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Description of *Culicoides paradoxalis* sp. nov. from France and Portugal (Diptera: Ceratopogonidae)

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Abstract

A new species, *Culicoides paradoxalis* Ramilo and Delécolle (Diptera: Ceratopogonidae), is described from specimens collected in France (Corsica and southeast region) and Portugal. This species resembles *Culicoides lupicaris* Downes and Kettle, and can be distinguished from this species and from *Culicoides newsteadi* Austen by its wing pattern, in addition to the absence of spines on the tarsomere 4 of female mid leg. In male, the presence of two appendices on the sternite 9 together with the absence of sensilla coeloconica on the flagellomere 11 is also useful to distinguish these three species. Separation from other members of the *Culicoides* subgenus is confirmed by the analysis of the Cytochrome Oxidase I (COI) mitochondrial marker.

Key words: *Culicoides*, Cytochrome Oxidase I, biting midges, taxonomy

Introduction

The emergence of *Culicoides*-borne viruses in Europe (Bluetongue virus, Schmallenberg virus) (Carpenter *et al.* 2009; Hoffmann *et al.* 2012; Doceul *et al*. 2013) in the past decade has regained interest on these small haematophagous biting insects. Bluetongue disease, a notifiable disease to OIE and European Commission Regulation (EC) n.º 1266/2007, introduces the obligation for the Member States to carry out bluetongue monitoring programs in the restricted zones and surveillance programs outside the restricted zones. These programs must include clinical, serological and entomological components. Therefore, the member states have started to implement entomological surveillance networks on a regular basis over the different territories. In this framework, the national surveillance network in France was set up from 2009 to 2012, collecting on a weekly or monthly frequency, *Culicoides* midges with UV light/suction trap in 160 farm sites, covering the whole continental and Corsica island territory (Balenghien *et al.* 2011). In Portugal, the authorities established a National Entomological Surveillance Program in 2005 (until 2012) covering mainland Portugal, the Azores and Madeira archipelagos (Ramilo *et al.* 2012). Light traps were operated one night per week throughout the year.

The intensive trapping of *Culicoides* across a significant proportion of Europe has not only allowed accurate mapping of geographic distribution and seasonal incidence but has also led to updated species lists being produced for many countries with only limited records of this genus (Meiswinkel *et al.* 2008; Patakakis *et al.* 2009; Ramilo *et al.* 2012). Two group species are widely distributed and abundant in Western Europe, namely the Obsoletus Group, belonging to the *Avaritia* subgenus, and the Pulicaris group, belonging to the *Culicoides* subgenus (Balenghien *et*

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according to region used (Meiswinkel *et al.* 2004; Pagès *et al.* 2009). As an example, phylogenetic tree obtained from ITS-2 sequences shows *C. lupicaris* and *C. pulicaris* (Linnaeus) clustered into the same clade (Meiswinkel *et al.* 2004), whereas phylogenetic tree obtained from COI sequences clusters *C. lupicaris* with *C. impunctatus* Goetghebuer and one form of *C. newsteadi* and *C. pulicaris* into a sister-group of *C. punctatus* (Meigen) (Pagès *et al.* 2009). Moreover, cryptic diversity has also been demonstrated within *C. pulicaris*, *C. fagineus* Edwards and *C. newsteadi* using the COI marker, which remains to be resolved (Pagès *et al.* 2009).

For the current study, *C. paradoxalis* can be distinguished from other species based initially on their unique wing pattern. Females of *C. paradoxalis* can also be differentiated from *C. newsteadi* and *C. lupicaris* by the absence of one spine on the tarsomere 4 of mid legs. The number and disposition of the clavate organs on the segment 3 of palpus are also useful anatomic characteristics to distinguish between these three species. It was observed that, when using COM, some sensilla coeloconica may be overlooked due to the limited resolution of this technique; with SEM the evaluation of the number and disposition of the same sensilla is more straightforward. Although the same pattern was always observed, only two specimens of each species were analyzed using SEM technique. Due to the possibility of intraspecific variation in the number of sensilla coeloconica, this feature must be used with caution. The presence of two to four sensilla coeloconica on the flagellomere 1 is also an important characteristic which may be diagnostic but requires further confirmation across specimens.

In the male, the aedeagus of the three related species are very similar. The two appendices on the sternite 9 for *C. paradoxalis* are absent in the other two species. In addition, the number of sensilla coeloconica differs between species: *C. paradoxalis* has these on flagellomeres 1, 12 and 13; *C. newsteadi* and *C. lupicaris* have these on flagellomeres 1, 11–13. The presence of sensilla coeloconica on flagellomere 11 is polymorphic for *C. newsteadi* and *C. lupicaris* (Delécolle, 1985).

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