

Distribution and assemblage structure of blackflies in the western Aures Mountains, Algeria (Diptera: Simuliidae)

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Besides their important ecological role in flowing waters, blackflies (Diptera: Simuliidae) may pose medical and veterinary risks. For seventeen months, we surveyed the blackflies of ten localities across the Aures Mountains, in the Saharan Atlas, Algeria, and recorded eight taxa (i.e. species, species groups or species complexes). High altitude sites were dominated by the *Simulium ornatum* (Meigen, 1818) group, whereas sites located on the southern slope of the Aures Mountains were occupied by the eurytopic *Simulium velutinum* (Santos Abreu, 1922) complex and the thermophilic, pollutant-tolerant *Simulium ruficorne* Macquart, 1838 'A' morphotype. Co-inertia analysis was used to determine the relationship between a species' abundance and habitat types. The co-inertia analysis revealed a likely co-structure between blackfly assemblages and measured environmental descriptors (water temperature, conductivity, current velocity, bed width, etc.) in sampled habitats. This confirmed the importance of altitude as a driver of blackfly distribution. Our results also showed that there has been an increase in anthropogenic pressures on the vulnerable freshwater biota of the Aures Mountains.

INTRODUCTION

Blackflies (Diptera: Simuliidae) are an important component of lotic ecosystems (Malmqvist et al. 2004). Their larvae and pupae develop in fresh, flowing water, with high levels of dissolved oxygen, while their adults are aerial. While a few species are entirely anthophilic, most adult blackfly females feed on birds, humans and other mammals (Crosskey 1990; Currie & Adler 2008). Consequently, blackflies may be a source of nuisance (Hansford & Ladle 1979; Tabatabaei et al. 2020; Sitarz et al. 2021), or act as vectors for pathogens, such as viruses, bacteria, protozoa and nematodes. These pathogens cause human onchocerciasis, eastern equine encephalitis, and other vector-borne diseases affecting mammals and birds (Brockhouse et al. 1993; Shelley & Coscarón 2001; Reeves & Nayduch 2002; Reeves et al. 2007; Barba et al. 2019). Therefore, the medical and veterinary importance of blackflies cannot be overstated (Adler et al. 2010; Watanabe 2014).

Blackflies are also considered the most diverse fauna of stream communities with more than 2401 species reported worldwide (Adler 2021). The Palearctic is considered the most species-rich biogeographic region with 700 recorded species (Currie & Adler 2008). In North Africa, 52 nominal species have been identified, with Morocco having the highest diversity (44 species), followed by Algeria (34 species), Tunisia (18 species), Libya (5 species) and Egypt (2 species) (Belqat et al. 2018).

In Algeria, several simuliid studies have focused mainly on surveys and relied largely on alpha taxonomy and species distributions (Belazzoug & Tabet-Derraz 1980; Gagneur & Clergue-Gazeau 1988). In contrast, recent studies that surveyed different Algerian regions, have tackled both taxonomy and bionomics (Djurdjura Mountains, central Algeria: Lounaci et al. 2000a, 2000b; Tafna River Basin, north-western Algeria: Boudghane-Bendiouis et al. 2014; Ahaggar, central Sahara: Cherairia & Adler 2018; Seybouse River Basin: Cherairia et al. 2014; and El Kala Highlands, north-eastern Algeria: Samraoui et al. 2021).

The present study, focusing on the Simuliidae of the western part of the Aures Mountains, aims to survey this important family in a poorly explored region. We also used multivariate analysis to analyse the response of community composition to environmental conditions by testing for possible co-structure between measured habitat characteristics and blackfly assemblages, in line with the habitat template concept (Southwood 1977; Townsend & Hildrew 1994).

MATERIALS AND METHODS

Study area

The Aures Massif, located at the eastern end of Algeria, is part of the Saharan Atlas Mountains (Figure 1). The north-east–south-west orientation of this mountain range led to the development of many valleys with in the alignment. The region covers three provinces, namely Batna, Khenchela and Biskra, an area of 2529 km². The climate in the Aures region varies between semi-arid, with cold winters in the northern part (Batna and Khenchela), to arid, with temperate winters in the southern part (Biskra). The area of study also included a protected park, namely the Belezma National Park, which harbours the Atlas cedar *Cedrus atlantica* (Endl.) Manetti ex Carriere, a coniferous tree endemic to Morocco and Algeria.

The collection of blackflies was part of a comprehensive study of macroinvertebrates of the region, carried out over a period of 17 months, from April 2018 to August 2019 (Dambri et al. 2020).

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Blackfly larvae and pupae were sampled monthly at ten localities (Table 1, Figure 1) using two methods. At each locality, an area of 100 m² was kick-sampled by walking across all microhabitats and samples collected using a dip-net (25 cm diameter, 500 µm mesh size). In addition, we sampled a random set of ten cobbles with an average size of 10 cm and collected larvae and pupae using entomological forceps. Sampling covered small to medium-sized mountainous streams with different degrees of ease of access. An additional set of four localities, not easily accessible, were sampled occasionally (Table 1, Figure 1). Larvae and pupae were fixed in ethanol or Carnoy's solution, with identification performed by Prof. Peter H Adler (Clemson University).

Physicochemical sampling and environmental data

For each sampling event, we recorded the physical and chemical parameters of the water in situ using a tape (water depth and river bed width) and multi-probes: conductivity, total dissolved solids (TDS), water temperature, and pH were measured using an Adwa AD32 tester and a HANNA HI1271 pH electrode. Water samples were transported in a cooler and the remaining parameters (i.e. NO₃, NO₂, NH₄, CO₃, HCO₃, Cl and O₂) measured within 48 hours in the laboratory. Water velocity was estimated using a floating cork stopper timed with a stopwatch. As recurrent droughts in intermittent streams resulted in missing data, only data (temperature, conductivity, TDS, etc.) recorded from November to February were presented/analysed.

Statistical analyses

A co-inertia analysis (CIA) was performed using the ade4 package (Dolédec & Chessel 1994; Dray et al. 2003) to test for co-structure between the blackfly assemblages and the measured environmental descriptors. The blackfly matrix was made up of the total abundance of each taxon at each site. Only regularly (monthly) sampled sites were included in the analysis. The vectorial correlation coefficient 'RV' of the CIA measured the overall correlation between the recorded taxa and the environmental descriptors. The RV ranged from 0 (all the taxa are independent of environmental variables) and 1 (perfect match). The significance of the RV coefficient was tested by performing a Monte-Carlo test (random permutation of the rows of both tables) (Dray et al. 2003). All statistical analyses

were performed using R software version 4.0.5 (R Development Core Team 2021).

RESULTS

A total of 479 specimens were identified and assigned to eight taxa (i.e. species, species groups or species complexes) during this study. The *Simulium ornatum* (Meigen, 1818) group was the most abundant and widespread taxon in the western Aures (Figure 2a). The *S. velutinum* (Santos Abreu, 1922) complex was also abundant and widespread, but to a lesser extent than *S. ornatum*. Three sites (i.e. Nafla, Maafa and Ghoufi) had four taxa present and were the most species-rich localities (Figure 2b).

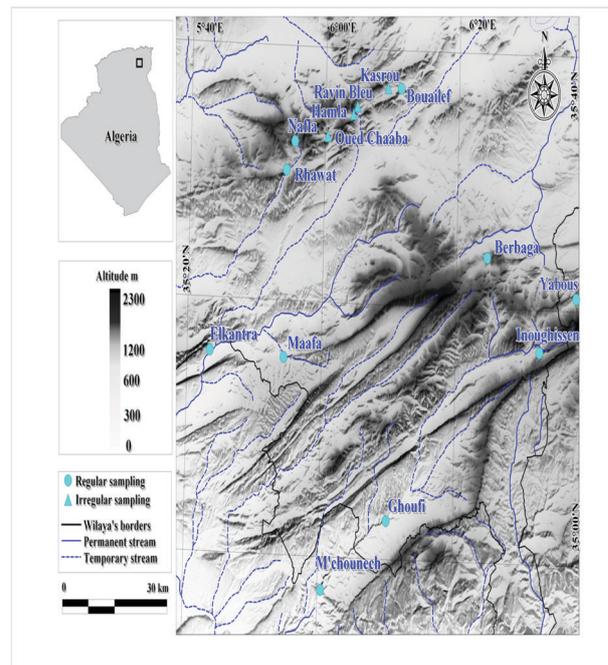


Figure 1. Location map of sampled sites in the western Aures Mountains, Algeria.

Table 1. Sampling sites for black flies in the Aures Mountains, Algeria.

Locality	Region	Alt. (m)	Latitude (N)	Longitude (E)	Substratum	Land use	Hydroperiod	Temperature (°C)	Conductivity (µS cm ⁻¹)
Bouailef	Fisdís	1060	35°37'01"	06°11'17"	Silt-mud, gravel	Forest	Permanent	25.7 ± 1.4	550.0 ± 215.7
Berbaga	Oued Taga	1445	35°24'01"	06°24'31"	Silt-mud, cobbles, gravel and boulders	Forest	Permanent	11.6 ± 1.3	462.3 ± 151.9
Inoughissen	Ichmoul	1670	35°16'42"	06°32'34"	Cobbles, gravel and boulders	Orchard	Temporary	8.9 ± 1.3	379.5 ± 116.4
Ghoufi	Arris	625	35°03'02"	06°10'04"	Sand and cobbles	Orchard	Temporary	11.7 ± 3.4	1388.5 ± 157.7
M'chounech	M'Chouneche	317	34°57'25"	06.00'29"	Sand and cobbles and boulders	Urban	Temporary	13.8 ± 1.3	1060.3 ± 68.8
Oued Ekehal	Yabous	1420	35°21'11"	06°38'35"	Cobbles, gravel and boulders	Forest	Permanent	N/A	N/A
Maafa	Ain Touta	932	35°15'33"	05°54'23"	Sand and cobbles	Orchard	Temporary	16.3 ± 1.0	352.3 ± 278.1
Rhawat	Hidoussa	1414	35°30'16"	05°54'18"	Silt-mud, gravel	Orchard	Permanent	14.3 ± 2.0	374.0 ± 152.8
Nafla	Hidoussa	1428	35°32'33"	05°55'30"	Silt-mud, gravel	Forest	Permanent	6.8 ± 2.1	281.0 ± 293.7
Oued Chaaba*	Oued Chaaba	1262	35°33'03"	06°00'22"	Silt-mud, gravel	Orchard	Temporary	N/A	N/A
Kasrou*	Fisdís	1154	35°37'03"	06°10'17"	Silt-mud, gravel and cobbles	Forest	Temporary	N/A	N/A
Hamla*	Condorcet	1289	35°34'06"	6°04'10"	Silt-mud, gravel	Forest	Temporary	N/A	N/A
El Kantra	Elkantra	571	35°15'51"	5°43'23"	Silt, cobbles and boulders	Urban	Temporary	15.5 ± 1.3	865.8 ± 526.2
Ravin Bleu*	Fisdís	1335	35°35'72"	6°04'55"	Silt-mud, fine gravel	Forest	Temporary	N/A	N/A

* sites that were sampled irregularly; Alt. = altitude.

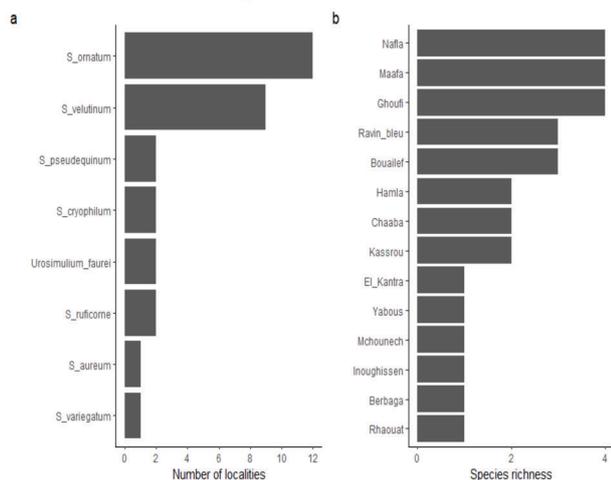


Figure 2. (a) Number of sites inhabited by the recorded taxa. (b) Species richness of the sampled sites.

Simulium (Simulium) ornatum (Meigen, 1818) group

The *S. ornatum* group was one of the two most common taxa in this study and was found at all the sites except M'Chouneche, Bouailef and El Kantra. The water temperature was high at the last two stations. The taxon tolerated a wide range of water temperatures, but seemed to avoid localities with high water conductivity and TDS (Table 2).

Prosimulium faurei Bertrand & Grenier, 1972

First record from the Aures Mountains. Our record from the Aures region is associated with a temporary stream (Maafa) at 932 m above mean sea level (AMSL). The substrate was sandy with cobbles and the water was clean with a low flow (0.24 m s^{-1}).

Simulium (Eusimulium) aureum (Fries, 1824) group

We recorded two larvae and one pupa from one small mountainous stream (Ravin Bleu) at 1335 m AMSL. The site was visited only once for reasons related to accessibility.

Simulium (Eusimulium) velutinum (Santos Abreu, 1922) complex

Our sampling showed that the *S. velutinum* complex had a wide distribution (i.e. Ghoufi, Maafa, Bouailef, Kassrou, Nafla, M'Chouneche, Hamla and Ravin Bleu) in the Aures region over a remarkable altitudinal range (i.e. 350–1700 m AMSL). *Simulium velutinum* s.l. has been shown to be made up of a complex of sibling species, based on the banding sequences of the polytene chromosomes from the larval salivary glands (Cherairia et al. 2014).

Simulium (Nevermannia) ruficorne Macquart, 1838 'A'

First record from the Aures Mountains. This taxon was collected from two sites (Bouailef and El Kantra) with a temperature range from 17.87–27.45 °C (Table 2). The species seemed to tolerate high values of water conductivity and TDS (Table 2). We based the identification of morphoform 'A' on gill structure (Cherairia et al. 2014).

Simulium (Nevermannia) cryophilum (Rubtsov, 1959) complex

First record from the Aures Mountains. We recorded it at three localities (i.e. Ghoufi, Maafa, and Kassrou). Characteristically, the taxon seemed to avoid localities with high water temperatures, but appeared to tolerate high water conductivity values (Table 2).

Simulium (Simulium) variegatum (Meigen, 1818) group

First record from the Aures Mountains. We collected *S. variegatum* larvae and pupae from two temporary streams (i.e. Maafa and Ravin Bleu) with low current velocity (0.24 m s^{-1} for Maafa).

Table 2. Mean \pm standard error (and maximum-minimum) for environmental factors at 14 sampling sites in the Aures Mountains, Algeria.

Species	n	Water temperature (°C)	Conductivity ($\mu\text{S cm}^{-1}$)	Total dissolved solids (mg L^{-1})	Stream depth (m)	Stream width (m)	Water velocity (m s^{-1})
<i>S. velutinum</i> complex	9	15.2 \pm 2.3 (7.0–27.5)	658.5 \pm 147.0 (326.4–1447.8)	328.2 \pm 76.0 (157.0–739.7)	0.2 \pm 0.1 (0.1–0.2)	2.0 \pm 0.6 (0.9–4.1)	0.2 \pm 0.04 (0.1–0.3)
<i>S. ornatum</i> group	10	13.9 \pm 1.1 (7.0–17.9)	549.1 \pm 115.6 (326.4–1447.8)	274.3 \pm 60.0 (157.0–739.7)	0.21 \pm 0.1 (0.1–0.8)	1.8 \pm 0.5 (0.9–3.2)	0.2 \pm 0.0 (0.1–0.4)
<i>S. ruficorne</i> 'A'	2	22.7 \pm 4.8 (17.9–27.5)	809.7 \pm 241.9 (567.9–1051.6)	402.1 \pm 118.2 (283.9–523.3)	0.1 \pm 0.0 (0.08–0.11)	2.0 \pm 0.5 (1.5–2.6)	0.2 \pm 0.0 (0.2–0.3)
<i>Prosimulium faurei</i>	1	17.8	394.2	190.3	0.8	1.1	0.2
<i>S. cryophilum</i> complex	3	13.4 \pm 3.3 (7.0–17.8)	817.7 \pm 321.2 (611.0–1447.8)	411.6 \pm 167.3 (305.0–739.7)	0.3 \pm 0.2 (0.1–0.8)	2.3 \pm 1.3 (0.9–5.0)	0.3 \pm 0.1 (0.2–0.4)
<i>S. variegatum</i> group	1	17.8	394.2	190.3	0.8	1.1	0.2
<i>S. pseudequinum</i>	2	14.5 \pm 0.9 (13.6–15.4)	921.0 \pm 526.9 (349.1–1447.8)	465.5 \pm 274.2 (191.3–739.7)	0.1 \pm 0.0 (0.1–0.1)	3.1 \pm 1.9 (1.1–5.0)	0.3 \pm 0.2 (0.1–0.4)
<i>S. aureum</i> group	1	NA	NA	NA	NA	NA	NA

Simulium (Wilhelmia) pseudequinum Séguy, 1921

We found it at two different localities (i.e. Ghoufi and Nafla) characterized by the presence of a large amount of macrophytes. In addition, the species seemed to tolerate high values of water conductivity and TDS (Table 2).

The CIA indicated the existence of a co-structure between the blackfly assemblages and the measured environmental descriptors (Figure 3a–f). The Monte-Carlo test using 10 000 replicates gave a marginally significant *p*-value (0.10) for the coefficient of vectorial correlation (RV = 0.42) for the CIA. This indicated a likely relationship between the distribution of black flies and the measured environmental descriptors. Axis 1, representing 96% of the total variance, separated high altitude sites from the low altitude Saharan sites, located on the southern flank of the Saharan Atlas. The high altitude sites were well oxygenated localities, with relatively deep water, dominated by taxa such as *S. ornatum* (Figure 4a). The low altitude sites were characterized by high water conductivity and temperature, high loads of total dissolved solids, nitrates and nitrites (Figure 4b) and were dominated by taxa such as *Simulium ruficorne* 'A' and the *S. velutinum* complex. Of lesser importance (3.5% of total inertia), Axis 2 organised the Saharan localities along increasing gradient of bed width.

DISCUSSION

In this study eight species, species complexes or groups were recorded in the western Aures, representing 23.5% of the known blackfly fauna of Algeria (Belqat et al. 2018). Four of the recorded taxa are new to the Aures [viz. *Prosimulium faurei*, *Simulium (Nevermannia) ruficorne* 'A', *Simulium (Nevermannia) cryophilum* complex and the *Simulium (Simulium) variegatum* group].

Previous records

The presence of the *S. ornatum* group has been recorded in several studies for localities across Algeria, namely the Batna Department and Bouïra Province (Edwards 1923), Biskra Province (Belazzoug & Tabet-Derraz 1980), the Oued El Haï Basin (Arigue et al. 2016), the Djurdjura Mountains (Lounaci et al. 2000b), the Tlemcen Mountains (Gagneur & Clergue-Gazeau 1988; Boudghane-Bendiouis et al. 2014), the Seybouse Basin (Cherairia et al. 2014), and El Kala, north-eastern Algeria (Samraoui et al. 2021).

Prosimulium faurei was previously found in the Tafna watershed in north-western Algeria (Gagneur & Clergue-Gazeau 1988). In north-eastern Algeria, it has been recorded in the Seybouse Basin (Cherairia et al. 2014) and the El Kala region (Samraoui et al. 2021).

The *Simulium (Eusimulium) velutinum* complex has been recorded in Algeria (Gagneur & Clergue-Gazeau 1988) from the Tlemcen Mountains in north-western Algeria and the Djurdjura Mountains (Lounaci et al. 2000a, 2000b). It was also recorded in the Seybouse Basin (Cherairia et al. 2014), the El Kala region (Samraoui et al. 2021) and the Aures region (Adler et al. 2015; Arigue et al. 2016).

Simulium (Nevermannia) ruficorne 'A' was previously recorded from several Algerian localities, especially those in the Sahara. It was found in habitats characterized by relatively high water temperatures (Cherairia & Adler 2018). It was also recorded in the Biskra area, Hoggar and Tassili N'Ajjer Mountains, Tlemcen Mountains, Seybouse Basin and El Kala region (Edwards 1923; Parrot 1949; Grenier & Clastrier 1960; Belazzoug & Tabet-Derraz 1980; Cherairia et al. 2014; Cherairia & Adler 2018; Samraoui et al. 2021).

The *Simulium (Nevermannia) cryophilum* complex is known to occur in eastern and western Algeria (Gagneur & Clergue-Gazeau 1988), the Djurdjura Mountains (Lounaci et al. 2000b; Haouchine & Lounaci 2012), the El Kala region (Samraoui et al. 2021) and the Tlemcen Mountains (Gagneur & Clergue-Gazeau 1988). Similarly, the *S. variegatum* group is well documented in

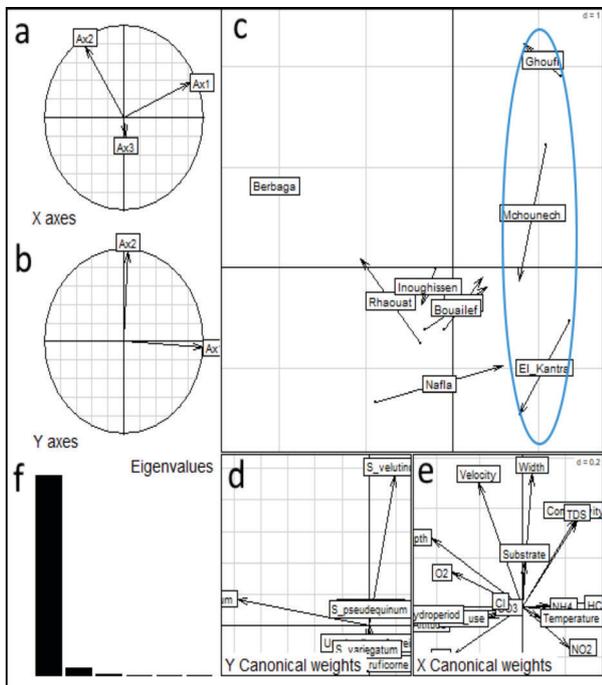


Figure 3. Results of co-inertia analysis (CIA) performed on the abundance of recorded blackflies and measured environmental descriptors. (a) Components of the standardized principal component analysis (PCA) of the environmental data set projected to the co-inertia axes. (b) Components of the centered principal component analysis of the blackflies data set projected onto the co-inertia axes. (c) Components of the environmental descriptors (base of arrows) and black fly assemblages (end of arrows). (d) Distribution of taxa on the F1 × F2 factorial plane for the CIA (Figure 3a). (e) Distribution of environmental descriptors on the F1 × F2 factorial plane (Figure 3b). (f) Histogram of eigenvalues for the CIA.

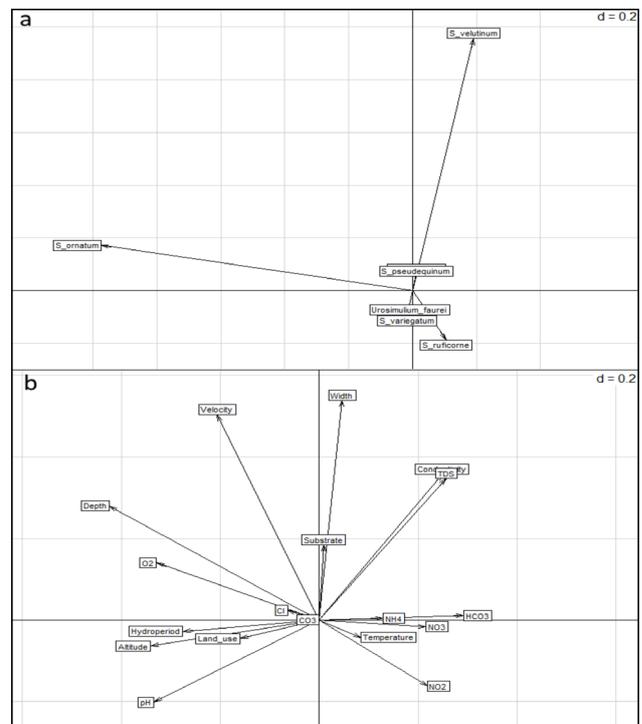


Figure 4. (a) Distribution of taxa on the F1 × F2 factorial plane for the CIA. (b) Distribution of environmental descriptors on the F1 × F2 factorial plane.

Algeria, with records for the Djurdjura Mountains (Lounaci et al. 2000b; Haouchine & Lounaci 2012) and Tlemcen Mountains (Clergue-Gazeau et al. 1991).

Simulium pseudequinum was first recorded from Batna, Biskra, Bouira and Constantine (Edwards 1923). Thereafter, the species was collected in western Algeria from the Tlemcen Mountains (Gagneur & Clergue-Gazeau 1988; Boudghane-Bendiouis et al. 2014) and Mascara Province (Parrot 1949). It was also recorded in the Djurdjura Mountains (Lounaci et al. 2000a, 2000b; Haouchine & Lounaci 2012), the Seybouse Basin (Cherairia et al. 2014), the El Kala region (Samraoui et al. 2021) and the Aures region (Belazzoug & Tabet-Derraz 1980; Oued El Haï Basin: Arigue et al. 2016).

Ecology

Two taxa, *S. ornatum* and *S. velutinum* were most widespread and abundant, dominating all other taxa recorded in the Aures. This result is similar to that obtained for the El Kala region (Samraoui et al. 2021) and the Tafna River Basin (Boudghane-Bendiouis et al. 2014), but differs from that obtained for the Seybouse River, where *S. pseudequinum* was the dominant taxon (Cherairia et al. 2014). *Simulium pseudequinum*, confined to only two sites in the Aures, is often a widespread lowland species, inhabiting streams with large bed width and high water conductivity, both in the Maghreb (Boudghane-Bendiouis et al. 2014; Cherairia et al. 2014; Samraoui et al. 2021) and the Iberian Peninsula (Gallardo-Mayenco & Toja 2002). The Seybouse River Basin may offer many opportunities for *S. pseudequinum*, which favours low-elevation sites, with a large river bed width and high water conductivity.

Likewise, larvae of *S. velutinum* were often dominant in the Aures, occupying lowland sites. A result consistent with records found elsewhere in Algeria (Boudghane-Bendiouis et al. 2014; Samraoui et al. 2021). This taxon is also known to tolerate waters with a high load of organic matter (Gallardo-Mayenco & Toja 2002). In fact, the large ecological amplitude of *S. velutinum* indicates the possible presence of cryptic species (Adler et al. 2015).

In contrast, *S. ornatum* presents a different case and it is worth noting that this taxon (group) may represent multiple cryptic species (Belqat et al. 2018), widely distributed in the western Mediterranean. The species *S. ornatum* is the most common and most frequently recorded species in Austrian streams and rivers (Ofenböck et al. 2002). Moreover, the ecology of this taxa seems to vary geographically. In the El Kala region, it is dominant and mainly recorded at downstream sites (Samraoui et al. 2021), while the taxon is clearly crenophilic or rhithrophilic in central and western Algeria and Morocco (Gagneur & Clergue-Gazeau 1988; Giudicelli et al. 2000; Boudghane-Bendiouis et al. 2014; this study). Elsewhere, *S. ornatum* was found in Pyrenean streams with slow currents, dominated by aquatic vegetation (Vinçon & Clergue-Gazeau 1993). Once again, these contradictory results would be consistent with the presence of a cryptic species complex.

Despite the low statistical power of the performed CIA, due to a small sample size, the results clearly indicate the importance of altitude in driving blackfly distribution in the Aures. This result is congruent with numerous other studies in Algeria (Boudghane-Bendiouis et al. 2014; Samraoui et al. 2021) and elsewhere (Giudicelli & Dakki 1984; Vinçon & Clergue-Gazeau 1993; Ya'cob et al. 2016). Undoubtedly, various environmental factors, such as temperature, dissolved oxygen, water flow, water depth, and stream width are correlated with altitude.

A good knowledge of the status and ecology of the freshwater biodiversity in the Maghreb is urgently needed, as climate change, interacting with other anthropogenic stressors, is fast disturbing freshwater communities. These disturbances

are leading to a precipitous decline in both the diversity and abundance of freshwater biota (Benslimane et al. 2019), with the conditions favouring widely distributed, thermophilic species (Morghad et al. 2019). High loads of stressors, such as nitrate and nitrites, common chemical contaminants that may negatively affect human health, are now routinely found in Maghrebian streams and rivers (Abdesselam et al. 2013; Aghzar et al. 2002). These groundwater contaminants are often associated with land use, with high levels of nitrates possibly a result of the use of fertilizers in apple orchards in the Aures, and geological factors. There is thus a need to monitor freshwater biodiversity, including taxa such as blackflies that may pose medical and veterinary risks.

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