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# Diversity and distribution of blackflies (Diptera: Simuliidae) of the Tormes river basin (western Spain)

**D. López-Peña[1*], J.D. Asís-Pardo[2], M. Portillo-Rubio[2] and R. Jiménez-Peydró[1]**

*[1]Laboratory of Entomology and Pest Control, Institut Cavanilles de Biodiversitat i Biologa Evolutiva (ICBiBE), Universitat de València (Estudi General), C/ Catedrático José Beltrán, 2, 46980 Paterna, Valencia, Spain; [2]Área de Zoología, Departamento de Biología Animal, Parasitología, Ecología, Edafología y Química Agrícola, Facultad de Biología, Universidad de Salamanca, Campus Miguel de Unamuno, C/ Licenciado Méndez Nieto, s/n, 37007 Salamanca, Spain; david.lopez@uv.es*

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**RESEARCH ARTICLE**

## Abstract

To enhance the partial knowledge on blackfly (Diptera: Simuliidae) fauna in Spain it is crucial to collect all the available data on this group to better understand their ecology and distribution over the years. This study presents data from samples collected along the Tormes river basin (western Spain) during the years 1988, 1989 and 1996 and then stored at the University of Salamanca. As a result of a research stay, a total of 19 species belonging to 3 genera (*Prosimulium, Metacnephia* and *Simulium*) and 5 *Simulium* subgenera (*Boophthora*, *Eusimulium*, *Nevermannia*, *Simulium* and *Wilhelmia*) were identified after examining 64% of the stored material, representing 35% of the species known from Spain. In addition, 14 species are reported for the first time in 3 provinces in the study area. Further sampling of blackflies is foreseen in the near future in order to check if the blackfly fauna composition determined in samples collected during the last two decades of the 20$^{th}$ century has changed. This aim gains in importance as a consequence of haematophagous species having been found, relevant for both public health and animal welfare, not only because of the discomfort caused by their bites but also because of their potential as vectors of disease-causing agents in animals and humans.

**Keywords:** Simuliidae, bioecology, geographical layout, human and animal health, Spain

## 1. Introduction

Simuliids, commonly known as blackflies, are nematocerous dipterans belonging to the Simuliidae family. Their study is of relevance for several reasons. First of all, they play an important role in food chains, because of their larvae, as filter feeding organisms (Adler and McCreadie, 1997; Crosskey, 1990, 1993; Kettle, 1995), are able to incorporate both organic and inorganic matter, thus constituting a key component in the recirculation of energy in stream food systems (Malmqvist *et al.*, 2004). Also, they represent an abundant food source, essential for other invertebrate and vertebrate organisms (Malmqvist *et al.*, 2004). Additionally, some species can be used as water quality indicators, while several are considered good environmental indicators to evaluate the ecological health of running watercourses (Ciadamidaro *et al.*, 2016; Kazanci and Ertunç, 2010). Finally, due to the fact that adult females of a few species, the anautogenous ones, require a *sine qua non* intake of bird and/or mammal blood to achieve development and maturation of the oocytes prior to egg laying, these blood-sucking species can act as vectors for disease-causing agents in animals and people (Adler *et al.*, 2004; Carter Centre, 1999), affecting health and well-being to a greater or lesser extent. Consequently, these agents, including viruses, blood protozoa and nematodes, can cause virosis, trypanosomiasis and filariasis (Cupp *et al.*, 1992; Kiszewski and Cupp, 1986) in their hosts. Simuliidae not only directly affect the health and well-being of humans, but also indirectly may cause economic losses in livestock production, agriculture, tourism and recreational activities (Adler *et al.*, 2004; Cupp and Cupp, 1997).

Accordingly, these anthropophilic species of medical importance (Hansford and Ladle, 1979), are responsible for painful and annoying bites which lead to bleeding wounds (Živković, 1970), and in several occasions also to allergic reactions, known as simuliotoxicosis (Crosskey, 1990), do to their saliva components. This saliva is made up of anaesthetic substances, which prevent the host from noticing the pain of the bite, vasodilators, which cause the increase in blood flow to the biting area, and anticoagulants and antithrombin substances, which limit blood coagulation; all of these favour the feeding process of the insect (Ribeiro and Francischetti, 2003). It is remarkable to underline that the blackfly bites clinical profile, as well as the concomitant pathology, is characterised by local tumefaction (inflammation), temporary temperature increase around the bite area, oedema, haemorrhage, intense itching (Burány *et al.*, 1972; De Villiers, 1987) and dermatitis (Krstić, 1966). In the worst cases, simuliid females may transmit pathogens which cause diseases such as human onchocerciasis and mansonellosis, but which so far have not been reported in Spain. Mansonellosis is caused by the filaria *Mansonella ozzardi* Manson 1897, a disease characterised as non-pathogenic, although it can produce erythematous skin rashes, headaches, fever and lymphadenitis (Bartoloni *et al.*, 1999). Human onchocerciasis, also known as river blindness, is caused by the species *Onchocerca volvulus* Leuckart 1983, has marked symptoms, including onchocercomata (subcutaneous nodules), blindness, skin depigmentation (leopard skin), thickened and hyperkeratotic skin (elephant skin), and lymphatic system complaints (CDC, 2019; Crosskey, 1993). The above-mentioned symptoms give rise to blindness and, therefore, work incapacitation (Evans, 1995), as well as social stigmatisation (Ovuga *et al.*, 1995).

Likewise, the zoophilic species have huge veterinary importance because of their effect on animal welfare. These haematophagous species can be harmful since they are capable of acting as vectors of pathogens and parasites such as viruses, protozoa, blood parasites and filarial nematodes. As a consequence of the associated dermatitis and resulting skin lesions (Gräfner, 1981), together with the resultant alterations in feeding and reproductive behaviour caused by the bites (Fredeen, 1977), negative impact on farming takes place, consisting of the reduction in meat and milk production (Anderson and Voskuil, 1963), as well as in egg and poultry production (Edgar, 1953). Apart from this, domestic livestock deaths (Boiteux and Noirtin, 1979; Fredeen, 1977) can occur as a result of toxaemia or anaphylactic and hypovolemic shock (Steelman, 1976) after massive attacks (Figueras *et al.*, 2011; Leclercq, 1987; Rivosecchi, 1986) caused by outbreaks when numberless adults emerge simultaneously (Ignjatović-Ćupina *et al.*, 2006; Živković and Burány, 1972).

In addition, the current climate change scenario urges to address the problem of vectors and public health. In fact, the World Health Organization (WHO), in its document 'Global Response for Vector Control 2017-2030' (WHO, 2017), highlights the role played by vectors, such as blackflies in the transmission of agents that cause disease in humans and represent a significant threat to populations.

For all these reasons, together with the fact that the knowledge of blackfly fauna is still very patchy in Spain, owing to the scarcity or the complete lack of studies in many hydrographic basins of the country, any past knowledge is of great relevance to better understand this dipteran family. Consequently, it is vital to have all the available insights into blackfly bioecology and life cycles, above all in order to be efficient in their population control to avoid the associated negative impact.

The present study provides information to deepen the bioecological knowledge of blackflies, as well as their geographical distribution, in the hydrographic basin of the Tormes river.

## 2. Materials and methods

The Tormes river is a tributary of the Duero river that rises in the Sierra de Gredos at an altitude of 1,585 metres above sea level (m a.s.l.) and flows into Los Arribes del Duero over 284 km. It is a river with a snow-pluvial regime characterised by irregularity and floods in the snowy and rainy seasons. However, its course is regulated both by the dams of Santa Teresa, Villagonzalo, Almendra and San Fernando reservoirs, as well as by the irrigation channels (Deputation of Avila, 2017; Sánchez-Herández, 2011).

The blackfly species from the samples stored at the University of Salamanca have started to be processed, catalogued and identified. However, some samples of pre-imaginal specimens are still under review. Thus, this contribution presents preliminary data obtained from samples collected sequentially along the Tormes river basin (western Spain) during the years 1988, 1989 and 1996 (Figure 1), already presented in a congress oral communication (López-Peña *et al.*, 2021b), but in a greatly extended and detailed way.

The samples were collected by Manuel Portillo Rubio and María Esther Martín Chamoso, collectors from the University of Salamanca, trying to cover both the central area and the two banks of the river. After locating the specimens in their larval and/or pupal stages, adhered to substrates such as boulders and/or plant structures, a representative sample of them was taken and introduced into plastic containers in 70% alcohol; the same process was carried out with small-sized stones and, when this not possible, several larvae were separated employing

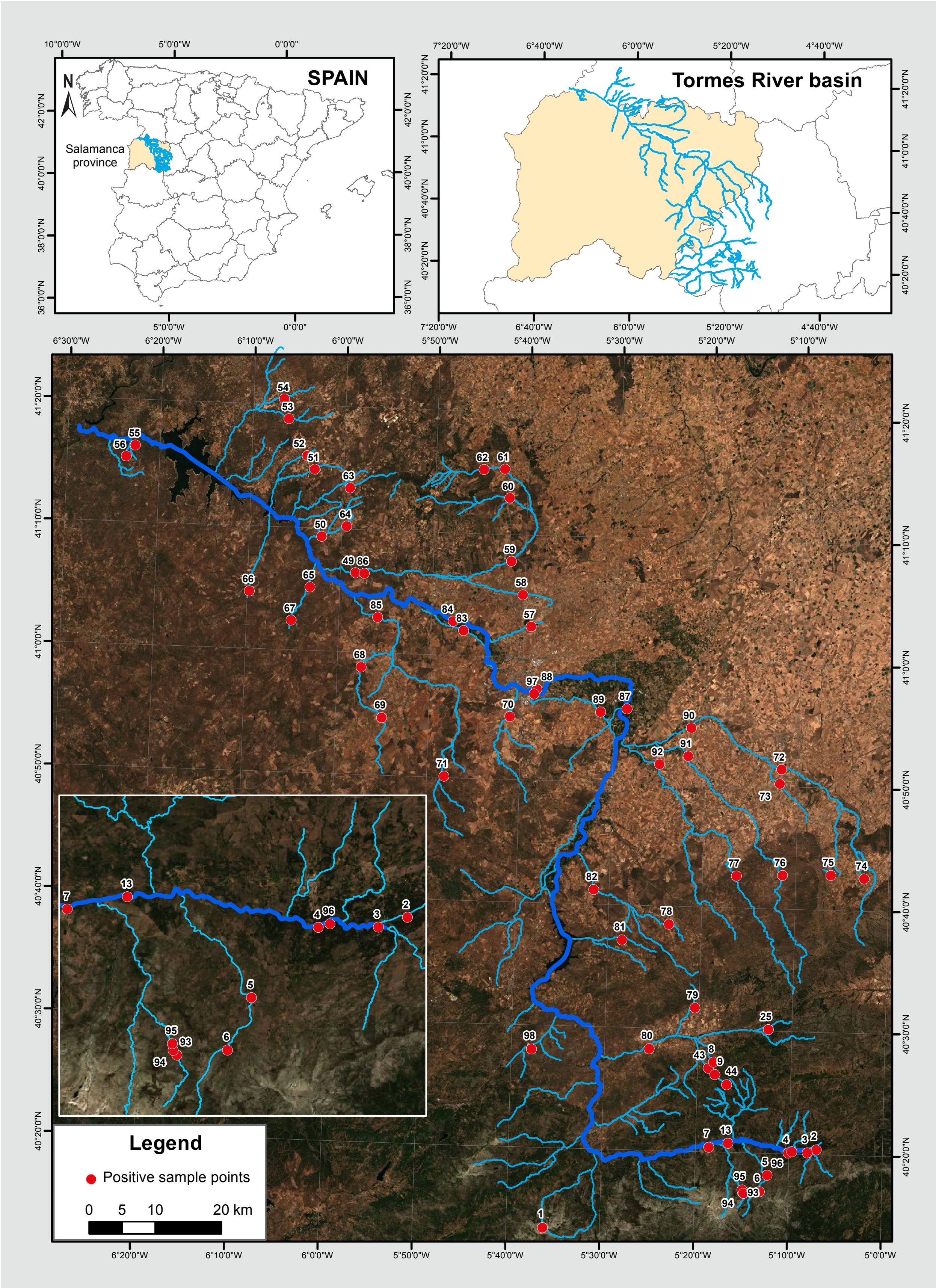


**Figure 1. Location of sampling stations in the hydrographic basin of the Tormes river. The numbers refer to collection sites listed in the tables.**

soft entomological forceps. The collectors, once in the laboratory, removed the bulk of the adhesion substrates and preserved the entomological material in tubes with 70% ethanol. It should be pointed out that the initial objective of the study was to carry out a faunistic survey of the blackfly species richness in the study area, the sampling effort not being standardised in terms of the duration of time collection. Nevertheless, the collectors always tried to dedicate a similar time to each sampling, although there is no exact record of time employed.

The data were recorded *in situ* in a field notebook whenever possible, these being the following: sample number, date, sampling location given in UTM coordinates (Universal Transverse Mercator), altitude (m), water temperature (°C), water speed (m/s) measuring the time taken by a cork structure to travel a distance of 5 metres, type of flow (very scarce, scarce, little, normal, abundant, very abundant), type of riverbed (stony, gravelly, sandy, silty, muddy), estimated water coloration (very clear, clear, darkened, yellowish, greenish) (Table S1). Once the samples were fixed in 70% alcohol, they were transported to the laboratory for further study.

In this study, 63 out of 98 samples were processed, removing the plant structures present in many of them after detaching the specimens adhered. Firstly, the associated macroinvertebrates were put to one side. Secondly, the blackfly larvae were separated from the pupae, placed in Petri dishes in 80% alcohol. Next, the immature larvae were separated from the mature ones; the identification of young larval instars is unreliable due to the fact that they do not yet possess the fully developed morphological structures used to carry out their taxonomic classification. Subsequently, the pupae and mature larvae were identified using taxonomic keys (Bass, 1998; González, 1997). Finally, both the immature larvae and the identified specimens in the mature larval and pupal stages were counted (Table S2). All of that was carried out using both Wild M8 Heerbrugg and Leica M80 10450630 stereomicroscopes.

The species distribution figures were created using the geographic information system (GIS) programme ArcMapTM 10.5, ESRI's ArcGIS® software (Redlands, CA, USA).

## 3. Results

In this study, 63 samples from a total of 98 have been reviewed, which means that 64% of the material has been examined so far and 36% will be in due course, and 19 species out of the 54 reported in Spain (Adler, 2021) have been identified, belonging to the genera *Prosimulium* Roubaud, 1906, *Metacnephia* Crosskey, 1969 and *Simulium* Latreille, 1802 and to 5 subgenera: *Boophthora* Enderlein, 1921; *Eusimulium* Roubaud, 1906; *Nevermannia* Enderlein, 1921; *Simulium* Latreille, 1802; *Wilhelmia* Enderlein, 1921. The species found are: *Metacnephia nuragica* Rivosecchi, Raastad & Contini, 1975; *Prosimulium latimucro* (Enderlein, 1925); *Prosimulium tomosvaryi* (Enderlein, 1921); *Simulium (Boophthora) erythrocephalum* (De Geer, 1776); *Simulium (Eusimulium) angustipes* Edwards, 1915; *Simulium (Eusimulium) aureum* Fries, 1824; *Simulium (Eusimulium) petricolum* (Rivosecchi, 1963); *Simulium (Eusimulium) velutinum* (Santos Abreu, 1922); *Simulium (Nevermania) carthusiense* Grenier & Dorier, 1959; *Simulium (Simulium) argyreatum* Meigen, 1838; *Simulium (Simulium) intermedium* Roubaud, 1906; *Simulium (Simulium) monticola* Friederichs, 1920; *Simulium (Simulium) ornatum* Meigen, 1818; *Simulium (Simulium) reptans* (Linnaeus, 1758) or *Simulium (Simulium) tuberosum* (Lundström, 1911); *Simulium (Simulium) trifasciatum* Curtis, 1839; *Simulium (Wilhelmia) equinum* (Linnaeus, 1758); *Simulium (Wilhelmia) lineatum* (Meigen, 1804); *Simulium (Wilhelmia) pseudequinum* Séguy, 1921; and *Simulium (Wilhelmia) sergenti* Edwards, 1923. Given the species *S. reptans* and *S. tuberosum*'s development stage of larva have strong morphological similarities such as branched anal gills, frontoclypeus spots pattern, shape and size of postgenal cleft as well as of the hypostomial teeth, all this, together with the fact that only one larvae specimen was collected during the samplings has made it difficult to identify this mature larva as one of these two species. In addition, the pupa stage of these two species is clearly differentiable, but not even one pupa was collected.

The species identified until now represent 35% of the simuliid species reported in Spain. Additionally, 14 species are recorded for the first time in several provinces in the study area: *M. nuragica*, *S. aureum*, *S. lineatum*, and *S. sergenti* in Salamanca, *P. latimucro*, *S. argyreatum*, *S. carthusiense*, *S. equinum*, *S. monticola*, *S. ornatum*, and *S. reptans*/*tuberosum* in Ávila, *S. intermedium* in Ávila and Salamanca, *S. petricolum* in Salamanca and Zamora, and finally *S. trifasciatum* in Ávila, Salamanca and Zamora, thus increasing their known distribution (López-Peña and Jiménez-Peydró, 2017a). Provincial distribution maps existing to date for those species have been updated (Figure 2. A-N).

Regarding the bioecological data compiled, *S. intermedium* and *S. ornatum* were the species present in the greatest number of sampling points, as well as the most abundant species in terms of number of larvae and pupae, followed by *S. velutinum*, *S. angustipes* and *S. petricolum*; concerning the number of mature larvae, those of *S. angustipes* and *S. velutinum* were more abundant than the mature ones of *S. intermedium*. On the other hand, the species with the lowest representation regarding presence at sampling points were *M. nuragica*, *P. latimucro*, *P. tomosvaryi*, *S. aureum*, *S. carthusiense*, *S. reptans*/*tuberosum* and *S. sergenti*; and in relation to the number of larval and/or pupal stages, these


**Figure 2. Provincial distribution maps of (A) *Metacnephia nuragica*, (B) *Prosimulium latimucro*, (C) *Simulium argyreatum*, (D) *S. aureum*, (E) *S. carthusiense*, (F) *S. equinum*, (G) *S. intermedium*, (H) *S. lineatum*, (I) *S. monticola*, (J) *S. ornatum*, (K) *S. petricolum*, (L) *S. reptans*, (M) *S. tuberosum*, (N) *S. sergenti*, and (O) *S. trifasciatum*. The cross sign indicates the Province names in which each species was found for the first time as a result of this research, and which are listed under each subfigure. The Provinces coloured in grey show where these species were reported prior to this study.**

were *M. nuragica*, *P. latimucro*, *S. aureum*, *S. carthusiense*, *S. pseudequinum*, *S. reptans*/*tuberosum* and *S. sergenti* with only one to three specimens in some samples (Table S3).

Likewise, as far the total number of pupae and mature larvae is concerned, *S. ornatum* and *S. intermedium* were the species that showed the greatest number for both categories, followed again by *S. angustipes*, *S. petricolum* and *S. velutinum*, while the lowest numbers of larvae and pupae were represented by *M. nuragica*, *P. latimucro*, *S. aureum*, *S. carthusiense*, *S. pseudequinum*, *S. reptans*/*tuberosum* and *S. sergenti* (Table S3).

It is a well-known fact that water temperature and altitude affect the distribution of blackfly species. In this regard, the data collected from the variables above show that some species tolerate very wide altitude ranges. For instance, *S. intermedium*, which was the species found in the widest altitude range, was collected in a range of 1,375 m. This species was followed by *S. ornatum* (range: 1,225 m), *S. trifasciatum* (1,200 m), *S. petricolum* (1,100 m), *S. angustipes* and *S. velutinum* (650 m), and *S. equinum* (600 m). Conversely, the species with the narrowest altitude range were *S. pseudequinum* (14 m), *S. erythrocephalum* (72 m), and *S. lineatum* (147 m) (Table S4).

The same occurred with temperature, concluding that most species tolerate fairly wide temperature ranges such as those of the species *S. velutinum*, *S. petricolum* and *S. angustipes* which were recorded in habitats with a temperature range width of 15.8, 15.7 and 15.2 °C respectively, and where the species with the widest temperature spectrum was *S. intermedium* with 16 °C. Likewise, other species showed a tolerance to slightly narrower ranges, such as the case of *S. ornatum* with an oscillation of 13.9 °C and *S. lineatum* and *S. trifasciatum* with 12.9 °C. Similarly, other species were found in habitats with a very low thermal oscillation, covering 5.8 °C in the case of *S. equinum*, 3.8 °C in that of *S. erythrocephalum* and 2 in that of *S. pseudequinum* (Table S4). In any case, the temperatures which are discussed correspond to the values recorded on the sampling dates, and so may be unrepresentative of the conditions experienced by the larvae over a season.

Regarding water flow speed, it has been observed that *S. petricolum* and *S. intermedium* were found in currents that oscillated between 1.56 and 1.15 m/s respectively, these species were the ones that tolerate higher oscillations width in the speed of the water. Next were the species *S. angustipes*, *S. ornatum* and *S. velutinum*, present in water flows with an oscillation of 0.85 m/s, *S. trifasciatum* at 0.78 m/s and *S. lineatum* at 0.73 m/s. Finally, the species found in water flows with the lowest oscillation were *S. equinum*, and *S. erythrocephalum* with 0.42 m/s and *S. pseudequinum* with 0.29 m/s, this last species being the one that tolerates less variation in water speed at its pre-imaginal habitats (Table S4). Therefore, the present study shows that most of the species are habitat generalists and occur along a wide range of ecological conditions.

Among the species identified in the study area, six of them (*S. equinum*, *S. erythrocephalum*, *S. lineatum*, *S. ornatum*, *S. pseudequinum*, and *S. reptans*) are considered of great importance for both human and animal health because of their haematophagic behaviour and potential as vectors of disease-causing pathogens. However, when the samples were collected, these species did not represent a great problem, due to their population sizes and distribution patterns. Although there are some doubts regarding the identification of specimens listed as *S. reptans* or *S. tuberosum*, an issue that will be solved in the near future, their abundances does not represent a risk, since only one specimen was collected at one sampling point (Table S3). Likewise, *S. erythrocephalum* and *S. pseudequinum* do not represent a considerable threat, this owing to their very low representation regardless the number of sample points where they were found, four and two respectively, and because of their low populations (Table S3). *Simulium equinum* showed low abundance populations, but with larger distribution if compared with the species mentioned above. *S. lineatum* was quite well distributed, being present in up to in 11 sampling points and presenting medium-sized populations in their pre-imaginal stages (Table S3). Finally, *S. ornatum*, was the blood sucking species with a fairly wide distribution, hardly ubiquitous at most of the sampling points, this species was recorded at 47 sampling stations. Apart from this, and based on the number of specimens present in the collection samples, it may be assumed that *S. ornatum* represented the dominant species, showing considerable population sizes (Table S3).

## 4. Discussion

Habitat characteristics as well as the ecological preferences of the blackfly species determine their distribution. Among these, the most relevant data regarding the ecology and distribution of the different species of simuliids are the altitude and the water temperature, since these affect the metabolism of the flies' immature stages and therefore regulate their life cycles. Furthermore, it is well known that the great diversity of identified species is due to different abiotic and biotic factors, among these being the wide range of altitude, in this study between 525 and 1,800 m a.s.l., which may explain this causal link a certain extent. Indeed, some authors have reported that elevation was one of the main factors regarding blackfly species distribution in their larvae and pupae stages of development (Ciadamidaro *et al.*, 2016; López-Peña *et al.*, 2020; Ya'cob *et al.*, 2016). Thus, the altitudinal distribution of the species is a very important factor to better understand the development of their life cycle. In this regard, the data provided by this study from the western area of Spain as well as those existing studies from the eastern part of the country contribute to obtaining a deeper understanding of the species listed below. *Simulium angustipes* had been found in sampling stations located between 300 and 400 m altitude (López-Peña and Jiménez-Peydró, 2018), while in the present study they were found between 700 and 1,350 m, enlarging its elevation range. *Simulium equinum* was reported from 200-700 m (López-Peña and Jiménez-Peydró, 2018), but in this study it appeared between 750 and 1,350 m, increasing in this way its known altitude range. *Simulium intermedium* had been found between 300 and 700 m (López-Peña and Jiménez-Peydró, 2018), and in this study between 525 and 1,900 m. *Simulium lineatum* had been found between 500 and 600 m (López-Peña and Jiménez-Peydró, 2018), and in this study between 675 and 822 m. *Simulium ornatum* had been found from 200 and 700 m (López-Peña and Jiménez-Peydró, 2018), while in this study it was sampled between 675 and 1,900 m. *Simulium petricolum* had been found between 400 and 600 m (López-Peña and Jiménez-Peydró, 2018), and in this study was found between 700 and 1,800 m. *Simulium pseudequinum* had been found between 200 and 700 m (López-Peña and Jiménez-Peydró, 2018), and in this study between 800-814 m. *Simulium trifasciatum* had been collected between 200 and 700 m (López-Peña and Jiménez-Peydró, 2018), and in this study between 700 and 1,900 m. *Simulium velutinum* had been found between 600 and 700 m (López-Peña and Jiménez-Peydró, 2018) and in this study between 700 and 1,350 m.

Considering species richness, 16 species had been identified in the Júcar Basin river (López-Peña and Jiménez-Peydró, 2017b), and 19 have been found in the present study in the Tormes Basin. However, if only the species present in the main rivers are considered, and not those from their tributaries, some differences and similarities appear. Comparing to the Segura river (Sánchez *et al.*, 2018) with just 1 species found or the Amadorio river (López-Peña and Jiménez-Peydró, 2020) with 2 species, 3 species have been found in the Jarama and Tajo rivers (Soriano *et al.*, 2019), 4 in the Ebro river (Ruiz-Arrondo *et al.*, 2017), 5 in the Guadalete (Gallardo-Mayenco and Toja, 2002), Júcar (López-Peña and Jiménez-Peydró, 2017b) and Perales (Soriano *et al.*, 2019) rivers, 6 in the Flumen (Villanúa-Inglada *et al.*, 2013) and Henares rivers (Soriano *et al.*, 2019), 7 in the Aller, Arganza, de la Pola, Esva, Jomezana, Nalón, Narcea, Naviego, Orle, Pigüeña (Puig *et al.*, 1984), Monegre (López-Peña and Jiménez-Peydró, 2020) rivers and Molinos stream (Ricoy-Llavero, 2015), 8 species in the Guadalhorce, Guadiaro (González *et al.*, 1987), Lozoya (Casado *et al.*, 1990), Anllóns, Eo, Lérez, Mandeo, Masma, Mera, Ouro, Pereira, Sar, Ulla and Umia (Lestón *et al.*, 2014), Manzanares (Soriano *et al.*, 2019) and Algar (López-Peña and Jiménez-Peydró, 2020) rivers and Orcera stream (Ricoy-Llavero, 2015), 9 in the Besós, Foix (González, 1985) and Cidacos (Ruiz and Rubio, 1999) rivers, while 10 species (*S. angustipes*, *S. argyreatum*, *S. equinum*, *S. erythrocephalum*, *S. intermedium*, *S. lineatum*, *S. ornatum*, *S. petricolum*, *S. trifasciatum* and *S. velutinum*) were identified in the present study in the Tormes river. This shows this is one of the most diverse rivers, after the Serpis river with 14 species (López-Peña and Jiménez-Peydró, 2020), Mijares (López-Peña and Jiménez-Peydró, 2018), Yeguas (González *et al.*, 1986) and Ter (Puig *et al.*, 1987) rivers with 13 species in each.

In our study, many generalist species able to tolerate wide ranges of abiotic and biotic variables have been identified, which can be used as indicator species of the water quality in their aquatic habitats. For instance, *S. angustipes* has been recorded from slightly polluted waters, although this species appears in waters with no pollution, as well as slightly, moderately and severely polluted rivers (Car *et al.*, 1995; Kazanci and Ertunç, 2010). *Simulium argyreatum* has been identified in non-polluted, very slight polluted and moderately polluted waters (Ertunç *et al.*, 2008). *Simulium lineatum* has been reported from slightly polluted waters (Kazanci and Ertunç, 2010; Šporka, 2003) but can be found also in moderately (Ertunç *et al.*, 2008) and severely polluted waters (Šporka, 2003). *Simulium ornatum* has been recorded in non-polluted or very slightly polluted waters (Car *et al.*, 1995) to moderately and severely polluted waters (Kazanci and Ertunç, 2010). *Simulium pseudequinum* can be found in slightly (Kazanci and Ertunç, 2010), moderately (Šporka, 2003; Ertunç *et al.*, 2008; Kazanci and Ertunç, 2010) and severely (Šporka, 2003) polluted waters. *Simulium trifasciatum* has been reported from non-polluted, very slight polluted (Car *et al.*, 1995) and moderately polluted waters (Ertunç *et al.*, 2008), but also in slightly polluted waters (Kazanci and Ertunç, 2010). However, further studies in the research area are needed to disentangle species bioecology.

The haematophagous species of medical and veterinary concern identified in the present study should be taken in great consideration due to the fact that they have been reported as the cause of bites and painful derived pathologies that endanger the health of both humans and animals. Concerning *S. erythrocephalum*, an anthropophilic species, cases of outbreaks and massive attacks on humans have been reported (Ignjatović-Ćupina *et al.*, 2006), with a negative impact on the public health services when they occur (López-Peña *et al.*, 2021a; Ruiz-Arrondo *et al.*, 2017; Soriano *et al.*, 2019), since the primary care department of health centres and hospitals can be overwhelmed, providing health care as a result of the bites received and the concomitant allergic reactions (Anonymous, 2013). What is more, the inconvenience caused by these insects to humans and animals can lead to economic depreciation in the tourist and livestock sectors. Moreover, S. *erythrocephalum* has been described in Europe as a vector species of the parasites *Onchocerca gutturosa* (Neumann, 1910) and *Onchocerca lienalis* (Stiles, 1892), which cause bovine onchocerciasis (Crosskey, 1990; Cupp, 1996). *Simulium ornatum*, which shows a preference for cattle as a host and which can also act as a vector of the same filariae as *S. erythrocephalum* (Crosskey, 1993), can also resort to humans. *Simulium equinum* shows preference for equine livestock to which it can transmit the filaria *O. lienalis* (Ham and Bianco, 1983b); *S. reptans* and *S. lineatum* can also transmit *O. lienalis* to cattle (Baužienė *et al.*, 2004; Cupp, 1996; Ham and Bianco, 1983a,b). Thus, it is important not to forget that some provinces of the study area, such as Salamanca, are regions of extensive cattle production that could be affected both by the bites of the haematophagous species of simuliids and by the pathogens transmitted by these. In addition, there is a theoretical possibility that *S. erythrocephalum* and *S. ornatum* could act as vectors of the *O. volvulus* to humans, since the development of microfilariae to the infective stage in these two blackfly species has previously been confirmed by experimental infection (Ham and Bianco, 1983a). Unfortunately, these species are gaining in importance and social repercussions in some areas of the country, among which stand Madrid (Soriano *et al.*, 2019), the Valencian Autonomous Region (Barberá *et al.*, 2018; López-Peña, 2018; López-Peña and Jiménez-Peydró, 2015) and Zaragoza (Ruiz-Arrondo, 2018; Ruiz-Arrondo *et al.*, 2012, 2014), where their distribution patterns are expanding together with their populations sizes. Hence the relevance of this study, and the foreseen possibility to check the current state of their populations as well as of their distribution in this region of the country.

## 5. Conclusions

Up to now, the study has reported 19 simuliid species in the area studied, although the final number might increase when all of the samples have been identified. Fourteen species have been first recorded in 3 provinces (Ávila, Salamanca and Zamora). Some haematophagous species have been detected (*S. erythrocephalum*, *S. ornatum*, *S. reptans*, *S. equinum*, *S. lineatum* and *S. pseudequinum*) of relevance for both public health and animal welfare. Likewise, valuable ecological information about the species and their distribution has been provided. The review has contributed to the improvement of bioecological knowledge of several species. New altitudinal ranges have been reported for the species *S. angustipes* (300-1,350 m), *S. equinum* (200-1,350 m), *S. intermedium* (300-1,900 m), *S. lineatum* (500-822 m), *S. ornatum* (200-1,900 m), *S. petricolum* (400-1,800 m), *S.pseudequinum* (200-814 m), *S. trifasciatum* (200-1,900 m), and *S. velutinum* (600-1,350 m).

Detailed bioecology knowledge of the different blackly species is *per se* of great importance, and even more so if one takes into account the potential that some of them have, such as causing damage and inconvenience to the health and well-being of people and animals. Therefore, to continue studying the abiotic and biotic variables that characterise their habitats is one of the future aims of the authors, since it is crucial to be able to establish the tolerance ranges of each species as well as optimal values for the correct development of their life cycle. In this way, it will be possible to make timely decisions to avoid situations that harm the health of human and animal populations.

Health problems at the human-animal-environment interface caused by zoonotic diseases still have significant repercussions on health and the economy (FAO, OIE, OMS, 2019). For some years now, a multidisciplinary approach has been adopted, championed by 'One Health', with the target of dealing with problems of public health, animal health (both domestic and wildlife) and the environment by adopting transnational, multisectoral and multidisciplinary approaches to achieve effectiveness not only at the local level, but also regionally and nationally, and even globally (FAO, OIE, OMS, 2019). Given that blackflies are responsible for endemic zoonotic diseases, and that as a consequence of the effects of globalisation and climate change they could also act as vectors of emerging zoonotic diseases, the study of their bioecology is absolutely essential to be able to anticipate their arrival, detect them early and provide an effective response.

## Supplementary material

Supplementary material can be found online at https://doi.org/10.52004/JEMCA2022.0002.

**Table S1.** Geographical data and hydrological parameters recorded at the blackfly breeding sites of the Tormes river basin during the sampling.

**Table S2.** Abundance of pre-imaginal stages of the species identified.

**Table S3.** Number of sampling points positive to simuliid species presence, maximum number of determined specimens (pupae and larvae) in a sampling point and total number of determined specimens (pupae and larvae).

**Table S4.** Altitude, water temperature and water velocity values and ranges recorded at the blackfly breeding sites of Tormes river basin.

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## Conflict of interest

All authors disclose that there is not any potential sources of conflict of interest among them.

## Data sharing

Authors agree to make the data available upon reasonable request. Considering that it is up to the authors to determine whether a request is reasonable.

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