



Simuliids (Diptera: Simuliidae) from the Madrid Region (Spain): update and new contributions

Simúlidos (Diptera: Simuliidae) de la Comunidad de Madrid (España): actualización y nuevas aportaciones

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ABSTRACT

The Simuliidae family in Spain has been studied irregularly: some enclaves have been widely investigated by both national and foreign experts, while others have been merely mentioned or addressed indirectly for various reasons. The Madrid Region in Spain has been recurrently investigated in this respect, yet many geographical areas remain to be studied regarding Simuliidae taxonomy, geographical distribution, and ecology. In this study, a thorough sampling was conducted based on searching for preimaginal stages of larva and pupa attached or fixed to any suitable support in different lotic water systems. The latter were chosen for their favourable conditions for hosting new and valuable information. The collected material was identified based on morphological characteristics. A total of 16 species were identified. The following is an update of the simuliid fauna and its altitudinal ranges from Spain's central geographical area, including seven new records for the Madrid Region: *Prosimulium rufipes* (Meigen, 1830); *Simulium (Nevermannia) naturale* Davies, 1966; *Simulium (Nevermannia) quasidocolletum* Crosskey, 1988; *Simulium (Nevermannia) urbanum* Davies, 1966; *Simulium (Simulium) trifasciatum* Curtis, 1839; *Simulium (Simulium) maximum* (Knoz, 1961); *Simulium (Simulium) variegatum* Meigen, 1818. Following the update, the species with the narrowest altitudinal distribution were: *S. (N.) naturale* and *S. (N.) urbanum*: 1843-1844 meters (m), while *Simulium (Nevermannia) cryophilum* (Rubtsov, 1959) and *Simulium (Simulium) intermedium* Roubaud, 1906 presented the widest distribution: 870-2000 m. In this study, *S. (N.) naturale* and *S. (N.) urbanum* also displayed the narrowest altitudinal range, with 1 m, whereas *S. (N.) cryophilum* presented the broadest, with 603 m. The present study contributes to the knowledge of Spanish blackflies generally and from the Madrid Region in particular, providing new insights into the species composition and their altitudinal distribution. It thus lays the foundations for future works.

Keywords: Simuliidae; Blackflies; Updated checklist; Public Health; Veterinary importance; Central Spain

RESUMEN

La familia Simuliidae ha sido estudiada de manera irregular en España, habiendo sido algunos enclaves ampliamente investigados por expertos tanto nacionales como extranjeros, y otros meramente abordados o no considerados directamente hasta el momento por diversas causas. La Comunidad de Madrid ha sido una de estas zonas recurrentemente investigadas, pero todavía hoy hay muchas partes cuya taxonomía, distribución geográfica, y ecología necesitan ser estudiadas. En este estudio, se ha realizado un muestreo basado en la búsqueda minuciosa de ejemplares preimaginales de larva y pupa adheridos o fijados en cualquier soporte en diferentes cuerpos de agua lóticos elegidos por sus condiciones favorables para albergar nueva y valiosa información. El material recolectado ha sido identificado atendiendo a caracteres morfológicos. Se ha obtenido un total de 16 especies. Esto ha supuesto una actualización de la simulidofauna y de sus rangos altitudinales del área central de España, con siete citas nuevas para la Comunidad de Madrid: *Prosimulium rufipes* (Meigen, 1830); *Simulium (Nevermannia) naturale* Davies, 1966; *Simulium (Nevermannia) quasidocolletum* Crosskey, 1988; *Simulium (Nevermannia) urbanum* Davies, 1966; *Simulium (Simulium) trifasciatum* Curtis, 1839; *Simulium (Simulium) maximum* (Knoz, 1961); *Simulium (Simulium) variegatum* Meigen, 1818. Las especies con distribución altitudinal más restringida, tras la actualización, han sido *S. (N.) naturale* y *S. (N.) urbanum*: 1843-1844 metros (m), mientras que *Simulium (Nevermannia) cryophilum* (Rubtsov, 1959) y *Simulium (Simulium) intermedium* Roubaud, 1906 han revelado la más extensa: 870-2000 m. En este estudio, *S. (N.) naturale* y *S. (N.) urbanum* también mostraron el rango altitudinal más reducido con 1 m, mientras que *S. (N.) cryophilum* mostró el más amplio con 603 m. Esta investigación arroja luz sobre el conocimiento de los simúlidos de España en general y de la Comunidad de Madrid en particular, aportando conocimientos nuevos sobre la composición de las especies y su distribución altitudinal, y sentando las bases para futuros trabajos.

Palabras clave: Simuliidae; Moscas negras; Catálogo actualizado; Salud Pública; Importancia veterinaria; España central

INTRODUCTION

According to Natura 2000 Network (Miteco, 2011), the geographical position, presence of orographic barriers (mountainous and marine), geological and climatic variety of the Iberian Peninsula, in conjunction with its insular territories, confer Spain as the country of the European Union with the greatest biological diversity. In fact, the biological richness of Spain with 85,000 species represents 54% of all species of flora and fauna of Europe. Moreover, this great diversity can be increased given that several families, as is the case of blackflies (Diptera: Simuliidae), have been succinctly studied in certain regions of the country, and even understudied in others.

This study is part of the taxonomic and phylogeographic research of the blackflies in the Madrid Region. From this perspective, the Madrid Region, due to its ecological characteristics and the rigorous review of all the previous studies carried out by the authors of the present paper in this part of Spain (Strobl, 1900, 1905; Czerny & Strobl, 1909; Arias Encobet, 1912; Grenier & Bertrand, 1954; Puig *et al.*, 1984; Casado, 1986; Casado *et al.*, 1990; González Peña, 1990; Vinçon & Clergue-Gazeau, 1993; González Peña, 1997; Crosskey & Crosskey, 2000; González Peña *et al.*, 2002; Belqat, & Garrido, 2008; Adler *et al.*, 2015; López-Peña, & Jiménez-Peydró, 2017; Ruiz-Arrondo *et al.*, 2018; Soriano *et al.*, 2019, 2020), was selected as the area to conduct this study.

The main objective of this study is to contribute to increasing the blackfly diversity information from the study area and to convey the remarkable results obtained as fruit of both, a fieldwork and a funded research stay to improve the taxonomic and distribution knowledge of the Spanish Simuliidae fauna which is still not sufficiently investigated in several regions of the country.

METHODOLOGY

Inspired by the works of Strobl (1900, 1905), Czerny & Strobl (1909), Grenier & Bertrand (1954), Puig *et al.* (1984), Casado *et al.* (1990), González Peña (1990), and Vinçon & Clergue-Gazeau, (1993), this study was designed following the same methodology of sampling collection employed by them. In that way, the 10 samples collected were conducted as explained in the following paragraphs.

The chosen breeding sites were explored by means of an active search for larvae and pupae of blackflies. Once they were detected, the way of collecting the specimens consisted of detaching them from plants growing near the riverbanks, the pebbles, stones, tree branches, and leaves in contact with the water with the help of entomological tweezers. Immediately, a portion of the mature larvae were put into tubes with modified Carnoy's solution for future chromosomal analyses, and other larvae and pupae were stored in plastic tubes with ethanol at 96.3% concentration.

The sampling took place in lotic environments of mountainous areas from the Region of Madrid (Spain) during May 2015 and May 2018, approximately on the same days (Table 1 and Figure 1).

The modified Carnoy's solution, a fixative agent for polytene chromosomes composed of 96.3% ethanol (C₂H₆O) and acetic acid (CH₃CO₂H) in proportions (3:1) was used in the field to fix the collected specimens, this liquid was changed *in situ* at least three times for a better DNA specimen conservation. Once in the laboratory, the ethanol and Carnoy's samples were stored in a -22 °C refrigerator of Department of Zoology located at the Faculty of Natural Sciences of Comenius University to preserve the DNA and the polytene chromosomes in the best possible conditions until the commence of the awarded scholarship.

The ethanol and Carnoy's solution were also used in the laboratory while and after processing the collected samples and identifying the specimens in order to keep the DNA in suitable conditions for future purposes.

Date	Locality	River	Longitude (W-E)	Latitude (N-S)	Altitude (m)
26/05/2015	Cercedilla	River de la Venta	-4.0683	40.7485	1,242
26/05/2015	Rascafría	Stream del Toril	-3.9459	40.8279	1,731
26/05/2015	Navacerrada	Stream de las Guarramillas	-3.9641	40.8090	1,843
26/05/2015	Navacerrada	Stream de las Guarramillas	-3.9645	40.8094	1,845
22/05/2018	Cercedilla	River de la Venta	-4.0683	40.7485	1,242
23/05/2018	Rascafría	Stream del Toril	-3.9459	40.8279	1,731
23/05/2018	Rascafría	Stream de las Guarramillas	-3.9648	40.8098	1,843
23/05/2018	Rascafría	Stream de las Guarramillas	-3.9648	40.8098	1,844
23/05/2018	Rascafría	Stream de la Laguna Grande	-3.9355	40.8324	1,606

Table 1. Data related to the study sampling points of simuliids in the Madrid Region, Spain.



Figure 1. Photographic representation of several of the blackfly breeding sites and sampling stations located in the municipality of Rascafría, Madrid, Spain. (Photographs: Matúš Kúdela, 23/5/2018).

The identification of the specimens was carried out using three stereomicroscopes (Leica Wild M8 445302 Plan 1.0x assisted with a cold light source Intralux[®] 4000-1 Volpi AG CH-8952 Schlieren 230V, P/N: 10250, S/N: 001072; Carl Zeiss SteREO Discovery.V12 GmbH 24V with PI 16X 444054-9000 and objective PlanApo S 1.0x FWD 60 mm; and Carl Zeiss AXIO Zoom.V16 GmbH 24V with PI 10x/23 444036-9000 and objective Apo Z 1.5x/0.37 FWD 30mm). Identification was based on the use of morpho-anatomic identification keys (Knoz, 1965; González Peña, 1997; Bass, 1998; Jedlička *et al.*, 2004; Rivosecchi *et al.*, 2007).

RESULTS

Recorded species

As a result, the daily work carried out during the stay enabled to report 16 species from the area of study pertaining to two genera (*Prosimulium* Roubaud, 1906 and *Simulium* Latreille, 1802), two subgenera (*Nevermannia* Enderlein, 1921 and *Simulium* Latreille, 1802), and three species-groups (*S. ornatum* species-group, *S. variegatum* species-group and *S. vernum* species-group) (Table 2).

Genus	Subgenus	Species-group	Species
<i>Prosimulium</i> Roubaud, 1906		<i>hirtipes</i> species-group	<i>P. hirtipes</i> (Fries, 1824)
			<i>P. latimucro</i> (Enderlein, 1925)
			<i>P. rufipes</i> (Meigen, 1830)
			<i>P. tomosvaryi</i> (Enderlein, 1921)
<i>Simulium</i> Latreille, 1802	<i>Nevermannia</i> Enderlein, 1921	<i>vernum</i> species-group	<i>S. (N.) carthusiense</i> Grenier & Dorier, 1959
			<i>S. (N.) cryophilum</i> (Rubtsov, 1959)
			<i>S. (N.) naturale</i> Davies, 1966
			<i>S. (N.) quasidocolletum</i> Crosskey, 1988
			<i>S. (N.) urbanum</i> Davies, 1966
			<i>S. (N.) vernum</i> Macquart, 1826
	<i>Simulium</i> Latreille, 1802	<i>ornatum</i> species-group	<i>S. (S.) intermedium</i> Roubaud, 1906
			<i>S. (S.) ornatum</i> Meigen, 1818
			<i>S. (S.) trifasciatum</i> Curtis, 1839
		<i>variegatum</i> species-group	<i>S. (S.) maximum</i> (Knoz, 1961)
<i>S. (S.) monticola</i> Friederichs, 1920			
<i>S. (S.) variegatum</i> Meigen, 1818			

Table 2. Taxonomic classification of the collected and identified simuliid species in the Madrid Region, Spain.

A total number of 1,381 individuals were processed, of which 358 were immature larvae (most larvae classified as *Simulium* sp. due to the fact that their incomplete development does not allow to

identify the specimens at the species level with the use of identification keys, based on morphological characters), 315 mature larvae belonging to 16 species, and 708 pupae of 11 species (Table 3).

Species	Nº of immature larvae	Nº of mature larvae	Nº of pupae
<i>P. hirtipes</i>	0	47	1
<i>P. latimucro</i>	0	7	45
<i>P. rufipes</i>	0	1	16
<i>P. tomosvaryi</i>	3	62	510
<i>S. carthusiense</i>	0	6	0
<i>S. cryophilum</i>	5	36	3
<i>S. intermedium</i>	0	30	1
<i>S. maximum</i>	0	4	0
<i>S. monticola</i>	4	62	79
<i>S. naturale</i>	0	5	3
<i>S. ornatum</i>	0	14	3
<i>S. quasidocolletum</i>	0	3	0
<i>S. trifasciatum</i>	0	1	0
<i>S. urbanum</i>	0	2	16
<i>S. variegatum</i>	0	4	0
<i>S. vernum</i>	0	31	31
<i>Simulium</i> sp.	346	0	0
Totals	358	315	708

Table 3. Specimens of each simuliid species recorded in the Madrid Region, Spain.

Taxonomic classification

The contribution of this study is seven first records for the Madrid Region of Spain: *P. rufipes*, *S. maximum*, *S. naturale*, *S. quasidocolletum*, *S. trifasciatum*, *S. urbanum*, and *S. variegatum*. Therefore, the knowledge of the Simuliidae of this Spanish Region, regarding its taxonomic classification and composition of 29 species has been updated as follows:

Order Diptera Linnaeus, 1758

 Infraorder Culicomorpha Hennig, 1948

 Superfamily Simuliodea Newman, 1834

 Family Simuliidae Newman, 1834

 Subfamily Parasimuliinae Smart, 1945

 Genus *Prosimulium* Roubaud, 1906

hirtipes species-group

Prosimulium hirtipes (Fries, 1824)

Prosimulium latimucro (Enderlein, 1925)

Prosimulium rufipes (Meigen, 1830)

Prosimulium tomosvaryi (Enderlein, 1921)

 Genus *Simulium* Latreille, 1802

 Subgenus *Boophthora* Enderlein, 1921

Simulium (Boophthora) erythrocephalum
(De Geer, 1776)

 Subgenus *Eusimulium* Roubaud, 1906

Simulium (Eusimulium) angustipes

 Edwards, 1915

Simulium (Eusimulium) petricolum

 (Rivosecchi, 1963)

Simulium (Eusimulium) rubzovianum

 (Sherban, 1961)

 Subgenus *Nevermannia* Enderlein, 1921

ruficornis species-group

Simulium (Nevermannia) angustitarse

 (Lundström, 1911)

Simulium (Nevermannia) ruficornis

 Macquart, 1838

vernum species-group

Simulium (Nevermannia) carthusiense

 Grenier & Dorier, 1959

Simulium (Nevermannia) costatum
Friederichs, 1920

Simulium (Nevermannia) cryophilum
(Rubtsov, 1959)

Simulium (Nevermannia) naturale Davies,
1966

Simulium (Nevermannia) quasidocolletum
Crosskey, 1988

Simulium (Nevermannia) urbanum
Davies, 1966

Simulium (Nevermannia) vernum
Macquart, 1826

Subgenus *Simulium* Latreille, 1802

ornatum species-group

Simulium (Simulium) intermedium

 Roubaud, 1906

Simulium (Simulium) ornatum Meigen,
1818

Simulium (Simulium) trifasciatum Curtis,
1839

tuberosum species-group

Simulium (Simulium) tuberosum
(Lundström, 1911)

variegatum species-group

Simulium (Simulium) argyreatum Meigen,
1838

Simulium (Simulium) maximum (Knoz,
1961)

Simulium (Simulium) monticola
Friederichs, 1920

Simulium (Simulium) variegatum Meigen,
1818

Subgenus *Wilhelmia* Enderlein, 1921

equinum species-group

Simulium (Wilhelmia) equinum
(Linnaeus, 1758)

Simulium (Wilhelmia) lineatum (Meigen,
1804)

Simulium (Wilhelmia) pseudequinum
Séguy, 1921

Simulium (Wilhelmia) sergenti Edwards,
1923

Species reports from the Madrid Region

Each of the species present in Madrid and the authors who reported their presence is presented in rigorous taxonomic order, based on the world blackflies taxonomic and geographical inventory of Adler (2022):

P. hirtipes: González Peña (1990), Soriano *et al.* (2020), and reported by the authors of this study.

P. latimucro: Grenier & Bertrand (1954), González Peña (1990), Soriano *et al.* (2020), and reported by the authors of this study.

P. rufipes: Reported by the authors of this study.

P. tomosvaryi: Strobl (1900, 1905), Arias Encobet (1912), and reported by the authors of this study. Strobl and Arias Encobet mentioned the presence of *S. fuscipes* (von Roser, 1840) in Madrid, which is a synonym of *P. tomosvaryi*.

S. erythrocephalum: Soriano *et al.* (2019, 2020).

S. angustipes: González Peña (1990).

S. petricolum: Grenier & Bertrand (1954). According to Crosskey and Crosskey (2000) and González Peña *et al.* (2002) the species *S. aureum* recorded in Madrid by Grenier and Bertrand (1954) must be considered as *S. petricolum*.

S. rubzovianum: Soriano *et al.* (2019, 2020). Soriano *et al.* (2019) cited in Madrid the presence of the species *Simulium (Eusimulium) velutinum* (Santos Abréu, 1922), which must be considered a synonym of the species *S. rubzovianum* since the publication of Adler *et al.* (2015), who agreed that the new concept of *S. velutinum* sensu stricto restricts the use of the name *velutinum* only to North Africa and the Canary Islands. Therefore, all specimens identified as *S. velutinum* prior to this publication in North Africa, the Mediterranean islands, Italy, Spain, the British Isles, Fennoscandia and Turkey, should be considered as *S. rubzovianum*.

S. angustitarse: Soriano *et al.* (2020).

S. ruficorne: Soriano *et al.* (2019, 2020).

S. carthusiense: Vinçon & Clergue-Gazeau (1993), Soriano *et al.* (2020), and reported by the authors of this study.

S. costatum: Vinçon & Clergue-Gazeau (1993) and Soriano *et al.* (2020). González Peña (1997) and González Peña *et al.* (2002) mentioned the contribution of Vinçon & Clergue-Gazeau (1993) in their works.

S. cryophilum: Grenier & Bertrand (1954), Vinçon & Clergue-Gazeau (1993), Soriano *et al.* (2020), and reported by the authors of this study. González Peña *et al.* (2002) agreed that *S. latipes* recorded in Madrid by Grenier and Bertrand (1954) must be considered as *S. cryophilum* due to a misidentification.

S. naturale: Reported by the authors of this study.

S. quasidecolletum: Reported by the authors of this study.

S. urbanum: Reported by the authors of this study.

S. vernum: Grenier & Bertrand (1954), Casado (1986), Casado *et al.* (1990), Soriano *et al.* (2020), and reported by the authors of this study.

S. intermedium: Grenier & Bertrand (1954), Vinçon & Clergue-Gazeau (1993), Soriano *et al.* (2020), and reported by the authors of this study. Grenier & Bertrand (1954) mentioned the presence of *S. ornatum* var. *nitidifrons* Edwards, 1920 in Madrid, which is a synonym of *S. intermedium*.

S. ornatum: Strobl (1900, 1905), Czerny & Strobl (1909), Arias Encobet (1912), Casado (1986), Casado *et al.* (1990), González Peña (1990), Soriano *et al.* (2020), and reported by the authors of this study.

S. trifasciatum: Reported by the authors of this study.

S. tuberosum: Grenier & Bertrand (1954), González Peña (1990), Soriano *et al.* (2020).

S. argyreatum: Grenier & Bertrand (1954), Casado (1986), Casado *et al.* (1990), Vinçon & Clergue-Gazeau (1993), Soriano *et al.* (2020).

S. maximum: Reported by the authors of this study.

S. monticola: Grenier & Bertrand (1954), González Peña (1990), Soriano *et al.* (2020), and reported by the authors of this study.

S. variegatum: Reported by the authors of this study.

S. equinum: Strobl (1900), Arias Encobet (1912), Grenier & Bertrand (1954), Puig *et al.* (1984), Casado (1986), Casado *et al.* (1990), González Peña (1990), Soriano *et al.* (2019, 2020).

S. lineatum: Grenier & Bertrand (1954), Casado (1986), Casado *et al.* (1990), González Peña (1990), Soriano *et al.* (2019, 2020). Grenier & Bertrand (1954) mentioned the presence of *S. salopiense* Edwards, 1927 in Madrid, which is a synonym of *S. lineatum*.

S. pseudequinum: Grenier & Bertrand (1954), Casado (1986), Casado *et al.* (1990), González Peña (1990), Soriano *et al.* (2019, 2020). Grenier & Bertrand (1954) mentioned the presence of *S. equinum* var. *mediterraneum* Puri, 1925 in Madrid, which is a synonym of *S. pseudequinum*.

S. sergenti: Ruiz-Arrondo *et al.* (2018), Soriano *et al.* (2019, 2020).

Altitude ranges of the species first recorded from Madrid

The breeding sites chosen by these species show similar altitude ranges, however, some nuances and slight differences are displayed. In the case of *S. trifasciatum* that only was collected at 1,606 metres above sea level (m a.s.l.) in the stream 'de la Laguna Grande' and in the case of *S. quasidocolletum* that was found at 1731 m in the stream 'del Toril'. Another relevant example is the narrow altitudinal range of *S. naturale* and *S. urbanum* which goes from 1843 to 1844 m a.s.l., both in the stream 'de las Guarramillas', followed by *S. maximum* that was recorded between 1731 and 1843 m in the stream 'de las Guarramillas' and another nameless stream. Finally, *S. variegatum* was the species with the widest altitudinal range, which varies from 1606 to 1844 m in the stream 'de las Guarramillas', stream 'de la Laguna Grande', and stream 'del Toril'.

Apart from this, and according to the literature, the species that was reported at the lowest altitude in Madrid Region was *S. sergenti*, which was collected at 571 m a.s.l. in Torrejón de Ardoz (Ruiz-Arrondo *et al.*, 2018), followed by *S. angustipes* at 1,075 m in Lozoya river (González Peña, 1990), *P. hirtipes* at 1235 m in Angostura stream, a tributary of Lozoya river (González Peña, 1990) and *P. latimucro*,

S. tuberosum, and *S. monticola* in Jarama river at 1300 m (González Peña, 1990), and *S. monticola* close to 'El Ventorrillo' Biological Station in 'Sierra de Guadarrama' at the same altitude (Grenier & Bertrand, 1954). In contrast, the species found at the highest altitude were *S. carthusiense* and *S. costatum*, both recorded at 2000 m in the vicinity of the Valdesquí Station located in the 'Sierra de Guadarrama' (Vinçon & Clergue-Gazeau, 1993). As a result of the present study, the altitudinal range of *P. hirtipes*, which was collected from 1242 to 1844 m in the river 'de la Venta', stream 'del Toril', stream 'de las Guarramillas' and stream 'de la Laguna Grande', has been increased, going from 1235 to 1844 m. The same situation has occurred with four more species: *P. latimucro*, which was sampled between 1731 and 1845 m in the stream 'de las Guarramillas', and its current altitudinal range goes from 1300 to 1845 m; *S. carthusiense* reported from 1606 to 1731 m in the stream 'del Toril' and the stream 'de la Laguna Grande', which means that its updated altitudinal range goes from 1606 to 2000 m; and *S. monticola* recorded between 1242 and 1843 m in the stream 'del Toril', stream 'de las Guarramillas', stream 'de la Laguna Grande' and river 'de la Venta', which represents its more updated altitudinal range.

DISCUSSION

Historic bibliographic species reports

Since the first report of blackflies in Spain by Antiga (1888), numberless studies have been published, standing out among them, from the taxonomic point of view, three species checklists. The first one was carried out by Dr. Gloria González Peña, a renowned Spanish expert on the study of blackflies in this country in collaboration with one of the leading experts in the study of this family of Diptera worldwide, the distinguished British entomologist of the Natural History Museum of London Roger Ward Crosskey and Dr. Marcos Báez Fumero from the University of La Laguna in Tenerife in whose work "*Catálogo de los Diptera de España, Portugal y Andorra*" they reported 51

species in Spain, 33 in Portugal and 19 in Andorra (González Peña *et al.*, 2002).

Later, Boutaina Belqat and Josefina Garrido from the universities of Abdelmalek Essaâdi (Morocco) and Vigo (Spain), respectively, provided a faunal and bibliographic checklist. This scientific contribution was of relevant importance because it allowed us to deepen our knowledge of the biogeographic distribution of Spanish species (Belqat & Garrido, 2008). A few years later, a new update on the status of the Spanish simuliid species came to light (López-Peña & Jiménez-Peydró, 2017) and contributed not only to its necessary taxonomic update but also to the incorporation of new records of numerous species from different regions of the country, mainly as a result of the doctoral thesis of the main author of the present paper (López-Peña, 2018), who increased the number of species reported from Madrid Region by 6 species, going from the 14 species reported by Belqat & Garrido (2008) to 20. Six years later, this work aims to update the status of the species recorded from Madrid Region as a result of an intense bibliographic search and a field investigation carried out in the Madrid enclaves likely to host the greatest diversity of simuliids in this Spanish region, with the aim of improving knowledge of this family of Diptera in the aforementioned territory.

Thus, this manuscript quotes all the records published so far for each species present in the Region of Madrid and adds seven new records obtained from this study (see the Results section).

Altitudinal records

Some species have been reported from different elevations by several authors. For example, the species *S. petricolum*, which reveals the minimum altitudinal range in the Madrid Region, has been found at 870 to 875 m. a.s.l. in the Guadarrama river (Grenier & Bertrand, 1954). The following species show an expanding altitudinal range respectively: the species that shows the narrowest altitudinal range is *S. vernum*, being discovered between 1104 and 1235 m in the Lozoya river (Casado *et al.*, 1990), but in this study was gathered from 1731 to 1845 m in the stream 'del Toril' and stream 'de

las Guarramillas', increasing its altitudinal range from 1104 to 1845 m; in second place, *S. equinum* depicts a range that fluctuates between 870 and 1104 m in different sections of the Lozoya river (González Peña, 1990; Casado *et al.*, 1990) and in the Guadarrama river (Grenier & Bertrand, 1954); the next one is *S. lineatum* which was recorded from 700 to 1075 m in the Guadarrama river (Grenier & Bertrand, 1954) and in the Lozoya river (González Peña, 1990; Casado *et al.*, 1990); the altitudinal range of *S. pseudequinum* goes from 650 to 1075 m in the Guadarrama river (Grenier & Bertrand, 1954), in distinct parts of the Lozoya river (González Peña, 1990; Casado *et al.*, 1990), and in the Jarama river (González Peña, 1990); the species *S. ornatum* shows a wider altitudinal range which varies from 650 to 1235 m in the Lozoya river (González Peña, 1990; Casado *et al.*, 1990), and in the Jarama river (González Peña, 1990). However, in this study *S. ornatum* has been recorded from 1239 to 1242 m in the river 'de la Venta', thus its altitudinal range has been increased fluctuating from 650 to 1242 m. The altitudinal range of *S. argyreatum* goes from 1075 to 2000 m in the Lozoya river (Casado *et al.*, 1990) and in the Valdesquí Station located in the 'Sierra de Guadarrama' (Vinçon & Clergue-Gazeau, 1993). Finally, the species with the widest altitudinal range are *S. cryophilum* and *S. intermedium*, which were recorded from 870 to 2000 m, the first one in the Guadarrama river (Grenier & Bertrand, 1954) and in the Valdesquí Station (Vinçon & Clergue-Gazeau, 1993), and the second one in the Guadarrama river (Grenier & Bertrand, 1954). Nonetheless, the records of these two species in this study do not modify their altitudinal range since *S. intermedium* was found at 1242 m in the river 'de la Venta' and *S. cryophilum* between 1242 and 1845 m a.s.l. in the stream 'del Toril', the stream 'de las Guarramillas', and the river 'de la Venta'.

Hematophagous species

In addition, nine species out of the 29 reported in this study are hematophagous, which are of great interest both to public health and animal welfare. It is noteworthy to underline the fact that

the species *S. argyreatum*, *S. variegatum* and *S. pseudequinum* are important from the veterinary point of view since the females of the two first species can bite a wide range of farm animals (Edwards, 1920; Zahar, 1951; Davies *et al.*, 1962; Davies, 1966; López-Peña & Cheke, 2023) and the ones of *S. pseudequinum* are inclined to feed from the blood of pigs, horses and cattle (Rivosecchi, 1978; Villanúa-Inglada *et al.*, 2013; López-Peña & Cheke, 2023). The most annoying and worrisome species for humans is *S. erythrocephalum* owing to the intense anthropophilic behaviour that its females depict (Živković, 1970; Rivosecchi, 1978; Ignjatović-Ćupina *et al.*, 2006; López-Peña & Cheke, 2023) and the “Blackfly fever” to which they can give rise, which mainly consists of fever, headache, joint pain, fatigue and inflammation of the lymphatic glands (Crosskey, 1993). Another species to bear in mind is *S. equinum* (Beaucournu-Saguez *et al.*, 1990; López-Peña & Cheke, 2023). The species *S. intermedium* and *S. ornatum* also tend to bite humans to acquire their blood to complete the development of their eggs (Crosskey, 1993; Davies, 1966; Živković, 1970; Rivosecchi, 1978; Ignjatović-Ćupina *et al.*, 2006; López-Peña & Cheke, 2023). Finally, *S. lineatum* (Beaucournu-Saguez *et al.*, 1990; Bernotienė, 2003; Baužienė *et al.*, 2004; López-Peña & Cheke, 2023) and *S. tuberosum* (Edwards *et al.*, 1939; Davies *et al.*, 1962; López-Peña & Cheke, 2023) show a tendency to bite mammals, including humans. Besides, the species *S. erythrocephalum* are prone to bite cattle (Wenk, 1981, 1987; Kettle, 1995; López-Peña & Cheke, 2023) and act as a vector of bovine onchocerciasis agents such as *Onchocerca gutturosa* (Neuman, 1910) (Crosskey, 1990; Ruiz-Arrondo *et al.*, 2017; López-Peña & Cheke, 2023) and *O. lienalis* (Johnston, 1921) (Mikhailyuk, 1967; Ham & Bianco, 1983; Cupp, 1996; López-Peña & Cheke, 2023). The females of *S. intermedium* may bite horses and cattle in order to suck their blood (Davies, 1966; López-Peña & Cheke, 2023). However, the females of *S. ornatum*, although may bite horses and pigs, they show a preference for cattle (Sutcliffe, 1986; Crosskey,

1993; López-Peña & Cheke, 2023), and they are able to transmit to them the same triggering agents of bovine onchocerciasis as *S. erythrocephalum*, that is *O. lienalis* and *O. gutturosa* (Eichler, 1973; Reid, 1979; López-Peña & Cheke, 2023). On the other hand, the females of the species *S. equinum* not only show a strong preference for the equine blood (Sutcliffe, 1986; Crosskey, 1993; López-Peña & Cheke, 2023) but also are able to transmit *O. lienalis* to them (Ham & Bianco, 1983; López-Peña & Cheke, 2023), and they also feed on cattle (Crosskey, 1993).

CONCLUSION

The results of this study contribute to increase the knowledge of blackflies in Spain and specifically in the Madrid Region, as well as to a better understanding of their geographic and altitudinal distributions. It is of vital importance to study and publish data on simuliids to deepen the knowledge of their ecology and biogeographic distribution. Such studies contribute to the protection programmes of the habitats of these species, to preserve their populations and to study both its phylogeny and its evolution over time in a scenario of climate change and global warming that can more intensely affect specialist species such as those that inhabit the mountainous areas of the Madrid Region.

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