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FIRST RECORD OF *CULICOIDES PARADOXALIS* RAMILO & DELÉCOLLE, 2013 (DIPTERA, CERATOPOGONIDAE) IN SPAIN

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ABSTRACT

The ceratopogonid *Culicoides paradoxalis* Ramilo & Delécolle, 2013 is recorded for the first time in Spain based on reliable morphological evidence according to the previous descriptions of other authors. A total of 438 females (349 nulliparous and 89 parous) and a single male were collected with CDC miniature light traps at three different livestock-associated locations in Extremadura Autonomous Community (Spain) in 2014. Most specimens were captured between June and August, suggesting a univoltine pattern for this species extended over summer and early autumn. Although the number of collections of *C. paradoxalis* is low in comparison with the dominant species, the occurrence of this species in monitoring surveillance programs should deserve specific attention in order to estimate the accurate ratio of potential vectors unmistakably. Interesting information about the period of flight and illustrated morphological features are presented for *C. paradoxalis* in the current paper.

Key words: Biting midges; Diptera; Ceratopogonidae; *Culicoides paradoxalis*; new record; Extremadura; Spain.

RESUMEN

Primera cita de *Culicoides paradoxalis* Ramilo & Delécolle, 2013 (Diptera, Ceratopogonidae) en España

Se cita por primera vez en España el ceratopogónido *Culicoides paradoxalis* Ramilo & Delécolle, 2013, basándose en evidencias morfológicas de acuerdo a las descripciones previas de otros autores. Un total de 438 hembras (349 nulíparas y 89 paras) y un macho se recolectaron con minitrampas de luz CDC en tres localidades ganaderas en la Comunidad Autónoma de Extremadura (España) en 2014. La mayor parte de los especímenes fueron capturados entre junio y agosto, mostrando un único período de vuelo que se extendió durante todo el verano y principios del otoño. Aunque el número de capturas de *C. paradoxalis* es reducido en comparación con los *Culicoides* dominantes, la aparición de esta nueva especie merece especial atención en los programas de vigilancia entomológica con el fin de estimar inequívocamente la proporción exacta de vectores potenciales. Se presenta en este artículo información de interés sobre el período de vuelo así como fotografías de las características morfológicas de *C. paradoxalis*.

Palabras clave: jejenes; Diptera; Ceratopogonidae; *Culicoides paradoxalis*; nueva cita; Extremadura; España.

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Introduction

Culicoides Latreille, 1809 biting midges (Diptera, Ceratopogonidae) cause a significant biting nuisance to livestock and humans and are the biological vectors of a wide range of internationally important pathogens of both veterinary and medical importance (Harrup *et al*., 2015). Moreover, certain species have become notorious for this activity because of their vast population density and persistent biting attacks, shaping public perception of the genus in many regions including northern Europe (Carpenter *et al*., 2013). In Spain, outbreaks of African horse sickness (AHS) (1966, 1987-1990), bluetongue disease (1956-1960, 2000-today) and more recently Schmallenberg virus (SBV) (2012) have led to a substantial economic damage into the livestock industry in past decades (Pérez de Diego *et al*., 2014), with mortality, loss of production, trade restrictions and expensive surveillance and vaccination programs.

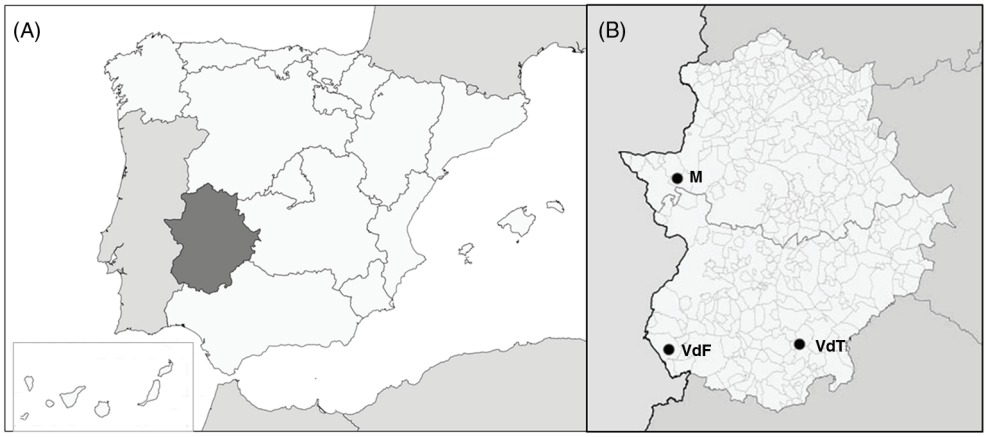
Since the first studies made by Strobl (1900) until today, there have been many contributions to the knowledge of these insects in our country. However, there was a turning point during the eighties, at which studies begun to be focused on the vectorial role (Mellor *et al*., 1983), leaving aside the purely faunal aspect of the past. Undoubtedly, the great veterinarian interest of *Culicoides* regarding the transmission of arbovirosis with an impact on livestock is the main reason why knowledge of these arthropods has advanced so much in recent years. The arrival of bluetongue virus (BTV) outbreaks in the new century contributed to an increased number of studies, and some incorporated the use of molecular techniques for the identification of certain closely related species (Ventura *et al*., 2005). To date, there are 81 species of *Culicoides* reported in Spain, divided in 9 subgenera, a miscellaneous group and a *nomen dubium* (Alarcón-Elbal & Lucientes, 2012). Thanks to this new finding the number of species rises to 82 and 28 in Spain and the Autonomous Community of Extremadura respectively (Sánchez Murillo *et al*., 2013).

Adult *Culicoides* are notable for their characteristic wing pigmentation pattern and distribution of wing macrotrichia, which can be used in most species as the main diagnostic features (Rawlings, 1996; Mathieu *et al*., 2012). Although the study of wing pattern is indeed the main tool in the taxonomy of the genus, identification based on morphological features is sometimes difficult because several cryptic species and species complexes are present (Pagès *et al*., 2009). In the particular case of subgenus *Culicoides*, taxonomy is still confused and observation of distinct intraspecific morphological variation has led to record of morphological cryptic diversity (Meiswinkel *et al*., 2004; Gomulski *et al*., 2006; Pagès *et al*., 2009; Muñoz-Muñoz *et al*., 2011). Therefore, the identification of these cryptic specimens to species level, or identification of other species, requires more detailed morphological study or even the use of molecular techniques (Nolan *et al*., 2007). Knowledge of which species could act as disease vectors, as well as their correct identification, remains essential to assess the real risk for disease transmission into disease-free areas (Augot *et al*., 2013).

In this contribution, we report the species *Culicoides paradoxalis* Ramilo & Delécolle, 2013, a new species recently described in France and Portugal (Ramilo *et al*., 2013), for the first time in Spain. At different localities, authors have collected significant numbers of *C. paradoxalis* specimens, which are morphologically close to *Culicoides lupicaris* and *Culicoides newsteadi*, but might be separated based on marked morphological observations. This work has as main objectives a) facilitate the reliable identification of *C. paradoxalis* based on morphological features observable under stereomicroscope (40x magnification) and b) to improve the knowledge of this species in terms of flight activity and gonotrophic status.

Material and methods

The Autonomous Community of Extremadura has been one of the regions in Spain most severely affected by bluetongue epidemics (Pascual-Linaza *et al*., 2014). Framed within a national surveillance program for BTV in Extremadura, CDC miniature light traps (Model 512; John W. Hock Company, Gainesville, Florida, USA) were used to capture adult *Culicoides* during 2014 in previously selected farms. A total of four locations (Alcollarín, Valencia de la Torres, Membrío and Villanueva del Fresno) were studied for *Culicoides* collection in the two provinces of Extremadura (mid-west, Spain). Only three locations were positive in capturing *C. paradoxalis* (Fig. 1A & 1B). Specimens of *Culicoides* were collected in a reusable plastic jar suspended below the fan of the trap, half-filled with water/ethanol (50%) and 1,2-propanodiol (50%) as an odorless collecting liquid. Light traps suspended near livestock farms were left overnight (set at dusk and retrieved at dawn), once a week. Adults were preserved in ethanol and subsequently cleaned, dissected and mounted on microscope glass slides using Hoyer's medium (50 ml of distilled water, 30 g of gum Arabic, 200 g chloral hydrate and 20 ml of glycerin) following the techniques described by González & Goldarazena (2011). Morphological identification was performed following the descriptions of Ramilo *et al*. (2013). Taxonomic features were photographed with a compound microscope Nikon Eclipse 80i coupled to a Canon Power Shot S-50 camera.



PROVINCE	•	MUNICIPICALITY	COORDINATES	HEIGHT	TOTAL
Cáceres	M	Membrío	29S 6646184379987	365	26♀♀
Badajoz	VdF	Villanueva del Fresno	29S 6637814258754	280	102♀♀ + 1♂
	VdT	Valencia de las Torres	30S 2418984257676	479	310♀♀

Fig. 1.— Study area, A) Autonomous Community of Extremadura and, B) sampling sites with presence of *Culicoides paradoxalis*. Data about locations are also added in the table included: UTM coordinates, height above sea level and detailed number of catches.

Fig. 1.— Área de estudio, donde A) Comunidad Autónoma de Extremadura y, B) puntos de muestreo con presencia de *Culicoides paradoxalis*. Se proporciona información sobre las localizaciones en la tabla incluida: coordenadas UTM, altura sobre el nivel del mar y número detallado de capturas.

Results and discussion

A total of 438 females and a single male were collected and voucher specimens were stored in the arthropods collection of the Parasitology Department, Regional Animal Health Laboratory of Extremadura (Spain).

In order to facilitate a quick identification of *C. paradoxalis* without needing to prepare slide-mounted specimens, authors suggest three anatomical characteristics which are species-specific for *C. paradoxalis* (Fig. 2A) to be distinguished from other sibling species of *Culicoides* subgenus, a) based initially on their unique wing pattern (Ramilo *et al*., 2013). The pigmentation of *C. paradoxalis* wing covers a large area of the wing (Fig. 2B) in comparison to morphologically related species (*C. lupicaris* and *C. newsteadi*). Anal cell and central part of the wing (medial cells) are quite covered by pigmentation, characteristics that usually occur in some members of subgenus *Oecacta* (e.g. *Culicoides catarneii*). Other features are required for a clear separation from *C. lupicaris* and *C. newsteadi*, such as b) absence of spine on the fourth tarsomere of mid legs (Fig. 2C), and c) sensory pits (clavate organs) of third segment of palp (Fig. 2D). As Ramilo *et al*. (2013) illustrated, the third segment of palpus shows a unique large, moderately deep depression with limited borders, which is markedly different from *C. newsteadi* and *C. lupicaris*.

Later species having individual depressions, giving an appearance of irregular borders that corresponds to small groups of shadow sensory pits.

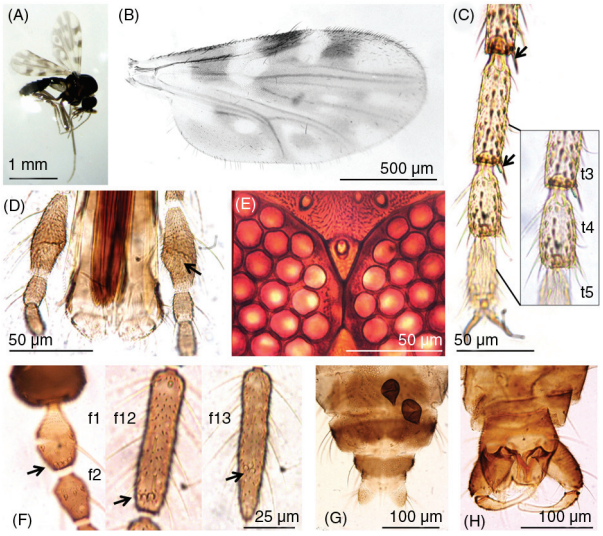


Fig. 2.— *Culicoides paradoxalis*, A) adult female, B) female wing pattern, C) tarsomeres 3 (t3), 4 (t4) and 5 (t5) of mid leg, arrows denote the spines, D) mouth parts and palpus, arrow denotes sensorial depression, E) eyes, F) antennal flagellomeres in female: 1 (f1), 2 (f2) and in male antennae: 12 (f12) and 13 (f13), arrows denote detail of sensilla coeloconica, G) female genitalia and, H) male genitalia.

Fig. 2.— *Culicoides paradoxalis*, donde A) hembra adulta, B) patrón alar femenino, C) tarsómeros 3 (t3), 4 (t4) y 5 (t5) de la pata medial, donde las flechas indican las espinas, D) piezas bucales y palpos, la flecha indicando la depresión sensorial, E) ojos, F) flagelómeros antenales de hembra: 1 (f1), 2 (f2) y de macho (f13), donde las flechas indican las sensilas celocónicas, G) genitalia femenina y, H) genitalia masculina.

There are other additional anatomical features that might be diagnostic. However, they are quite similar across other species of the subgenus. Eyes contiguously joined for a short distance (1-2 facets), a characteristic with high intraspecific variation across specimens (Fig. 2E). Four sensilla coeloconica on flagellomere 1 were observed in most of the specimens studied (Fig. 2F). Female genitalia have two functional pyriform spermathecae, subequal with a small neck, plus one rudimentary spermatheca with digital shape (Fig. 2G).

Males are typically rare in field collections (González, 2014), and their identification is usually based on genitalia supported by wing pattern. A single male of this species was collected with genitalia characteristics compatible with *C. paradoxalis* (Fig. 2H), as Ramilo *et al*. (2013) pointed out. Additionally, sensilla coeloconica distribution differs between species: *C. paradoxalis* has sensilla coeloconica on flagellomeres 1, 12 and 13 (Fig. 2F); *C. newsteadi* and *C. lupicaris* on flagellomeres 1, 11-13. The presence of sensilla coeloconica on flagellomere 11 is polymorphic for *C. newsteadi* and *C. lupicaris* (Delécolle, 1985).

Regarding seasonal collections, most European species produce between one generation (univoltine) and several generations annually (multivoltine) (Sarto i Monteys & Saiz-Ardanaz, 2003; Miranda *et al*., 2003; González *et al*., 2013a, 2013b). Seasonal dynamics of *Culicoides* are determined by climatic factors, mainly rainfall and temperature, depending on each species, but also by chemical characteristics of breeding sites (Uslu & Dik, 2010; Venail *et al*., 2012). *Culicoides paradoxalis* displayed a high peak of activity with maximum abundance in July and declined during early autumn (Fig. 3). This suggests a univoltine pattern for this species in contrast to *C. lupicaris*, which is a bivoltine species with peak activity from May to August and from October to November in the Basque Country region (González *et al*., 2013a). These seasonal dynamics have also been observed for *C. pulicaris* in Sardinia (Foxi *et al*., 2011) and the Basque Country (González, 2014), where the species shows a first generational peak (late April/early May), on average larger than the second one (late summer/early autumn). In Extremadura, in contrast, *C. pulicaris* shows a single peak of activity in June and July, according to current and historical data (Sánchez Murillo *et al*., 2011).

Gonotrophic status examination revealed that 74,5% were nulliparous and 25,5% parous, with no specimens belonging to gravid or blood-engorged stages. The high number of nulliparous females is characteristic of the summer season when more midges emerge (Lysyk, 2007). The estimation of the physiological stages in a population is important for various reasons, particularly because the determination of the parous gonotrophic stage in epidemiological studies serves to give an idea of the potential capacity of these midges for transmitting virus (EFSA, 2008) as well as an indicator of the population age structure (Mullens & Schmidtmann, 1982).

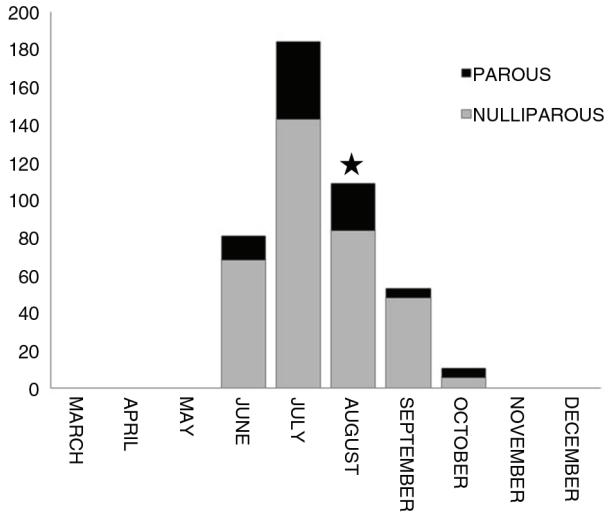


Fig. 3.— Annual flight activity of *Culicoides paradoxalis* in Extremadura. Bars correspond to total captures gathered monthly in the three sampling sites over 2014 and black star represents the single male collected.

Fig. 3.— Actividad anual de vuelo de *Culicoides paradoxalis* en Extremadura. Las barras corresponden con las capturas totales mensuales en los tres puntos de muestreo durante 2014 y la estrella representa el único macho capturado.

Culicoides species of subgenus *Avaritia* (*Culicoides obsoletus, Culicoides scoticus, Culicoides imicola, Culicoides chiopterus* and *Culicoides dewulfi*) are thought to be the primary vectors of BTV and SBV in the Mediterranean region, based on abundance and host preference, vector competence studies, and isolation or detection of virus in field-collected midges (see Garros *et al*., 2014). Similarly, species of subgenus *Culicoides* are implicated as vectors of BTV (Caracappa *et al*., 2003; Vanbinst *et al*., 2009; Romón *et al*., 2012) and SBV (De Regee *et al*., 2012; Elbers *et al*., 2013; Balenghien *et al*., 2014). In Europe, more recently, some members of subgenus *Culicoides* are increasingly being implicated as suspected vectors, such as *Culicoides punctatus* (Larska *et al*., 2013) and *Culicoides nubeculosus* (Balenghien *et al*., 2014), while *C. imicola* is considered to be the main vector of BTV and AHS in Spain (Calvete *et al*., 2008).

Considering that *C. paradoxalis* is a member of the *pulicaris* group, closely related to species that play a role in BTV and SBV, their accurate identification to species level is crucial in epidemiological investigations, as great differences in vectorial capacity are found even between these species. However, morphological identification of *Culicoides* is considered challenging, requiring significant expertise and is restricted to relatively few entomologists in Europe (Koenraadt *et al*., 2014). Unfortunately, due to the large number of samples that taxonomists and specialists have to face daily, this species has most probably been notoriously

overlooked in field-based studies, because of its resemblance to other members of subgenus *Culicoides*. Re-examination of stored specimens in preparations and/or those preserved in alcohol could provide more details about its presence in other regions of Spain.

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