300 m a.s.l.) (Fig. 1A).

The leech was fixed in formaldehyde 37 % and later preserved in 70 % ethanol. The specific identity was examined based on the external morphology (annulation pattern) and the shape of the genital atrium using a stereomicroscope (Novex). Photographs were made using a camera (Canon EOS 400 D), and macro-photographies was carried out with a macro lens (×10). The size was determined by a ruler in millimetres.

The specimen is stored in the Museum of Zoology of the University of Navarra, with the identification number MZNA 721188.

Study area

The Señorío de Bértiz Natural Park (Natura 2000 cod. ZEC ES2200017) is located in the north-eastern of Navarra province, on the western edge of the Pyrenees. The park includes approximately 20 km^2 of mixed temperate forest. The tree community is composed primarily of European beech (*Fagus sylvatica* L.), common oak (*Quercus robur* L.), Pyrenean oak (*Quercus pyrenaica* Willd.) and sweet chestnut (*Castanea sativa* Mill.).

There are numerous intermittent creeks scattered around the park, and most of them flow into the Suspiro stream (a tributary of the Bidasoa river). Due to the natural characteristics and the low anthropic impacts on the basin, the Suspiro stream and its tributaries can be considered as pristine ecosystems (Fig. 1B). In such a hydrographic net, it is possible to find numerous native species of fish (e.g., *Salmo salar* Linnaeus, 1758; *Cottus aturi* (Freyhof, Kottelat & Nolte, 2005)), amphibians (e.g., *Salamandra salamandra* (Linnaeus, 1758)), and invertebrates (e.g., *Cordulegaster boltonii* Donovan, 1807; *Agriotypus armatus* Curtis, 1832).

Sampling protocol

Benthic invertebrates were collected on 2 July 2010 along the upper stretch of Suspiro stream (Bidasoa River Basin, Navarra) as part of the project *"Estudios de monitorización integrada en una cuenca forestal del Pirineo"* conducted by the group of Biodiversity Data Analytics and Environmental Quality (BEQ), Department of Environmental Biology (University of Navarra). To collect freshwater invertebrates, we used a hand net with a mesh size of 500 µm. Furthermore, we conducted a visual analysis of the area to detect the animals with elusive behaviour. Physicochemical parameters as temperature, pH and dissolved oxygen concentration were also measured in situ with a multiparametric probe. In addition, to better characterise the habitat, other environmental

Figure 2. *T. subviridis*: habitus; specimen from Spain, Navarra. (Photo: C. Grosser). T. subviridis*: habitus; ejemplar recogido en España, Navarra. (Foto: C. Grosser).*

parameters such as average width and depth were measured, and substrate size were estimated.

RESULTS

Stream characterisation

Physicochemical measurements obtained from the stream stretch were as follow: temperature: 16.9 °C; pH: 7.6; conductivity: 98 µS/m; O_2: 9.5 mg/l; O_2: 100 %. Moreover, the stretch was characterised by a width of approximately three meters and a deep of 30-40 cm. The riverbed substrate was basically composed of medium-sized stones, cobbles, and isolated sandy areas.

Figure 3. *T. subviridis*: annulation; specimen from Spain, Navarra. (Photo: C. Grosser). T. subviridis*: annulation; ejemplar recogido en España, Navarra. (Foto: C. Grosser).*

Species Identification

The specimen has been clearly identified based on the following morphological characters:

1. Very large leech. The preserved and contracted specimen had a length of 132 mm and a maximum body width of 18 mm. In addition, a considerable size also has been found for the caudal sucker: 10 mm long and 13 mm wide (Fig. 2).
2. Dorsal and ventral surfaces were smooth, without prominent papillae (Fig. 2).
3. Colour of the preserved specimen was a uniform whitish grey (Fig. 2).
4. From lateral view the annulation is heteronomous (Fig. 3). A medium body somite was subdivided into seven narrow and two broadened annuli. Furthermore, the broadened annuli were slightly subdivided.
5. The genital pores were separated by 7.5 annuli. Only *T. subviridis* has this large distance between the genital pores among the European Erpobdellids. The male genital pore was situated in the furrow of annuli, whereas the female genital pore was located on the annulus.
6. The genital atrium had a large body with very short cornua. The cornua were strongly curved to the ventral side (Fig. 4A). The shape

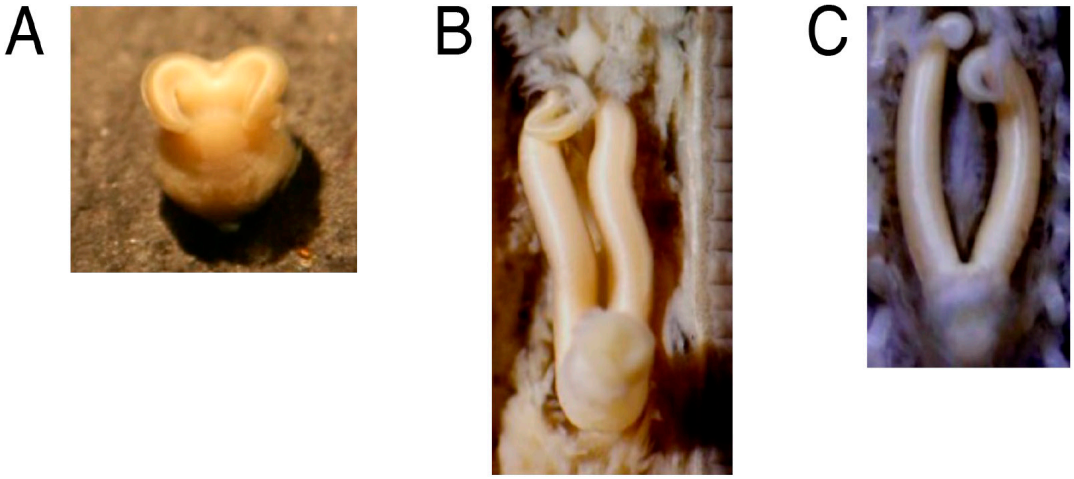

Figure 4. Genital atrium. A: *T. subviridis*, specimen from Spain, Navarra; B: *T. danastrica*, specimen from Serbia (Grosser & Epshtein 2009); C: *T. taunensis*, specimen from the river Wisper, Taunus/Germany (Grosser 2015). (Photos: C. Grosser). *Atrium del aparato genital. A:* T. subviridis*, ejemplar recogido en España, Navarra.; B:* T. danastrica*, ejemplar recogido en Serbia (Grosser & Epshtein 2009); C:* T. taunensis*, ejemplar recogido en el rio Wisper, Taunus/Germany (Grosser 2015). (Fotos: C. Grosser).*

of this atrium is very similar to specimens from the French Pyrenees, published by Nesemann & Neubert (1999).

DISCUSSION

In the present study, we reported the first valid record for the species *Trocheta subviridis* for the Iberian Peninsula. This record is particularly interesting because, to date, it represents the most western extreme of the distribution of such species. Historically, the morphological identification of the genus *Trocheta* is considered a challenge due to the lack of evident morphological characters and the high number of cryptic species (Khomenko et al., 2020). The species *T. subviridis* is an example of that kind of phenotypical traits variation. This species presents quite different morphological characters compared to the most representative congeners of the genus *Trocheta* as *T. danastrica* Stschegolew, 1938 (Fig. 4B), and *T. taunensis* Grosser, 2015 (Fig. 4C). Indeed, the species *T. subviridis* is characterised by short cornua, contrary to the other species of the same genus that show long cornua of the genital atrium. However, nowadays, molecular techniques coupled with the study of leech phylogeny have proven to be good allies for the investigation of their systematics, and many open questions about the taxonomy of these invertebrates are constantly solving (e.g., *Trocheta blanchardi* (Khomenko et al., 2020)).

To conclude, we want to remark, that investigating and retrieving taxonomic information about invertebrates are challenging because the study about these organisms is often neglected in advantage of more charismatic animals. Moreover, the literature concerning their biology and ecology is often scarce and confined to local or old publications difficult to consult for the general public. However, in the last years, several studies in scientific journals are turning on the light about the countless threats and the high rate of extinction affecting invertebrates worldwide (e.g., Eisenhauer, Bonn, & Guerra (2019); Borrell (2012); Cardoso et al., (2020)). Therefore, we consider crucial the sharing of records of primary invertebrate data, since only by sharing this basic scientific information we can improve our knowledge about the actual distribution and ecological preferences of these animals, increasing our possibilities to develop more efficient strategies to preserve their biodiversity.

Future research to determine the persistence of this species is recommended.

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