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**Article**

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# The genus *Aphelocheirus* Westwood, 1833 (Hemiptera: Aphelocheiridae) in the Iberian Peninsula

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## Abstract

This study updates current knowledge of the relationships and geographical distributions of the species from the genus *Aphelocheirus* in the Iberian Peninsula which, until now, have been involved in nomenclatural and taxonomic confusion. The morphological and molecular analyses presented in this study confirm the taxonomic validity of *A. murcius* and the absence of *A. aestivalis* in the Iberian Peninsula. The shape of the left paramere emerged as the only valid characteristic to differentiate both species. Specimens of *Aphelocheirus* from northern Spain, previously identified as *A. murcius*, formed an independent lineage genetically distinct to *A. murcius* and *A. aestivalis*, although morphologically indistinguishable from *A. murcius*.

**Key words:** morphology, DNA identification, taxonomic status, *Aphelocheirus*, Hemiptera, Iberian Peninsula

## Introduction

*Aphelocheirus* Westwood belongs to the order Hemiptera Linnaeus, 1758, suborder Prosorrhyncha Sorensen, Campbell, Gill & Steffen-Campbell, 1995, infraorder Gerromorpha Popov, 1971, superfamily Naucoroidea Leach, 1815 and family Aphelocheiridae Fieber, 1851. Aphelocheiridae is related to family Naucoridae Leach, 1815, but with some clear morphological differences: a thinner, longer rostrum and much less developed front femurs than in Naucoridae (Fig. 1). Its populations show wing polymorphism dominating micropterus form, at least in the south-east of Iberian Peninsula (Millán *et al*. 1988).

In the Iberian Peninsula, the main habitats of the genus *Aphelocheirus* are middle and upper reaches of streams and rivers (Fig. 2). The species of this genus seem to be nocturnal, hiding under rock substrates during the daytime (Andersen & Weir 2004). They are predacious, using their front legs and long rostrum to consume preys, usually mayfly and caddisfly larvae (Nieser *et al*. 1994). The life cycle of *A. aestivalis* (Fabricius 1794) was described by Larsen (1927, 1931) in Sweden, being three years long.

In the Palearctic Region there are nearly 60 species of *Aphelocheirus* (Aukema & Rieger 1995) inhabiting streams and lakes with a depth greater than 10 meters. Along the Western Palearctic Region four species of *Aphelocheirus* appear. In a large extent of Europe, only occur *A. aestivalis*, which is widely distributed in medium sections of well-oxygenated and conserved rivers. In the Iberian Peninsula, three species of *Aphelocheirus* have been cited: *A. murcius* Nieser & Millán, 1989 and *A. occidentalis* Nieser & Millán, 1989, both Iberian endemics, as well as an *A. aestivalis*, for which exist only old and unconfirmed records. The fourth species, *A. rotroui* Bergevin, 1925, is endemic of Morocco. This species is known by a male macropterus specimen from the National Park of Tazza, collected at night with a light-trap (Bergevin 1925), and unfortunately not available for our study.

The two Iberian endemic species described (Nieser & Millán 1989) have clear differences between them. Conversely, *A. aestivalis*, with a European distribution and the only species of this genus cited to the Iberian Peninsula until 1989, is very similar to *A. murcius* and some taxonomic specialists showed reasonable doubts about the valid-

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ity of this latter species (M. Baena pers. com.). Thus, a review of the morphological characteristics and genetic differentiation of species is necessary to elucidate this controversy. Therefore, the main goal of the present work is to revise and diagnose genetically and morphologically the species of the genus occurring in the Iberian Peninsula. The results of this revision will allow us to (1) elucidate the taxonomic validity of *A. murcius*; (2) to determine the real presence of *A. aestivalis* in Iberian Peninsula; and (3) to update the current knowledge of the relationships and geographical distributions of the species, which, in view of the nomenclatural and taxonomic confusion, is outdated.

**FIGURE 1**. *Aphelocheirus aestivalis*. With flattened body and long rostrum.

## Material and methods

**Data set.** An exhaustive bibliographic review of the genus *Aphelocheirus* in the Iberian Peninsula (Fernández 1982; Nieser & Montes 1984; Murillo 1985; Millán *et al*. 1988; Nieser & Millán 1989; Aukema & Rieger 1995; López *et al*. 1995; Nieser *et al*. 1994; Millán *et al*. 2002; Salamanca *et al*. 2002; Miguélez & Valladares 2006), private collections of specimens (D.T. Bilton, A. Castro, D. Miguélez, A. Mellado, I. Ribera and E. Rico collections), and own field surveys were used.

**Morphological analysis.** Material examined previously identified as *A. occidentalis*: *Paratype*: 1 ♂, 12/07/1987, Umia River, Portas, Pontevedra (Spain), leg. A. Cordero. Department of Ecology and Hydrology, University of Murcia.

*Other material*: 2 ♀, 08/05/1993, Áncora River, Viana do Castelo (Portugal), leg. D. T. Bilton; 1 ♀, 24/07/1998, Áncora River, Viana do Castelo (Portugal), leg. I. Ribera; 1 ♂, 25/07/2000, Estena River, Toledo (Spain), leg. P. Aguilera; 1 ♂, 30/07/2001, Bronova River, Guadalajara (Spain), leg. M. Navarro; 1 ♂ and 2 ♀, 13/05/2002, Pusa River, Toledo (Spain), leg. J. L. Moreno; 1 ♂ and 1 ♀; 21/05/2002, Sorbe River, Guadalajara (Spain), leg. J. L. Moreno; 1 ♂ and 4 ♀, 21/05/2002, Bornova River, Guadalajara (Spain), leg. J. L. Moreno; 1 ♂, 22/05/2007, Lil-

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las River, Guadalajara (Spain), leg. F. Picazo; 1 ♂, 20/05/2009, Navia River, Lugo (Spain), leg. A. Mellado; 2 ♀, 09/05/2009, Lamas River, Lugo (Spain), leg. A. Mellado; 1 ♂ and 1♀, 09/09/2009, Lamas River, Lugo (Spain), leg. A. Mellado; 1 ♂ and 1♀, 09/09/2009, Navia River, Asturias (Spain), leg. A. Mellado.

**FIGURE 2.** Typical habitat of *Aphelocheirus*. Mundo river (Albacete).

Material examined previously identified as *A. murcius*: *Paratype*: 1 ♂, 20/03/1983, Mundo River, La Altera, Albacete (Spain), leg. Montes *et al*. Department of Ecology and Hydrology, University of Murcia.
*Other material*: 2 ♂, 13/06/1988, Urola River, Guipúzcoa (Spain), leg. E. Rico; 1 ♂, 27/04/1997, Tus River, Albacete (Spain), leg. I. Ribera; 2 ♂ and 2 ♀, 19/09/2001, Tus River, Albacete (Spain), leg. J. L. Moreno; 1 ♂ and 4 ♀, 30/08/2007, Genal River, Málaga (Spain), leg. by Castro *et al*.; 12 ♂ and 4 ♀, 09/07/2008, Ebro River, Burgos (Spain), leg. D. Miguélez & L. F. Valladares; 2 ♂ and 2 ♀, 09/04/009, Tus River, Albacete (Spain), leg. P. Abellán & N. Martínez; 2 ♀, 28/04/2009, Mundo River, Albacete (Spain), leg. A. Millán & J.A. Carbonell; 3 ♂ and 3 ♀, 03/08/2010, Tus River, Albacete (Spain), leg. Belmar *et al*.; 9 ♂ and 6 ♀, 02/09/2010, Ebro River, Burgos (Spain), leg. Millán *et al*.

Material examined previously identified as *A. aestivalis*: 1 ♂ and 3 ♀, 03/06/1995, Lessay (France), leg. J. F. Elder; 2 ♂ and 11 ♀, 12/04/2001, Fozzano (Corsica), leg. J. F. Elder; 1 ♂ and 1 ♀, 29/09/2001, Arzano (France), leg. J. F. Elder; 3 ♂ and 3 ♀, 30/04/2009, West Dorset (England), leg. F. Picazo; 1 ♂ and 2 ♀, 02/05/2009, Nort Devon (England), leg. F. Picazo (all specimens preserved in 70% ethanol except 2009 specimens preserved in absolute ethanol). Furthermore, we studied dry preserved material (12 specimens: 9 ♂ and 3 ♀) from different localities of French Manche, Orne and Côtes-d´Armor (leg. J. F. Elder): 1 ♀, 17/07/1978, Lessay; 2 ♂, 22/06/1992, La Feuillie; 1 ♂, 17/05/1997, Kairon; 1 ♀, 21/05/1997, Montpinchon; 2 ♂, 08/05/2000, Vidouville; 1 ♂ and 1 ♀, 06/08/2006, Candol; 1 ♂, 14/06/2008, La Courbe; 1 ♂, 24/09/2000, Saint Céneri le Gérei; 1 ♂, 26/05/2006, Saint-Hervé.

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**Molecular analysis.** To elucidate the taxonomic validity of *A. murcius*, the phylogenetic relationships between this species and *A. aestivalis* were studied. A total of 11 specimens of both species were selected from 10 localities (Table 1). 5 populations of *A. aestivalis* from France and England as well as populations from throughout the complete known distribution range of *A. murcius* were sampled. Populations of *A. occidentalis* from the centre of the Iberian Peninsula were used as an outgroup taxa. Samples were collected in the field and kept in absolute ethanol until processing. DNA was extracted using Invisorb Spin Tissue Mini Kit (Invitek, Berlin, Germany). One mitochondrial (5' end of cytochrome c oxidase subunit 1; cox1) gene fragment was sequenced using C1-J-2183 and L2-N-3014 primers (Simon *et al*. 1994). Sequencing was done using the ABI PRISM BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and sequenced products were electrophoresed on ABI 310 and 3700 automated sequencers (Applied Biosystems). They were then assembled and edited with Sequencher 4.7 (GeneCodes Corporation) and submitted to GenBank (see Table 1 for Accession numbers).

**TABLE 1.** Material used in the molecular analysis, with species, locality, country, number of individuals (N. ind.), collector (Leg.), DNA voucher and GenBank Accesion Number (Accession n.). *A.* sp. corresponds to individuals previously identified as *A. murcius*.

| Species | Locality | Country | N. ind. | Leg. | DNA voucher | Accession n. |
|---|---|---|---|---|---|---|
| *A.* sp | Ebro river (Pesquera de Ebro, Burgos) | Spain | 1 | A. Millán *et al.* | IBE-AB337 | HQ738307 |
| *A.* sp | Ebro river (Pesquera de Ebro, Burgos) | Spain | 1 | A. Millán *et al.* | IBE-AB339 | HQ738308 |
| *A. aestivalis* | La Drôme river (Balleroy, Calvados) | France | 1 | J.F. Elder | IBE-AB292 | HQ738309 |
| *A. aestivalis* | Le Noireau river (Berjou, Orne) | France | 1 | J.F. Elder | IBE-AB293 | HQ738310 |
| *A. aestivalis* | La Sienne (S. Denis le Gast, La Manche) | France | 1 | J.F. Elder | IBE-AB291 | HQ738311 |
| *A. aestivalis* | L´Airou (Ver, La Manche) | France | 1 | J.F. Elder | IBE-AB270 | HQ738312 |
| *A. aestivalis* | Taw river (Umberleigh, Nort Devon) | England | 1 | F. Picazo | IBE-PA310 | HQ738313 |
| *A. murcius* | Genal river (Algatocín, Málaga) | Spain | 1 | A. Castro *et al.* | IBE-AB271 | HQ738314 |
| *A. murcius* | Guadaiza river (S.de Alcántara, Málaga) | Spain | 1 | A. Castro *et al.* | IBE-AB294 | HQ738315 |
| *A. murcius* | Tus river (Yeste, Albacete) | Spain | 1 | P. Abellán & N. Martínez | IBE-PA301 | HQ738316 |
| *A. murcius* | Mundo river (La Alfera, Albacete) | Spain | 1 | A. Millán & J.A. Carbonell | IBE-PA302 | HQ738317 |
| *A. occidentalis* | Bornova river (Gascueña de Bornova, Guadalajara) | Spain | 1 | J.L. Moreno | IBE-AB272 | HQ738318 |

The optimal evolutionary model was estimated prior to analysis with jModel-Test (Posada 2008). Bayesian analyses (BA) were conducted on a combined data matrix with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). MrBayes ran for $10*10^6$ generations using default values, saving trees each 100. ''Burn-in" values were established after visual examination of the standard deviation plot of the split frequencies between two simultaneous runs. An additional phylogenetic approach was calculated for comparative purposes, using Maximum Likelihood (ML) with a genetic algorithm implemented in RaxML 7.0.3 (Stamatakis 2006) using an estimated GTR+gamma model for the combined sequence and the default settings. Node support was measured with posterior probabilities in MrBayes, and 1000 bootstrap replicates using the rapid RaxML bootstrapping algorithm (Stamatakis *et al*. 2008).

## Results

### Phylogenetic relationships

Both reconstruction methods (Bayesian probabilities and Maximum Likelihood) produced similar tree topology, with comparable support for most nodes (Fig. 3). *A. murcius* from southern populations and *A. aestivalis* appeared in two independent and well-supported clades, forming a monophyletic group with strong support. In turn, this

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group was a sister of a clade (designed as *Aphelocheirus sp*. in Fig. 3) containing individuals from the northern Iberian Peninsula previously morphologically assigned to *A. murcius* (Carbonell & Millán 2010).

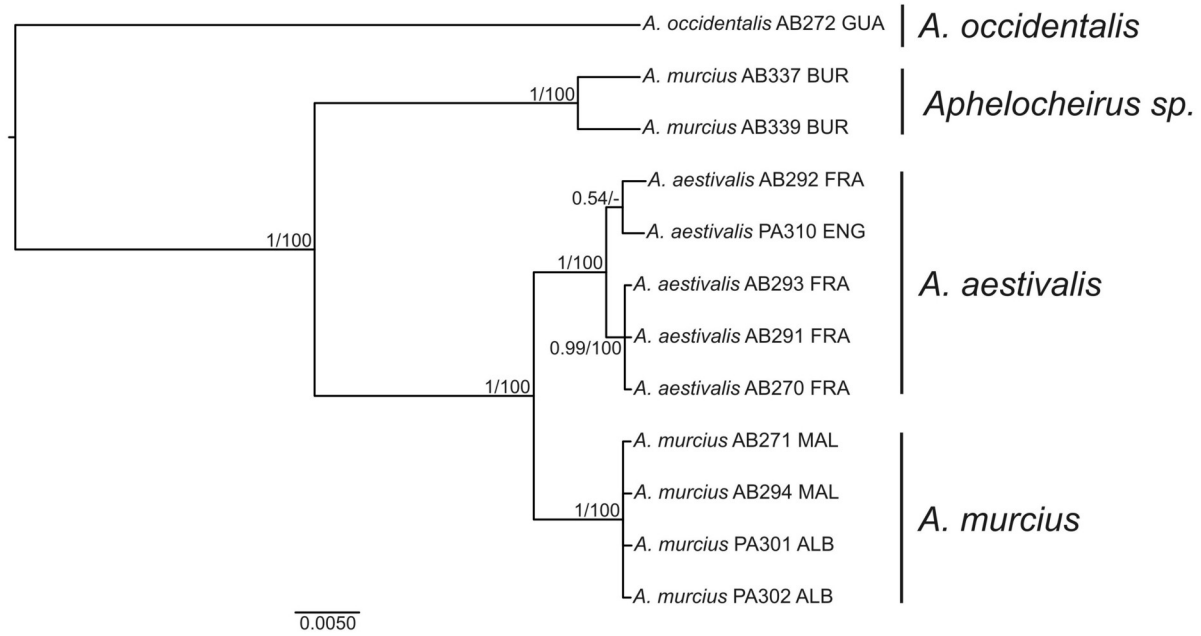


**FIGURE 3.** Phylogram obtained with MrBayes and mitochondrial data. In nodes, Bayesian posterior probabilities / boostrap support values in RaxML (when >50%).

## Morphological diagnosis

### *Aphelocheirus occidentalis* Nieser & Millán, 1989

**Short description:** water bug with a flat, oval body and length between 9.2 and 9.8 mm (Fig. 4A). The principal difference between other *Aphelocheirus* species is the form of the abdominal conexives 3 to 5, which are very long and pointed. Colour: head, including antennae, yellowish, eyes blackish, anterior and posterior margin dorsally blackish. Dorsally dark greyish thorax and abdomen with narrow yellowish margins. Yellow legs. Genitalia: both parameres with rounded heel (Fig. 4C,D). Penis without lateral spines in apical part (Fig. 4B).

### *Aphelocheirus murcius* Nieser & Millán, 1989

**Short description:** water bug with a flat oval body and length between 8.1 and 9.1 mm (Fig. 5A). Colour: head, including antennae, yellowish, eyes blackish. Dorsally greyish thorax and abdomen, usually with broad yellowish lateral bands and yellowish marks in pronotum and posterio of abdomen. Yellow legs. Genitalia: heel of left paramere pointed, right paramere long and thin with rounded heel (Fig. 5C,D). Penis with lateral spines in apical part (Fig. 5B).

### *Aphelocheirus* sp. (north Iberian populations)

**Short description:** Specimens from Northern Iberia previously assigned to *A. murcius*. Water bug with a flat oval body and length between 8.1 and 9.1 mm. Colour: head, including antennae, yellowish, eyes blackish. Dorsally greyish thorax and abdomen, sometimes with broad yellowish lateral bands and yellowish marks in pronotum and posterio of abdomen. Yellow legs. Genitalia: heel of left paramere pointed (Fig. 6A), right paramere long and thin with rounded heel . Penis with lateral spines in apical part.

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These specimens do not present any clear morphological differences with those from populations of *A. murcius* of South-east of Iberian Peninsula. After genitalia study we did not find significant differences in the shape of left paramere (Fig. 6A,B).

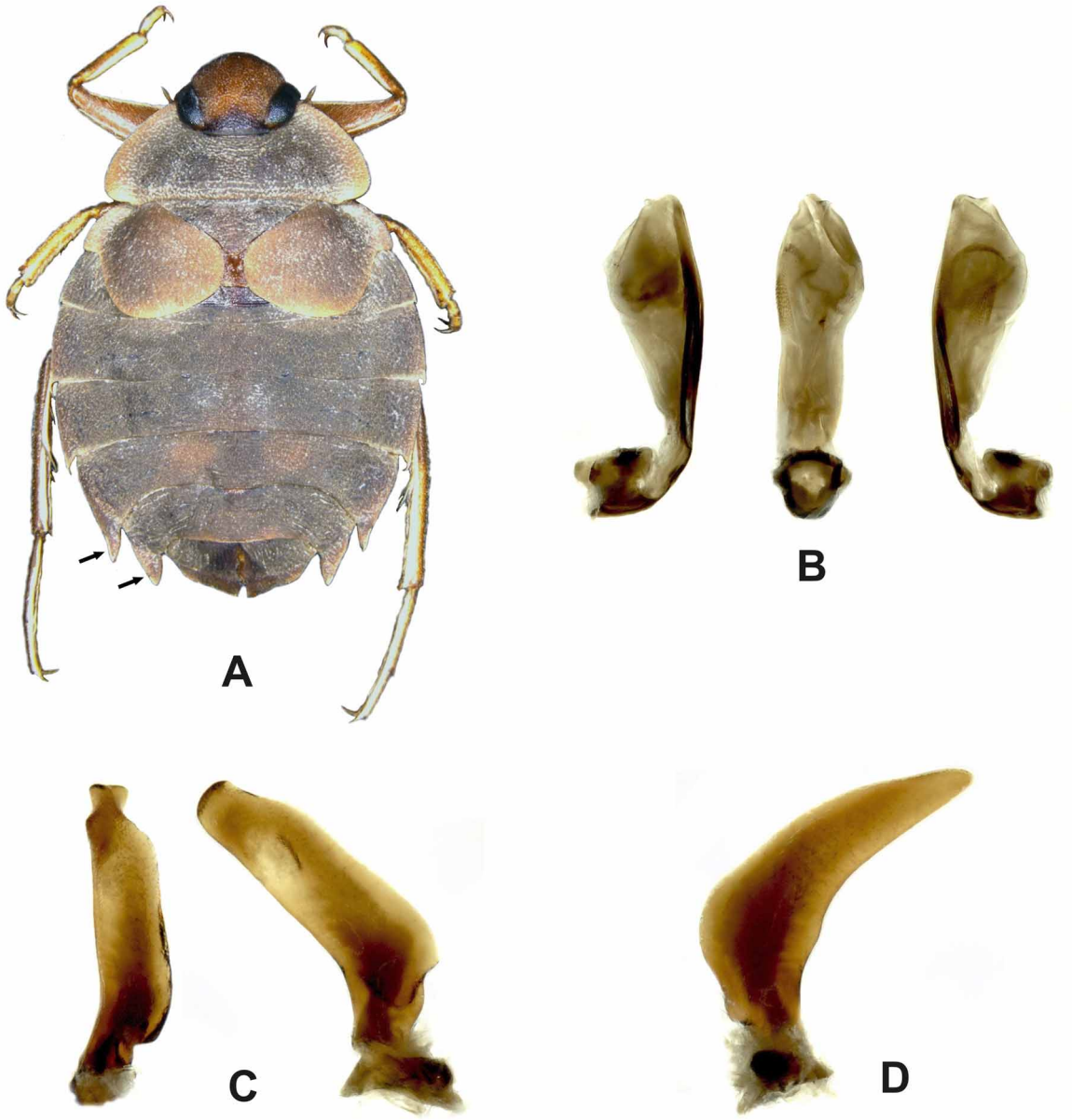


**FIGURE 4.** *Aphelocheirus occidentalis*. A) habitus; B) penis (ventral and lateral view); C) Left paramere (dorsal and lateral view); D) right paramere.

### *Aphelocheirus aestivalis* (Fabricius, 1794)

**Short description:** water bug with a flat, oval body and length between 8.5 and 10 mm (Fig. 7A). Colour: head, including antennae, yellowish, eyes blackish. Dorsally greyish thorax and abdomen, sometimes with broad yellowish lateral bands and yellowish marks in pronotum and abdomen. Yellow legs. Genitalia: both parameres with rounded heels (Fig. 7C,D). Penis with lateral spines in apical part (Fig. 7B).

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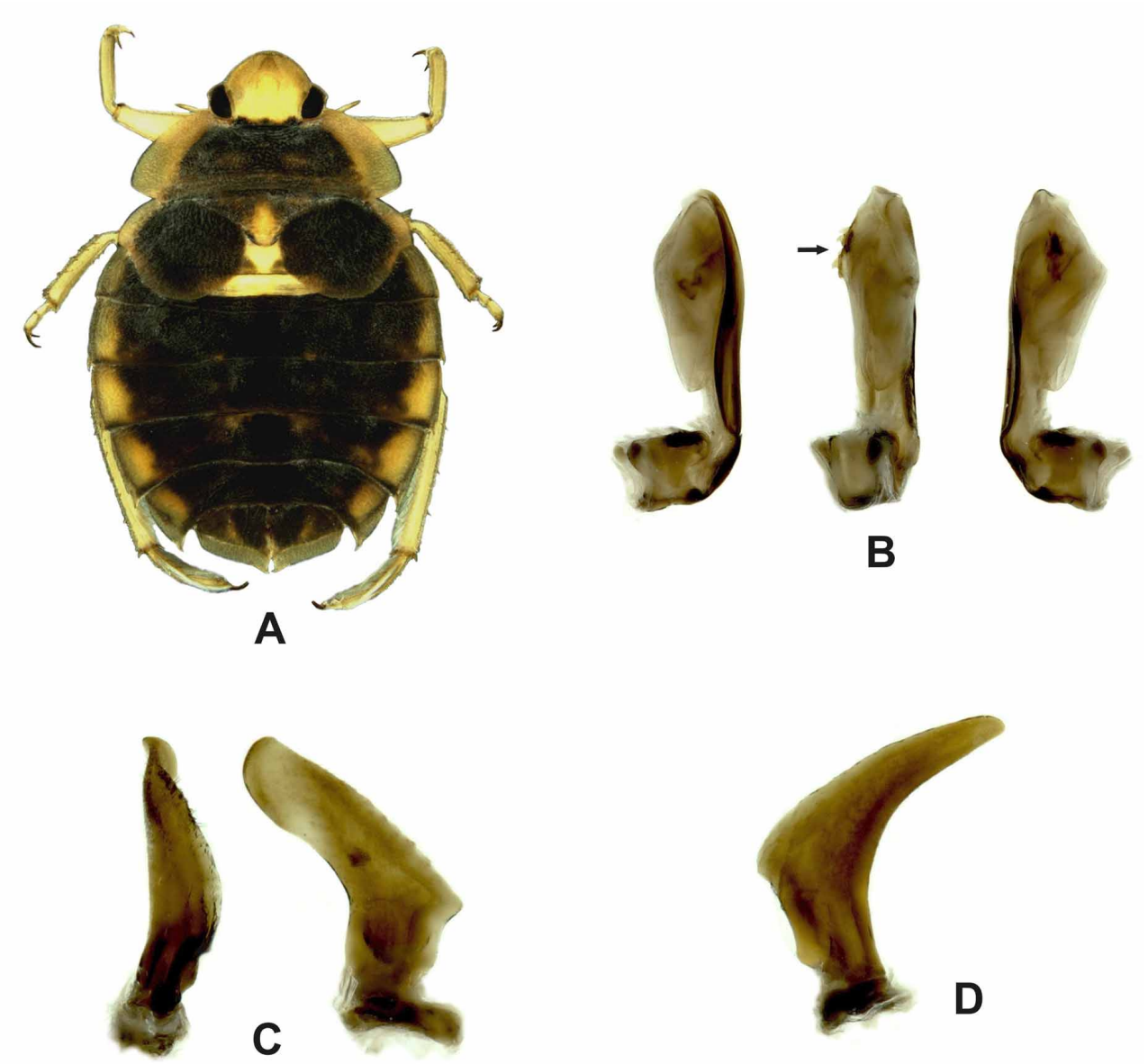


**FIGURE 5.** *Aphelocheirus murcius*. A) habitus; B) penis (ventral and lateral view); C) Left paramere (dorsal and lateral view); D) right paramere.

## Iberian distribution

***A. occidentalis*:** Iberian endemic of north and central Spain and north of Portugal: A Coruña, Ourense, Pontevedra, Lugo, Asturias, León, Madrid, Toledo, Guadalajara, Viana do Castelo and Vila Real (Fig. 8). Table 2 shows the Iberian records of *A. occidentalis*.

**1st record in the Iberian Peninsula:** Nieser & Millán (1989).

***A.murcius*:** Iberian endemic distributed in south and south-east of the Iberian Peninsula: Albacete, Cádiz and Málaga (Fig. 8). Table 3 shows the Iberian records of *A. murcius*.

**1st record for the Iberian Peninsula:** Segura River basin, Nieser & Millán (1989).

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**TABLE 2.** Iberian records of *A. occidentalis*.

| Country | Province | Locality | River | Date | Collected by | U.T.M. | Alt. (m) |
|---|---|---|---|---|---|---|---|
| Spain | Pontevedra | Pontevedra | Almofrei | 29/08/1984 | A. Cordero | 29TNG39 | 40 |
| Spain | Pontevedra | Pontevedra | Fontanes | 02/07/1985 | A. Cordero | 29TNH30 | 160 |
| Spain | Pontevedra | Portas | Umia | 26/07/1986 | A. Cordero | 29TNH21 | 60 |
| Spain | Pontevedra | Portas | Umia | 12/07/1987 | A. Cordero | 29TNH21 | 60 |
| Spain | A Coruña | Sigueiro | Tambre | 10/1983 | J. Murillo | 29TNH45 | 200 |
| Spain | A Coruña | Oroso | Lengüelle | 10/1983 | J. Murillo | 29TNH46 | 200 |
| Spain | Ourense | Viana del Bollo | Bibey | 11/05/1987 | N. Nieser | 29TPG56 | 800 |
| Spain | Lugo | Navia de Suarna | Navia | 20/05/2009 | A. Mellado | 29TPH65 | 350 |
| Spain | Lugo | Navia de Suarna | Lamas | 09/05/2009 | A. Mellado | 29TPH66 | 500 |
| Spain | Lugo | Navia de Suarna | Lamas | 09/09/2009 | A. Mellado | 29TPH66 | 500 |
| Spain | Lugo | Navia de Suarna | Rao | 20/05/2009 | A. Mellado | 29TPH66 | 300 |
| Spain | Asturias | Ibias | Navia | 09/09/2009 | A. Mellado | 29TPH66 | 450 |
| Spain | León | Albares de la Ribera | Boeza | 31/08/1994 | F. García-Criado | 29TQH12 | 700 |
| Spain | León | San Miguel de las Dueñas | Boeza | 31/08/1994 | F. García-Criado | 29TQH01 | 570 |
| Spain | León | Puente de Domingo Florez | Cabrera | 15/06/2005 | D. Miguélez | 29TPG89 | 370 |
| Spain | León | Castroquilame | Cabrera | 16/06/2005 | D. Miguélez | 29TPG89 | 420 |
| Spain | León | Llamas de Cabrera | Cabrera | 16/06/2005 | D. Miguélez | 29TPG99 | 600 |
| Spain | Madrid | Villamantilla | Perales | 1987-1989 | T. López *et al.* | 30TVK06 | 450 |
| Spain | Madrid | El Pardo | Manzanares | 1987-1990 | T. López *et al.* | 30TVK38 | 575 |
| Spain | Guadalajara | Gascueña de Bornova | Bornova | 30/07/2001 | M. Navarro | 30TVL95 | 1000 |
| Spain | Guadalajara | Gascueña de Bornova | Bornova | 21/05/2002 | J.L. Moreno | 30TVL95 | 1000 |
| Spain | Guadalajara | Gascueña de Bornova | Bornova | 08/05/2009 | J.L. Moreno | 30TVL95 | 1000 |
| Spain | Guadalajara | Valverde de los Arroyos | Sorbe | 21/05/2002 | J.L. Moreno | 30TVL85 | 1075 |
| Spain | Guadalajara | Cantalojas | Lillas | 22/05/2007 | F. Picazo | 30TVL76 | 1350 |
| Spain | Toledo | Hontanar | Estena | 25/07/2000 | P. Aguilera | 30SUJ67 | 720 |
| Spain | Toledo | Hontanar | Estena | 08/07/2008 | A. Millán *et al.* | 30SUJ68 | 700 |
| Spain | Toledo | Hontanar | Pusa | 13/05/2002 | J.L. Moreno | 30SUJ57 | 860 |
| Spain | Toledo | Los Navalucillos | Pusa | 09/07/2008 | A. Millán *et al.* | 30SUJ57 | 860 |
| Portugal | Vila Real | Vila Pouca de Aguiar | Corgo | 13/05/1987 | N. Nieser | 29TPF18 | 700 |
| Portugal | Viana do Castelo | Vila Praia de Áncora | Áncora | 08/05/1993 | D.T. Bilton | 29TNG12 | 25 |
| Portugal | Viana do Castelo | Montaria | Áncora | 24/07/1998 | I. Ribera | 29TNG22 | 300 |

***Aphelocheirus sp.*:** Iberian endemic lineage distributed in the north of the Iberian Peninsula: Álava, Guipúzcoa and Burgos (Fig. 8). There is an old record of *A. aestivalis* from Asturias (Fernández, 1982) which could belong to this lineage due to the limestone calcareous nature of river where it was found. However, the material was unable to be reviewed (see discussion section). Table 4 shows the Iberian records of *Aphelocheirus* sp.

**1st record for the Iberian Peninsula:** North basin, Millán *et al*. (2002).

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**TABLE 3**. Iberian records of *A. murcius*.

| Country | Province | Locality | River | Date | Collected by | U.T.M. | Alt. (m) |
|---|---|---|---|---|---|---|---|
| Spain | Albacete | Molinicos | Mundo | 11/10/1982 | C. Montes *et al.* | 30SWH66 | 750 |
| Spain | Albacete | La Alfera | Mundo | 20/03/1983 | C. Montes *et al.* | 30SWH66 | 850 |
| Spain | Albacete | Yeste | Tús | 21/07/1983 | C. Montes *et al.* | 30SWH54 | 780 |
| Spain | Albacete | Yeste | Tús | 22/07/1983 | C. Montes *et al.* | 30SWH55 | 650 |
| Spain | Albacete | La Alfera | Mundo | 30/04/1987 | N. Nieser | 30SWH66 | 750 |
| Spain | Albacete | Yeste | Tús | 27/04/1997 | I. Ribera | 30SWH44 | 820 |
| Spain | Albacete | Yeste | Tús | 19/09/2001 | J.L. Moreno | 30SWH44 | 820 |
| Spain | Albacete | Yeste | Tús | 09/04/2009 | P. Abellán & N. Martínez | 30SWH44 | 800 |
| Spain | Albacete | La Alfera | Mundo | 28/04/2009 | A. Millán & J.A. Carbonell | 30SWH66 | 750 |
| Spain | Albacete | Yeste | Tús | 03/08/2010 | O. Belmar *et al.* | 30SWH44 | 820 |
| Spain | Albacete | Yeste | Tús | 03/08/2010 | O. Belmar *et al.* | 30SWH54 | 780 |
| Spain | Albacete | La Alfera | Mundo | 05/08/2010 | O. Belmar *et al.* | 30SWH66 | 750 |
| Spain | Cádiz | Jerez de la Frontera | Majaceite | verano 2000 | J.C. Salamanca *et al.* | 30STF65 | 130 |
| Spain | Málaga | Algatocín | Genal | 30/08/2007 | A. Castro *et al.* | 30STF94 | 260 |
| Spain | Málaga | San Pedro de Alcántara | Guadaiza | 19/08/2009 | A. Castro *et al.* | 30SUF24 | 300 |

**TABLE 4**. Iberian records of *Aphelocheirus* sp.

| Country | Province | Locality | River | Date | Collected by | U.T.M. | Alt. (m) |
|---|---|---|---|---|---|---|---|
| Spain | Álava | Ozaeta | Barrundia | 18/05/1988 | E. Rico | 30TWN45 | 560 |
| Spain | Guipúzcoa | Aizarnazabal | Urola | 13/06/1988 | E. Rico | 30TWN68 | 20 |
| Spain | Guipúzcoa | Venta de Janoy | Bidasoa | 02/11/1988 | E. Rico | 30TWN68 | 60 |
| Spain | Guipúzcoa | Endarlatza | Bidasoa | 03/11/1988 | E. Rico | 30TXN09 | 5 |
| Spain | Burgos | Espinosa de los Monteros | Trueba | 21/03/2006 | D. Miguélez *et al.* | 30TVN57 | 775 |
| Spain | Burgos | Valle de Sedano | Ebro | 07/07/2008 | D. Miguélez *et al.* | 30TVN34 | 700 |
| Spain | Burgos | Valle de Sedano | Ebro | 07/07/2008 | D. Miguélez *et al.* | 30TVN33 | 650 |
| Spain | Burgos | Valle de Sedano | Ebro | 07/07/2008 | D. Miguélez *et al.* | 30TVN43 | 630 |
| Spain | Burgos | Valle de Sedano | Ebro | 07/07/2008 | D. Miguélez *et al.* | 30TVN44 | 630 |
| Spain | Burgos | Junta de Traslaloma | Ebro | 08/07/2008 | D. Miguélez *et al.* | 30TVN44 | 620 |
| Spain | Burgos | Villarcayo de Merindad | Ebro | 10/07/2008 | D. Miguélez *et al.* | 30TVN54 | 590 |
| Spain | Burgos | Frías | Ebro | 10/07/2008 | D. Miguélez *et al.* | 30TVN73 | 525 |
| Spain | Burgos | Pesquera de Ebro | Ebro | 02/09/2010 | A. Millán *et al.* | 30TVN43 | 650 |

***A. aestivalis*:** Western half of the Palearctic Region except Iberian Peninsula, North Africa, Arabic Peninsula and south-western Asia (Aukema & Rieger 1995) (Fig. 11). All the records from the Iberian Peninsula must to be referred to the species listed above.

## Discussion

These results update current knowledge of the relationships and geographical distributions of the species of the genus *Aphelocheirus* in the Iberian Peninsula which, until now; have been involved in nomenclatural and taxonomic confusion. First, *A. occidentalis* presents clear external differences, such as colour pattern and very long conexives, which permit it to be distinguished easily from other *Aphelocheirus* species. *A. occidentalis* is restricted to the centre and west of the Iberian Peninsula within the Hercynian region (see Ribera, 2000 for details about biogeographic regions in the Iberian Peninsula), and seems to prefer rivers with siliceous substrates.

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On the other hand, *A. murcius* and *A. aestivalis* are both morphologically and ecologically quite similar, and it is more difficult to distinguish between them. Nevertheless, the different left paramere shape and molecular analyses presented in this study confirm the taxonomic validity of *A. murcius*.

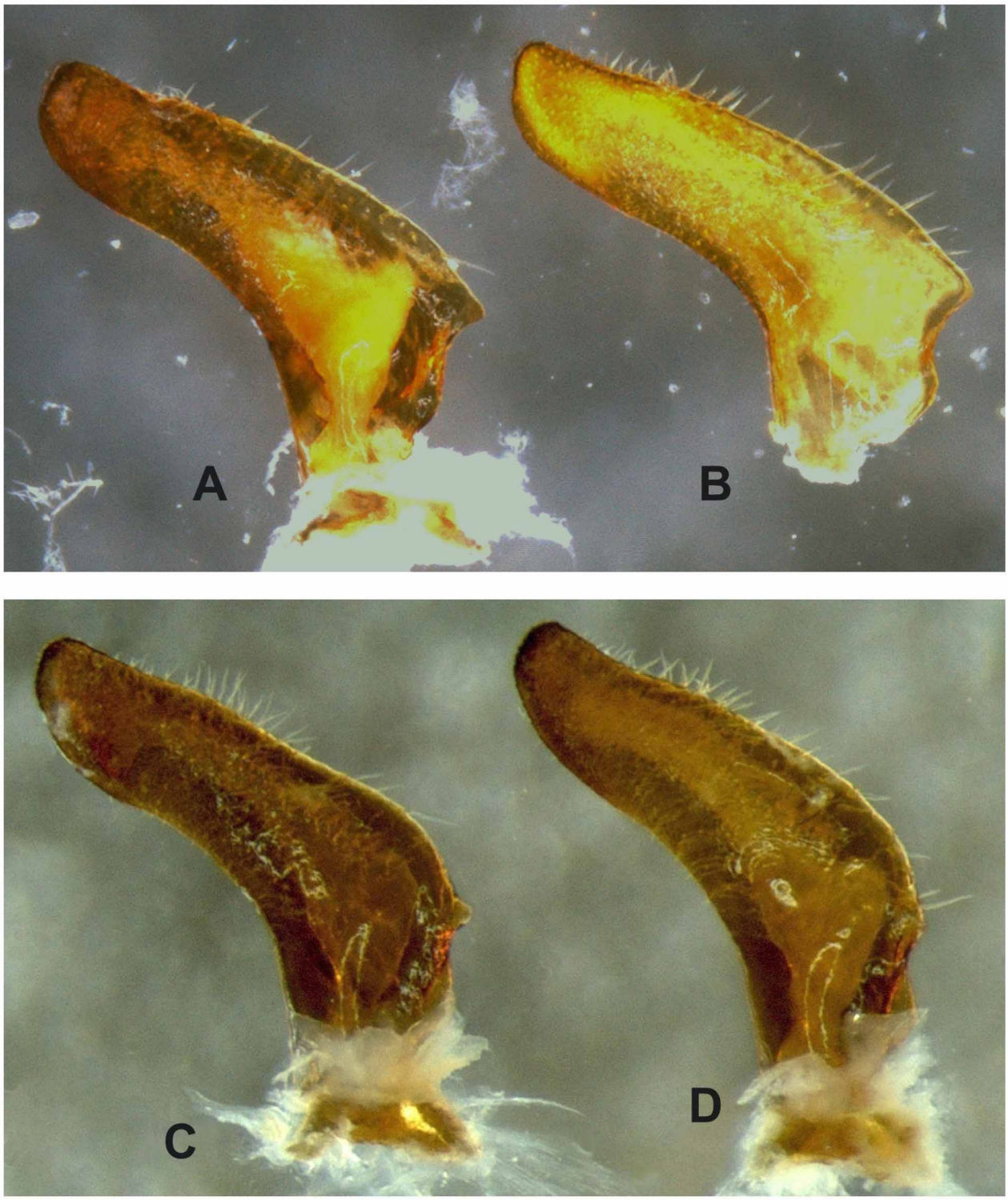


**FIGURE 6.** Left paramere of A) *Aphelocheirus* sp.; B) *A. murcius*; C) *A. aestivalis* (La Manche, France); D) *A. aestivalis* (Corsica).

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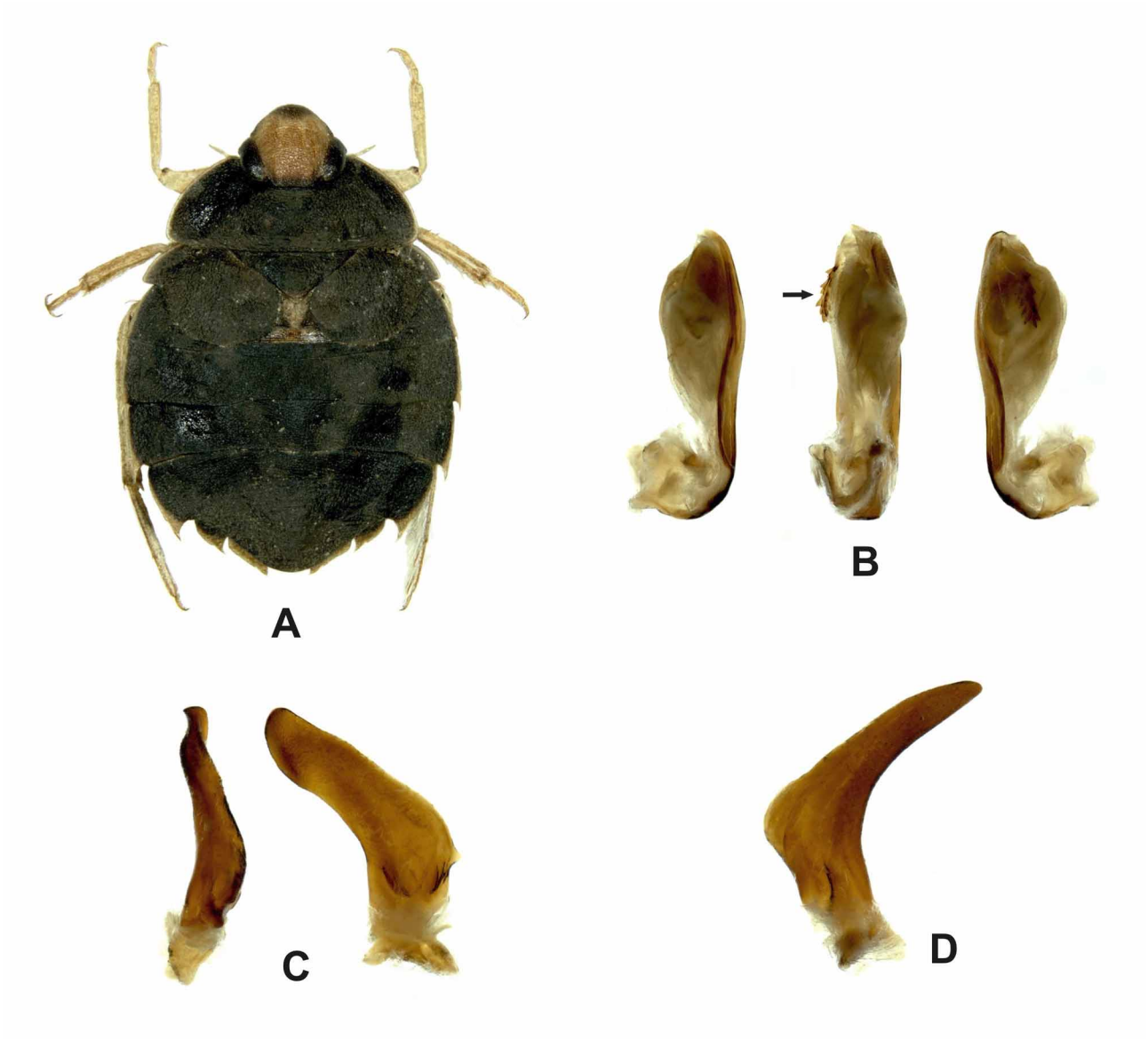


**FIGURE 7.** *Aphelocheirus aestivalis*. A) habitus; B) penis (ventral and lateral view); C) Left paramere (dorsal and lateral view); D) right paramere.

Nieser & Millán (1989) distinguish several morphological differences between both species which were used in the description of *A. murcius*: (i) *A. murcius*: yellowish head and antennae with broad, yellowish lateral bands in thorax and abdomen, slightly pronounced lateral edge of embolium in micropterous, and genitalia with pointed left paramere heel; (ii) *A. aestivalis*: yellowish head and antennae without broad yellowish lateral bands in thorax and abdomen, very pronounced lateral edge of embolium in micropterous, and genitalia with a rounded left paramere heel.

After a comparative morphological analysis of recent material from both species, two common colour patterns in *A. murcius* and *A. aestivalis* were found: (i) yellowish head and antennae, greyish thorax and abdomen with large, yellowish lateral bands and yellowish marks in the pronotum and posterior half of abdomen (Fig. 9A,C,D); and (ii) yellowish head and antennae, dark greyish thorax and abdomen with very thin yellowish lateral bands (Fig. 9B,E); the yellowish marks can extend to dominate the greyish colour. These two patterns are independent of the specimen's sex and are present in the same population. The study of the embolium also did not show differences between the species, since individuals with a very pronounced lateral embolium edge and individuals with a less pronounced edge were found in populations of both species (Fig. 10). Analysis of the genitalia did not show differences in penis or right paramere. However, unambiguous differences were found in the shape of the left paramere

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heel, which was clearly pointed in *A. murcius* and rounded in *A. aestivalis*. This difference was corroborated in all studied individuals, with the exception of one specimen of *A. aestivalis* from the French Manche, which presented a little tooth in the heel of the left paramere (Fig. 6C), although obviously different to those from *A. murcius* (Fig. 6B). Hence, after analysing the morphological characteristics between *A. murcius* and *A. aestivalis*, it was found that most of them are too confusing or inadequate to differentiate between the species. It was observed that only the genitalia, notably the heel shape of the left paramere, presented a clear difference among all studied individuals.

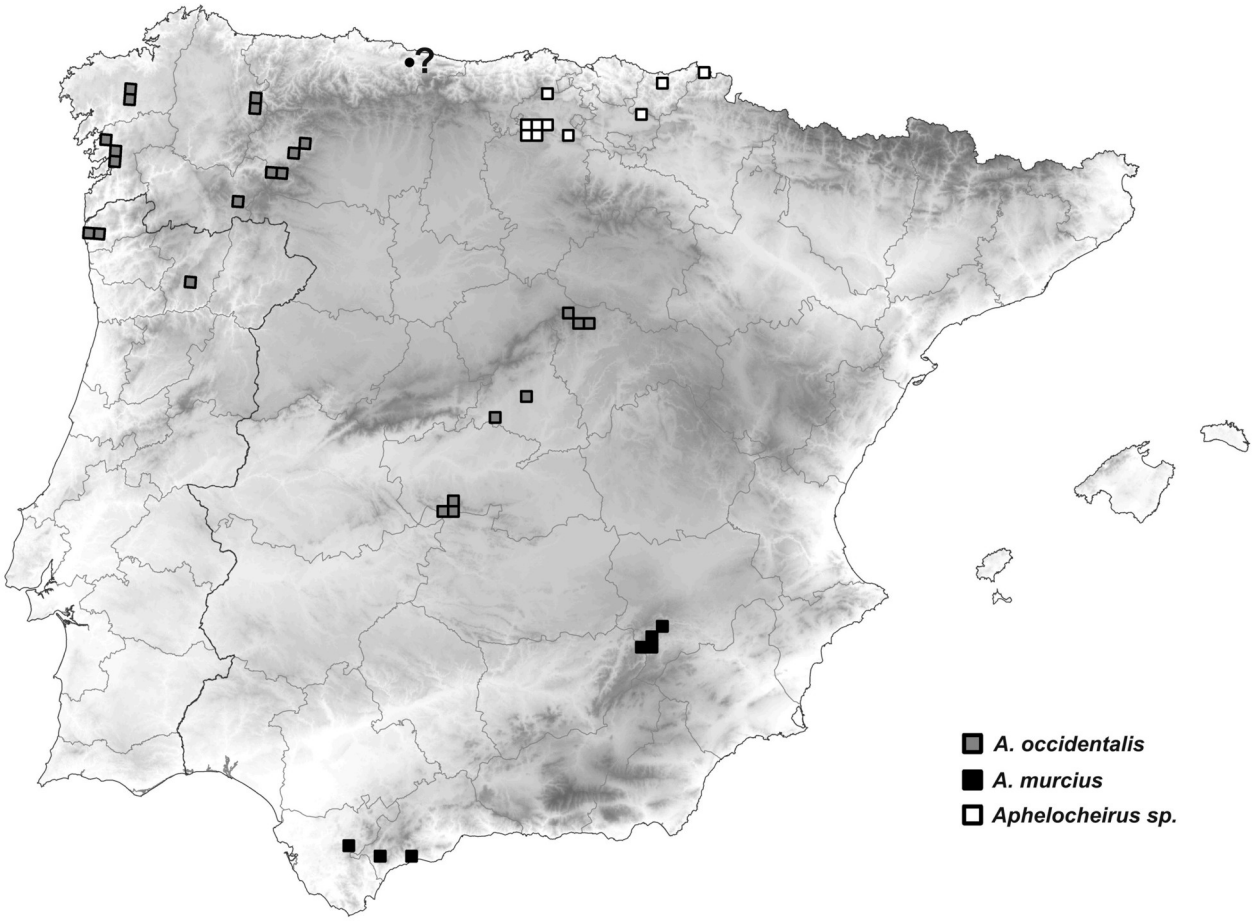


**FIGURE 8.** Distribution map of Iberian species of genus *Aphelocheirus*.

Following to the review of records and study of material of the genus *Aphelocheirus* in the study area, the presence of the species *A. aestivalis* in the Iberian Peninsula must be rejected. The first record of *Aphelocheirus* in the Iberian Peninsula was cited as *A. aestivalis* (Seabra, 1926) and documented in the Tras-os-Montes region of Portugal. This record, and those that followed, such as Seabra (1939) and Murillo (1985), seem to be incorrect. Although material could not be reviewed, according to the specimens analysed in this study in areas near to those referred to by Seabra (1926, 1939) and Murillo (1985), all the records of *Aphelocheirus* from the northwestern Iberian Peninsula may correspond to *A. occidentalis*. Elsewhere in the peninsula, there is no doubt that the revised specimens from the top section of the Segura and Ebro basins, southern Spain and the Basque Country do not belong to *A. aestivalis*. Some doubts exist regarding the identity of an old record of *A. aestivalis* from Asturias (Fernández, 1982) because this material also could not be reviewed. Nevertheless, the limestone nature of the river basin where it was located may indicate the presence of the *Aphelocheirus sp.* lineage in this region (see below). Therefore, *A. aestivalis* occurs in the western half of the Palearctic Region, except in the Iberian Peninsula, North Africa, Arabic Peninsula and southwestern Asia.

Molecular relationships between *A. murcius* and *A. aestivalis* showed three well-supported clades in the Iberian Peninsula (Fig. 11). Southern specimens of *A. murcius* (where this species was described) and those of *A. aestivalis* represent two independent sister lineages, which present a genetic divergence of around 1,5 %. This divergence is similar, for example, to those found among morphologically well-characterised endemic Iberian

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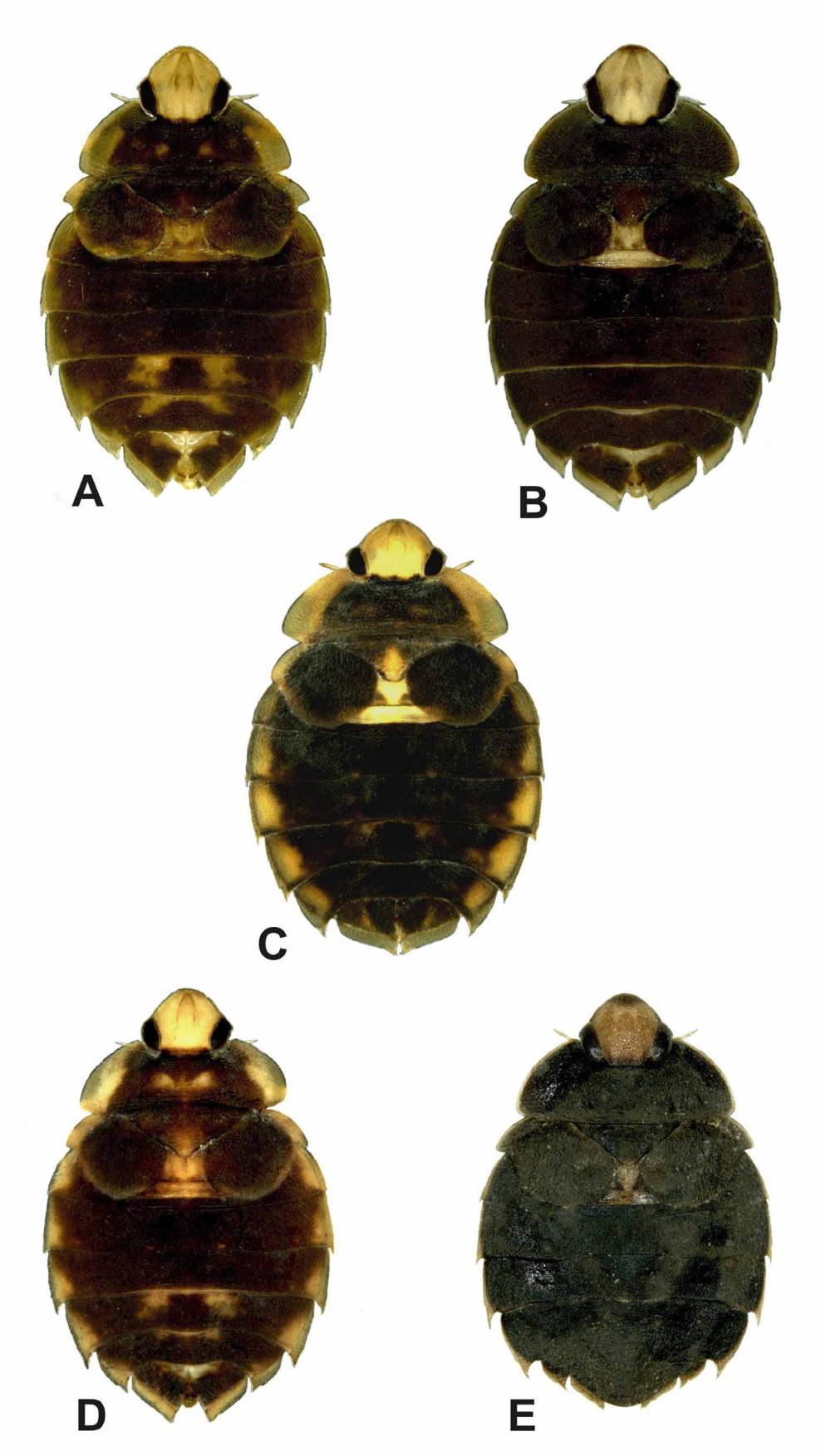


**FIGURE 9.** Color patherns of A,B) *Aphelocheirus sp.* (Ebro river, Burgos); C) *Aphelocheirus murcius* (Mundo river, Albacete); D) *Aphelocheirus aestivalis* (Corsica); E) *Aphelocheirus aestivalis* (La Manche, France).

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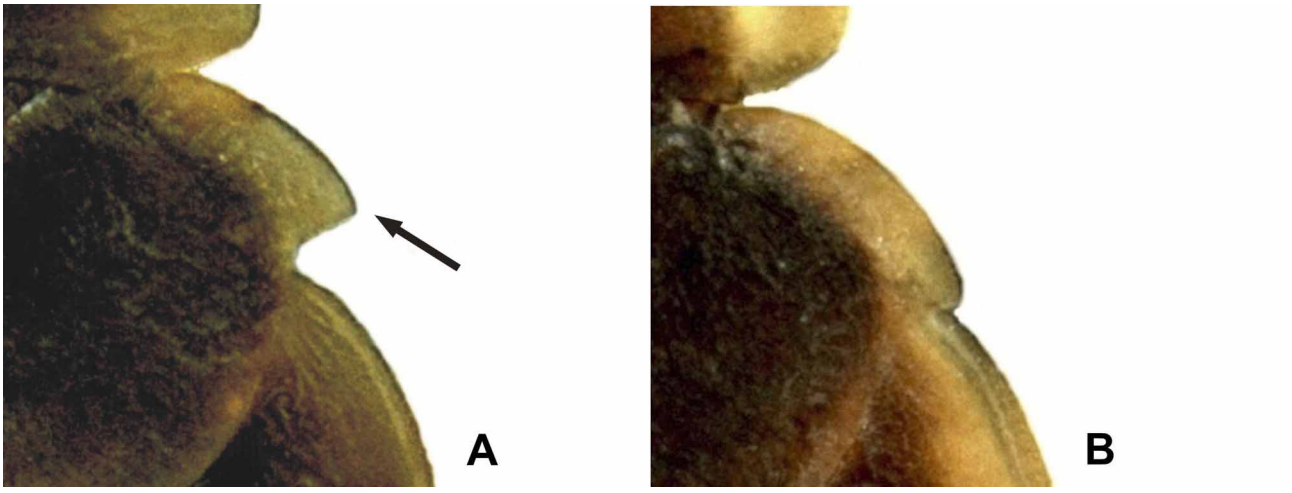


**FIGURE 10.** Lateral edge of embolium. A) strongly pronounced in *A. murcius*; B) weakly pronounced in *A. aestivalis*.

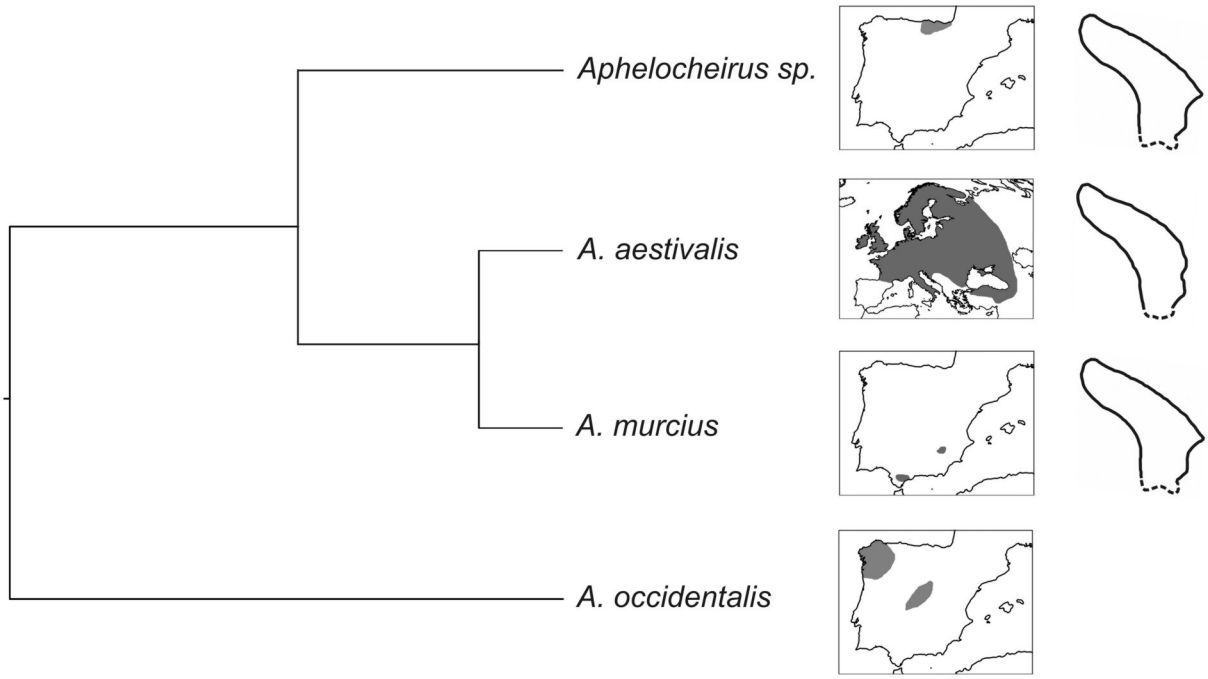


**FIGURE 11.** Phylogenetic relationships, distribution maps and left parameres of *Aphelocheirus sp*., *A. aestivalis*, *A. murcius* and *A. occidentalis* (no left paramere showed).

species of water beetle species (Ribera & Vogler 2004). The genetic differentiation of both species is in agreement with the morphological differences found between them (notably the shape the left paramere), and confirm the taxonomic validity of *A. murcius*.

Unexpectedly, specimens of *Aphelocheirus* from Northern Spain, previously identified as *A. murcius* according to morphology (Carbonell & Millán 2010), formed an independent sister lineage to the clade encompassing *A. murcius* (in southern populations) and *A. aestivalis*, with a divergence of around 5,6 %. Despite the strong genetic divergence of this lineage, consistent morphological differences between specimens within this lineage and those of *A. murcius* (both lineages share the pointed shape of the left paramere) could not be found. In fact, this lack of clear morphological differences between both entities resulted in the assignation of *Aphelocheirus* specimens from the north of the Iberian Peninsula to *A. murcius* in previous studies. Similarly, ecological preferences do not seem to be divergent between the lineages, who occupy similar microhabitats in limestone rivers. Since *A. aestivalis* and *A. murcius* are recognised as separate species, then populations of this lineage can be considered also as a distinct

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species. However, extreme caution should be exercised when describing taxa from molecular differences exclusively (but see Cook *et al*. 2010), and the lack of clear morphological differences, together with the lack of additional genetic data from other molecular markers and information concerning ecological differentiation, prevents us from naming this lineage as a new taxa. The potential taxonomic implications of these results, still difficult to reconcile with our current knowledge of morphological variation within *Aphelocheirus*, will be the focus of future research aided with the analysis of nuclear markers, additional European taxa, and the ecology of the different populations of the species.

Recent studies have considered *A. murcius* and *A. occidentalis* to be endangered in the Iberian Peninsula due to their limited geographical ranges and high habitat specificity (Carbonell & Millán 2010). The assignation of the *Aphelocheirus* specimens from northern Spain to a distinct evolutionary entity suggests a new scenario to describe the distribution and conservation status of the genus *Aphelocheirus* in Iberian Peninsula. As a result, a new endangered Iberian endemism has been incorporated, and, at the same time, the geographical range of *A. murcius* has been reduced. Thus, *A. murcius* would be restricted to the south and southeast of the Iberian Peninsula within the Southeast biogeographical region (see Ribera 2000), and the northern lineage would be restricted to northern Iberia, while both species inhabit rivers with calcareous substrates.

Further studies including phylogenetic (using additional molecular markers like nuclear genes), morphological (focusing on microstructures and internal morphology), physiological, ecological and biogeographical approaches are necessary to understand the origin and evolution of species within the *Aphelocheirus* genus.

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