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From molecular hypotheses to valid species: description of three endemic species of *Baetis* (Ephemeroptera: Baetidae) from the Canary Islands

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Abstract. *Baetis* (*Rhodobaetis*) *canariensis* s.l. was considered to be the most common species of mayfly (Ephemeroptera) in running waters of the Canary Islands. Recent studies using mitochondrial genetic markers suggested that what was considered a single species was in fact composed of four closely related, but distinct species. Here we present the results of comprehensive morphological analysis of specimens from Tenerife, Gran Canaria, La Palma, and La Gomera that confirms the validity of the four species based on small but consistent differences in some characters. Three of these are new species and are described herein at the larval stage. Each of the four species appears to be restricted to a single island. The loss of freshwater habitats on the islands has led to a drastically diminished distribution and these species are largely restricted to protected areas in national parks. All four species must be considered endangered and are very sensitive sentinels of the state of conservation of running waters.

Key words. *Baetis gomerensis*, *B. palmensis*, *B. tenerifensis*, conservation, DNA barcoding, La Gomera, La Palma, Macaronesia, mayflies, new species, Tenerife.

1. Introduction

The Canarian archipelago is formed by seven major islands and some small islets. All of them have a volcanic origin and were formed at different times. The most eastern islands (Lanzarote and Fuerteventura) are the oldest (20.4–20.6 million years ago (mya)). The "middle islands" of Gran Canaria, Tenerife, and La Gomera emerged between 14.5 and 10.5 mya. The case of Tenerife is the most complex as it was originally composed of three independent islands (Roque del Conde 10.5 mya; Teno 7.4 mya; and Anaga 6.5 mya) (Anguita & Hernán 2000; Guillou et al. 2004). The three edifices were connected later on by volcanic activities (< 3.2 mya) creating the 3718 m high volcano El Teide (Geyer & Martí 2010). These activities had a strong impact on the geology of the island and of course on its biota. The most western islands (La Palma and El Hierro) are the youngest (1.7 and 1.1 mya, respectively). Due to their geographic position, the different islands have distinct climates. The Gulf Stream has a significant impact on the western islands (La Palma, La Gomera, and El Hierro); the vegetation is well developed with the presence of laurisilva forest at high and middle altitudes. The climate gets drier toward the West, Fuerteventura and Lanzarote being arid to semi-arid. Gran Canaria and Tenerife present intermediate climate. Only the four islands Tenerife, Gran Canaria, La Palma, and La Gomera have permanent running waters (Fig. 1).

Various taxonomic groups have been studied in an effort to better understand the colonisation and speciation

processes on the Canary Islands. A number of different colonisation pathways have been identified in different groups, including multiple colonisations, single colonisation with stepping stones dispersal as well as colonisation from islands to mainland (aquatic insects, spiders, birds as well as several families of plants) (Kelly et al. 2001; Ribera et al. 2003; Emerson & Oromi 2005; Dietzen et al. 2008; Fernandez-Palacios & Whittaker 2008; Steinbauer et al. 2016; Rutschmann et al. 2017; Valente et al. 2017).

Because of their low dispersal capacity (see below), mayflies are considered to be an excellent group for biogeographical studies (Monaghan et al. 2005). For mayflies of lotic habitats (i.e., rivers and streams), a colonisation of the archipelago from Europe possibly via Madeira or from North Africa followed by speciation within and among islands is the most plausible scenario (Rutschmann et al. 2014). However, it remains open to what extent the island-endemic species distribution pattern (see below) might be driven by ecological or morphological diversification.

Mayflies are the oldest order of winged insects; the order presently encompasses about 3500 species belonging to 42 families. Mayflies are merolimnic with strictly freshwater dependent larval stages, while subimaginal and imaginal stages are aerial; imagos only live from a few hours to a few days (Barber-James et al. 2008). The family Baetidae is widely distributed and is, with around 1000 known species, one of the most diversified families in both tropical and temperate regions, including remote volcanic islands such as La Réunion, Vanuatu, Guam and the Fiji islands (Flowers 1990; Gattolliat 2004; Gattolliat & Staniczek 2011). In the Palaearctic the genus *Baetis* Leach, 1815 colonizes a wide range of habitats and diversified to 84 species. It is scarcer and less diversified in the Afrotropical region, except in South Africa where *Baetis harrisoni* Barnard, 1932 is widely distributed and potentially represents a complex of species (Pereira-Da-Conceicoa et al. 2012). The systematics of this genus is still being studied and subject to debate. In the western Palearctic, *Baetis* was divided into eleven species groups comprising 26 species (Müller-Liebenau 1969), and some of the species groups are now considered as true genera, while others are treated as subgenera (Fujitani et al. 2003; Sroka 2012). Based on the latest systematics, the species originally described as the *rhodani* species group sensu Müller-Liebenau (1969) are now placed in the subgenus *Rhodobaetis* Jacob, 2003 (Jacob 2003; Godunko et al. 2004; Sroka et al. 2012b; Godunko et al. 2015). Apomorphies of the subgenus *Rhodobaetis* were first defined by Jacob (2003) who considered the presence of spatulas on tergites as well the peculiar posterior margins of abdominal tergites as the unique characters to define the subgenus *Rhodobaetis* at the larval stage. Godunko et al. (2004) compiled a list of nineteen larval and seven imaginal characters to distinguish the West Palaearctic species (Table 1). This set of characters was subsequently used to describe or re-describe species in Eastern Europe, Central Asia and North Africa (Bekettov & Godunko 2005; Soldán et al. 2005; Soldán & Godunko 2006; Gattolliat et al. 2008; Gattolliat & Sartori 2008; Soldan & Godunko 2008; Sroka et al. 2012a). Moreover, Godunko et al. (2004) completed the subgeneric diagnosis by adding the shape of spatulas as well as their presence also on antennal segments (mainly on the pedicel), upper face of femora and paraproct as diagnostic characters. Furthermore, species attributed to *Rhodobaetis* often possess spines on the margins of gills (Gattolliat & Sartori 2008). This character is sufficient to indicate subgeneric placement but is not fully conclusive as some species, including *B. canariensis*, do not possess such spines (Müller-Liebenau 1971).

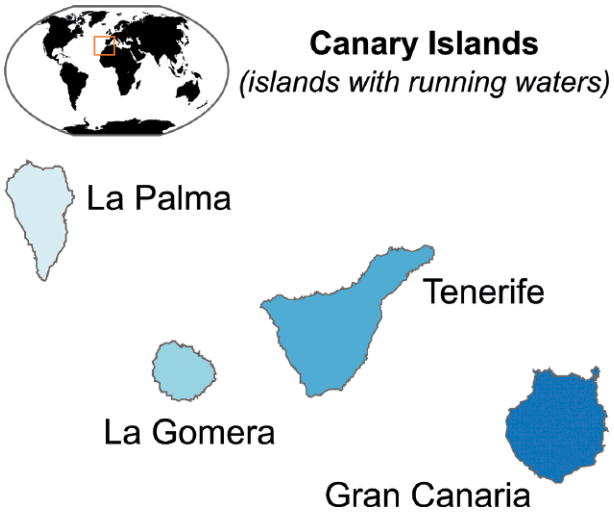

Fig. 1. Overview of the Canary Islands, including the islands with running waters. Their colors are also used in Fig. 2.

The subgenus *Rhodobaetis* presently encompasses about 40 species with a presumably highest diversity in Central Asia (Godunko et al. 2004, 2015). Its diversity in Central and Western Europe is likely higher than previously thought as genetic studies revealed a number of distinct evolutionary lineages that might correspond to different species (Williams et al. 2006; Lucentini et al. 2011; Sroka 2012; Gattolliat et al. 2015; Bisconti et al. 2016).

The genus *Baetis* is known from the Canary Islands since the end of the 19th century where supposedly the same species as in continental Europe, *Baetis* (*Rhodobaetis*) *rhodani* (Pictet, 1843) was reported (Eaton 1871; McLachlan 1882; Brauer 1900). One Canarian lineage was subsequently described as *Baetis nigrescens* Navás, 1931, the first species of the genus associated with the Canary Islands (Navás 1931). This species was later on assigned to the *lutheri* species group (Müller-Liebenau 1969) and reported from the Iberian Peninsula and Algeria (Müller-Liebenau 1971, 1974). Müller-Liebenau (1971) comprehensively investigated the Canarian mayfly fauna based on material she collected in the years 1966 and 1968. She confirmed the presence of *B. nigrescens* on Gran Canaria and reported the species also from La Gomera. Besides this, she demonstrated that specimens of the *rhodani* group from the Canary Islands

not only differ morphologically from the continental specimens, but rather represent two distinct species that she described as *B. canariensis* Müller-Liebenau, 1971 and *B. pseudorhodani* Müller-Liebenau, 1971. *Baetis canariensis* was later reported from Gran Canaria, La Gomera, La Palma and Tenerife (Alba-Tercedor et al. 1987).

Recently, a study of the diversity and evolutionary history of the *Baetis* species on Madeira and the Canary Islands was undertaken using a molecular phylogenetic approach (Rutschmann et al. 2014). Therein, genetic evidence, i.e. species hypotheses inferred using the Generalized Mixed Yule Coalescent (GMYC) model analysis (Pons et al. 2006; Fujisawa & Barraclough 2013), indicated the presence of seven distinct taxa within the subgenus *Rhodobaetis*, four of them corresponding to the concept of *B. canariensis* sensu Müller-Liebenau (1971) (hereafter *B. canariensis* s.l.) and three to *B. pseudorhodani* sensu Müller-Liebenau (1971). Moreover, each of the four delimited lineages within *B. canariensis* s.l. seemed to be confined to a single island. A preliminary morphological analysis based on the characters proposed by Godunko et al. (2004) corroborated differentiation between the lineages but no formal description or even diagnosis was proposed in the previous study (Rutschmann et al. 2014).

To fill this gap, we present herein an integrative taxonomic framework of *B. canariensis* s.l. In particular, we propose species hypotheses, including tree-based and distance-based species delimitation methods in combination with a new morphological diagnosis. Further, we describe three new endemic species. Thus, we perform a rare out-of-the-dark integrative species description, following previous molecular delimitation of evolutionary lineages (Pante et al. 2015). The four species share a set of distinctive characters such as the strong reduction of the first pair of gills, the absence of spines on the margin of all gills, and a rather uniform colouration especially of the abdominal tergites (Müller-Liebenau 1971).

2. Material and methods

Rhodobaetis individuals were collected from larval aquatic habitats using kick-samples on the islands of Tenerife, Gran Canaria, La Palma, and La Gomera in March 2007, March 2008, January 2009 and March 2014 (Fig.1; see also Rutschmann et al. 2014). Samples were preserved in 99% ethanol in the field and stored at 4°C in the laboratory until further study. For molecular analyses, DNA was extracted from a total of 101 whole specimens, using the NucleoSpin® 96 (Macherey-Nagel, Düren, Germany) tissue kit. Digestion with protein kinase K was performed overnight and the voucher material (i.e., remains of the exoskeleton) were collected before proceeding with the DNA extraction and stored in 75% ethanol for morphological analyses.

We analysed the DNA barcoding gene cytochrome *c* oxidase subunit 1 (*cox1*). Standard polymerase chain reactions (PCR) amplifications were performed with the primer pair LCO1490 + HCO2198 (Folmer et al. 1994). The PCR products were custom-purified and sequenced at Macrogen (Amsterdam, The Netherlands). Forward and reverse sequences were assembled and edited using Geneious R7 v.7.1.3 (Biomatters Ltd.). Multiple sequence alignment was performed with MAFFT v.7.050b (L-INS-i algorithm; Katoh & Standley 2013). The alignment was checked for the occurrence of stop codons and indels using Mesquite v.2.75 (Maddison & Maddison 2011). Identical haplotypes were removed using collapsetypes_v4.5.pl (Chesters 2013).

In order to provide a comprehensive taxon sampling for the species delimitation, we analysed *cox1* sequences from 274 specimens. We included available *Rhodobaetis* specimens from Macaronesia (i.e., *B. enigmaticus*, *B. pseudorhodani*; Rutschmann et al. 2014), and from neighbouring geographical areas (i.e., *B. ingridae*). In addition, we included specimens of the subgenus *Rhodobaetis* from the European and African mainland since the taxonomic status of this species is unclear (but see Gattolliat & Sartori 2008, and above), including several recently delimited putative species (Rutschmann et al. 2014; Bisconti et al. 2016). In detail, we thus used 73 newly sequenced *B. canariensis* s.l. and *B. pseudorhodani* s.l. specimens from the Canary Islands (GenBank acc. nos. MH940352–MH940415), 26 specimens from Algeria (GenBank acc. nos. MH940326–MH940351), and two specimens from Corsica (GenBank acc. nos. MH976799–MH976800). Additionally, we included 41 specimens from Rutschmann et al. (2014), and 131 specimens from Williams et al. (2006), Lucentini et al. (2011), and Sroka (2012). As an outgroup, we included the damselfly *Euphaea formosa* (NC014493).

Species hypotheses were proposed based primarily on the GMYC (Pons et al. 2006; Fujisawa & Barraclough 2013) model as has been applied for mayflies in previous studies (e.g., Vuataz et al. 2011, 2013; Rutschmann et al. 2014) and shows complete congruence with nuclear markers in Baetidae (Monaghan et al. 2009; Rutschmann et al. 2017). We used an ultrametric gene tree as input, reconstructed using the methods of Rutschmann et al. (2014) except that we used BEAST v2.4 (Bouckaert et al. 2014) and applied an HKY + Γ model of evolution on each codon position. We ran three inferences, whereby all runs converged and all parameters reached effective sample sizes >300. Single-threshold GMYC analysis was conducted based on the resulting tree using the Splits package (http://r-forge.r-project.org/projects/splits/; Ezard et al. 2014) for R v.3.3.2 (R Core Team 2016). We used the single-threshold GMYC model as it has been found to outperform the multi-threshold version (Esselstyn et al. 2012; Fujisawa & Barraclough 2013; Talavera et al. 2013).

For comparison, we also used the species delimitation methods PTP (Poisson Tree Processes; Zhang et al. 2013) and ABGD (Automatic Barcode Gap Discovery;

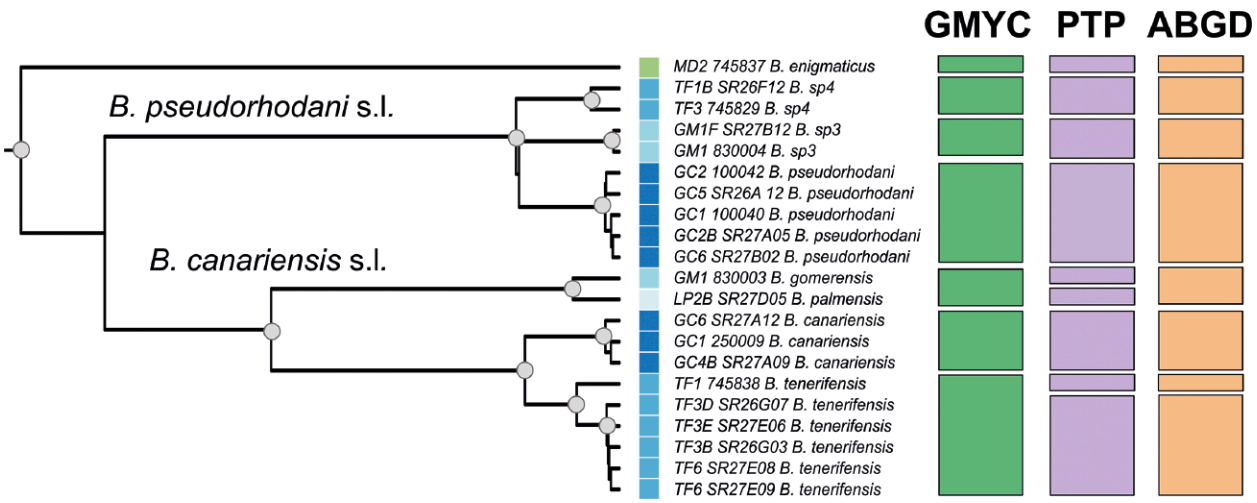

Fig. 2. Molecular species delimitation of the Canarian and Madeiran representatives of the *Rhodobaetis* group (i.e., *B. pseudorhodani* s.l. and *B. canariensis* s.l.), using the Generalized Mixed Yule Coalescent (GMYC, FUJISAWA & BARRACLOUGH 2013), Poisson Tree Processes (PTP, ZHANG et al (2013)), and Automatic Barcode Gap Discovery (ABGD, PUILLANDRE et al. (2012)). The phylogenetic tree shows the topology of the cox1 gene tree used for the GMYC method. Filled circles indicate well-supported nodes (Bayesian posterior probability ≥ 0.95). Terminal labels represent unique haplotypes, indicating sampling sites, sample voucher, and morphological assignment. *B. pseudorhodani* species hypotheses are indicated after RUTSCHMANN et al. (2014) as sp3, sp4.

PUILLANDRE et al. 2012). The input tree used for PTP was a gene tree inferred with raxml-ng v.0.5.1b (https://github.com/amkozlov/raxml-ng; KOZLOV 2017) using ten random starting trees, 100 bootstrap replicates, and a GTR + Γ model. We used the single PTP method with default settings (i.e., p-value 0,001) as available on the webserver (http://mptp.h-its.org/#/tree; accessed on 22.iv.2018). The ABGD method was performed on the webserver (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html; accessed on 22.iv.2018) using as input the multiple sequence alignment of the unique haplotypes (see above), default values for the prior intraspecific divergences, relative gap width, distance distribution, and a Jukes-Cantor (JC69) model. We used the distance metric JC69 as this was previously found to produce more conservative species hypotheses (KEKKONEN & HERBERT 2014; KEKKONEN et al. 2015).

Morphological analyses were performed on all the vouchers derived from specimens used for DNA extraction. All specimens were dissected and entirely mounted on slides in Euparal medium. Drawings and pictures of body parts were made using an Olympus BX51 stereoscopic microscope with a camera lucida or a digital camera Olympus SC50. Extended-depth-of-focus images were obtained using the software Stream Basic1.9.4. Photographs of the whole larval body were taken with the Visionary LK system (Dun., Inc., USA). Pictures and drawings were subsequently enhanced with Adobe Photoshop™CC2015.

The morphological terminology used in the description follows GODUNKO et al. (2004, 2015) and GATTOLLIAT & SARTORI (2008). The use of the term "spatulas" is restricted to setae exclusive to the subgenus *Rhodobaetis* (fig. 22 in GODUNKO et. 2004; figs. 12–14 in GATTOLLIAT & SARTORI 2008). Scales cover most of the body of *Rhodobaetis* species. They are generally no longer visible (mainly detached form the body) in Canadian balsam and other mounting liquid, and are best seen on dry slides of the empty cuticle (KLUGE et al. 2018). Often only the scale bases remain visible, the shape of which are of taxonomic importance (fig. 22 in GODUNKO et al. 2004). The right and left mandibles possess two sets of incisors: the outer set has a lateral position while the inner set has a medial position; the two sets are almost completely fused in the left mandible. Teeth of incisors are counted from outside to inside (lateral to medial) for both mandibles. The spines on the costal margin of gills are present in almost all the species of the subgenus; their shape, colour and abundance are also of taxonomic importance (fig. 17 in GATTOLLIAT & SARTORI 2008). The lateral extension of the paraproct is often called cercotractor. The leg orientation follows the attempt of HUBBARD (1995: fig. 2) to standardize the description of mayflies.

The holotypes and parts of the paratype series of the new species are housed in the Museum of Zoology, Lausanne, Switzerland (MZL). Additional paratypes are deposited in the Museum für Naturkunde, Berlin, Germany (MFN).

3. Results

3.1. Molecular reconstruction

The results from the three species delimitation methods we used were very similar, ranging from 27 to 31 putative species based on the analysis of 167 unique *cox1*-haplotypes. The GMYC model (χ^2: 52.51, $P < 0.001$) delineated 27 species hypotheses for *Rhodobaetis*, consisting of 19 distinct clusters and eight singletons (Figs. 2, 3). The

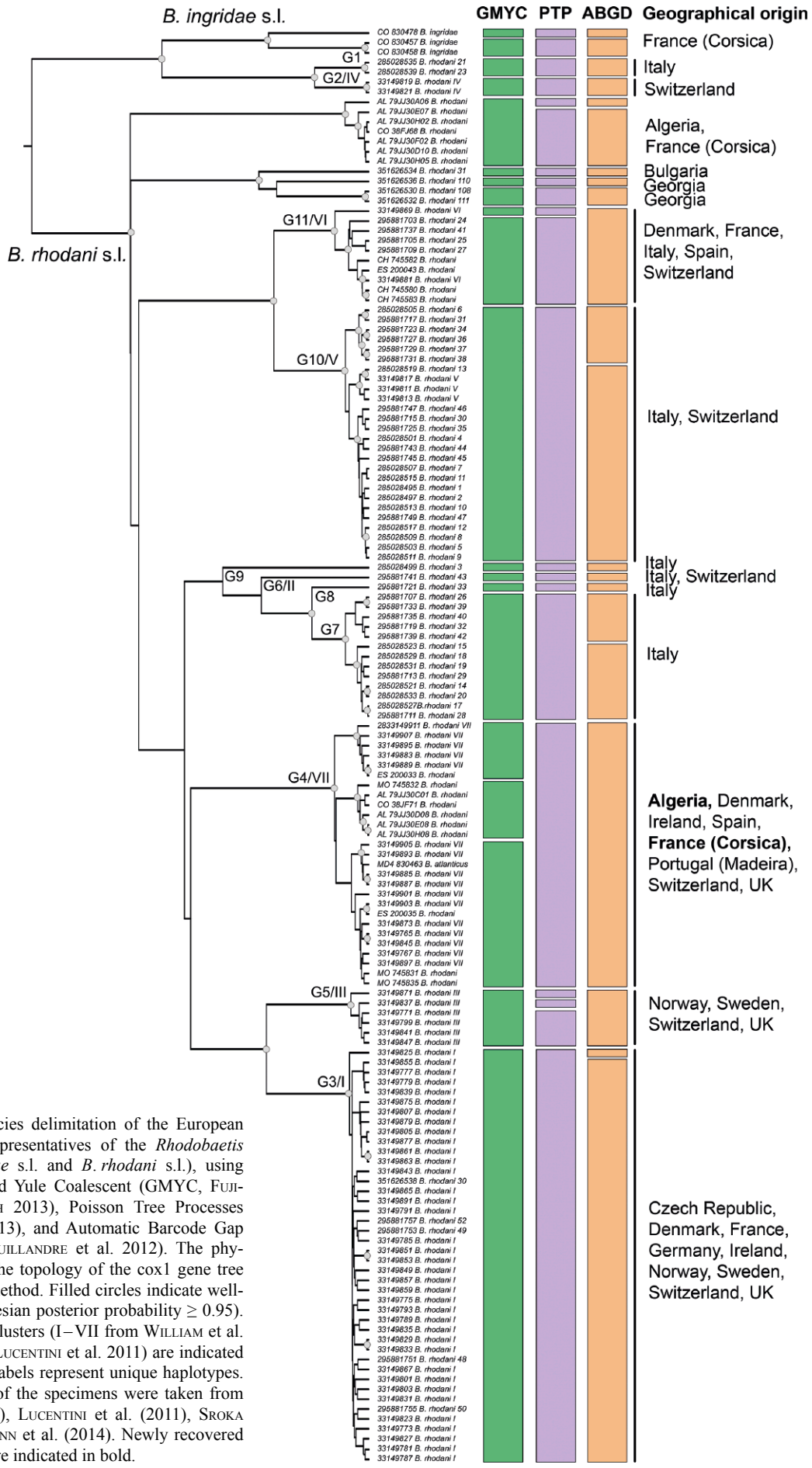

Fig. 3. Molecular species delimitation of the European and North African representatives of the *Rhodobaetis* group (i.e., *B. ingridae* s.l. and *B. rhodani* s.l.), using the Generalized Mixed Yule Coalescent (GMYC, Fujisawa & Barraclough 2013), Poisson Tree Processes (PTP, Zhang et al 2013), and Automatic Barcode Gap Discovery (ABGD, Puillandre et al. 2012). The phylogenetic tree shows the topology of the cox1 gene tree used for the GMYC method. Filled circles indicate well-supported nodes (Bayesian posterior probability ≥ 0.95). Previously identified clusters (I–VII from Williams et al. 2006; G1–G11 from Lucentini et al. 2011) are indicated on the tree. Terminal labels represent unique haplotypes. Geographical origins of the specimens were taken from Williams et al. (2006), Lucentini et al. (2011), Sroka (2012), and Rutschmann et al. (2014). Newly recovered geographical origins are indicated in bold.

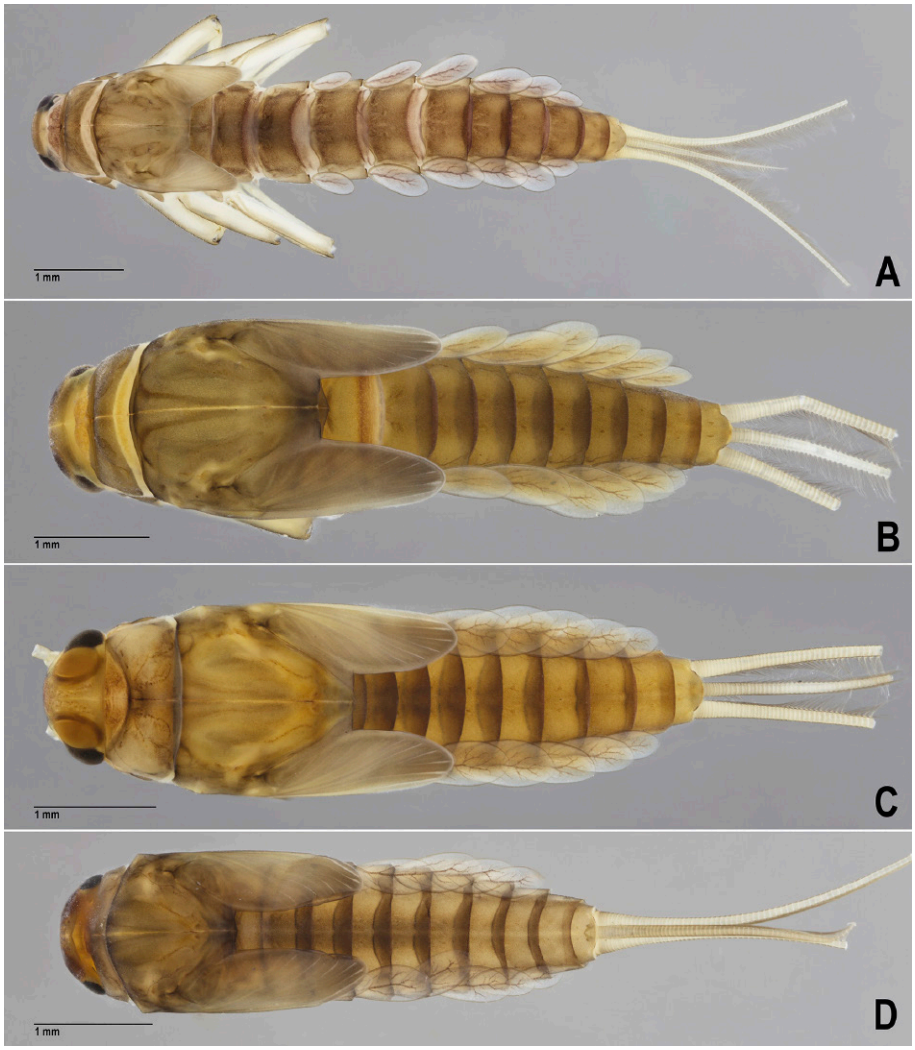

Fig. 4. Habitus of *Baetis* (*Rhodobaetis*) *canariensis* s.l. **A**: *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **B**: *Baetis* (*Rhodobaetis*) *palmensis* n.sp. **C**: *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. **D**: *Baetis* (*Rhodobaetis*) *canariensis* s.s.

95% confidence interval (CI, defined as 2 log likelihood units) ranged from 27 to 31 species hypotheses. The PTP model recovered 31 species hypotheses, including a best score coalescent rate of 903.75 and the ABGD method supported 29 species hypotheses (Figs. 2, 3).

On the Canary Islands, the molecular species assignment suggested between six and eight *Rhodobaetis* species hypotheses, including three (GMYC), four (ABGD), and five (PTP) species hypotheses for *B. canariensis* s.l., and three species hypotheses (GMYC, PTP, ABGD) for *B. pseudorhodani* s.l. (Fig. 2). Within *B. canariensis* s.l., PTP and ABGD supported two distinct species hypotheses for *B. tenerifensis*, comprising a singleton from the northern part of the island (i.e., Anaga), and a cluster from the southern part (i.e., Adeje and Vilaflor). PTP recovered *B. gomerensis* and *B. palmensis* as distinct singletons whereas the two other models (GMYC, ABGD) recovered them as a single species hypothesis.

All species delimitation methods recovered one endemic species hypothesis on Madeira (*B. enigmaticus*) and two endemic species hypotheses on Corsica (i.e., three specimens of *B. ingridae* that were recovered as two distinct species hypotheses). *Baetis rhodani* s.l. was split up by the different species delimitation methods into 18 (GMYC, 95% CI 18–21) or 19 (PTP, ABGD) species hypotheses (Fig. 3). Our analysis recovered all clades previously defined by WILLIAMS et al. (2006) (i.e., I–VII) and LUCENTINI et al. (2011) (i.e., G1–G11). Additionally, we recovered two species hypotheses for the clades G3 (ABGD), G7 (ABGD), G10/V (ABGD), and G11/VI (GMYC, PTP), and three species hypotheses for the clades G4/VI (GMYC) and G5/III (PTP).

3.2. Systematics

3.2.1. *Baetis* (*Rhodobaetis*) *gomerensis* Gattolliat & Sartori, n.sp.

Description of larva. ***Male larva length***: body 5.1–5.4 mm; cerci broken but >3.5 mm. In smaller specimens cerci slightly shorter than body and median caudal filament length less than half of cerci length. ***Female larva length***: body 4.8–7.3 mm; cerci broken but >4.5 mm. In smaller specimens cerci slightly shorter than body and median caudal filament length less than half of cerci length. ***Colouration*** (in pure alcohol) (Fig. 4A): Head medium brown except dark brown between ocelli and

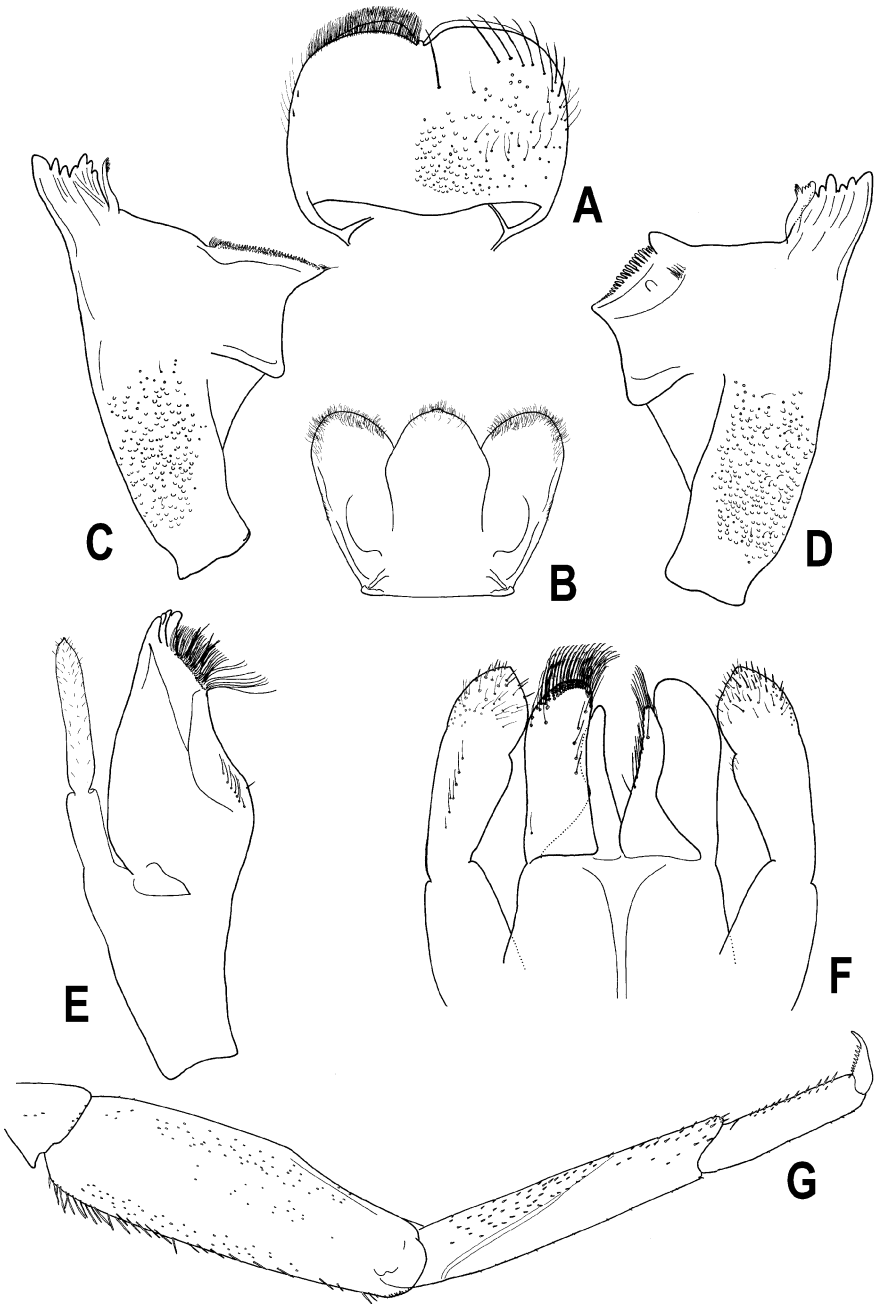

Fig. 5. Mouthparts of *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **A**: labrum; left: ventral view; right: dorsal view. **B**: hypopharynx. **C**: right mandible. **D**: left mandibular. **E**: right maxilla. **F**: labium; left: dorsal view; right: ventral view. **G**: foreleg.

yellow under central ocelli; turbinate eyes orange brown; antennae yellow. Prothorax medium brown almost without clear pattern; meso- and metathorax medium brown, darker along main sutures. Legs: femora light brown with a proximal yellow mark, tibiae light brown except yellow around tibio-patellar suture, tarsi and claws light brown. Terga (Fig. 4A) light brown darker distally with generally two symmetrical brown dark brown spots; no pattern and no terga darker or lighter than other. Sterna I–V yellow without pattern; sterna VI–IX light brown without pattern. Cerci light brown without annulations or stripes.

Head: Antenna: scape with scale bases and a few spatulas. Pedicel with scale bases and 12–14 spatulas near distal margin. Labrum (Fig. 5A) rounded with distal margin somewhat straight, width/length ratio 1.51–1.58; dorsal face of labrum covered with scale bases, with an arc subparallel to distal margin formed by 1+6–8 long and stout setae, roughly arranged in 1 row, short and fin setae scattered on surface; distal margins bordered with long and feathered setae. Hypopharynx (Fig. 5B) with simple lingua covered distally with small thin setae, lingua without clear mark; superlingua as long as lingua. Right mandible (Fig. 5C) outer set with outer tooth as broad as the 2 smaller teeth combined (Fig. 7A), inner set with 4 teeth, second tooth bigger than others, inner margin slightly crenulate; row of thin setae on outer margin of outer of set absent; prostheca elongated and slender with thin setae on inner margin distally; no setae between prostheca and mola; apex of mola (= most distal portion of mola) with a brush of abundant setae: proximal half of mandible with thin setae and abundant

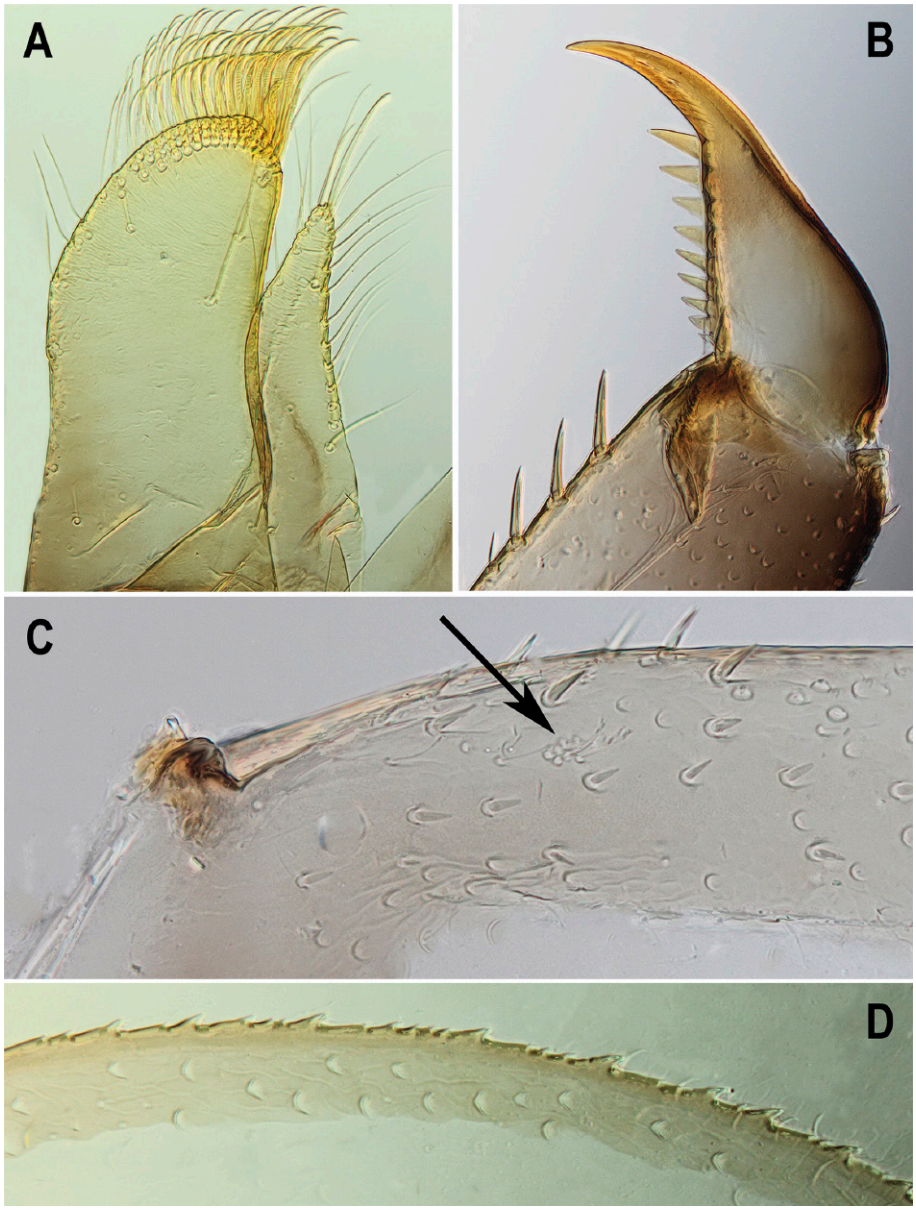

Fig. 6. Diagnostic features of *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **A**: glossa and paraglossa. **B**: claw. **C**: forefemora: ventral margin proximally (black arrow: villopore). **D**: apical margin of gill IV.

scale bases. Left mandible (Fig. 5D) with 6 main teeth and 1 additional smaller tooth, outer tooth as broad as the 2 following combined; prostheca with 4–5 denticles and an elongated comb-shaped structure; no setae between prostheca and mola; no setae at apex of mola; proximal half of mandible with thin setae and abundant scale bases. Maxillae (Fig. 5E) with 4 short teeth, none of them opposed to others; apico-medially with one row of medium setae ending to 7–10 long simple setae, second row compound of 2 spine-like bifid dentisetae and 6–8 long setae, base of lacinia with a row of 6–8 long setae; palp 2-segmented, shorter than galea-lacinia, segment I slightly shorter than segment II, segment II with short rounded projection and a small scale at tip, segments I and II with scattered thin setae. Labium (Fig. 5F) with glossae clearly shorter than paraglossae; glossae triangular with broad base, inner margin and distal half of outer margin with long setae, dorsal face with a few scattered short thin setae and a single long seta medially; apex of paraglossae with three rows of long curved feathered setae and a single very stout setae distoventrally, ventral face with 1 row of 4 long setae, dorsal face with 2 long thin setae distally (Fig. 6A); labial palp 3-segmented (Figs. 5F, 8A); segment I slightly shorter than segments II and III combined; segment II slightly expanded apico-laterally, with 7–12 medium setae, arranged in longitudinal row in some specimens; segment III conical and asymmetrical, almost as broad as long, inner margin convex, with scattered stout setae (Fig. 8A).

Thorax: Forelegs (Fig. 5G) completely covered with scale bases; dorsal margin of femora with medium apically rounded setae (Fig. 9A), abundant proximally and more scarce distally; short and stout setae scattered close to dorsal margin but not arranged in row; dorsoapical setal patch formed by 2 pointed setae, apex with several short setae; ventral margin with abundant scattered short and stout setae, villopore strongly reduced to a few minute setae (Fig. 6C); lateral surface with scattered short se-

tae. Tibiae dorsally with a row of minute setae and lacking short thin setae; ventrally and laterally with medium stout setae, more abundant proximally to tibio-patellar suture. Tarsi dorsally with a few short setae, without thin setae; ventrally with a row of 13–15 short to medium pointed setae and a few additional short pointed setae; claws (Fig. 6B) hooked, with 1 row of 10–12 acute teeth increasing in length toward the apex; subapical setae absent but setal bases visible.

Abdomen: Tergites (Fig. 10A) slightly shagreened and covered with scale bases; spatulas rare limited to posterior margin or close to posterior margin on tergites I–II, abundant on tergites III–VI, very abundant on tergites VII–IX; posterior margin of tergites I smooth, triangular spines limited to lateral portion on tergites II and III, spines present but sometimes variable size on tergites IV and V, abundant on tergites VI–IX. Lateral margin of segments III–IX with short lanceolate spines (Fig. 11A). Sternites with scale bases; spatulas absent on sternites I–IV, rare on sternites V and VI, abundant on sternites VII–IX; posterior margin smooth with few spatulas and friction structures laterally, without spines (Fig. 11A). Gills (Fig. 12A): ochre, margins brown; costal and anal margins with double crenation, lacking spines (Fig. 6D); scale bases closed to margins. Gill I extremely reduced, 0.2 × gill IV, without tracheation. Gill II slightly reduced, 0.7 × gill IV, almost symmetrical with a central tracheation. Gills III–VII relatively slender (2.1 × longer than broad), secondary tracheation poorly developed. Paraproct (Fig. 13A) with abundant scale bases and a few thin setae, 9–13 lanceolate spatulas of various sizes mainly present near posterior margin; margin with 15–20 spines much smaller laterally; postero-lateral extension (cercotractor) with scale bases on apical half, margin with 20–25 irregular medium spines. Cerci with patch of thin setae on inner margins, distal half covered with scale bases, posterior margin with triangular spines; median caudal filament similar to cerci except a patch of setae present on both sides.

Differential diagnosis. Gill I extremely reduced, Gills II–VII elongated slender with well-visible simple central tracheation almost without distinct ramification (Fig. 12A). Right mandible (Figs. 5C, 7A) with outer tooth as broad as other teeth of outer set combined. Segment III of labial palp (Fig. 8A) almost as broad as long, asymmetrical (inner margin more convex). Dorsal margin of femora (Fig. 9A) with apically rounded setae. Tergites VII–IX with abundant spatulas, posterior margin with triangular spines; sternite IX (Fig. 11A) with abundant spatulas on surface and along posterior margin. Paraproct (Fig. 13D) with restricted number of spatulas, spines along margin of cercotractor only slightly smaller than those of paraproct. The distribution should be also considered as a relevant character to identify the species, as *B. gomerensis* is only known from La Gomera.

Derivatio nominis. This species is named after La Gomera, from where the species was collected.

Material examined. ***Holotype***: One female larva (GBIFCH00280786); La Gomera (GM1E); Parque National de Garajonay, Barranco del Cedro, El Cedro; Coord. 28.13556/–17.21435; Alt. 822 m; 08.III.2014; Leg. S. Rutschmann & H. Detering. (MZL) — ***Paratypes***: 20 larvae (GBIFCH00280829); La Gomera (GM1E); same data as holotype. (MFN). 22 larvae in ethanol (GBIFCH00280782) and 2 larvae on slides (GBIFCH00465072; GBIFCH00465073); La Gomera (GM1B); Parque National de Garajonay, Barranco del Cedro, El Cedro; Coord. 28.127/–17.221; Alt. 900 m; 07.III.2014; Leg. S. Rutschmann & H. Detering. (MZL). 13 larvae; La Gomera (GM1C); Parque National de Garajonay, Barranco del Cedro, El Cedro; Coord. 28.12961/–17.22019; Alt. 920 m; 07.III.2014; Leg. S. Rutschmann & H. Detering. (MFN). 16 larvae in ethanol (GBIFCH00280783) and 2 larvae on slides (GBIFCH00465074; GBIFCH00465075); La Gomera (GM1D); Parque National de Garajonay, Barranco del Cedro, El Cedro, artificial channel; Coord. 28.13011/–17.21953; Alt. 920 m; 07.III.2014; Leg. S. Rutschmann & H. Detering. (MZL). 4 larvae in ethanol (GBIFCH00280829) and 2 larvae on slides (GBIFCH00465076; GBIFCH00465077); La Gomera (GM1F) Parque National de Garajonay, Barranco del Cedro, El Cedro; Coord. 28.12603/–17.22081; Alt. 906 m; 08.III.2014; Leg. S. Rutschmann & H. Detering. (MZL). 19 larvae in ethanol (GBIFCH00280852) and 1 larva on slide (GBIFCH00465079); La Gomera (GM2); Parque National de Garajonay, Barranco del Cedro, El Cedro; Coord. 28.12695/–17.22070; Alt. 907 m; 12.III.2008; Leg. M. Sartori & P. Derleth. (MZL). 15 larvae in ethanol (GBIFCH00280828) and 2 larvae on slides (GBIFCH00465080; GBIFCH00465081); La Gomera (GM3); Parque National de Garajonay, Barranco de Monteforte, Emerita de Nuestra Señora de Guadalupe; Coord. 28.12882/–17.20936; Alt. 686 m; 07.III.2014; Leg. S. Rutschmann & H. Detering. (MZL). 2 larvae on slides (GBIFCH00465082; GBIFCH00465083); La Gomera (GM6); Barranco de Arure, El Guro; Coord. 28.10682/–17.326; Alt. 173 m; 08.III.2014; Leg. S. Rutschmann & H. Detering. (MZL).

3.2.2. *Baetis* (*Rhodobaetis*) *palmensis* Gattolliat & Sartori, n.sp.

Description of larva. ***Male larva length***: body 6.2–6.8 mm; cerci 4.5–4.7 mm; median caudal filament 2.4–2.7 mm. ***Female larva length***: body 6.5–8.2 mm; cerci 4.5–5.5 mm; median caudal filament 2.9–3.1 mm. ***Colouration*** (in pure alcohol) (Fig. 4B): Similar to *B. gomerensis*.

Head: Antenna: scape with scale bases and a few thin setae. Pedicel with scale bases and 7–9 spatulas. Labrum (as in Fig. 5A) width/length ratio 1.55–1.65; dorsal face of labrum with an arc subparallel to distal margin formed by 1+6–7 long stout setae arranged in 1 row, short thin setae scattered proximally; distal margins bordered with long and feathered setae. Hypopharynx as in *B. gomerensis* (Fig. 5B). Right mandible (Fig. 7B) outer set with outer tooth as broad as the 2 smaller teeth combined, inner set with 4 teeth, second tooth bigger than others, inner margin slightly crenulate; other characters as in Fig. 5C. Left mandible with 6 main teeth and 2 additional smaller teeth, outer tooth as broad as the two following combined; other characters as in Fig. 5D. Maxillae as in *B. gomerensis* (Fig. 5E). Labium with glossae and paraglossae as in *B. gomerensis* (Fig. 6A); labial palp segment I slightly shorter than segments II and III combined; segment II slightly expanded apico-laterally, with an oblique row of 6–7 medium setae; segment III slender, conical, almost symmetrical, longer than broad, with scattered stout setae (Fig. 8B).

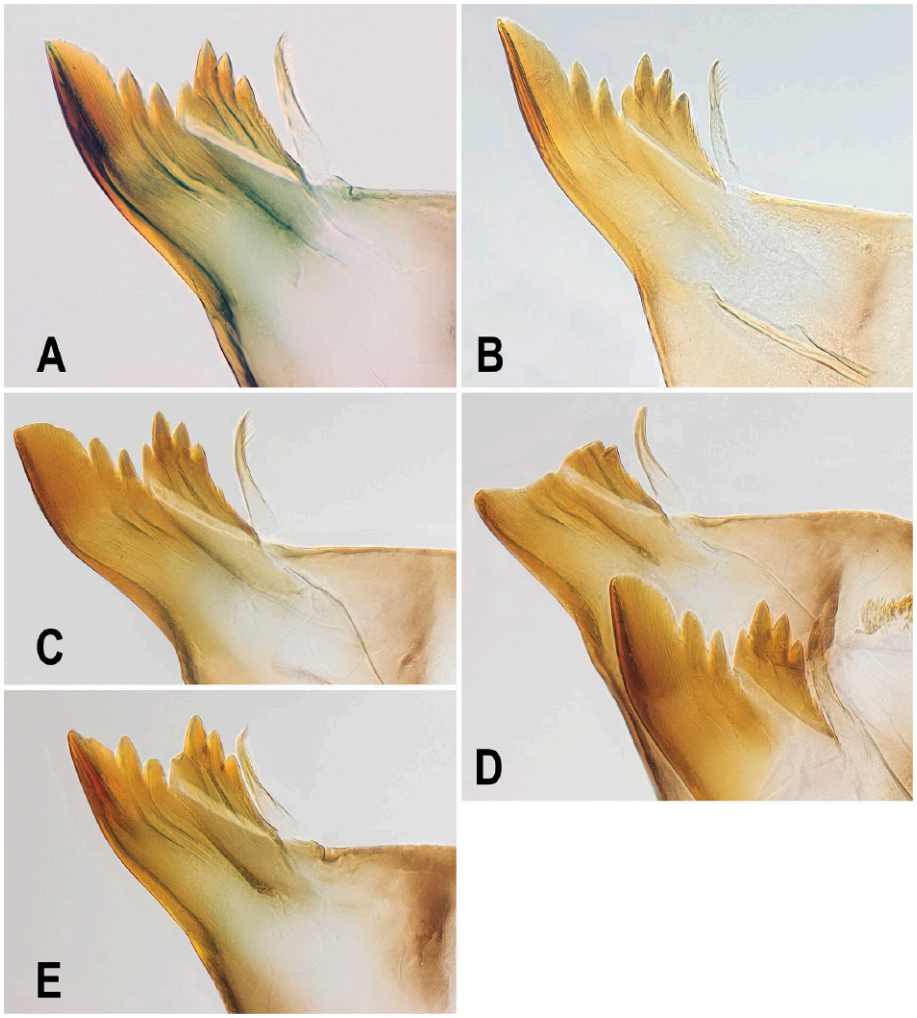

Fig. 7. Canines of right mandible of *Baetis* (*Rhodobaetis*) *canariensis* s.l. **A**: *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **B**: *Baetis* (*Rhodobaetis*) *palmensis* n.sp. **C**: *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. **D**: *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. (specimen ready to moult). **E**: *Baetis* (*Rhodobaetis*) *canariensis* s.s.

Thorax: Forelegs completely covered with scale bases. Dorsal margin of femora with medium apically blunt setae (Fig. 9B); abundant proximally and more scarce distally; short stout setae scattered close to dorsal margin but not arranged in row; dorsoapical setal patch formed by 2 medium apically blunt setae, several short setae distally; ventral margin with scattered short stout pointed setae, villopore reduced to a patch of 6–8 thin setae; lateral surface with scattered short setae. Tibiae dorsally with a row of minute setae lacking short thin setae; ventrally and laterally with medium stout setae, more abundant proximally near tibio-patellar suture. Tarsi dorsally with a few short setae but without thin setae; ventrally with a row of about 15 short to medium pointed setae and a few additional short pointed setae; claws as in *B. gomerensis* (Fig. 6B).

Abdomen: Tergites (Fig. 10B) shagreened and covered with scale bases; spatulas rare, limited to posterior margin or close to posterior margin, slightly more abundant on tergites VII–IX; posterior margin of tergites I smooth, triangular spines limited to lateral portion on tergites II and III, spines present but still irregular on tergite IV, abundant and generally pentagonal on tergites V–IX. Lateral margin of segments III–IX with medium lanceolate spatulas. Sterna with scale bases; spatulas absent on sternites I–IV, rare on sternites V and VI, abundant on tergites VII–IX; posterior margin of sternites I–VIII smooth without spatulas and spines, with friction structures laterally; posterior margin of sternite IX (Fig. 11B) with a few irregular triangular spines. Gills (Fig. 12B): ochre, margins brown; costal and anal margins with double crenation, lacking spines (as in *B. gomerensis* (Fig. 6D)); scale bases closed to margins. Gill I much reduced, 0.3 × gill IV, without tracheation. Gill II slightly reduced, 0.8 × gill IV, almost symmetrical with a central tracheation with abundant ramifications. Gills III–VII relatively broad (twice longer than broad), secondary tracheation well developed. Paraproct (Fig. 13B) with scale bases and a few thin setae, 4–6 lanceolate spatulas mainly present near posterior margin (rarely 7–8); margin with 17–25 spines; postero-lateral extension (cercotractor) with a few scale bases, margin with 15–25 irregular spines, central spines generally broad. Cerci and median caudal filament as in *B. gomerensis*.

Differential diagnosis. Gill I much reduced, Gills II–VII asymmetrical with a central tracheation well divided (Fig. 12B). Right mandible with outer tooth incurved, as broad as other teeth of outer set combined (Fig. 7B). Segment III of labial palp (Fig. 8B) slender, symmetri-

cal, apical nipple well marked. Dorsal margin of femora (Fig. 9B) with apically blunt setae. Tergites VII–IX with scarce spatulas, posterior margin with pentagonal spines; sternite IX (Fig. 11B) with abundant spatulas on surface, posterior margin with a few triangular spines. Paraproct (Fig. 13B) with a few spatulas, cercotractor with relatively broad spines medially. The distribution should be also considered as a relevant character to identify the species, as *B. palmensis* is only known from La Palma.

Derivatio nominis. This species is named after La Palma, from where the species was collected.

Material examined. ***Holotype***: One female larva (GBIFCH00280779); La Palma (LP1B); Parque National de la Caldera de Taburiente, Barranco de Las Anguistas, confluence Río Taburiente; Coord. 28.72455/–17.876; Alt. 771 m; 10.III.2014; Leg. S. Rutschmann & H. Detering. (MZL). — ***Paratypes***: 26 larvae in ethanol (GBIFCH00280780), 32 larvae in ethanol and 4 larvae on slides (GBIFCH00465089; GBIFCH00465090; GBIFCH00465091; GBIFCH00465092); same data as holotype. (MFN + MZL). 16 larvae in ethanol (GBIFCH00280824) and 2 larvae on slides (GBIFCH00465087; GBIFCH00465088); La Palma (LP1); Parque Nacional de la Caldera de Taburiente, Río Taburiente; Coord. 28.728611/–17.873333; Alt. 800 m; 29.I.2009; Leg. M. Sartori & M. Báez. (MZL). 30 larvae in ethanol (GBIFCH00280784) and 1 larva on slide (GBIFCH00465093); La Palma (LP2); Parque Nacional de la Caldera de Taburiente, Río Taburiente (above confluence); Coord. 28.730833/–17.871111; Alt. 810 m; 29.I.2009; Leg. M. Sartori & M. Báez. (MZL). 10 larvae in ethanol (GBIFCH00280831), 15 larvae in ethanol and 4 larvae on slides (GBIFCH00465094; GBIFCH00465095; GBIFCH00465096; GBIFCH00465097); La Palma (LP2B); Parque National de la Caldera de Taburiente, Barranco del Ciempiés; Coord. 28.715/–17.901; Alt. 1040 m; 10.III.2014; Leg. S. Rutschmann & H. Detering. (MFN + MZL). 30 larvae in ethanol (GBIFCH00280827) and 3 larvae on slides (GBIFCH00465098; GBIFCH00465099; GBIFCH00465100); La Palma (LP3); Barranco del Cempiés, Breitos; Coord. 28.716111/–17.901388; Alt. 1040 m; 29.I.2009; Leg. M. Sartori & M. Báez. (MZL). — ***Other material***: One female imago (GBIFCH00280781), La Palma (LP1B); Parque National de la Caldera de Taburiente, Barranco de Las Anguistas, afluente Río Taburiente; Coord. 28.72455/–17.876; Alt. 771 m; 10.III.2014; Leg. S. Rutschmann & H. Detering. (MZL)

3.2.3. *Baetis* (*Rhodobaetis*) *tenerifensis* Gattolliat & Sartori, n.sp.

Description of larva. ***Male larva length***: body 6.1–6.6 mm; cerci broken; median caudal filament broken, > 4.5 mm. ***Female larva length***: body 6.7–9.1 mm; cerci broken; median caudal filament 3.1–3.5 mm. ***Colouration*** (in pure alcohol) (Fig. 4C): Similar to *B. gomerensis*.

Head: Antenna: scape with scale bases and a few thin setae. Pedicel with scale bases and 10–14 spatulas. Labrum width/length ratio 1.43–1.50; dorsal face of labrum with an arc subparallel to distal margin formed by 1+5–6 long stout setae arranged in 1 row, few short fin setae scattered proximally; distal margins bordered with long and feathered setae. Hypopharynx as in *B. gomerensis* (Fig. 5B). Right mandible: outer set with outer tooth broader than the 2 smaller teeth combined, inner incisor with 4 teeth, second tooth bigger than others, inner margin slightly crenulate (Fig. 7C,D); other characters as in as in *B. gomerensis*. Left mandible with 6 main teeth and 2 additional smaller teeth, outer tooth broader than the two following combined; other characters as in *B. gomerensis*. Maxillae as in *B. gomerensis* (Fig. 5E). Labium with glossae and paraglossae as in *B. gomerensis* (Fig. 6A); labial palp segment I shorter than segments II and III combined; segment II slightly expanded apicolaterally, with an oblique row of 6–9 medium setae; segment III (Fig. 8C) conical, almost symmetrical, inner margin almost straight, slightly longer than broad, with scattered stout setae and short thin setae.

Thorax: Forelegs completely covered with scale bases. Dorsal margin of femora (Fig. 9C) with setae, very abundant proximally and more scarce distally, proximally slender, pointed setae, getting broader and rounder to almost spatulated form in distal part of femora; short and stout setae scattered close to dorsal margin roughly arranged in 2 rows; dorsoapical setal patch formed by 2 medium apically rounded setae, several short setae distally; ventral margin with scattered short stout pointed setae, villopore reduced to a patch of 6–10 thin setae; lateral surface with scattered short setae. Tibiae dorsally with a row of very tiny setae and without short thin setae; ventrally and laterally with medium stout setae, more abundant proximally to tibio-patellar suture. Tarsi dorsally with a few short setae but without thin setae; ventrally with a row of about 12 short to medium pointed setae and a few additional shorter pointed setae; claws as in *B. gomerensis* (Fig. 6B).

Abdomen: Tergites (Fig. 10C) shagreened and covered with scale bases; spatulas present on posterior margin of tergites II–IX, posterior margin of tergite I entirely smooth, few triangular spines on tergites II and IV, small and slender spines present on tergites V–IX. Lateral margin of segments VII–IX with medium lanceolate spatulas. Sternites slightly shagreened with scale bases; spatulas absent on sternites I–VI, rare on sternites VII, very abundant on sternites VIII–IX; posterior margin of sternites I–IX smooth without spines (Fig. 11C), with friction structures laterally. Gills (Fig. 12C): ochre, medially tainted brown, margins brown; costal and anal margins with double crenation, lacking spines (as in *B. gomerensis*, Fig. 6D); scale bases closed to margins. Gill I reduced, 0.4 × gill IV, with tracheation. Gill II slightly reduced, 0.8 × gill IV, almost symmetrical with a central tracheation with abundant ramifications. Gills III–VII relatively broad, secondary tracheation well developed. Paraproct (Fig. 13C) with scale bases and thin setae, 12–15 lanceolate spatulas of various size; margin with 19–25 spines; postero-lateral extension (cercotractor) scale bases, margin with 18–27 irregular, generally broad, long spines (longest spines subegal to spines of margin of paraproct). Cerci and median caudal filament as in *B. gomerensis*.

Differential diagnosis. Gill I reduced but with tracheation well visible and ramified. Gills II–VII asymmetrical with distinctly ramified central tracheation, medially tainted brown (Fig. 12C). Right and left mandibles with outer tooth broader than other teeth of outer set com-

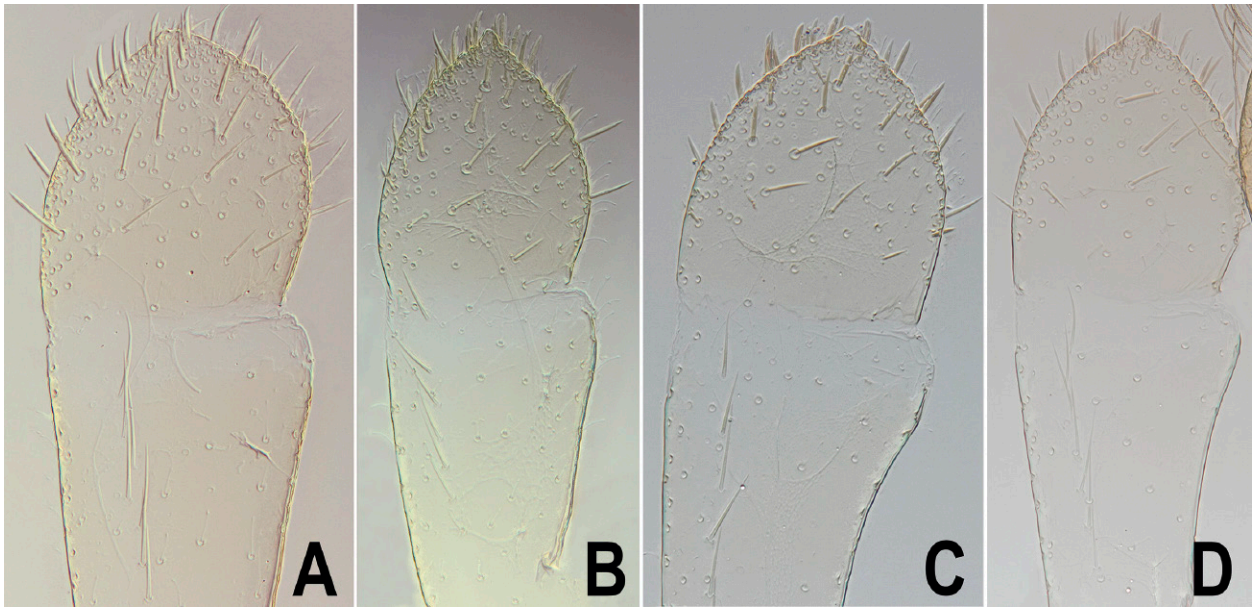

Fig. 8. Labial palp of *Baetis* (*Rhodobaetis*) *canariensis* s.l. **A**: *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **B**: *Baetis* (*Rhodobaetis*) *palmensis* n.sp. **C**: *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. **D**: *Baetis* (*Rhodobaetis*) *canariensis* s.s.

bined (Fig. 7C,D). Segment III of labial palp almost symmetrical (inner margin almost straight) (Fig. 8C). Dorsal margin of femora with slender apically pointed setae (Fig. 9C). Tergites VII–IX with abundant spatulas, posterior margin with triangular spines; sternite IX (Fig. 11C) with abundant spatulas on surface and rare or absent along posterior margin, posterior margin almost without triangular spines. Paraproct (Fig. 13C) with part of stout spines of cercotractor almost as long as spines of the margin of paraproct. The distribution should be also considered as a relevant character to identify the species, as *B. tenerifensis* is only known from Tenerife.

Derivatio nominis. This species is named after Tenerife, from where the species was collected.

Material examined. ***Holotype***: One female larva (GBIFCH00280787); Tenerife (TF6); Barranco del Río, Vilaflor; Coord. 28.19299/–16.57216; Alt. 1428 m; 12.III.2014; Leg. S. Rutschmann & H. Detering. (MZL). — ***Paratypes***: 34 larvae in ethanol (GBIFCH00280833) and 4 larvae on slides (GBIFCH00465112; GBIFCH00465113; GBIFCH00465114; GBIFCH00465115); Tenerife (TF6); same data as holotype. (MZL). 1 larva on slide (GBIFCH00465101); Tenerife (TF1); Barranco de Afur, Afur; Coord. 28.555/–16.250556; Alt. 170 m; 04.IV.2007; Leg. M. Báez. (MZL). 23 larvae in ethanol; Tenerife (TF3B); Barranco del Infierno, Adeje; Coord. 28.13296/–16.7108; Alt. 479 m; 22.III.2014; Leg. S. Rutschmann, H. Detering & M. Báez. (MFN). 30 larvae in ethanol and 2 larvae on slides (GBIFCH00465103; GBIFCH00465104); Tenerife (TF3C); Barranco del Infierno, Adeje; Coord. 28.13741/–16.70285; Alt. 571 m; 22.III.2014; Leg. S. Rutschmann, H. Detering & M. Báez. (MFN). 35 larvae in ethanol (GBIFCH00280788) and 3 larvae on slides (GBIFCH00465105; GBIFCH00465106; GBIFCH00465107); Tenerife (TF3D); Barranco del Infierno, Adeje; Coord. 28.13339/–16.70518; Alt. 522 m; 22.III.2014; Leg. S. Rutschmann, H. Detering & M. Báez. (MZL). 10 larvae in ethanol (GBIFCH00280832) and 3 larvae on slides (GBIFCH00465108; GBIFCH00465109; GBIFCH00465110); Tenerife (TF3E); Barranco del Infierno, Adeje, artificial channel; Coord. 28.13297/–16.71102; Alt. 487 m; 22.III.2014; Leg. S. Rutschmann, H. Detering & M. Báez. (MZL). 14 larvae in ethanol (GBIFCH00280834) and 1 larva on slide (GBIFCH00465110); Tenerife (TF4); Barranco del Infierno, below waterfall; Coord. 28.136833/–16.702724; Alt. 560 m; 22.III.2007; Leg. M. Sartori & P. Derleth. (MZL). 31 larvae in ethanol (GBIFCH00280851) and 1 larva on slide (GBIFCH00465111); Tenerife (TF5); Barranco del Infierno, 200 m below waterfall; Coord. 28.135755/–16.704559; Alt. 540 m; 22.III.2007; Leg. M. Sartori & P. Derleth. (MZL)

3.2.4. *Baetis* (*Rhodobaetis*) *canariensis* Müller-Liebenau, 1971

Differential diagnosis. Gill I reduced with tracheation well visible but not divided, Gills II–VII almost symmetrical with a central tracheation, medially brown tainted (Fig. 12D). Right mandible with outer tooth narrower than other teeth of outer set combined (Fig. 7E). Segment III of labial palp almost symmetrical (Fig. 8D). Dorsal margin of femora (Fig. 9D) with spatulate apically rounded setae. Tergites IV–IX with scarce spatulas, posterior margin with pentagonal spines (Fig. 10D); sternite IX (Fig. 11D) with scarce spatulas on surface and along posterior margin, posterior margin with few spines. Paraproct (Fig. 13D) with restricted number of spatulas, spines along margin of cercotractor much smaller than spines of margin of paraproct. The distribution should be also considered as a relevant character to identify the species, as *B. canariensis* is only known from Gran Canaria.

Material examined. ***Paratypes***: 3 larvae; Gran Canaria; Baranco de la Mina; Alt. 1150 m; 19. März 1968; Leg. I. Müller-Liebenau. (Zoological Museum of Hamburg ZMH). — ***Other material***: 31 larvae in ethanol (GBIFCH00280805) and 3 larvae on slides (GBIFCH00465117; GBIFCH00465118; GBIFCH00465119); Gran Canaria (GC1); Barranco de los Cernícalos, Telde; Coord. 27.965/–15.496111; Alt. 822 m; 25.I.2009; Leg. M. Sartori & M. Báez. (MZL). 1 larva in ethanol (GBIFCH00280848) and 1 larva on slide (GBIFCH00465120); Gran Canaria (GC2); Barranco de La Mina, Las Lagunetas; Coord. 28.000833/–15.585; Alt.

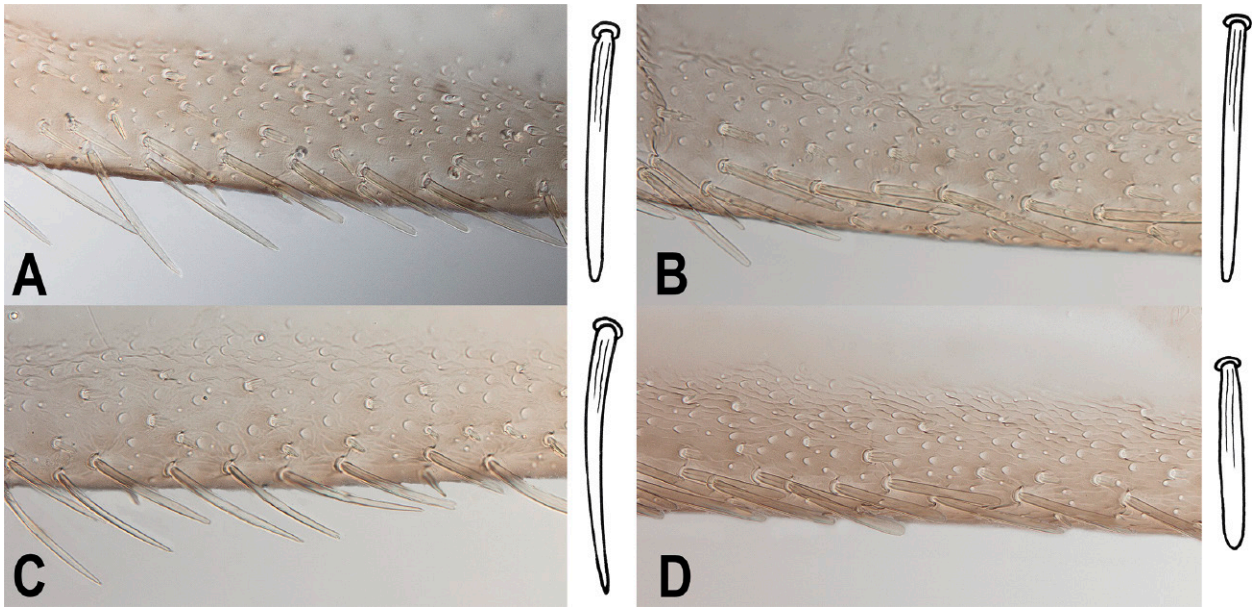

Fig. 9. Dorsal margin of forefemora of *Baetis* (*Rhodobaetis*) *canariensis* s.l. **A**: *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **B**: *Baetis* (*Rhodobaetis*) *palmensis* n.sp. **C**: *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. **D**: *Baetis* (*Rhodobaetis*) *canariensis* s.s.

1180 m; 25.I.2009; Leg. M. Sartori & M. Báez. (MZL). 1 larva in ethanol (GBIFCH00280849); Gran Canaria (GC3); Barranco de La Mina, Utiaca; Coord. 28.018056/–15.557222; Alt. 790 m; 25.I.2009; Leg. M. Sartori & M. Báez. (MZL). 20 larvae in ethanol (GBIFCH00280806) and 1 larva on slide (GBIFCH00465121); Gran Canaria (GC4); Barranco de La Mina, Las Lagunetas; Coord. 27.999444/–15.586389; Alt. 1220 m; 26.I.2009; Leg. M. Sartori & M. Báez. (MZL). 1 larva in ethanol and 1 larva on slide (GBIFCH00465122); Gran Canaria (GC4B); Barranco de La Mina, Las Lagunetas; Coord. 27.99954/–15.58643; Alt. 1222 m; 14.III.2014; Leg. S. Rutschmann & H. Detering. (MFN + MZL). 12 larvae in ethanol (GBIFCH00280850) and 1 larva on slide (GBIFCH00465123); Gran Canaria (GC5B); Barranco de los Cernícalos, La Breña; Coord. 27.96419/–15.49903; Alt. 837 m; 15.III.2014; Leg. S. Rutschmann & H. Detering. (MZL). 24 larvae in ethanol (GBIFCH00280804) and 2 larvae on slides (GBIFCH00465124; GBIFCH00465125); Gran Canaria (GC6); Barranco de los Cernícalos, Lomo Magullo, artificial channel; Coord. 27.97831/–15.48112; Alt. 512 m; 15.III.2014; Leg. S. Rutschmann & H. Detering. (MZL).

4. Discussion

A number of studies using mitochondrial DNA have reported *Rhodobaetis* to be a complex of species hypotheses (Williams et al. 2006; Lucentini et al. 2011; Sroka 2012; Rutschmann et al. 2014; Gattolliat et al. 2015; Bisconti et al. 2016; Múrria et al. 2017). A DNA barcoding project of European mayflies has also shown that *Baetis rhodani* s.l. is composed of many mitochondrial clusters recognized as species hypotheses by GMYC, some of which are widely distributed and sympatric, with others being endemic to restricted geographical areas (Fig. 3; S. Rutschmann unpubl. data.). Based on widespread agreement between mitochondrial markers and unlinked nuclear markers in other Baetidae (Rutschmann et al. 2017), we assume these to be valid species hypotheses. Nonetheless, most of the species hypotheses remain difficult to separate morphologically and therefore have never been formally described, even using the set of characters established to discriminate the species of *Rhodobaetis* (Godunko et al. 2004). In comparison, Canary Islands, with a restricted number of species hypotheses and a rather simple geographic distribution, are therefore an easy-to-handle study case.

The Canarian *Rhodobaetis* clearly form two *cox1* lineages: one corresponding to *B. canariensis* s.l., including the three species newly described herein, and the other to *B. pseudorhodani* s.l., in agreement with earlier results (Rutschmann et al. 2014). Within *B. canariensis* s.l. the applied species delimitation methods resulted in two rather small inconsistencies, including *B. gomerensis* and *B. palmensis* (split: PTP, grouped: GMYC, ABGD) and the split of *B. tenerifensis* into two species hypotheses (PTP, ABGD). The species hypotheses for *B. pseudorhodani* s.l. agree for all methods and are congruent with the ones recovered by Rutschmann et al. (2014). Notably, the inconsistencies within *B. canariensis* s.l. are associated with the species hypotheses represented by the smallest numbers of specimens. Tang et al. (2014) found a general tendency of species hypotheses to be more split when fewer specimens were included. Interestingly, the highest number of species hypotheses for *B. canariensis* s.l. was recovered using PTP. In previous studies, PTP was found to be as accurate, if not more, than GMYC (Zhang et al. 2013; Tang et al. 2014) and to outperform the latter method when few species are involved (Luo et al. 2018). In contrast to our previous results (Rutschmann et al. 2014), the separation of *B. gomerensis* and *B. palmensis* was not supported when using GMYC. Tree-based methods, in particular GMYC, are quite sensitive to the priors and parameters used to construct the ultrametric tree (Talavera et al. 2013; Tang et al. 2014). In compari-

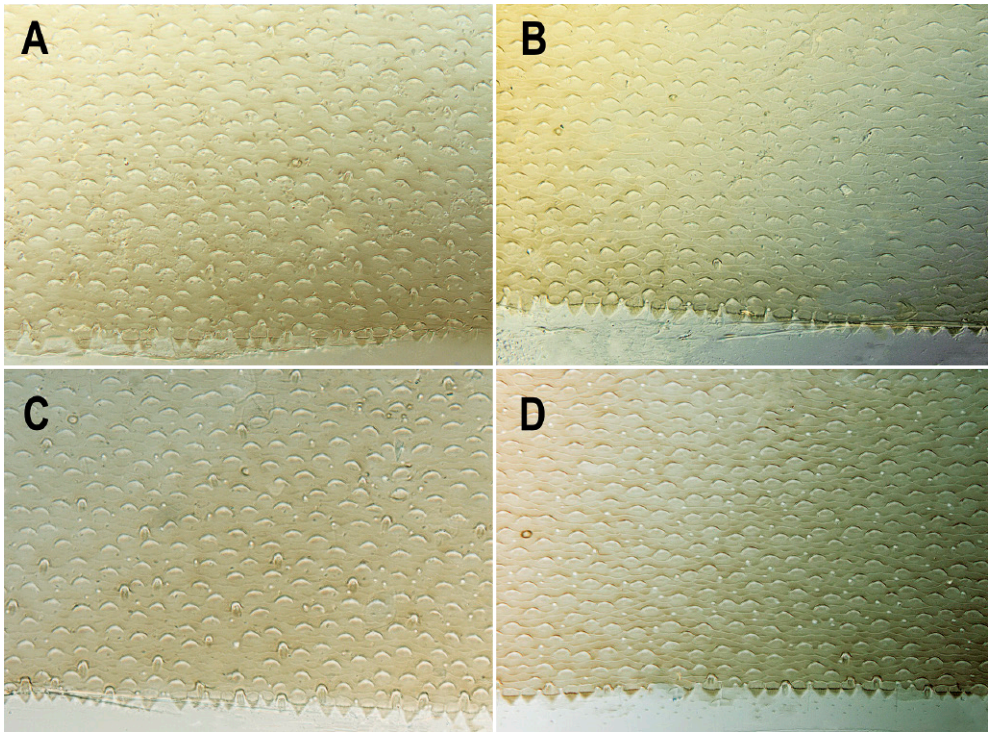

Fig. 10. Posterior margin of tergite IV of *Baetis* (*Rhodobaetis*) *canariensis* s.l. **A**: *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **B**: *Baetis* (*Rhodobaetis*) *palmensis* n.sp. **C**: *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. **D**: *Baetis* (*Rhodobaetis*) *canariensis* s.s.

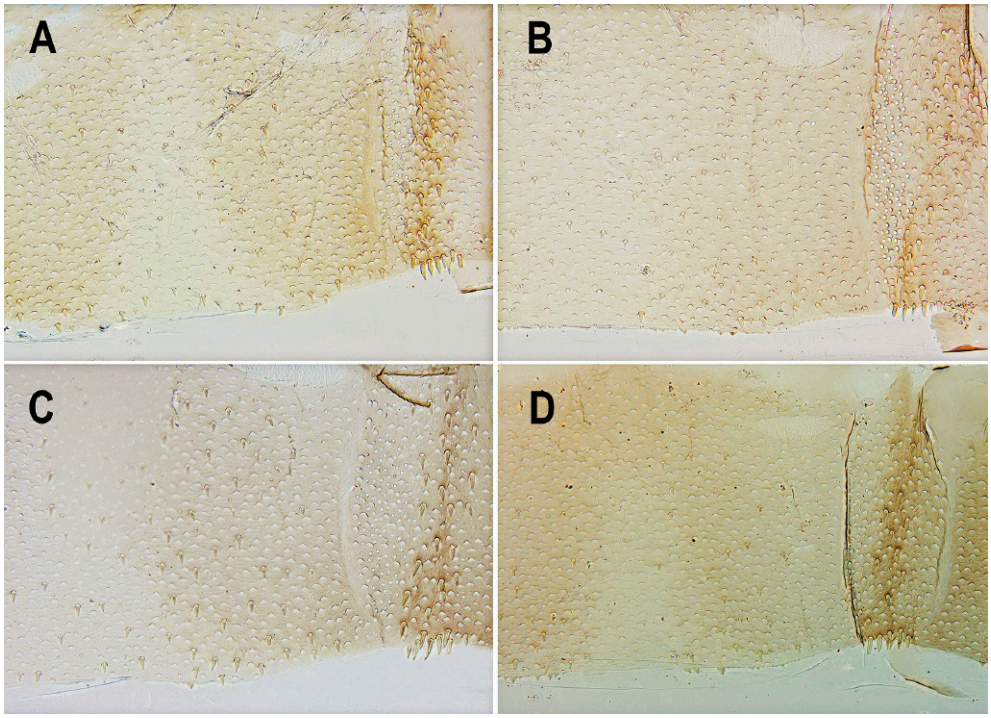

Fig. 11. Posterior margin of sternite IX of *Baetis* (*Rhodobaetis*) *canariensis* s.l. **A**: *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **B**: *Baetis* (*Rhodobaetis*) *palmensis* n.sp. **C**: *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. **D**: *Baetis* (*Rhodobaetis*) *canariensis* s.s.

son to RUTSCHMANN et al. (2014), the set of specimens included here was slightly different (i.e., including additional Canarian specimens and *B. rhodani* s.l. specimens from North Africa), and the sequence partitioning and choice of model of molecular evolution differed as a result. Notably, *B. gomerensis* and *B. palmensis* seem to present very little genetic variability as, for both, all specimens belonged to a single haplotype. This could be due to insufficient sampling, despite our sequencing specimens collected from different localities and different streams (see also below). As all Canarian species – and in particular the ones on La Palma and La Gomera – occur in rather isolated areas (see

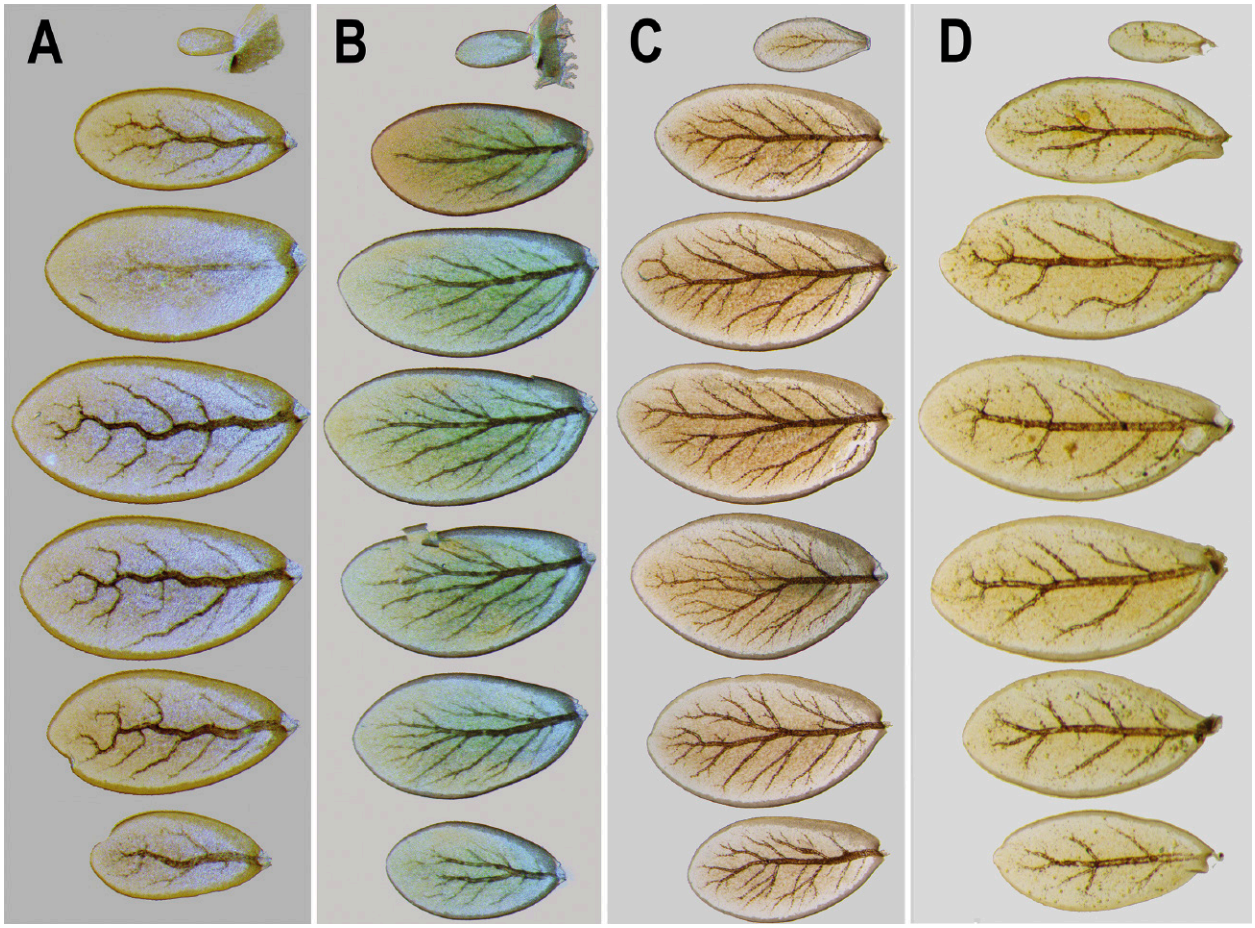

Fig. 12. Gills I–IX of *Baetis* (*Rhodobaetis*) *canariensis* s.l. **A**: *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **B**: *Baetis* (*Rhodobaetis*) *palmensis* n.sp. **C**: *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. **D**: *Baetis* (*Rhodobaetis*) *canariensis* s.s.

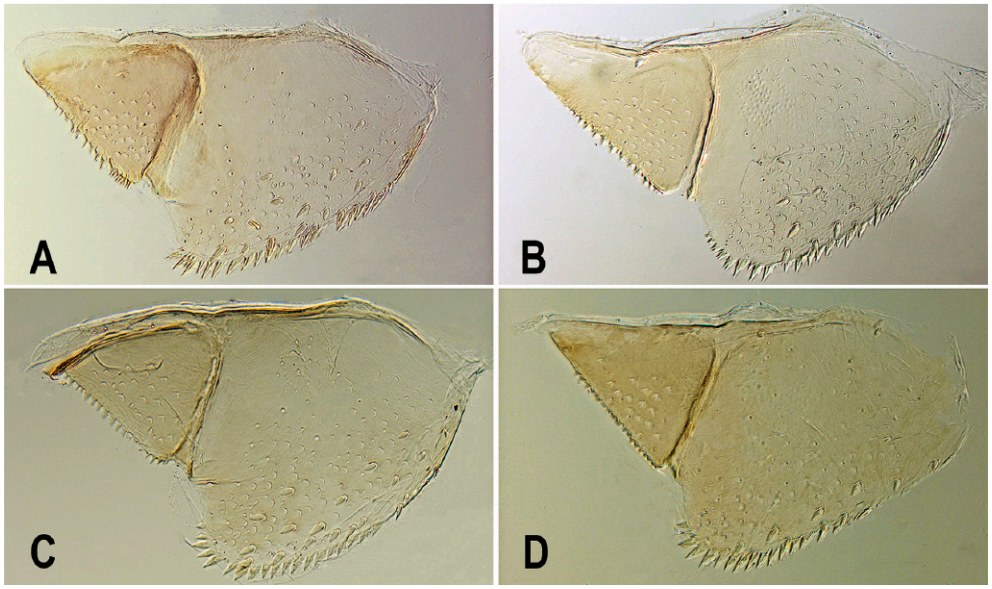

Fig. 13. Paraproct and postero-lateral extension (cercotractor) of *Baetis* (*Rhodobaetis*) *canariensis* s.l. **A**: *Baetis* (*Rhodobaetis*) *gomerensis* n.sp. **B**: *Baetis* (*Rhodobaetis*) *palmensis* n.sp. **C**: *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. **D**: *Baetis* (*Rhodobaetis*) *canariensis* s.s.

also below), a low genetic variability might be expected. Alternatively, *B. gomerensis* and *B. palmensis* may be very closely related, including a very recent dispersal event between the two neighbouring islands. The potential species hypothesis of *B. tenerifensis* for the northern part (i.e., Anaga) is interesting. In particular this matches volcanic activities on Tenerife, supporting within-island phylogeographic patterns. In fact several plant and ani-

mal taxa include sister lineages endemic to the Tenerife palaeo-islands (Juan et al. 2000; Mairal et al. 2015 and references therein; Brown et al. 2017).

We recovered very similar species hypotheses as previous studies for *B. rhodani* s.l. and *B. ingridae* s.l. (Williams et al. 2006; Lucentini et al. 2011; Rutschmann et al. 2014). Our extended taxon sampling recovered two to three North African species hypotheses. In particular, we found a previously unknown clade, distributed in Algeria and Corsica, comprising one (GMYC) or two species hypotheses (PTP, ABGD), and a separate North African/Corsican species hypothesis within the formerly detected clade G4/VII (GMYC, PTP) (Lucentini et al. 2011; Rutschmann et al. 2014). In comparison to Rutschmann et al. (2014), we generally detected fewer species hypotheses when using the GMYC method, including G3/I, G7, and G10/V that were inferred as one species hypothesis, but with the exception of G4/VII. For Corsica, our data support the growing evidence of several *B. rhodani* s.l. species hypotheses (Gattolliat et al. 2015; Bisconti et al. 2016).

Species delimitation methods can be misleading if species are not sampled adequately (Lohse 2009; Papadopoulou et al. 2009). In particular, undersampling can result in inappropriate delimitations, leading to either underestimation of species hypotheses or overestimation due to apparent genetic structuring (Papadopoulou et al. 2009). For cryptic species with a wide or unknown distribution range, such as *B. rhodani* s.l. (Gattolliat & Sartori 2008), it can be difficult to assess whether sampling is adequate. For the species newly described herein, assessing distribution and sampling seems easier considering their island-endemic distributions, although species range, population structuring (i.e., possibly for *B. tenerifensis*) and population size (i.e., as evidenced for *B. gomerensis* and *B. palmensis*) influence adequate taxon sampling (Bergsten et al. 2012; Hamilton et al. 2014).

The use of species delimitation methods based on single-locus data (i.e., gene tree) can provide useful information for the survey of biodiversity patterns (Monaghan et al. 2009; Esselstyn et al. 2012; Ratnasingham & Hebert 2013; Tang et al. 2014). Nonetheless, its use is questionable (Knowles & Carstens 2007; Dupuis et al. 2012). In particular, the sole use of mitochondrial DNA is problematic for species with mitonuclear or cytonuclear discordance (Toews & Brelsford 2012; Papakostas et al. 2016; Ivanov et al. 2017), including mitochondrial pseudogenes in nuclear DNA (NUMTs) (Song et al. 2008; Leite 2012). Species delimitation based on multi-locus data (i.e., species tree) generally result in more reliable species hypotheses because these methods can incorporate discordances between loci (Maddison 1997; Yang & Rannala 2010). In particular this applies when species are not recovered as monophyletic clades based on gene trees due to among-gene differences in lineage sorting or in recent speciation events (Funk & Omland 2003; Mallo & Posada 2016). However, a recent study on species hypotheses, comparing single (i.e., *cox1*) and multilocus data (i.e., 59 nuclear DNA markers) recovered corresponding species hypotheses for closely related species of *Cloeon dipterum* s.l. (Rutschmann et al. 2017). While species delimitation methods entirely based on single-locus data and on small numbers of specimens do pose interpretational risks, the combination of molecular and morphological data provides suitable criteria for species description (DeSalle et al. 2005).

Morphologically, the four species *B. canariensis*, *B. gomerensis*, *B. palmensis* and *B. tenerifensis* are very similar and share several important characters, notably the reduction of the first pair of gills and the absence of spines on the gill margin (Fig. 12). These two characters are rather unusual within *Rhodobaetis* and can explain why Müller-Liebenau (1971) first considered them to be a single species. *Baetis gomerensis* and *B. palmensis* present the highest reduction of the first pair of gills (Fig. 12), comparable mandibles (Fig. 7A,B), and similar setation of the dorsal margin of femora (Fig. 9A,B) (Table 1). They mainly differ by the shape of the labial palp (more slender in *B. palmensis*: Fig. 8B) and tergites VII–IX with scarce spatulas in *B. palmensis* (Fig. 10B). The gills I of *Baetis tenerifensis* and *B. canariensis* are less reduced than in the two previous species (Fig. 12C,D). *Baetis tenerifensis* can be identified by the broader outer incisive of both mandibles (Fig. 7C,D) and cercotractor with stouter and longer spines along the margin (Fig. 13C). *Baetis canariensis* was the single species originally documented by Müller-Liebenau (1971). We can easily recognise that the material used for the illustrations (fig. 19E,F,J in Müller-Liebenau 1971) was collected in Gran Canaria because segment III of the labial palp is almost symmetrical (Fig. 8D) and the setae on the dorsal margin of femora are spatulate (Fig. 9D). Despite meticulous observation of the different specimens on slides and other specimens in ethanol, we were unable to see the subapical setae on claws. Traces of insertions of setae may be seen in some case (Fig. 6B), but no setae are visible as in fig. 19K in Müller-Liebenau (1971).

The present study clearly shows that the search for relevant and usable morphological characters to support species hypothesis revealed by molecular tools remains a great challenge. Most of the traditional characters used to separate species (see for example the key to *Baetis* species in Müller-Liebenau 1969) were not suitable for separating these very similar species. Interpretation of the discriminating characters (Table 1) requires long-term experience and observation of several specimens with a high-performance binocular or SEM images. We hope that the present study will encourage taxonomists to take molecular putative species out of the dark taxonomy by providing a formal description.

A major aim of this revision is related to the conservation of these endemic species. Rather than having one species (namely *B. canariensis*) spread over four islands, our results show that each island possesses its own endemic species. Therefore their current status should be investigated rapidly by authorities. Comparison with the data extracted from the literature (Müller-Liebenau 1971; Alba-Tercedor et al.

Table 1. Morphological characters to distinguish *Baetis* (*Rhodobaetis*) *canariensis*, *Baetis* (*Rhodobaetis*) *gomerensis* n.sp., *Baetis* (*Rhodobaetis*) *palmensis* n.sp. and *Baetis* (*Rhodobaetis*) *tenerifensis* n.sp. following the diagnoses proposed by Godunko et al. (2004) for West Palaearctic species of the subgenus *Rhodobaetis*. [1] From Godunko et al. (2004); [2] character observed at final instar of larval stage.

N°	Characters	*Baetis canariensis*	*Baetis gomerensis*	*Baetis palmensis*	*Baetis tenerifensis*
	Larva				
1	Pedicel: shape of scales	Elongated, rounded apically	Elongated, spatulated, apically pointed	Medium, apically rounded	Medium, apically rounded
2	Scape: shape of scales	Elongated, rounded apically	Elongated, spatulated, apically pointed	Medium, apically rounded	Medium, spatulate, apically rounded
3	Labrum: mean width/length ratio	1.52 – 1.60	1.51 – 1.58	1.55 – 1.65	1.43 – 1.50
4	Labrum: number of long submarginal setae	1 + 5 – 7	1 + 6 – 8	1 + 6 – 7	1 + 5 – 6
5	Maxillary palps: apical part of distal segment	1 rudimentary scale	1 rudimentary scale	1 rudimentary scale	1 rudimentary scale
6	Paraglossae: number of regular row of bristles	3	3	3	3
7	Labial palp segment III	Almost symmetrical	Conical and asymmetrical	Slender, conical, almost symmetrical	Conical, almost symmetrical
8	External margin of femora: shape of bristles	Spatulate setae	Medium apically rounded setae	Medium blunt setae	Medium apically pointed (broader in distal part of femora)
9	Claw: number of strong teeth	10 – 12	10 – 12	10 – 12	10 – 12
10	Claw: presence of apical setae	Traces of insertion	Traces of insertion	Traces of insertion	Traces of insertion
11	Surface of terga: shape of scales	Medium, apically rounded	Short, apically blunt	Short, apically blunt	Short, apically blunt
12	Posterior margin of terga III – VI: presence of triangular spines	Abundant and generally pentagonal	Abundant, sometimes irregular	Abundant and generally pentagonal	Small and slender (rare on tergites III and IV)
13	Shape of gills III – V	Slightly asymmetrical, relatively slender	Slightly asymmetrical, relatively slender	Broad and asymetrical	Broad and asymetrical
14	Spines of external margin of gills	Absent	Absent	Absent	Absent
15	Paraproct plate: number of marginal spines	16 – 19	15 – 20	17 – 25	19 – 25
16	Paraproct plate: shape of spatulas	Medium, lanceolated	Medium, lanceolated, apically pointed	Medium, lanceolated, apically pointed	Medium, lanceolated, apically pointed
17	Terminal filament length: relative to cerci length	1/2 – 2/3	< 1/2	1/2 – 2/3	< 1/2
	Male imago				
18	Turbinate eyes: facetted surface	Light yellow to brownish[1]	Orange brown[2]	Orange brown[2]	Orange brown[2]
19	Shaft of turbinate eyes	Yellowish without ring[1]	—	—	—
20	Coloration of thorax	Brown[1]	—	—	—
21	Hindwing: number of veins	3[1]	—	—	—
22	Basal segment of forceps	Approximately square[1]	—	—	—
23	Segment I of forceps	With mainly suparallel margins and strong inner projection apically[1]	—	—	—
24	Segment II of forceps: widened part	1/2 – 2/3 segment length[1]	—	—	—
25	Segment II of forceps: inner margin	Clearly concave[1]	—	—	—
26	Segment III of forceps	Oval[1]	—	—	—

1987; Malmqvist et al. 1993, 1995; Nilsson et al. 1998), as well as our unpublished data, we suspect all these species to be highly endangered. *Baetis tenerifensis* is known from three streams and probably disappeared from five others during the last century. Streams that still harbour that species are located either at high altitudes (1400 m asl) or in nature reserves at middle altitude. *Baetis canariensis* is currently known with confidence from only two streams at 800 – 1200 m asl, whereas in the 20th century it was recorded in eight watercourses. *Baetis palmensis* is only known from the Río Taburiente and its tributaries in the Caldera de Taburiente National Park (700 – 1100 m asl), and already disappeared from downstream the caldera where it was common in the 1970s. *Baetis gomerensis* was mainly found in two streams of the Parque National de Garajonay between 800 and 900 m asl, whereas in the 1970s the species was also recorded from five other streams at lower altitudes.

Overall, freshwater ecosystems on the Canary Islands are unique and under tremendous stress due to water ex-

tractions for domestic and agricultural use; tourism also greatly increases global water consumption. Pollution can have important impact especially at low elevations. These endangered species now only survive at high altitudes in protected areas, in particular the laurel forests that are a unique ecosystem in the west Mediterranean biome.

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***Baetis (Rhodobaetis) gomerensis* Gattolliat & Sartori, 2018:** http://zoobank.org/urn:lsid:zoobank.org:act:4033F770-7B3C-46A4-8F1C-F433E4423C8D

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File 1: gattolliat&al-canaryrhodobaetis-asp2018-electronicsupplement-1.pdf — **Fig. S1.** Molecular species delimitation of the European and North African representatives of the *Rhodobaetis* group (i.e., *Baetis pseudorhodani* s.l., *Baetis canariensis* s.l., and *Baetis rhodani* s.l.), using the Generalized Mixed Yule Coalescent (GMYC, FUJISAWA & BARRACLOUGH 2013), Poisson Tree Processes (PTP, ZHANG et al. 2013), and Automatic Barcode Gap Discovery (ABGD, PUILLANDRE et al. 2012). The phylogenetic tree shows the topology of the cox1 gene tree used for the GMYC method. Previously identified clusters of *Baetis rhodani* s.l. are indicated on the tree (i.e., I–VII from WILLIAM et al. 2006; G1–G11 from LUCENTINI et al. 2011). Terminal labels represent unique haplotypes.