Zootaxa 1957: 52–68 (2008)
www.mapress.com/zootaxa/

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Revision of Madeiran mayflies (Insecta, Ephemeroptera)

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Abstract

A comprehensive revision of the mayflies (Ephemeroptera) inhabiting the island of Madeira (Portugal) is presented, based on newly collected specimens and DNA sequence analysis. Disregarding a report of a single *Centroptilum* female subimago that is very likely a misidentification, the Madeira mayfly fauna encompasses three species, all belonging to the Baetidae. Previous reports of the continental species *Cloeon dipterum* Linnaeus, 1758 are erroneous, as all examined specimens belong to the new species *Cloeon peregrinator* n.sp. Gattolliat and Sartori. *Baetis atlanticus* Soldán & Godunko, 2006 is by far the most common and abundant species on the island. A second new species, *B. enigmaticus* n.sp. Gattolliat and Sartori is established for larvae presenting rheophilous adaptations similar to those of *B. pseudorhodani* Müller-Liebenau, 1971 from the Canary Islands.

Key words: Madeira, *Cloeon dipterum*, *Baetis rhodani*, revision, new species

Introduction

The earliest known reference to Ephemeroptera from the island of Madeira (Portugal) is attributed to Hagen (1865), who mentioned two species of Baetidae, *Cloe dipterum* (Linnaeus, 1758) and a new species, *Cloe maderensis* Hagen, 1865. Eaton (1871) later proposed the synonymy of *Cloe maderensis* with *Baetis rhodani* (Pictet, 1843). More than 75 years later, *Cloeon dipterum* and *Baetis rhodani* were again confirmed as the only two species occurring on the island (Brinck & Scherer 1961). A third species, *Centroptilum* sp., was mentioned based on a single female subimago (Classey 1966). In her revision of European *Baetis* species, Müller-Liebenau (1969) confirmed the presence of *B. rhodani* in Madeira, and again in a separate study of the mayflies from the Canary Islands (Müller-Liebenau 1971). Malmqvist (1988) was the first to provide ecological data for *B. rhodani* on Madeira, followed by studies of distribution and ecology by Stauder (1991; 1995). In addition to *C. dipterum* and *B. rhodani*, Stauder (1991) also reported the occurrence of *B. pseudorhodani* Müller-Liebenau, 1971, a species described from the Canary Islands, in two streams on Madeira. These four species were included in the first comprehensive catalogue of Madeiran freshwater macroinvertebrates (Hughes *et al.* 1998). Most recently, Soldán & Godunko (2006) suggested that *B. pseudorhodani* on Madeira was a misidentification, and proposed the name *Baetis atlanticus* for this species.

 Accepted by M. Hubbard: 30 Oct. 2008; published: 5 Dec. 2008

Thus, prior to the present study, the mayflies from Madeira were thought to be composed of:

Cloeon dipterum (Linnaeus, 1758)
Centroptilum sp.
Baetis rhodani (Pictet, 1843) = *B. maderensis* (Hagen, 1865)
Baetis atlanticus Soldán & Godunko, 2006 = *B. pseudorhodani* sensu Stauder, 1991 nec Müller-Liebenau, 1971

Within the context of a larger research programme on the colonization of islands by mayflies (Gattolliat 2004; Monaghan *et al.* 2005), our study of Atlantic Islands began in 2005. With the aim of reconstructing evolutionary relationships to decipher the colonization patterns of these insects using DNA markers, our sampling has led to the collection of all previously recognized species from Madeira except for *Centroptilum* sp. Because no *Centroptilum* have been caught despite an extensive sampling programme, we suspect that the mention of a *Centroptilum* female subimago is probably a misidentification of a *Baetis* female, hence it will not be treated here. The objective of the current project is to clarify the taxonomic confusion that has arisen in the mayfly fauna of Madeira over the last 150 years. In particular we want to know if the continental species *Baetis rhodani* and *Cloeon dipterum* occur in Madeira. Of importance is also to check the status of the synonymy made by Soldán & Godunko (2006) between *B. atlanticus* and *B. pseudorhodani* sensu Stauder (1991) since some discrepancies appear between the two descriptions.

The species *Baetis rhodani* is widespread in the West Palaearctic region and some very closely related species have been recently described (Thomas 1999; Godunko *et al.* 2004a; Godunko *et al.* 2004b; Beketov & Godunko 2005; Soldán *et al.* 2005). A phylogenetic analysis of mitochondrial DNA revealed a number of highly divergent lineages, strongly suggesting that *B. rhodani* represents a complex of sibling species (Williams *et al.* 2006). We therefore compared *Baetis* species from Madeira with the neotype of *B. rhodani* (Versoix River, tributary of Lake of Geneva, Switzerland) (for details see Gattolliat & Sartori 2008).

The holotypes and some of the paratypes are housed in the Museum of Zoology, Lausanne, Switzerland. Other paratypes are deposited in the Museu Municipal do Funchal (História Natural), Funchal, Madeira (Portugal).

Descriptions

Cloeon peregrinator n.sp. Gattolliat and Sartori

Cloe diptera sensu Hagen 1865 pro parte nec Linnaeus, 1758
Cloeon dipterum sensu Eaton, 1871, 1885 pro parte
Cloeon dipterum sensu Brinck & Scherer, 1961
Cloeon dipterum sensu Stauder, 1991, 1995

Material observed: Holotype: 1 larva; Madeira; Funchal, Jardim Botanico (ponds); 32°39’43”N / 16°53’44”W; Alt. 270m; 17.04.2006. S.J. Hughes coll.

Paratypes: 27 larvae (3 on slides); same data as holotype.

1 larva on slide; Madeira; R. do Alecrim, Road to Rabacal; 32°45’13’’N / 17°07’46’’W; Alt. 1280m; 1.12.2005. P. Derleth & M. Sartori coll.

2 female imagos; Madeira; Funchal; 32°38’30’’N / 16°55’36’’W; Alt. 70m.; 2.12.2005. P. Derleth & M. Sartori coll.

1 male imago; Madeira; Funchal (Rua dos Netos); 32º39’03.48’’N / 16º54’37.75’’W; Alt 36m; 10.02.2008. S.J. Hughes & J.P. Abreu coll.

Larva

Female larva length: body 5.7–6.6 mm; cerci 5.0–5.7 mm; median caudal filament 4.6–5.4 mm.

Male larva length: body 5.5–6.1 mm; cerci 4.9–5.4 mm; median caudal filament 4.6–5.1 mm.

Colouration. Head yellow with two longitudinal brown stripes with vermiform marks. Male turbinate eyes honey brown. Antennae yellow. Thorax medium amber brown with symmetrical darker spots apico-medially of each segment. Legs generally uniformly ecru, in some specimens with a small brown dot in middle of lateral margin of femora. Tergal coloration yellow and brown. Pattern rather similar between different terga: medially brown and laterally yellow; terga I, IV, VII and IX generally lighter. Sterna ecru without pattern or coloration. Cerci yellow with broad brown stripe at 2/3 of length, brown annulations every four segments.

Head. Dorsal surface of labrum (Fig. 1) apically with long and stout setae not arranged in a row, finer setae proximally; ventral surface with 9 small pointed setae; distal and lateral margins bordered with long and simple setae.

Hypopharynx (Fig. 2) with lingua rounded, covered apically only with minute setae; superlingua as long as lingua.

Right mandible (Fig. 3) with two sets of incisors, outer with 4 denticles and inner with 3 denticles; prostheca with 4 elongated denticles; tuft of very abundant long setae between prostheca and mola.

Left mandible with two almost completely fused sets of incisors; outer set with 5 denticles, inner set with 4 denticles (Fig. 4); prostheca with 3 denticles and an elongated comb-shaped structure; tuft of numerous setae between prostheca and mola.

Maxillae (Fig. 5) with 4 hooked teeth, none of them opposed to others; one row of medium subequal setae and one row of spine-like slightly feathered setae; palp 3-segmented, segments II and III partially fused, segment I approximately 0.7 x length of segments II and III combined, segment II approximately 1.8 x length of segment III.

Labium (Fig. 6) with glossae subequal to paraglossae; margin of glossae with short and stout setae, ventrally with long and thin setae; paraglossae falcate with dorsally and ventrally a row of long and stout setae parallel to inner margin and scattered thin setae, stout and long setae apically; labial palp 3-segmented; segment I rectangular and elongated, 0.8 x length of segments II and III combined; segment II expanded apically, with a dorsal oblique row of 5 pointed setae; segment III broad and clavate, distal margin with stout pointed setae.

Thorax. Forelegs (Fig. 7). Femora dorsally and ventrally with numerous short and pointed setae; dorsoapical setal patch formed by 3 small setae.

Tibiae with numerous short to medium pointed setae; subproximal arc of thin setae present.

Tarsi almost bare dorsally; ventral and upper margins with numerous small to medium pointed setae, none of them pectinate; tarsal claws (Fig. 8) hooked and elongated, approximately 0.5x length of tarsi, with 2 rows of about 17 acute teeth increasing in length toward the apex; subapical setae absent.

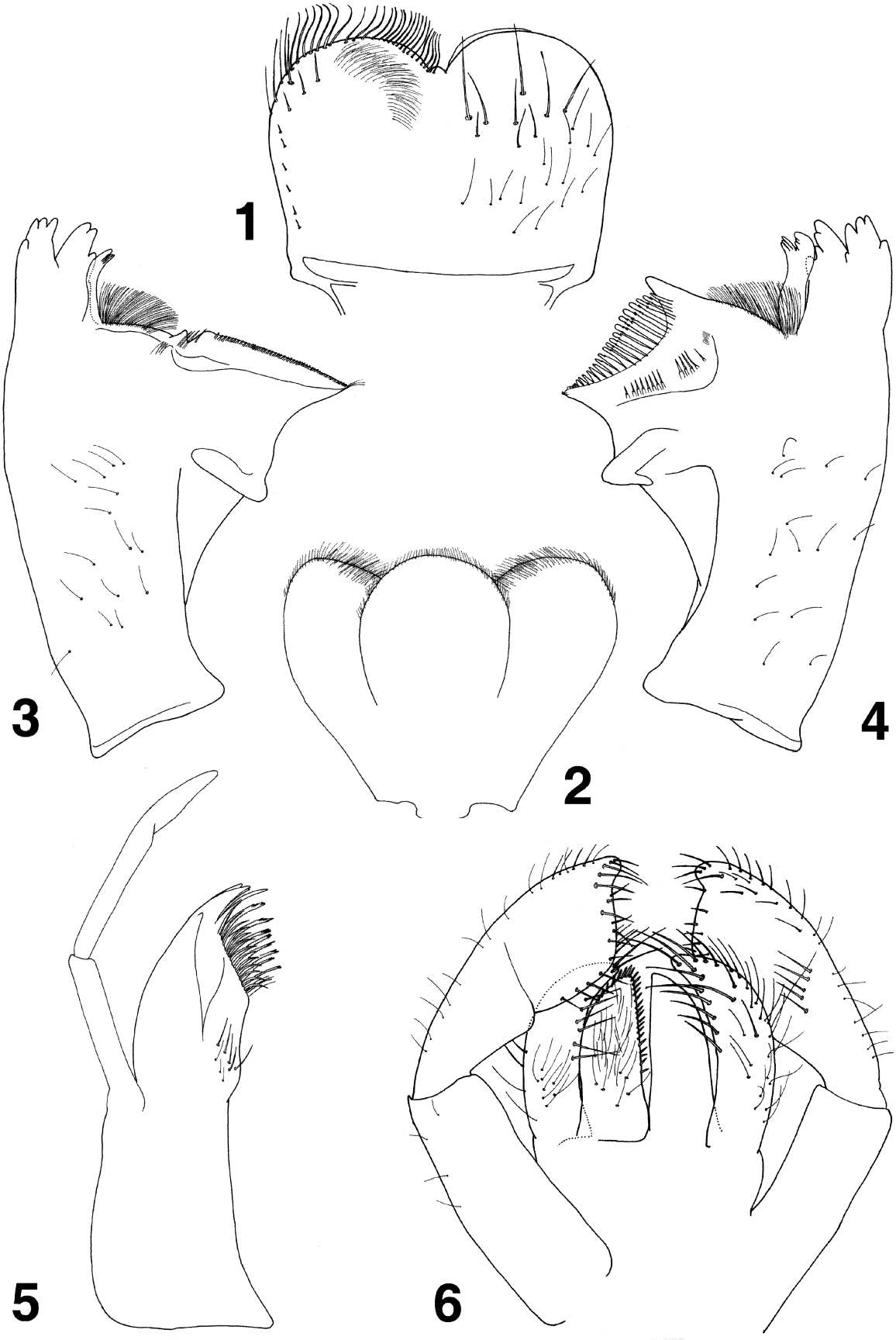
Abdomen. Terga (Fig. 10) with scale bases; posterior margin with long acute spines. Sterna without setae or scale bases, distal margin smooth.

Lateral margin of abdominal segment VIII with about 8 spines; lateral margin of abdominal segment IX with about 8 spines; lateral margins of segments I–VII without spines (Fig. 11).

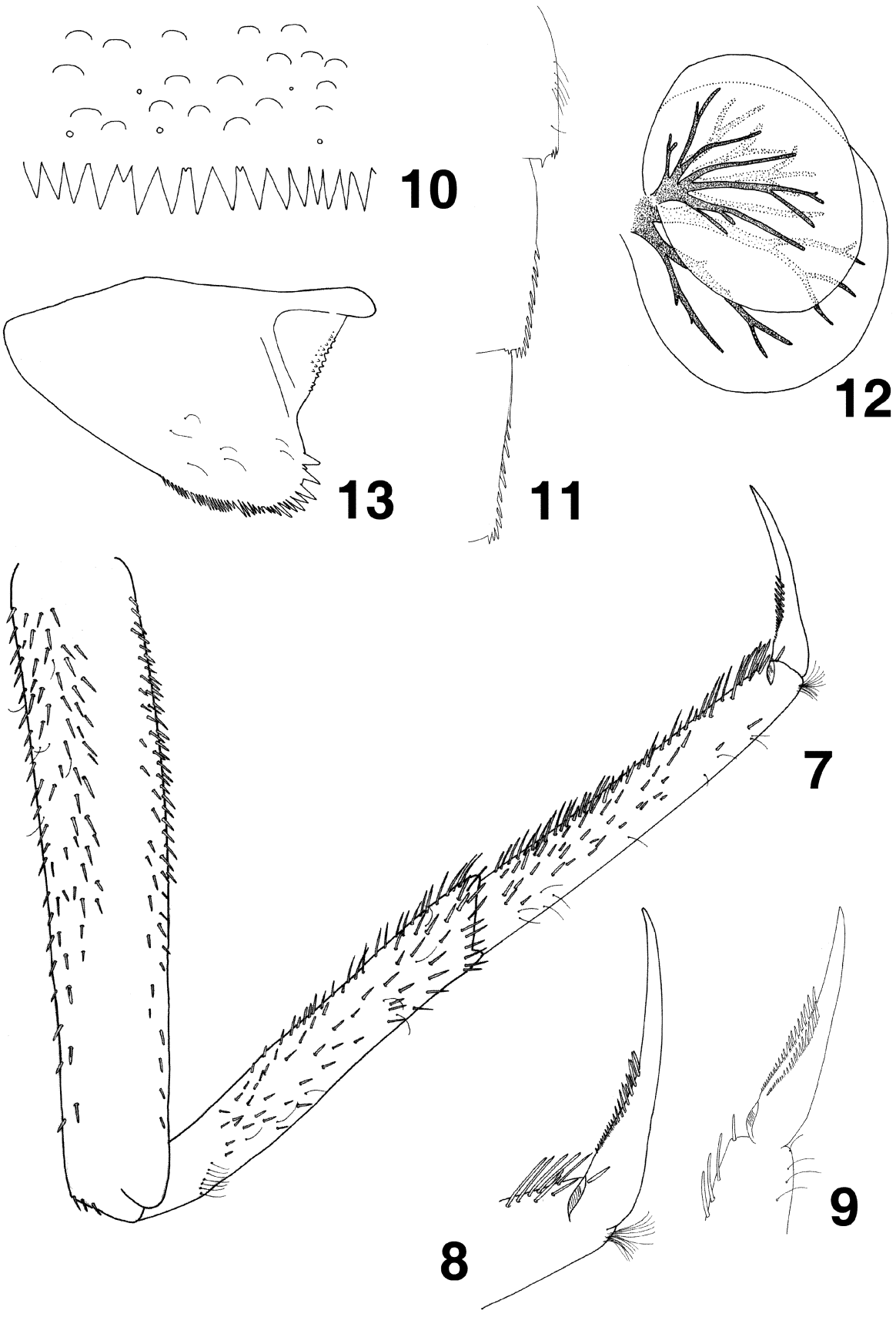
Gills (Fig. 12) Gills (Fig. 12) on segments I to VI with two lamellae, upper lamella rounded and well developed; gill VII single.

Paraproct (Fig. 13) without scales or scale bases, with only a few thin setae; margin with numerous slender and pointed spines, apico-lateral ones more developed; postero-lateral extension without scale bases, margin with three rows of minute spines.

Cerci with abundant thin setae on inner margins in basal half, apical half extremely thin and without setae; median caudal filament similar to cerci except thin setae on both margins.



FIGURES 1–6. Larval structures of *Cloeon peregrinator*: 1, labrum (left: ventral; right: dorsal). 2, hypopharynx. 3, right mandible. 4, left mandible. 5, right maxilla. 6, labium.



FIGURES 7–13. Larval structures of *Cloeon peregrinator* (except fig. 9: *Cloeon dipterum*): 7, foreleg. 8, tarsal claw (*Cloeon peregrinator*). 9, tarsal claw (*Cloeon dipterum*). 10, distal margin of abdominal tergum IV. 11, lateral margin of abdominal segments VII–IX. 12, gill IV. 13, paraproct.

Female imago

Length: body 8.1 mm; forewings 7.7 mm; cerci 13.2 mm.

Head: light brown with limits of sclerites dark brown (Fig. 14). Eyes grey with two black transverse stripes. Thorax. Prothorax medium brown with central symmetrical longitudinal lighter stripes and two brown broad lateral lines. Meso and metathorax dark brown. Forewings with costal, subcostal and distal half of first radial areas brown with hyaline windows; veins and cross-veins coppery brown, except cross veins of the costal, subcostal and first radial area hyaline; pterostigma with 4 to 5 hyaline cross-veins (Figs. 14–15). Leg colouration: femora yellow with a brown median spot, tibiae and tarsi yellow. Abdominal terga yellow with a symmetrical red brown blade-shaped mark laterally and an interrupted median orange brown stripe (Fig. 14). Abdominal sterna yellow with symmetrical longitudinal brown stripes (Fig. 15). Cerci yellow with alternatively broad and narrow dark brown annulations.

Male imago

Length: body 6.5 mm; forewings 5.9 mm; cerci broken.

Head and thorax dark brown. Turbinated eyes deep orange (Fig. 16). Forewings hyaline except pterostigma area. Leg coloration yellow, femora with a brown median spot. Abdominal terga yellow with a symmetrical red brown blade-shaped mark laterally and an interrupted faded orange brown stripe medially; terga VIII to X uniformly dark brown. Abdominal sterna yellow with symmetrical longitudinal brown stripes laterally, faded on terga V and VI. Cerci yellow with alternatively broad and narrow dark brown annulations. Male genitalia (Fig. 17) with forceps clearly separated at the base; segment I of gonopods conical only partially fused with segment II; segment II apically moderately expanded; segment III short and rounded. Broad triangular genital plate.

Discussion

Cloeon peregrinator is morphologically very similar to the European species included in *Cloeon dipterum s.l.* (Sowa 1975), in particular the mouthparts, the setation of the legs and the paraprocts of the larvae. The colouration of the forewing (Figs. 14–15) and the dorsal and ventral patterns of the abdomen are all recognized as useful characters to identify the female imagos of *Cloeon.* The basal segments of male forceps are clearly separated, the genital plate is triangular. Again, these characters are almost identical between *C. peregrinator* and *C. dipterum s.l.* The strong morphological similarities explain why previous workers considered the material collected in Madeira as belonging to the continental species. However, at the larval stage, *C. peregrinator* can be identified by the spines restricted to the lateral margins of the abdominal segments VIII and IX (Fig. 11) as well as the long and slender tarsal claws with denticles limited to the proximal half (Fig. 8). In *C. dipterum s.l.,* spines are always present on lateral margins of segment V to IX and the tarsal claws are shorter with denticles reaching at least the 2/3 of the length of the claw (Fig. 9). *Cloeon peregrinator* can be easily separated from species of *Cloeon simile* group *sensu* Sowa (1980) by the number of segments of the maxillary palp (Fig. 5), the relative size of the upper lamella of gills (Fig. 12), the pigmentation of the female forewing, the distance between the basal segments of the male forceps and the shape of the male genital plate (Fig. 17).

Several African species of *Cloeon* present spines restricted to the lateral margin of the abdominal segments VIII and IX (Gillies 1980; 1985). Among them only *Cloeon scitulum* Kimmins, 1955, *Cloeon smaeleni* Lestage, 1924 and *Cloeon tanzaniae* Gillies, 1985 possess a 3-segmented maxillary palp and a clavate labial palp. *Cloeon scitulum* and *C. tanzaniae* have female forewing unpigmented and abdominal pattern significantly different from *C. peregrinator. Cloeon peregrinator* and *C. smaeleni* are rather similar both at larval and imaginal stage; *C. smaeleni* differs from *C. peregrinator* by having a broader labial palp (Fig. 6 in Gattolliat & Rabeantoandro 2002), and alternatively long and short spines along the distal margin of abdominal terga (Fig. 9 in Gattolliat & Rabeantoandro 2002); the two species also slightly differ in the ventral and dorsal abdominal pattern (Fig. 13 in Gillies 1980).

FIGURES 14–15. Female imago of *Cloeon peregrinator*: 14, dorsal view. 15, ventral view.

Only *Cloeon dipterum* and *Cloeon saharense* have been reported from North Africa (Soldán & Thomas 1983; Thomas 1998). Larvae of *C. saharense* do not present spines on the lateral margin of any segment and female imagos have hyaline forewings.

One female imago and five larvae of *Cloeon* from Madeira were sequenced for mitochondrial cytochrome-oxidase b (*cob*); a number of shared nucleotide substitutions allow us to unambiguously diagnose the female imago and larvae as belonging to the same species. The similarity of the abdominal pattern between male and female imagos clearly indicates the conspecificity.

Etymology

peregrinator is a noun from Latin origin meaning “the one who made a long journey”

***Baetis enigmaticus* n.sp. Gattolliat and Sartori**

B. pseudorhodani sensu Stauder 1991, nec Müller-Liebenau, 1971

Material observed: Holotype: 1 larva (on slide); Madeira; R. Frio; Sito B; 32°43'19.19"N / 16°53'23.92"W; Alt. ca. 1200m; 27.05.2004. N. Pestana coll.

Paratypes: 1 larva (on slide); same data as holotype.

1 larva (on slide); Madeira; Fajã da Nogueira; R. Ametade; 32°44’43”N / 16°54’33”W; Alt. ca. 700–800m; 7.04.2007. S.J. Hughes & J.P. Abreu coll.

Larva

Female larva length: body 5.7 mm; cerci 5.2 mm; median caudal filament 0.5 mm.

Colouration. Antennae brown, darker apically. Legs brown, femora with a yellow dot proximally, dorsal margin of femora, tibiae and tarsi dark brown. Tergal colouration brown with four central dark brown spots. Pattern rather similar between different terga. Sterna light brown without pattern. Cerci brown to dark brown without annulations or stripe.

Head. Antenna (Fig. 31): scape almost bare. Pedicel with short apically rounded scales more abundant near distal margin.

Labrum distinctly large (width/length ratio 1.75–1.85); dorsal face of labrum (Fig. 18) with an arc subparallel to distal margin formed by 1 + 7 long and stout setae, about 7 finer setae laterally, short and fin setae scattered proximally; ventral surface with 4 fin pointed setae disto-laterally; distal and lateral margins bordered with long and feathered setae.

Hypopharynx (Fig. 19) with lingua with a weakly marked central lobe, covered apically with minute setae; superlingua triangular.

Right mandible (Fig. 20) with two sets of incisors partially fused, outer set with 4 denticles external one highly developed and inner ones poorly developed, inner set with 4 poorly developed denticles; row of thin setae on outer margin of outer of incisors; prostheca elongated and slender; no setae between prostheca and mola.

Left mandible (Fig. 21) with two almost completely fused sets of incisors; outer set with 2 denticles external one highly developed and inner one poorly developed, inner set with 4 poorly denticles; prostheca with 1 main and 4 small denticles and an elongated comb-shaped structure; no setae between prostheca and mola.

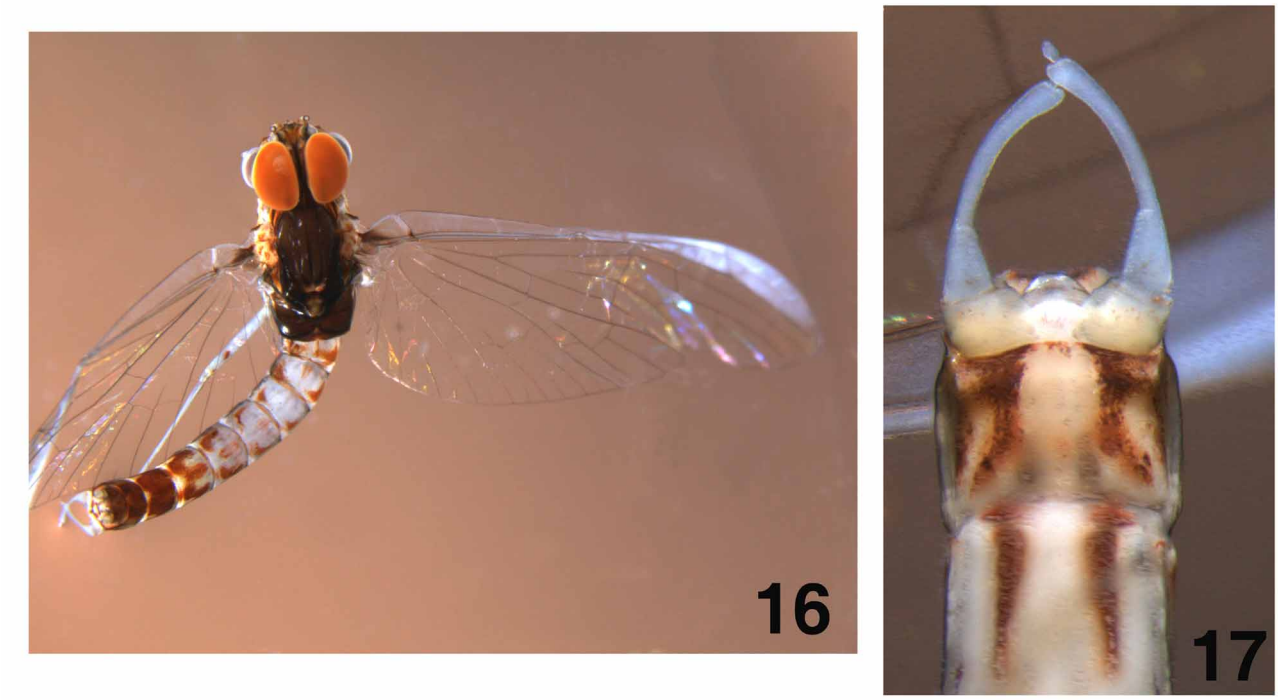
Maxillae (Fig. 22) with 4 short teeth, none of them opposed to others; one row of medium subequal setae and one row of 2 spine-like setae ending with long setae increasing in length toward apex; palp 2-segmented shorter than galea-lacinia, segment I slightly shorter than segment II, segment II with a nipple apically (Fig. 23).

Labium (Fig. 24) with glossae clearly shorter than paraglossae; glossae triangular with broad base, inner margin and distal half of outer margin with medium setae; paraglossae with three rows of long and curved setae apically, 1 row of medium setae parallel to the inner margin; labial palp 3-segmented; segment I 0.8 x length of segment 2 and 3 combined; segment II slightly expanded apically, with a oblique row of 5 thin setae; segment III ogival, with short and pointed setae apically.

Thorax. Forelegs (Fig. 25). Dorsal margin of femora with medium apically rounded setae, in several rows proximally but scarce apically; dorsoapical setal patch formed by several tiny and rounded setae; ventrally with scale bases and minute setae; upper face with numerous scale bases.

Tibiae with a row of tiny setae and a row of short and very thin setae dorsally; ventrally with tiny setae; upper face with tiny setae and small thin setae; tibio-patellar suture present.

Tarsi with a row of short and very thin setae dorsally; ventrally with a row of short and pointed setae; laterally small thin setae; tarsal claws (Fig. 26) hooked, with 1 row of about 11 acute teeth increasing in length toward the apex; two subapical setae present.



FIGURES 16–17. Male imago of *Cloeon peregrinator*: 14, dorsal view. 15, genitalia (ventral view).

Abdomen. Terga (Fig. 27) with scale bases and with a few spatulas and setae; posterior margin with blunt spines, row of spatulas parallel to the distal margin. Sterna with very thin setae along distal margin and scattered on surface, without scale bases, distal margin smooth.

Gills (Fig. 28) on segments I to VII symmetrical and elongated; tracheation well marked; area around margin smoked brown; thin setae all around margins, stout setae absent.

Paraproct (Fig. 29) with thin setae and without scales or scale bases; margin without spines, row of spatulas parallel to the distal-margin; postero-lateral extension with a few thin setae and without scale bases, margin without spines.

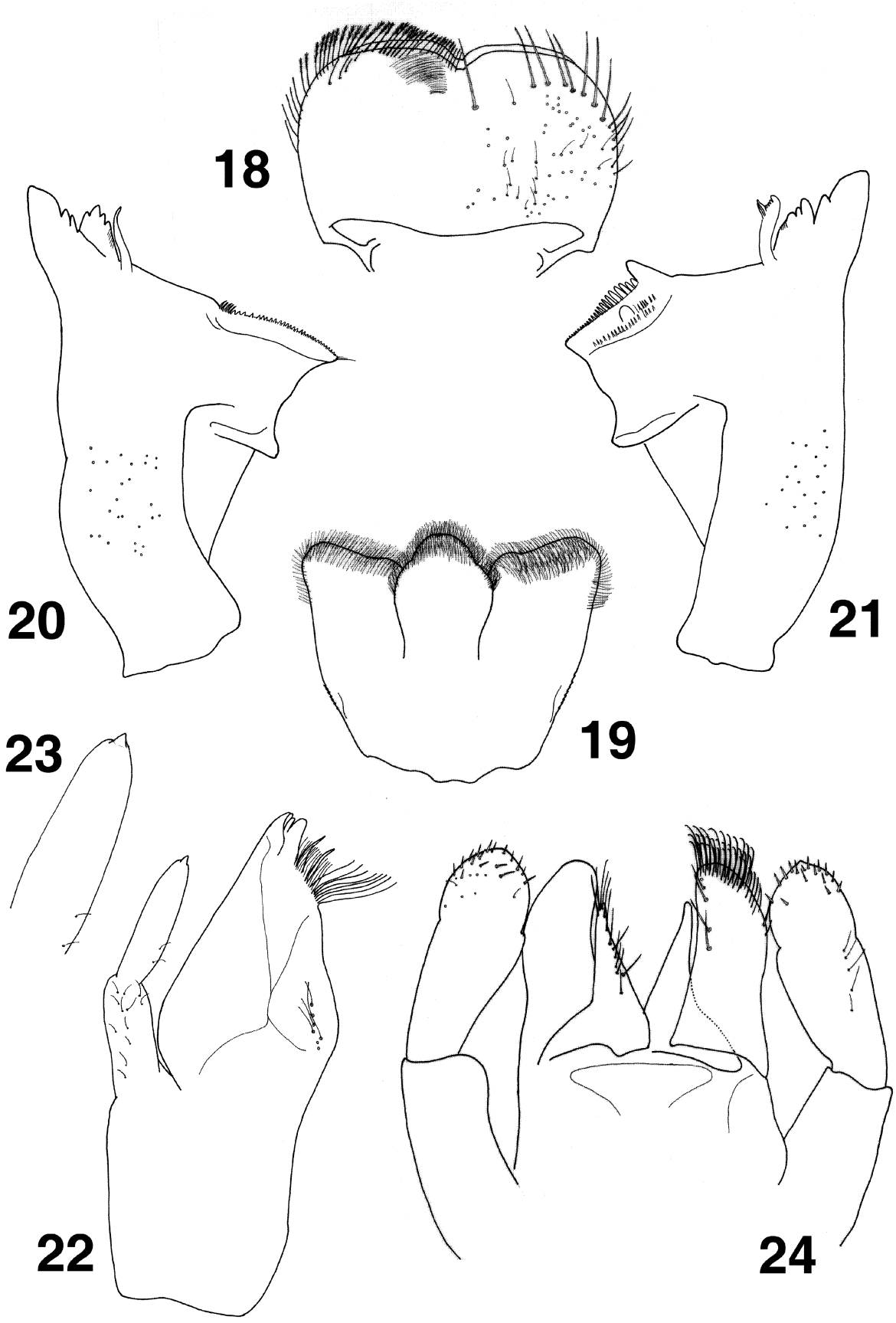
Cerci with patch of thin setae on inner margins; median caudal filament (Fig. 30) similar to cerci except thin setae almost absent.

Male and female imagos

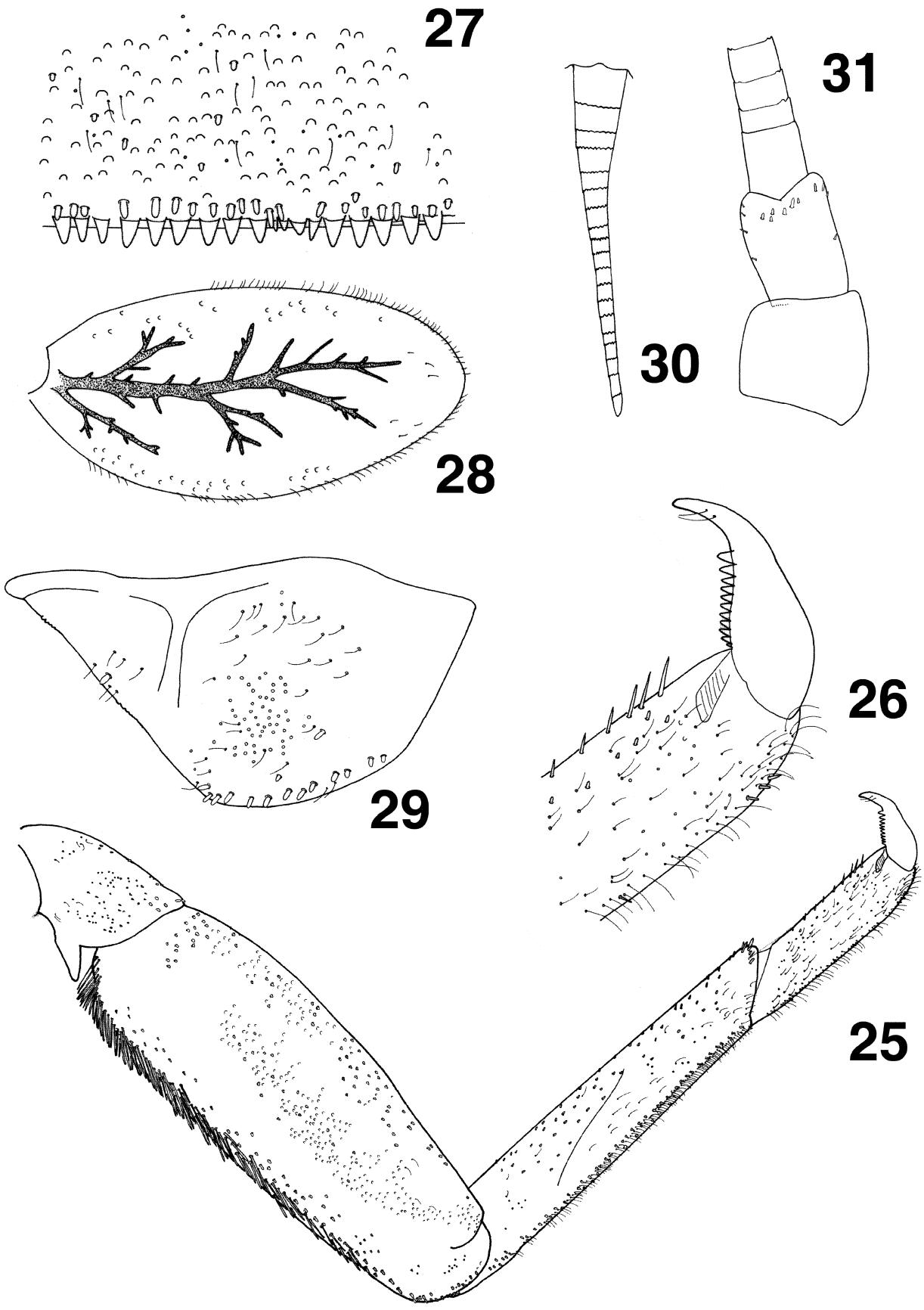
Unknown

Discussion

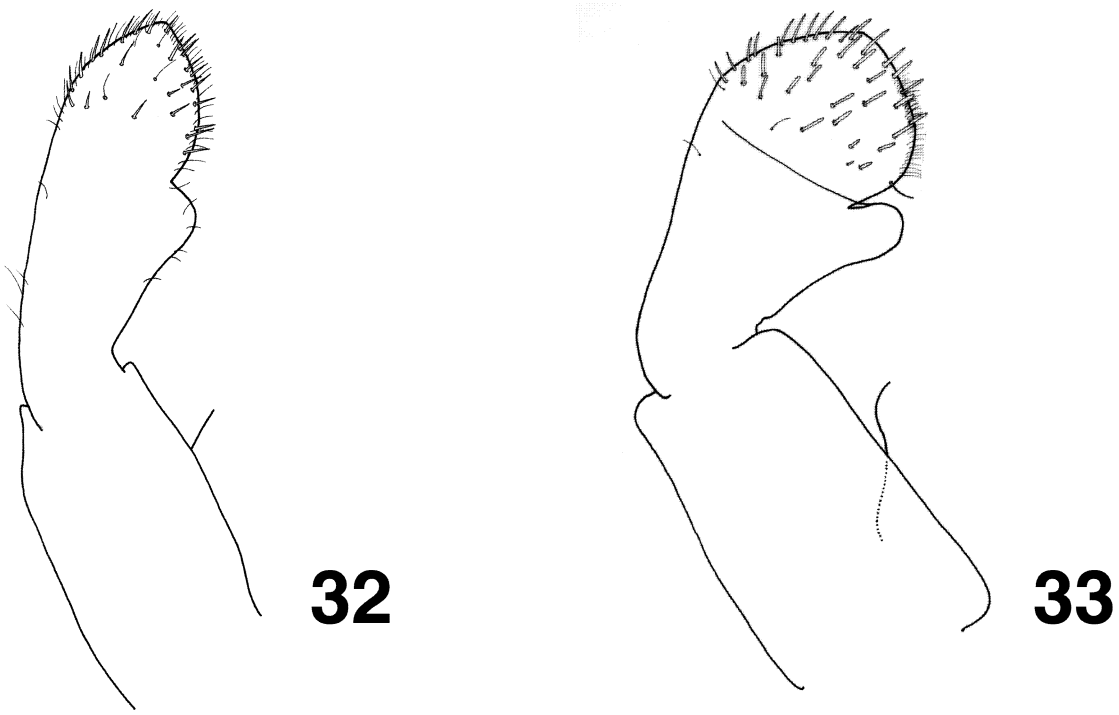
Baetis enigmaticus can be easily separated from *B. rhodani* and related species by its rheophilic adaptation: flattened body, strongly reduced median caudal filament (Fig. 30) and setae on the dorsal margin of legs thin and abundant (Fig. 25). Other morphological characters allow the identification of *B. enigmaticus*: external denticle of both mandibles highly developed and other denticles reduced (Figs. 20–21), maxillary clearly shorter than galealacinia (Fig. 22), segment II and III of the labial palp relatively slender (Fig. 24), presence of setae at the apex of tarsal claw (Fig. 26), oval and slender gills without spines on margins (Fig. 28). The rheophilic adaptation as well as all the other characters listed above show that *B. enigmaticus* is probably closely related to *B. pseudorhodani* described from the Canary Islands (Müller-Liebenau 1971). Direct observation of larvae from Madeira and Tenerife (Canary Islands), as well as the study of the original description of *B. pseudorhodani*, confirm that the two species can only be separated by minute characters: pigmentation of the gills restricted to an area around the margin in *B. enigmaticus* and to the central area in *B. pseudorhodani*; pedicel



FIGURES 18–24. Larval structures of *Baetis enigmaticus*: 18, labrum (left: ventral; right: dorsal). 19, hypopharynx. 20, right mandible. 21, left mandible. 22, right maxilla. 23, tip of maxillary palp. 24, labium.



FIGURES 25–31. Larval structures of *Baetis enigmaticus*: 25, foreleg. 26, tarsal claw. 27, distal margin of abdominal tergum IV. 28, gill IV. 29, paraproct. 30, median caudal filament. 31, detail of antenna (scape and pedicel).



FIGURES 32–33. labial palps of *Baetis* spp: 32, *Baetis atlanticus*. 33, *Baetis rhodani*.

with a restricted number of scales in *B. enigmaticus* (Fig. 31) but very abundant in *B. pseudorhodani*; tergites with scale bases and a few spatulas but without scale in *B. enigmaticus* (Fig. 27), most scale bases with triangular scales as well as spatulas in *B. pseudorhodani* (Fig. 26 in Müller-Liebenau 1971); finally the paracercus is similarly reduced in the two species, but the lateral setae are almost completely absent in *B. enigmaticus* (Fig. 30) and still normally developed in *B. pseudorhodani*. Müller-Liebenau (1971) illustrated the tarsal claw with several setae at the apex (Fig. 25j in Müller-Liebenau 1971); in the material we examined from Tenerife, we only observed two setae as in *B. enigmaticus.* This character seems not to be constant enough to separate the two species.

B. enigmaticus is a rather uncommon species: it has been found only in Ribeira da Ametade, Fajã da Nogueira, a protected area with indigenous laurissilva vegetation. Historically, this species was only reported by Stauder (1991) under the name *B. pseudorhodani.* She considered this species as very rare as she collected only 19 larvae in two different sites in summer 1988 and spring 1989; in comparison she found between 200 and 500 specimens of *B. "rhodani"* in each site. The nymphs were found mainly on large smooth rocks surrounded by fast water, under stones in riffle and above small waterfall. *B. enigmaticus* and *B. "rhodani"* co-occur more or less frequently. In their large amount of material from different areas of Madeira, Soldán and Godunko (2006) did not successfully collect any larvae of *B. enigmaticus* and concluded that Stauder probably misidentified part of material belonging to *Baetis rhodani* or *Baetis atlanticus* (Soldán & Godunko 2006). However, Stauder's description of the larva especially of the colour (dark brown to black) and general habitus (flattened body with widely spread legs) clearly indicate that what she identified as *B. pseudorhodani* belongs in fact to the new species *B. enigmaticus* and not to *B. atlanticus*. The material collected by Stauder is not housed at Museu Municipal de História Natural of Funchal; unfortunately we were unable to locate and examined it.

Etymology

enigmaticus is an adjective from Latin origin meaning "mysterious" in reference to the elusive presence of this species in Madeira.

***Baetis atlanticus* Godunko & Soldán, 2006**

Baetis rhodani sensu Eaton, 1871, Brinck & Schäfer, 1961, Stauder, 1991, 1995, nec Pictet, 1843

Material observed: 65 larvae (1 larva on slide), 4 larval exuviae, 1 female imago (TOPOTYPES *B. atlanticus*); Madeira; R. do Inferno; up stream old road; Alt. 20m; 15.04.2006. S.J. Hughes coll.

5 larvae (1 larva on slide); Madeira; El Pico das Pedras; 300 m before Queimadas; unnamed tributary; 32°46’57’’N / 16°54’17’’W; Alt. 930m; 30.11.2005. P. Derleth & M. Sartori coll.

10 larvae (5 larvae on slides), 1 female imago; Madeira; Levada do Norte at Encumeada; 32°45’15”N / 17°01’09”W; Alt. 1027m; 15.04.2006. S.J. Hughes coll.

46 larvae (4 larvae on slides), 1 female imago; Madeira; R. do Alecrim; Paul da Serra; 32°45’05”N / 17°06’36”W; Alt. 1405m; 15.04.2006. S.J. Hughes coll.

37 larvae (1 larva on slide), 2 female imagos; Madeira; R. Sao Vicente; Senhora de Fatima; ca 100 m upstream beach; 32°48’24”N / 17°02’51”W; Alt. 15 m; 15.04.2006. S.J. Hughes coll.

50 larvae (2 larvae on slides), 1 female subimago; Madeira; Fajã da Nogueira; R. Ametade; 32°44’43”N / 16°54’33”W; Alt. ca. 700–800 m; 16.04.2006. S.J. Hughes coll.

45 larvae, 41 larval exuviae, 1 female subimago; same locality; 17.04.2006. S.J. Hughes.

36 larvae; same locality; 22.11.2006. I. Martins coll.

30 larvae; same locality; 25.11.2006. I. Martins coll.

76 larvae; same locality; 09.12.2006. S.J. Hughes coll.

33 larvae; same locality; 7.04.2007. S.J. Hughes & J.P. Abreu coll.

26 larvae, 18 larval exuviae; same locality; 8.04.2007. S.J. Hughes & J.P. Abreu coll.

18 larvae, 1 female imago; same locality; 05.02.2008. S.J. Hughes & J.P. Abreu coll.

25 larvae; same locality; 06.02.2008. S.J. Hughes & J.P. Abreu coll.

31 larvae; same locality; 9.12.2000. S.J. Hughes coll.

23 larvae; Madeira; Ametade trib.; Alt. ca 500m; 16.04.2006. S.J. Hughes coll.

13 larvae; same locality; 7.04.2007. S.J. Hughes & J.P. Abreu coll.

92 larvae (1 larva on slide), 25 larval exuviae, 1 female imago, 2 female subimagos; Madeira; R. das Cales; 32°43’02”N / 16°54’30”W; Alt. 1100 m; 17.04.2006. S.J. Hughes.

7 larvae, 1 female subimago; same locality; 04.02.2008. S.J. Hughes & J.P. Abreu coll.

26 larvae, 1 female subimago; same locality; 08.02.2008. S.J. Hughes & J.P. Abreu coll.

26 larvae; same locality; 18.11.2006. S.J. Hughes coll.

61 larvae; same locality; 17.02.2007. S.J. Hughes & J.P. Abreu coll.

12 larvae; Madeira; R. das Cales, Chão de Lagoa, Parque Ecológico de Funchal (PEF); 32°43’11” N / 16°54’31”W; Alt. 1500 m; 08.02.2008. S.J. Hughes & J.P. Abreu coll.

34 larvae, 1 female subimago; Madeira; R. das Cales, Chão de Lagoa; 32°43'2.28"N / 16°54'29.77"W; Alt 1479m; 17.02.2007. S.J. Hughes & J.P. Abreu coll.

18 larvae; Madeira; R. Frio; 32°43'19.19"N / 16°53'23.92"W; Alt ca. 1200m; 9.12.2006. S.J. Hughes coll.

32 larvae; Madeira; Cabeço Meio da Serra; 32°42’37”N / 16°51’33”W; Alt. 1135 m; 9.04.2007. S.J. Hughes & J.P. Abreu coll.

Diagnosis

Baetis atlanticus, described in detail at all stages by Soldán & Godunko (2006), can be identified by the following characters: labrum with width/length ratio 1.35–1.45, with an arc subparallel to distal margin formed by 1 + 6–9 long and stout setae. Labial palp (Fig. 32) relatively slender, segment II slightly expanded apically, segment III almost symmetrical. Tarsal claw without apical setae. Thorax and abdomen medium brown with yellowish pattern. Gills elongated, slightly asymmetrical, with distinct but poorly developed tracheation, dorsal margin with abundant spines.

Discussion

In their study of the subgenus *Rhodobaetis* in Madeira, Soldán & Godunko (2006) recognized the presence of two species on the island: *Baetis atlanticus* and *B. rhodani.* They considered the presence of a rheophilic species of *Baetis* stated by Stauder (1991; 1995) as a misidentification. They indicated that *B. atlanticus* is distributed all over the main island of Madeira, but restricted to lower altitude (below 650 m) while *B. rhodani* lives in mountain streams. In middle altitude, the two species may co-occur. We examined larvae from different areas of Madeira including specimens from the type locality of *Baetis atlanticus* and material from high altitude. It appears clearly that no morphological characters allow the separation of the different populations in different species. The larvae examined from Madeira in the present study perfectly match the description of *Baetis atlanticus* made by Soldán and Godunko (2006). Using genetic variation measured with cob sequences, there were no characters (nucleotides) that would permit the diagnosis of discrete groups from within the island-wide sample. Consequently, both morphological and molecular approaches indicate that, besides the rheophilic species *B. enigmaticus*, only one species of *Baetis* occurs in Madeira.

As shown in Gattolliat & Sartori (2008), Godunko *et al* (2004b)'s concept of *B. rhodani* differs slightly from the type material. Consequently, the characters given for distinguishing *B. atlanticus* and *B. rhodani* need to be re-examined. The characters used by Godunko *et al* (2004b) to separate the different species within the subgenus *Rhodobaetis* are coded for the specimens from Madeira and for *B. rhodani* and *B. canariensis* (table 1). The presence/absence of strong spines on the dorsal margin of gills and the presence/absence of apical setae on the tarsal claws are the easiest characters to separate larvae from Madeira from *B. canariensis.* The larvae collected in Madeira are very similar to *B. rhodani* from the type locality in Geneva Switzerland. However, they differ in the shape of the labial palp (degree of development of apico-lateral expansion of segment II, shape of segment III (Figs. 32–33)), the shape and setation of the labrum and the abdominal pattern (see table 1). Consequently, specimens from Madeira do not belong to *B. rhodani* or *B. canariensis*, but to a sibling species. Using sequence data, there were 45 fixed character differences between the 10 individuals of *B atlanticus* and 3 individuals of *B. rhodani*.

Baetis maderensis was originally established for male imagos collected in Madeira (Hagen 1865). As stated above, the material examined from Madeira differs from *Baetis rhodani*. As a result, the synonymy of *B. maderensis* with *B. rhodani* made by Eaton (1871) is incorrect and *B. maderensis* should be considered as a valid species. The problem is that two species of *Rhodobaetis* occur in Madeira, and one of them *(B. enigmaticus)* is unknown at the imaginal stage. It is therefore possible that *B. atlanticus* could be a junior synonym of *B. maderensis*, but a lower probability also exists that *B. enigmaticus* could be a junior synonym of *B. maderensis.* Moreover, specific differences among male imagos of *Rhodobaetis* are tiny and less reliable than those found on larvae, and we can expect the male imagos of the two species occurring in Madeira to be hardly discernible. Because this problem is not likely to be solved in the near future, and since *B. atlanticus* and *B. enigmaticus* are well described and characterized at the larval stage, we propose to keep these epithets. Since *B. maderensis* has not been used as a valid name after 1899 (article 23.9.1.1. ICZN 1999), it should be considered as a *nomen oblitum* as soon as the conditions of article 23.9.1.2. are met.

Affinities and the differential diagnosis of *B. atlanticus* are discussed in detail in Soldán & Godunko (2006). They suggested possible affinities of *B. atlanticus* with *B. canariensis, B. ingridae* Thomas & Soldán, 1987 from Corsica and *B. braaschi* Zimmermann, 1980 from Crimea. An ongoing molecular phylogenetic study (Monaghan *et al.*, in prep.) will probably greatly help to clarify affinities among the subgenus *Rhodobaetis*

B. atlanticus is by far the most common species in Madeiran streams. Accurate biological data are presented in Soldán & Godunko (2006), except the concept of altitudinal vicariance is not valid.

TABLE 1. Morphological characters used to distinguishing West Palaearctic species of the subgenus *Rhodobaetis* (according to Godunko *et al.* 2004b) coded for *Baetis atlanticus, B. rhodani* and *B. canariensis*.

N°	Characters	*Baetis atlanticus* Godunko & Soldán, 2006	*Baetis rhodani* (coding according to Gattolliat & Sartori, 2008)	*Baetis canariensis* BC (coding according to Godunko *et al.* 2004b)
	Larva			
1	Pedicel : shape of scales	Elongated and rounded apically	Elongated and blunt	Elongated rounded or bluntly pointed apically
2	Scape : shape of scales	Small and blunt	Small and wide	Rounded or pointed
3	Labrum : mean width/length ratio	1.35–1.45	1.55–1.65	1.50
4	Labrum : number of long submarginal setae	1 + 6–9	1 + 8–10	1 + 5–7
5	Maxillary palps : apical part of distal segment	With one small pointed scale	With one pointed scale	With one rudimentary scale
6	Paraglossae : number of regular row of bristles	3	3	3
7	Labial palp	Almost symmetrical	Asymmetrical and wide	Relatively symmetrical
8	External margin of femora : shape of bristles	Long and rounded apically	Long and blunt	Long and rounded apically
9	Tarsal claw : number of strong teeth	10–13	12–14	9–12
10	Tarsal claw : presence of apical setae	Absent	Absent	Present
11	Surface of terga : shape of scales	Slightly longer than wide, pointed apically	Mainly absent. When present wide, triangular and blunt apically	Bluntly pointed apically
12	Posterior margin of terga III–VI: presence of triangular spines	Present	Present	Present
13	Shape of gills III–V	Strongly asymmetrical	Slightly asymmetrical	Slightly asymmetrical
14	Spines of external margin of gills	Present	Present	Absent
15	Paraproct plate : number of marginal teeth	8–12	15–25	At least 15
16	Paraproct plate : shape of scale (=spatulas)	Elongated, pointed apically	Elongated, rounded apically	Pointed
17	Terminal filament length : relative to cerci length	> 3/4	> 3/4	1/2–1/3
	Male imago			
18	Turbinate eyes : facetted surface	Dark orange	Orange	Light yellow to brownish
19	Shaft of turbinate eyes	Orange with a basal orange-grey or orange-violet ring	Dark orange with a basal brown ring	Yellow without ring
20	Coloration of thorax	Yellowish brown to brown	Medium to dark brown	Brown
21	Hindwing : number of veins	3	3	3
22	Basal segment of forceps	Slightly elongated	Elongated	Approximately square
N°	Characters	*Baetis atlanticus* Godunko & Soldán, 2006	*Baetis rhodani* (coding according to Gattolliat & Sartori, 2008)	*Baetis canariensis* BC (coding according to Godunko *et al.* 2004b)
23	Segment I of forceps	With inner projection apically	With inner projection apically	With mainly subparallel margins and strong inner projection apically
24	Segment II of forceps : widened part	1/2–2/3 segment length	Almost not widened	1/2–2/3 segment length
25	Segment II of forceps : inner margin	Straight to slightly concave	Concave	Clearly concave
26	Segment III of forceps	Oval, sometimes with truncate inner margin	Rounded as long as broad	Oval

mtDNA sequences

Molecular characters were obtained by PCR amplification and sequencing of mitochondrial cytochrome-oxidase b (*cob*) using standard procedures with primers cb3 (5'-GAG GAG CAA CTG TAA TTA CTA A) and cb4 (5'-AAA AGA AAR TAT CAT TCA GGT TGA AT). We sequenced individuals of *Cloeon peregrinator* (GenBank accessions AM941016-AM941020) and *Baetis atlanticus* (GenBank accessions AM941010-AM941015) but were unable to amplify sequences from 2 specimens of *B. enigmaticus*, most likely due to low DNA quality. We employed a character-based phylogenetic approach to test hypotheses of species status, using the criterion of diagnosibility with corroboration from multiple lines of evidence (DNA, morphology, geography) sensu DeSalle *et al.* (2005).

Acknowledgements

Many thanks to João Paulo Pestana Abreu for valuable field assistance in Madeira. Thanks also to the Parque Natural da Madeira and the Parque Ecológico do Funchal for authorising collections in protected areas. Constructive discussion with Roman Godunko and Tomas Soldán improved our manuscript. Financial support for trips to Madeira was provided by Museum of Zoology at Lausanne. MTM was supported by BBSRC (UK) grant BBS/B/04358 and the Museum of Zoology at Lausanne.

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