Limnetica, 45(1): 000-000 (2026). DOI: 10.23818/limn.45.06

A study of blackfly (Diptera: Simuliidae) samples from the Cidacos River in Soria and La Rioja provinces (Spain)

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Received: 25/09/24 Accepted: 28/02/25 Available online: 25/03/25

ABSTRACT

A study of blackfly (Diptera: Simuliidae) samples from the Cidacos River in Soria and La Rioja provinces (Spain).

The study of simuliids in Spain is of the utmost importance, not only because of the key role they play in food chains both as agents recirculating matter and as a source of food for countless organisms, but also due to the negative impact that females of blood-sucking species have on animal and human health and welfare, and the economic damage that they can cause to health, veterinary, agriculture and tourism sectors. Therefore, a deeper knowledge of blackflies is crucial. To this purpose, the specimens collected in 1997 along the Cidacos River from two Spanish provinces were studied. The objective of this study has been to improve the knowledge of this family in Spain. As a result, 12 species of the genus *Simulium* have been identified, belonging to four subgenera and classified into four species groups. Three species are first records for La Rioja and one species for Soria. The elevational and water temperature ranges have been described for each one of the species. The sampled stations with the highest species richness have been indicated and the species composition of several sampling stations has been analysed. The species with the highest and lowest relative abundances are described in detail and provincial distribution maps of the four species recorded for the first time in the area studied area are provided. The species whose females require obligatory intake of blood to develop their eggs are highlighted, as well as the harm due to each species with this type of feeding behaviour.

KEY WORDS: Blackflies, species diversity, altitude, water temperature, provincial distribution, haematophagy, Spain.

RESUMEN

Estudio de muestras de mosca negra (Diptera: Simuliidae) del río Cidacos en las provincias de Soria y La Rioja (España).

El estudio de los simúlidos en España es de suma importancia, no solo por el rol clave que desempeñan en las cadenas tróficas como agentes recirculantes de materia y como fuente de alimento para innumerables organismos, sino también por el impacto negativo que las hembras de las especies succionadoras de sangre desencadenan en el bienestar y la salud animal y humana, y por los perjuicios económicos que pueden ocasionar en los sectores de salud, veterinaria, agricultura y turismo. Por ello, un conocimiento más profundo de las moscas negras es crucial. Para ello, se estudiaron los ejemplares *colectados en 1997 en el río Cidacos, procedentes de dos provincias españolas estudiadas. El objetivo de este estudio ha sido mejorar el conocimiento que se tiene de esta familia en España. Como resultado, se han identificado 12 especies del género* Simulium*, pertenecientes a cuatro subgéneros y clasificadas en cuatro grupos de especies. Tres especies son primeras citas para La Rioja y una especie para Soria. Se han descrito los rangos altitudinales y de temperatura del agua para cada especie. Se han indicado las estaciones muestreadas con mayor y menor riqueza de especies y se han analizado las composiciones de especies de varias estaciones de muestro. Se describen en detalle las especies con las mayores y menores abundancias relativas y se proporcionan los mapas de distribución provincial de las cuatro especies registradas por primera vez en el área estudiada. Se destaca tanto las especies cuyas hembras requieren la ingesta obligada de sangre para desarrollar sus huevos, como hecho hincapié en los perjuicios debido a cada especie con este tipo de comportamiento alimenticio.*

PALABRAS CLAVE: *Moscas negras, diversidad de especies, altitud, temperatura del agua, distribución provincial, hematofagia, España.*

INTRODUCTION

The study of blackflies (Diptera: Simuliidae) in Spain has intensified over time and address a variety of topics such as description of new species, taxonomy, ecology, fauna, distribution, catalogues and inventories, identification keys and guides, pathogenic agents, parasites, genetics, and sanitary and veterinary importance (López-Peña & Jiménez-Peydró, 2019). However, these topics have been addressed unevenly across Spain's terrain, with some regions receiving little attention while others have been intensively investigated. For example, the uniprovincial Autonomous Region of La Rioja and the province of Soria, a part of the region of Castile and Leon, which share a border have had a similar number of studies carried out in their territories: three in La Rioja (Vinçon & Clergue-Gazeau, 1993, Martínez Ruiz & Portillo Rubio, 1999, Ruiz-Arrondo et al., 2022) and three in Soria (Strobl, 1905, González Peña, 1990, Ruiz-Arrondo et al., 2018).

Martínez Ruiz and Portillo Rubio studied a small part of the material that they collected in 1997; the great bulk of the specimens captured remained stored at the University of Salamanca until 2022 when they were examined to obtain new insights on this dipteran family and the study area.

The objectives of this study are to increase the knowledge of blackfly species present in Spain by examining all the specimens not yet studied, shed light on the species richness and ecology of simuliids from the sampled area, gain new insights into their geographical distribution and elevational and water temperature ranges of the habitats sampled, and detect the presence of haematophagous species important from a health and veterinary point of view.

METHODOLOGY

Area of study: Geography, hydrology, and geology

The Cidacos River rises in the Puerto de Oncala, in the Sierra de Montes Claros, also known as Sierra de Alba (MAPA), at 1400 m above sea level (m. a.s.l.) in the north of the province of Soria close to the municipality of Los Campos (Martín Escorza, 1999). Its course meanders through the Soria municipalities of Villar del Río and Yanguas before entering La Rioja, which it crosses from south to north, passing through the municipal territories of Enciso, Arnedillo, Herce, Arnedo, Quel, Autol and Calahorra. In Calahorra, and at an elevation of 320 m (Martín Escorza, 1999), the Cidacos River discharges its waters into the Ebro River.

In the area, the Cidacos river basin, which has a southwest-northeast orientation (Martín Escorza, 1999), is 710.52 km^2 according to Martín Escorza (1999), and 719.22 km^2 according to the Ebro Hydrographic Confederation (CHEBRO). Its flow rate is 85.17 hm^3/year (CHEBRO). However, during most of the year, the river's flow is low, and the streams that feed it are completely dry (Martín Escorza, 1999). The Cidacos River's length, has been estimated variously at 79 km (IGN, 1995), 80 km (Gonzalo Moreno, 1968,

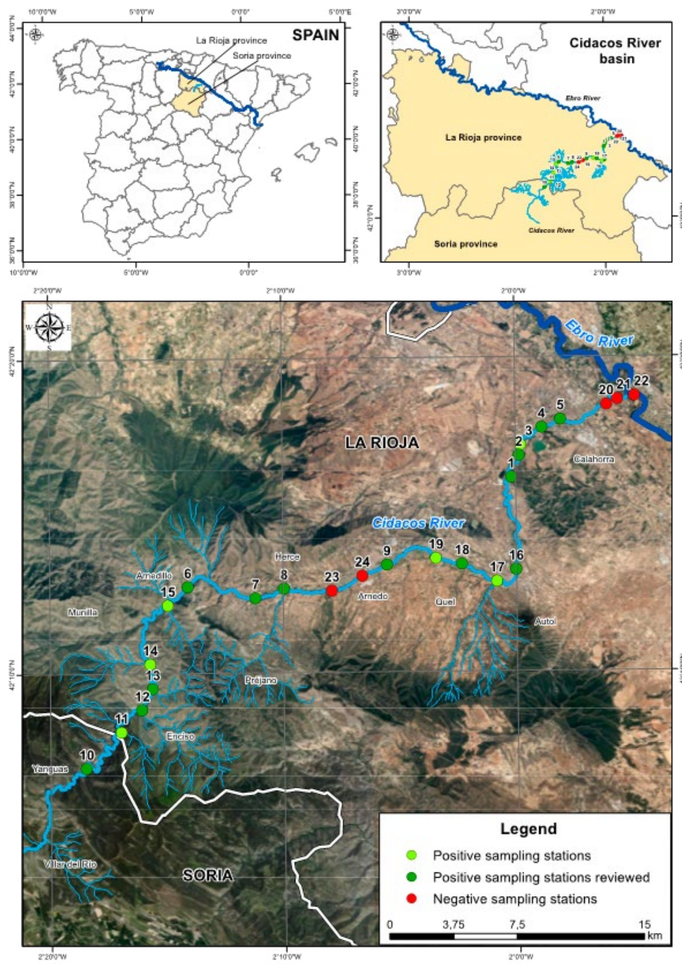

Figure 1. Geographical positioning of the province of Salamanca within Spain (left side). Location of Cidacos river basin along with the sampling stations (right side). Detailed depiction of the river, its tributaries, and each sampling station (below). The numbers correspond to the sample codes, which can be found in the accompanying tables. *Posicionamiento geográfico de la provincia de Salamanca dentro de España (lado izquierdo). Ubicación de la cuenca del río Cidacos junto con las estaciones de muestreo (lado derecho). Representación detallada del río, sus afluentes y cada estación de muestreo (abajo). Los números corresponden a los códigos de muestra, que se pueden encontrar en las tablas adjuntas.*

Gonzalo, 1985), 82.5 km (Martín Escorza, 1999), 83.89 km (Sánchez Lozano, 1894), 85 km (Espasa-Calpe, 1930), and 91 km (Abad León, 1978). The most recent estimate is 88.56 km (Martín Escorza, 2018).

The Cidacos River is characterised by an irregular pluvial regime (Angulo et al., 2020), with a Mediterranean tendency (Lopo et al., 2006) and strong floods that provide its watercourse with a flow of up to 100 m^3/second (Angulo et al., 2020). However, the flow is not permanent and tends to dry up several times during the year (Angulo et al., 2020) due to both an extensive the demand for water to irrigate crops (Lopo et al., 2006) and the geological sediments of certain sectors that favour water infiltration (Martín Escorza, 1999). Consequently, the route of the river basin is notable for the presence of rocky cliffs and river canyons shaped by both fluvial influences and wind erosion (MAPA). As a result, the average slope of this river is 14% (Sánchez Lozano, 1894) to 13.2% (Martín Escorza, 1999).

The substratum of the river between Yanguas and Enciso is made up of outcrops of sedimentary rocks of marine origin, folded and formed of quartzite, limestone and sandstone, with the river

Table 1. Location data of the studied sampling stations. *Datos de ubicación de las estaciones de muestreo estudiadas.*

Sampling station code	Municipality	Province	Autonomous Region	Date	Elevation (m)	Water Tª (ºC)
R1	Calahorra	La Rioja	La Rioja	04/1997	390	13
R2	Calahorra	La Rioja	La Rioja	04/1997	380	13
R4	Calahorra	La Rioja	La Rioja	04/1997	350	15
R5	Calahorra	La Rioja	La Rioja	04/1997	350	17
R6	Arnedillo	La Rioja	La Rioja	05/1997	650	10
R7	Santa Eulalia Somera (locality belonging to the municipality of Arnedillo)	La Rioja	La Rioja	05/1997	625	13
R8	Herce	La Rioja	La Rioja	05/1997	601	16
R9	Arnedo	La Rioja	La Rioja	05/1997	535	16
R10	La Vega	Soria	Castilla y León	06/1997	900	10
R12	Enciso	La Rioja	La Rioja	06/1997	817	16
R13	Enciso	La Rioja	La Rioja	07/1997	815	14
R16	Autol	La Rioja	La Rioja	08/1997	458	22
R18	Villa de Quel	La Rioja	La Rioja	08/1997	500	20

running wedged between valleys in a "V" shape (Martín Escorza, 1999). In Arnedo and Arnedillo, it is made up of continental sedimentary rocks composed of conglomerates (Martín Escorza, 1999), deformed in an anticline fold (Angulo et al., 2020), which have caused fluvial erosion, giving rise to a valley with a flat-floored profile (Martín Escorza, 1999).

Sampling stations, sample collection, and specimen identification

To study the diversity of simuliid species in the Cidacos River, 24 sampling stations were chosen. The stations were not equidistant as the distance between them depended on the accessibility of the body of water (Fig. 1). The physical and hydrological characteristics of the watercourse, such as stagnant water with a eutrophic appearance and a low concentration of dissolved oxygen and a current with a very high flow and speed which prevented the establishment of immature stages of blackflies, indicated the absence of simuliids in five of the sampled stations. The remaining 19 sampling stations were positive for simuliid larvae and pupae. Specimens from six of these stations were scarce and were fully studied by Martínez Ruiz and Portillo Rubio (1999). The material sampled was more abundant in the remaining 13 stations, and after selecting a representative proportion of each sample, the rest was stored in the Zoology Area of the University of Salamanca; these samples were investigated in the present study.

The stations were sampled between April and August 1997. The sampling consisted of a prior search and subsequent direct capture of the specimens detected on different substrates, including structures and surfaces of inorganic (rocks, stones and boulders), organic (vegetation growing in the centre of the river and its banks), and anthropic (plastics) origin. After detecting the presence of simuliids, physical-chemical variables, such as the location of each sampling station (the municipality, province and autonomous region), date of collection, altitude (m) and water temperature (ºC) were recorded (Table 1).

The samples were fixed *in situ* in 70% ethanol by the collectors. Once in the laboratory, and after selecting a representative sample, the rest of the unanalysed specimens were stored in plastic jars with 70% ethanol. The samples were processed to eliminate all particles and plant remains, with the aim of cleaning the morphological structures used for identifying specimens. After processing and cleaning, identification keys based on morphological characters (Knoz, 1965, González Peña, 1997, Bass, 1998, Jedlička et al., 2004, Rivosecchi et al., 2007, Kúdela et al., 2022) were used to identify specimens observed under stereoscopic optical microscopes (CARL ZEISS, 4981713, West Germany, 47 52 00 – 9901, 4 objectives and 10× eyepieces), assisted in the first instance with an external amber light source (SCHOTT KL 1500, 230 V, 50 60 Hz, 200 W, Halogenlampe 15 V 150 W, Germany) and then with a white light ring (AmScope, Led – 56S), and a stereo microscope (Leica S Apo Stereozoom 1.0–8.0×, 10× eyepieces/23 LEICA 10447137, PN: 10 450 828, SN: 1123180003, Leica Microsystems (Switzerland) Ltd. CH-9435 Heerbrugg, Singapore) assisted with a Leica ring light (Leica LED 3000 RL58mm, PN: 10 450 271, S/No: 6228655, Input: 33.0V–0.45A, Leica Microsystems (Switzerland) Ltd. CH-9435 Heerbrugg, Indonesia). Lastly, the specimens identified were placed in plastic tubs with 80% ethanol for preservation and labelled with the sampling station number and the name of the species.

Figure creation

The location figures of the area studied, along with the provincial distribution maps of the species identified, have been generated using the Geographic Information System (GIS) software ArcMapTM 10.5 (ArcGIS®, ESR, Redlands, CA, USA).

RESULTS

Species documented for the study area

The 13 samples collected from the Cidacos River, which were not subjected to previous analysis, has revealed 7630 specimens of blackfly, all in preimaginal stages: 6335 immature larvae, 629 mature larvae, and 666 pupae (Table 2). All specimens belong to the genus *Simulium* Latreille, 1802, classified into four subgenera (*Boophthora* Enderlein, 1921, *Eusimulium* Roubaud, 1906, *Simulium* Latreille, 1802, and *Wilhelmia* Enderlein, 1921), the last two being represented by specimens from four species-groups (*bezzii* species-group, *ornatum* species-group, *variegatum* species-group, and *equinum* species-group). This study has identified 12 species: *Simulium* (*Boophthora*) *erythrocephalum* (De Geer, 1776); *Simulium* (*Eusimulium*) *angustipes* Edwards, 1915; *Simulium* (*Eusimulium*) *petricolum* (Rivosecchi, 1963); *Simulium* (*Eusimulium*) *rubzovianum* (Sherban, 1961); *Simulium* (*Simulium*) *bezzii* (Corti, 1914); *Simulium* (*Simulium*) *intermedium* Roubaud, 1906; *Simulium* (*Simulium*) *ornatum* Meigen, 1818; *Simulium* (*Simulium*) *trifasciatum* Curtis, 1839; *Simulium* (*Simulium*) *variegatum* Meigen, 1818; *Simulium* (*Wilhelmia*) *equinum* (Linnaeus, 1758); *Simulium* (*Wilhelmia*) *lineatum* (Meigen, 1804); *Simulium* (*Wilhelmia*) *pseudequinum* Séguy, 1921 (Table 3).

As a result, four species have been recorded for the first time from the study area, three for La Rioja (*S. erythrocephalum*, *S. petricolum*, *S. trifasciatum*), and one for Soria (*S. variegatum*). As a consequence, their documented Spanish geographical distribution (López-Peña & Jiménez-Peydró, 2017) have been expanded and updated (Fig. 2). As for *S. erythrocephalum*, this species has been reported from 17 provinces and nine Autonomous Regions, *S. petricolum* from 20 provinces and the same number of autonomous regions that the previous species, *S. trifasciatum* from 21 provinces and 11 Autonomous Regions, and *S. variegatum* from 24 provinces and also from 11 Autonomous Regions, being the one with the widest distribution of these four species.

Relative abundance of species

In La Rioja, the mature larvae of *S. intermedium* had the highest relative abundance, with a total of 191 individuals, followed by those of *S. pseudequinum* with 179 individuals. This result is partly explained by the wide distribution of *S. intermedium* and *S. pseudequinum* in 12 and 10 of the studied sampling stations, respectively. In contrast, the species with the lowest relative abundance of mature larvae were *S. trifasciatum* with only one specimen and *S. lineatum* with eight larvae. In the case of pupae, *S. intermedium* showed the highest abundance, with a total of 226 specimens, followed by *S. pseudequinum* with 106 pupae. However, the lowest pupal abundance was seen for *S. erythrocephalum* with one specimen and *S. trifasciatum* with seven pupae.

In Soria, *S. equinum* showed the lowest rel-

Table 2. Recorded specimens of each species and sampling station. E*jemplares registrados de cada especie y estación de muestreo.*

Sampling station code	Species	No. of immature larvae	No. of mature larvae	No. of pupae
R1	*S. angustipes*		0	3
	S. bezzii		1	3
	S. equinum		1	6
	S. intermedium		56	19
	S. lineatum		1	1
	S. ornatum		8	1
	S. petricolum		7	4
	S. pseudequinum		5	11
	S. velutinum		8	2
	Simulium sp.	655		
R2	*S. bezzii*		14	43
	S. equinum		20	7
	S. intermedium		53	52
	S. lineatum		7	0
	S. pseudequinum		30	19
	Simulium sp.	305		
R4	*S. angustipes*		0	1
	S. bezzii		7	17
	S. equinum		10	1
	S. intermedium		28	51
	S. ornatum		7	15
	S. petricolum		2	3
	S. pseudequinum		85	41
	Simulium sp.	1291		
R5	*S. bezzii*		1	3
	S. intermedium		18	26
	S. ornatum		0	2
	S. petricolum		0	5
	S. pseudequinum		0	2
	S. trifasciatum		0	1
	S. velutinum		1	0
	Simulium sp.	102		
R6	*S. equinum*		0	1
	S. ornatum		9	2
	S. pseudequinum	1 ? (with helminth)	17	3
	S. variegatum		11	3
	Simulium sp.	643		
R7	*S. bezzii*		2	3
	S. equinum		0	1
	S. intermedium		1	1
	S. lineatum		0	1

Cont.

Cont.

Sampling station code	Species	No. of immature larvae	No. of mature larvae	No. of pupae
	S. ornatum		4	3
	S. pseudequinum		13	11
	S. trifasciatum		1	0
	S. variegatum		0	2
	S. velutinum		1	0
	Simulium sp.	1218		
R8	*S. bezzii*		3	5
	S. equinum		0	1
	S. erythrocephalum		0	1
	S. intermedium		19	5
	S. lineatum		0	1
	S. ornatum		7	5
	S. pseudequinum		8	3
	S. trifasciatum		0	1
	S. velutinum		1	0
	Simulium sp.	348		
R9	*S. bezzii*		1	8
	S. intermedium		3	2
	S. ornatum		4	2
	S. pseudequinum		5	5
	S. trifasciatum		0	1
	S. velutinum		1	0
	Simulium sp.	98		
R10	*S. equinum*		1	0
	S. intermedium		8	2
	S. ornatum		3	4
	S. variegatum		5	3
	Simulium sp.	277		
R12	*S. bezzii*		0	1
	S. equinum		1	2
	S. intermedium		1	35
	S. ornatum		2	26
	S. trifasciatum		0	3
	S. variegatum		0	17
	S. velutinum		2	0
	Simulium sp.	121, 2 (with helminths, *S.* (*Eusimulium*) sp.)		
R13	*S. equinum*		0	3
	S. intermedium		6	26
	S. ornatum		5	23
	S. trifasciatum		0	1
	S. variegatum		3	26
	Simulium sp.	720		

Cont.

Cont.

Sampling station code	Species	No. of immature larvae	No. of mature larvae	No. of pupae
R16	*S. angustipes*		13	18
	S. intermedium		4	9
	S. ornatum		0	3
	S. petricolum		30	31
	S. pseudequinum		12	8
	S. velutinum		44	12
	Simulium sp.	527	1	0
R18	*S. angustipes*		1	0
	S. intermedium		2	0
	S. pseudequinum		4	3
	Simulium sp.	20		

ative abundance of mature larvae, while *S. intermedium* showed the highest. In contrast, *S. intermedium* had the lowest abundance of pupae with only two specimens, while *S. ornatum* had the highest with four specimens. In the case of the sum of specimens of each stage of development (mature larvae and pupae) from La Rioja and Soria, *S. erythrocephalum* had the lowest relative abundance with one specimen, followed by *S. trifasciatum* with eight specimens (Table 4). In contrast, the species with the highest abundances was *S. intermedium* with 427 specimens, followed by *S. pseudequinum* with 285, *S. ornatum* with 135, and *S. bezzii* with 112 individuals (Table 4). Because immature larvae do not yet possess the morphological characters used for the species identification, we classified them at the level of genus; thus, the total number of immature larvae was 6325, with 6048 in La Rioja and 277 in Soria (Table 4). In the case of species with the highest abundances, it is worth noting that *S. bezzii* showed the highest relative abundance at sample station R2, with 57 specimens collected (14 mature larvae and 43 pupae), and the lowest abundance at station R12, with one pupa (Table 2). *Simulium intermedium* showed the highest abundance at sampling R7, with 105 individuals (53 mature larvae and 52 pupae), and the lowest at stations R7 with two specimens (one mature larva and one pupa) and at R18 with two mature larvae (Table 2). *Simulium ornatum* showed the highest abundances at sampling stations R12 with 28 specimens (two mature larvae and 26 pupae) and R13 with the same number of individuals (five mature larvae and 23 pupae), while the lowest abundance was recorded for R5 with two pupae (Table 2). Finally, *S. pseudequinum* showed the highest abundance at station R4 with 126 specimens (85 mature larvae and 41 pupae) and the lowest at station R5 with two pupae (Table 2).

Elevational and water temperature ranges of the species recorded for the first time in La Rioja and Soria

The four species reported for the first time in the study area, three in La Rioja and one in Soria, showed some similarities and differences in elevation. For instance, because *S. erythrocephalum* was found solely at sampling station R8 in the surroundings of the municipality of Herce in La Rioja, it is known only from habitats located at 601 m in this study (Tables 1 and 2). However, as *S. petricolum* and *S. trifasciatum* were found in several sampling stations, their elevational ranges varied from 108 m (350 m to 458 m) for the former to 467 m (350 m to 817 m) for the latter; *S. trifasciatum* showed the widest elevational range out of these three species (Table 1 and 2). In Soria, *S. variegatum* was discovered in the waters of only one sampling station, R10, close to the municipality of La Vega; therefore, this species is known only at an elevation of 900 m (Table 1 and 2), which represents the highest elevation determined in this study.

The water temperature range of the aquatic habitats of *S. erythrocephalum* in La Rioja and *S. variegatum* in Soria was 16 ºC and 10 ºC, respectively (Tables 1 and 2), while that of the other two species from La rioja fluctuated, between 13 ºC and 17 ºC for *S. trifasciatum* and 13 ºC and 22 ºC for *S. petricolum*, which means that the latter had the widest range (9 ºC) (Tables 1 and 2).

Simuliid distribution and species richness in the upper, middle and lower courses of the Cidacos River

The results obtained for the altitudinal profile of the area studied, which ranges between 350 and 900 m, indicate the habitat requirements of the species identified which have been categorised into five groups: (1) species whose breeding sites are in the upper stretch of the Cidacos River, between 625 and 900 m, an example being *S. variegatum* which was present in all the sampling stations located within this elevational range; (2) species that inhabit the middle course, between 458 and 625 m, such as *S. erythrocephalum*, found at 601 m; (3) species that tend to occupy the lower course, between 350 and 458 m, e.g. *S. petricolum* (350–458 m) and *S. angustipes* (350–500 m); (4) species whose habitat is found in both the lower and the middle reaches, e.g. *S. lineatum* (380–625 m) and of *S. pseudequinum* (350–650 m); and (5) generalist species with broad tolerance of factors, such as elevation and water temperature, found in the upper, middle, and lower reaches of the river, i.e. *S. bezii*, *S. trifasciatum*, and *S. rubzovianum* living between 350 and 817 m and *S. equinum*, *S. intermedium*, and *S. ornatum* found between 350 and 900 m.

Of the sampling stations surveyed, R18, located in the middle course of the river, was the least species rich, with only three species cohabiting at this site (Table 2). However, R7 and R8 located in the middle course, as well as R1 located in the lower course, have shown the highest species richness with nine species, but with different compositions; for example, *S. variegatum* appeared only in R7, *S. erythrocepalum* only in R8, and *S. angustipes* and *S. petricolum* only in R1 (Table 2). In the upper course, the sampling stations R6 and R10 have four species, with their

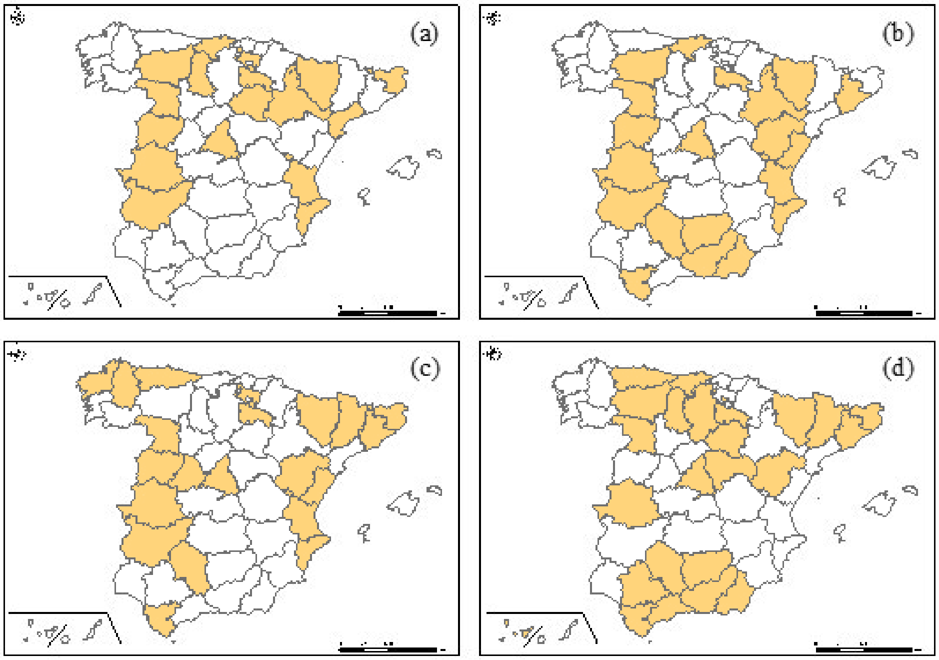

Figure 2. Provincial distribution maps of the 4 species reported for the first time from the study area: *S. erythrocephalum* (a), *S. petricolum* (b), *S. trifasciatum* (c) for La Rioja, and *S. variegatum* (d) for Soria. *Mapas de distribución provincial de las 4 especies reportadas por primera vez para el área de estudio:* S. erythrocephalum *(a),* S. petricolum *(b),* S. trifasciatum *(c) para La Rioja y* S. variegatum *(d) para Soria.*

species compositions differentiated by the presence of *S. intermedium* in R10 and *S. pseudequinum* in R6; R13 had five species; and R12 had seven species (Table 2). In the middle course, stations R9 and R16 showed the cohabitation of six species but their species compositions were different; R9 is characterised by the presence of the species *S. bezzii*, and *S. trifasciatum* and R16 by the species *S. angustipes* and *S. petricolum* (Table 2). In the lower course, station R2 showed five species; R4 and R5 had seven species, although the stations showed different species complexes, with *S. angustipes* and *S. equinum* recorded in R4 and *S. trifasciatum* and *S. rubzovianum* found in R5 (Table 2). Similarly, station R12 has shown species in common with R4, such as *S. equinum*, and with R5, such as *S. trifasciatum* and *S. rubzovianum*; however, *S. variegatum* was only present at R12 make this sampling site different from R4 and R5 species (Table 3). Stations R2 and R13 shared two species, *S. equinum* and *S. intermedium*, of the five detected, but their species complexes could be differentiated by the presence of *S. ornatum*, *S. trifasciatum* and *S. variegatum* in R13 and *S. bezzii*, *S. lineatum* and *S. pseudequinum* in R2 (Table 2).

Haematophagous species

Of the 12 species identified, nine are notable for their blood-sucking feeding behaviour, and the targeting of the blood of avian and mammalian hosts, including humans. These species, *S. bezzii*, *S. equinum*, *S. erythrocephalum*, *S. intermedium*, *S. lineatum*, *S. ornatum*, *S. petricolum*, *S. pseudequinum*, and *S. variegatum*, showed differences in distribution and population size between sampling stations along the Cidacos River, reflecting varying levels of risk, threat and harm to wildlife, livestock, and human populations in proximity to their breeding sites. For instance, despite its anthropophilic tendencies, *S. erythrocephalum* is of least concern compared to the other species because of its limited presence and small population size (Table 4). In contrast, *S. lineatum* and *S. petricolum*, found in four of the 13 sampling stations, and *S. variegatum*, found in five (Table 4), pose an intermediate risk owing to their constrained distributions and moderate population sizes, indicated by a total of 11, 82, and 70 specimens of mature larval and pupa stages, respectively, (Table 4). *Simulium bezzii* and *S. equinum* present a greater risk due to their distribution in eight sam-

Table 3. Taxonomic classification of the identified blackfly specimens. *Clasificación taxonómica de los ejemplares identificados de mosca negra.*

Genus	Subgenus	Species-group	Species
Simulium Latreille, 1802	*Boophthora* Enderlein, 1921		*Simulium* (*Boophthora*) *erythrocephalum* (De Geer, 1776)
	Eusimulium Roubaud, 1906		*Simulium* (*Eusimulium*) *angustipes* Edwards, 1915
			Simulium (*Eusimulium*) *petricolum* (Rivosecchi, 1963)
			Simulium (*Eusimulium*) *rubzovianum* (Sherban, 1961)
	Simulium Latreille, 1802	*bezzi* species-group	*Simulium* (*Simulium*) *bezzi* (Corti, 1914)
		ornatum species-group	*Simulium* (*Simulium*) *intermedium* Roubaud, 1906
			Simulium (*Simulium*) *ornatum* Meigen, 1818
			Simulium (*Simulium*) *trifasciatum* Curtis, 1839
		variegatum species-group	*Simulium* (*Simulium*) *variegatum* Meigen, 1818
	Wilhelmia Enderlein, 1921	*equinum* species-group	*Simulium* (*Wilhelmia*) *equinum* (Linnaeus, 1758)
			Simulium (*Wilhelmia*) *lineatum* (Meigen, 1804)
			Simulium (*Wilhelmia*) *pseudequinum* Séguy, 1921

pling stations (Table 4) and their larger populations indicated by a total of 112 and 55 specimens of mature larvae and pupae, respectively (Table 4). *Simulium onatum*, with 135 larval and pupal specimens collected from 11 sampling stations, and *S. pseudequinum*, with 285 larval and pupal specimens collected from 10 stations (Table 4), indicated the largest population sizes. Lastly, *S. intermedium* represents the greatest threat, being the most widely distributed across 12 of the 13 stations sampled (Table 4) and boasting the largest population, with a total of 427 mature larvae and pupae sampled (Table 4).

DISCUSSION

Species records for Soria and La Rioja

Previous studies on La Rioja recorded the presence of 14 species of simuliids: *S. angustipes*, *S. bezzii*, *S. equinum*, *S. intermedium*, *S. lineatum*, *S. ornatum*, *S. pseudequinum*, *S. rubzovianum*, *S. variegatum* (Martínez Ruiz & Portillo Rubio, 1999), *Simulium* (*Nevermannia*) *brevidens* (Rubtsov, 1956), *Simulium* (*Nevermannia*) *carthusiense* Grenier & Dorier, 1959, *Simulium* (*Nevermannia*) *costatum* Friederichs, 1920 (Vinçon & Clergue-Gazeau, 1993), *Simulium* (*Nevermannia*) *cryophilum* (Rubtsov, 1959), and *Simulium (Simulium*) *monticola* Friederichs, 1920 (Vinçon & Clergue-Gazeau, 1993, Ruiz-Arrondo et al., 2022). This study recorded 17 blackfly species, owing to the identification of preimaginal specimens of three species: a pupa of *S. erythrocephalum* in the municipality of Herce; several mature larvae and pupae of *S. petricolum* in four sampling stations (R1, R4, R5 and R16); and a mature larva and various pupae of *S. trifasciatum* in six sampling stations (R5, R7, R8, R9, R12 and R13). Likewise, only 12 species of simuliids were known according to the bibliographic records for the Castilian-Leonese province of Soria: *Metacnephia blanci* (Grenier & Theodorides, 1953), *S. angustipes*, *Simulium* (*Simulium*) *argyreatum* Meigen, 1813, *S. bezzii*, *S. equinum*, *S. erythrocephalum*, *S. lineatum*, *S. pseudequinum*, *Simulium* (*Wilhelmia*) *sergenti* Edwards, 1923 (González Peña, 1990), *S. intermedium* (Strobl, 1905, Ruiz-Arrondo et al., 2018), *S. monticola* (Ruiz-Arrondo et al., 2018), and *S. ornatum* (González Peña, 1990, Ruiz-Arrondo et al., 2018). As a result of this study, this list has been increased to 13 species, with the identification of *S. variegatum* in the municipality of La Vega. It should be noted that for La Rioja, Vinçon and Clergue-Gazeau's (1993) records

Table 4. Total relative abundance of immature and mature larvae, as well as pupae of each species, taking into account all the sampling stations in which they have been collected. *Abundancia relativa total de larvas inmaduras y maduras, así como de pupas de cada especie teniendo en cuenta todas las estaciones de muestreo en las que han sido colectadas.*

No. of sampling stations	Species	No. of immature larvae	No. of mature larvae	No. of pupae	Total number
4 (R1, R2, R16, R18)	*S. angustipes*		14	22	36
8 (R1, R2, R4, R5, R7, R8, R9, R12)	*S. bezzii*		29	83	112
9 (R1, R2, R4, R6, R7, R8, R10, R12, R13)	*S. equinum*		33	22	55
1 (R8)	*S. erythrocephalum*		0	1	1
12 (R1, R2, R4, R5, R7, R8, R9, R10, R12, R13, R16, R18)	*S. intermedium*		199	228	427
4 (R1, R2, R7, R8)	*S. lineatum*		8	3	11
11 (R1, R4, R5, R6, R7, R8, R9, R10, R12, R13, R16)	*S. ornatum*		49	86	135
4 (R1, R4, R5, R16)	*S. petricolum*		39	43	82
10 (R1, R2, R4, R5, R6, R7, R8, R9, R16, R18)	*S. pseudequinum*		179	106	285
6 (R5, R7, R8, R9, R12, R13)	*S. trifasciatum*		1	7	8
5 (R6, R7, R10, R12, R13)	*S. variegatum*		19	51	70
7 (R1, R5, R7, R8, R9, R12, R16)	*S. rubzovianum*		58	14	72
13 (R1, R2, R4, R5, R6, R7, R8, R9, R10, R12, R13, R16, R18)	*Similium sp.*	6325			6325

of *S. brevidens*, and *S. costatum* were contrasted and validated by González, Crosskey and Báez, (2002), and Vinçon and Clergue-Gazeau's (1993) records *S. carthusiense*, and *S. cryophilum* were confirmed by Crosskey and Crosskey (2000). It is also important to note that Martínez Ruiz and Portillo Rubio's (1999) record of the *Simulium* (*Eusimulium*) *velutinum* (Santos Abréu, 1922) for this region of Spain is currently considered to be *S. rubzovianum* because of Adler et al.'s (2015) work which proved the presence of *S. velutinum* in Spain only in the Canary Islands and confirmed that all the records of this species from the rest of the Spanish territory must be considered to be *S. rubzovianum*.

Likewise, the record of *S. intermedium* in the province of Soria was originally reported by Strobl (1905) as *Simulium* (*Simulium*) *reptans* (Linnaeus, 1758) and this record from the municipality of Medinaceli was later cited by Arias Encobet (1912). However, Crosskey and Crosskey (2000) considered that the adults identified by Strobl (1905) as *S. reptans* were *S. intermedium*. Strobl's (1905) record of *Simulium* (*Byssodon*) *maculatum* (Meigen, 1804), which was quoted by Arias Encobet in his 1912 work, was considered by Crosskey and Crosskey (2000) to be an erroneous assignment of adult specimens which must have belonged to the species *S. equinum* or another related species of the subgenus *Wilhelmia*. Nonetheless, it is recommended that the record of *S. maculatum* is referred to as *Simulium* (*Wilhelmia*) spp. since the specimens collected by Strobl (1905) seem to be lost and, therefore, cannot be studied to elucidate to which species they belong (personal communication, Professor Peter H. Adler). González Peña's (1990) records of *M. blanci*, *S. bezzii*, *S. equinum*, *S. lineatum*, *S. ornatum*, *S. pseudequinum* and *S. sergenti* for Soria were also verified by Crosskey and Crosskey (2000).

Elevational ranges observed for species in this study and in the literature

The field and laboratory work conducted in the present study allowed the reporting of three new species for La Rioja as well as the nine species reported previously by Martínez Ruiz and Portillo Rubio (1999) for this region of Spain. Based on the results of this study, the elevations range of *S. angustipes* and *S. lineatum* of 480 m and 601 m, respectively, for this region of the Cidacos River reported by Martínez Ruiz and Portillo Rubio (1999) were updated to 350–500 m for the first species and 380–625 m for the second species. The elevational ranges of the other five species reported in the literature but not found in this study remain the same for this region: 1500 m for *S. brevidens* after it was detected in a nameless lesser tributary of the Oja or Glera River (Which is a tributary of the Ebro River) in the Sierra de la Demanda; 2000 m for *S. carthusiense* and *S. costatum* from the same nameless tributary of the Oja or Glera River (Vinçon & Clergue-Gazeau, 1993); and 1340-–2000 m for *S. cryophilum* and *S. monticola* after their discovery in the Rá River in the Sierra Cebollera Natural Park (minimum elevation) (Ruiz-Arrondo et al., 2022) and in the nameless lesser tributary of the Oja or Glera River (maximum elevation) (Vinçon & Clergue-Gazeau, 1993). Of these 14 species, *S. angustipes* has the narrowest range at 150 m; followed by *S. variegatum* at 192 m; *S. lineatum* at 245 m; *S. pseudequinium* at 300 m; *S. bezzii*, *S. equinum*, *S. intermedium*, *S. ornatum* and *S. rubzovianum* at 467 m; and *S. cryophilum* and *S. monticola*, the species with the widest elevational range, at 660 m.

In Soria, *S. equinum* was reported for the Duero River at 860 m and the Ucero River, a tributary of the Duero River, at 980 m (González Peña, 1990), and this reported range remains up to date. The same situation occurs with *Simulium ornatum* was also reported at 890 m for the Ucero River (González Peña, 1990) and at 929 m for the de la Casa or Val River (Ruiz-Arrondo et al., 2018); this range also remains up to date for Soria. However, *S. intermedium*, was reported for de la Casa or Val River at 929 m (Ruiz-Arrondo et al., 2018) and at 900 m in this study; therefore, its current range is 900–929 m. Furthermore, the elevation data available for of the other nine species reveal four species at a single altitude: *S. angustipes* and *S. sergenti* were reported at 900 m and *S. argyreatum* at 1010 m for the Duero River (González Peña, 1990) and *S. monticola* at 1156 m for a nameless stream (Ruiz-Arrondo et al., 2018). The elevation ranges of the other five species are as follows: *M. blanci* at 860–900 m (González Peña, 1990), *S. bezzii* and *S. pseudequinum* at 860–980 m (González Peña, 1990), *S. erythrocephalum* at 890–1010 m (González Peña, 1990), and *S. lineatum* at 900–980 m (González Peña, 1990), all from the Duero River. Therefore, the species with the narrowest elevational range is *S. intermedium* at 29 m, followed by *S. ornatum* at 39 m, *M. blanci* at 40 m, *S. lineatum* at 80 m, and *S. bezzii*, *S. equinum*, *S. erythrocephalum* and *S. pseudequinum* at 120 m.

Water temperature ranges observed for species in this study and in the literature

Data on the abiotic factor, river water temperature, are limited for La Rioja; in fact, of the 14 species that were known before this study, information on temperature was available only for *S. angustipes*, reported in waters at 22 ºC (Martínez Ruiz & Portillo Rubio, 1999) and *S. lineatum*, reported in waters at 16 ºC (Martínez Ruiz & Portillo Rubio, 1999). In the present study, we found *S. angustipes* in waters at 13–20 ºC, a difference of 9 ºC, and *S. lineatum* at 13–16 ºC, a difference of 3 ºC, the narrowest range of all the species reported for La Rioja (Tables 1 and 2). Therefore, the water temperature ranges of seven more species are provided in increasing order of thermal amplitude: *S. bezzii* (13–16 ºC) which showed the same range of 3 ºC as did *S. lineatum* and, therefore, shared with the latter the state of having the narrowest water temperature range; *S. equinum* and *S. variegatum* (10–16 ºC) (Tables 1 and 2) with a range of 6 ºC; *S. intermedium* and *S. rubzovianum* (13–22 ºC) (Tables 1 and 2) with a range of 9 ºC, equal to that of *S. angustipes*,; and *S. ornatum* and *S. pseudequinum* (10–22 ºC) (Tables 1 and 2) presenting the widest range of 12 ºC.

No previous data are available on water temperature for the province of Soria; therefore, based on a single location (R10-La Vega), new data are provided for three (*S. equinum*, *S. intermedium* and *S. ornatum*) of the 13 species reported so far for this region.

Haematophagous species

Nine species identified in this study have emerged as significant concerns for public health, veterinary, agriculture, and tourism sectors (López-Peña, 2019, López-Peña, Lis-Cantín & Jiménez-Peydró, 2019). The bites inflicted by obligate haematophagous blackfly females, which cause not only discomfort but also symptomatic effects of varying severity, lead to a decline in the well-being of both humans and animals, as well as an increased burden on primary healthcare systems and economic losses for livestock, agriculture, and tourism sectors (López-Peña, 2019, López-Peña & Jiménez-Peydró, 2021). *Simulium petricolum*, which belongs to the *Eusimulium* subgenus, is distinguished by its preference for avian hosts and its capacity to act as a potential vector of parasitic protozoa, such as *Trypanosoma* spp. and *Leucocytozoon* spp. (Adler et al., 2015), transmitted to several bird species (Bennett, 1961, Hunter et al., 1997, Votýpka et al., 2002). In addition, mammalophilic species, such as *S. bezzii*, are noteworthy for their role in transmitting the myxomatosis virus (Joubert & Monet, 1975) and for being generally indiscriminate in their choice of mammalian hosts (Joubert & Monet, 1975). Similarly, *S. variegatum* is highlighted for feeding on a spectrum of farm animals (Davies et al., 1962, López-Peña & Cheke, 2023).

However, certain species exhibit a preference for specific groups of mammals to obtain their blood meals. For instance, *S. erythrocephalum* is prone to bite cattle (Wenk, 1981, 1987, Kettle, 1995, López-Peña & Cheke, 2023) and humans (Živković, 1970, Rivosecchi, 1978, Crosskey, 1993, Ignjatović-Ćupina et al., 2006, Lopez-Rock & Check, 2023). Similarly, blood-sucking by *S. lineatum* has also been documented for cattle (Beaucournu-Saguez et al., 1990, Bernotienė, 2003, Baužienė et al., 2004, López-Peña & Cheke, 2023) and humans (Cunze et al., 2024), indicating its mammalophilic and anthropophilic tendencies. In addition, *S. erythrocephalum* is capable of transmitting two pathogens, *Onchocerca gutturosa* (Neumann, 1910) (Crosskey, 1990, Ruiz-Arrondo et al., 2017, López-Peña & Cheke, 2023) and *Onchocerca lienalis* (Stiles, 1892) (Mikhailyuk, 1967, Ham & Bianco, 1983b, Cupp, 1996, López-Peña & Cheke, 2023) responsible for bovine onchocerciasis. Likewise, *S. lineatum* can also transmit *O. lienalis* to cattle (Ham

& Bianco, 1983a, 1983b, Baužienė et al., 2004, López-Peña & Check, 2023).

Other species show a slightly broader feeding spectrum that includes equine blood in addition to cattle and human blood. *Simulium equinum* stands out, as its name implies, for its primary host preference. It feeds on equine livestock (Sutcliffe, 1986, Crosskey, 1993, López-Peña & Cheke, 2023), cattle (Crosskey, 1993, López-Peña & Cheke, 2023), and humans (Beaucournu-Saguez et al., 1990, Lopez-Rock & Check, 2023). Its veterinary importance lies in its role as a vector of the endoparasitic nematode *O. lienalis* transmitted to equine hosts (Ham & Bianco, 1983b, López-Peña & Cheke, 2023). Another species that feeds on the blood of both equine and bovine livestock (David, 1966, López-Peña & Cheke, 2023), as well as of human (Crosskey, 1993, López-Peña & Cheke, 2023), is *S. intermedium*.

Lastly, the mammalophilic species with a tendency to obtain their blood meals from equines, bovids and suids are *S. pseudequinum* (Rivosecchi, 1978, Villanúa-Inglada et al., 2013, López-Peña & Cheke, 2023) and *S. ornatum* (Malmqvist et al., 2004, López-Peña & Cheke, 2023). Observations of blackflies obtaining blood meals from horses and cattle were reported by Zahar (1951) and David (1966); blackfly bites can affect cattle well-being (Sutcliffe, 1986, Wenk, 1981, Crosskey, 1993, Kettle, 1995, López-Peña & Check, 2023). *Simulium ornatum* can act as a potential vector of *O. gutturosa* in bovid hosts (Steward, 1937, Eichler, 1973, Bianco et al., 1980, Trees et al., 1987, López-Peña & Cheke, 2023) and of *O. lienalis* also in bovine livestock (Ham & Bianco, 1893b, Cupp, 1996, López-Peña & Check, 2023, Ignjatović et al., 2006, López-Peña & Check, 2023, Cunze et al., 2024). In addition, *S. ornatum* displays anthropophilia (David, 1966, Živković, 1970, Rivosecchi, 1978, Crosskey, 1993, Ignjatović et al., 2006, López-Peña & Cheke, 2023, Cunze et al., 2024).

CONCLUSION

This study increases the understanding of simuliids in Spain by elucidating their species richness in the provinces of Soria and the Autonomous Region of La Rioja. It provides comprehensive insights into their geographical distribution, elevational preferences, and water temperature tolerances. The fieldwork conducted in the Cidacos river basin led to the reporting of 12 species, of which, three were first records for La Rioja and one was a first record for Soria. Of the 13 sampling stations studied, stations R1, R7 and R8 showed the highest species richness, with a total of nine species each, while station R18 showed the lowest species richness, with three species. *Simulium intermedium* had the highest relative abundance, followed by *S. pseudequinum*, *S. ornatum*, and *S. bezzii*, while *S. erythrocephalum* had the lowest abundance, followed by *S. trifasciatum*. Elevational and water temperature ranges have been provided for some species, and updated for others. Likewise, provincial distribution maps of the species recorded for the first time in the study area have also been provided. Finally, the importance of the nine species with haematophagous species that obtain blood meals from wildlife, domestic and farm animals, and humans has been discussed. Thus, the results obtained contribute significantly to the improvement of the knowledge of simuliids in the area studied and in Spain in general, as well as emphasising the pressing need to continue studying their environmental role and possible detrimental effects on animal and human health, and the associated economic drawbacks.

ACKNOWLEDGEMENTS

The authors express their profound thankfulness to the CIAPOS/2021/116 grant for the recruitment of postdoctoral research staff (APOSTD/2022) co-funded by the Conselleria d'Innovació, Universitats, Ciència i Societat Digital of the Generalitat Valenciana and the European Social Fund, as well as to Josep Daniel Asís-Pardo for their invaluable and selfless help, advice and guidance.

AUTHOR CONTRIBUTIONS

D.L.P.: Processing and identification of samples, Data processing, Fund acquisition, Preparation of the original draft, Review, Editing, and Validity; M.S.P.R.: Conceptualization, Sampling methodology, samples collection, Project supervision, Review, and Validity; A.L.C.: Technical support, Figures creation, Review, and Validity; J.V.F.G.: Fund acquisition, Review, Editing, and Validity.

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