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First report of *Cloeon vanharteni* Gattolliat & Sartori, 2008 (Baetidae, Ephemeroptera) in the Maghreb

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Abstract

Cloeon vanharteni Gattolliat & Sartori, 2008 was newly discovered in the framework of our study of Ephemeroptera in the Draa basin, located in the southern region of the High Atlas in Morocco. This discovery is rather unexpected as the species was never reported outside the Arabian Peninsula and Levant; it is thus the first record for the Maghreb. The identification was based on morphological evidence and confirmed by the mitochondrial COI barcode.

Key Words

COI, Drâa basin, Distribution, Mayflies, Morocco

Introduction

Morocco by its geographical position in the northwest of Africa is part of the Maghreb (region that includes five countries in North Africa: Morocco, Algeria, Tunisia, Libya, and Mauritania). Research on macroinvertebrates and Ephemeroptera in the Maghreb has primarily focused on Morocco, Algeria, and Tunisia, while comparatively less research has been conducted in Libya and Mauritania (Gattolliat et al. 2023). In Morocco, the studies of Ephemeroptera began with the work of Lestage and Kimmins (Lestage 1925; Kimmins 1938), that were then taken up by Navás (1929), and following these studies, a first faunistic list of ten species was established (Gattolliat et al. 2023). After a period of interruption, the study

of mayflies resumed in the late 1970s, with the work of Dakki (1978) and then Dakki and El Agbani (1983), who completed this list with 16 additional species, resulting in a total of 26 species distributed in the different Moroccan regions (Gattolliat et al. 2023).

Starting in the 1980s, several hydrobiological studies were conducted in the different Moroccan rivers (Dakki and Giudicelli 1979; Dakki and Thomas 1986; Thomas and Bouzidi 1986; Ouahsine and Lavandier 1988; Qninba et al. 1988; Vitte and Thomas 1988; Vitte 1991; El Alami et al. 2000; El Bazi et al. 2017; Khadri et al. 2017; Mabrouki et al. 2017), which has allowed to further enrich the species list for Morocco and led to the discovery of species new to science, considerably increasing the list of Ephemeroptera species in Morocco.

The most recent compilation of Ephemeroptera species in Morocco was carried out by El Alami et al. (2022b) who established a list of 54 species belonging to 26 genera and 10 families distributed as follows: The Rif with 37 species, the Middle Atlas with 31 species, the High Atlas with 30 species, Eastern Morocco with 24 species, and finally the Central Plateau with 19 species. In this list, there are 18 species currently considered endemic to Morocco and nine endemic to the Maghreb region (El Alami et al. 2022b). Finally, the recent description of *Prosopistoma maroccanum* El Alami, Benlasri & Sartori, 2022 from the northern slope of the High Atlas (El Alami et al. 2022a) and *Centropitulum alamaiae* Kaltenbach, Vuataz & Gattolliat, 2022 from the Rif (Kaltenbach et al. 2022) has increased the number of mayfly species in Morocco to 56. However, none of these studies focused on the south of Morocco.

Morocco is characterized by four mountain ranges: the Rif, which borders the Mediterranean in the north and extends to the Atlantic coast, and the Atlas Mountains in the center of the country, which extend on a southwest-northeast axis to the Algerian border in the northeast. The Atlas Mountain is divided into three chains: the Middle Atlas, the High Atlas, and the Anti-Atlas.

Depending on the part of Morocco, the climate varies between Mediterranean and Atlantic. It presents a dry and hot season from May to September, and a cold season from October to April (Houssni et al. 2020); indeed, the mountain chains separate vast regions that are part of very differentiated climatic zones. The north of the country is characterized by a Mediterranean climate, while the areas located in the south of Morocco and southeast of the Atlas Mountains are marked by a Saharan climate. The presence of the sea attenuates the temperature differences, moderates the seasons, and increases the humidity of the air in the coastal regions. The mountainous regions benefit from a sub-humid to humid climate (Bouaicha and Benabdelfadel 2010). The rainfall is marked by strong annual and decennial variability.

During an ongoing project investigating benthic macroinvertebrates in the southern Draa basin, our sampling revealed the presence of *Cloeon* larvae that morphologically and genetically differ from members of the *Cloeon* group *dipterum* previously documented from Morocco. Further analysis proved that the larvae unambiguously belong to *Cloeon vanharteni* Gattolliat & Sartori, 2008. This unexpected observation was confined to a single salty stream. This species was originally described from the United Arab Emirates (UAE hereafter; Gattolliat and Sartori 2008). It was then reported in the Middle East in Israel and the Palestinian Authority in arid and semi-arid areas (Yanai et al. 2020), in running water habitats, and in brackish ponds in Jordan (Alhejoj et al. 2020, 2023). In the Maghreb countries however, it has never been reported. Therefore, the aim of the present paper is to provide detailed morphological characteristics of the species, to compare the COI barcode of the Moroccan population with other populations and species, and to discuss its ecology and biogeographic distribution.

Material and method

As part of the study of macroinvertebrates in the Draa basin, the first author sampled 17 sites from multiple streams throughout the basin (Fig. 1) that differ in altitude, temperature and conductivity (Benlasri et al. 2022). The Draa basin is located between the southern slopes of the High Atlas Mountains in Morocco and extends southwards into the Sahara. It is divided into three sub-basins: the Upper, Middle, and Lower Draa (Fig. 1). The sub-basin of the Upper Draa is the drainage area of Atlas Mountains feeding the Mansour Eddehbi reservoir. Here we focus on three sampling sites in the west zone of the Upper Draa basin: Ounilla 1, a left tributary of Oued El Maleh which is not salty; Ounilla 2, a salty right tributary of Oued El Maleh with a high conductivity, which hosted specimens of *Cloeon vanharteni*; Oued El Maleh, referred to as the “salty stream” in Arabic, characterized by a lower flow and temperate water (Fig. 2, Table 1). Sampling was conducted in March and June 2022 using a 0.20 m × 0.25 m Surber sampler with a mesh size of 500 µm. To ensure comprehensive collection of macroinvertebrates, twenty spots covering all microhabitats were sampled within each site. Water conductivity was measured using WTW MultiLine® Multi 3510 IDS device. The samples were placed in tubes with 96% alcohol and sorted in the laboratory under a binocular magnifier to separate and identify species.

To complement our morphological investigations, we sequenced a 658 bp fragment of the mitochondrial gene cytochrome oxidase subunit 1 (COI hereafter) for two specimens of *Cloeon vanharteni* (one from our newly discovered site in Morocco and the other from Israel), three specimens of *Cloeon peregrinator* Gattolliat & Sartori, 2008 and one specimen of *Procloeon stagnicola* Soldán & Thomas, 1983 (all from Morocco). The *Cloeon peregrinator* sequences were chosen for comparison, as this species represents the only other genetically confirmed lineage of this genus in Morocco (El Alami et al. 2022a, Gattolliat et al. 2023). The *Procloeon stagnicola* sequence was used as the outgroup (Table 3). Sanger sequencing procedures were carried out at three locations: Duisburg-Essen, Germany using standard protocols (Suppl. material 1); Lausanne, Switzerland; and Tel Aviv, Israel. In Lausanne and Tel Aviv, the non-destructive DNA extraction method from Vuataz. (Vuataz et al. 2011) was used. Polymerase Chain Reaction (PCR) and sequencing were conducted according to the methodology described by El Alami (El Alami et al. 2022a) for Lausanne, and by Yanai (Yanai et al. 2018) for Tel Aviv. To augment our molecular dataset, we downloaded all COI sequences associated with *Cloeon vanharteni* available on the GenBank database as of June 8, 2023, resulting in three additional records from Israel (Yanai et al. 2020), for a total of nine sequences in our COI dataset. All sequences were aligned using MAFFT (Katoh et al. 2019) with default settings as implemented in Jalview 2.11.2.6 (Waterhouse et al.

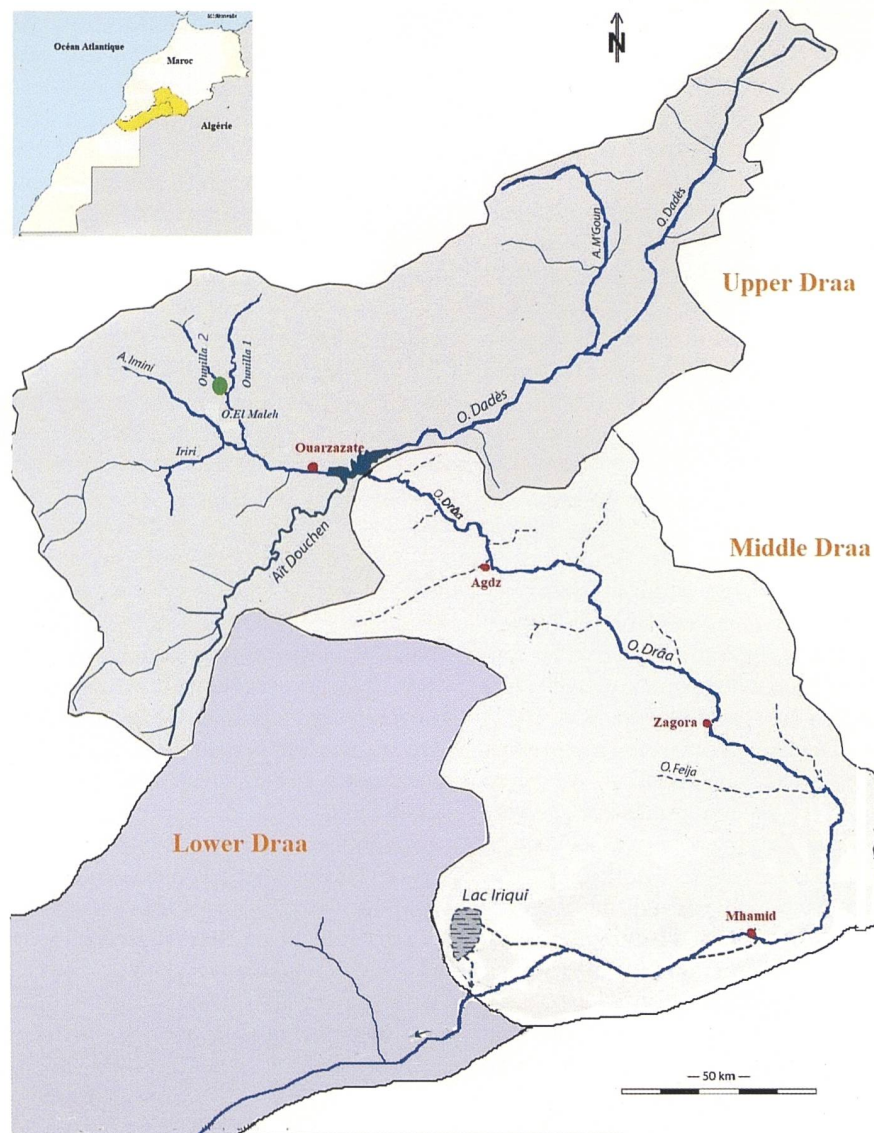


Figure 1. The 17 sampling sites in Draa basin with the location of the site hosting *Cloeon vanharteni* in the west zone of the Upper Draa basin (green dot).

Table 1. Location and collection dates of study sites, with water conductivity specified.

Site Name	Coordinates Latitude, Longitude	Alt (m)	Date	Conductivity [$\mu\text{S}/\text{cm}$]	Water temperature $^{\circ}\text{C}$
Ounilla 2 salty	31,09406, -7,148652	1318	12/03/2022	19330	22.3
			26/06/2022	19660	33.7
Ounilla 1 fresh	31,094021, -7,14659	1311	12/03/2022	3890	18.3
			26/06/2022	2590	30.3
Oued El Maleh	31,011, -7,10006	1229	12/03/2022	10570	17.1
			26/06/2022	12100	24.3

2009). The number of parsimony-informative sites of the alignment was calculated in Mega 10.2.4 (Kumar et al. 2018; Stecher et al. 2020).

To explore and visualize the COI evolutionary divergence, we employed both pairwise genetic distances and gene tree approaches. COI pairwise distances were calculated using the `dist.dna` function from the `ape` 5.7-1 package (Paradis and Schliep 2019) in R 4.3.0 (R Core

team 2023), selecting the raw model and the pairwise deletion option, corresponding to uncorrected p-distances (see Srivathsan and Meier 2012) with missing data removed in a pairwise way. Mean, minimum and maximum distances within and between species were calculated using the `ddply` function from the `plyr` 1.8.8 package (Wickham 2011). We also applied the species delimitation method ASAP (Assemble Species by

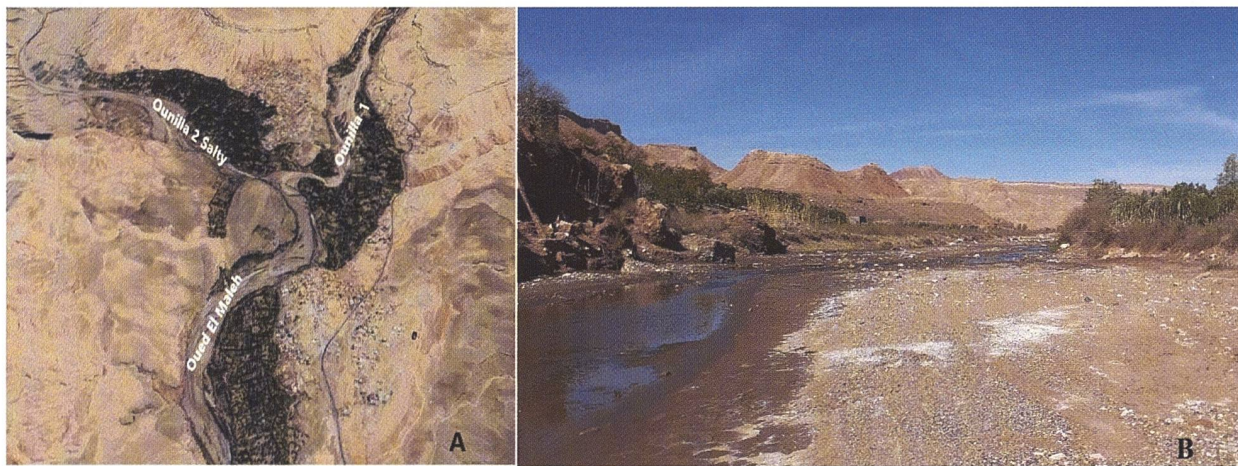


Figure 2. Photographic views of the study sites. **A.** Aerial view of the three study sites (from google Earth); **B.** downstream view of Ounilla 2 salty site.

Automatic Partitioning; (Puillandre et al. 2021) to our COI dataset using the webserver available at <https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html>. This distance-based method is similar to the popular ABGD (Automatic Barcode Gap Discovery) (Puillandre et al. 2012) approach but has the advantage of providing a score that specifies the most likely species delimitation. We calculated genetic distances using simple p-distances and selected the species delimitation hypothesis associated to the best asap-score.

Prior to reconstructing the COI gene tree, the best evolutionary model (GTR+I) was selected based on the second-order Akaike information criterion (AICc) (Hurvich and Tsai 1989) implemented in JModelTest 2.1.10 (Darriba et al. 2012) with five substitution schemes, six gamma categories and default values for all other parameters. To account for different substitution rates among COI codon positions, we analysed our data set in two partitions, one with first and second codon positions, and the other with third positions (1 + 2, 3). Bayesian inference analysis was performed in MrBayes 3.2.7a (Ronquist et al. 2012). Two independent analyses of four MCMC chains run for three million generations with trees sampled every 1'000 generations were implemented, and 300'000 generations were discarded as a burnin after visually verifying run stationarity and convergence in Tracer 1.7.2 (Rambaut 2007). Visualization and editing of the 50% majority rule consensus tree were conducted in iTOL 6.7.5 (Letunic and Bork 2021).

Results

Water conductivity was high in Ounilla 2 salty (19330 and 19660 $\mu\text{S}/\text{cm}$), while it was lower in Ounilla 1 fresh (3890 and 2590 $\mu\text{S}/\text{cm}$). After the confluence of the two streams that forms the stream Oued El Maleh (Fig. 2A), the conductivity remained high (10570 and 12100 $\mu\text{S}/\text{cm}$; Table 1).

Sampling at the three study sites allowed us to identify seven Ephemeroptera species, including 24 nymphs of *C. vanharteni* (Table 2). The material is deposited at the Museum of Natural History of Marrakech (four *C. vanharteni* nymphs on slide).

Diagnosis

At the larval stage, *Cloeon vanharteni* can be distinguished from other species of *Cloeon* by the absence of hindwings pads; lateral spines present on tergites VIII and IX (sometimes also VII; Fig. 3); maxillary palp 2-segmented (Fig. 4); elongated claws with two rows of abundant short teeth (Fig. 5); gills with double lamellae, upper lamella roughly half of the size of the lower lamella.

At imaginal stage, by the absence of colouration of the costal and subcostal areas of female forewing; forceps of male imago with segment I and II clearly separated, male subgenital plate apically flattened (Gattolliat and Sartori 2008).

Table 2. Number of individuals per species collected in the three study sites.

Site	Date	Collected species						
		<i>Cloeon vanharteni</i>	<i>Cloeon peregrinator</i>	<i>Cloeon simile</i>	<i>Procloeon stagnicola</i>	<i>Caenis luctuosa</i>	<i>Caenis pusilla</i>	<i>Baetis pavidus</i>
Ounilla 2 salty	12/03/2022	24	16	32	28	46	0	0
	26/06/2022	0	0	3	3	8	0	2
Ounilla 1 fresh	12/03/2022	0	0	1	0	130	26	256
	26/06/2022	0	0	0	0	62	14	140
Oued El Maleh	12/03/2022	0	0	0	0	12	0	22
	26/06/2022	0	0	2	0	80	0	185

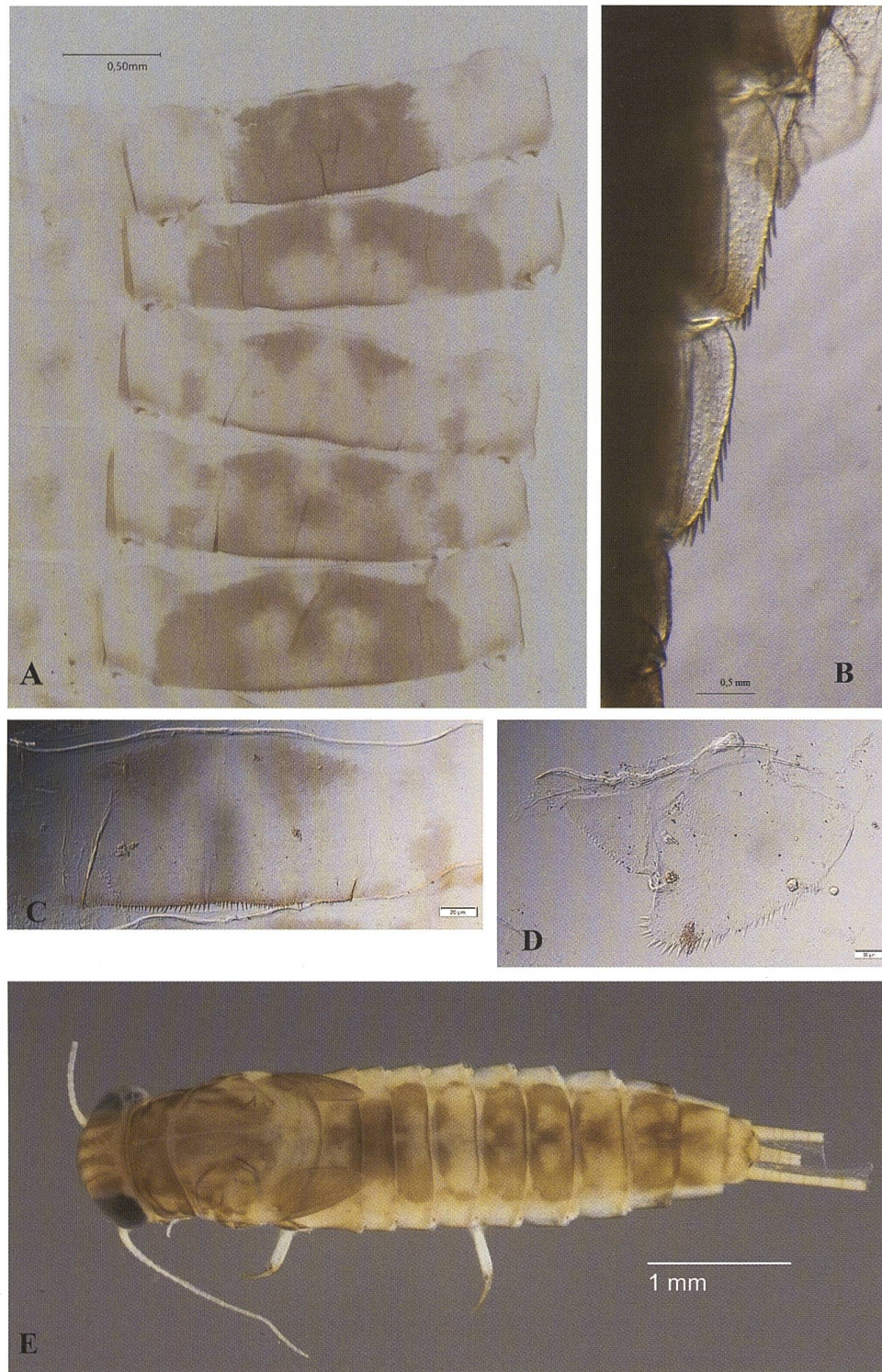


Figure 3. *Cloeon vanharteni* Gattolliat & Sartori, 2008, larva habitus and morphology. **A.** Abdominal tergites; **B.** Abdominal lateral spines; **C.** Tergite IV; **D.** Paraproct; **E.** Dorsal view of the larva.

Molecular analysis

The COI ingroup dataset was >96% complete and included 17% of parsimony informative sites. The five *Cloeon vanharteni* sequences, including the sequence from Morocco, formed a strongly supported monophyletic COI lineage, identified as a distinct species in the

ASAP delimitation analysis (Fig. 6). Similarly, the three *Cloeon peregrinator* sequences were recovered as a distinct, strongly supported monophyletic lineage. The mean p-distance within the *C. vanharteni* COI lineage was 1.6% (range 0–2.5%), while it was 0.5% (range 0–0.8%) within the *C. peregrinator* lineage. The mean p-distance between both lineages was 17.8% (range 17.3%–18.3%).

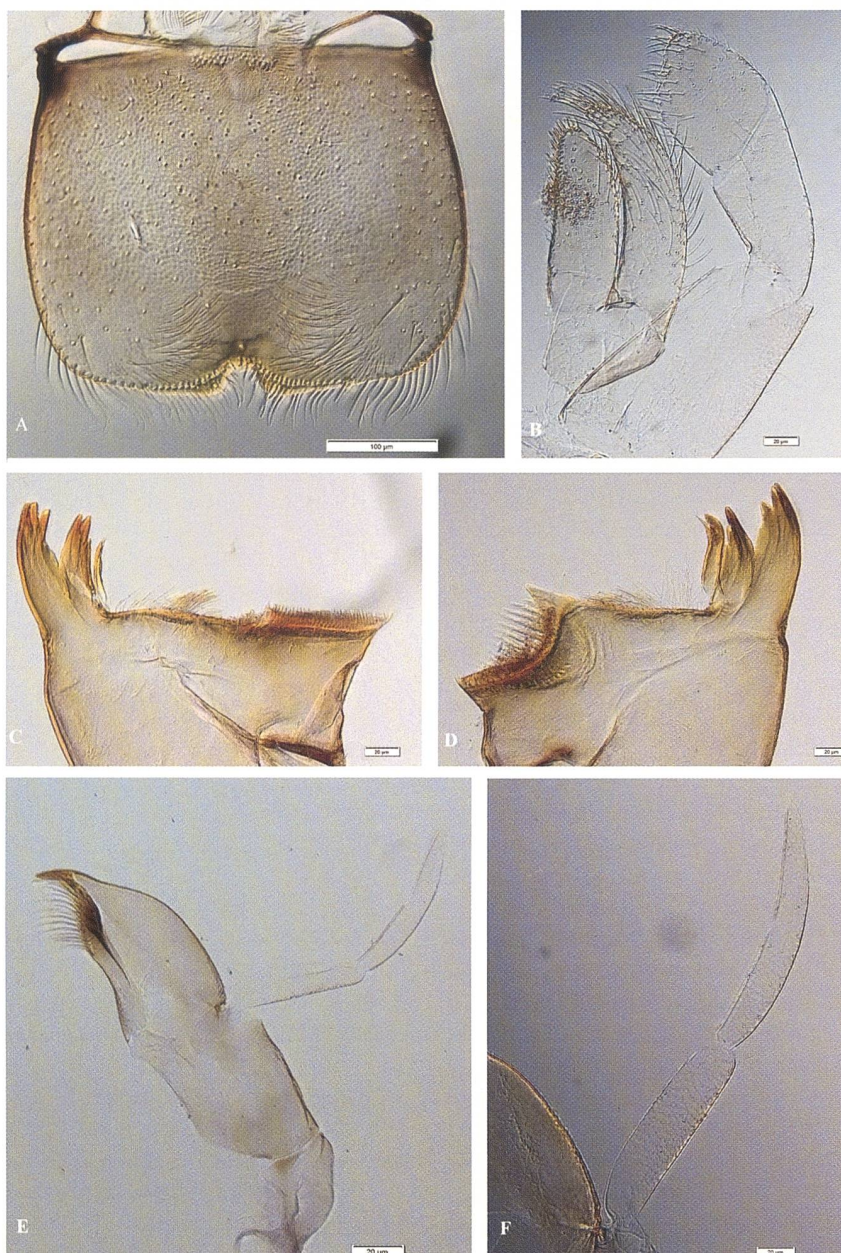


Figure 4. *Cloeon vanharteni* Gattolliat & Sartori, 2008, larva mouth parts. **A.** Labrum; **B.** Labrial palp; **C.** Left mandible; **D.** Right mandible; **E.** Maxilla; **F.** maxillar palp.

Table 3. Newly sequenced nymph specimens for this study, with collection information and accession numbers (OR codes: from GenBank; MZBM codes: from BOLD).

Specimen catalogue no	Species	Country	Locality	GPS coordinates	Date	Collector	Accession ID
CLS_OC220312_03	<i>Cloeon vanharteni</i>	Morocco	Ounilla salty steam	31,09406, -7,148652	12.iii.2022	M. Benlasri	MZBM701-23
B229	<i>Cloeon vanharteni</i>	Israel	Arugot stream	31.46165, 35.35542	25.vi.2014	Z. Yanai	OR345160
GBIFCH01137417	<i>Cloeon peregrinator</i>	Morocco	Oukaïmeden	31.197900, -7.858033	29.v.2014	J. Bojková & T. Soldán	OR345163
GBIFCH01137407	<i>Cloeon peregrinator</i>	Morocco	Ait Mansour	29.547722, -8.873694	31.v.2014	J. Bojková & T. Soldán	OR345162
CLS_SO220311_03	<i>Cloeon peregrinator</i>	Morocco	Ounilla salty steam	31,09406, -7,148652	11.iii.2022	M. Benlasri	MZBM702-23
GBIFCH01122671	<i>Procloeon stagnicola</i>	Morocco	Gorges Oued El Abid	32.066111, -6.677806	18.v.2014	J. Bojková & T. Soldán	OR345161

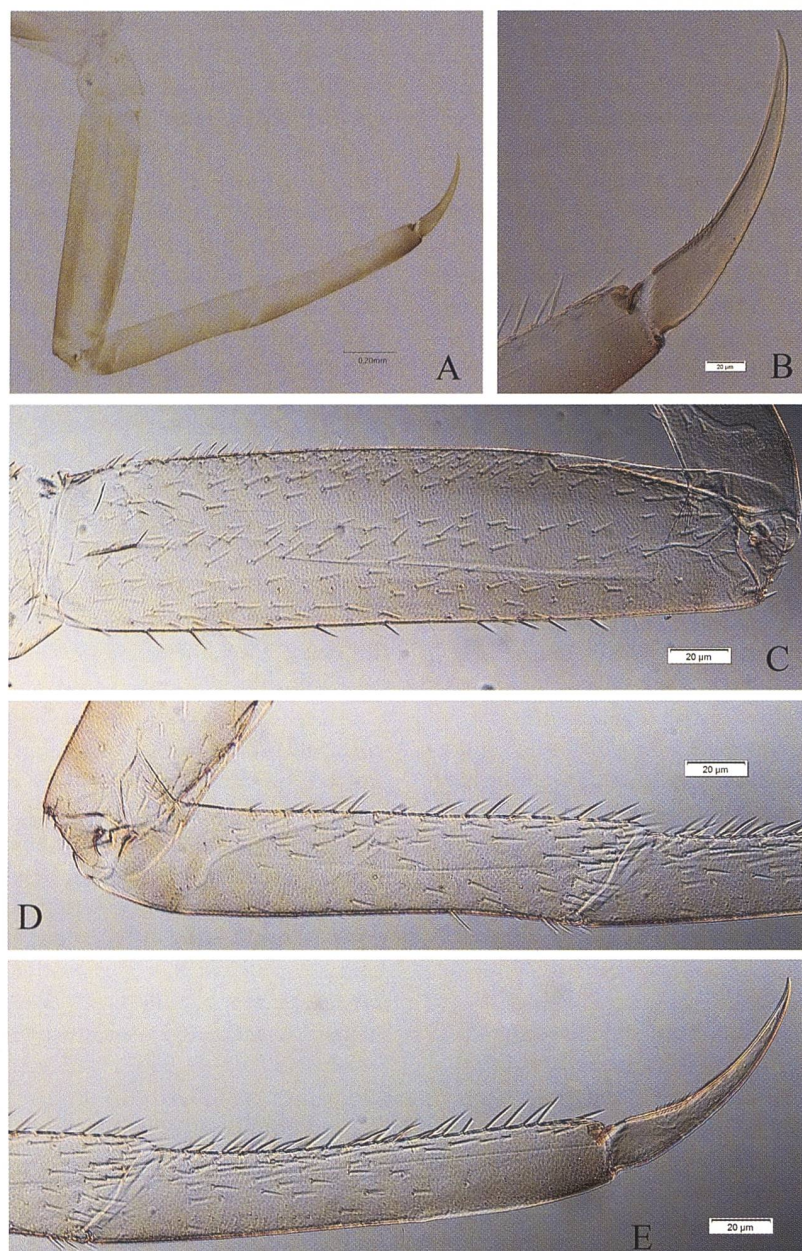


Figure 5. *Cloeon vanharteni* Gattolliat & Sartori, 2008, larva foreleg. **A.** Foreleg; **B.** Claw; **C.** Forefemur; **D.** Foretibia; **E.** Foretarsus and claw.

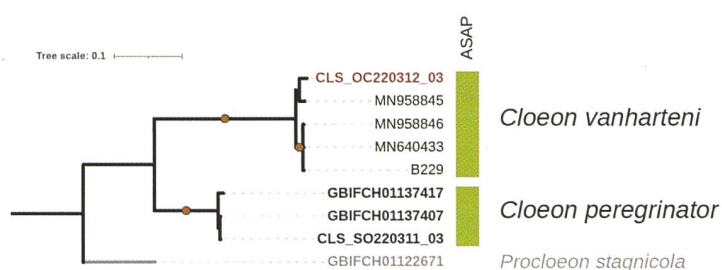


Figure 6. Bayesian majority-rule consensus COI tree including *Cloeon vanharteni* and *C. peregrinator*. Tips labelled with MN codes indicate sequences retrieved from GenBank, other codes represent newly obtained sequences from our samples. Bolded labels indicate sequences derived from Moroccan specimens, with the *C. vanharteni* Moroccan specimen highlighted in red. The B229 code represents a sequence originating from a specimen collected in Israel. Colored vertical boxes indicate species delimitation according to the ASAP method, with the corresponding species name next to each box. Circles on branches indicate Bayesian posterior probabilities > 0.95. The outgroup branch (*Procloeon stagnicola*) is presented in grey, along with its corresponding tip label and species name.

Discussion

Cloeon vanharteni has elongated claws with two rows of abundant small teeth and spines on the lateral margin of last abdominal segments. It clearly belongs to the Cloeoninae; this subfamily encompasses in the Maghreb the five genera *Centropilum* Eaton, 1869, *Cheleocloeon* Wuillot & Gillies, 1993, *Cloeon* Leach, 1815, *Procloeon* Bengtsson, 1915 and *Similicloeon* Kluge & Novikova, 1992 (Gatolliat et al. 2023). *Cloeon vanharteni* possesses bilamellated gills and lateral spines on last tergites, the two characters clearly indicating that it does not belong to *Centropilum*. The labial palp is clavate and does not present any thumb-like projection, separating it from *Cheleocloeon*. The upper lamella is around half of the lower lamella and cerci do not have conspicuous lateral spines, therefore the species does not belong to either *Similicloeon* or *Procloeon* (Gatolliat et al. 2023).

Cloeon vanharteni can be easily separated from all the other species of *Cloeon* previously reported from the Maghreb by the 2-segmented maxillary palp (3-segmented in all the other species). The species of the group *dipterum* (*Cloeon dipterum* and *Cloeon peregrinator*) possess elongated claws with two rows of teeth increasing in length towards the apex, the two rows reaching at least the half of the length of the claw; in *C. vanharteni*, all teeth are short, and the two rows reach at most the first third of the length of the claw. *Cloeon saharense* differs from all the other known species of *Cloeon* by the absence of spines on the lateral margins of last abdominal tergites.

The genus *Cloeon* is one of the most diverse genera of mayflies (Gatolliat et al. 2023). It encompasses 75 species (Salles et al. 2014), including 23 species reported from Africa (Gatolliat 2002). This genus presents a great potential of dispersion among the mayflies, even on some remote islands such as the Azores in the North Atlantic Ocean. This genus colonizes all kinds of still and standing habitats: ponds, lakes, stagnant waters, lentic habitats in streams, as well as in artificial habitats (Salles et al. 2014).

Cloeon vanharteni was described in 2008, when it was identified in a dry region of the UAE. Subsequent citations of this species in Israel, the Palestinian territories, and Jordan (Yanai et al. 2020) mainly concerns arid and semi-arid areas, such as the Jordan Valley, the Dead Sea region, and the central Negev desert (Alhejoj et al. 2020; Yanai et al. 2020). It is the most abundant mayfly to colonize newly formed dolines near the Dead Sea.

In Morocco, this species was collected in a single semi-arid area characterized by cold winters and hot summers, in a salty stream with a low flow, and a moderate temperature; its bottom is formed by pebbles, gravel, and sand. Despite conducting sampling efforts, carried out in these sites between 2020 and 2023, across multiple streams with lower conductivity (Irir, Ait Douchene, and Ounilla 1) within the same basin (Fig. 1), we did not find

the species in any other sites not even in the neighbouring stream that bears the same name but where the conductivity is low. This species seems to be resistant to high conductivity and summer temperatures.

The discovery of *Cloeon vanharteni* in the Maghreb is rather surprising but it makes sense if we consider the sub-desertic distribution and the poor knowledge of the still and standing freshwater habitats in this area: in the whole Maghreb, standing waters are poorly sampled in comparison to running waters. Additionally, *Cloeon* represents the genus of mayflies that possesses the greatest capacity for dispersal, primarily due to the female adult mayflies having a lifespan of over two weeks (Salles et al. 2014).

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Responsibility for the content of this publication rests with the authors.

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Supplementary material 1

Methods DNA barcoding Duisburg-Essen

Authors: Mokhtar Benlasri

Data type: docx

Explanation note: The method used for the barcoding at the university of Duisburg-Essen to confirm the identification

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