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Fauna of simuliids (Diptera: Simuliidae) of the Tormes River hydrographic basin in central-western Spain and their responses to abiotic factors in their habitats

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A R T I C L E I N F O

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A B S T R A C T

Delving into knowing the blackfly (Diptera: Simuliidae) species composition of Spain and their ecological requirements is crucial, due to their instrumental role in natural food webs as intermediaries in the flow of energy in aquatic ecosystems, and because of the haematophagic behaviour displayed by females of several species. The present study has analysed the abundance and distribution of the larvae and pupae of blackfly species in 105 sampling stations located in lotic water bodies of the provinces of Ávila, Salamanca, and Zamora of the Tormes River basin. The study has allowed to identify 24 species: 17 from Ávila, 13 from Salamanca, and seven from Zamora, classified in three genera (*Metacnephia, Prosimulium*, and *Simulium*), and five subgenera (*Boophthora, Eusimulium, Nevermannia, Simulium, Wilhelmia*). The results include four first records for Ávila province: *Simulium* (*Nevermannia*) *armoricanum* Doby & David, 1961, *Simulium* (*Nevermannia*) *cryophilum* (Rubtsov, 1959), *Simulium* (*Nevermannia*) *naturale* Davies, 1966, and *Simulium* (*Simulium*) *variegatum* Meigen, 1818. Among them, *S. armoricanum* shows the widest elevational range (900-1650 m a.s.l.), *S. naturale* shows the widest water temperature range (6-15 °C), and *S. cryophilum* stands out for inhabiting very fast waters. Blackfly communities were best discriminated by elevation and temperature. Our analyses allowed us to estimate the optima and tolerance ranges of the described species, with *S. argygreatum, S. reptans, P. latimucro* and *P. tomosvaryi* standing out for their preference for altitudes above 1000 m a.s.l., although with wide tolerance ranges. In terms of temperature, these two *Prosimulium* species were best represented at the coldest waters.

1. Introduction

In the last decades, the taxonomic study of the Simuliidae family has experienced a significant increase regarding the faunistic of the group, which has provided the identification of numerous new species in several regions of the world (Adler, 2024) such as Britain (England) and Ireland (Bass and Brockhouse, 1990), United States (Adler and Huang, 2022), Brazil (Hamada et al., 2010), Nepal (Takaoka et al., 2020), Peninsular Malaysia (Takaoka et al., 2011), Germany (Seitz and Adler, 2017), Austria (Seitz et al., 2021), China (An et al., 2022), Thailand (Srisuka et al., 2023), Algeria (Adler et al., 2024), or Morocco (Belqat and Adler, 2024). Likewise, efforts derived from scientific sampling campaigns have revealed the presence of certain species in places where they had not been recorded until now. A clear example of this has been the report of the North African species *Simulium* (*Eusimulium*) *mellah* Giudicelli et al., 2000 in Almería (Spain) (Ruiz-Arrondo et al., 2023), which represents the first record of this species in Europe, and the European species endemic to Sardinia (Italy) *Simulium* (*Nevermannia*) *ichnusae* Rivosecchi & Contini, 1994 in African territory, particularly in Algeria (Haouchine et al., 2024). These two discoveries have led to an increase in the biogeographic distribution of both species, as well as the change in cataloging from endemism to pseudoendemism in the case of

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S. ichnusae.

In Spain, the study of blackflies has also been notably intensified from different perspectives, highlighting faunal studies as a valuable source of information about the species present in Spain in general (Grenier and Bertrand, 1954; Doby et al., 1964; Beaucournu-Saguez, 1975a, 1975b; González Peña, 1990; Vinçon and Clergue-Gazeau, 1993; González Peña et al., 2002), being the most complete and up-to-date work on species diversity and geographical distribution that of López-Peña and Jiménez-Peydró (2017b), and particularly in many autonomous regions such as Andalusia (González Peña et al., 1986, 1987; Crosskey & Crosskey, 2000; Ricoy Llavero, 2015), Aragón (Villanúa-Inglada et al., 2013), Balearic Islands (Crosskey, 1991), Canary Islands (Becker, 1908; Crosskey and Báez, 2004), Catalonia (González Peña, 1985, 1990), Madrid (López-Peña et al., 2024a), Valencia (Lestón Outón, 2012; López-Peña and Jiménez-Peydró, 2017a, 2018, 2020; López Peña, 2018), Extremadura (Martínez and Portillo, 1999b), Galicia (Lestón et al., 2014), or La Rioja (Martínez Ruiz and Portillo Rubio, 1999a) among others, which have also studied in more detail the species altitudinal distributions and ranges of tolerance to abiotic variables, that have substantially leaded to improve the knowledge of their ecology.

In this regard, the present study, which completes some preliminary results conveyed in 2022 (López-Peña et al., 2022a), contributes to both increasing knowledge of the composition of species that inhabit many rivers and streams in the southwest of the Autonomous Region of Castilla y León, an area that traditionally has been scarcely studied, and delves into their optima and tolerances regarding the main physical-chemical characteristics of their breeding and development sites.

2. Material and methods

2.1. Study area and sampling collection

The presence of blackfly species was examined over the years 1988, 1989, and 1996 by Manuel Portillo Rubio and María Esther Martín Chamoso, personnel of Universidad de Salamanca, in a total of 105 sampling stations in order to know both the species composition and their ecological preferences along the hydrographic basin of the Tormes River through the provinces of Ávila, Salamanca, and Zamora (Fig. 1).

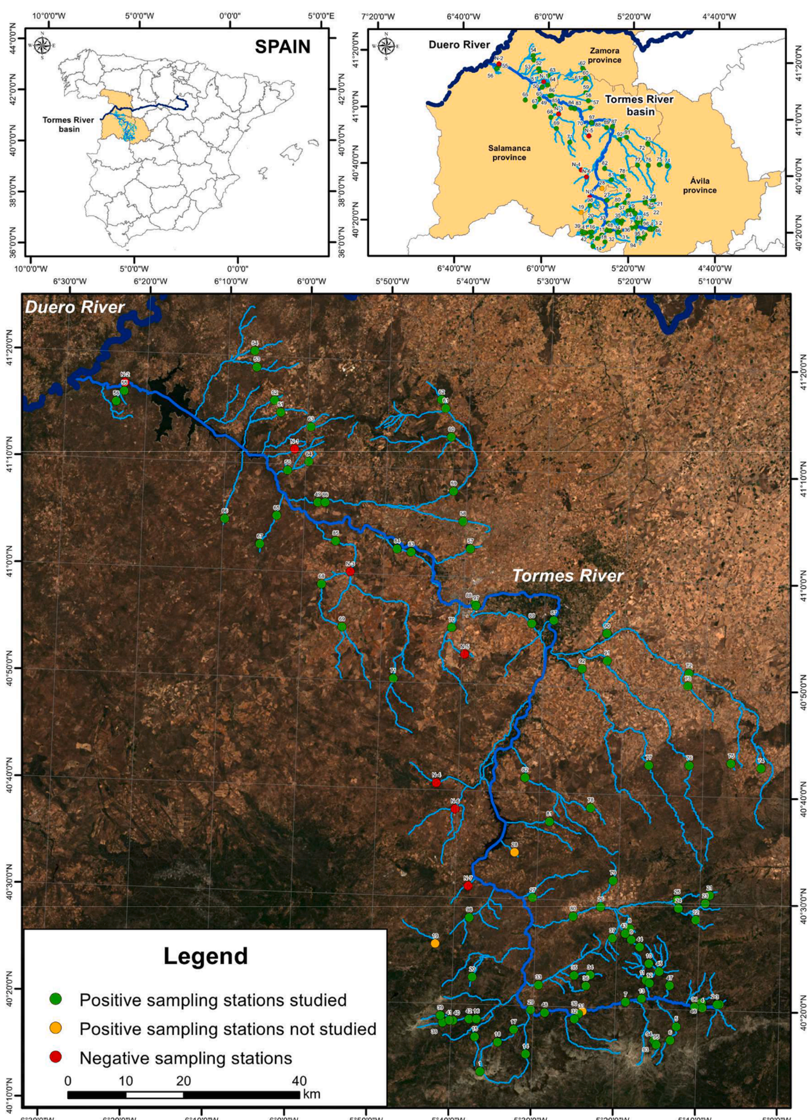

Fig. 1. Geographical location of the province of Salamanca within Spain (left side), the delineation of the Tormes River basin and the placement of sampling stations (right side). Accurate portrayal of the river and its tributaries with the precise positions of each sampling station (below). The numeric identifiers correlate with the accompanying tables.

This river, a 284-km tributary of the Duero River, originates in the Sierra de Gredos at 1,585 metres above sea level (m a.s.l.) and features a snow-pluvial regime marked by irregular flows and seasonal floods (Deputation of Ávila, 2017; Sánchez-Herández, 2011). The field work was conducted attempting to cover the four seasons: Winter (February), Spring (April, May, June), Summer (July), Autumn (October, November) (Table 1). The collectors collected the samples focusing on the central area and both riverbanks. Specimens in larval or pupal stages were located on substrates like boulders or plants, then sampled and preserved in 70% alcohol. The sampling effort was standardised, and a similar time was generally dedicated to each sampling. Whenever possible, *in situ*, data were measured and written down in a field notebook by quantitative or by qualitative scales, including sample number, UTM coordinates (Universal Transverse Mercator), date, elevation (m), width (m), depth (cm), water temperature (°C), water velocity (m/s or descriptive scale (from slow to very fast)), sort of flow (very scarce, scarce, little, normal, abundant, very abundant), estimated water coloration (very clear, clear, darkened, yellowish, greenish), and kind of riverbed (stony, gravelly, sandy, silty, muddy) (Tables S1 and S2). The coordinates and the elevation were measured by means of a GPS, the width and depth with a measuring tape, the water temperature with a thermometer, and the water velocity measuring the time it takes for a cork structure to cover a distance of 5 metres.

2.2. Samples processing and species identification

Once in the laboratory, some representative specimens from each sampling station were selected to carry out projects for academic purposes. The rest of the material collected at each prospected station, that is, more than 90%, was stored in 70% ethanol. The present study, carried out within the framework of a postdoctoral research project, has focused on processing the stored and not previously studied samples with the aim of expanding the knowledge on simuliids' distribution in Spain, analysing in detail both the diversity of blackfly species present in the study area, as well as the ecology of each of them. To this end, the stored samples have been processed, first removing all those adhesion substrates and particles that made the identification process difficult, such as plant structures (parts of leaves and branches), small stones, and mud deposited on the bodies and structures of the larvae and pupae used for their identification, using soft entomological forceps.

Most of the prospected places provided representative blackfly samples, whose specimens in both mature larva and pupa stages were separated from the rest of the collected entomological material in order to count and identify them to the species level by means of morphological-based keys (Knoz, 1965; González Peña, 1997; Bass, 1998; Jedlička et al., 2004; Rivosecchi et al., 2007; Kúdela et al., 2022) and using both Wild M8 Heerbrugg and Leica M80 10450630 stereomicroscopes.

Hence, if someone studies the simuliids of this study area again, historical data such as those shown in this study are keystone to being able to compare them with species present-day distributions and current ecological conditions, which will be useful to understand possible changes that have occurred in species diversity, habitat or environmental factors of their aquatic breeding sites over time in response to the framework of climate change and variations in land use among other factors.

2.3. Data analysis

We examined correlations between environmental variables at the sampling stations. Only pairs of variables for which Pearson's r was less than 0.8 were retained for principal component analysis (PCA), which was used to explore differences between sampling stations on the normalised values of measured environmental variables. Prior to further analyses, we built up a simuliid species richness accumulation curve to confirm that sampling was exhaustive enough. The curve was derived by randomly ordering samples from the species by sample dataset and searching for asymptotical behaviour. A total of 100 random permutations of sampling units order were performed for each sampling site following a subsampling without replacement procedure as suggested by Gotelli and Colwell (2001).

Then we performed a Canonical Correspondence Analysis (CCA; Borcard et al., 2011) using the log abundance data of mature larvae (immature larvae could not be identified morphologically with sufficient taxonomic resolution) and pupae of the identified species. A previous Detrended Canonical Correspondence Analysis (DCCA) provided support of the unimodal model response of blackfly species to the gradients defined by the environmental variables (i.e., the maximal length of the first two axes of the DCCA ordination > 3 SD; Leps and Smilauer, 2003) and justified the choice of CCA as the appropriate method of analysis to explore the relationship between the blackfly species assemblage and environmental variables. For the CCA, a forward selection procedure was used to identify the environmental variables that best explained the variability in blackfly assemblages at the sampling stations. The significance of the environmental variables entered at each step was inferred from Monte Carlo permutation tests (999 permutations, p-value < 0.05).

Finally, Weighted Average (WA) regression (Birks, 1995) was used to estimate the distribution of the mature larval and pupal stages of the blackfly species identified in the study along each of the environmental gradients of the variables retained after the forward selection in the CCA. For each species, WA regression estimated the value with the highest probability of occurrence with respect to a quantitative environmental variable, based on the weighted mean of the values of the variable of interest at those sampling stations where the species was present. Weights were the relative abundances of each species at each sampling station.

All statistical analyses were carried out using the free software R version 4.4.0 from The R Foundation for Statistical Computing (R Development Core Team, 2024; The Comprehensive R Archive Network). PCA was performed using the *prcomp* function from package 'stats'. DCCA and forward-selection CCA analyses were performed using, respectively, the *decorana, cca* and *ordistep* functions from the 'vegan' package (Oksanen et al., 2022). The *specaccum* function from this same package was used to build up the species accumulation curve. WA regression and associated plots were performed using the functions *optima, tolerance* and *caterpillarplot* from the 'analogue' package (Simpson and Oksanen, 2024).

2.4. Creation of maps

The figures with the location of the sampling stations, as well as the distribution maps of the species mentioned for the first time in the studied area, have been created using the geographic information system (GIS) program ArcMapTM 10.5, ESRI's ArcGIS ® software (ESRI,

Table 1
Months in which blackfly specimens were collected during the years 1988, 189 and 1999 are indicated by a plus sign (+).

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1988							+			+	+	
1989		+			+		+					
1999				+	+	+						

2017).

3. Results

3.1. Species occurrence

The entomological material processing of the 33 sampling stations (Table S1), which come to complete the study of all the samples collected from the study area, have revealed a total number of 4943 specimens (3999 immature larvae, 370 mature larvae, and 574 pupae). As a result, it has been recorded the presence of 17 species (Table 2), which belongs to 2 genera (*Prosimulium* (Roubaud, 1906), *Simulium* Latreille, 1802), 4 subgenera (*Eusimulium* (Roubaud, 1906), *Nevermannia* Enderlein, 1921, *Simulium* Latreille, 1802, *Wilhelmia* Enderlein, 1921), and 5 species-groups (*equinum, hirtipes, ornatum, variegatum, vernum*) (Table 2), being 16 of them reported from Ávila and 3 from Salamanca (Table S1 and Table S3). In addition to that, 4 species arise as first records for the province of Ávila: *Simulium* (*Nevermannia*) *armoricanum* Doby & David, 1961, *Simulium* (*Nevermannia*) *cryophilum* (Rubtsov, 1959), *Simulium* (*Nevermannia*) *naturale* (Davies, 1966), and *Simulium* (*Simulium*) *variegatum* Meigen, 1818.

The study of these 33 samples has allowed us to obtain a complete picture of all the sampling carried out in the research plan. Therefore, in the whole area of study, 98 sampling stations out of the 105 prospected resulted to be positive in presence of blackflies while the other 7 were negative (Fig. 1). As a result of the study of these 98 samples, 24 species have been identified, 17 from Ávila, 13 from Salamanca, and 7 from Zamora (Table 3), which belong to 3 genera (*Metacnephia* Crosskey, 1969, *Prosimulium, Simulium*), 5 subgenera (*Boophthora* Enderlein, 1921, *Eusimulium, Nevermannia, Simulium, Wilhelmia*), and 6 species-groups (*equinum, hirtipes, ornatum, reptans, variegatum, vernum*).

Table 2
Taxonomic classification of the species identified from the 33 samples processed.

Genus	Subgenus	Species-group	Species
Prosimulium (Roubaud, 1906)		*hirtipes* species-group	*P. hirtipes* (Fries, 1824)
			P. tomosvaryi (Enderlein, 1921)
Simulium Latreille, 1802	*Eusimulium* (Roubaud, 1906)		*S.* (*Eusimulium*) *angustipes* Edwards, 1915
			S. (*Eusimulium*) *petricolum* (Rivosecchi, 1963)
			S. (*Eusimulium*) *rubzovianum* (Sherban, 1961)
	Nevermannia Enderlein, 1921	*vernum* species-group	*S.* (*Nevermannia*) *armoricanum* Doby & David, 1961
			S. (*Nevermannia*) *carthusiense* (Grenier and Dorier, 1959)
			S. (*Nevermannia*) *cryophilum* (Rubtsov, 1959)
			S. (Nevermannia) naturale Davies, 1966
			S. (*Nevermannia*) *vernum* Macquart, 1826
	Simulium Latreille, 1802	*ornatum* species-group	*S.* (*Simulium*) *intermedium* (Roubaud, 1906)
			S. (*Simulium*) *ornatum* Meigen, 1818
			S. (*Simulium*) *trifasciatum* Curtis, 1839
		variegatum species-group	*S.* (*Simulium*) *argyreatum* Meigen, 1838
			S. (*Simulium*) *monticola* Friederichs, 1920
			S. (*Simulium*) *variegatum* Meigen, 1818
	Wilhelmia Enderlein, 1921	*equinum* species-group	*S.* (*Wilhelmia*) *equinum* (Linnaeus, 1758)

3.2. Updating of the geographical distribution of several species

The distribution maps of the four species recorded for the first time for the province of Ávila have been enlarged and updated (Fig. 2).

Therefore, it is concluded that with respect to the last update of the Simuliid species checklist of Spain and their respective distributions by provinces (López-Peña and Jiménez-Peydró, 2017b), the geographical distribution of the species *S. armoricanum* includes 6 provinces and 4 autonomous regions, the distribution of *S. cryophilum* extends across 25 provinces and 13 autonomous regions, that of *S. naturale* covers 5 provinces and 3 autonomous regions, and finally that of *S. variegatum* spans 26 provinces and 11 autonomous regions.

3.3. Characterization of pre-imaginal habitats of blackfly species in the Tormes River hydrographic basin and ecological preferences

The habitats for mature larvae and pupae of the identified blackfly species in the Tormes River hydrographic basin were characterised using a PCA on elevation, water temperature, depth, width and water velocity. PCA was restricted to 48 sampling stations, where all these environmental variables were registered. The first two axes of the ordination explained 32.89% and 25.60% of the total variance, respectively, among the sampling stations (Fig. 3). PCA1 was positively related to water velocity (loading = 0.718) and elevation (loading = 0.681). PCA2 was positively related to water temperature (loading = 0.746) and channel width (loading = 0.600).

The species accumulation curve for the 48 studied sampling stations is shown in Fig. 4. It showed an asymptotic behaviour confirming that sampling effort was high enough. Then, a constrained ordination via CCA aimed to relate blackfly species assemblages with environmental variables. The CCA explained a significant percentage of total inertia (22.6%) in the variability of the assemblages of larvae and pupae ($F = 2.464$; df = 5, 42; p-value = 0.023). Further, a small subset of environmental variables was enough to retain most of the explained variability after applying forward selection. Hence, blackfly pre-imaginal assemblages were mainly explained by a first CCA axis positively related to elevation and negatively to temperature, introduced sequentially (Fig. 5a). The rest of variables appeared to have a minor effect. Blackfly species in Fig. 5a are placed at the centroids (weighted average) of the sampling stations where each species occurred. Clearly, *S. argyreatum, P. latimucro, P. tomosvaryi* and *S. reptans* appeared to occur at high elevation sampling stations. On the other hand, *S. equinum* and *S. erytrocephalum* are the species with higher temperature optima and preferentially found in low land sampling stations. The rest of blackfly species were ordered along the elevation gradient as evidenced after zooming in the central set of sampling points (Fig. 5b).

Optima and tolerance values for elevation and temperature (the two environmental variables retained after sequential CCA) provided more comprehensive information regarding niche specificity of the pre-imaginal stages of blackfly species in the Tormes hydrographic basin (Fig. 6). In general, all species were collected at sampling points of a certain elevation, given the geographical characteristics of the hydrographic base studied. However, the following species stand out: *P. latimucro, P. tomosvaryi, S. argyreatum* and *S. reptans* had their optimum above 1000 m a.s.l. The first two species were found across a wide elevational range, whereas the latter two species were recorded from single sampling stations. Regarding water temperature, *S. aureum, S. equinum, S. erythrocephalum, S. lineatum, S. pseudequinum* and *S. sergenti* had optima above 20 °C. On the contrary, *P. latimucro* and *P. tomosvaryi* had optima at colder waters (below 15 °C) and in accordance with their wide altitudinal distribution also seem well adapted to thermal variation.

As mentioned above, the analyses in this section were restricted to only those sampling points where data were available for all

Table 3
Simuliid species identified per province from the samples collected at the 98 positive sampling stations.

Species	Province	Species	Province	Species	Province
P. hirtipes	Ávila	*S. reptans*	Ávila	*S. pseudequinum*	Salamanca
P. latimucro	Ávila	*S. rubzovianum*	Ávila	*S. rubzovianum*	Salamanca
S. angustipes	Ávila	*S. trifasciatum*	Ávila	*S. sergenti*	Salamanca
S. argyreatum	Ávila	*S. variegatum*	Ávila	*S. trifasciatum*	Salamanca
S. armoricanum	Ávila	*M. nuragica*	Salamanca	*S. angustipes*	Zamora
S. carthusiense	Ávila	*S. angustipes*	Salamanca	*S. intermedium*	Zamora
S. cryophilum	Ávila	*S. aureum*	Salamanca	*S. lineatum*	Zamora
S. equinum	Ávila	*S. equinum*	Salamanca	*S. ornatum*	Zamora
S. intermedium	Ávila	*S. erythrocephalum*	Salamanca	*S. petricolum*	Zamora
S. monticola	Ávila	*S. intermedium*	Salamanca	*S. rubzovianum*	Zamora
S. naturale	Ávila	*S. lineatum*	Salamanca	*S. trifasciatum*	Zamora
S. ornatum	Ávila	*S. ornatum*	Salamanca		
S. petricolum	Ávila	*S. petricolum*	Salamanca		

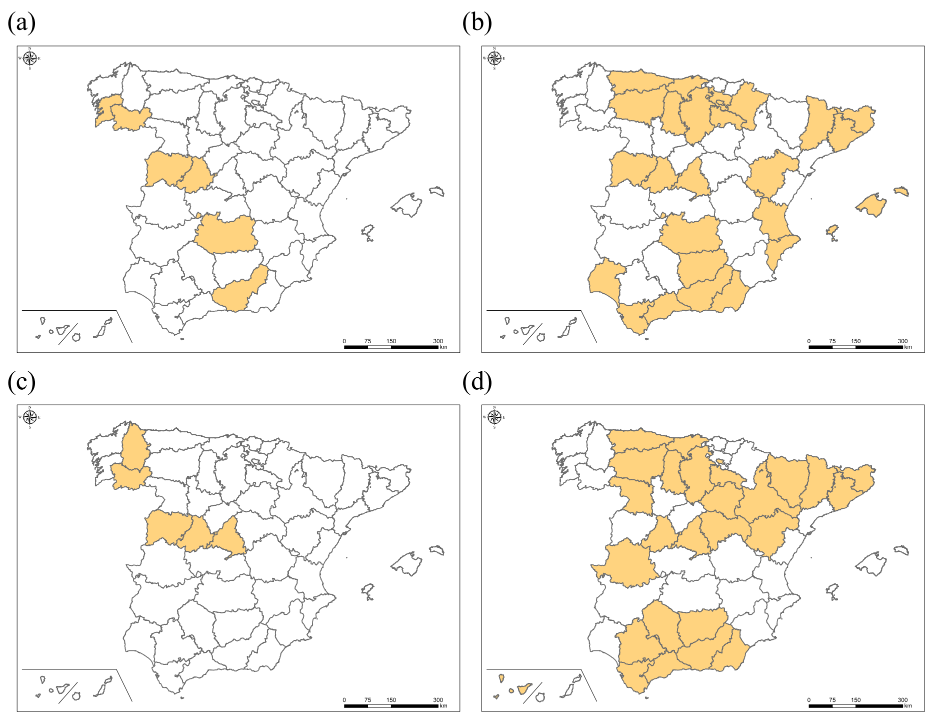

Fig. 2. Update of the distribution maps of the species: (a) *S. armoricanum*, (b) *S. cryophilum*, (c) *S. naturale*, and (d) *S. variegatum* after their identification for the first time in the province of Ávila.

environmental variables under study. This fact ruled out many sampling points and consequently excluded several species from our analyses. Some of these species are first descriptions in the region. That is why in the following section we wanted to provide specific data for these species with respect to the environmental characteristics that could be collected from their sampling points.

3.4. Elevation, water temperature and water velocity ranges of the species recorded for the first time in the province of Ávila

The present study provides valuable insights regarding the ranges of the environmental variables that significantly affect the presence and distribution of the four species recorded for the first time for the province of Ávila. Although not as comprehensive as the previous section, this information depicts instrumental knowledge about the ecology of these species. With respect to the elevation range where these species were discovered during this study, *S. variegatum* was recorded from a single site, particularly at sampling station 29 (Table S3), located at 900 m a.s.l. (Table S2), the other three displayed an elevation range where *S. naturale* showcases the narrowest value ranging from 1100 to 1650 m a.s.l., followed by *S. cryophilum* which varied from 950 to 1650 m, and *S. armoricanum*, that showed the widest one ranging from 900 to 1650 m a.s.l. (Table S2 and Table S3). Our data show that these four species occur in a similar elevation range to the higher elevation species in the previous section.

Regarding water temperature, *S. variegatum* was found at 5 °C in a channel nearby the Tormes River at Los Llanos de Tormes, while the other three species, although have similar ranges, showed some nuances. *Simulium cryophilum* exhibited the smallest thermal range (5-9 °C), followed by *S. armoricanum* with a thermal amplitude of 5 °C (5-10 °C), and *S. naturale* showed the widest thermal range with 9 °C (6-15 °C) (Table S2 and Table S3). Interestingly, all these species occurred in the coldest waters in this study, below even the ranges described in the previous section. Besides that, the species *S. cryophilum* was found at very fast waters (Table S2 and Table S3).

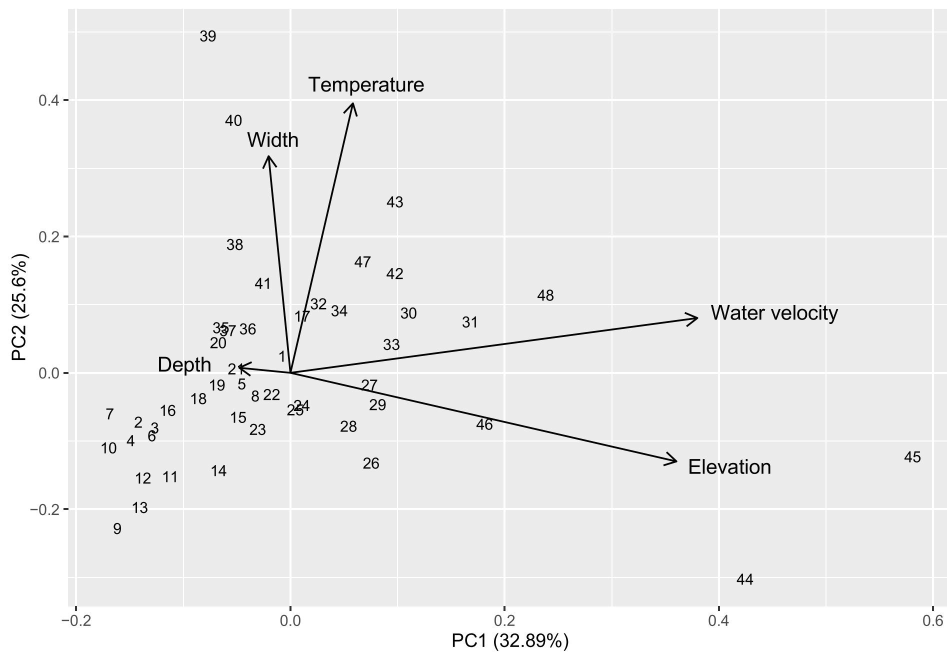

Fig. 3. Principal Components Analysis (PCA) ordination plot showing the multivariate variation among sampling points in terms of environmental variables. Vectors indicate the direction and strength of each environmental variable to the overall distribution. The first two principal axes explained 68 % of the variance.

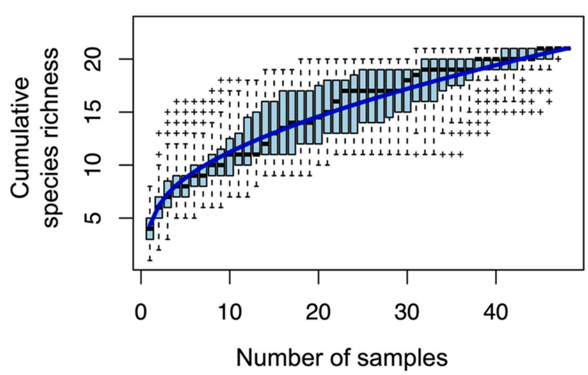

Fig. 4. Species accumulation curve showing average cumulative simuliid species richness (in blue) against the number of samples for which environmental parameters were measured. Boxplots show the variance of species richness estimates from 100 permutations with samples added in random order (black bars indicate the median, and box limits indicate first to third quartile, with outliers indicated by crosses).

4. Discussion

4.1. Species occurrence

In the partial study of samples collected in the Tormes River basin (López-Peña et al., 2022a), 19 species were reported in. However, in the present study, and after processing the total number of samples collected, 5 more species have been reported, and 4 species have been recorded for the first time for Ávila province.

In addition to this, the thorough bibliographical consultation, together with the results obtained from the present study have allowed to conclude the presence of a total of 22 species for Ávila province, of 27 species for Salamanca province, and of 20 species for Zamora province, that is, 31 species considering the three provinces (Table 4). In this way, the most updated list of species to date for the three provinces studied has been provided.

Apart from this, the findings of this research, as previous studies already did in the past, have contributed to know the regional diversity of blackfly species in several hydrographic basins of Spain. In this way, the Tormes river basin rises as the catchment area with the highest species richness with 24 species of simuliids reported, followed by the water basins of the Júcar River with 16 species (López-Peña & Jiménez-Peydró, 2017a), Serpis River with 14 species (López-Peña & Jiménez-Peydró, 2020), Mijares (López-Peña & Jiménez-Peydró, 2018), Ter (Puig et al., 1987) and Yeguas (González et al., 1986) rivers with 13 species, Palancia River with 12 species (López-Peña et al., 2020), and Turia River with 11 species (López-Peña et al., 2020).

4.2. Update of the elevation and water temperature ranges of several species recorded from the studied area

The available information coming from the literature, together with the data obtained in this study have allowed to compile valuable knowledge regarding the ranges for abiotic factors such as the elevation, the water temperature, the water velocity, and the depth of the water column of the simuliid breeding sites, as well as to complete the absent value for some species (Table S4). Apart from this, the elevation and water temperature ranges of several species have been updated (Table S4). For instance, in Ávila, as for the elevation range, the ranges of *S. angustipes* (950-1550 m a.s.l.), *S. argyreatum* (1200-1800 m a.s.l.), *S. carthusiense* (925-1550 m a.s.l.) and *S. rubzovianum* (850-1650 m a.s.l.) have been enlarged and updated (Table S4). In like manner, the ranges of the following species have been created since only a single datum was previously known: *P. hirtipes* (925-1650 m a.s.l.), *P. tomosvaryi* (950-1650 m a.s.l.), *S. equinum* (1300-1350 m a.s.l.) and *S. vernum* (1100-1550 m a.s.l.) (Table S4). Regarding the water temperature, something similar has happened, in this way the ranges of the following species have been enlarged: *S. intermedium* (5-23 °C), *S. ornatum* and *S. rubzovianum* (5-23 °C), *S. trifasciatum* (6-23 °C) (Table S4). Others have been created because of the existence of a single datum: *P. hirtipes* (5-20 °C), *S. argyreatum* (10-17 °C), *S. carthusiense* (7-14.8 °C), and *S. monticola* (6-20 °C) (Table S4). By the same token, we provide the range of the species *P. tomosvaryi* (5-20 °C), and a value for the species *S. vernum* (10 °C) (Table S4).

In addition to that, it is worthwhile to underline that previous reports

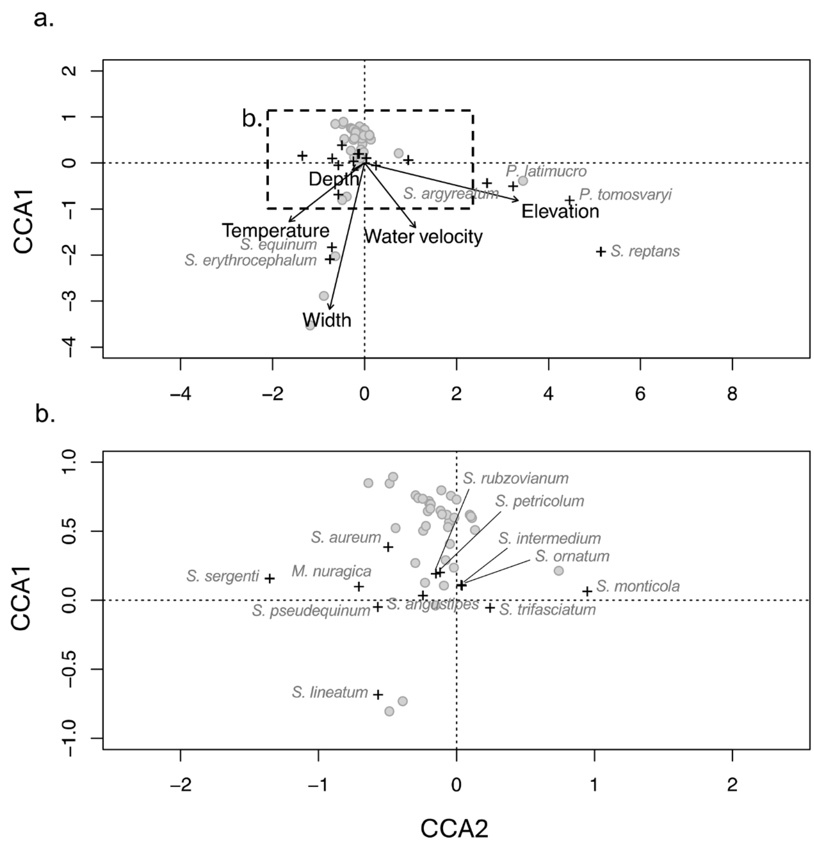

Fig. 5. (a) Canonical correspondence analysis (CCA) ordination biplot of blackfly pupae and larvae assemblages in the sampling sites, represented by grey circles. Scores for simuliid species are represented by black crosses. Species scores are at the centroid (weighted average) of the sampling points where each species occurs. Vectors indicate the direction and strength of environmental variables to the overall distribution. Environmental variables were selected using forward selection and Monte Carlo permutation tests (see ''Materials and methods''). (b) Zooming on the rectangular area delimited by a dashed line with standardized names for simuliid species.

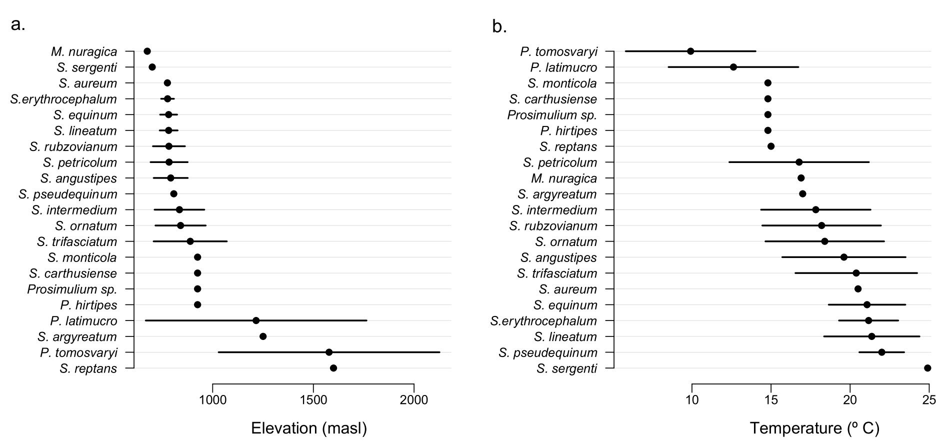

Fig. 6. Optima (modes) and tolerances (error bars) for (a) elevation and (b) temperature for the pupae and larvae of blackfly species found in this study. Species are arranged on the y axis by increasing optima for each variable.

conducted in Spain already explained and continue supporting the specific habitat associations of several species of simuliids with the diverse abiotic factors of their aquatic pre-imaginal habitats such as elevation and temperature. For instance, Martínez and Portillo (1999a), Crosskey and Crosskey (2000) and López-Peña et al. (2022b) pointed out that the elevation was the most determining abiotic factor in the distribution patterns of the immature forms of the simuliid species that these authors found.

With respect to the water temperature, Nascimento et al. (2007) indicated that this variable is widely associated with the distribution of preimaginal blackfly species of a Brazilian river, and López-Peña et al. (2020) emphasised that the water temperature also plays a relevant role in blackfly distribution and in shaping their species assemblages. Other authors also highlighted the importance of the elevation and water temperature variables in the simuliid assemblages, and therefore in their geographical distribution (McCreadie & Adler, 2006, 2014; Ya'cob

Table 4
Taxonomic classification of the species identified both in this and in previous studies conducted in the provinces of Ávila, Salamanca, and Zamora (Central-Western Spain). For more information, see Table S4 in supplementary material.

Genus	Subgenus	Species-group	Species
Metacnephia Crosskey, 1969			*M. blanci* (Grenier and Theodorides, 1953)
			M. nuragica Rivosecchi, Raastad and Contini, 1975
Prosimulium (Roubaud, 1906)		*hirtipes* species-group	*P. hirtipes* (Fries, 1824)
			P. latimucro (Enderlein, 1925)
			P. tomosvaryi (Enderlein, 1921)
Simulium Latreille, 1802	*Boophthora* Enderlein, 1921		*S.* (*Boophthora*) *erythrocephalum* (De Geer, 1776)
	Eusimulium (Roubaud, 1906)		*S.* (*Eusimulium*) *angustipes* Edwards, 1915
			S. (*Eusimulium*) *aureum* Fries, 1824
			S. (*Eusimulium*) *petricolum* (Rivosecchi, 1963)
			S. (*Eusimulium*) *rubzovianum* (Sherban, 1961)
	Hellichiella Rivosecchi & Cardinali, 1975	*congareenarum* species-group	*S.* (*Hellichiella*) *latipes* (Meigen, 1804)
	Nevermannia Enderlein, 1921	*ruficorne* species-group	*S.* (*Simulium*) *lundstromi* (Enderlein, 1921)
		vernum species-group	*S.* (*Nevermannia*) *armoricanum* Doby and David, 1961
			S. (*Nevermannia*) *carthusiense* (Grenier and Dorier, 1959)
			S. (*Nevermannia*) *cryophilum* (Rubtsov, 1959)
			S. (*Nevermannia*) *naturale* Davies, 1966
			S. (*Nevermannia*) *vernum* Macquart, 1826
	Simulium Latreille, 1802	*argenteostriatum* species-group	*S.* (*Simulium*) *hispaniola* Grenier and Bertrand, 1954
		bezzii species-group	*S.* (*Simulium*) *bezzii* (Corti, 1914)
		ornatum species-group	*S.* (*Simulium*) *intermedium* (Roubaud, 1906)
			S. (*Simulium*) *ornatum* Meigen, 1818
			S. (*Simulium*) *trifasciatum* Curtis, 1839
		reptans species-group	*S.* (*Simulium*) *reptans* (Linnaeus, 1758)
		variegatum species-group	*S.* (*Simulium*) *argyreatum* Meigen, 1838
			S. (*Simulium*) *monticola* Friederichs, 1920
			S. (*Simulium*) *variegatum* Meigen, 1818
	Wilhelmia Enderlein, 1921	*equinum* species-group	*S.* (*Wilhelmia*) *equinum* (Linnaeus, 1758)
			S. (*Wilhelmia*) *lineatum* (Meigen, 1804)
			S. (*Wilhelmia*) *pseudequinum* (Séguy, 1921)
			S. (*Wilhelmia*) *sergenti* Edwards, 1923
Urosimulium Contini, 1963			*U. faurei* (Bernard et al., 1972)

et al., 2016). Indeed, by way of an example, the species *S. equinum* that was found in this study, was associated with high elevation and with cold waters by López-Peña et al. (2022b).

The water velocity also arose as an important factor in the establishment and distribution of blackflies in this study, a fact that was also proved by McCreadie et al. (2005) in Nearctic and Neotropical regions, by Figueiró et al. (2012) in Brazilian streams and by López-Peña et al. (2020) in streams of Eastern Spain. In fact, and by way of an example, the distribution of the species *M. blanci* and *S. bezzii* reported from Salamanca province by González Peña (1990) and López-Peña et al. (2024b) each in order, were correlated with high waterflow velocities by López-Peña et al. (2020) in the Valencian Autonomous Region.

5. Conclusions

The study of 33 sample stations in the Tormes river basin revealed 17 new species for the area (16 in Ávila and 3 in Salamanca) from the genera *Prosimulium* and *Simulium*, including four first records for Ávila (*S. armoricanum, S. cryophilum, S. naturale*, and *S. variegatum*). A total of 24 species were identified across 98 positive sites, with distributions spanning Ávila, Salamanca, and Zamora. Key findings include *S. armoricanum* exhibiting the broadest elevation range (900–1650 m), *S. naturale* thriving in the widest temperature range (6–15 °C), and *S. cryophilum* preferring fast-flowing waters.

The study confirmed prior knowledge about species' ecological preferences, such as *Prosimulium* favoring high-elevation waters and species like *S. pseudequinum, S. lineatum*, and *S. sergenti* being thermophilic. However, many species were found at single sites, underscoring the need for expanded spatial and temporal sampling to improve understanding of critical areas harboring species of biosanitary importance.

Glossary

Principal Component Analysis (PCA): A dimensionality reduction and machine learning method used to simplify a large data set into a smaller set while still maintaining significant patterns and trends.

Canonical Correspondence Analysis (CCA): A multivariate method to elucidate the relationships between biological assemblages of species and their environment.

Detrended Canonical Correspondence Analysis (DCCA): An efficient ordination technique when species have bell-shaped response curves or surfaces with respect to environmental gradients.

Biosanitary: Word used to describe something that uses biological science in medical developments.

Haematophagic behaviour: Is the practice by certain animals of feeding on blood since this is an accessible fluid rich in nutritious proteins and lipids.

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CRediT authorship contribution statement

David López-Peña: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Manuel Salvador Portillo-Rubio:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Eduardo Moisés García-Roger:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Formal analysis. **Álvaro Lis-Cantín:** Writing – review & editing, Visualization, Validation, Formal analysis. **José Vicente Falcó-Garí:** Writing – review & editing, Visualization, Validation, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2024.107516.

Data availability

All the data used appears both in the tables of the manuscript and supplementary material files.

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