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First Report of *Culicoides* Biting Midges (Diptera: Ceratopogonidae) Attacking People in Italy, With the Description of Extreme Larval Breeding Sites and Diurnal Activity of *Culicoides riethi*

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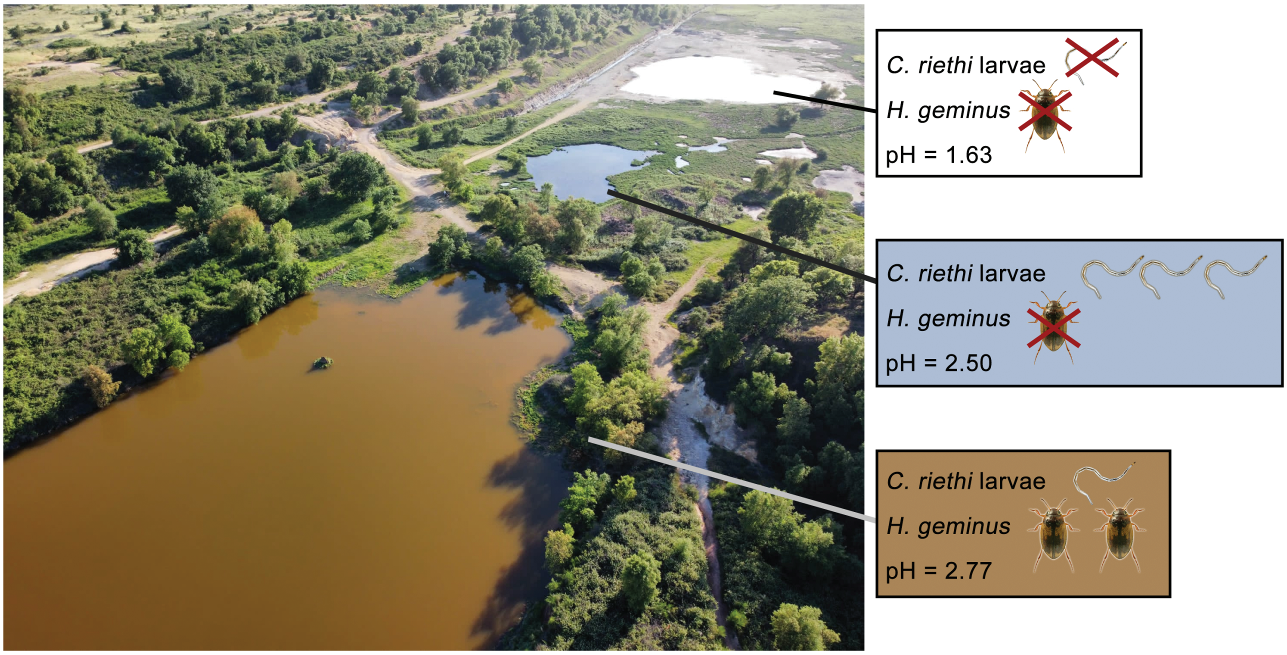
Abstract

Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) play a paramount role in medical and veterinary entomology worldwide, particularly as vectors of pathogens which cause animal diseases. Biting midges are also infamous for the nuisance they provoke to people involved in outdoor activities. Nonetheless, attacks to man by midges from any *Culicoides* species have not been reported in Italy. An entomological investigation was performed following repeated attacks to man in a nature park near Rome (central Italy). The study area is a natural degassing zone, characterized by widespread hazardous gas emissions of CO_2 and H_2S, with several water bodies including permanent lakes, ponds, and pools. The biting midge *C. riethi* Kieffer, 1914 was very active during daytime in the period April–June. The species has been identified as responsible for attacks on people in the area. An in-depth analysis of the extreme environmental conditions revealed the ability of larvae to thrive in several water bodies, characterized by an extremely low pH and a high concentration of sulfates.

Graphical Abstract



View of the area from WB_b (white lake top right) to WB_d with reported the pH value of each water body (WB), the presence/absence and density of *Culicoides riethi* larvae and *Hydroglyphus geminus* along the pH gradient. Larval density is highest where pH levels do not allow the development of its possible predator.

Key words: biting midges, breeding sites, Ceratopogonidae, nuisance, extreme environment

Worldwide, biting midges in genus *Culicoides* (Diptera: Ceratopogonidae) play a paramount role as vectors of viruses of veterinary relevance, such as bluetongue, African horse sickness, and Schmallenberg viruses (Linley et al. 1983, Elbers et al. 2013, Mullen and Murphree 2019, Purse et al. 2015). In addition, they are also infamous for the nuisance they provoke to people, causing economic hampers to touristic and outdoor activities (Hendry and Godwin 1988). Problems linked to *Culicoides* spp. attacking people are reported from tropical and temperate areas of the world (Linley and Davies 1971, Hendry 2011, Carpenter et al. 2013). Particularly, *Culicoides impunctatus* Goetghebuer, 1920 in Scottish Highlands, represent a major problem for tourism and outdoor activities (Blackwell and Page 2003). In Italy, episodes of attacks on people by midges of the genus *Culicoides* have never been reported or described in literature.

Preimaginal stages of biting midges are able to thrive in several aquatic and ground habitats (Sprygin et al. 2015). Examples include lakes, ponds, thermal water bodies, temporary swamps, swampy meadows, rotting fruits, and decaying organic substrates of animal origin (Snow et al. 1957, Breeland 1960, Williams 1964, Smith 1965, Glukhova 1989). *Culicoides* (*Monoculicoides*) *riethi* Kieffer, 1914, is a mammofilic species with a widespread Palearctic distribution, not proven biological vector of any disease agent (Purse et al. 2015). This species has been reported once as responsible of attacks to people (Sprygin et al. 2015).

The present paper reports the results of an entomological investigation following repeated biting midges' attacks on people in a natural area nearby Rome (central Italy). Identification of the responsible species as *C. riethi*, insights on larval breeding sites and diurnal activity of this species are reported, with the description of extreme environmental conditions.

Materials and Methods

The attacks to people by biting midges occurred in the Solforata of Pomezia (Rome Municipality) and were reported to the entomologists of the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri" (IZSLT) in April 2021. Park visitors claimed they were swarmed by midges, which bit them viciously. The attacks occurred in full daylight and were so intense to impede leisure outdoor activities. The reaction consisted in localized itching and burning sensation, which lasted few hours. Following this report, IZSLT entomologists investigated the issue, in order to identify the responsible species.

Study Area

The Solforata of Pomezia (41.705118°N, 12.537676°E) is known for its gas emissions due to geothermal activity and the presence of natural sulphurous waters (Minissale et al. 1997) (Fig. 1A). The morphology in the area has been greatly modified by human activity, with the formation of artificial small lakes and seasonal ponds due to mining excavations (Carapezza et al. 2012). Considering both natural ponds and artificial lakes, five water bodies (WBs) can be identified in the area, hereafter named from WB_a to WB_e (Fig. 1B). Gas manifestations consist of high CO_2-rich emissions (97.6 %), with minor content of N_2 (1.28 %) and H_2S (1.07 %), discharged mainly from WB_b and a nearby watercourse (Minissale et al. 1997). People

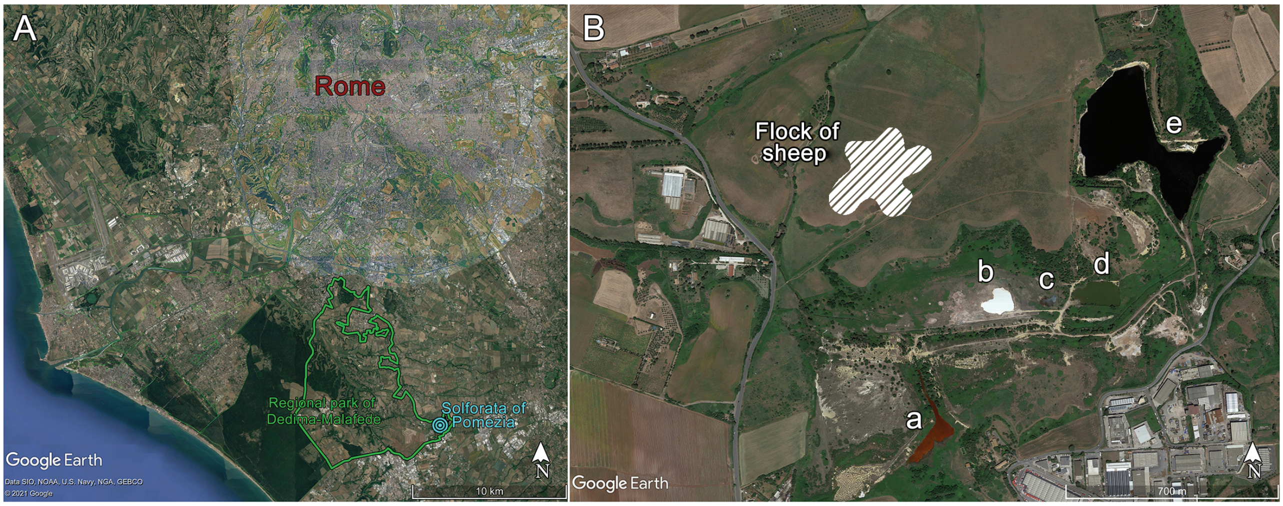


Fig. 1. Satellite imagery. In (A) the regional park of Decima-Malafede, southern of Rome, with highlighted the location of Solforata (bottom right circle); in (B), identified by lower case letters, the five WBs present in the Solforata area: the red lake and the white lake (WB_a and WB_b), two small lakes (WB_c and WB_d), and the inundated basin of the old mining zone (WB_e). See Carapezza et al. (2012) for the CO_2 and H_2S soil flux maps of the area.

frequently visit the Solforata, being a few kilometers apart from Rome, inside the Decima-Malafede regional park. A swampy zone with plenty of small pools extends from WB_b to WB_d and is characterized by the highest concentration of CO_2 and H_2S. Particularly, WB_b has no vegetation on its banks, fine coatings and crusts of sulphur and sulphates, and several gas bubbling vents (Voltaggio and Spadoni 2009, Carapezza et al. 2012). The other lakes have lower concentrations of sulphur and are surrounded by riparian flora characterized by *Agrostis canina*, well adapted to these extreme conditions (Miglietta et al. 1993, Barnes et al. 1997).

Sampling Methods

Adult and larval *Culicoides* were sampled in the period April–June 2021. Mud from each WB was collected to investigate emergence of adults. During surveys, midges were collected by human-landing using entomological aspirators, to identify the involved species and to study its presence and biting activity during the day, from dawn to dusk. An Onderstepoort black-light trap was set on 11 June near WB_b, to investigate the potential nocturnal activity of the species. The trap worked from dusk to dawn with favorable weather conditions (minimum temperature ~ 17°C).

Midge identification was performed analyzing female wing pattern, number and duct length of spermathecae, and male genitalia morphology (Mathieu et al. 2010, 2012). Genitalia were cleared in Amman's lactophenol for 24 h, mounted and observed under compound microscope. Collection of larvae was performed in early May from each WB and surrounding ponds, using a dipper. To calculate larval density, 900 ml of water were collected the same day from each WB, wherever at least a swimming larva was observed. Mud samples were collected from the banks of each WB (~50 cc), from the soil below pools and from the banks of a small pond (ϴ = ~2 m) and brought to the laboratory, kept in plastic containers at room temperature under natural L/D cycle and moistened, to allow adults emergence. Sex ratio of specimens emerging in laboratory was then calculated.

To analyze the composition of the benthic community, macroinvertebrates were sampled from each WB by means of kick and sweep technique, employing an aquatic hand-net. A physicochemical characterization of the waters of the five WBs was carried out. Water samples were filtered with a 0.45-µm nylon filter and stored at 4°C in 15-ml polyethylene centrifuge tubes. The total dissolved solids (TDS) were measured at 180°C using a static oven and glass pyrex beaker. The cations (Fe^{3+}, Al^{3+}, Pb^{2+}, Ni^{2+}, Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}) were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES); the anions ($SO_4^{=}$, Cl^-, F^-, Br^-, NO_3^-) were determined by ionic chromatography (IC). The total acidity was determined by manual titration with strong base (NaOH) and the equivalence points were determined either potentiometrically or with visual indicators.

Results

During human-landing surveys, authors observed midges swarming around, alighting and biting. After a clearly visible engorging process, the blood was observed pooling on the skin. One of the authors reported a hypersensitivity reaction with the production of red, slightly elevated areas of swelling that continued to itch for 5 d.

Specimens collected by human-landing were identified as *C. riethi* females, relying on wing pattern and on the elongated shape of the duct of the single spermatheca. The observation of male genitalia, in particular the morphology of the ninth sternite, of specimens emerged in the laboratory confirmed that *C. riethi* was the only found biting midge species.

Regarding diurnal activity, via the human-landing, it was possible to ascertain that *C. riethi* was active throughout the whole day. Attacks occurred in the morning, by midday, in the afternoon, and in the last 2 h prior to the sunset. Only 15 specimens were caught by means of Onderstepoort black-light trap (12 females and 3 males), even if swarms were observed a few minutes before sunset. The species was widely distributed in the area, since adults were present nearby each WB.

Active larvae were clearly visible swimming in free water at the borders of the WBs, ponds, and pools. With the exception of WB_b, which had the highest sulphur concentration and no vegetation, larvae were present in many small ponds as well as in four out of five WB. The highest larval density was recorded in the WB_c, whereas the lowest density was found in WB_d (Table 1).

Adults failed to emerge from mud samples of WB_a, WB_c, and WB_d. Pupae were not observed and adults did not emerge from the mud of WB_b. Up to 367 midges emerged from 20 cm^3 of mud from

Table 1. Larval density recorded in each WB with relative area calculated from satellite imagery and summary of the physicochemical properties

WB id	No. of larvae/900 ml	WB area (m^2)	pH	Conduct. (µS/cm)	Fixed residue (mg/liter)	mg/liter														meq/liter			Imbal. %
						Fe	Al	Pb	Ni	Na	K	Ca	Mg	SO_4	HCO_3	Cl	F	Br	NO_3	Total acidity	Σ cations	Σ anions	
WB_a	15	756.61	3.10	2,040	1,547	231.10	235.9	2.323	2.083	50.65	56.73	132.5	41.29	2,523	abs	22.9	8.6	abs	15.6	25.3	52.40	53.91272474	−1.4
WB_b	0	319.93	1.63	17,650	15,010	209.40	315.6	2.405	2.125	62.15	81.2	146.9	52.07	7,117	abs	50.9	8.6	abs	9.1	97.5	62.83	150.3060642	−41
WB_c	90	14.37	2.50	2,300	1,744	147.20	187.8	2.460	2.183	54.82	44.88	179.1	47.24	2,098	abs	42.5	13.6	abs	7.4	26.3	45.24	45.7423493	−0.5
WB_d	5	421.96	2.77	5,370	4,073	86.08	192.7	2.460	2.198	69.38	53.11	162.8	39.63	1,968	abs	90.6	7.1	abs	6.9	19.5	41.91	44.0406868	−2.5
WB_e	21	1,799.74	2.27	11,710	9,958	479.50	1,217.0	5.911	5.695	115.8	104.5	282	145.1	7,006	abs	38.8	3.9	abs	9.4	150.9	195.05	147.4097087	14.1

Conduct. = conductivity; Imbal. = imbalance.

the small pond (ϴ = ~2 m) in an 8-d interval, with a 1/1.5 male/female ratio. Mud collected from the pools produced 20 adult midges, in a 1/1 male/female ratio.

The benthic community of each WB was poorly diversified. Besides *C. riethi* larvae, just Chironomidae larvae of the *Chironomus plumosus* complex (Insecta, Chironomidae) and adults of *Hydroglyphus geminus* (Coleoptera, Dytiscidae) were collected from WB_d. Chironomidae and *C. riethi* larvae were also abundant in small ponds and pools around WB_c, where gas bubbling vents occurred. WB_b resulted to be azoic.

Results of the physicochemical water analysis are shown in Table 1. The anion HCO_3^- was absent in extremely low pH conditions. It must be noted the extremely low pH in all WBs, a very high conductivity, a great amount of sulfates and, among cations, of Fe^{3+} and Al^{3+}, that characterize this site as an extreme environment.

Discussion

To the best of our knowledge, this is the first report of attacks to people by midges of the genus *Culicoides* in Italy. Furthermore, peculiar is the occurrence of the attacks during daytime, even in full daylight, considering that previous authors reported a negative relationship between *C. riethi* captures and amount of solar radiation (Walgama and Lysyk 2019). Swarms of *C. riethi* attacked people and authors during field surveys, mainly between 10:00 a.m. and 7:00 p.m. Around midday, the swarms were consistent and active in many different sites within the park, thus highlighting a widespread activity. Some of the visitors claimed that at times the nuisance became so intense it forced them to leave the area. Our surveys confirmed that in the study area and in the period of the attacks, April–June, *C. riethi* behaved as a diurnal species, attacking from the morning to a few minutes after sunset, with an almost nil night activity.

The only large mammals present in the area are sheep, around 200 animals, grazing 300 m far from the ponds and WB_b to WB_d. It is plausible that local midge population sustains mainly on these hosts, given the mammophily of this species (Tomazatos et al. 2020).

Because of the chemistry of the water, the described larval breeding sites appear exceedingly interesting. Due to the number of larvae and pupae observed in lakes and ponds, it is possible to hypothesize that the peculiar water characteristics (Table 1) of the area are particularly favorable to this species. All other species found in the benthic community are extremely tolerant. Larvae of the *C. plumosus* complex can survive in polluted environments, hypoxia and even anoxia, and high concentration of H_2S (Nagell and Landall 1978). *Hydroglyphus geminus* is the most euryecious Italian Dytiscidae, frequent in all kinds of aquatic sites, ranging from rice paddies to rainwater pools and brackish water. Neither mosquito larvae nor other aquatic organisms, such as tadpoles or freshwater fishes were seen or sampled in the same waters. Hence, *C. riethi* would be able to thrive in a prohibitive habitat and lack of competitors and/or predators during the immature stages could be one of the factors allowing this species to become so abundant. Particularly interesting is the gradient of extreme conditions from WB_b, azoic and with no vegetation, to WD_d, characterized by the presence of dense vegetation, *Culicoides* larvae, and their possible predators. WB_c, lying in between these WBs and exhibiting an intermediate pH, acidity, and Fe concentration where bubbling water indicated high gas emissions presented a scattered *A. canina* cover and was characterized by the highest midges larval density where no predators were observed. Indeed, *C. riethi* was reported as one of the most abundant species of biting midges fauna in anoxic habitat of the Siberian forests and steppe, characterized by shallow water ponds and slow-flow streams with turbid water rich in organic matter (Mirzaeva 1969). However *C. riethi* was also found in meadows surrounded by dense shrubbery with temporary pools (Nielsen et al. 1971), which is similar in features to our study site. To the best of our knowledge, this is the first description of *Culicoides* sp. breeding sites characterized by waters and soil so rich in sulphur that it is defined hazardous (Carapezza et al. 2012).

Finally, it must be noted that, despite the extreme chemicophysical water properties of all WBs, *C. riethi* larvae and a very simplified benthic community are still present. Where pH dropped below 2 (WB_b), animal life was absent. This is in agreement with the threshold limit of pH for the animal life (Rothschild and Mancinelli 2001).

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