Reports of Baetidae (Ephemeroptera) species from Tafna Basin, Algeria and biogeographic affinities revealed by DNA barcoding

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Abstract

Background

The Mediterranean basin is known to be the cradle of many endemic species. Within mayflies (Insecta, Ephemeroptera), North African species belonging to the family Baetidae remain poorly known and, traditionally, affinities to European fauna were proposed. Recent studies, based on molecular reconstructions, showed closer relationships to Mediterranean islands fauna.

New information

Baetidae were sampled from North-West Algerian wadis (Tafna basin) and involved in COI barcoding reconstructions. Seven species were identified. The subgenus Rhodobaetis is represented by Baetis atlanticus known previously from Macaronesian islands, Europe and Morocco and the Maghrebian endemic Baetis sinespinosus. Specimens, previously
identified as *Cloeon cf. dipterum*, correspond to *Cloeon peregrinator* and, until now, only reported from Macaronesia. Besides the confirmation of endemism of some species, such as *Procloeon stagnicola* and *B. sinespinosus*, our molecular study showed quite original results for relationships between European, insular and Algerian species. *Baetis maurus* stood out as a North African endemic sister clade to an Iberian clade. Furthermore, we found clear interspecific distances between Algerian and European clades for *A. cf. sinaica* and *B. cf. pavidus*, suggesting the presence of cryptic species in Algeria. However, additional studies are needed, as, for the moment, no clear morphological characters were found to separate the different clades and support them as valid species.

**Keywords**

Mayflies, *Baetis*, *Rhodobaetis*, *Cloeon*, DNA Barcoding, COI, endemism, Algeria

**Introduction**

The family Baetidae has a cosmopolitan distribution and represents a quarter of the Ephemeroptera diversity worldwide both at generic and specific levels (Barber-James et al. 2008, Gattolliat and Nieto 2009, Sartori and Brittain 2015). The genera *Baetis* Leach, 1815 and *Cloeon* Leach, 1815 have the largest distribution amongst the family and encompass, respectively, 152 and 74 species (Sartori and Brittain 2015, Jacobus et al. 2019). In Europe, *Baetis* was originally divided into eleven species groups (Müller-Liebenau 1969), of which some are now considered as valid genera (*Alainites* Waltz and McCafferty, 1994; *Labiobaetis* Novikova and Kluge, 1987; *Nigrobaetis* Novikova and Kluge, 1987) or subgenera (*Patites* Thomas and Dia, 1999; *Rhodobaetis* Jacob, 2003) (Jacob 2003, Novikova and Kluge 1987, Thomas and Dia 2000, Waltz et al. 1994).

The subgenus *Rhodobaetis* (corresponding to the *Baetis rhodani* group) presently encompasses 43 species, some of them being amongst the most common and abundant mayflies. While some species are widely distributed (e.g. *Baetis rhodani* (Pictet, 1843), *Baetis atlanticus* Soldán and Godunko, 2006), others present a presumably restricted distribution, such as endemic to a single Canary Island (*Baetis palmensis* Gattolliat and Sartori, 2018; *B. tenerifensis* Gattolliat and Sartori, 2018; *B. gomerensis* Gattolliat and Sartori, 2018) or known from a restricted area (*Baetis chelif* Soldán, Godunko and Thomas, 2005 or *Baetis sinespinosus* Soldán and Thomas, 1983 in Algeria) (Gattolliat et al. 2018b, Soldán et al. 2005). Previous molecular studies, based on COI, revealed a high number of independent lineages, which may correspond to cryptic undescribed species (Bisconti et al. 2018, Bisconti et al. 2016, Gattolliat et al. 2015, Gattolliat et al. 2018b, Sroka 2012, Williams et al. 2006).

The species delimitation within the genus *Cloeon* Leach, 1815 (sensu Kluge 2016b) is also highly problematic. Most of the reports of the widely-distributed *Cloeon dipterum* (Linnaeus, 1761) or *Cloeon cognatum* Stephens, 1835 must be considered with caution as several
independent lineages are hidden behind these concepts (Rutschmann et al. 2014, Rutschmann et al. 2017).

Thomas (1998) proposed the first preliminary checklist of mayflies from North Africa, including 25 Baetidae species. Eleven species are endemic to North Africa: Centroptilum algericum Eaton, 1899; Baetis sinespinosus; Cloeon saharense Soldán and Thomas, 1983; Nigrobaetis numidicus (Soldán and Thomas, 1983); Nigrobaetis rhithralis (Soldán and Thomas, 1983); Procloeon stagnicola Soldán and Thomas, 1983; Cheleocloeon dimorphicum (Soldán and Thomas, 1985); Baetis berberus Thomas, 1986; Alainites oukaimeden (Thomas and Sartori, 1992); Alainites sadati Thomas, 1994 and Baetis chelif.

Eleven species are originally described from Algeria, ten of them between 1983 and 1986 (Soldán and Thomas 1983a, Soldán and Thomas 1983b, Soldán and Thomas 1985) and no new taxa have been described since 2005 (Soldán et al. 2005). Ten Central European species were reported from Maghreb, but all these identifications should be taken with caution. Remaining species present West Mediterranean distribution including North Africa and Iberian Peninsula (Baetis punicus Thomas, Boumaiza and Soldán, 1983; Baetis maurus Kimmins, 1938) or Italian peninsula and South of France (Baetis pavidus Grandi, 1949).

Recently, molecular reconstructions involving Baetidae were conducted for different projects, in particular for the origin of Macaronesian and Corsican mayflies fauna (Gattolliat et al. 2015, Gattolliat et al. 2018b, Rutschmann et al. 2014, Rutschmann et al. 2017). Despite being not directly focused on North Africa, they included specimens from Tunisia, Algeria and Morocco. These preliminary results for North Africa underlined important links between North African and Macaronesian faunas (in the case of Cloeon and Baetis). The discovery in Tunisia of a species of Leptophlebiidae, assumed as endemic to Sardinia (Zrelli et al. 2011), also confirmed possible connections between Italy and Maghreb, mainly during crucial geological events, such as the Messinian Salinity Crisis (Gattolliat et al. 2015).

North African species of Labiobaetis and Cheleocloeon Wuillot and Gillies, 1993 have most probably an Afrotropical origin as they are mainly diversified in this area. A dozenspecies of Cheleocloeon are described in Afrotropics, while the genus is only represented in the Palearctic by a single Maghrebian species (Cheleocloeon dimorphicum) and one in the Arabian Peninsula, Cheleocloeon soldani Gattolliat and Sartori, 2008 (Gattolliat and Sartori 2008, Kluge 2016a). Despite also being present in Central Europe, Labiobaetis is mostly diversified in tropical areas, as proven by its high diversity in Afrotropics (Gattolliat 2001, Lugo-Ortiz and McCafferty 1997) and South East Asia (Kaltenbach and Gattolliat 2018, Kaltenbach and Gattolliat 2019, Kaltenbach and Gattolliat 2020). While a part of the Afrotropical species present a very restricted distribution (Gattolliat 2001, Lugo-Ortiz and McCafferty 1997), a recent molecular reconstruction proved that specimens from Comoro Islands, South Africa and Arabian Peninsula form a monophyletic clade corresponding to Labiobaetis glaucus (Agnew, 1961) (Gattolliat et al. 2018a).

The present study is the first molecular analysis for Algerian mayflies using the cytochrome oxidase subunit I (COI) region for species delimitation. The main aims are to clarify the
status of the different species of Baetidae present in North West Algeria, especially for Central European species assumed to occur in Maghreb. We also want to clarify the species delimitation in some groups with potential cryptic species and significant difficulties to identify, based on morphological characters only. Finally, we want to understand the affinities between Maghrebian and neighbouring fauna.

**Materials and methods**

**Sampling**

We investigated twelve sampling sites, all located in the Tafna basin in North-West Algeria (Fig. 1); a detailed description of this area is presented in Benhadji et al. (2019). The Baetidae specimens used for the molecular study are listed in Table 1. They were collected by using a Surber net between April and October 2016, then preserved in 99% ethanol and stored at cold and stable temperature (4°C). A total of 52 larvae were identified at the generic or specific level, based on morphological characters (Benhadji et al. 2019), including *Rhodobaetis* spp. (26 specimens), *B. cf. pavidus* (13 specimens), *B. maurus* (four specimens), *Acentrella cf. sinaica* (one specimen), *C. cf. dipterum* (four specimens) and *P. stagnicola* (three specimens) (Table 1). Specimens and DNA extractions are housed in the collections of the Museum of Zoology, Lausanne, Switzerland.

| Taxa                  | Sites | Codes         | Genbank accession |
|-----------------------|-------|---------------|-------------------|
| *Baetis sinespinosus* | SK1   | RB_ALG_A06    | MT800078          |
|                       | SK1   | RB_ALG_D07    | MT800085          |
|                       | SK1   | RB_ALG_E07    | MT800086          |
|                       | SK1   | RB_ALG_F05F   | MT800093          |
|                       | SK1   | RB_ALG_G05F   | MT800095          |
|                       | SK1   | RB_ALG_H05F   | MT800096          |
|                       | CH0   | RB_ALG_D01F   | MT800091          |
|                       | CH1   | RB_ALG_B03    | MT800080          |
|                       | CH1   | RB_ALG_F02    | MT800087          |
|                       | CH1   | RB_ALG_H02    | MT800089          |
|                       | CH1   | RB_ALG_A03F   | MT800090          |
|                       | CH1   | RB_ALG_G02F   | MT800094          |

Table 1.

List of COI sequenced specimens with Genbank accession number. For sites code, see Benhadji et al. (2019). Each specimen is identified by alphanumeric codes. The two first letters indicate the taxonomic group (RB: *Rhodobaetis*; BP: *Baetis pavidus*; BM: *Baetis maurus*; AC: *Acentrella cf. sinaica*; CO: *Cloeon peregrinator*; PC: *Procloeon stagnicola*). ALG indicates the country (i.e. Algeria). The following letter (A to H) with number (1 to 8) indicate the position of the well on the PCR plate. An “F” is added at the end of some codes when only the forward amplification was successful.
| Taxa          | Sites | Codes   | Genbank accession |
|--------------|-------|---------|-------------------|
|              | CH4   | RB_ALG_B04 | MT800081          |
|              | CH4   | RB_ALG_C04 | MT800083          |
|              | IOM   | RB_ALG_F04 | MT800088          |
|              | TGB   | RB_ALG_C10 | MT800084          |
|              | TGB   | RB_ALG_D10F | MT800092        |
|              | TFF   | RB_ALG_A10 | MT800079          |
|              | TFF   | RB_ALG_B10 | MT800082          |
| Baetis atlanticus | SK1   | RB_ALG_C07 | MT800053          |
|              | CH0   | RB_ALG_C01F | MT800059         |
|              | KH1   | RB_ALG_C08 | MT800054          |
|              | KH1   | RB_ALG_D08 | MT800055          |
|              | KH1   | RB_ALG_E08 | MT800056          |
|              | KH1   | RB_ALG_H08 | MT800058          |
|              | KH1   | RB_ALG_F08 | MT800057          |
|              | KH1   | RB_ALG_G08F | MT800060        |
| Baetis cf. pavidus    | SK1   | BP_ALG_F07 | MT800069          |
|              | SK1   | BP_ALG_G07 | MT800071          |
|              | CH1   | BP_ALG_B02 | MT800062          |
|              | CH1   | BP_ALG_C02 | MT800063          |
|              | CH1   | BP_ALG_D02 | MT800065          |
|              | CH1   | BP_ALG_E02 | MT800067          |
|              | CH4   | BP_ALG_A02 | MT800061          |
|              | IOM   | BP_ALG_C05 | MT800064          |
|              | TGA   | BP_ALG_D09 | MT800066          |
|              | TGA   | BP_ALG_E09 | MT800068          |
|              | TFF   | BP_ALG_F09 | MT800070          |
|              | TFF   | BP_ALG_G09 | MT800072          |
|              | TFF   | BP_ALG_H09 | MT800073          |
| Baetis maurus      | CH1   | BM_ALG_G01 | MT800074          |
|              | CH1   | BM_ALG_H10 | MT800075          |
|              | SK1   | BM_ALG_D05F | MT800076       |
|              | SK1   | BM_ALG_E05F | MT800077       |
| Acentrella cf. sinaica | IOM   | AC-ALG-A05 | MT800052          |
| Cloeon peregrinator   | CH0   | CO_ALG_B01F | MT800100         |
|              | CH1   | CO_ALG_C03 | MT800097          |
|              | CH1   | CO_ALG_D03 | MT800098          |
**COI gene amplifications**

We performed DNA extraction using DNeasy Blood & Tissue kit (QIAGEN) and BioSprint 96 extraction robot (Qiagen) by soaking each specimen in buffer and proteinase K at 56°C for an overnight incubation. The mitochondrial DNA cytochrome oxidase c subunit I gene (COI) was amplified using the primers LCO1490 and HCO2198 (Folmer et al. 1994) with an initial denaturation temperature of 98°C for 30 sec followed by a total of 37 cycles with denaturation temperature of 98°C for 10 sec, an annealing temperature of 50°C for 30 sec and an extension at 72°C for 30 sec, final extension at 72°C for 2 min. We checked if the amplification was successful using agarose gel electrophoresis, then we purified PCR products and prepared bi-directional sequencing using the same primers LCO1490 and HCO2198.
COI gene trees

We corrected and edited forward and reverse sequencing reads using Bioedit, then we assembled each of the two complementary sequences using Codon Code Aligner (demo mode) and obtained sequence alignments (Suppl. materials 4, 5, 6, 7, 8, 9) using Jalview 2.10.1 via Mafft alignment as in Vuataz et al. (2011). We aligned sequences of each taxon with analogue genus or species selected from Genbank database (GenBank 2019) or BoldSystem database (BOLD 2019). For Rhodobaetis, we added sequences corresponding to the known haplogroups of Baetis rhodani (Gattolliat et al. 2015, Gattolliat et al. 2018b, Lucentini et al. 2011, Williams et al. 2006). For B. maurus, we selected all available sequences of B. maurus and several of B. gr. alpinus. For the remaining taxa, in addition to conspecific sequences, we incorporated outgroups sequences (for example, Cloeon simile Eaton, 1870, C. praetextum Bengtsson, 1914 and C. smaeleni Lestage, 1924 to C. cf. dipterum reconstruction). To delimit the haplogroups, we used ABGD, Automatic Barcode Gap Discovery (Puillandre et al. 2012).

To reconstruct the trees, we used Mega version 10.0.4; we chose the best evolutionary model using the AICc criteria (Akaike 1974), then we set to run a Maximum Likelihood bootstrap analysis with 1000 normal bootstrap replicates.

Checklist of the Baetidae species of the Tafna basin (North-West Algeria)

Order Ephemeroptera Hyatt & Arms, 1890

Family Baetidae Leach, 1815

Genus Acentrella Bengtsson, 1912

Acentrella cf. sinaica Bogoescu, 1931

Genus Baetis Leach, 1815

Baetis (Rhodobaetis) atlanticus Soldán and Godunko 2006

Baetis maurus Kimmins, 1938

Baetis cf. pavidus Grandi, 1949

Baetis (Rhodobaetis) sinespinosus Soldán and Thomas, 1983

Genus Cloeon Leach, 1815

Cloeon peregrinator Gattolliat and Sartori, 2008

Genus Procloeon Bengtsson, 1915

Procloeon stagnicola Soldán and Thomas, 1984
Analysis

Species delimitations

Based on the molecular analysis, seven Baetidae species were recognised or confirmed in the Tafna basin sites.

Subgenus *Rhodobaetis*

We obtained 11 haplogroups of *Rhodobaetis* (Suppl. material 1, Table 2) with the 27 Algerian sequences differentiating into two haplogroups (Fig. 2): a) RB_Gp1, a strongly supported monophyletic clade (97% BS) also containing Macaronesian and Iberian sequences from *B. atlanticus*; b) RB_Gp2, a monophyletic clade with a strong bootstrap support (98%) containing only Algerian sequences of *B. sinespinosus*. RB_Gp1 and RB_Gp2 have, respectively, 1.65% and 0.48% of intraspecific distance. RB_Gp1 and RB_Gp2 are poorly supported as a monophyletic clade (25% BS); the two haplogroups present an interspecific distance of 15.2%.

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**Figure 2.**

Maximum Likelihood tree including representative of *Rhodobaetis* using TN93 (+G+I) model; only bootstrap supports (BS) higher than 70% are indicated on the corresponding branch.
We obtained two haplogroups of *Baetis cf. pavidus* (Fig. 3, Table 3): a highly-supported clade gathering Algerian specimens and two sequences from southern France (BP_Gp1) and a second clade (BP_Gp2) which is composed by *Baetis pavidus* from Italy. Both haplogroups are highly supported as sister clades. BP_Gp1 possesses a very low intraspecific distance (0.2%) and high interspecific distance with its sister clade BP_Gp2 (11%).

### Table 2.
Distances within (in bold) and between *Rhodobaetis* haplogroups. RB-Gp1: *Baetis (Rhodobaetis) atlanticus*; RB-Gp2: *Baetis (Rhodobaetis) sinespinosus*. Haplogroups with Algerian sequences are underlined.

|        | RB_Gp1 | RB_Gp2 | RB_Gp3 | RB_Gp4 | RB_Gp5 | RB_Gp6 | RB_Gp7 | RB_Gp8 | RB_Gp9 | RB_Gp10 | RB_Gp11 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|
| RB_Gp1 | 0.02   |        |        |        |        |        |        |        |        |         |         |
| RB_Gp2 | 0.15   | 0.004  |        |        |        |        |        |        |        |         |         |
| RB_Gp3 | 0.16   | 0.14   | n/c    |        |        |        |        |        |        |         |         |
| RB_Gp4 | 0.15   | 0.14   | 0.12   | 0.004  |        |        |        |        |        |         |         |
| RB_Gp5 | 0.15   | 0.14   | 0.14   | 0.10   | 0.01   |        |        |        |        |         |         |
| RB_Gp6 | 0.17   | 0.17   | 0.18   | 0.18   | 0.20   | n/c    |        |        |        |         |         |
| RB_Gp7 | 0.18   | 0.16   | 0.17   | 0.14   | 0.16   | 0.12   | n/c    |        |        |         |         |
| RB_Gp8 | 0.20   | 0.19   | 0.17   | 0.17   | 0.20   | 0.14   | 0.12   | 0.01   |        |         |         |
| RB_Gp9 | 0.18   | 0.16   | 0.15   | 0.17   | 0.20   | 0.19   | 0.18   | 0.21   | 0.003  |         |         |
| RB_Gp10| 0.19   | 0.17   | 0.17   | 0.16   | 0.17   | 0.19   | 0.18   | 0.19   | 0.10   | 0.01    |         |
| RB_Gp11| 0.26   | 0.24   | 0.23   | 0.23   | 0.25   | 0.22   | 0.23   | 0.25   | 0.23   | 0.22    | 0.002   |

### Baetis cf. pavidus

We obtained two haplogroups of *Baetis cf. pavidus* (Fig. 3, Table 3): a highly-supported clade gathering Algerian specimens and two sequences from southern France (BP_Gp1) and a second clade (BP_Gp2) which is composed by *Baetis pavidus* from Italy. Both haplogroups are highly supported as sister clades. BP_Gp1 possesses a very low intraspecific distance (0.2%) and high interspecific distance with its sister clade BP_Gp2 (11%).

### Table 3.
Distances within (in bold) and between *Baetis cf. pavidus* (BP_Gp1-BM_Gp2), *Baetis alpinus* (BP_Gp3) and *Baetis lutheri* haplogroups (BP_Gp4-BP_Gp7). Haplogroup with Algerian sequences is underlined.

|        | BP_Gp1 | BP_Gp2 | BP_Gp3 | BP_Gp4 | BP_Gp5 | BP_Gp6 | BP_Gp7 |
|--------|--------|--------|--------|--------|--------|--------|--------|
| BP_Gp1 | 0.002  |        |        |        |        |        |        |
| BP_Gp2 | 0.11   | 0.003  |        |        |        |        |        |
| BP_Gp3 | 0.21   | 0.23   | 0.004  |        |        |        |        |
| BP_Gp4 | 0.22   | 0.24   | 0.23   | n/c    |        |        |        |
| BP_Gp5 | 0.23   | 0.25   | 0.24   | 0.08   | 0.006  |        |        |
| BP_Gp6 | 0.23   | 0.25   | 0.25   | 0.15   | 0.15   | 0.008  |        |
| BP_Gp7 | 0.24   | 0.24   | 0.24   | 0.23   | 0.22   | 0.23   | n/c    |
We delineated three *B. maurus* haplogroups (Suppl. material 2, Table 4). Our sequences (BM_Gp1) belong to a strongly-supported monophyletic haplogroup identified as *Baetis maurus* (Fig. 4). This haplogroup is highly supported as the sister clade of specimens identified also as *B. maurus* (BM_Gp2 and BM_Gp3), but coming from Spain; the distances between these clades, all identified as *B. maurus*, are of interspecific level (15-16%).

Table 4.

Distances within (in bold) and between *Baetis maurus* (BM_Gp1-BM_Gp3) and *Baetis* cf. *alpinus* haplogroups (BM_Gp4-BM_Gp7). Haplogroup with Algerian sequences is underlined.

|        | BM_Gp1 | BM_Gp2 | BM_Gp3 | BM_Gp4 | BM_Gp5 | BM_Gp6 | BM_Gp7 |
|--------|--------|--------|--------|--------|--------|--------|--------|
| BM_Gp1 | 0      |        |        |        |        |        |        |
| BM_Gp2 | 0.16   | 0.003  |        |        |        |        |        |
| BM_Gp3 | 0.15   | 0.16   | 0.007  |        |        |        |        |
| BM_Gp4 | 0.24   | 0.24   | 0.23   | 0.043  |        |        |        |
| BM_Gp5 | 0.26   | 0.24   | 0.21   | 0.23   | 0.06   |        |        |
| BM_Gp6 | 0.24   | 0.23   | 0.22   | 0.25   | 0.19   | 0.06   |        |
| BM_Gp7 | 0.25   | 0.26   | 0.22   | 0.25   | 0.19   | 0.19   | 0.04   |
Acentrella cf. sinaica

The reconstruction divided Acentrella sequences into 6 haplogroups (Fig. 5, Table 5). The single sequence from Algeria was recovered as an independent clade (AC_Gp5) distant at least 19% from other clades. AC_Gp6 haplogroup is the sister clade of AC_Gp5; it contains A. sinaica sequences from France and Italy gathered with a very strong BS (100%).

Table 5.
Distances within (in bold) and between Acentrella haplogroups. AC-Gp1: Acentrella nadineae. AC-Gp2: Acentrella parvula. AC-Gp3: Acentrella turbida. AC-Gp4: Acentrella laponica. AC-Gp5: Acentrella cf. sinaica. AC-Gp6: Acentrella sinaica. Haplogroup with Algerian sequence is underlined.

|         | AC_Gp1 | AC_Gp2 | AC_Gp3 | AC_Gp4 | AC_Gp5 | AC_Gp6 |
|---------|--------|--------|--------|--------|--------|--------|
| AC_Gp1  | 0      |        |        |        |        |        |
| AC_Gp2  | 0.19   | 0.011  |        |        |        |        |
| AC_Gp3  | 0.23   | 0.21   | 0      |        |        |        |
| AC_Gp4  | 0.22   | 0.22   | 0.24   | 0.002  |        |        |
| AC_Gp5  | 0.25   | 0.25   | 0.26   | 0.22   | n/c    |        |
| AC_Gp6  | 0.26   | 0.25   | 0.24   | 0.25   | 0.19   | 0.003  |
**Cloeon peregrinator**

We obtained 11 haplogroups from the reconstruction (Suppl. material 3, Table 6) including six highly supported *C. cf. dipterum* haplogroups. The clade containing all haplogroups of *C. dipterum* s.l. is a monophyletic clade with a high BS (99%).

**Table 6.**
Distances within (in bold) and between *Cloeon* haplogroups. CO_Gp1-CO-Gp6: *Cloeon dipterum* sl. CO_Gp2: *Cloeon peregrinator*. Haplogroup with Algerian sequences is underlined.

|        | CO_Gp1 | CO_Gp2 | CO_Gp3 | CO_Gp4 | CO_Gp5 | CO_Gp6 | CO_Gp7 | CO_Gp8 | CO_Gp9 | CO_Gp10 | CO_Gp11 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|
| CO_Gp_1  | 0.002  |        |        |        |        |        |        |        |        |         |         |
| CO_Gp_2  | 0.09   | 0.002  |        |        |        |        |        |        |        |         |         |
| CO_Gp_3  | 0.10   | 0.10   | 0.002  |        |        |        |        |        |        |         |         |
| CO_Gp_4  | 0.11   | 0.11   | 0.07   | 0.012  |        |        |        |        |        |         |         |
| CO_Gp_5  | 0.11   | 0.11   | 0.08   | 0.08   | 0.021  |        |        |        |        |         |         |
| CO_Gp_6  | 0.09   | 0.09   | 0.1    | 0.1    | 0.1    | 0.002  |        |        |        |         |         |
| CO_Gp_7  | 0.15   | 0.15   | 0.17   | 0.17   | 0.17   | 0.16   | 0.16   | 0.01   |        |         |         |
| CO_Gp_8  | 0.18   | 0.19   | 0.19   | 0.19   | 0.20   | 0.20   | 0.20   | 0.15   | 0.00   |         |         |
| CO_Gp_9  | 0.19   | 0.21   | 0.2    | 0.19   | 0.19   | 0.20   | 0.20   | 0.20   | 0.22   | 0.013   |         |
| CO_Gp_10 | 0.17   | 0.19   | 0.17   | 0.18   | 0.18   | 0.19   | 0.19   | 0.20   | 0.11   | 0.005   |         |
| CO_Gp_11 | 0.17   | 0.19   | 0.16   | 0.17   | 0.17   | 0.17   | 0.17   | 0.18   | 0.18   | 0.19    | n/c     |

**Figure 5.**
Maximum Likelihood tree including a representative of *Acentrella* spp using the General Time Reversible model (+I); only bootstrap supports (BS) higher than 70% are indicated on the corresponding branch.
The CO_Gp2 haplogroup, which includes four sequences from Algeria and sequences of *C. peregrinator* from Madeira (type locality) and Gran Canaria (Fig. 6), is highly supported as a monophyletic haplogroup (100%). This haplogroup has a low intraspecific distance (0.2%) and high interspecific distance with all the other haplogroups of *C. dipterum* s.l., for instance with CO_Gp1 with which it has the least distance (8.9%). Consequently, the sequences from Algeria and Gran Canaria belong to *C. peregrinator*.

![Figure 6](image-url)

**Procloeon stagnicola**

In this reconstruction, six haplogroups (Fig. 7, Table 7) were obtained. *Procloeon stagnicola* from Algeria (PC_Gp1) forms a well-supported monophyletic haplogroup. PC_Gp1 has a high interspecific distance from PC_Gp2 (16.2%), which corresponds to the closest European species *Procloeon bifidum* (Bengsston, 1912) and from the remaining sequences (from 16% to 23.7%).
**Table 7.**
Distances within (in bold) and between *Procloeon* haplogroups (PC_Gp1-PC_Gp5) and *Centroptilum luteolum* group (PC_Gp6). PC-Gp1: *Procloeon stagnicola*. PC-Gp2 – PC-Gp3: *Procloeon bifidum*. PC-Gp4: *Procloeon pennulatum*. Haplogroup with Algerian sequences is underlined.

|        | PC_Gp1 | PC_Gp2 | PC_Gp3 | PC_Gp4 | PC_Gp5 | PC_Gp6 |
|--------|--------|--------|--------|--------|--------|--------|
| PC_Gp1 | 0.006  |        |        |        |        |        |
| PC_Gp2 | 0.16   | 0.027  |        |        |        |        |
| PC_Gp3 | 0.18   | 0.19   | n/c    |        |        |        |
| PC_Gp4 | 0.19   | 0.19   | 0.20   | 0.016  |        |        |
| PC_Gp5 | 0.19   | 0.20   | 0.21   | 0.20   | 0.037  |        |
| PC_Gp6 | 0.22   | 0.23   | 0.24   | 0.24   | 0.23   | 0.000  |

**Figure 7.**
Maximum Likelihood tree including a representative of *Procloeon* spp using the General Time Reversible model (+G+I); only bootstrap supports (BS) higher than 70% are indicated on the corresponding branch.

**Discussion**

The different trees we obtained allowed us to better understand the composition of Algerian Baetidae. Based on our analysis, we can link the Algerian lineages with their sister-groups, calculate the maximum and minimum distances and evaluate which lineages may represent putative species.
Maghrebian endemic species

*Baetis (Rhodobaetis) sinespinosus* Soldán and Thomas, 1983

*Rhodobaetis* is a subgenus of *Baetis* and corresponds to the concept of *Baetis rhodani* species-group (Jacob 2003, Müller-Liebenau 1969). It is widely distributed in all West Palearctic streams (Gattolliat et al. 2015). Three species of *Rhodobaetis* are reported from Algeria, two of them are endemic: *Baetis chelif* and *Baetis sinespinosus* (Bauernfeind and Soldán 2012, Thomas 1998). A representative of *B. rhodani* s.l. is also reported (Thomas 1998). As noticed by Sroka (2012), it is generally difficult to find morphological characters to support the molecular species delimitation within *Rhodobaetis*. In the present case, the absence of a single rudimentary scale on the tip of the maxillary palp and the presence of four rows of setae at the apex of the paraglossae indicate that the clade RB_Gp2 corresponds to *B. sinespinosus*. *Baetis sinespinosus* is a well-supported monophyletic clade and presents high interspecific distances with all the other European and Mediterranean species. According to our data and reports from literature, this species seems to be endemic to Algeria. However, its presence in nearby countries, such as Tunisia and Morocco, will not be surprising as several populations were provisionally identified as *Baetis rhodani* s.l. (Mabrouki et al. 2017, Thomas 1998, Zrelli et al. 2016) and the present study is based on material collected very close to the border of Morocco.

*Procloeon stagnicola* Soldán and Thomas, 1983

Our results showed a high interspecific distance between the Algerian clade and its European sister species *Procloeon bifidum*; thus, it confirms the validity of *Procloeon stagnicola*. This latter differs from *P. bifidum* especially by the flat and rounded bristles on the labrum margin; the pointed apex of the gills with an extremely reduced second lamella and also by the lateral margins of the abdominal segments which possess spines from segment V to IX. The species was originally described from Algeria (Soldán and Thomas 1983a) and was subsequently discovered in Tunisia (Boumaiza and Thomas 1995). The report of *Procloeon bifidum* from Morocco (El Alami et al. 2000) may be a misidentification and may also concern *P. stagnicola*. Identification of material collected by the last author (Jean-Luc Gattolliat) and stored in the MZL collection confirmed the presence of this species in Morocco (unpublished data). This species should be therefore considered as endemic to Maghreb.

*Baetis maurus* Kimmins, 1938

*Baetis maurus* is a representative of the *Baetis alpinus* species-group. The species is considered as an Atlanto-Mediterranean element (Bauernfeind and Soldán 2012). It was originally described from Morocco (Kimmins 1938), then reported from the Iberian Peninsula (Alba-Tercedor 1982, Müller-Liebenau 1974). Algerian haplotypes present high distances with presumably conspecific specimens from Spain (Murria et al. 2017), as well as with the other Euro-Mediterranean species belonging to the *B. alpinus* species-group. Our results tend to prove that *B. maurus* is, in fact, a Magrebian endemic species (originally described from Morocco) and that at least one sister undescribed species occurs...
in the Iberian Peninsula. These preliminary results must be confirmed by sequencing additional populations from Spain and Maghreb and by morphological evidence. With the presence of a second rudimentary row of denticles on claws, both Maghrebian and Iberian populations possess a unique morphological character amongst the genus *Baetis* (Thomas et al. 1983).

*Acentrella cf. sinaica* Bogoescu, 1931

*Acentrella sinaica* is a South and Central European species (Bauernfeind and Soldán 2012). It was reported from Tunisia (Boumaiza and Thomas 1995) and then from Algeria (Mebarki et al. 2017). In his checklist of North African mayflies, Thomas (1998) considered the specific identification as possibly incorrect, referring maybe to *Acentrella almohades* Alba-Tercedor & El Alami 1999 described from Morocco (Alba-Tercedor and El Alami 1999). The important distances between our unique haplotype and those from France and Italy tend to confirm that Algerian specimens do not belong to *A. sinaica*. Unfortunately, no sequence is available for *A. almohades*. We refrain to attribute the specimens from Algeria to *A. almohades*, as important distinctive characters do not match between our specimens and the original description. Especially, the Algerian specimens possess long setae along the dorsal margin of the femora (similar to *A. sinaica*), while *A. almohades* present much shorter ones (Alba-Tercedor and El Alami 1999). There is, therefore, some probability that our specimens represent an undescribed species from North Africa. Additional sequences and close morphological studies are needed to confirm this hypothesis.

Western Mediterranean species

*Baetis cf. pavidus* Grandi, 1949

*Baetis pavidus* is a Western Mediterranean species and was originally described from Italy and then reported from the Maghreb (Bauernfeind and Soldán 2012, Jacob 2003). In Algeria and Tunisia, it is one of the most common and abundant species at low to middle elevation and is rather tolerant to pollution and low oxygenation (Benhadji et al. 2019, Boumaiza and Thomas 1995). The Algerian haplotypes present low genetic distance with specimens from southern France, confirming the link for this species between North African and South European populations. The sequences from Sicily, Italy (BP_Gp2) were not assigned to *Baetis pavidus* with certainty by Tenchini et al. (2018) and may represent an undescribed species close to *B. pavidus* or, alternatively, may be correctly associated with *B. pavidus* and the specimens from Algeria and from South of France represent a new species.

Widely-distributed species

*Cloeon peregrinator* Gattolliat and Sartori, 2008

*Cloeon peregrinator* was first considered as an endemic species from Madeira (Gattolliat et al. 2008), then was also found on the Canary Islands (Rutschmann et al. 2017). It belongs to the *Cloeon dipterum* species-group from which it can be separated by minute morphological characters (Gattolliat et al. 2008). Algerian haplotypes present a low genetic
distance with Madeiran specimens which prove their conspecificity. This discovery is rather surprising as *C. peregrinator* was first thought to be an insular endemic. Molecular studies showed mayflies are able to colonise islands, even for such taxa with presumably low dispersal capacity (Monaghan et al. 2005, Rutschmann et al. 2014). The origin of the species cannot yet be proven and two scenarios can be proposed: either a colonisation of Macaronesia from Continental Europe, then a speciation process on the islands and a subsequent colonisation of North Africa or, alternatively, a colonisation of North Africa by a European lineage followed by a speciation process and subsequently a colonisation of Macaronesia.

**Baetis atlanticus** Soldán and Godunko, 2006

Bauernfeind and Soldán (2012) stated that *Baetis atlanticus* is a *Rhodobaetis* species endemic to Madeira, while Rutschmann et al. (2014) showed later that the species has a much wider distribution. They pointed out its European or North-African (Morocco) origin and suggested a recent colonisation of Madeira. *Baetis atlanticus* is widely distributed in Atlantic Europe as proven by the recent reports of the species from the United Kingdom (Macadam et al. 2018). This species remains difficult to distinguish morphologically from other *Rhodobaetis* species (Soldán and Godunko 2006). The main discriminating character between *B. atlanticus* and the North-African endemic *B. sinespinosus* is the number of regular rows of long setae at the apex of the paraglossae (the usual three rows in *B. atlanticus* and four rows in *B. sinespinosus*) and the apex of the maxillary palp (with one typical small apical scale in *B. atlanticus* and without the apical scale in *B. sinespinosus*). The genetic distances amongst Macaronesian, Iberian and Algerian specimens unequivocally confirm the conspecificity of the different populations. The ecological preference of *B. atlanticus* in the Tafna catchment is similar to that of the lowlands of Madeira (Soldán and Godunko 2006): larvae prefer coarse substrate composed of rocks, cobbles or pebbles in low, moderate to fast current velocities; they were less abundant and only present in relatively-preserved sites at higher altitude (CH0; CH1; KH1; SK1; IOM). In comparison, *B. sinespinosus* larvae have a much wider ecological range and are more pollution resistant; they are also highly abundant and present in all sampling sites with various substrates and velocities.

**Concluding remarks**

We summarise the state of the knowledge and the implication of the present study for the Baetidae fauna of North-West Algeria in Table 8. As we mostly found interspecific distances between Algerian and European lineages, our results generally highlighted and confirmed the high endemism of North African Baetidae. According to the present knowledge, the endemism may be restricted to Algeria or to the Maghreb, even for species that were supposed to present West Palearctic distribution (Western Europe and North Africa). The link with the Iberian Peninsula is less strong than expected, as no species included in the study is shared only between the two areas. From a genetic point of view, only one case of sister-species was found (*Baetis maurus*). *Baetis cf. pavidus* is the only species with a West Mediterranean distribution, as the same species occurs in Algeria and South of
France. Finally, our study confirms the presence of Macaronesian and Atlantic species in Maghreb and, therefore, endorses the preliminary results of Rutschmann et al. (2014), Rutschmann et al. (2017).

| Operational Taxonomic Units | Implications from this study |
|-----------------------------|------------------------------|
| Acentrella cf. sinaica      | New unnamed species in North Africa |
| Baetis maurus               | Endemic to North Africa\textsuperscript{1,2}; new unnamed species in Iberian Peninsula |
| Baetis cf. pavidus          | North Africa and South of France\textsuperscript{1,2} |
| Baetis (Rhodobaetis) atlanticus | First report for North Africa; known from Macaronesia and Atlantic Europe\textsuperscript{1,3} |
| Baetis (Rhodobaetis) sinespinosus | Confirmation of North African endemism\textsuperscript{1} |
| Cloeon peregrinator         | First report for North Africa; known from Macaronesia\textsuperscript{1} |
| Procloeon stagnicola        | Confirmation of North African endemism\textsuperscript{1} |

The next steps will be to sequence more specimens from different areas of Algeria and also from Morocco and Tunisia to confirm the monophyly of the different North African clades. The results, especially the validation of the new species hypotheses, need to be confirmed by integrative methods. Only morphological evidence and more mitochondrial or nuclear genes can validate the specific status of these clades. Our study may have implications outside of North Africa, as our results suggest that one or two lineages, previously supposed to belong to *Baetis maurus*, may represent new species in Spain, as well as the presumably non-conspecificity of the French and Italian lineages of *Baetis pavidus*.

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**Supplementary materials**

**Suppl. material 1: Complete Maximum Likelihood tree including representative of *Rhodobaetis* using TN93 (+G+I) model** [doi](https://doi.org/10.1051/limn/1983010)

- **Authors:** Nadhira Benhadji, Michel Sartori, Karima Abdellaoui Hassaine & Jean-Luc Gattolliat
- **Data type:** Biomolecular tree
- **Download file** (9.65 MB)
Suppl. material 2: Complete Maximum Likelihood tree including representative of *Baetis maurus* using TN93 (+G+I) model

Authors: Nadhira Benhadji, Michel Sartori, Karima Abdellaoui Hassaine & Jean-Luc Gattolliat  
Data type: Biomolecular tree  
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Suppl. material 3: Complete Maximum Likelihood tree including representative of *Cloeon* spp. using General Time Reversible model (+G+I)

Authors: Nadhira Benhadji, Michel Sartori, Karima Abdellaoui Hassaine & Jean-Luc Gattolliat  
Data type: Biomolecular tree  
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Suppl. material 4: RB_Sequence alignment

Authors: Nadhira Benhadji, Michel Sartori, Karima Abdellaoui Hassaine & Jean-Luc Gattolliat  
Data type: Nucleotide sequences  
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Suppl. material 5: BP_sequence alignment

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Suppl. material 6: BM_sequence alignment

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Suppl. material 7: AC_sequence alignment

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Suppl. material 8: CO_sequence alignment

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Data type: Nucleotide sequences  
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Suppl. material 9: PC_sequence alignment

Authors: Nadhira Benhadji, Michel Sartori, Karima Abdellaoui Hassaine & Jean-Luc Gattolliat  
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