

An approach to telmophagous Nematocera (Ceratopogonidae, Psychodidae, and Simuliidae) of Spain, with emphasis on its medical and veterinary importance

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ABSTRACT

The Nematocera are a suborder of Diptera which historically have influenced human history more than any other arthropod group. In Spain, four families show a hematophagous behaviour: one is solenophagous (Culicidae), feeding directly on blood vessels, and three are telmophagous (Ceratopogonidae, Psychodidae, and Simuliidae), feeding on blood that pools at the site where their mouthparts have formed a laceration. Although mosquitoes rank first in importance, the telmophagous are also of great interest. We update the status of these nematocera in Spain through a transdisciplinary approach, discussing about the main characteristics of each family, the situation of the main vector-borne diseases, especially during the 21st century, and the most relevant species or species groups from a medical and veterinary perspective. To date, 84 species of *Culicoides* biting midges (Ceratopogonidae), 14 species of sand flies (Psychodidae), and 53 species of black flies (Simuliidae) have been reported in Spain. *Culicoides imicola* and the *Obsoletus* complex stand out as the most important biting midges, as they are incriminated in the transmission of bluetongue and Schmallenberg virus; *Phlebotomus perniciosus* and *Phlebotomus ariasi* are widespread vectors of *Leishmania infantum*; and *Simulium erythrocephalum* and the *Simulium ornatum* s.l. can be considered the most annoying pests for humans and livestock. The authors urge to increase research capacity in Spain in order to address several health challenges arising from the presence of telmophagous Nematocera in particular, and of blood-sucking arthropods in general.

Indexing terms: Biting midges. Sand Flies. Black Flies. Vector-Borne Diseases. Spain.

RESUMEN

Los Nematocera son un suborden de dípteros que históricamente han influido en la historia de la humanidad más que cualquier otro grupo de artrópodos. En España, cuatro familias presentan un comportamiento hematofago: una es solenofaga (Culicidae), alimentándose directamente de los vasos sanguíneos, y tres son telmofagas (Ceratopogonidae, Psychodidae y Simuliidae), alimentándose de la sangre que se acumula en el lugar donde sus piezas bucales han formado una laceración. Aunque los mosquitos ocupan el primer lugar en importancia, los telmofagos también son de gran interés. Se actualiza el estado de estos nematóceros en España mediante un enfoque transdisciplinar de las principales características de cada familia, la situación de las principales enfermedades transmitidas por estos, especialmente durante el siglo XXI, y las especies o grupos de especies más relevantes desde una perspectiva médica y veterinaria. Hasta la fecha, se han descrito en España 84 especies de jejenos (Ceratopogonidae), 14 especies de flebotomos (Psychodidae) y 53 especies de moscas negras (Simuliidae). *Culicoides imicola* y el complejo *Obsoletus* destacan como los jejenos más importantes, ya que están incriminados en la transmisión de la lengua azul y el virus de Schmallenberg; *Phlebotomus perniciosus* y *Phlebotomus ariasi* son vectores generalizados de *Leishmania infantum*; y *Simulium erythrocephalum* y *Simulium ornatum* s.l. pueden considerarse las plagas más molestas para el ser humano y el ganado. Los autores instan a aumentar la capacidad de investigación en España para hacer frente a diversos retos sanitarios derivados de la presencia de Nematocera telmofagos en particular, y de artrópodos hematofagos en general.

Palabras clave: Jejenos. Flebotomos. Moscas Negras. Enfermedades Transmitidas Por Vector. España.

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RESUMO

A Nematocera é uma subordem da Diptera que historicamente influenciou a história humana mais do que qualquer outro grupo de artrópodes. Na Espanha, quatro famílias apresentam um comportamento hematófago: uma é solenófaga (Culicidae), alimentando-se diretamente dos vasos sanguíneos, e três são telmofágicas (Ceratopogonidae, Psychodidae e Simuliidae), alimentando-se de sangue que se acumula no local onde suas partes bucais formam uma laceração. Embora os mosquitos sejam os primeiros em importância, os telmófagos também são de grande interesse. Se atualizam o status destes nematóceros na Espanha através de uma abordagem transdisciplinar sobre as principais características de cada família, a situação das principais doenças transmitidas por vetores, especialmente durante o século XXI, e as espécies ou grupos de espécies mais relevantes do ponto de vista médico e veterinário. Até o momento, 84 espécies de maruins do gênero *Culicoides* (Ceratopogonidae), 14 espécies de flebotomíneos (Psychodidae) e 53 espécies de borrachudos (Simuliidae) foram relatadas na Espanha. *Culicoides imicola* e o complexo *Obsoletus* destacam-se como os maruins mais importantes, pois são implicados na transmissão da língua azul e do vírus Schmallenberg; *Phlebotomus perniciosus* e *Phlebotomus ariasi* são vetores amplamente conhecidos de *Leishmania infantum*; e *Simulium erythrocephalum* e *Simulium ornatum* s.l. podem ser considerados as pragas mais incômodas para os seres humanos e o gado. Os autores impõem à aumentar a capacidade de pesquisa na Espanha a fim de enfrentar vários desafios de saúde decorrentes da presença de Nematocera telmofágica em particular, e de artrópodes sugadores de sangue em geral.

Termos de Indexação: Maruins. Flebotomíneos. Borrachudos. Doenças Transmitidas Por Vetores. Espanha.

INTRODUCTION

The Diptera are classically divided into two suborders, Nematocera and Brachycera, both of which have blood-feeding flies species. Worldwide, the Nematocera suborder includes the sand flies (Psychodidae) and the Culicomorpha families: mosquitoes (Culicidae), biting midges (Ceratopogonidae), frog feeding flies (Corethrellidae) and black flies (Simuliidae); whereas the Brachycera suborder comprises horse flies and deer flies (Tabanidae), louse flies or keds (Hippoboscidae), and stable and horn flies (Muscidae) [1,2]. Hematophagous Diptera can be regarded as ectoparasites that feed on the blood of their hosts when they are in temporary contact with them [3]. Specifically, the suborder Nematocera consists of four important families of obligate blood-feeders: one solenophagous family (Culicidae), which insert their modified mouthparts into the blood vessel to extract the blood, and three telmophagous families (Ceratopogonidae, Psychodidae, and Simuliidae), which cut the epidermis and create a small pool of blood that they suck [4]. These pool-feeders are more likely to be involved in mechanical transmission than vessel-feeding Diptera because a larger surface area of their mouthparts is in contact with the bloodmeal [5]. However, these telmophagous are usually unable to bite through clothes, even if these are made of thin material [6].

The Nematocerans historically have impacted human history more than any other arthropod, not only through their unpleasant and disturbing behaviour, but more importantly through their role in the transmission of some pathogens, including many of medical and veterinary importance [7]. Undoubtedly, the family Culicidae ranks first in importance, on account of their vast number and wide distribution of species as well as their role as vectors of a huge variety of pathogens to vertebrates, including viruses, bacteria, and parasites. However, the three families of pool-feeders, which are tiny in size and are commonly known as ‘midges’ (along with other families of non-hematophagous Nematocera), are also considered of major interest due to the nuisance they cause and as transmitters of disease-causing organisms. The economic importance of these insects cannot be underestimated since they cause direct monetary losses and humans incur expenditures for their control [8].

Epidemiologically, the prevalence of several vector-borne diseases appears to be associated with global changes, which includes not only climate change, but also land use and land cover changes, deforestation, biodiversity loss, and the spreading of invasive alien species, among others [9,10]. In addition, the changes resulting from globalisation, such as global shipping and intercontinental trade, create new opportunities for invasive vectors and pathogens to spread [11]. Due to Spain is located near Africa (the shortest distance across the Strait of Gibraltar is 14.4 km), and is a stopping-off point for humans and other animal reservoirs (e.g., migratory birds), and also due to its changing climate conditions (the country is one of the areas most affected by global warming), the impact of hematophagous Nematocera is notorious in the country [12].

Herein, remarks are provided in general (worldwide) and detailed information on the status of this non-culicid hematophagous Nematocera (Ceratopogonidae, Psychodidae, and Simuliidae) in Spain is succinctly updated through a transdisciplinary approach, targeting the main characteristics of each group, the historical and current occurrence of the diseases carried by them, as well as the most important species and groups of species from a medical and veterinary perspective. It is noteworthy that this manuscript is an approach to a topic and not a comprehensive review.

FAMILY CERATOPOGONIDAE

Worldwide. Among ceratopogonids, only the females of the genera *Austroconops*, *Culicoides*, *Leptoconops* and those belonging to the subgenus *Lasiohelea* of the genus *Forcipomyia* are hematophagous [13]. However, the relevance of this family is essentially given by the genus *Culicoides*, which are commonly called ‘biting midges’.

These nematocerans are among the world’s smallest blood sucking insects, with the adults ranging from 1 to 4 mm in length, with wings that are usually patterned with light and dark markings, a morphological characteristic of primary importance in species diagnosis [14]; however, in plane wing female species the identification needs a more exhaustive morphological study of various features (mostly palpi, distribution of *sensilla coeloconica* in antennae, and spermathecae, among others), and genitalia in the case of males [15]. Molecular techniques are also very valuable to separate sibling species or species groups [16]. Regarding their bioecology, *Culicoides* species are relatively poor flyers and usually disperse only a few hundred meters from their breeding sites [17]. Most *Culicoides* species have a crepuscular periodicity, peaking mainly at dusk and dawn. Despite some specimens have been found resting in the inner walls of farm-holdings (stables, farms, etc.), resting sites of *Culicoides* are fairly unknown and still remain as an unsolved mystery about these midges. Larval habitats are usually defined as subaquatic (or aquatic) humidity-rich and enriched in animal or vegetal organic matter, and may cover a wide range of natural and artificial substrates such as freshwater marshes, shallow margins of ponds, swamps, beaches, bogs, peat lands, tree holes, leaf litter, animal manure, rotting and fallen fruits, among others [18] (figure 1).

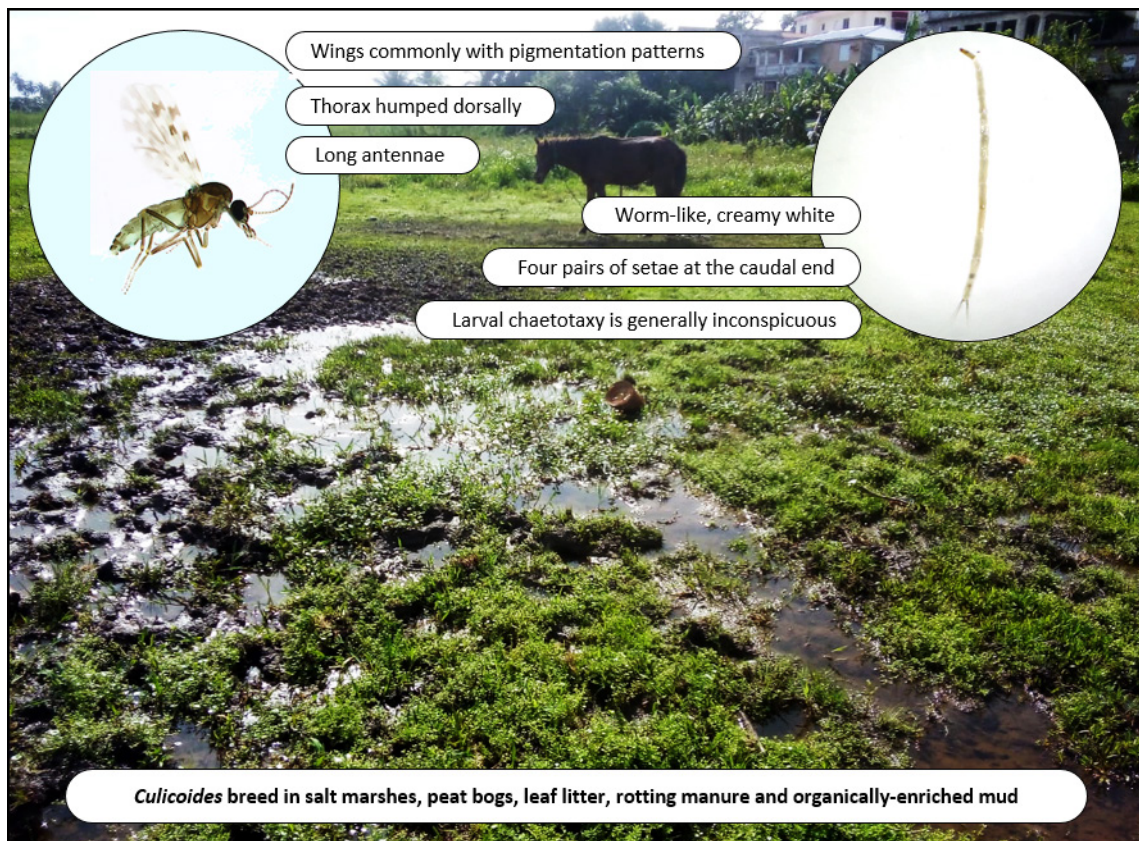


Figure 1. Main characteristics of adults, larvae and breeding sites of *Culicoides* biting midges.

These dipterans have the major veterinary relevance as vectors of internationally important arboviruses of livestock, such as the African horse sickness virus (AHSV), bluetongue virus (BTV) and Schmallenberg virus (SMV), among others [19]. Bites may also cause hypersensitivity reactions, mostly in equines but also in ruminants, a condition known as 'sweet itch' [20]. The only arbovirus identified as being primarily transmitted by *Culicoides* to and between humans is the Oropouche virus (OROV), which is currently endemic to certain regions of South and Central America. Only a small number of *Culicoides* species have a significant injurious impact on human existence. Nevertheless, even in the case of species known to play a role in transmitting pathogens, they remain the least studied of the major Dipteran vector groups [21].

Spain. The first known BTV outbreak in the Iberian Peninsula took place in 1956 [22]. The BTV-10 serotype responsible for this Spanish outbreak was related to an African strain and it was first detected in Portugal. It was caused probably by *Culicoides* specimens transported by wind from affected regions of Africa [23]. This long-distance dispersion has been postulated according to different mathematical models based on analysis of wind and/or dust/pollen in the air, but it was also proved by the collection of *Culicoides* biting midges by means of a helium-filled blimp at the high of approx. 200 m above ground level over the UK [24]. BTV disease was eradicated from Spain in 1960 as a result of a national strategy that included movement restriction, vaccination, and slaughtering [25,26]. Regarding AHSV, the disease occurred in several North African countries in 1965, spreading across the Strait of Gibraltar into Spain in 1966 again via possible wind dispersal of infected midges [27]. Further AHSV outbreaks occurred between 1987 and 1990 in the country, the infection having been introduced into the region by a group of zebras from Namibia [28].

In the beginning of the 21st century, BTV-2 was notified in the Balearic Islands. As a consequence of this detection, a national eradication and surveillance programme was implemented in order to facilitate the early detection of any further circulation of the disease and to eradicate it by vaccination of domestic ruminants with an attenuated vaccine. After that, four BTV serotypes have been detected in peninsular Spain: i) BTV-1 (detected for the first time in 2007); ii) BTV-4 (detected for the first time in 2004 and, after its eradication, detected again in 2010); and iii) BTV-8 (first detected in 2008, eradicated in 2013, and then detected again in 2020). In the 2021-2022 season, circulation of BTV-1 and BTV-4 has been detected in the country, mostly in sentinel sheep flocks [29]. Spain reported the first outbreaks of SMV in sheep in 2012 [30] and in cattle in 2013 [31] in southern and central regions of the country, respectively. Although no further cases have been reported to date, SBV circulation has been detected regionally in cattle. However, there is evidence of widespread endemic circulation among wild ruminants in mainland Spain in the recent years [32]. Interestingly, there have been no incidence reports of *Culicoides* bites on humans in Spain. In contrast, several studies in certain European countries have reported that *Culicoides* fed opportunistically on humans, but most likely due to their tiny size their bites go unnoticed. The bites of *Culicoides* biting midges are described as painful and itchy, with clinical symptoms that range from a small reddish bump and a burning sensation at the bite spot to local irritations that cause significant itching [33]. It is noteworthy that *Leptoconops* (other genus of the family Ceratopogonidae) has also been recorded causing major discomfort to humans in some specific habitats. However, there are very scant information available about these midges in Spain [34].

The first report of *Culicoides* biting midges in Spain dates back to 1900 [35]. Up to date, 84 species have been recorded in the country [36-38]. *Culicoides imicola* Kieffer, 1913 and *Culicoides obsoletus* species complex (henceforth *Obsoletus* complex) are considered the most important vectors for the transmission of arbovirus. Bluetongue had been historically related with the species *C. imicola*, an Afrotropical ceratopogonid well-distributed across the dry Mediterranean area. This *Culicoides* species inhabits the the central and southern Spain and the Balearic Islands but is infrequent in the north, which is more humid [39], peaking its population in the September-October period [40]. Sporadically, some specimens were captured along the Ebro Valley and along the North-eastern Mediterranean coast [41], even occasionally in the Basque Country region [42] (Annex 1A). On the other hand, the well widespread *Obsoletus* complex, which comprise the sibling species *Culicoides obsoletus* sensu stricto (Meigen, 1818) and *Culicoides scoticus* (Downes & Kettle, 1952) has been identified as the main responsables for the transmission of BTV and SMV in the Western Palearctic region [43]. This fact suggests that they are able to adapt to a wider range of eco-climatic conditions in comparison to *C. imicola*. The highest densities of the *Obsoletus* complex have been recorded in Northern Spain [44], but is also prominent in

other communities in the centre of the country, such as Castilla-La Mancha, where its adult populations peak in May-June and then in mid-October [40] (Annex 1B). Other common and well-distributed species such as *Culicoides punctatus* (Meigen, 1818), *Culicoides newsteadi* Austen, 1921, *Culicoides nubeculosus* (Meigen, 1830), and the *Culicoides pulicaris* species complex were also found positive to BTV, but their role in the transmission is uncertain, in line with the studies conducted in Italy [45-47]. In addition, *Culicoides circumscriptus* Kieffer, 1918 and *Culicoides paolae* Boorman, 1996 have been recorded as the most notorious vectors in the transmission dynamics of *Haemoproteus* parasites in certain bird species of Andalusia [48]. A brief summary of the taxonomic status, breeding sites, distribution, vector status and trophic preferences can be consulted in table 1.

Table 1. Summary of ecological aspects and vector status of the two most important *Culicoides* (Diptera: Ceratopogonidae) species in Spain. Main information retrieved from González [2].

Species group	Taxonomic remarks	Habitat	Local distribution	Vector status	Biting preferences
<i>Culicoides</i>					
Obsoletus complex	<i>Culicoides obsoletus</i> and <i>Culicoides scoticus</i> belong to the Obsoletus complex; females are morphologically inseparables while the genitalia of males are clearly different.	Farm environments (fresh and composted manure, maize silage residues, rotting vegetation, faeces-contaminated straw, organically enriched soil in stable yards, and wet soil rich in organic matter in shaded habitats) and wild habitats (moist forest leaf litter and marsh edges with vegetation).	Widespread in the entire Iberian Peninsula, Balearic Islands and Canary Islands.	Confirmed vector of BTV and suspected of SVB*	Mostly mammals (domestic and wild mammals), rarely humans.
<i>Culicoides imicola</i>	It species belongs to the <i>C. imicola</i> species complex containing 10 species in Africa but <i>C. imicola</i> is a unique species in Spain.	Farm-environments (moist organically enriched soil, organic stable environments and faeces-contaminated straw).	Abundant in the central and south regions of the Iberian Peninsula Balearic Islands and Canary Islands.	Confirmed vector of BTV and AHSV, suspected of SVB*	Mostly mammals (domestic and wild mammals), rarely humans.

* BTV confirmed by RT-PCR and AHSV by virus isolation [129,130]. SVB detection in *Culicoides* has been confirmed in other European countries.

FAMILY PSYCHODIDAE

Worldwide. Of the five psychodid subfamilies, only those belonging to Phlebotominae have piercing mouthparts capable of taking blood. These flies are commonly called ‘sand flies’. Currently, a conservative approach based on practical criteria has led to the subdivision of the Phlebotominae into six genera: three genera from the Old World (*Phlebotomus*, *Sergentomyia*, and *Chinius*) and three from the New World (*Lutzomyia*, *Brumptomyia*, and *Warileya*) [49].

These nematocerans are tiny insects, with adults having a length of 1.5 to 3.5 mm, a hairy appearance, brownish or grayish colored, and long, stilt-like legs. Morphological identification of phlebotomine sand flies rely on the examination of internal structures, such as both the spermathecae and pharynx in females, and the genitalia in males, so it is mandatory to prepare slide-mounted specimens to achieve species level determination [50]. Nevertheless, the use of the COI genetic marker for species identification confirmation has become more widely used, especially in females with hardly visible structures [51,52]. Regarding its bioecology, sand flies are weak fliers, with a characteristic short hopping flight, travelling no more than a few hundred metres from their breeding sites. They are mostly crepuscular and nocturnal biters. Unlike most biting Diptera, and as their common name implies, the development of sand flies takes place in terrestrial biotopes rather than aquatic microhabitats. Larvae are mainly scavengers, feeding on organic matter such as fungi, decaying leaves, animal faeces, tree holes, crevices, caves, and burrows, among others [50]. Unfortunately, larval sites are overall poorly studied, most likely due to the wide and scattered breeding sites which makes this kind of studies tough and very unsuccessful (figure 2).



Figure 2. Main characteristics of adults, larvae and breeding sites of sand flies.

In relation to its importance in the transmission of pathogens, sand flies are the natural vectors of *Leishmania* spp. (Kinetoplastida: Trypanosomatidae) and arboviruses (*Phlebovirus*, *Vesiculovirus*, and *Orbivirus*) worldwide [54,55], and *Bartonella bacilliformis* in South America [56]. Leishmaniosis is probably the most important endemic zoonotic disease in the Mediterranean basin. There are three main clinical forms, and the visceral form is the most serious form of the disease, being fatal if untreated. Domestic dogs are considered the main reservoir for human infection by the protozoan parasite *Leishmania infantum* [57].

Spain. Leishmaniosis is an endemic zoonosis caused by *L. infantum*, which is found nearly everywhere in the Iberian Peninsula and Balearic Islands. In humans, this neglected tropical disease has traditionally been regarded as a hypoendemic infection mostly affecting children. In the 1980s, it emerged as a threat to people carrying HIV, at least until highly active antiretroviral therapy became available in the late 1990s. This significantly reduced the number of patients coinfecting with AIDS and *L. infantum*, although they remained at a greater risk [58]. On the other hand, canine leishmaniosis (CanL) is an endemic and dynamic disease with an overall seroprevalence and transmission risk that varies according to local environmental and climatic conditions [59].

Since 2009, an epidemic outbreak of human leishmaniosis has been developing in the southwest of Madrid, causing the largest community outbreak in recent times in Europe [60]. Although dogs are the main reservoir host of *L. infantum*, the surveillance system for CanL did not detect any increase in prevalence during this period. In fact, environmental changes motivated by the construction of green parks in a large urban setting have led to an increased density of hares, a newly incriminated lagomorph reservoir that sustained a large sand fly population [60-62]. Also, other synanthropic animals have been attributed a role in the transmission cycle, such as Norway rats [63] and cats [64]. The leishmaniosis circulation in this region of Madrid remains active but with lower density of sand flies. Regarding *Phlebovirus*, there are indications of the recent presence of Toscana, Granada, Naples, Sicily, Arbia, and Arrabida-like

viruses in humans, animals, and sandflies. Nevertheless, only human cases associated with the Toscana virus have been identified. Although some cases of this virus disease can be serious, most are not, so the overall impact of the Toscana virus disease for the Spanish population is considered low and very low for the rest of the sandfly-borne phleboviruses [65]. Regarding sand fly bites, they are often painful and cause small red bumps and blisters but often remain unnoticed. These bumps and blisters can become itchy, infected, or cause dermatitis or skin inflammation and can persist for days or weeks [66].

The first report of sand flies in Spain dates back to 1909 [67]. Up to date, 14 species have been recorded for the country [68,69], among which several are recorded as confirmed or suspected vectors of *L. infantum* [70]. Only *Phlebotomus perniciosus* Newstead, 1911 and *Phlebotomus ariasi* Tonnoir, 1921 are proven vectors of leishmaniosis in the territory [71]. *Phlebotomus perniciosus* is abundant and well-distributed in practically the whole country, with the exception of the province of Vizcaya (Basque Country) and probably also absent in the three easternmost islands of the Canary Islands (Annex 1C) [68]. Its density is greater in the driest areas with warmer temperatures, predominantly in the so-called meso- and thermo-Mediterranean areas, where it has two peaks of abundance, one in spring and early summer and one in autumn. On the other hand, *Ph. ariasi* is also an abundant species present in most of the Spanish territory with a few exceptions [68] (Annex 1D). This species is better adapted to colder areas and altitudes above 600-900 m (supra-Mediterranean area), showing activity from May to October, but with a peak of abundance only in August. In Spain, these two vectors are well-adapted to peri-urban residential areas containing detached houses with gardens and dogs, the main domestic reservoir of the parasite [71-73]. Other interesting species is *Phlebotomus mascittii* Grassi, 1908, which has never been proven to be a vector of leishmaniosis, but the recent detection of *Leishmania* spp. DNA in specimens collected from other European countries such as Austria may support its possible role in the transmission [74]. This sand fly species is found in Northern Spain, including the Catalan provinces of Barcelona and Girona, and the Autonomous Community of Cantabria and the Basque Country [52]. Although *L. infantum* is the only species present in Spain to date, it must be taken into account that some other Spanish species are capable of transmitting *Leishmania tropica* (which is also present in neighbouring Portugal) and *Leishmania major*, such as *Phlebotomus sergenti* Parrot, 1917 [75] and *Phlebotomus papatasi* (Scopoli, 1786) [76], respectively. In fact, traditionally the latter is also implicated as vector of phleboviruses. However, in the last decade, these viruses have been detected in other species distributed on Spanish territory [(*Ph. perniciosus*, *Ph. papatasi*, *Ph. sergenti*, *Phlebotomus longicuspis* Nitzulescu, 1930, and *Sergentomyia minuta*

Table 2. Summary of the ecological aspects and vector status of the two most important sand fly (Diptera: Psychodidae) species in Spain. Main information retrieved from González [2].

Species group	Taxonomic remarks	Habitat	Local distribution	Vector status	Biting preferences
Psychodidae					
<i>Phlebotomus perniciosus</i>	Males have a very characteristic <i>aedeagus</i> valves, which have the terminal end bifurcated into two unequal tips (fork shaped). Spermathecae (body and neck) and ducts can be used to separate female species from sibling species.	Specific larval sites are unknown. Found in peridomestic and wild environments. In Italy, <i>Ph. perniciosus</i> was observed emerging in high numbers from an abandoned concrete structure used as the roof of an animal shelter.	A widespread and abundant species in the Iberian Peninsula (except in Vizcaya), and the Balearic Islands.	Confirmed vector of <i>L. infantum</i> and other different phleboviruses*.	A wide variety of vertebrates, mainly mammals (including humans) but also birds. Blood intakes derived mainly from hares, humans and to a lesser extent cats.
<i>Phlebotomus ariasi</i>	Males possess a characterised <i>aedeagus</i> with valves apically dilated (club-shaped). Spermathecae (body and neck) and ducts can be used to separate female species from sibling species.	Peridomestic and wild habitats. Specific larval sites are unknown in Spain. In other countries, <i>Ph. ariasi</i> has been recorded breeding in leaf litter, rubbish, under the roofs of sheep sheds and in burrows of field mice (genus <i>Apodemus</i>).	Widely distributed in the Iberian Peninsula and the Balearic Islands, but absent in some parts of the northwest of the Iberian Peninsula.	Confirmed vector of <i>L. infantum</i> and other different phleboviruses*.	Feed on a wide variety of vertebrates, mainly mammals (including humans) but also birds, showing opportunistic tendencies.

* Confirmed by PCR, culture and microscopy/histology [63,65,70, 128]

(Rondani, 1843)], indicating that sand flies have low specificity for phleboviruses [55]. A brief summary of the taxonomic status, breeding sites, distribution, vector status and trophic preferences can be consulted in table 2.

FAMILY SIMULIIDAE

Worldwide. Two subfamilies comprise the family Simuliidae. The most primitive subfamily, Parasimuliinae, includes females without biting mouthparts, and comprises four described and one undescribed species endemic to the Pacific Northwest. The subfamily Simuliinae contains all remaining species. *Simulium* is the the largest genus and comprises many of the species with economic importance [77]. Members of this family are commonly called ‘black flies’ or ‘buffalo gnats’.

Adult black flies are small dipterans that measure 1 to 5 mm in length with short antennae, broad wings, stout-bodied and hump-backed appearance that range in colour from black to various shades of grey or yellow. The morphological uniformity of black flies creates difficulty for the identification of species together with the fact that adult specimens should be stored in dry (and not in ethanol) in order to be in good condition for identification [78]. For this reason, a holistic approach to identification is typically used, relying on characteristics mainly of larvae, pupae, and adults (males and females), and also the polytene chromosomes and new molecular methods, as well as distributional and ecological information [77-79]. Regarding its bioecology, many species are generally strong flyers moving long-distances away from their breeding sites. They are essentially diurnal dipterans, feeding in open and sunny locations [80]. The breeding sites of immature stages of black flies are restricted mainly to flowing freshwater such as rivers, creeks, streams and irrigation ditches, where the filter-feeding larvae often play a key ecological role, being an important group of macroinvertebrates used as bioindicators of water quality [81]. Black fly larvae and pupae attach themselves to solid, usually smooth substrates such as rocks, aquatic or emergent vegetation, and logs, but also to artificial substrates [82] (figure 3).

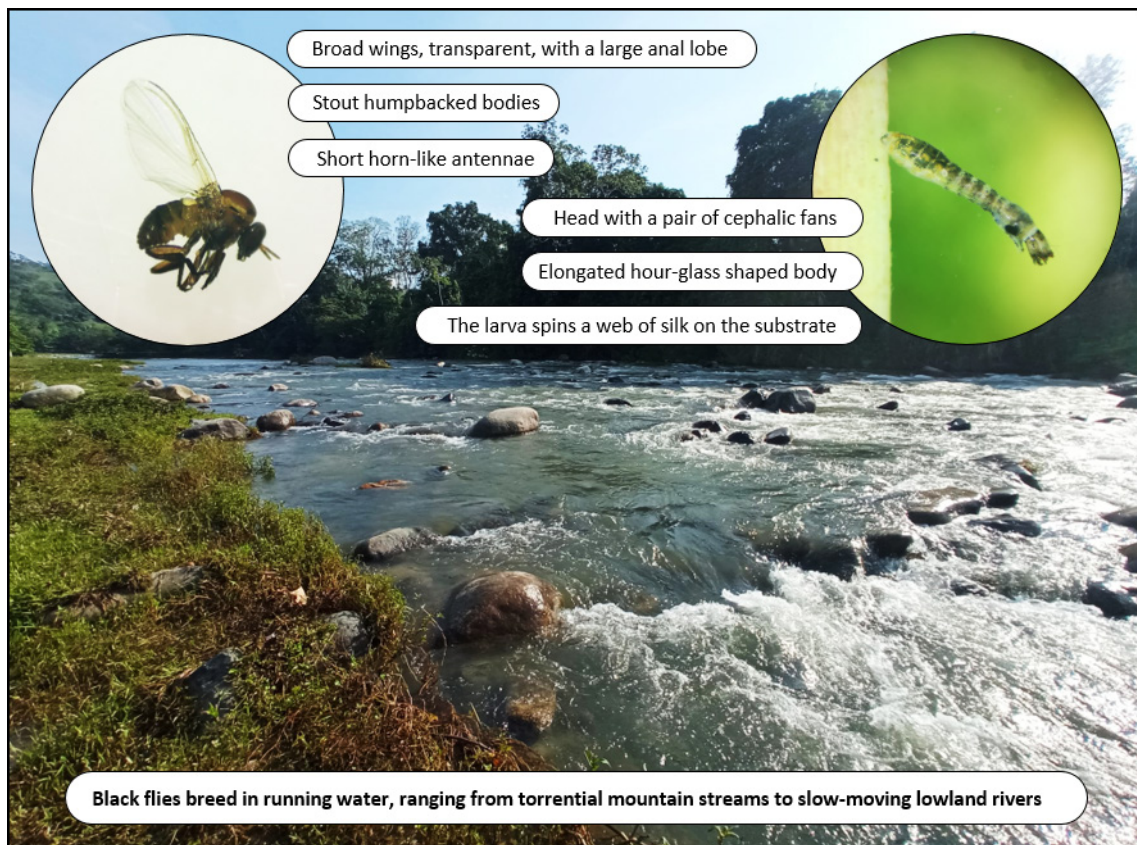


Figure 3. Main characteristics of adults, larvae and breeding sites of black flies.

Simulids are among the most annoying blood-feeding dipterans, causing severe irritation and distress to poultry, wild birds, animals, and humans globally [83]. These insects are also vectors for viruses, bacteria, protozoans, and filarial nematodes of medical and veterinary importance. Among the filarial nematodes, the public health significance of *Onchocerca volvulus* cannot be overemphasized, being the causative organism of the dreadful and debilitating disease onchocerciasis, also named 'river blindness'. This neglected tropical disease occurs mainly in Africa, with additional foci in South America, crossing the border between the Bolivarian Republic of Venezuela and Brazil [84]. *Mansonella ozzardi* causes filariasis, which affects humans exclusively but is not pathogenic [85]. Simulids can also mechanically transmit myxomatosis to rabbits [86].

Spain. The circulation of filariae of veterinary importance with zoonotic potential has been sporadically recorded in the country, such as the nematode *Onchocerca lupi* [87] and *Onchocerca flexuosa* [88]. These microfilariae are theoretically transmitted by simulids (also might be by *Culicoides*) to wild and domestic animals, but limited evidences of the proven vectors are provided in Europe [88,89]. However, no endemic simulid-borne human disease has been reported in the country to date. Therefore, the main concern of black flies is the annoyance caused by its bites to both humans [90] and livestock [91]. In fact, their painful bites can cause allergic reactions in sensitive animals (called 'simuliotoxicosis'), and are among the few blood-sucking arthropods that can kill animals by exsanguination during massive attacks [92]. Despite these reactions have not yet been published in Spain, similar episodes might be occurring in sheep flocks in the region of Aragon (Ruiz-Arrondo, personal communication). Black flies have become very relevant pests in certain Spanish regions since the beginning of 21st century, particularly in Aragon, Catalonia, Madrid, and the Valencian Community [90,93-95], although other regions have also reported problems associated to these insects during the last years. Medical attention towards simulid bites has been frequent over the last decade in these regions, particularly in the city of Zaragoza, where hundreds of people are attended due to the bites of black flies that are breeding in the Ebro river since passing through the Cathedral-Basilica of Our Lady of the Pillar [90]. Bites are characterised by itching, localized swelling, inflammation eruptions of pruritic papules, vesicles, intense pruritus, and erythematous wheals [96]. Other more severe reactions may occur after a bite, such as systemic reactions that includes headache, nausea, fever, weakness, and swollen lymph nodes commonly known as 'black fly fever' [77], and even severe anaphylactic reactions that may require hospitalization [97].

The first report of black flies in Spain dates back to 1888 [98]. Up to date, 53 species have been found in the country [99], with *Simulium erythrocephalum* (De Geer, 1776) recorded as the main anthropophilic species causing the majority of the problems [100]. This species could act as potential vector of *O. volvulus* and even cause the so-called black fly fever [84]. *Simulium erythrocephalum* shows a scattered distribution most likely conditioned by the lack of studies in several regions of Spain. This species is not present in Andalusia, the Canary Islands and the Balearic Islands [101] (Annex 1E). Further studies are needed to define the spatial and temporal dynamics of *S. erythrocephalum* in Spain, although the population has been found to increase between May and June and then decline in the warmer months [100]. Anthropophilic behaviour has also been reported in the *Simulium ornatum* species group (henceforth Ornatum group) [102]. In Spain, this species group includes at least *Simulium ornatum* sensu stricto Meigen, 1818 and other undefined morphospecies, *Simulium intermedium* Roubaud, 1906, and *Simulium trifasciatum* Curtis, 1839 [103], being the latter the species with the most restricted distribution, but present throughout the country except in the islands and some central parts of the Iberian Peninsula [100] (Annex 1F). On the other hand, other species within of the subgenus *Wilhelmia* such as *Simulium equinum* (Linnaeus, 1758) and *Simulium lineatum* (Meigen, 1804) have also been found feeding on humans, but these are not commonly their natural hosts [100]. Regarding their mammophilic habits, *S. erythrocephalum*, *S. ornatum* s.l. and *S. lineatum* also fed on cattle, *S. equinum* fed on cattle and equines, while *Simulium pseudequinum* Séguy, 1921 fed on cattle, equines, and pigs. All three species generate great discomfort and stress on animals, and led to huge economic losses [85,104]. A brief summary of the taxonomic status, breeding sites, distribution, vector status and trophic preferences can be consulted in table 3.

Table 3. Summary of the ecological aspects and vector status of the two most important black fly (Diptera: Simuliidae) species in Spain. Main information retrieved from González [2].

Species group	Taxonomic remarks	Habitat	Local distribution	Vector status	Biting preferences
Simuliidae					
<i>Simulium erythrocephalum</i>	Both adults and pupae might be used for accurate identification. Particularly, males have a characteristic genitalia and pupae is composed of 6 thin gills.	Biotopes of medium-low river sections, settling indiscriminately on petrified and sandy substrates with or without vegetation, with a low oxygen concentration and low to moderate velocity water currents. It is a characteristic species of the lower reaches of rivers.	Relatively frequent in central and northern Europe, with the Iberian Peninsula being its southern limit of distribution. Cited only in some autonomous regions.	Unclear in Spain *. <i>Onchocerca</i> spp. in livestock.	Mammophilic (domestic animals such as cattle and horses). It is a highly anthropophilic species.
<i>Simulium ornatum</i> s.l.	The taxonomic status of the Ornatum group is very complex. <i>Simulium ornatum</i> s.l. includes <i>S. ornatum</i> s.s. and other still undefined morphotypes. Within the Ornatum group there is also included at least other two species (<i>S. trifasciatum</i> and <i>S. intermedium</i>).	It is typical in torrents and large rivers with stable currents in low and medium altitude stretches, with a very variable altitudinal distribution (200-1,400 m). It can be found in environments with very different conditions, especially depending on the degree of mineralisation and eutrophication, benefiting in conditions of slight eutrophication.	Distribution still incomplected. Most likely present in most of the Iberian Peninsula and absent in the islands.	Unclear in Spain *. Local vector of <i>O. gutturosa</i> in cattle from England. Possibly vector of trypanosomes, haemosporidia, and microfilariae.	Mammophilic (cattle and humans). Aggressive behaviour to humans.

* Without conclusive data. *Onchocerca flexuosa* has been detected in deer from northern Spain but the specific vectors harbouring the nematodes are still unknown [88].

CONCLUDING REMARKS

To date, 84 species of *Culicoides* biting midges, 14 species of phlebotomine sand flies and 53 species of black flies have been recorded in Spain. However, it must be pointed out that exists controversy with regard to taxonomic status of some species, particularly (but not exclusively) in relation to the existence of cryptic species complexes which are very common in the order Diptera. Furthermore, some other species are known but still unnamed (particularly in the family Simuliidae), and additional species undoubtedly remain to be discovered. In this regard, DNA-based methods for species identification which have been extensively employed in the last few years, will be very important to decipher the taxonomy of these groups in the future.

In relation to the distribution of these nematocerans, the Spanish Bluetongue National Surveillance Program provided very valuable knowledge of the distribution and phenology of *Culicoides* vectors throughout the country [39] but also of many other species, which have helped in detecting new records at both regional and national scale [36,105,106]. In addition, the entomological catches obtained from this program have considerably increased the knowledge of the Spanish sand flies [52,68], as the miniature CDC UV-light traps, which operate from dusk until dawn, allowed these Nematocera to be collected along with other insects with nocturnal habits such as some mosquito species [108]. Unfortunately, these passive trapping methods did not collect black flies as they are primarily diurnal insects and require other attraction baits (e.g. carbon dioxide) to be lured. This is the major reason to explain that the black flies distribution knowledge is more limited as require direct collection of immature stages in water courses and/or a source of carbon dioxide coupled to suction traps, which is usually expensive, bulky, short-life heavy and frequently difficult to obtain [108]. This fact, together with the absence of a significant role of simuliids as vectors in Spain, makes their distribution and phenology the least known within the three telmophagous families. Presumably, as more systematic sampling is carried out in more autonomous communities, bioecological knowledge about Spanish black flies will greatly increase, as recently seen in the Basque Country [103].

Spain is considered one of the most vulnerable countries to climate change within the European Union due to its unique geographic location and socio-economical characteristics [109]. In this changing context, rapid shifts in the geographic distribution of blood-feeding Nematocera species, including incursion into new regions, can have major ecological and economic impacts and even completely alter the way we live. The most high-profile recent examples of this phenomenon in Spain have been reported in the family Culicidae, such as the invasive species *Aedes albopictus* (Skuse, 1894), which, since its detection in Catalonia in 2004, has shown a pattern of colonisation from the coastal area to inland in the mainland [110]. This rapid expansion in their distribution, associated mostly with global trade and water-filled containers have not generally been reported for telmophagous Nematocera. However, geographical dispersion can occur to adjacent territories following environmental changes that result in suitable conditions for survival, reproduction, and establishment. This appears to be the case of the vector *C. imicola* in Spain, where populations of this species colonized new habitats further north of the original distribution range [39]. However, long-range dispersal of some *Culicoides* species have been reported in Spain associated with transport by winds from Northern Africa [111]. There is also evidence of changes in the distribution of several species such as *Ph. perniciosus*, *Ph. ariasi*, and *Ph. mascittii*, which are expanding their range into more northerly and higher altitude places in some European countries [112], supported by changes in the eco-climatic conditions. In addition, there is evidence that human-induced environmental changes may strongly influence the patterns and distribution of sand fly vectors (e.g. the outbreak of human leishmaniosis of Madrid) [113]. In relation to black flies, their recent expansion into metropolitan areas is not clear but most likely conditioned by different factors, including the improvement of water quality in river basins thanks to the current regulations in force controlling discharges of substances from water treatment plants, industrial factories, and livestock farming, among others [114]. Remarkably, improving water quality around cities also improves the conditions for these filtering organisms to proliferate in these metropolitan areas, which also take advantage of the dispersion of certain species of macrophytes that are their most suitable substrates for immature fixation [104].

The three telmophagous families within Nematocera order breed on a wide variety of biotopes, which are often scattered and/or are inaccessible, so vector control is not easily accomplished and evidently there is no silver bullet. In addition, applying adulticides for vector control is unlikely to be environmentally acceptable and its use should be restricted to extreme circumstances or in epidemic outbreaks where exists a significant risk to public health. Therefore, in order to minimize *Culicoides* breeding sites, certain hygienic measures should be taken, such as the removal of animal litter, remove manure from the farm setting, dry or cover it with canvas, reduce the silage residues or treat it with methods such as composting and acidification [115]. Another feasible option of reducing the contact between vectors and livestock includes the use of chemically treated net barriers placed around sheds and/or yards where livestock is confined nightly or permanently, although is genuinely used only with pure-bred horses [115]. However, none of the previous measures are recorded as highly efficient to prevent adult *Culicoides* species. Vaccination appears to be the only effective tool to limit disease spreading [116].

Regarding phlebotomine sand flies, vector control is constrained by the inherent difficulties associated with their non-aquatic breeding sites, which are not susceptible to large-scale insecticidal treatment programmes. Public health interventions to control these dipterans in the leishmaniosis outbreak in Madrid required an integrated strategy which combined vector surveillance with environmental interventions in order to eliminate sand fly breeding and resting sites. In addition, the widespread application of insecticides across the target environment is short-lived and inefficient. Actions against reservoir species, mainly the culling of hares and rabbits and also destroying their nesting grounds, indirectly decreased the vector population. The use of insecticide impregnated dog collars has been extensively studied and used as an effective tool in reducing the incidence of CanL in many countries [117]. In Spain, the results were not promising using insecticide-impregnated dog collars, but in combination with pipettes treatment provided a better protection [118].

In the case of simuliids, the most efficient and environmentally acceptable technique to suppress black fly populations is the use of *Bacillus thuringiensis* var. *israelensis* (*Bti*) in rivers and streams where the immature stages are developing. In this sense, a better understanding of the effects of the chemical and physical parameters of each water course, and consequently on larvicidal activity, is critical to the optimal operational use of *Bti*-based larvicides [119]. In addition, taking into account the great importance of macrophytes that act as support for the preimaginal stages,

mechanically removing the hydrophytes and helophytes from the lotic water courses has proven to be an effective control strategy in some Spanish cities [95]. New model traps that consist of interception adhesive-coated blue and black striped fabric squares (similar to those called Esperanza window traps) were tested for the collection of black flies in adult stage in Spain showing promising results (Ruiz-Arrondo, personal communication). Regarding cultural control measures, although telmophagous do not usually breed in domestic environments, as some species of synanthropic mosquitoes do, it is crucial to promote citizen awareness of the risks and facilitate access to preventive measures through didactic approaches to these topics of general interest [120].

Further laboratory and field-based studies on dispersion, survival, population densities, vector-parasite interactions, vectorial capacity, and host preference may reveal more valuable information on these blood-feeders in Spain. For this purposes, rearing of Diptera vectors under confined laboratory conditions is essential for investigating the above aspects [121]. However, the requirements of many species are challenging and many attempts to rear telmophagous species have failed, with the exception of some species of *Culicoides* (*C. imicola* and *C. nubeculosus*) [122] and *S. erythrocephalum* [123]. There is no evidence of the establishment of laboratory colonies of *Culicoides* biting midges or black flies in Spain. However, sand flies rearing protocols are better studied and need less requirements. At least two Spanish colonies of *Ph. perniciosus* has been successfully established, which has allowed the possibility of study leishmaniosis in depth [124].

In addition to the implementation of new tools for the accurate identification of these non-culicid Nematocera, the study of the factors causing their spread across new areas and the importance of surveillance of arthropod-borne pathogens and their vectors in the implementation of more adapted and eco-friendly control strategies, require further research in order to fill these gaps in knowledge. In fact, for many species most aspects of their ecology and behaviour remain undefined, which hampers future control efforts. Deciphering these questions requires a One Health approach. Therefore, anticipating, preventing, detecting, and controlling vectors and diseases by combining expertise across multiple fields of study and outreach are essential to make a progress [125].

The aforementioned remarks reinforce the critical need to increase research capacity in the country to address this and other health challenges arising from the presence of hematophagous arthropods, which most probably will arrive in the not-too-distant future. However, in order to sustain and further the advances that were already made, political will must be enhanced and investment in vector research should be indispensably increased.

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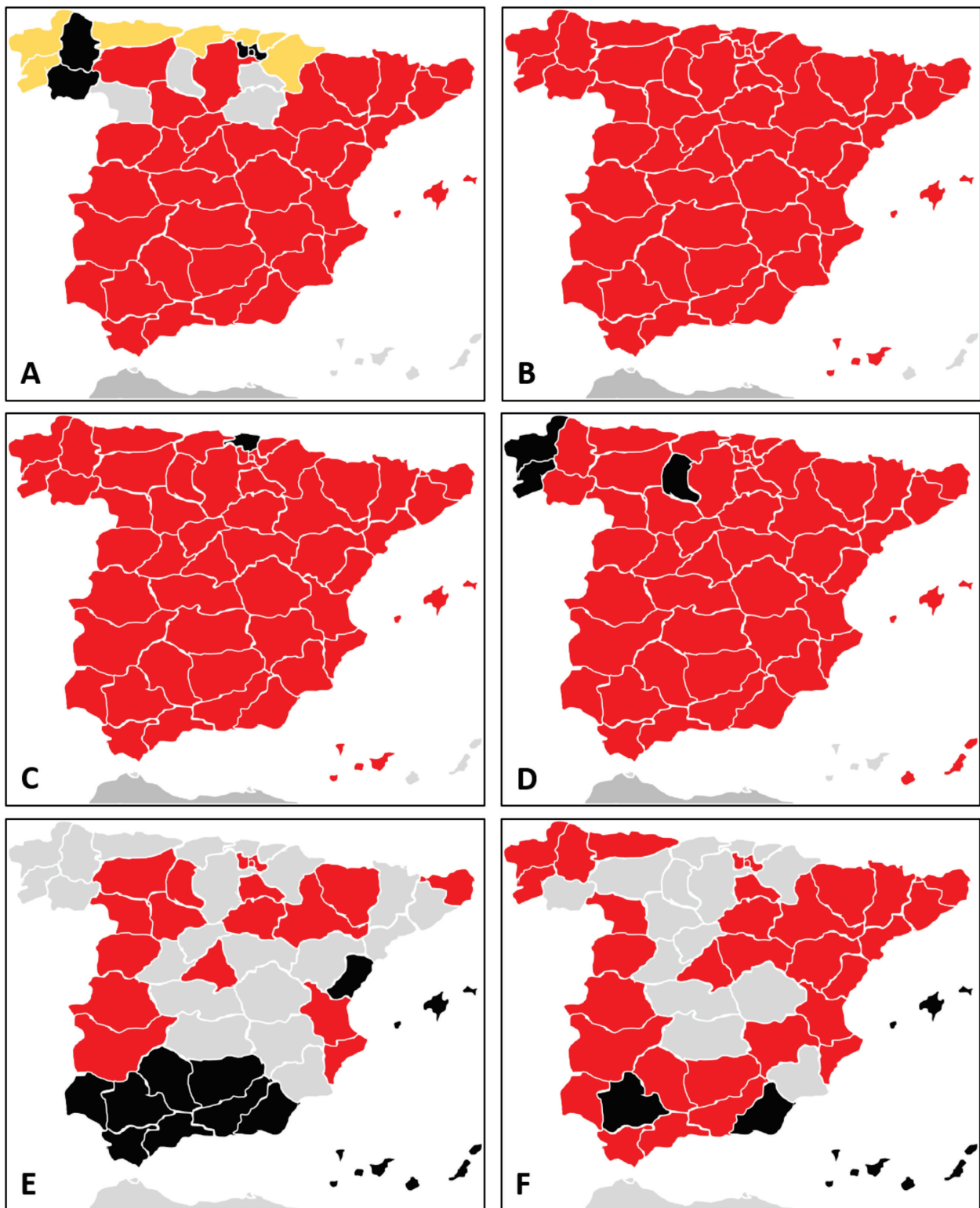
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Annex 1. Distribution maps of the most important vector species of telmophagous Nematocera in Spain. A) *Culicoides imicola*; B) Obsoletus complex (*C. obsoletus* s.s. and *C. scoticus*); C) *Phlebotomus perniciosus*; D) *Phlebotomus ariasi*; E) *Simulium erythrocephalum*; and, F) *Simulium ornatum* s.l. Information retrieved and updated from Bravo-Barriga et al. [68], López-Peña & Jiménez-Peydró [101], ECDC [126] and MARM [127].



Legend: Red = Present; Orange = Sporadic presence; Black = Absent; Grey = No data or unknown.

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