

# The *Hydropsyche instabilis* group (Trichoptera: Hydropsychidae) on the Iberian Peninsula: evolutionary relationships, new species, taxonomical controversies, and a key to larvae

CARMEN ZAMORA-MUÑOZ<sup>\*,1</sup>, CESC MÚRRIA<sup>2</sup>, NÚRIA BONADA<sup>2</sup> & MARCOS A. GONZÁLEZ<sup>3</sup>

<sup>1</sup> Departamento de Zoología, Facultad de Ciencias, Universidad de Granada, C/Severo Ochoa s/n, 18071-Granada, España; Carmen Zamora-Muñoz [czamora@ugr.es] — <sup>2</sup> Grup de Recerca Freshwater Ecology and Management (FEM), Departament de Biologia Evolutiva, Ecologia i Ciències Ambientals, Facultat de Biologia, Institut de Recerca de la Biodiversitat (IRBio), Universitat de Barcelona (UB), Diagonal 643, 08028-Barcelona, Catalonia, Spain; Cesc Múrria [cmurria@ub.edu], Núria Bonada [bonada@ub.edu] — <sup>3</sup> Departamento de Zoología, Genética y Antropología Física, Facultad de Biología, Universidad de Santiago de Compostela, Campus Universitario Sur, 15782-Santiago de Compostela, España; Marcos A. González [marcos.gonzalez@usc.es] — \* Corresponding author

Accepted 09.ii.2017.

Published online at [www.senckenberg.de/arthropod-systematics](http://www.senckenberg.de/arthropod-systematics) on 5.iv.2017.

Editor in charge: Karl Kjer

## Abstract

The *Hydropsyche instabilis* group is a diverse Palearctic group composed of more than 30 species, many of them local endemics. The exact number of species in the Mediterranean Basin remains ambiguous, mainly due to the high variability of the genitalia and the lack of in-depth taxonomic studies in some areas. In this paper, we describe the adult male and the larva of a new species from the Iberian Peninsula (*Hydropsyche solerorum* sp.n.), and provide information about the ecological requirements of the larvae. We also discuss the identity of a species, provisionally designated as *Hydropsyche* cf. *spiritoi*. The morphology of the imago genitalia does not allow us to distinguish it from *H. spiritoi*, but the two forms show some larval morphological differences, molecular divergence at the mtDNA gene *cox1*, and, apparently, non-overlapping geographical distributions. In addition, we present a complete molecular phylogeny for the mtDNA gene *cox1* of the currently known Iberian species of the *instabilis* group to determine the evolutionary position of *H. solerorum*, *H. cf. spiritoi*, and the two other endemic species *H. ambigua* and *H. fontinalis*. Finally, we provide an update of the identification key for the known larvae of the genus *Hydropsyche* on the Iberian Peninsula.

## Key words

Caddisflies, distribution, diversity, Mediterranean streams, imago.

## 1. Introduction

The diverse *Hydropsyche instabilis* group comprises more than 30 described species distributed across the Western Palearctic (MALICKY 2004). Most of them are endemic, inhabit headwater habitats (GRAF et al. 2008; MÚRRIA et al. 2012), and present a strong genetic structure among

mountain ranges (MÚRRIA et al. 2013). However, the exact number of species remains ambiguous, mainly because of the morphological variability of the genitalia within some species and the lack of in-depth taxonomic studies in some areas of their geographical distribution (MALICKY 2001).

In Europe, the *instabilis* group is composed of a few species of wide distribution, and many others regionally restricted and limited to small areas along the Eastern (Greece, Cyclades and Dodecanese Islands, Crete, and Bulgaria) and Western Mediterranean Basin (Iberian Peninsula, France, Italy, Sicily, and Corsica; MALICKY 2001, 2004; SIPAHLER 2010).

Of the 21 *Hydropsyche* species recorded on the Iberian Peninsula (GONZÁLEZ & MARTÍNEZ 2011), seven belong to the *instabilis* group: *H. ambigua* and the species identified as *H. spiritoi* are distributed in northern Spain; *H. pictetorum* is distributed in the north and center of the Iberian Peninsula; *H. fontinalis* and *H. infernalis* are endemics of southern Spain; and *H. instabilis* and *H. silta-lai* are distributed throughout the Iberian Peninsula (BONADA et al. 2004, 2008; GONZÁLEZ & MARTÍNEZ-MENÉNDEZ 2008; GONZÁLEZ et al. 1992; ZAMORA-MUÑOZ et al. 1995). The phylogeny using mitochondrial and nuclear genes of 19 species of the genus *Hydropsyche* encountered in the Mediterranean region of the Iberian Peninsula and Morocco provided strong evidence that the members of the *instabilis* group form a single clade, including two undescribed, distinct genetic clusters, *Hydropsyche* sp. 1 and *Hydropsyche* sp. 2 (MÚRRIA et al. 2012).

In this paper, we describe the adult male and the larva of a new species, *Hydropsyche solerorum* sp.n. (*Hydropsyche* sp. 2 in MÚRRIA et al. 2012). In addition, we provide strong evidence for larval morphological differentiation and high genetic divergence of the species designated *Hydropsyche* cf. *spiritoi* (*Hydropsyche* sp. 1 in MÚRRIA et al. 2012), compared to the morphotype of *H. spiritoi* from Italy and a couple of populations located in La Provence (France). Adults of *Hydropsyche spiritoi* were recorded from Spain by GONZÁLEZ & MARTÍNEZ-MENÉNDEZ (2008) but larval characteristics of Iberian specimens needed a deeper study (BONADA et al. 2004). We also present an exhaustive phylogeny for the mtDNA gene *cox1* of the *instabilis* group, considering more species than those in MÚRRIA et al. (2012), for assessing the evolutionary position and the molecular distinctiveness of *H. solerorum*, *H. cf. spiritoi* and two species previously considered doubtful. MALICKY (2005) suggested that the endemic Iberian species *H. ambigua* and *H. fontinalis* could be synonyms of *H. tenuis* and *H. spiritoi*, respectively. Both the morphological distinctiveness and molecular differentiation will help resolve controversies surrounding the validity of these species. Finally, we use our results to update the identification key for the known larvae of the genus *Hydropsyche* on the Iberian Peninsula (ZAMORA-MUÑOZ et al. 1995).

## 2. Materials and methods

### 2.1. Specimen collection

The majority of species of the *instabilis* group used here were collected and sequenced by MÚRRIA et al. (2012, 2013). From these collections, we used specimens from 24 sites on the Iberian Peninsula and from three sites in Morocco (Electronic Supplement Table S1). Additionally, three specimens of *H. cf. spiritoi* from the north-eastern Iberian Peninsula, four specimens of *H. spiritoi* from Sicily (Italy) and five from La Provence (France) were collected and sequenced (Electronic Supplement Table S1). In order to resolve controversies about the validity of the endemic Iberian species *H. ambigua* (see MALICKY 2005), five specimens of *H. ambigua* from the Iberian Peninsula and one specimen of *H. tenuis* from France were collected and sequenced (Electronic Supplement Table S1). Finally, additional samplings of *H. solerorum* sp.n. from three sites and *H. cf. spiritoi* from two sites were collected in order to obtain a more complete understanding of the morphological variability of these species (Electronic Supplement Table S2).

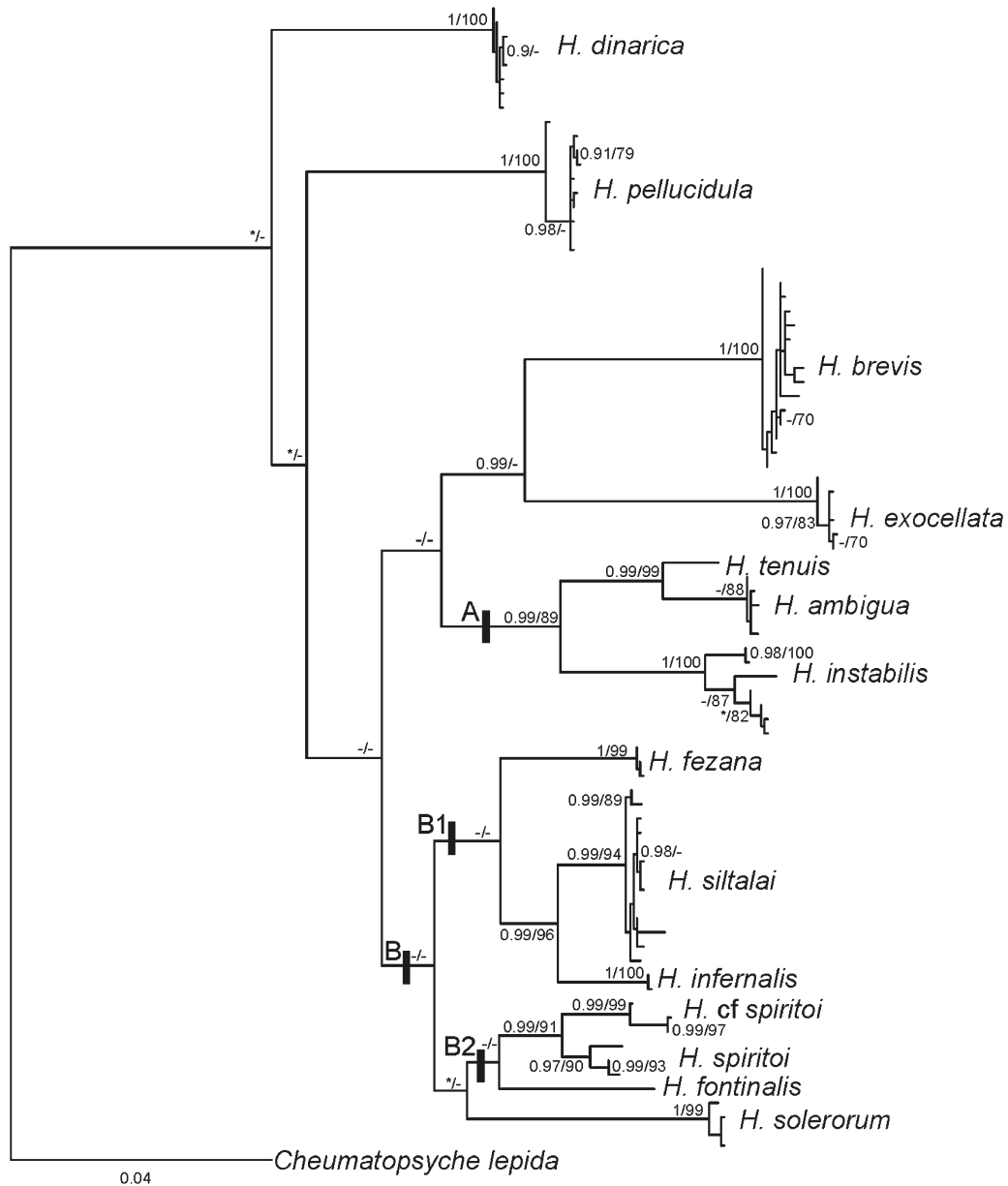
Larvae and pupae were obtained by sampling all aquatic habitats with a kick net of 250 mm mesh size. Adults were obtained by sweeping riparian vegetation with an entomological net or using a UV-light trap. Collected larvae, pupae, and adults were preserved in 96% ethanol for the molecular analyses.

Specimens and type material are deposited in the collections of Marcos González at the Department of Zoology, University of Santiago de Compostela and C. Zamora-Muñoz collection at the Department of Zoology, University of Granada (Spain; [http://cccn.ugr.es/colecciones/col\\_zamora.php](http://cccn.ugr.es/colecciones/col_zamora.php)), as indicated in the material examined.

### 2.2. Morphological study

Characterization of the larvae was based on the presence or absence of gills on abdominal segment VII and the pattern of the head and thoracic structures of the 5<sup>th</sup> instar larvae, such as the shape and the colouration pattern of the frontoclypeal apotome and posterior prosteronites, and the shape and size of submentum lateral parts (as in ZAMORA-MUÑOZ et al. 1995, 2002). Photographs of the larvae were taken using a stereo-microscope (Motic SMZ-168, with 10-50X) with a built-in camera (Moti-cam 2300).

In order to identify adults and pupae, genitalia were digested in a 10% KOH solution, placed in a glycerin solution or resin (DMHF), and observed under a stereoscope or microscope. Photographs of the adults were taken using a microscope Olympus CH-40 with a built-in camera (Canon Eos 70D) and Helicon Focus software



**Fig. 1.** Phylogenetic relationships among 10 species of *Hydropsyche* within the *instabilis* group (clades A and B), four species from two other *Hydropsyche* groups and outgroup taxa included in this study, based on the Maximum-likelihood analysis of unique haplotypes of mtDNA *cox1* gene. Branch support is indicated as follows: Bayesian inference posterior probability (> 0.95) / maximum likelihood bootstrap (> 70), – refers to clades that appeared in the analysis but with support values below levels indicated above, \* refers to groups contradicted by the analysis.

for combining 20–50 frames in one focussed image. The genital terminology used in this paper follows GONZÁLEZ & BOTOSANEANU (1985).

### 2.3. Molecular analyses

The mitochondrial DNA Cytochrome c Oxidase subunit 1 gene (*cox1*) was sequenced. DNA was extracted from the abdominal segments I–V using Promega WizardSV extraction plates. Partial DNA sequences of *cox1* (592 bp) were obtained following (MÚRRIA et al. 2010). All unique

*cox1* haplotypes were aligned by eye because no length differences and gaps were found. *Cheumatopsyche lepida* (Pictet) (GenBank accession number JQ687925) was included as an outgroup together with four species from two other *Hydropsyche* groups (GenBank accession numbers JQ687909, JQ687910, KF255617–KF255622, KF255639–KF255648, JQ687918, JQ687919, KF255604–KF255616, JQ687914, JQ687915, KF255623–KF255626).

Maximum likelihood searches were conducted with RAxML 7.0.4 (STAMATAKIS 2006) under the GTR +  $\Gamma$  nucleotide substitution models. The best trees were selected from 100 multiple inferences, and clade support was

assessed by means of 1000 nonparametric bootstrap resampling replicates of the original matrix. Bayesian inference was conducted using MrBayes 3.2.5 (RONQUIST & HUELSENBECK 2003). The best-fit partitioning schemes and models of molecular evolution for the three codon positions were selected using Partition Finder (LANFEAR et al. 2012) based on AIC (Akaike Information Criterion), and individual models of nucleotide substitution were specified for each codon partition. Two independent runs with four simultaneous Markov chain Monte Carlo (MCMC) chains (one cold and three heated), each with random starting trees, were carried out simultaneously, sampling 1000 generations until the standard deviation of the split frequencies of these two runs dropped below 0.01 (10 million generations). Tracer 1.4 (<http://evolve.zoo.ox.ac.uk/>) was used to ensure that the MCMC chains had reached stationarity by examining the effective sample size (ESS) values and to determine the correct number of generations to discard as burn-in. The two phylogenetic analyses were run remotely at the CIPRES Science Gateway (MILLER et al. 2010).

### 3. Results

#### 3.1. Molecular analysis

We provided nine new unique *cox1* haplotype sequences for the *instabilis* group (GenBank accession numbers KY656176, KY656184, KY656198, KY656201, KY656204, KY656207, KY678901–KY678903, Electronic Supplement Table S1). Maximum likelihood searches and Bayesian inference clustered all studied species belonging to the *instabilis* group into the two clades A and B, and therefore the *instabilis* group is not monophyletic in the *cox1* gene tree (Fig. 1). This split agrees with a taxonomic character used to classify larvae: presence (clade A) versus absence (clade B) of gills on the abdominal segment VII.

In clade A, the endemic species of the north of the Iberian Peninsula, *H. ambigua*, was closely related to *H. tenuis*, and both species were sister to *H. instabilis*. Across its wide geographical distribution on the Iberian Peninsula, the morphospecies *H. instabilis* showed high intraspecific structure. The most basal lineage of *H. instabilis* was distributed in the south of Spain, whereas the two other lineages were distributed across the north-east of the Iberian Peninsula (see site locations in Electronic Supplement Table S1; MÚRRIA et al. 2013).

The highly supported clade, formed by *H. siltalai* and *H. infernalis*, was sister to *H. fezana* in clade B1. Some specimens (larvae and adults), morphologically identified as *H. infernalis*, from the southeast of the Iberian Peninsula (KF255651–KF255654, HM134823 in Electronic Supplement Table S1), were clustered within

*H. siltalai*. This inconsistency was accompanied by high intraspecific morphological variability of *H. siltalai* along its geographical range.

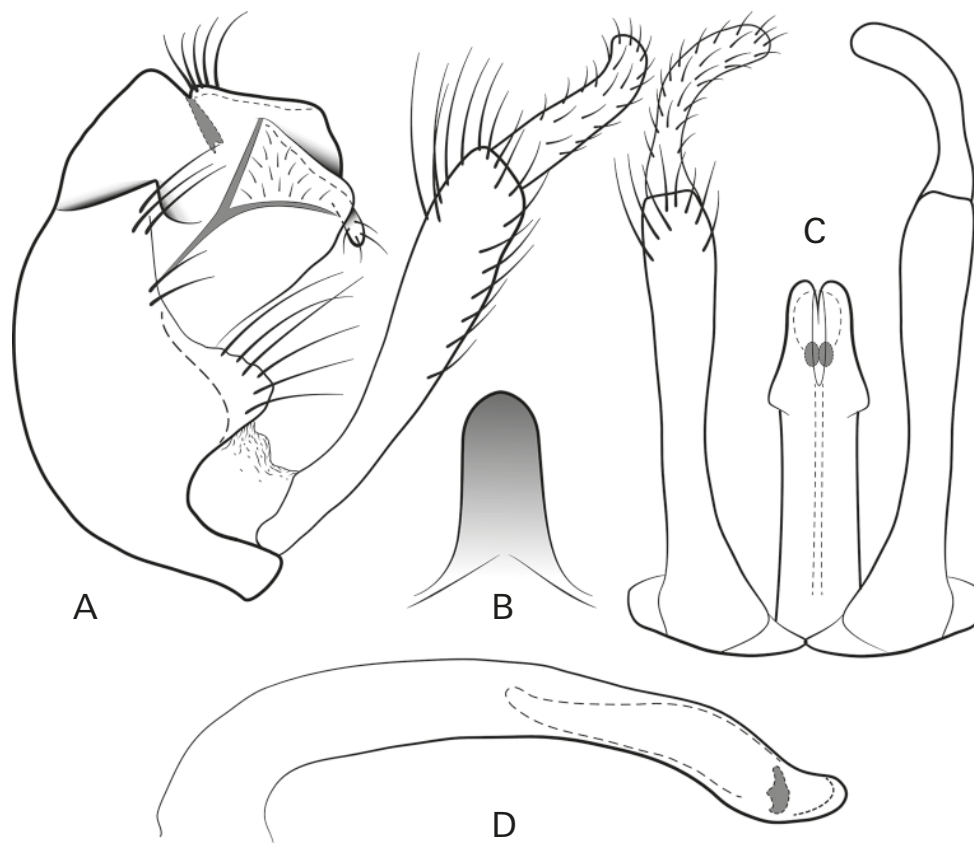
In clade B2, individuals of *H. cf. spiritoi* and *H. spiritoi* were located in two distinct *cox1* haplotype clusters, which were highly supported. Moreover, the specimens previously identified as *H. spiritoi* on the Iberian Peninsula (GONZÁLEZ & MARTÍNEZ-MENÉNDEZ 2008) clustered with the *H. cf. spiritoi* specimens collected in the north-east of the Iberian Peninsula (MÚRRIA et al. 2013; Electronic Supplement Table S1). *Hydropsyche cf. spiritoi* and *H. spiritoi* were closely related to *H. fontinalis*, which is endemic to the south of the Iberian Peninsula. The new species *H. solerorum* was highly supported, but its placement was in conflict between the two model-based phylogenetic analyses and received very low support, and not resolved using only the *cox1* gene.

#### 3.2. Species descriptions

##### 3.2.1. *Hydropsyche solerorum* Zamora-Muñoz & González sp.n.

**Imago description** (Fig. 2). Antennae, palps, and wings light brown. Length of male anterior wings 0.78–0.95 mm. **Male genitalia**: Dorsal depressions of segments IX and X both very well delimited and deep, but that of segment IX slightly greater than that of segment X (Fig. 2A). Dorsal keel of segment IX, in dorsal view (Fig. 2B), narrow and digitiform, slightly constricted at apex; in lateral view (Fig. 2A) almost straight (gently turned upward in some specimens). Dorsal folds (wings) of segment X (Fig. 2A) as long as dorsal keel, narrow and obliquely directed ventrally; digitiform appendages rather short and thin, in lateral view (Fig. 2A) almost straight and slightly curved inward. Harpago of inferior appendages short (ratio harpago/coxopodite 1 : 2.3), slender, almost equal in breadth, slightly and regularly incurved and rounded at apex (Fig. 2A,C); in lateral view, apex gently curved inward (Fig. 2A). Phallic apparatus, in lateral view (Fig. 2D), slightly curved in proximal part, straight in middle part with markedly sinuous dorsal margin, moderately recurved downwards and preapically sinuous; seen from below (Fig. 2C), apical part short, broadly triangular, narrowing towards tip; lateroapical projections large but slightly rounded.

**5<sup>th</sup> instar larva description** (Fig. 3). **Head**: In dorsal view (Fig. 3A–C) rectangular, slightly longer (1.15–1.60 mm, mean = 1.35, n = 28, S.E. = 0.02) than wide (1.06–1.50 mm, mean = 1.27, n = 28, S.E. = 0.02). Cephalic capsule brown in colour, lighter in specimens from Pereilas River (Fig. 3A) and darker in specimens from Caballos and Horcajos Rivers (Fig. 3B). Frontoclypeal apotome wide, pentagonal, with a slight indentation at about the level of the lateral light spots (above the epistomal sulcus). Anterior margin of frontoclypeus straight, posterior tip slightly



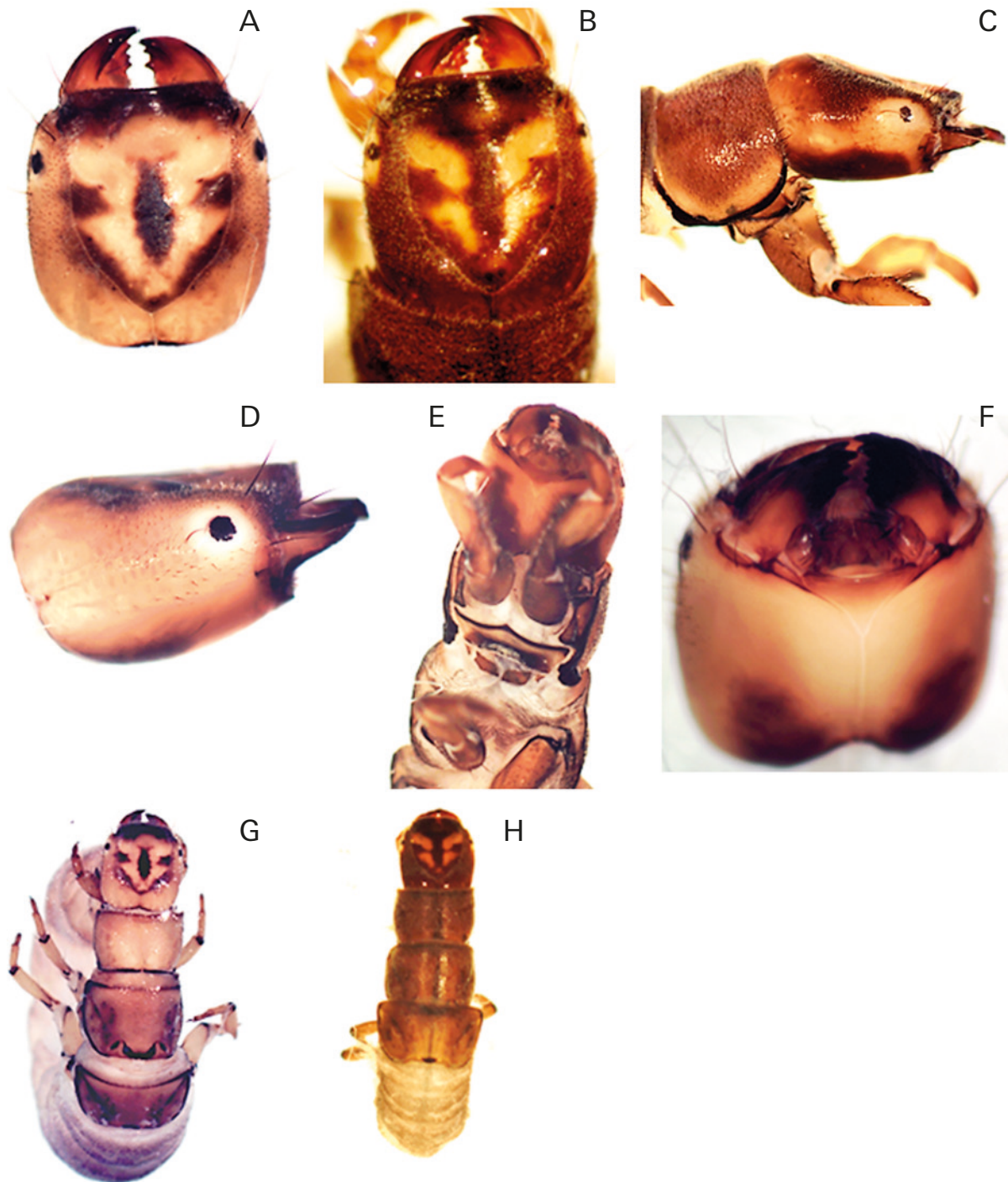
**Fig. 2.** *Hydropsyche solerorum* imago, male genitalia: **A:** lateral; **B:** dorsal keel of segment IX, dorsal; **C:** inferior appendages and phallus, ventral; **D:** phallus, lateral.

pointed. Frontoclypeal anterior light spot variable: absent to large, then fused with median spots (Fig. 3A,B). Lateral light spots above epistomal sulcus elongate and oriented obliquely. Posterior light spot (formed by the light lateral spots under epistomal sulcus and the aboral light spot) V-shaped. Lateral and posterior spots clearly joined in lighter specimens (Fig. 3A) and nearly in darker ones (Fig. 3B). Dorsally, dark area of head extending lateroventrally until middle part leaving a light portion (Fig. 3B,C). In lighter specimens dark area reduced to edges of frontoclypeus (Fig. 3A,D). Ventral area of head variably patterned and shaped (Fig. 3E,F). In highly pigmented individuals the darker area extending on stridulation lines but never reaching anterior head margin or middle suture (Fig. 3E). Submentum with lateral lobules of intermediate size, shorter and wider than long and narrow (Fig. 3F); a : b ratio about 0.16–0.25 (mean = 0.21, n = 27, S.E. = 0.004). **Thorax:** Coloration of pronotum lighter than that of meso- and metanotum (Fig. 3G), except in very highly pigmented specimens (Fig. 3H). Median posterior prosternites dark-brown and irregularly oblong or elongated; clearly visible lateral ones, similar in colour and separated from median region (Fig. 3E). **Abdomen:** Abdominal gills absent on segment VII.

**Differential diagnosis and remarks.** Adults of the new species are similar to *H. fontinalis*, *H. fezana* and, especially, to *H. klefbecki*. The first two species are endemic

in southern Spain and North Africa, respectively, and the third is endemic in the Italian Central-S-Apennines from Umbria to Calabria, and of Sicily (CIANFICONI & CORALINI 2010). Differences between *H. fezana* and *H. fontinalis* and the new species are obvious, especially when comparing the morphology of the distal part of the phallus, both in lateral and ventral view (see MALICKY 2004): it is more markedly sinuous in lateral view and broadly triangular and narrowing towards the tip in the new species.

The genitalia of the new species is very similar to that of *H. klefbecki*. We have carefully compared our specimens with the available figures of male genitalia of *H. klefbecki* (see TJEDER 1946: fig. 1; BOTOSANEANU & MARINKOVIC 1966: fig. 12; MALICKY 2004: p. 138) and directly with some males from Italy (Apennines and Sicily, leg. De Pietro). The most obvious characteristic to distinguish the adults of these two species are: the dorsal keel, in dorsal view, is much broader (almost twice) in *H. klefbecki*; the dorsal folds (wings) of the X segment, in lateral view, are sinuate, forming rounded lobes in *H. klefbecki* (smaller and obliquely directed ventrally in the new species); in lateral view, the digitiform appendages of *H. klefbecki* are longer and obliquely directed upward. The harpago of the inferior appendages is slenderer and it is regularly incurved in the new species, not dilated and abruptly incurved at the apex as in *H. klefbecki*. The phallic apparatus is not so strongly curved at the base in



**Fig. 3.** *Hydropsyche solerorum* larva: **A,B:** cephalic capsule, dorsal; **C:** cephalic capsule and prothorax, lateral; **D:** cephalic capsule, lateral; **E:** cephalic capsule, ventral & posterior prothorax; **F:** cephalic capsule, ventral; **G,H:** head and thorax, dorsal.

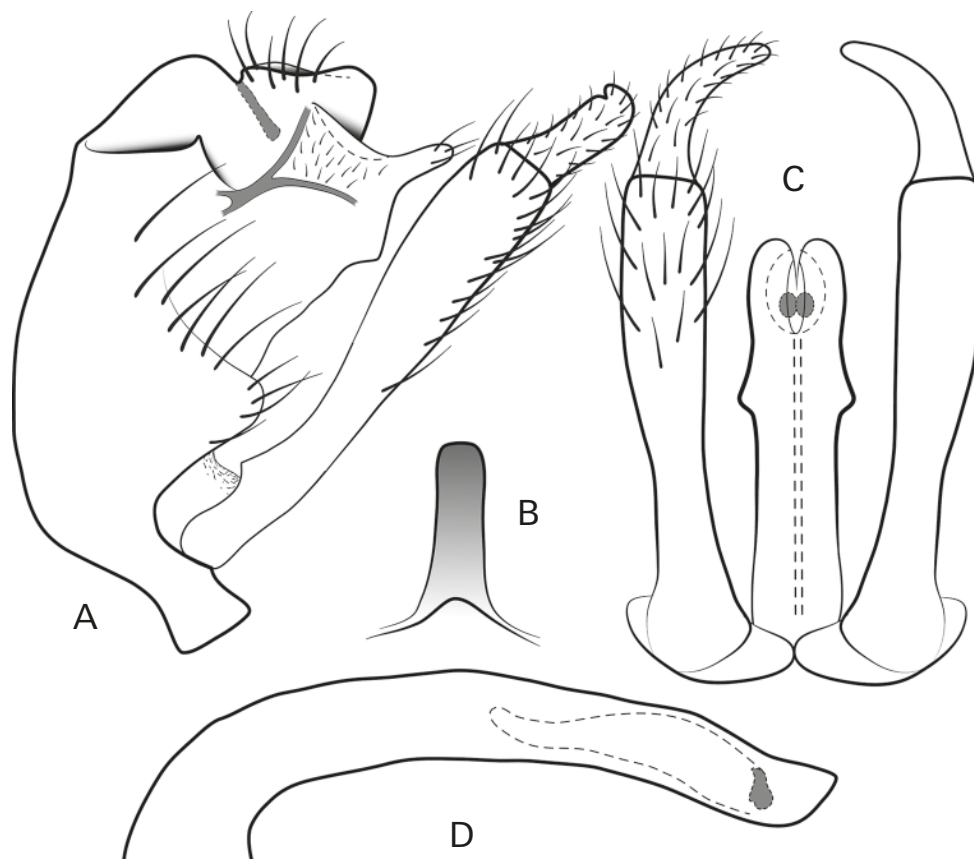
the new species as in *H. klefbecki*; in both species the apical part, in ventral view, is short, broadly triangular and narrowing towards the tip, whereas in lateral view, the apical part is much more sharply bent downward before the apex in *H. klefbecki*. Furthermore, the males of the new species are apparently slightly smaller than *H. klefbecki*: the length of their anterior wings is 0.8–0.9 mm and 10–11 mm, respectively.

In addition, it should be noted that the larvae of these two species are also clearly distinguished; they belong to

two different groups characterized by the presence (*H. klefbecki*, see DE PIETRO 1999) or absence (*H. solerorum* sp.n., see above) of abdominal gills on the segment VII.

**Etymology.** The species is dedicated to Juan, Carmen and Cristina Soler, husband and daughters of the first author, with thanks for frequent help in caddisfly samplings.

**Larval habitat and ecological requirements.** This species was mainly distributed in the southern part of the



**Fig. 4.** *Hydropsyche* cf. *spiritoi* imago, male genitalia: **A:** lateral; **B:** dorsal keel of segment IX, dorsal; **C:** phallus and inferior appendages, ventral; **D:** phallus, lateral.

Iberian Peninsula. Specimens were found in permanent headwaters of low-altitude streams (altitude range 200–400 m a.s.l.). Some sites drained over peridotites, whereas others drained over calcareous geology. All sites had very good ecological status and well-developed riparian vegetation.

**Material examined. Adults:** HOLOTYPE, ♂, Pereilas River (Coín, Málaga, SPAIN), 7-viii-2013, leg. O. Gavira. PARATYPES: 4♂, 12,13-v-2013; 2♂, 17-vi-2013; 2♂, 7,8-vii-2013; 1♂, 11-viii-2013, all leg. O. Gavira, from the same locality as the holotype. Caballos River (Tolox, Málaga, SPAIN), 2♂, 4-viii-2007, leg. C. Zamora-Muñoz. **Larvae:** PARATYPES: 2 4<sup>th</sup> instar larvae and 6 5<sup>th</sup> instar larvae, Caballos River (Tolox, Málaga, SPAIN), 21-v-2003, 6-viii-2003, 19-xi-2003, leg. CEDEX; 03-v-2011, leg. O. Gavira; 08-viii-2012, leg. N. Bonada; 9 5<sup>th</sup> instar larvae, Horcajos River (Tolox, Málaga, SPAIN), 03-v-2011, leg. O. Gavira et al.; 2 5<sup>th</sup> instar larvae, Pereilas River (Coín, Málaga, SPAIN), 18-iv-2011, leg. O. Gavira et al.; 5 4<sup>th</sup> instar larvae and 11 5<sup>th</sup> instar larvae, Corcho River (Coín, Málaga, SPAIN), 18-iv-2011, leg. O. Gavira et al.

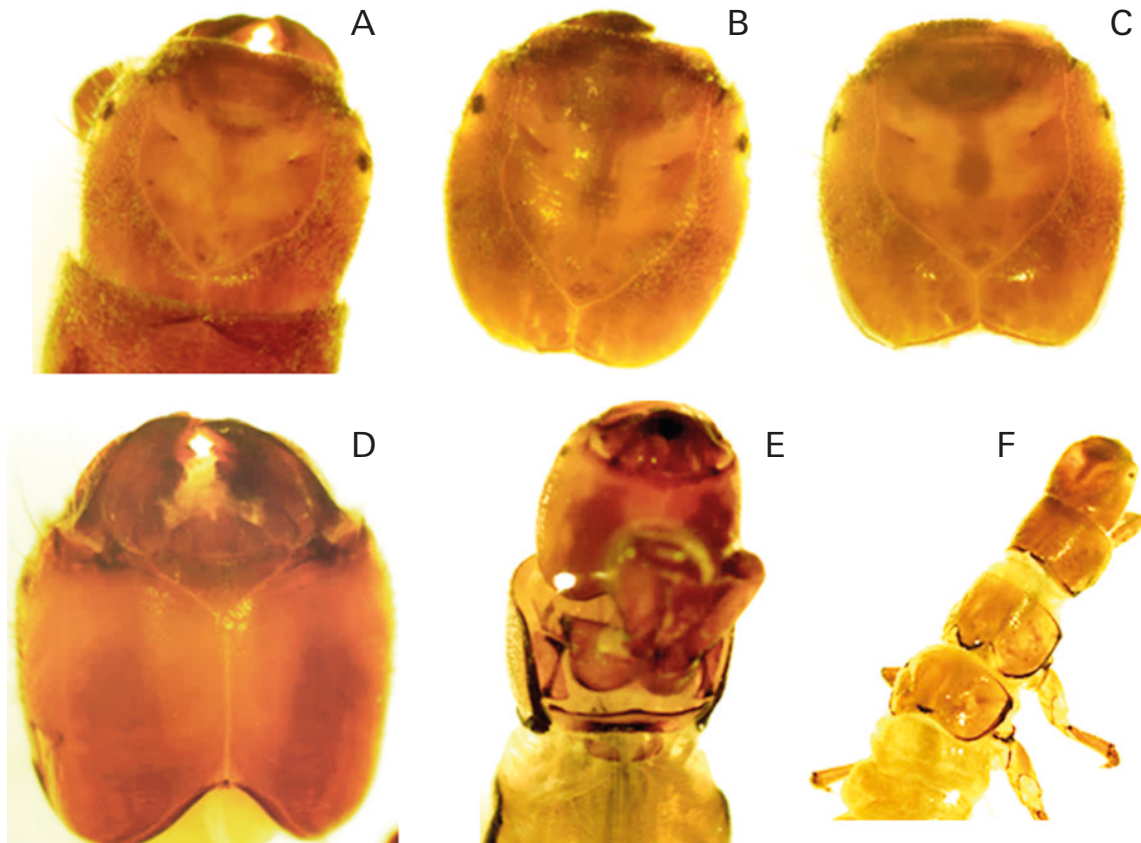
Formal arrangements have been made for the eventual deposit of types. The holotype (ref. number: 122) and 41 paratypes (6 imagines, ref. numbers: 123–128; 35 larvae, ref. numbers: 129–136) are deposited in the C. Zamora-Muñoz collection. Five paratypes are deposited in the M.A. González collection.

### 3.2.2. *Hydropsyche* cf. *spiritoi*

**Imago description** (Fig. 4). Antennae, palps, legs and wings pale brown; veins of the wings darker. Length

of male anterior wings 9.0–10.5 mm. **Male genitalia:** Dorsal depressions of segments IX and X both well delimited and deep; that of segment X slightly shorter but deeper than of segment IX (Fig. 4A). Dorsal keel of segment IX, in dorsal view (Fig. 4B), very narrow and digitiform; in lateral view, directed dorsally (Fig. 4A). In lateral view (Fig. 4A), dorsal folds (wings) of segment X sinuate, forming rounded lobes at apical portion. Digitiform appendages moderately long, slightly directed upward. Inferior appendages with ratio hapago/coxopodite 1:2.7; harpago rather broad and long; coxopodite very short and thin, becoming narrower on distal portion (Fig. 4A,C). Phallic apparatus, in lateral view (Fig. 4D), slightly curved in its proximal part, moderately and regularly recurved downwards to apex and slightly sinuous preapically. In ventral view (Fig. 4C), apical part long and gently rounded at tips; lateroapical projections large and triangular.

**5<sup>th</sup> instar larva description** (Fig. 5). **Head:** In dorsal view (Fig. 5A–C) rectangular, slightly longer (1.57–1.73 mm, mean=1.64, n=9, S.E.=0.02) than wide (1.47–1.66 mm, mean=1.56, n=9, S.E.=0.02). Cephalic capsule brown in colour, often darker than meso- and metathorax. Frontoclypeal apotome wide, pentagonal, with anterior margin straight, posterior tip pointed or slightly rounded, very conspicuous epistomal sulcus (Fig. 5A,B). In very dark specimens, light spots



**Fig. 5.** *Hydropsyche* cf. *spiritoi* larva: **A,B,C**: cephalic capsule, dorsal; **D**: cephalic capsule, ventral; **E**: cephalic capsule, ventral & posterior prosternites; **F**: head and thorax, dorsal.

of apotome barely distinguished (Fig. 5C). Frontoclypeal anterior light spot often absent, when present never joined to lateral spots (Fig. 5A–C). Posterior light spot (formed by the light lateral spots under the epistomal sulcus and the aboral light spot) V-shaped, although aboral spot is often blurred (Fig. 5A,B). Lateral light spots (above the epistomal sulcus) and posterior light spot always joined. With a conspicuous dark Y or T-shaped patch in central area of apotome (Fig. 5B,C,F). In ventral view (Fig. 5D,E), darker area extends on stridulation lines but never reaching anterior head margin or middle suture. Submentum with long and narrow lateral lobes (Fig. 5D); a:b ratio about 0.16–0.21 (mean=0.18, n=10, S.E.=0.005). **Thorax:** Colouration of pronotum darker than that of meso- and metanotum, often as dark as cephalic capsule (Fig. 5F). Median posterior prosternites dark-brown and irregularly oblong or elongated; clearly visible lateral ones, lighter in colour and separated from median region (Fig. 5E). **Abdomen:** Abdominal gills absent on segment VII.

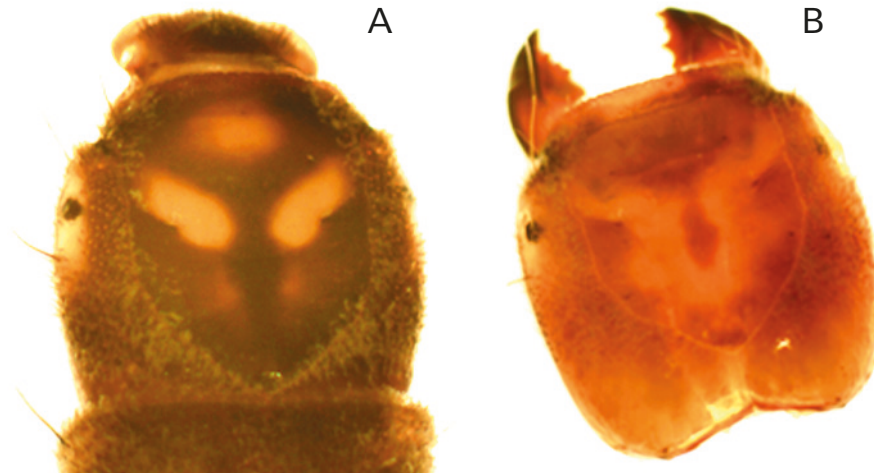
**Differential diagnosis and remarks.** Some Spanish specimens from Aragón were identified as *H. spiritoi* by GONZÁLEZ & MARTÍNEZ (2008). In fact, the male genitalia of these specimens is apparently identical to that of the Italian and French specimens of this species (see MORETTI 1991: fig. V; COPPA et al. 2016). However, the molecular analyses of new specimens (adults and larvae)

collected in northern and northeastern Spain (La Rioja and Barcelona) show the possibility that they belong to a different and new species. Nevertheless, in this paper (see discussion) we have acted cautiously, maintaining for the Spanish populations the provisional designation of *H. cf. spiritoi*.

In the Iberian context, adults of *H. cf. spiritoi* are morphologically similar to those of *H. fontinalis*. Differences between *H. spiritoi* (and as consequence *H. cf. spiritoi*) and *H. fontinalis* have been well discussed and figured by ZAMORA-MUÑOZ et al. (2002): the apex of the phallus is more elongated in *H. spiritoi*, and the phallic teeth, in ventral view, are slightly less prominent in *H. fontinalis*. However, the molecular analysis showed that both species belong to different, but closely related clades in clade B2 (Fig. 1).

**Larval habitat and ecological requirements.** This species was mainly distributed along the east coast of the Iberian Peninsula (*Hydropsyche* gr. *instabilis* in BONADA et al. 2004, 2008). Specimens were found in headwaters and midstream reaches (altitude range 400–1300 m a.s.l.) of calcareous and sedimentary basins (conductivity around 0.001 S/cm, pH > 8), with very good to good ecological status and well-developed riparian vegetation (BONADA et al. 2004). Some of the sites where larvae and adults were collected dried out in summer or maintained very low discharge.





**Fig. 6.** *Hydropsyche spiritoi* larva: **A,B:** cephalic capsule, dorsal, of two larvae from La Provence (France).

**Material examined. Adults:** 4♂, Mora de Rubielos (Teruel, SPAIN), 04-viii-1978, leg. V. Monserrat; 3♂, Bailo (Huesca, SPAIN), 11-vi-1983, leg. V. Monserrat; 1♂, Monasterio de Piedra (Zaragoza, SPAIN), 06-v-1989, leg. González; 1♂, Alinyà River (Alinyà, Lleida, SPAIN), muestra 75, vii-2000, leg. N. Bonada; 12♂, Marganell River (Marganell, Barcelona, SPAIN), 21-viii-2009, leg. N. Bonada; 2♂, Jalón de Cameros (La Rioja, SPAIN), 07-vii-2015, leg. M. González. Specimens are deposited in the M.A. González collection. **Pupae:** 1♂, Pontons River (Pontons, Barcelona, SPAIN), 09-viii-2001; 1♂, Coaner River (Coaner, Barcelona, SPAIN), 14-viii-2001, all leg. N. Bonada. Specimens are deposited in the M.A. González collection. **Larvae:** 1 5<sup>th</sup> instar larva, Gaià River (Gaià, Barcelona, SPAIN), 01-viii-1996; 1 5<sup>th</sup> instar larva, Sant Cugat River (Sant Cugat del Racó, Barcelona, SPAIN), 07-viii-1996; 1 5<sup>th</sup> instar larva, Can Relat River (Sentforas, Barcelona, SPAIN), viii-1996; 2 5<sup>th</sup> instar larvae, Pontons River (Pontons, Barcelona, SPAIN), viii-1996; 1 5<sup>th</sup> instar larva, Cornet River (Sant Salvador de Guardiola, Barcelona, SPAIN), 08-viii-1996; 1 5<sup>th</sup> instar larva, Vilanova River (Avinyó, Barcelona, SPAIN) 09-viii-1996, all leg. M. Rieradevall; 1 5<sup>th</sup> instar larva, Pontons River (Pontons, Barcelona, SPAIN), 25-vii-2000; 1 5<sup>th</sup> instar larva, Coaner River (Coaner, Barcelona, SPAIN), 27-vii-1999; 1 5<sup>th</sup> instar larva, L'Albareda River (Fontrubí, Barcelona, SPAIN) 24-vii-2001, all leg. N. Bonada. Specimens are deposited in the C. Zamora-Muñoz collection.

#### 4. Discussion

Our results provide morphological and genetic evidence for the existence of one new species of *Hydropsyche* of the *instabilis* group. Moreover, we present a complete phylogeny for the *cox1* gene of the currently recognized Iberian species of this group, which clarifies the evolutionary relationship among them and resolves controversies about the validity of two previously described species (*H. fontinalis* and *H. ambigua*). Our phylogenetic tree is highly concordant with previous taxonomic classifications based on male genitalia (MALICKY 2004) and a molecular phylogenetic tree for *Hydropsyche* species from France (STATZNER et al. 2010).

Male adults of *H. solerorum* can be easily distinguished from the other Iberian species of the *instabilis*

group by the aspect, in ventral view, of the distal part of the phallus, which is short, broadly triangular and narrowing towards the tip. The larva of *H. solerorum* has a slightly triangular frontoclypeal apotome and this characteristic differentiates its larva from those species with clear wide and pentagonal apotomes (Table 1). Although the larva of *H. spiritoi* has a slightly elongated apotome, the lateral margins are parallel (DE PIETRO 1999; Table 1 and Fig. 6), while in *H. solerorum* the anterior third is always wider than the posterior third, with a slight indentation at about the level of the lateral light spots. Furthermore, the larva of *H. solerorum* and *H. fontinalis* can be distinguished by the presence of an aboral light spot in the apotome of the former species, and lateral and aboral light spots may often be joined (Table 1).

Although male adults of *H. cf. spiritoi* and *H. spiritoi* cannot be morphologically distinguished and were thought to be the same morphospecies (GONZÁLEZ & MARTÍNEZ-MENÉNDEZ 2008), they form two distinct *cox1* haplotype clusters that correspond to fixed morphological character differences in the larvae. Larvae of *H. cf. spiritoi* often lack an oral light spot, and the aboral and lateral light spots are indistinct. Moreover, the lateral light spots under the epistomal sulcus extend to the posterior vertex, following the lower arm of the Y- or T-shaped central patch. In contrast, *H. spiritoi* presents an aboral light spot that is often joined to lateral spots under the epistomal sulcus (DE PIETRO 1999; Table 1), except in very dark specimens (DE PIETRO 1999: fig. 10B; Fig. 6A). It is worth mentioning that some French specimens of this species not only lack an oral light spot, but also have a wide pentagonal frontoclypeal apotome (Fig. 6B). Notably, the known distributional ranges of the two forms do not overlap, i.e., *H. spiritoi* in Sicily and the Italian mainland (MALICKY 2013) and La Provence in France (COPPA et al. 2016), and *H. cf. spiritoi* in northeastern and eastern Spain (BONADA et al. 2004; GONZÁLEZ & MARTÍNEZ-MENÉNDEZ 2008; present results). These results suggest that these forms are geographically isolated representatives of the same lineage that can have cryptic species (i.e., species classified as a single nominal species, mostly due to seemingly identical genitalia morphology, BICKFORD et al. 2007), which

**Table 1.** Diagnostic features of the European *Hydropsyche instabilis* group lacking gills on the abdominal segment VII, according to ZAMORA-MUÑOZ et al. (1995, 2002), DE PIETRO (1999), KARAOUZAS (2009) and this paper.

Species	Shape of fronto-clypeal apotome	Oral light spot	Aboral light spot	Oral and lateral light spots	Lateral and aboral light spots	Submentum
<i>H. infernalis</i>	Wide/pentagonal, posterior tip slightly pointed	Often present	Present	Often joined	Often joined (V-shaped)	Short, wide lateral lobes
<i>H. fontinalis</i>	Nearly triangular, posterior tip pointed	Variable	Absent	Never joined	Never joined	Long, narrow lateral lobes
<i>H. peristerica</i>	Wide/pentagonal, posterior tip rounded	Present	Present	Often joined	Always joined (V-shaped)	Long, elongated lateral lobes
<i>H. siltalai</i> (morph-1)	Wide/pentagonal, posterior tip rounded	Present	Present	Often joined	Never joined	Short, wide lateral lobes
<i>H. siltalai</i> (morph-2)	Wide/pentagonal, posterior tip slightly pointed	Present	Present	Often joined	Often joined (V-shaped)	Short, wide lateral lobes
<i>H. cf. spiritoi</i>	Wide/pentagonal, posterior tip pointed	Often absent	Blurred	Never joined	Joined (V-shaped)	Long, narrow lateral lobes
<i>H. spiritoi</i>	Slightly elongated, with parallel lateral margins, posterior tip pointed	Variable	Present/blurred	Often joined	Often joined (V-shaped)	Short, wide lateral lobes
<i>H. solerorum</i>	Slightly triangular, posterior tip slightly pointed	Variable	Present	Often joined	Often joined (V-shaped)	Short, wide lateral lobes

is not rare in caddisflies and the discovery of which has increased in recent years, facilitated by the use of molecular techniques (e.g., PAULS et al. 2010; PREVIŠIĆ et al. 2014). In order to assert whether it should be considered a new species, additional morphological characters, DNA sequences of non-mitochondrial genes and geographical analyses, especially at edge distribution of the two forms on both sides of the Pyrenees, are needed.

Regarding the validity of *H. fontinalis*, this species was considered doubtful by MALICKY (2005) based on morphological similarities of male genitalia to *H. spiritoi*. However, morphological and genetic differences reported here, in ZAMORA-MUÑOZ et al. (2002) and MÚRRIA et al. (2012), resolve any doubt about the validity of *H. fontinalis*, as was advanced by GONZÁLEZ & MARTÍNEZ-MENÉNDEZ (2008).

In relation to the identity of the Iberian *H. ambigua*, MALICKY (2005) pointed out the possibility of it being a synonym of *H. tenuis*. However, a close comparative analysis of the descriptions and figures of male genitalia of both species (*H. ambigua*: see GONZÁLEZ & BOTOSANEANU 1985: figs. 9–16; *H. tenuis*: see TOBIAS 1972: figs. 52–57; BOTOSANEANU & SCHMID 1973: fig. 3 and NEU & TOBIAS 2004: fig. 47) shows that both species clearly differ in the shape of the carina of the IX segment (larger in *H. tenuis*), the digitiform appendages (smaller in *H. tenuis*) and, especially, in the details of the shape of the apex of the phallus in ventral view (it is more elongate and the phallic teeth are rounded and less prominent in *H. tenuis*). Occasionally larvae of *H. ambigua* show a variable colouration pattern with light spots of the apotome quite similar to those of *H. tenuis* (NEU & TOBIAS 2003) and in the same locality (e.g. Nansa in Electronic Supplement Table S1) we have found larvae with head patterns corresponding to both species (HapC19 to *H. ambigua* and HapC42 to *H. tenuis*). Moreover, *H. ambigua* is limited to the north of the Iberian Peninsula, whereas *H. tenuis* is located in central Europe, France, and Italy (MALICKY

2013; C. Múrria, unpublished data). Finally, *cox1* haplotypes of these two species were genetically distinct in terms of pairwise distances. Overall, we provided several morphological and molecular evidences for the validity of these species.

Molecular analyses of intraspecific variability of *cox1* gene revealed that some specimens morphologically identified as *H. infernalis* were clustered together within the clade of *H. siltalai*. This result may indicate some degree of hybridization or introgression between the two forms, resulting in the non-monophyletic mitochondrial lineages but also more morphological variability. Since only a single molecular marker was sequenced, we cannot be confident that *H. infernalis* and *H. siltalai* are not hybridizing. *Hydropsyche siltalai* is a widespread European species that is scarcely distributed in southern Spain (MÚRRIA et al. 2013), where the closely phylogenetically related species, *H. infernalis*, is predominant (BONADA et al. 2004; GONZÁLEZ et al. 1992; ZAMORA-MUÑOZ et al. 1995). Characteristics of the male genitalia of these specimens fit well within those of *H. infernalis*, and adult males have an intermediate size between *H. infernalis* and *H. siltalai*. Concerning the morphological characters of larvae, the spot pattern of the apotome has been traditionally used to distinguish the full-grown larvae of both species in areas where they are not known to coexist (BONADA et al. 2004; ZAMORA-MUÑOZ et al. 1995, 2002): in *H. siltalai* it is U-shaped (morph-1, Table 1), whereas in *H. infernalis* it is V-shaped. However, this pattern is highly variable on the Iberian Peninsula. As occurs in northwestern Spain (VIEIRA-LANERO 2000), the light aboral spot of the frontoclypeal apotome in the specimens from the southeastern part of the Iberian Peninsula, phylogenetically close to *H. siltalai*, is more frequently V- than U- shaped. Thus, *H. siltalai* larvae with the V-shaped morph (morph-2, Table 1) cannot be distinguished from other Iberian species with this characteristic, as *H. infernalis* and *H. cf. spiritoi*. Based on these results, special

care should be taken with the morphological recognition and identification of these two species on the Iberian Peninsula, not only with regard to larval characteristics but also with regard to male genitalia, especially when they occur sympatrically in some localities.

Lastly, although the high intraspecific genetic variability of *H. instabilis* throughout the Iberian Peninsula, the morphological characteristics of larvae are undifferentiated between populations. Sequenced specimens were mainly early-instar larvae with diagnostic larval characteristics not fully developed. Therefore, genetic differentiation is more strongly related to population dynamics that resulted in intraspecific genetic structure than speciation events or morphological differentiation.

The description of the larvae of *H. cf. spiritoi* and *H. solerorum* enlarges the group of Palearctic species lacking gills on the abdominal segment VII. Up to now, this subgroup of the *H. instabilis* group included six European species: *H. fontinalis*, *H. fumata*, *H. infernalis*, *H. peristerica*, *H. siltalai*, and *H. spiritoi*, and three North African species: *H. fezana*, *H. morla*, and *H. obscura* (ALLAYA 2003; DAKKI & TACHET 1987; KARAOUZAS 2009; MALICKY & LOUNACI 1987; DE PIETRO 1999; ZAMORA-MUÑOZ et al. 1995, 2002). There is no information available about diagnostic larval features of *H. fumata*. Below we provide an updated identification key for full-grown larvae of Iberian *Hydropsyche*, which incorporates these species and the newly observed morphological variability in the larvae of previously described species (BOURNAUD et al. 1982; PITSCH 1993; ZAMORA-MUÑOZ et al. 1995; VIEIRA-LANERO 2000; VIEIRA-LANERO et al. 2001; ZAMORA-MUÑOZ et al. 2002; MÚRRIA et al. 2010).

## 5. Updated key for full-grown larvae of Iberian Peninsula

- 1 Head with a deep transverse depression at the frontoclypeal apotome and with a careen at eye level (ZAMORA-MUÑOZ et al. 1995: fig. 11c). Maximum width of apotome at eye level (ZAMORA-MUÑOZ et al. 1995: fig. 11a,b) ..... ***H. tibialis* McLachlan**
- 1' Without these characteristics ..... 2
- 2 Gills absent on abdominal segment VII ..... 3
- 2' Gills present on abdominal segment VII ..... 7
- 3 Apotome with a light aboral spot ..... 4
- 3' Apotome with a blurred light aboral spot or without it ..... 5
- 4 Apotome with a light aboral V- or Y-shaped spot. Oral and aboral light spots of the apotome joined or almost so (ZAMORA-MUÑOZ et al. 1995: fig. 4a,c; Fig. 3A,B) ..... 6
- 4' Apotome with a light aboral U-shaped spot. Oral and aboral light spots of the apotome never joined (ZAMORA-MUÑOZ et al. 1995: fig. 10) ..... ***H. siltalai* Döhler** (morph-1)
- 5 Apotome with a blurred light aboral spot (Fig. 5A–C). Frontoclypeal apotome wide, pentagonal, with the posterior tip pointed or slightly rounded (Figs. 5A–C, 6A) ..... ***H. cf. spiritoi***
- 5' Apotome without a light aboral spot (ZAMORA-MUÑOZ et al. 2002: fig. 12). Frontoclypeal apotome narrowed posteriorly, nearly triangular, with the posterior tip very pointed (ZAMORA-MUÑOZ et al. 2002: figs. 11, 12) ..... ***H. fontinalis* Zamora-Muñoz & González**
- 6 Apotome nearly triangular, with anterior third wider than posterior third (Fig. 3A,B) ..... ***H. solerorum* sp.n.**
- 6' Apotome wide, with parallel lateral margins (ZAMORA-MUÑOZ et al. 1995: fig. 4a,c; DE PIETRO 1999: fig. 10; Fig. 6B) ..... ***H. infernalis* Schmid** and ***H. siltalai*** (morph-2)
- 7 Anterior edge of the apotome convex ..... 8
- 7' Anterior edge of the apotome straight or slightly concave ..... 11
- 8 Posterior prosternites indistinct and uniformly pale ..... 9
- 8' Posterior prosternites clearly visible (lightly pigmented but the medial regions darker than the lateral ones) ..... 10
- 9 Apotome with two light patches more or less developed (oral and aboral) and separated by a dark spot never situated on the cibarian muscles (ZAMORA-MUÑOZ et al. 1995: fig. 8a) ..... ***H. contubernalis* McLachlan**
- 9' Apotome with a light continuous patch or interrupted by a dark transversal spot on the insertion of the cibarian muscles (ZAMORA-MUÑOZ et al. 1995: fig. 5a–c) ..... ***H. teruela* Malicky**
- 10 Light spots (oral, aboral and laterals) of the apotome completely or almost joined, with a dark T-shaped figure in the centre. Lateral sclerites of head, in dorsal view, with a brownish area of granular appearance (ZAMORA-MUÑOZ et al. 1995: fig. 12) ..... ***H. exocellata* Dufour**
- 10' Light and dark spots of the apotome often indistinct and variable, but always in centre of apotome, a longitudinal dark spot between two light ones. No brownish area of granular appearance on the head (BOURNAUD et al. 1982: fig. 11 as *H. dissimulata*) ..... ***H. modesta* Navás**
- 11 Pronotum and mesonotum with a dark longitudinal band in the centre; on the pronotum this band usually joins with a transversal band on the anterior edge ..... 12
- 11' Longitudinal bands absent on the pronotum and mesonotum, although in rare cases present only on the pronotum, never joining with a transversal band on the anterior edge on the pronotum ..... 14
- 12 Apotome almost entirely light in colour, only edges and pretentorinae dark (ZAMORA-MUÑOZ et al. 1995: fig. 3b) ..... ***H. brevis* Mosely**
- 12' Apotome dark with only some distinctly lighter spots ..... 13

- 13 Submentum with short, wide lateral lobes. Apotome with three separate aboral round spots or, elongated and fused to form a V figure (ZAMORA-MUÑOZ et al. 1995: figs. 3a,c,d) ..... *H. brevis* Mosely
- 13' Submentum with relatively long, narrow lobes. Apotome with a light aboral spot and two indistinct lateral ones (ZAMORA-MUÑOZ et al. 1995: fig. 13) ..... *H. lobata* McLachlan
- 14 Apotome with a light U-shaped aboral spot (ZAMORA-MUÑOZ et al. 1995: fig. 14) ..... *H. instabilis* (Curtis)
- 14' Light aboral spot of the apotome different in form or absent ..... 15
- 15 Lateral parts of the submentum short and wide; ratio  $a : b > 0.21$  (about 0.22–0.26) ..... 16
- 15' Lateral parts of the submentum long and narrow; ratio  $a : b < 0.21$  (about 0.12–0.20) ..... 17
- 16 Head colouration very light; oral area of the apotome bright ..... *H. bulbifera* McLachlan
- 16' Head colouration and oral area of the apotome equally dark (ZAMORA-MUÑOZ et al. 1995: fig. 15) ..... *H. ambigua* Schmid
- 17 Apotome wide, pentagonal (width of anterior edge roughly similar to width of the posterior third) ... 18
- 17' Apotome narrow, triangular (width of anterior edge clearly wider than the posterior third) ..... 20
- 18 Apotome with two rounded light spots on the epistomal sulcus (ZAMORA-MUÑOZ et al. 1995: fig. 7) ..... *H. dinarica* Marinkövic
- 18' Apotome with two elongated light spots on the epistomal sulcus ..... 19
- 19 Apotome with parallel lateral margins and a rounded posterior tip. A distinct curved edge across the cibarian muscle attachment spots, very conspicuous in lateral view (ZAMORA-MUÑOZ et al. 1995: fig. 13) ..... *H. lobata* McLachlan
- 19' Apotome slightly narrower to eye-level, posterior margins concave and the posterior tip pointed. Without a curved edge across the cibarian muscle attachment spots (VIEIRA-LANERO 2000: figs. 28i,k) ..... *H. urgorri* González & Malicky
- 20 Medial and lateral regions of posterior prosternites forming a continuous, distinct elongated structure; lateral regions usually similar in colour to the medial regions or slightly lighter (PITSCH 1993: fig. 133) ..... *H. angustipennis* (Curtis)
- 20' Medial regions of posterior prosternites irregularly squarish or oblong; lateral region lighter and less distinct than medial regions ..... 21
- 21 Head colouration very dark, with a wide longitudinal dark band on either side of the coronal suture (which leaves two narrow, light areas in the posterolateral regions of the head; MÚRRRIA et al. 2010: figs. 2b, 4). Submentum dark brown, homogeneously coloured (MÚRRRIA et al. 2010: fig. 2f) or at least in the anterior part (MÚRRRIA et al. 2010: fig. 2c) ..... 22
- 21' Head colouration lighter, without a wide longitudinal dark band on either side of the coronal suture (two broad, light areas in the postero-lateral regions

of the head instead; MÚRRRIA et al. 2010: fig. 2a and most haplotypes of fig. 3). Submentum light brown in colour (caramel; MÚRRRIA et al. 2010: fig. 2e) or with an intermediate colouration (dark brown in the anterior part and light brown in the posterior part; MÚRRRIA et al. 2010: fig. 2g)

..... *H. pellucidula* (Curtis)

[Lightly pigmented form of this species matches that described under the name of *H. pictetorum* Botosaneanu & Schmid by GARCÍA DE JALÓN 1983.]

22 Submentum with a triangular protuberance in the centre of the anterior margin (MÚRRRIA et al. 2010: fig. 2c,d) ..... *H. incognita* Pitsch

22' Submentum without a triangular protuberance in the centre of the anterior margin (MÚRRRIA et al. 2010: fig. 2f) ... *H. iberomaroccana* González & Malicky

## 6. Acknowledgments

We are very grateful to Asociación Jara, J. Alba-Tercedor, N. Benas, F. Cianficconi, R. De Pietro, G. Coppa, M. El Alami, L. Gallo, O. Gavira, T. Gutiérrez, T. Herrera, L. Lucadamo, J.L. Moreno, A. Pujante, M. Ordeix, J. Ortiz, J.M. Poquet, N. Prat, T. Puntí, M. Rieradevall, M.M. Sánchez-Montoya, B. Launay, T. Detry, M. Toro and other members of the GUADALMED I & II projects and FEM research group for providing individuals, to J. Martínez for helping in digitizing figures of adults, and to Juan, Carmen and Cristina Soler for helping during field sampling. This research received support from the SYNTHESYS Project (<http://www.synthesys.info/>) financed by the European Community Research Infrastructure Action under the FP6 'Structuring the European Research Area' Programme (GB-TAF-1797) and an international cooperation project between Spain and Morocco (70/04/P/E). This paper is also a part of the outcome of projects dealing with macroinvertebrates (HID98-0323-C05, REN2001-3438-C07 and CGL2007-60163/BOS), funded by the Spanish Ministry of Education and Science/FEDER (Fondo Europeo de Desarrollo Regional). CM was supported by a Beatriu de Pinós postdoctoral fellowship (BP-DGR-2011) from Agència de Gestió d'Ajuts Universitaris i de Recerca, Catalunya. We appreciate very much the detailed revisions and valuable comments of Dr Karl Kjer and Dr Roger Blahnik, as well as the suggestions and editorial corrections of Dr Klaus Klass that improved the manuscript.

## 7. References

- ALLAYA W. 2003. Description de cinq larves de Trichoptères du genre *Hydropsyche* récoltées en Tunisie. – *Braueria* **30**: 21–22.
- BICKFORD D., LOHMAN D.J., SODHI N.S., NG P.K.L., MEIER R., WINKER K., INGRAM K.K., DAS I. 2007. Cryptic species as a window on diversity and conservation. – *Trends in Ecology and Evolution* **22**: 148–155.
- BONADA N., ZAMORA-MUÑOZ C., EL ALAMI M., MÚRRRIA C., PRAT N. 2008. New records of Trichoptera in reference mediterranean-climate rivers of the Iberian Peninsula and north of Africa: Taxonomical, faunistic and ecological aspects. – *Graellsia* **64**: 189–208.
- BONADA N., ZAMORA-MUÑOZ C., RIERADEVALL M., PRAT N. 2004. Trichoptera (Insecta) collected in Mediterranean river basins

- of the Iberian Peninsula: Taxonomic remarks and notes on ecology. – *Graellsia* **60**: 41–69.
- BOTOSANEANU L., SCHMID F. 1973. Les Trichoptères du Muséum d'Histoire naturelle de Genève. – *Revue Suisse Zoologie* **80**(1): 221–256.
- BOURNAUD M., TACHET H., PERRIN P.F. 1982. Les Hydropsychidae (Trichoptera) du Haut-Rhône entre Genève et Lyon. – *Annales de Limnologie* **18**: 61–80.
- CIANFICCONI F., CORALLINI C. 2010. Trichopteran fauna in a region of Central-Southern Italy: Molise. – *Denisia* **29**: 81–104.
- COPPA G., BONADA N., DATRY T., GONZÁLEZ M., LAUNAY B., LE GUELLEC G., MÚRRIA C., ZAMORA-MUÑOZ C. 2016. First record of *Hydropsyche spiritoi* Moretti, 1991 in France [Trichoptera, Hydropsychidae]. – *Ephemera* **17**: 43–46.
- DAKKI M., TACHET H. 1987. Les larves d'*Hydropsyche* du Maroc (Trichoptères, Hydropsychidae). Pp. 25–28 in: BOURNAUD M., TACHET H. (eds), Proceedings of the 5<sup>th</sup> International Symposium on Trichoptera. Series Ent. – Dr W. Junk Publishers, Dordrecht, The Netherlands.
- GARCÍA DE JALÓN D. 1983. Contribución al conocimiento de las larvas del género *Hydropsyche* (Trichoptera) ibéricas. Pp. 275–285 in: Actas I Congreso Ibérico de Entomología. – Asociación Española de Entomología, León.
- GONZÁLEZ M., MARTÍNEZ J. 2011. Checklist of the caddisflies of the Iberian Peninsula and Balearic Islands (Trichoptera). – *Zoosymposia* **5**: 115–135.
- GONZÁLEZ M., TERRA L.S., GARCÍA DE JALÓN D., COBO F. 1992. Lista faunística y bibliográfica de los Tricópteros (Trichoptera) de la Península Ibérica e Islas Baleares. – Asociación Española de Limnología, Madrid. 105 pp.
- GONZÁLEZ M.A., BOTOSANEANU L. 1985. Étude de trois espèces de *Hydropsyche* Pictet d'Espagne décrites par F. Schmid (Insecta: Trichoptera). – *Bulletin Zoologisch Museum Amsterdam* **10**: 89–95.
- GONZÁLEZ M.A., MARTÍNEZ-MENÉNDEZ J. 2008. Observaciones sobre los tricópteros de la península ibérica. X: Tricópteros de Aragón (NE de España) (Insecta: Trichoptera). – *Boletín de la Sociedad Entomológica Aragonesa* **43**: 187–192.
- GRAF W., MURPHY J., DAHL J., ZAMORA-MUÑOZ C., LOPEZ-RODRIGUEZ M.J. 2008. Distribution and ecological preferences of European freshwater organisms, Volume 1, Trichoptera (SCHMIDT-KLOIBER A. & HERING D. eds). – Pensoft, Sofia.
- KARAOUZAS I. 2009. The larva of *Hydropsyche peristerica* Botosaneanu & Marinković-Gospodnetić, 1968 (Trichoptera: Hydropsychidae), with some notes on its ecology. – *Aquatic Insects* **31**: 139–143.
- LANFEAR R., CALCOTT B., HO S.Y., GUINDON S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. – *Molecular Biology and Evolution* **29**(6): 1695–1701.
- MALICKY H. 2001. Ein Beitrag zur Kenntnis der Arten der *Hydropsyche instabilis* – Verwandtschaft im östlichen Mittelmeergebiet (Trichoptera, Hydropsychidae). – *Linzer Biologische Beiträge* **33**: 489–518.
- MALICKY H. 2004. Atlas of European Trichoptera / Atlas der Europäischen Köcherfliegen / Atlas des Trichoptères d'Europe. – Springer, Dordrecht.
- MALICKY H. 2005. Ein kommentiertes Verzeichnis der Köcherfliegen (Trichoptera) Europas und des Mittelmeergebietes. – *Linzer Biologische Beiträge* **37**: 533–596.
- MALICKY H. 2013. Fauna Europaea: Trichoptera. Fauna Europaea versión 2.6.2. – Available from: <http://www.faunaeur.org/> (February 28, 2015).
- MALICKY H., LOUNACI A. 1987. Beitrag zur Taxonomie und Faunistik der Köcherfliegen von Tunesien, Algerien und Marokko (Trichoptera). – *Opuscula Zoologica Fluminensia* **14**: 1–20.
- MILLER M.A., PFEIFFER W., SCHWARTZ T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 in: Proceedings of the Gateway Computing Environments Workshop (GCE). – New Orleans.
- MORETTI G. 1991. Nouvelles espèces et sous-espèces de Trichoptères Italiens et exemples de variabilité des populations des Alpes meridionales et des Apennins. Pp. 385–402 in: TOMASZEWSKI C. (ed.), Proceedings of the 6<sup>th</sup> International Symposium on Trichoptera. – Adam Mickiewicz University Press, Poznan.
- MÚRRIA C., BONADA N., ARNEDO M.A., PRAT N., VOGLER A.P. 2013. Higher  $\beta$ - and  $\gamma$ -diversity at species and genetic levels in headwaters than in mid-order streams in *Hydropsyche* (Trichoptera). – *Freshwater Biology* **58**: 2226–2236.
- MÚRRIA C., BONADA N., ARNEDO M.A., ZAMORA-MUÑOZ C., PRAT N., VOGLER A.P. 2012. Phylogenetic and ecological structure of Mediterranean caddisfly communities at various spatio-temporal scales. – *Journal of Biogeography* **39**: 1621–1632.
- MÚRRIA C., BONADA N., RIBERA C., PRAT N. 2010. Homage to the Virgin of Ecology, or why an aquatic insect unadapted to desiccation may maintain populations in very small, temporary Mediterranean streams. – *Hydrobiologia* **653**: 179–190.
- NEU P.J., TOBIAS W. 2003. Digitale Schlüssel zur Bestimmung der in Deutschland vorkommenden Hydropsychidae (Insecta: Trichoptera), CD-Version 200703a.
- NEU P.J., TOBIAS W. 2004. Die Bestimmung der in Deutschland vorkommenden Hydropsychidae (Insecta: Trichoptera). – *Lauterbornia* **51**: 1–68.
- PAULS S.U., BLAHNIK R.J., ZHOU X., WARDWELL C.T., HOLZENTHAL R.W. 2010. DNA barcode data confirm new species and reveal cryptic diversity in Chilean *Smicridea* (*Smicridea*) (Trichoptera: Hydropsychidae). – *Journal of the North American Benthological Society* **29**: 1058–1074.
- DE PIETRO R. 1999. Identification of the larvae of *Hydropsyche* species from Sicily and peninsular Italy (Trichoptera, Hydropsychidae). – *Archiv für Hydrobiologie Supplement* **121**: 91–117.
- PITSCH T. 1993. Zur Larvaltaxonomie, Faunistik und Ökologie mitteleuropäischer Fließwasser-Köcherfliegen (Insecta: Trichoptera). – Technische Universität Berlin, Berlin. 322 pp.
- PREVIŠIĆ A., GRAF W., VITEČEK S., KUČINIĆ M., BÁLINT M., KERESZTES L., PAULS S.U., WÄRINGER J. 2014. Cryptic diversity of caddisflies in the Balkans: The curious case of *Ecclisopteryx* species (Trichoptera: Limnephilidae). – *Arthropod Systematics and Phylogeny* **72**: 309–329.
- RONQUIST F., HUELSENBECK J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. – *Bioinformatics* **19**: 1572–1574.
- SIPAHLER F. 2010. Studies on the males of the *Hydropsyche instabilis* group in Turkey, with the description of nine new species (Trichoptera: Hydropsychidae). – *Munis Entomology & Zoology* **5**: 830–844.
- STAMATAKIS A. 2006. RAXML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. – *Bioinformatics* **22**: 2688–2690.
- STATZNER B., DOUADY C.J., KONECNY L., DOLÉDEC S. 2010. Unraveling phylogenetic relationships among regionally co-existing species: *Hydropsyche* species (Trichoptera: Hydropsychidae) in the Loire River. – *Zootaxa* **2556**: 51–68.
- TOBIAS W. 1972. Zur Kenntnis europäischer Hydropsychidae (Insecta: Trichoptera), I. – *Senckenbergiana Biologica* **53**(1/2): 59–89.
- VIEIRA-LANERO R. 2000. Las larvas de los Tricópteros de Galicia (Insecta: Trichoptera). – Departamento de Biología Animal, Facultad de Biología, Universidad de Santiago de Compostela. 611 pp.
- VIEIRA-LANERO R., GONZÁLEZ M., COBO F. 2001. The larva of *Hydropsyche urgortii* González & Malicky, 1980. – *Spixiana* **24**: 141–146.
- ZAMORA-MUÑOZ C., ALBA-TERCEDOR J., GARCÍA DE JALÓN D. 1995. The larvae of the genus *Hydropsyche* (Hydropsychidae; Trichoptera) and keys for the identification of species of the Iberian Peninsula. – *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* **68**: 189–219.
- ZAMORA-MUÑOZ C., GONZÁLEZ M., PICAZO-MUÑOZ J., ALBA-TERCEDOR J. 2002. *Hydropsyche fontinalis*, a new species of the *instabilis*-group from the Iberian Peninsula (Trichoptera, Hydropsychidae). – *Aquatic Insects* **24**: 189–197.

---

## Electronic Supplement File

at <http://www.senckenberg.de/arthropod-systematics>

**File 1:** zamoramunoz&al-iberianhydropsyche-asp2017-electronicsupplement.doc. – **Table S1.** Haplotype composition (GenBank accession number) for each species. For each site, basin, stream, altitude and geographical coordinates are shown; also site location in MÚRRIA et al. (2013) is given. – **Table S2.** Complete locality information of individuals morphologically described. For each site, basin, stream and/or locality, altitude and geographical coordinates are shown.

---

## Zoobank registrations

at <http://zoobank.org>

**Present article:** <http://zoobank.org/urn:lsid:zoobank.org:pub:C75D7075-7070-4FF8-BE9D-D84109F1044D>

***Hydropsyche solerorum* Zamora-Muñoz & González, 2017:**  
<http://zoobank.org/urn:lsid:zoobank.org:act:77B751C4-6897-4AD4-816C-2703531EB596>