

Blackflies (*Simulium* spp.) attacks on humans and animals in Rome and surrounding areas (Central Italy)

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Abstract

Background. Due to their abundance in some environments, the blood-sucking habit and the involvement in the transmission of several diseases, blackflies of the genus *Simulium* (Diptera: Simuliidae) can be considered among the most annoying biting arthropods.

Methods. Following repeated attacks to humans and animals, entomological investigations were carried out in green areas of Rome and surroundings. Site and period of attacks were reported, together with the human and animal reactions to the bites.

Results. Four *Simulium* species have been identified through morphological or molecular analysis: *Simulium intermedium*, *Simulium lineatum*, *Simulium lundstromi* and *Simulium ornatum* (complex). Larval breeding sites were identified in small moats, where a high density of blackflies larvae and pupae was revealed.

Conclusions. Being able to thrive in highly polluted water, even in few mm depth watercourses, some blackfly species are widely distributed in the area and their bites caused mild to severe reactions to humans and animals.

Key words

- Simuliosis
- blood-sucking arthropods
- Sanger sequencing
- DNA barcoding
- cryptic diversity

INTRODUCTION

Blackflies (Diptera: Simuliidae) of the genus *Simulium* are biting midges infamous worldwide for their direct attacks to men and animals and for their role as vectors of pathogens of outstanding relevance in human and veterinary medicine. Biting activity of the females of this genus in man can elicit allergic reactions known as "black-fly fever" or simuliotoxicosis, characterised by itching, haemorrhage and oedema, at times needing medical intervention [1-5]. Similar allergic reactions can occur in bovines, and cattle mortalities due to *Simulium* spp. attacks have been recorded in many European countries and North America [6-8]. Furthermore, animals under attack from blackflies can be considerably irritated, with consequent reduction of feeding and mating activities and damage to production [9, 10]. Wounds provoked by blackflies can facilitate infection by secondary pathogens.

Simulium spp. are vectors of viruses, protozoa and helminths. From a veterinary point of view, the most relevant pathogens they transmit are avian protozoa of the genus *Leucocytozoon*, responsible for leucocytozoonosis, an infection of wild and domestic birds with a Holarctic distribution [11], and filarial nematodes of the genus *Onchocerca*, affecting cattle and equids [12,

13]. To the same genus, it belongs *Onchocerca volvulus*, the greatest public health problem involving blackflies, responsible for human onchocerciasis or river blindness in the tropics. Onchocerciasis is among the more relevant parasitosis affecting man worldwide, with an estimate 17 million people infected in Africa and tropical America [14].

Simulium sp. larvae breed in running water, from snow melting streams to large rivers close to the mouth [15, 16]. Environmental heterogeneity of Italy allows the presence of suitable habitats for a rich fauna of this genus, with 70 recorded species [17]. In our Country, attacks by blackflies to men and animals have been historically recorded in many Regions, with several species involved [18]. Nevertheless, studies regarding these flies are quite scanty in Italy, with few, old reports of attacks [18] and studies dealing with Simuliidae as indicators of river environmental quality [15]. These insects, despite their relevance, are rather unfamiliar to most people, hence problems related to *Simulium* sp. attacks to humans and animals go often unnoticed or unreported and the responsible of the problem unidentified.

A problem due to *Simulium* sp. attacks to people living near Rome was first brought to the attention of the Authors in 2013, when *S. intermedium* (*ornatum* group

[19]), was responsible of repeated attacks to people living in a villa close to the village of Castel Gandolfo, 15 km southeast from Rome. From then on, many other cases have been brought to our attention, regarding both man and animals. We here describe in detail these cases and, when possible, the larval breeding sites.

MATERIALS AND METHODS

Study area and specimens collection

Samples were collected in the southeast of Rome urban area and around the Lake Albano (22 km south-east from Rome city centre) from 2013 to 2021. The six cases summarized hereafter concern people that contacted the Laboratory of Entomology of the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana “M. Aleandri” (IZSLT) and brought to our attention their problems related to human or animals attacked by unidentified insects. Following these reports, surveys were carried out to identify the species involved in the attacks and the putative larval breeding sites. Adult blackflies were sampled using an entomological net and an aspirator or collected using traps of the model Italian Mosquito Trap (IMT) (a modified CDC light trap) lured with 1 kg of dry ice (CO₂). Larvae were sampled from stones and water vegetation during watercourses inspection. Samples were stored in vials with 70% ethanol and 5% glycerol until molecular identification. When molecular identification was not possible, larvae and adult specimens were morphologically identified [20]. Blackflies species identification often relies on a holistic approach, where morphological characteristics of eggs, larvae, pupae, males and females are used. Hence, molecular analysis was performed whenever the morphological identification of black flies posed critical issues.

Molecular identification

Adult blackflies were treated with lysis buffer and genomic DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Molecular specific identification was conducted with barcode sequences obtained by amplification of a 710 bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI). The polymerase chain reactions (PCRs) were performed using the Folmer primers (LCO1490 and HCO2198) [21]. PCR product underwent an enzymatic clean-up ExoSAP-IT and amplicons were Sanger sequenced on a 3500 Series Genetic Analyzer with BigDye Terminator chemistry (Applied Biosystems, USA) using the same primers. Sequence data analysis and trimming was performed using the CLC DNA workbench software version 5.7.1. The resulting sequences were compared with the sequences deposited in GenBank using BLAST [22].

RESULTS

All the inspected sites resulted positive for the presence of *Simulium* sp. (Figure 1). In five cases (only exception: case 3) identification to species level was possible, highlighting the presence of *S. intermedium*, *S. lineatum*, *S. lundstromi* and *S. ornatum* (complex).

Cases description

1. *Attacks to people (Castel Gandolfo, Rome; June 2013)* – Attacks by blood-sucking midges occurred in a country house, 15 km southeast from Rome (350 m asl). People were attacked while in the garden of the villa in daytime. Bites involved mainly legs, provoking a considerable discomfort. During a survey, blackflies flying around the legs of the Authors and resting in the hedge bordering the garden were sampled. The specimens were morphologically identified as *S. intermedium*, within the *ornatum* group. In the proximity of the villa, no possible larval breeding site was identified, the closer stream being that described in the following case.

2. *Attacks to dogs (Marino, Rome; 2018-2021)* – In October 2018, the owner of a dog training field situated 1 km apart from the country house of case 1 (330 m asl) brought to IZSLT some specimens of small biting midges that were seriously threatening his work of dog trainer, due to massive attacks to the dogs in the training field. Some of the dogs were so seriously bitten and presented so severe consequences, even with a case of systemic shock, that owners refused to bring them again for training, thus impeding the working activity. The trainer referred about swarms attacking dogs simultaneously, mainly to legs and belly, in full daylight. The attacks went on also during winter, whenever sunshine warmed up the lawn. During a survey at the end of October 2019, it was possible to ascertain the presence of active blackflies in the lawn and swarms around nearby reeds. No larval breeding site was identified in the proximities of the training field. During subsequent surveys (March and April 2021), larvae were collected and pupae observed inspecting two sites along a nearby

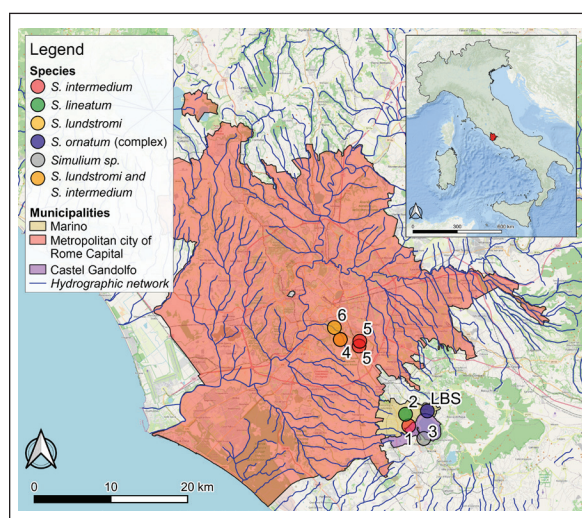


Figure 1

Map of the reported cases of *Simulium* spp. attacks to men and animals. Numbers indicate the cases on the Alban Hills (1-3) and within the Rome urban area (4-6). Blue lines indicate the hydrographic network downloaded from SINANET, the Environmental Information Systems developed and managed by ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale, www.sinanet.isprambiente.it/it). Blue circle indicates the larval breeding site (LBS) highlighted in Marino municipality (Pietrare moat).

small moat, described in *Larval breeding sites* section. Ten adult blackflies were collected with an entomological net and an aspirator while flying and alighting on one of the Authors in the dog-training field. Molecular analyses allowed identifying adults as *S. lineatum*, within the *equinum* group, and larvae from the nearby moat as *S. ornatum* (complex).

3. *Attack to cattle (Castel Gandolfo, Rome; August 2019)* – A veterinary managing a cattle dairy farm at the fringe of the village of Castel Gandolfo (426 m asl) referred of insects heavily bothering free-ranging young cattle and provoking open wounds, possibly favouring papilloma-virus infection. All the cattle in fact presented lesions provoked by this virus, first appeared three years before, concurrently with the first attacks by these biting insects. From then on, insects were always present at the farm and papillomatous lesions, regarding mainly teats, became more frequent and serious. During a survey, many specimens of *Simulium* sp. were sampled while feeding on cattle. Biting activity almost exclusively concentrated along the ridge of the belly. Cattle resulted to be bothered by the presence of these insects and very brittle. During the survey it was not possible to identify any larval breeding site and no potential breeding sites were highlighted, neither studying satellite images of the zone. Unfortunately, DNA amplification did not yield any results due to bad conditions of the specimens sampled in this locality, neither it was possible to carry out additional surveys in this farm.

4. *Attack to golfers (Appia Antica, Rome; October 2019, July 2021)* – The Local Health Authorities reported to the Authors that since three years people playing golf in a club within Rome urban area (40 m asl) were occasionally attacked by small insects, mainly at the ankle. The bites provoked serious reactions to two women, one of them, presenting systemic allergic reaction with glottis oedema, required medical intervention. The Almone River, known as producing blackflies [15], runs through the golf course, nearby the site where the attacks occurred. Golfers reported further attacks in the late afternoons of July 2021, especially on legs and ankles, even if wearing long trousers. Two specimens were

caught with an IMT in July 2021 and identified as *S. intermedium* and *S. lundstromi* by molecular analysis.

5. *Nuisance to people (Parco degli Acquedotti, Rome; autumn 2019, spring 2021)* – Two kilometres away from the golf course of case 4 (60 m asl), nuisance to people caused by biting midges was reported in a green area, the Parco degli Acquedotti. During a survey in November 2019, adult *Simulium* sp. were sampled using an entomological net and an aspirator. A second survey, aimed at looking for breeding sites was carried out in April 2021. Blackfly larvae and pupae were found in a moat and a ditch, respectively inside and outside the Park. In late spring 2021, further attacks to people doing gymnastics were reported to the Authors. The collected larvae, from both watercourses, were identified by molecular analyses as *S. intermedium*.

6. *Attacks to people (Caffarella Park, Rome; summer 2020)* – Around 1 km away from the golf club of case 4 (20 m asl), a couple reported attacks at ankles and lower legs in a large grassy area of the Caffarella Park. They referred the presence of flying midges around them. They both complained by the attack but apart from small wheals with a haemorrhagic centre and an intense pruritic condition, no further reaction occurred. The attacks occurred from June to the end of July, 100 m from the Caffarella moat and 260 m from the Almone river. The event was reported to the Authors and a survey was carried out in March 2021 to identify the larval breeding site (see *Larval breeding sites* section). The collected larvae were identified by molecular analyses as *S. lundstromi*.

Larval breeding sites

Survey nearby cases 1, 2 and 3 – A larval breeding site of *S. ornatum* was identified along a moat running through the villages of Marino and Ciampino (southeast from Rome, 340 m asl) (Table 1). The moat was investigated looking for the breeding sites of the species responsible for the attacks to people, dogs and cattle, respectively of cases 1, 2 and 3. In two inspected sites along the watercourse, even where the water was only few millimetres deep, several larvae at different developmental

Table 1
Summary of the surveys data together with the results on species identification. Identification was carried out by means of molecular analysis (*) or morphological identification (**). Details of the larval breeding site (LBS) of Pietrare moat (Marino municipality) were here reported due to its proximity to three cases (cases 1-3)

Case N.	Coordinates	Species	Collected (c) or observed (o) life stage	Coverage (%)	Identity (%)
1	41.75009 N, 12.63610 E	<i>Simulium intermedium</i>	Adult** (c)	NA	NA
2	41.76364 N, 12.63099 E	<i>Simulium lineatum</i>	Adult* (c)	100	99.67
3	41.73533 N, 12.65963 E	<i>Simulium</i> sp.	Adult (o)	NA	NA
4	41.85269 N, 12.53391 E	<i>Simulium intermedium</i>	Adult* (c)	100	100.00
		<i>Simulium lundstromi</i>		100	96.42
5	41.84304 N, 12.55833 E	<i>Simulium intermedium</i>	Adult (o), larvae* (c)	100	99.84
5	41.84866 N, 12.55936 E	<i>Simulium intermedium</i>	Larvae* (c)	100	99.73
6	41.86461 N, 12.51960 E	<i>Simulium lundstromi</i>	Adult (o), larvae* (c), pupae (c)	98	97.12
LBS (nearby cases 1-3)	41.76744 N, 12.66524 E	<i>Simulium ornatum</i>	Larvae* (c) and pupae (o)	100	98.87

stages and pupae were observed, despite the high level of pollution due to urban sewages [23]. Larvae and pupae were attached to stones and construction debris, as well as to the rocks of the riverbed.

Survey nearby cases 4 and 6 – During a survey in March 2021, two streams were identified as larval breeding sites for *S. lundstromi* in the Caffarella Park: the Almone river and the Caffarella moat. Both watercourses are perennial and present medium to bad ecological conditions, considering both the Extended Biotic Index [24] and river functionality index [25], as reported by [26]. The survey was carried out nearby the site where the attacks to humans occurred in 2020. Larvae and pupae were collected from different substrates: stones and clay roof tiles on the riverbed and plant stems, mainly cane (*Arundo donax* L., 1753). *S. lundstromi* larvae were found in both watercourses, even where the moat was only few centimetres deep. Inspecting the underwater surface of few cane stems in the Almone river, up to 50 cm length of the stems were covered by *S. lundstromi* pupae, with a density of ~10/cm².

Survey nearby case 5 – The last survey, carried out inside and outside the Acquedotti Park, confirmed two watercourses as larval breeding sites of *S. intermedium* (Table 1), both tributaries of the Almone river [27]. Both watercourses are characterized by bad ecological conditions and polluted water, considering their Extended Biotic Index and the low diversity value of the macroinvertebrate community [23].

DISCUSSION

All identified species were previously reported from Rome urban area and surroundings [28]. The species belonging to the *ornatum* group are well known for their attacks to people in Italy [29, 30]. On the other hand, this is the first report of attacks to people by *S. lineatum*. Given the proximity of *S. lundstromi* larval breeding sites to the areas where attacks to people occurred in the Caffarella Park, this species is the putative responsible of the nuisance described in case 6. Nevertheless, an analysis of adult biting blackflies is still needed to confirm its involvement.

Simulium sp. attacks to people and animals in Rome and the surrounding area seem to have become more frequent in the last few years. Although Authors have been working for 20 years in a public health institution in Rome (IZSLT), point of reference for sanitary entomology for people and other health institutions, only since the last few years people experiencing problems related to blackflies contacted them. Indeed, since the first case in 2013, these requests became more frequent. These findings suggest that further ecological investigations could be useful to pinpoint potential imbalances in the aquatic community (e.g., reduction of larvae and pupae predators), which could have led to adults' proliferation. Blackflies are a neglected group of insects, unknown to most part of people and identifiable only by expert entomologists. Hence, presumably many similar cases remain unrecorded. People contacting Authors usually refer to "mosquitoes", "small biting insects", even "grass mites" as being the origin of their problems and only after surveys and accurate study of each case, Au-

thors were able to identify blackflies as responsible of the nuisance. Interestingly, all cases occurred in proximities of watercourses belonging to the same aquifer, part of the Alban Hills hydrogeological unit, and southeast from Rome urban area [23, 27, 31]. The high levels of underground circulation generate groups of peripheral water springs and perennial streams [32, 27], suitable habitats for several *Simulium* species. These watercourses have been subjected to several flow managements during centuries [32], maybe increasing habitat suitability [33]. *S. intermedium*, due to its high ecological plasticity [15, 34], in many cases replaced other *Simulium* species, when environment underwent anthropic changes, such as elimination of trees and hedges from streams margins, disappearance of free-ranging cattle and progressive pollution of waters [15, 18, 29, 35]. Indeed, this is often the dominant group of species in polluted, eutrophic streams, with basins dominated by agricultural or urban use and it has been already reported in literature as the dominant group in Lazio Region [15, 36]. *S. ornatum* (complex) resulted one the most frequent species in urban areas and in highly polluted waters [15], but it is also present in less disturbed watercourses, where can be associated with *S. lineatum* (*equinum* group). Our findings confirm the extreme ecological plasticity of this species group, capable to thrive even in highly polluted watercourses. Our results confirm the punctual distribution of *S. lundstromi* [28] and its preference for plain watercourses [20] and the wider distribution of *S. intermedium*, present in both plain and hilly sites. Among the species-groups and species present in Central Italy, *S. ornatum* group and *S. intermedium* in particular have been frequently reported as biting people, at time being very aggressive and causing considerable nuisance [29, 34, 37]. Peculiar biological trait of this group of species is the attack to the lower body parts of the victims, confirmed in our reports, where dogs and cattle were attacked to the belly and people mostly at legs and ankles. Blackflies control is a hard target to achieve and is possible, although difficult, only by means of microbial insecticide on larval stages, usually *Bacillus thuringiensis* var. *israeliensis* [38, 39]. Given the low effectiveness of larval breeding sites treatments, which need to be frequently repeated in lotic watercourses, it was very difficult for the Authors to give any suggestion to face the problem. In the case of the dog-training field, it was suggested to cut reeds along the border, given that this vegetation could act as a visual cue attracting flying *Simulium* individuals. It cannot be excluded that flying individuals, maybe originating from the Pietrare moat (1 km away), might have been attracted by the long row of reeds as indicator of the possible presence of a moat and, once landed on the reeds, started attacking dogs. In the case of the golf course, due to the limited number of players complaining for the nuisance, club manager considered not necessary any kind of intervention. In this case, Authors gave indications to the interested persons about individual protection measures, such as the use of repellents and of long trousers and sockets when playing on the course. Finally, in the Caffarella and in the Acquedotti Parks, where the larval breeding sites were identified, considering the limited number of people

complaining for blackflies attacks and the environmental value of the two parks, no control treatments were performed. Even if not demonstrable, very interesting is the possibility that blackflies bites were the point of entry of papillomavirus in cattle (case 3). In this case, even though indirectly, besides being a nuisance, blackflies would have provoked a real damage to the animals and to farm production. Indeed, papillomatous lesions were in some cases so severe to prevent the milking and some of these animals had to be removed from the herd.

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Authors' contributions

Conceptualization, FR, AM and CDL; entomological surveys, FR, DS, AM and CDL; molecular analysis, ELD and MI; writing-original draft preparation, FR, ELD, MI and CDL; writing-review and editing, FR

and CDL. All Authors have read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding Author, FR, upon reasonable request.

Conflict of interest statement

The Authors declare that they have no competing interests.

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