

## New evidences on the presence of *Aphelocheirus aestivalis* in the Iberian Peninsula, its ecology and description of two northeastern Iberian populations

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Received: 17/05/17

Accepted: 15/07/19

### ABSTRACT

#### New evidences on the presence of *Aphelocheirus aestivalis* in the Iberian Peninsula, its ecology and description of two northeastern Iberian populations

The waterbug *Aphelocheirus aestivalis* is herein recorded for the first time in Northeastern Iberian Peninsula, constituting the first citation of the family Aphelocheiridae in the region. Yet, later on in 1989, the description of three new endemic Iberian species belonging to *Aphelocheirus* implied the assumption that *A. aestivalis* was absent in the Iberian Peninsula. Here, we show strong evidences of the presence of this species by the collection of individuals in two isolated stream reaches located 36 km apart, in the two Mediterranean rivers Llobregat and Ter. The Iberian specimens studied showed slight differentiation from other European specimens in the structures of male internal genital. Photographs of the habitus of the male and female are provided. More than 25 years of study of these rivers show that *Aphelocheirus* sp. is confined solely to a short stretch in both rivers. Our study thus suggests that the distribution of *A. aestivalis* in the Iberian Peninsula should be revised and further investigated, and warns of urgent measures to improve, or at least preserve, the habitat and the hydrological conditions rivers Llobregat and Ter to protect the species.

**Key words:** water bugs, faunistics, Iberian fauna, stream ecology, Mediterranean streams, aquatic macroinvertebrates

### RESUMEN

#### Nuevas evidencias de la presencia de *Aphelocheirus aestivalis* en la Península Ibérica, su ecología y descripción de las poblaciones del noreste ibérico

La familia Aphelocheiridae se registra por primera vez en el noreste de la Península Ibérica tras la captura de *Aphelocheirus aestivalis*. Tras la descripción de tres especies de *Aphelocheirus* endémicas de la Península Ibérica, se descartó que *A. aestivalis* formara parte de la fauna ibérica. Nuestro hallazgo pone de manifiesto lo contrario, sugiriendo que la distribución de *A. aestivalis* en la Península Ibérica debería revisarse. El material estudiado presenta ligeras diferencias con respecto a ejemplares de otras localidades europeas en las estructuras genitales internas masculinas. Se proporcionan fotografías del hábito del macho y de la hembra. Los ejemplares fueron encontrados en dos localidades de los ríos Llobregat y Ter, distantes 36 km entre sí. Más de 25 años de estudio en ambas cuencas apuntan que *Aphelocheirus* sp. se halla confinada a un corto trecho de esos ríos, y por tanto se requieren medidas urgentes para preservar el hábitat y las condiciones hidrológicas con el fin de proteger la especie.

**Palabras clave:** chinches acuáticos, faunística, fauna ibérica, ecología fluvial, ríos mediterráneos, macroinvertebrados acuáticos

## INTRODUCTION

The family Aphelocheiridae is distributed in the Eastern Hemisphere (Polhemus & Polhemus, 2008) and includes a single genus, *Aphelocheirus* Westwood, 1833. This genus has around 100 species (Xie & Liu, 2014), 30 of which in the Palearctic Region (Kanyukova, 1995; Aukema *et al.*, 2013). *Aphelocheirus aestivalis* (Fabricius, 1794) is the most common and widely distributed species across Europe. In the Iberian Peninsula, *A. aestivalis* was reported several times during the 20<sup>th</sup> century (Lindberg, 1929; Seabra, 1926, 1939, 1941; Pardo, 1933; Gómez Menor, 1956; Serrao & Azevedo, 1970; Nieser, 1978; Baena, 1980; Fernández, 1982; Murillo, 1985; Lucas, 1988; Grijalbo, 1991; López *et al.*, 1995; Casado *et al.*, 1990; López, 1998; Millán *et al.*, 2002; Sánchez-Hernández, 2011), and was included in the current checklist (Nieser & Montes, 1984), catalogue (González, com. pers. in Baena & Vázquez, 1986) and keys (Nieser *et al.*, 1994; the authors suggest confirmation for *A. aestivalis*, a species included in the key). However, in 1989 the three Iberian endemic *Aphelocheirus* species *Aphelocheirus murcius* Nieser & Millán, 1989, *Aphelocheirus occidentalis* Nieser & Millán, 1989 were described, and an innominate *Aphelocheirus* sp. considered (Carbonell *et al.*, 2011) which apparently replaced the original records of *A. aestivalis*. Thus, since 1989 the presence of *A. aestivalis* has been questioned (Nieser & Millán, 1989; Nieser *et al.*, 1994) or explicitly rejected (Carbonell & Millán, 2010; Carbonell *et al.*, 2011; Millán *et al.*, 2016) in the Iberian Peninsula. The main criticism on the presence of *A. aestivalis* in the Iberian Peninsula was that ancient citations were based on poorly studied specimens, which in fact should belong to any of the three Iberian endemic species (Nieser & Millán, 1989).

Although several studies of *Aphelocheirus* were published dealing with its distribution (Bracken, 1974; Zivic *et al.*, 2007), behavior (Lemb & Maier, 1996), life cycle (Papáček, 2012; Papáček & Soldán, 2008) or physiology (Thorpe & Crisp, 1947a, 1947b, 1947c; Messner *et al.*, 1986), many aspects of its ecology and conservation biology are still to be addressed. Species

belonging the genus *Aphelocheirus* inhabit lakes and the upper and mid sections of streams with unperturbed environmental conditions, making them good indicators of water quality and ecological status (De Brabander *et al.*, 1992; Pardo *et al.*, 2014). Moreover, *Aphelocheirus* spp. may consistently contribute to food webs as food resource for freshwater fishes (Sánchez-Hernández & Cobo, 2011). Unfortunately, the preferred habitat of *Aphelocheirus* species are at conservation risk mostly by anthropogenic reasons such as water pollution, hydromorphological changes, habitat fragmentation and water over-extraction (Papáček, 2012; Stoianova *et al.*, 2018), which are even more critical in Mediterranean streams submitted to an important water stress in summer (Filipe *et al.*, 2013). While streams located in protected areas are still in good biological condition, the middle and lowland section of most of the Mediterranean streams have been heavily modified and receive untreated wastewater (Prat & Munné, 2000), highly impeding the presence of many macroinvertebrate species, including *Aphelocheirus* bugs (Fortuño *et al.*, 2018). Moreover, it is accepted that *A. aestivalis* disperses mainly through the watercourses (Papáček, 2012). In fact, only micropterous individuals of Iberian *Aphelocheirus* species have been captured (Carbonell & Millán, 2010), and therefore their poor dispersal abilities could increase their vulnerability in case of habitat loss. As a result, Carbonell & Millán (2010) included all the Iberian *Aphelocheirus* spp. in the Spanish Red List as Vulnerable taxa (IUCN criteria). Similarly, *A. aestivalis* is included in the Red List of several European countries, for instance in Flanders (Maes *et al.*, 2019), or the Check Republic (Papáček, 2012).

Rivers in Catalonia are in general relatively short, narrow and highly menaced to drought during hot Mediterranean summers (Múrria *et al.*, 2010). Many of those streams were highly transformed since the end of 19<sup>th</sup> century during the industrialization process and more recently by urbanization. Llobregat and Ter rivers basins are paradigmatic examples. At the end of 19<sup>th</sup> century, a system of weirs diverting water through canals to textile-mills, and throwing it back to the river and soon after diverted again to the follow-

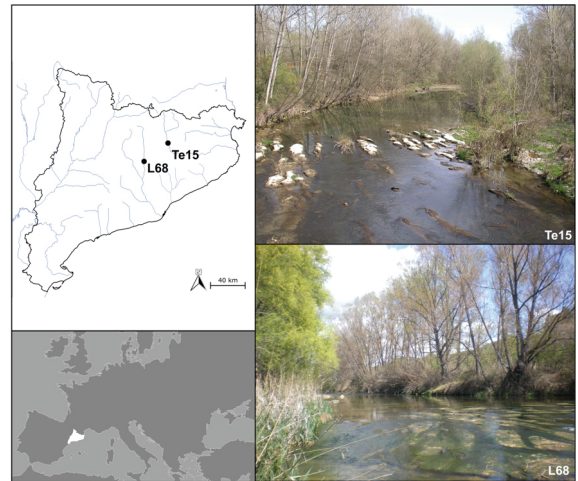
ing textile-mill, resulted in highly altered water flow. In addition to hydrological issues, treated or untreated sewage waters from domestic and industrial uses decreased the water quality, which has been recovered during the last two decades when waste water treatment plants have been built. Since half of the 20<sup>th</sup> century, the textile mills were transformed into hydroelectric power stations, and water diversion continued until present with variable but severe effects on freshwater macroinvertebrate communities (Prat & Rieradevall, 2006). Nevertheless, the residual flows below the weirs may maintain acceptable-good hydrological and environmental conditions that should be considered as refuges for biota. In order to evaluate the ecological quality of Llobregat river, the first ecological study along the river was performed in 1979, but since 1994 the middle river sections of the Llobregat and Ter rivers have been routinely biomonitored as part of the program called “Qualitat Ecològica dels Rius de la Província de Barcelona” (www.ub.edu/barcelonarius). As a result, more than 50 sites located along the 208 km of the Ter and 175 km of the Llobregat were sampled following the same protocol (Prat *et al.*, 2012).

Here, we examined the specimens collected during the project “Qualitat Ecològica dels Rius de la Província de Barcelona”. We report the family Aphelocheiridae in the north-eastern Iberian Peninsula for the first time, and the presence of *A. aestivalis* that was in the last decades considered absent from the Iberian Peninsula. We include morphological and genital descriptions, illustrations of its habitus and an ecological characterization of its habitat.

## MATERIALS AND METHODS

### Field sampling and habitat characterization

The project “Qualitat Ecològica dels Rius de la Província de Barcelona” provides a long-term species record series with samples collected regularly (spring and summer) from 1994 until present. Additionally, samples collected in the Llobregat river during 1979-80 were also available. In this project, the specimens of *Aphelocheirus* were collected using a circular hand net of



**Figure 1.** Location of Catalonia in Western Europe. Geographical distribution and habitat of sites L68 and Te15 where *Aphelocheirus aestivalis* was found (North-East Iberian Peninsula). Localización de Cataluña en Europa occidental. Distribución geográfica y hábitat de las localidades L68 y Te15 donde *Aphelocheirus aestivalis* ha sido hallada (Noroeste Peninsular).

250  $\mu$ m mesh net and were usually preserved in 10 % formaldehyde solution, as required for the current monitoring protocol (Prat *et al.*, 2012). *Aphelocheirus* was found only in two river reaches that were located in the middle sections of the Llobregat and Ter rivers (L68, Balsareny; and Te15, Torelló; Fig. 1). In their middle sections, these two rivers are separated by low-elevation topographic barriers and thus could constitute a contact zone between them in case of overflow.

In order to determine the ecological conditions of these river reaches, we analyzed water temperature, electrical conductivity, oxygen (concentration and percentage of saturation) and pH were determined using a multi-parametric digital probe YSI® Pro Plus. Water samples were filtered through glass fiber filters (GF/F; Whatman, Maidstone, UK), transported to the laboratory on ice, and finally frozen for subsequent analysis. Major anions (chloride, sulfate, nitrite and nitrate) were analyzed by high-pressure liquid chromatography and ammonium and soluble reactive phosphorus concentrations were estimated using standard colorimetric methods. We also calculated the river flow (l/s) measuring the river section (width x depth) and current velocity of water with a digital

anemometer Schiltknecht® MiniAir2. Additionally, we used the Fluvial Habitat Index (IHF, Pardo *et al.*, 2004) to determine the physical habitat structure, and the Quality of Riparian Forest Index (QBR, Munné *et al.*, 2003) to characterize the riparian habitat.

### Specimens study

In the laboratory, specimens were cleansed with distilled water prior to dry mounting. All adults were glued on 10 x 21 mm cardboard mounting cards (dry mounted) and individually pinned, whereas juveniles were piled up under the same needle. Individuals were examined under a Leica MZ160A (10–115X) and Leica MZ 125 binocular stereoscopes. Specimens fixed in formaldehyde (specified in the data label) did not show the typical colouration of the species, because of fixation. Male genitalia were dissected and genital structures were glued on the same card as the specimen. In all adult specimens, biometric characters useful in taxonomy (i.e., total length, total width, leg segment lengths) were measured. Photographs of the habitus and male genitalia were taken using a Nikon DXM1200 and LEICA DFC 450 cameras attached to the stereoscope. A series of 10 to 30 photographs were combined and image processed with the Helicon Focus 6.2.2. image-stacking and processed using Photoshop CS5.

The acronyms of collections where specimens are deposited are as follows: CRBA, Centre de Recursos de Biodiversitat Animal, Universitat de Barcelona, Spain; JRC, Jordi Ribes Collection (deposited in CRBA); MGC, Marta Goula Collection; MRCC, Marcos Roca-Cusachs Collection.

## RESULTS

### Material studied

In the list below, double dash (//) separate the contents of different labels, and sample dash (/) indicates lines within the same label. All together 33 specimens were considered.

(1M, CRBA) Riu Llobregat, Balsareny, / Catalunya, Península Ibèrica / 41.85943 N, 1.87998 E / 11<sup>th</sup> May 2016 / M. Roca-Cusachs,

P. Fortuño & N. Prat leg. // MRCAPHEL04 // *Aphelocheirus aestivalis* // deposited at Centre de Recursos de la Biodiversitat Animal with code: CRBA-54282. (1F, MRCC); Riu Llobregat, Balsareny, / Catalunya, Península Ibèrica. / 41.85943 N, 1.87998 E / 11<sup>th</sup> May 2016. / M. Roca-Cusachs, P. Fortuño & N. Prat leg. // MRCAPHEL01; // *Aphelocheirus aestivalis* (1F, MRCC): same locality / 11<sup>th</sup> May 2016. / M. Roca-Cusachs, P. Fortuño & N. Prat leg. // MRCAPHEL02; (1F, MRCC): same locality / 11<sup>th</sup> May 2016. / M. Roca-Cusachs, P. Fortuño & N. Prat leg. // MRCAPHEL03; (1M, MGC): same locality / 13<sup>th</sup> April 2010 / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* (1F, CRBA): same locality / 13<sup>th</sup> April 2010. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* // deposited with code CRBA-54283; (1F, MGC): same locality / 10<sup>th</sup> June 2009. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis*; (1F, MRCC): same locality / 10<sup>th</sup> June 2009. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis*; (1M, MRCC): same locality / 10<sup>th</sup> June 2009. / P. Fortuño & N. Prat leg. // fixed in formaldehyde; (1F, CRBA): same locality / 7<sup>th</sup> May 2008. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // deposited with code CRBA-54284; (1M, MRCC): Riu Ter, Torelló, Catalunya, / Península Ibèrica. / 41.01196 N, 2.25640 E. / 30<sup>th</sup> May 2016. // MRCAPHEL05 // *Aphelocheirus aestivalis*; (1 juvenile, CRBA): Riu Llobregat, Balsareny, / Catalunya, Península Ibèrica. / 41.85943 N, 1.87998 E. / 25<sup>th</sup> April 2013. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* // in etOH 70 %, deposited with code CRBA-54290; (4 juveniles, CRBA): same locality / 18<sup>th</sup> July 2012. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* // dry mounted, deposited with code CRBA-54287; (4 juveniles, CRBA): same locality / 24<sup>th</sup> April 2012. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* // dry mounted, deposited with code CRBA-54286; (2 juveniles, CRBA): same locality / 27<sup>th</sup> July 2011. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* // in etOH 70 %, de-



posited with code CRBA-54288; (1 juvenile, CRBA): same locality / 3<sup>rd</sup> May 2011. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* // in etOH 70 %, deposited with code CRBA-54289; (2 juveniles, CRBA): same locality / 10<sup>th</sup> June 2009. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* // dry mounted, deposited with code CRBA-54285; (3 juvenile, CRBA): same locality / 15<sup>th</sup> July 2008. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* // in etOH 70 %, deposited with code CRBA-54292; (2 juvenile, CRBA): same locality / 7<sup>th</sup> May 2008. / P. Fortuño & N. Prat leg. // fixed in formaldehyde // *Aphelocheirus aestivalis* // in etOH 70 %, deposited with code CRBA-54291; (1M, CRBA): Kl. Schierensee / Umg. Kiel / H. H. Weber leg./ 17.10.47 // *Aphelocheirus / aestivalis* F. / H. H. Weber det. 195. From JRC; (1M, CRBA): Silesia / Lucina / 20.VII.1952 // *Aphelocheirus / aestivalis* F.(F.) / Štusák det. 68. From JRC;

Specimens with code CRBA are deposited at the Centre de Recerca de Biodiversitat Animal (CRBA) at the Faculty of Biology, University of Barcelona with collection codes shown on the data label information mentioned herein.

### Description of *A. aestivalis* from Northeast Iberian Peninsula

A detailed description of *A. aestivalis* micropterous specimens collected is given, remarking the characters which are slightly different to the specimens revised from out of the Iberian Peninsula. In general, the variation of most features between Iberian and non-Iberian specimens is very weak. Specimens fixed in formaldehyde lost the typical colouration of the species, as may be seen in Figures 2f and 2g, but the morphology was unaltered. Measures are given in mm.

**General habitus.** Micropterous. Length, 9.6 (9.1-10). Width, 7.1 (6.8-7.5). Overall appearance ovate (Fig. 2), flattened. Dorsum mostly dark brown with contrasting yellow head, legs, margins of thorax, abdomen and two light markings on pronotum and abdomen. Ventral surface brownish.

**Head.** Yellow. Head punctuate, punctuation

finer in the middle of head; greatest head width at anterolateral corners of eyes, 2.2 (2.1-2.2); with sparse, short setae surrounding eyes. Head length 1.6 (1.1-1.8). Eyes black, semi-rectangular, anteriorly divergent, external margins gently sinuate and slightly pointed outwards near the anterior end. Interocular distance between anteromedial corners of eyes 1.7 (1.6-1.7), between posteromedial corners of eyes 1.2 (1.1-1.3). Eye length 0.9 (0.8-0.9). Eye width 0.5 (0.4-0.5). Venter concolour with dorsal colouration, and covered with sparse, thin, short setae. Antennae pale, 4-segmented. Length of antennal segment: 5-7-14-17. Labrum evenly rounded anteriorly. Rostrum concolour with head, not surpassing middle of mesocoxae. Four visible rostral segments, total length: 3.7 (3.3-3.7).

**Pronotum.** General colour brown. Yellow at lateral band and along two markings at center; very finely tuberculate; sparse, yellowish very short shiny setae; posterior margin slightly concave, nearly straight, sometimes narrowly yellow coloured; pronotum width 5.2 (4.9-5.2), length 1.3 (1.1-1.3). Prosternum carinate at midline. Propleuron brown; posteromedial corner sharply acuminate, directed ventromedially. Mesosternum light brown, sparsely covered with small setae. Mesosternum with small, but well-developed, mid-ventral process.

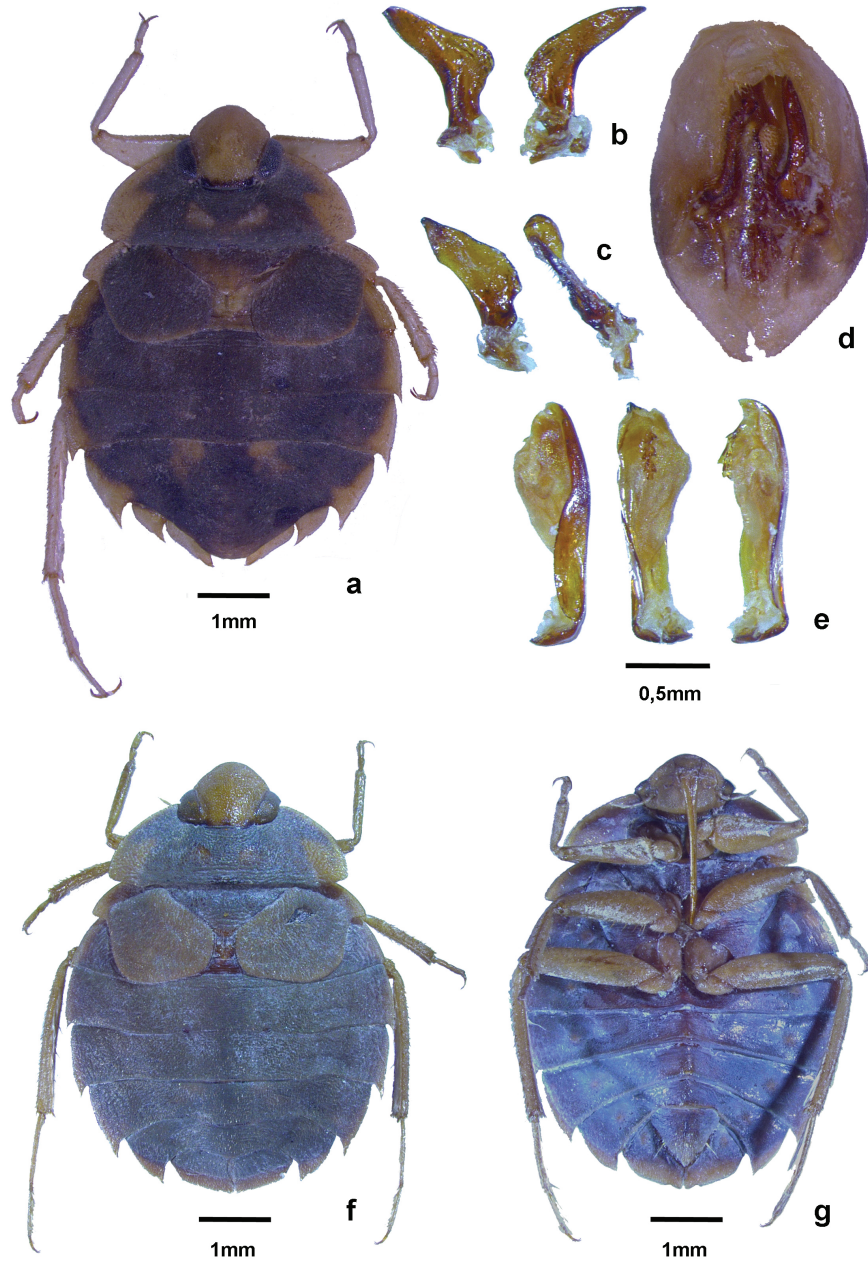
**Scutellum.** Triangular; brown with yellowish apex; very finely tuberculate; sparse, yellowish very short shiny setae; anterior margin straight, posterior apex slightly rounded; length 0.9 (0.9-1.1) mm, width 2.9 (2.5-2.9).

**Hemelytra.** Very finely granulate with fine longitudinal markings, brown, lighter at anterolateral corners and posterior margin; anterolateral corners produced in a semi right angle; rounded posteriorly; slightly separated medially; distance of separation 0.8 (0.4-0.8); hemelytra width 6 (5.5-6), length 2.1 (1.8-2.1).

**Legs.** Yellow, legs segments rod-like, or somewhat angular. Mesal surface of all coxae and trochanters with short shiny setae. Coxae with dense row of stout elongate setae on mesoapical margin. Claws concolour with legs, gently curved, tips darker. Profemurs with anterolateral surface covered in a dense, short, shiny pilosity. Lengths of profemur, protibia, protarsomeres

1–3: 2.3, 1.9, 0.1, 0.25, 0.4. Mesofemora and mesotibia covered in thin setae and short thin spines. Mesotibia with stout peg like spines on apex. Lengths of mesofemur, mesotibia, mesotarsomeres 1–3: 2.4, 1.9, 0.1, 0.3, 0.4. Metafemora

with scattered, stout setae throughout. Metatibia and metatarsus with dense row of swimming hairs continuously distributed and short scattered spines; metatibia and metatarsus apex with circlet of stout spines, swimming hairs lighter coloured



**Figure 2.** *Aphelocheirus aestivalis* a) Male dorsal habitus; b) Right paramere; c) Left paramere; d) Genital capsule; e) Aedeagus; f) Female dorsal habitus; g) Female ventral habitus. *Aphelocheirus aestivalis* a) Hábito dorsal del macho; b) Parámetro derecho; c) Parámetro izquierdo; d) Cápsula genital; e) Edeago; f) Hábito dorsal de la hembra; g) Hábito ventral de la hembra.

than legs. Metatarsomer1 inconspicuous. Lengths of metafemur, metatibia, metatarsomeres 2–3: 3.1, 3.5, 1.6, 0.9.

**Abdomen.** Brown with yellowish margins, extended in posterolateral acute processes in all visible segments. Tergites very finely granulate, with very short, yellowish shiny setae scattered throughout. Lateral margins narrowly glabrous. Posterolateral angle of segment II very slightly acuminate; III–V acuminate with progressively larger, broadly triangular spiny process; VI–VII produced and pointed with scattered recumbent setae. Male abdominal tergite V large, covering most of tergites VI to IX, segment IX reduced. Female abdominal tergites V and VI normal shaped, VII divided in two valves with two medio-caudal points. Sterna generally pruinose throughout. Spiracular rosettes on sterna II–VII reduced and 3/4 distance from midline to lateral margin. Hydrostatic organ on abdominal sternite II large, ovate, laterally compressed, length 0.5, with semi rounded borders.

**Genitalia.** Male genital capsule light yellowish, apex broadly pointed; heel of left paramere arrow shaped, paramere thickened at center, and subtly constraint, somewhat pointed at apex; right paramere elongated, with pointed apex and rounded heel, parameres lacking a thick fringe of hairs; aedeagus with 3 pairs of robust spines in apical section of interior margin and ending in a prominent fang shape process. Female operculum pointed shaped with lateral hair tufts consisting in a brush of long, dense hairs orientated backwards. Apical pilosity not developed into a real tuft.

**Ecology.** The sites where specimens of *A. aestivalis* were found belong to water bodies typified as “Mediterranean rivers with High Flow” (ACA, 2010). The reaches where the specimens were collected are relatively wide (up to 50 meters) with a substrate formed by large rocks, stones, boulders, gravel and sand, which is not always the common niche occupied by *A. aestivalis* (Giordano, 2018). The altitude of the sites is, respectively, 285 (L68) and 483 (T15) m a.s.l. The water flows seasonally with low discharge in summers and maximum in spring and autumn with an annual average of 7.07 m<sup>3</sup>/s (L68) and 15.18 (T15) m<sup>3</sup>/s (ACA, 2000). Available data of the biomonitoring program since 2002 show conductivity ranging between 250 and

1000 µS/cm and average values of nitrate of 0.98 (L68) and 0.69 (T15) mg N-NO<sub>3</sub>/L, phosphorous of 0.07 (L68) and 0.04 (T15) mg P-PO<sub>4</sub><sup>3-</sup>/L, temperature of 14.8 (L68) and 18.1 (T15) °C and pH of 8.28 (L68) and 8.35 (T15). The presence of macrophytes was common, mainly *Potamogeton pectinatus* (L.) Böerner in the bed of the river that can develop into large mats, due to moderate levels of phosphorous content in the waters (some eutrophication), and *Phragmites australis* (Cav.) Trin. ex Steud. and *Typha* sp. on the banks. Finally, according to the Water Framework Directive (WFD), the ecological status since 2002 ranged between moderate and good, but occasionally very good, as riparian forest quality at both sites is moderate and the biological indices ranged between good and very-good (Prat *et al.*, 2016) (Fig. 1).

## DISCUSSION AND CONCLUSION

The *A. aestivalis* specimens found at the Ter and Llobregat are the first record of the family Aphelocheiridae in Catalonia (Goula *et al.*, 2010; Ribes *et al.*, 2004; 2008), and more extensively in Northeastern Iberian Peninsula. Moreover, this record reconfirms the species in the Iberian fauna (Carbonell & Millán, 2010; Carbonell *et al.*, 2011; Millán *et al.*, 2016). According to Millán and collaborators (2016), the only reliable characters are head silhouette, spiny processes in the lateral margin of abdominal segments, and male genitalia. Following Carbonell *et al.* (2011), Papáček (2012) and Millán *et al.* (2016), the males of *A. aestivalis* collected in the Northeastern Iberian Peninsula have clear distinctive characters in the genitalia. The left paramere is lanceolate at the apex (truncate or rounded in the other species), its body is approximately as long as base (body is longer than base in the other western Palaearctic species), right paramere shows a heel (as in European *A. aestivalis* and *A. murcius*), its interior profile is angulated (curved in these latter two species), and the aedeagus is provided with lateral spines in apical part (as found for the Iberian *A. murcius* and *Aphelocheirus* sp., and the European *A. aestivalis*), ending in a prominent fang shape process (exclusive character for the Northeastern Iberian Peninsula populations). Thus, we believe the Northeastern Iberian Peninsula specimens

belong to *A. aestivalis*, and differences in male genitalia have to be interpreted as variations within the species (A. Millán com. pers.).

Findings of *A. aestivalis* in Northeastern Iberian Peninsula indicate the need for a rigorous revision of the taxonomy of the genus *Aphelocheirus* in the Iberian Peninsula and its current distribution. The controversy is distinguishing the records attributed to *A. murcius* and *A. occidentalis*, which progressively enlarge their distribution as more studies have been published (i.e., Miguélez & Valladares, 2010) from record of *A. aestivalis*, or *vice versa*. For instance, specimens captured in the rivers Perales and Manzaneres (López *et al.*, 1995) and Lozoya (Casado *et al.*, 1990) in the Madrid province were attributed to *A. aestivalis*, but this assignation have to be rechecked because Madrid is in the joint point of the distribution range of *A. murcius* and *A. occidentalis*. Similarly, *A. aestivalis* has recently been reported in the province of Ávila in the frame of a macroinvertebrate study (Sánchez-Fernández, 2011), largely after *A. occidentalis* was stated. *A. occidentalis* is morphologically similar to *A. aestivalis*, thus adding more ambiguity on the distribution of *Aphelocheirus* species in the Iberian fauna. The intraspecific variability in morphology is not exclusive for the Iberian specimens, *A. aestivalis* has been largely reported across Europe and an extensive review revealed morphological divergences across its distribution range (Papáček, 2011).

In the Llobregat and Ter rivers, more than 25 years of continuous monitoring are available for 52 sites across the entire basins (Prat & Rieradevall, 2006). During these years, *Aphelocheirus* specimens were collected almost every year only in one isolated site in each basin categorized in “good ecological status”, which indicates isolated but stable populations. Presumably, those specimens could belong to *A. aestivalis*, but unfortunately, the majority of those samples were lost. These sites are among the few reaches in the Llobregat and Ter rivers where the water flow has not been or is scarcely diverted for hydroelectrical purposes and did not receive direct inputs from sewage water. Therefore, the minimum annual water flow (> 1 m<sup>3</sup>/s) at this site is guaranteed and provides enough water

renewal to maintain the oxygen levels close to saturation at the substrate, which is important for cutaneous and plastron respiration of *Aphelocheirus* (Thorpe, 1950). On the contrary, most of the reaches upstream and downstream from the localities where *A. aestivalis* is found are unsuitable as most of the water flow is diverted out from the river, a fact that is especially critical during dry summers in Mediterranean streams (Gasith & Resh, 1999; Filipe *et al.*, 2013), with water flow limited to 100 l/s. The low water flow in summer produces water stagnation, which together with the high temperature, decrease the oxygen concentration in water, leading to sulphate reduction to sulphide under large rocks, and this effect is increased when reaches received direct inputs from treated (now) or untreated (in the past) sewage water. As a result, *A. aestivalis* cannot refuge under the stones and boulders during daytime. Specially critical is species conservation in the Llobregat river because downstream from the locality the physico-chemical condition of water is unsuitable for *A. aestivalis* requirements due to the runoff from huge salt mines activity, resulting in a strong increase of conductivity (~2200 µS/cm), and other water pollutants (Cañedo-Argüelles *et al.*, 2015). Moreover, the upstream La Baells reservoir (102 m. high dam, 11 km of length and an average depth of 35 m.) releases relatively cold water from near the bottom of the reservoir that may limit upstream dispersion of *A. aestivalis* in summer. Temperature and pH conditions in the localities where *A. aestivalis* was collected are within the range already known in other localities, but the species can accept more acid and cold conditions (Papáček, 2012; Stoianova *et al.*, 2018; Giordano, 2018). In any case, the persistent and intensive monitoring of both Ter and Llobregat rivers suggests that the narrow distribution of *Aphelocheirus* is not associated directly to low sampling intensity, instead is likely the species response to unappropriated water environmental conditions.

In conclusion, the specimens of *A. aestivalis* were found in isolated, preserved and unique reaches in the highly modified Llobregat and Ter rivers basins. The strong habitat specificity of the species and its highly limited dispersal abilities



explain its fragmented distribution, which leads to the risk of population disappearance. Under those circumstances, *A. aestivalis* should be considered together with the other Iberian *Aphelocheirus* endemics to be added in the red list of threatened invertebrates. Moreover, Mediterranean rivers are some of the most threatened aquatic ecosystems worldwide (Abellán *et al.*, 2005; Filipe *et al.*, 2013; Sánchez-Fernández *et al.*, 2008) because of the ongoing urban, industrial and agricultural pressures that critically reduce their water flow, biological condition and ecological quality, thus the localities where the species has been found are under threat. Although it has been proved that *A. aestivalis* responds to habitat restoration by means of adding wood debris (Brugmans *et al.*, 2015), the protection of the fragile ecosystems of the genus *Aphelocheirus* in the Iberian Peninsula is compulsory by maintaining the water flow in summer at least at the same level as we have today. On the other hand, the high score of Aphelocheiridae (10) attributed by the Biological Monitoring Working Party (BMWP) (Bartram & Balance, 1996) does highlight its great sensitivity and the need of preserving the few habitats and niches where the species was found.

## ACKNOWLEDGEMENTS

We would like to thank all researchers that collaborated in the biomonitoring program “Qualitat Ecològica dels Rius de la Província de Barcelona” since 1994, and the Diputació de Barcelona for funding the program through several projects (ECOBILL, ECOSTRIMED and CARIMED). To A. Millán for valuable comments, and together with J.A. Carbonell for facilitating all the bibliography requested and their helpful advice. To R. Sites (University of Missouri) for his critical revision of the manuscript that improved the final version. To M.Á. Vázquez and F. Faracci for critical reading of a previous manuscript.

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