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DESCRIPTION OF THREE NEW SPECIES OF THE GENUS *ACHONDROSTOMA* ROBALO, ALMADA, LEVI & DOADRIO, 2007 (ACTINOPTERYGII, LEUCISCIDAE) IN THE IBERIAN PENINSULA

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ABSTRACT

Three new species, *Achondrostoma garzonorum* sp. nov., *Achondrostoma asturicense* sp. nov. and *Achondrotoma numantinum* sp. nov. are described on the basis of morphological and genetic characters. *Achondrostoma garzonorum* sp. nov. is restricted to the Cuerpo de Hombre and Alagón rivers (Tajo Drainage, Alagón sub-basin) and Corneja river (Duero Drainage, Tormes sub-basin) in western Spain. *Achondrostoma garzonorum* sp. nov. can be distinguished from other *Achondrostoma* species through a combination of morphometric, meristic and genetic characters: 43-49 ($\bar{x} = 45.9$; $M_d = 46$) canaliculate scales on the lateral line; 6-7 ($\bar{x} = 6.8$; $M_d = 7$) scales above the lateral line; 4-5 ($\bar{x} = 4.4$; $M_d = 4$) scales below the lateral line; a high caudal peduncle in proportion to the body depth; 5-5 pharyngeal teeth, narrow and high coronoid process of dentary and 3 autapomorphies, none of them transversions, in the mitochondrial cytochrome *b* gene. *Achondrostoma asturicense* sp. nov. inhabits the sub-basin of the Esla river in the Duero Drainage of Spain. *Achondrostoma asturicense* sp. nov. can be differentiated from other *Achondrostoma* species through a set of morphometric, meristic and genetic characters: 38-44 ($\bar{x} = 40.9$, $M_d = 41$) canaliculate scales on the lateral line; 5-6 ($\bar{x} = 5.8$, $M_d = 6$) scales above the lateral line; 3-4 ($\bar{x} = 3.1$, $M_d = 3$) scales below the lateral line; a narrow skull; 5-5 pharyngeal teeth; and 13 autapomorphies in the mitochondrial cytochrome *b* gene, one of which is a transversion. *Achondrostoma numantinum* sp. nov. inhabits the Duero Drainage in Spain except for Esla and Sabor sub-basins and Corneja river within the Duero Drainage. *Achondrostoma numantinum* sp. nov. can be distinguished from other *Achondrostoma* species through a combination of morphometric, meristic and genetic characters: 43-48 ($\bar{x} = 45.1$; $M_d = 45$) canaliculate scales on the lateral line; 6-8 ($\bar{x} = 6.9$; $M_d = 7$) scales above the lateral line; 4-5 ($\bar{x} = 4.3$; $M_d = 4$) scales below the lateral line; a wide skull; low caudal peduncle in proportion to the body depth; 5-5 pharyngeal teeth, and one autapomorphy, no transversion, in the mitochondrial cytochrome *b* gene.

Keywords: Taxonomy, Iberian Peninsula, Achondrostoma, Cypriniformes, Leuciscidae, genetics, morphology.

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RESUMEN

Descripción de tres nuevas especies del género *Achondrostoma* Robalo, Almada, Levi & Doadrio, 2007 (Actinopterygii, Leuciscidae) en la Península Ibérica

Se describen tres nuevas especies, *Achondrostoma garzonorum* sp. nov., *Achondrostoma asturicense* sp. nov. y *Achondrotoma numantinum* sp. nov., sobre la base de caracteres genéticos y morfológicos. La distribución de *Achondrostoma garzonorum* sp. nov. se restringe a los ríos Alagón y Cuerpo de Hombre (cuenca del Tajo, subcuenca del Alagón) y Corneja (cuenca del Duero, subcuenca del Tormes), oeste de España. *Achondrostoma garzonorum* sp. nov. se diferencia de otras especies del género *Achondrostoma* que viven en la Península Ibérica por una combinación de caracteres morfométricos, merísticos y genéticos, como 43-49 ($\bar{x} = 45.9$; $M_d = 46$) escamas canaliculadas en la línea lateral; 6-7 ($\bar{x} = 6.8$; $M_d = 7$) escamas por encima de la línea lateral; 4-5 ($\bar{x} = 4.4$; $M_d = 4$) escamas por debajo de la línea lateral, un pedúnculo caudal alto en comparación a la anchura del cuerpo; un alto y estrecho proceso coronoideo del dentario; 5-5 dientes faríngeos y 3 autapomorfias, ninguna de ellas transversiones, para el gen mitocondrial citocromo *b*. *Achondrostoma asturicense* sp. nov. vive en la subcuenca del río Esla dentro de la cuenca del Duero. *Achondrostoma asturicense* sp. nov. se diferencia de otras especies del género *Achondrostoma* que viven en la Península Ibérica por una combinación de caracteres morfométricos, merísticos y genéticos, como 38-44 ($\bar{x} = 40.9$, $M_d = 41$) escamas canaliculadas en la línea lateral; 5-6 ($\bar{x} = 5.8$, $M_d = 6$) escamas por encima de la línea lateral; 3-4 ($\bar{x} = 3.1$, $M_d = 3$) escamas por debajo de la línea lateral, un cráneo estrecho, 5-5 dientes faríngeos y 13 autapomorfias, una de ellas una transversión para el gen mitocondrial citocromo *b*. *Achondrostoma numantinum* sp. nov. vive en los ríos de la cuenca del Duero excepto en las subcuencas de los ríos Esla y Sabor y en el río Corneja. *Achondrostoma numantinum* sp. nov. se diferencia de otras especies del género *Achondrostoma* que viven en la Península Ibérica por una combinación de caracteres morfométricos, merísticos y genéticos, como 43-48 ($\bar{x}$ = 45.1; M_d = 45) escamas canaliculadas en la línea lateral; 6-8 ($\bar{x}$ = 6.9; M_d = 7) escamas por encima de la línea lateral; 4-5 ($\bar{x}$ = 4.3; M_d = 4) escamas por debajo de la línea lateral; cráneo ancho; pedúnculo caudal estrecho en comparación con la anchura del cuerpo; 5-5 dientes faríngeos, y una autapomorfia, no transversión, para el gen mitocondrial citocromo *b*.

Palabras clave: Taxonomía, Península Ibérica, Achondrostoma, Cypriniformes, Leuciscidae, genética, morfología.

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Introduction

The Iberian Peninsula harbours a large number of local freshwater fish endemisms, mainly belonging to the family Leuciscidae. Close to 80% of the species of this family inhabiting the Iberian Peninsula are local endemisms (Doadrio, 2002; Doadrio *et al.*, 2011). A salient feature of the Iberian freshwater fish fauna is the presence of few lineages with numerous species per lineage. This is the case for the lineage corresponding to the former genus *Chondrostoma*, which is comprised of more than 30 species widespread throughout Europe and near Asia, of which 15 are endemic of the Iberian Peninsula (Doadrio & Carmona, 2003a; Coelho *et al.*, 2005; Robalo *et al.*, 2005a, 2005b; Doadrio *et al.*, 2011). In a revision of the genus *Chondrostoma*, Robalo *et al.* (2007) identified six major clades well supported both by morphological and genetic characters, and recognized five new genera. Three of these genera are Iberian endemics (*Achondrostoma*, *Iberochondrostoma* and *Pseudochondrostoma*) while a fourth (*Parachondrostoma*) is mostly Iberian (eastern Spain) but with a single species distributed mainly in France (*P. toxostoma*). These lineages separated approximately 11 MYA and most radiation occurred in the Iberian Peninsula (Doadrio & Carmona, 2004; Robalo *et al.*, 2007).

The genera *Parachondrostoma* and *Pseudochondrostoma* contain large-sized potamodromous species (Doadrio, 2002). For this reason, their populations tend to be more uniform and less genetically structured. In contrast, species of the *Iberochondrostoma* and *Achondrostoma* genera are small sedentary fish and thus different sections of a single drainage show genetic differentiation (Doadrio, 2002; Corral-Lou *et al.*, 2021). This situation has complicated the taxonomy of these genera, and new species continue to be described (*I. lusitanicum* Collares-Pereira, 1980; *I. oretanum* Doadrio & Carmona, 2003b; *I. almacai* Coelho, Mesquita & Collares-Pereira, 2005; *A. oligolepis* Robalo, Doadrio, Almada & Kottelat, 2005; *A. occidentale* Robalo, Almada, Sousa, Moreira & Doadrio, 2005; *A. salmantinum* Doadrio & Elvira, 2007; *I. olisiponense* Gante, Santos & Alves, 2007) including some awaiting formal description (Robalo *et al.*, 2007).

Taxonomic problems in the genus *Achondrostoma* had already been detected in a molecular study by Zardoya & Doadrio (1998). These authors found that the two species recognized at the time in this clade, *A. arcasii* and *A. oligolepis* (= *C. macrolepidotum*) did not form reciprocally monophyletic groups. Some populations of *A. arcasii* were closer to *A. oligolepis*, indicating that *A. arcasii* was a polyphyletic entity that required further examination. In subsequent phylogenetic analyses of fishes of the genus *Achondrostoma* (Robalo *et al.*, 2006, 2007), the authors were repeatedly faced with the same problem, and *A. arcasii* was noted to comprise at least six different lineages that had been grouped under the same name (Doadrio *et al.*, 2021). Moreover, these lineages occupy distinct geographical areas and the divergence time among them has been estimated to vary from 2 to 7 MYA, depending on the pair of clades compared (Robalo *et al.*, 2006, 2007).

In this paper, three new species are described corresponding to populations previously ascribed to *A. arcasii* from Western Spain (Tajo and Duero drainages), Northwestern Duero Drainage (Esla sub-basin) and Central-Eastern Duero Drainage. Besides being geographically distant, these three locations present high morphological and genetic differentiation in their *A. arcasii* populations.

Material and methods

The morphometric and meristic study of *Achondrostoma arcasii* was based on the analysis of

56 individuals from Western Spain: 26 from Alagón River (Tajo Drainage), El Tornadizo, Salamanca and 30 from the Corneja River (Duero Drainage), San Bartolomé de Corneja, Ávila. Ninety-nine individuals from Central-Eastern Duero: 32 from Cega River Lastras de Cuellar, Segovia; 21 from Pedro River, Cuevas de Ayllón, Soria, 20 from Voltoya River, 6 individuals from Labajos, Ávila and 14 individuals from Urraca Miguel, Ávila and 26 from Odra River, Villasendino, Burgos. Thirty-seven individuals from Northwestern Duero: 21 from Tera River, Ribadelago, Zamora and 16 of Peces and Garandones lakes, Galende, Zamora. One hundred eighteen individuals of *A. arcasii* specimens from Mediterranean basin: 60 from Ebro Drainage: 25 from the Zirauntza River, Egino, Alava and 35 from the Queiles River, Tulebras, Navarra; 28 individuals from Mijares Drainage, Mijares River, Olba, Teruel and 30 from Turia Drainage, Alfambra River, Aguilar de Alfambra (Teruel) and 2 syntypes deposited in the collections of the Naturhistorisches Museum Wien NMW 50646:1,5 from the Queiles River, Tudela. Thus, a total of 310 specimens of *A. arcasii*: 56 from Western Spain; 99 from Central-Eastern Duero; 37 from Northwestern Duero and 118 from Mediterranean basin were studied to the morphometric analyses.

The material analyzed to the osteological study is showed in Appendix 1.

Holotypes and paratype series of the three new species have been deposited in the Museo Nacional de Ciencias Naturales (MNCN-CSIC Spain).

Morphology

Twenty-three morphometric measurements (in mm) and ten meristic variables were recorded from digital photographs using TpsDig v.1.4 (Rohlf, 2003). The following abbreviations were used for morphometric and meristic characters: TL, total length; SL, standard length; HL, head length; HH, head high; PrOL, preorbital length; ED, eye diameter; PsOL, postorbital length, IO, interorbital distance; PrDD, predorsal distance; PrPD, prepectoral distance; PrVD, preventral distance; PrAD, preanal distance; CPL, caudal peduncle length; APL, anal peduncle length; DFL, dorsal fin length; DHL, dorsal fin height; PFL, pectoral fin length; VFL, ventral fin length; AFL, anal fin length; AHL, anal fin height; CFL, caudal fin length; BD body depth; BLD body least depth; LLS, lateral line scale rows; SRA, scale rows above lateral line; SRB, scale rows below lateral line; D, dorsal fin rays; A, anal fin rays; P, pectoral fin rays; V, ventral fin rays; C, caudal fin rays; RPT, right pharyngeal teeth; LPT, left pharyngeal teeth; Vr, Vertebrae. After constructing the measurement matrix, Burnaby's method was used to correct for size effect. The Burnaby method removes the effects of a within population size-factor from between-group morphometric analyses through an orthogonal projection procedure (Burnaby, 1966). All analyses were conducted with the corrected matrix. Morphometric and meristic characters were analysed independently. To identify the variables that contributed most to the variation among populations, one principal component analyses (PCA) were performed using the covariance matrix for morphometric characters. Statistical analyses were carried out using PAST software (Hammer *et al.*, 2001).

Osteological characteristics were investigated through of cleared and stained specimens (Taylor, 1967); X-ray computer tomography (CT) scan and digital dissection using VGStudio MAX v2.2 (Volume Graphics, http://www.volumegraphics.com) and dry skeletons preserved in the MNCN collections.

Additionally, we increased the samples of pharyngeal teeth through dissection of preserved alcohol specimens (Appendix 1).

Due to osteological differences found in the wide of the skull, we took the minimum interorbital distance to a subset of n = 78 with a digital caliper of 0.1 mm of precision.

Institutional acronyms: MNCN Museo Nacional de Ciencias Naturales; NMW Naturhistorischen Museum Wien.

Genetic analyses

141 specimens from different populations of *Achondrostoma arcasii*, 44 specimens of *A. oligolepis,* 17 specimens *A. occidentale* and 2 specimens of *A. salmantinum* were analysed throughout their geographic ranges (Appendix 1). In these analyses, we examined sequences of the entire mitochondrial cytochrome b gene (*MT-CYB*), using previously published data (Robalo *et al.*, 2006, Doadrio *et al.*, 2021) deposited in GenBank and new sequences data obtained from the DNA and Tissue Collection at the National Museum of Natural Sciences of Madrid (MNCN–CSIC). The new sequences data has been deposited in the GenBank data base (Appendix 2).

Total genomic DNA was extracted from fin-clip tissue using the Qiagen DNeasy® Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA), following the manufacturer's protocol. For each specimen, the complete *MT-CYB* region (1140 bp) was amplified. Primers and protocols used for PCR for *MT-CYB* amplification followed Corral-Lou *et al.* (2019). PCR products were purified with ExoSAP-IT (USB Cleveland, OH, USA) and then sequenced on a 3730xl DNA Analyzer by Macrogen Europe Inc. (http://www.macrogen.com). Two different phylogenetic analyses were performed using Bayesian inference (BI) implemented in MrBayes v. 3.2 (Ronquist *et al.*, 2012) and Maximum Likelihood implemented in the IQ-tree online web server from the Vienna University (http://iqtree.cibiv.univie.ac.at/; Trifinopoulus *et al.*, 2016). ModelFinder, implemented in the previous IQ-Tree web server (Kalyaanamoorthy *et al.*, 2017) and the Bayesian Information Criterion (Schwarz, 1978) were used to estimate the evolutionary model that best fitted the data. The selected evolutionary model was TN+F+G4. The Bayesian analysis was performed with two simultaneous independent runs each with four Markov chain Monte Carlo (MCMC), which were run for 5×10^7 generations. The first 25% of generations were removed as *burn-in*. Posterior probability (pp) values were used to assess the reliability of the phylogenetic hypothesis. The accuracy of the Maximum Likelihood phylogeny was evaluated with the UltraFast Bootstrap method (1000 replicates) (Minh *et al.*, 2013). Two sequences of *Achondrostoma salmantinum* were used as outgroup (Appendix 2). Uncorrected p-distances among *Achondrostoma* populations were calculated for the *MT-CYB* gene using Mega X (Kumar *et al.*, 2018). Only nodes with a posterior probability (pp) of 0.95 and bootstrap of 70, or higher, in at least one analysis are considered as statistically supported.

To assess the phylogeographic structure among the haplotypes which are present in the morphological groups studied (Western Spain, Central-Eastern Duero, Northwestern Duero and Mediterranean drainages) and in the species of the genus *Achondrostoma*, we reconstructed haplotype networks using the Median-joining algorithm (Bandelt *et al.*, 1999) as implemented in the program *Pop*Art (Leigh & Bryant, 2015).

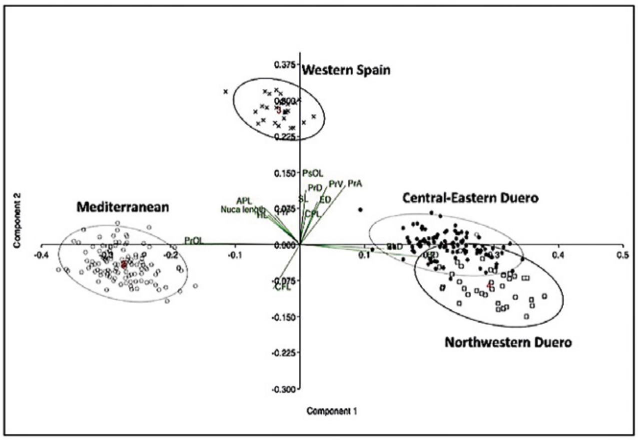

Fig. 1.– Variables that most contributed to the PC 1 (PrOL, PsOL, BD) and PC 2 (PsOL). Symbols: Squares Northwestern Duero drainage population, Esla sub-basin. Dots, Central-Eastern Duero drainage populations. X, Populations from Western Spain, Corneja (Duero) and Alagón (Tajo) subdrainages. Circles, populations from the Mediterranean drainages Ebro and levantine drainages (Mijares and Turia). Abbreviations are defined in Materials and Methods.

Fig. 1.– Variables que más contribuyen al ordenamiento en el PCA. Simbolos: Cuadrados, población del Noroeste del Duero, subcuenca del Esla. Puntos, poblaciones del centro y este del Duero. Equis, poblaciones del oeste de España, subcuencas del Corneja (Duero) y Alagón (Tajo). Círculos, poblaciones del mediterráneo, cuenca del Ebro y Levante (Mijares y Turia). Las abreviaturas están descritas en el epígrafe de material y métodos.

Results and discussion

Comparison of morphology among populations

Due to the sexual dimorphism of *Achondrostoma* (Rojo & Ramos, 1987), we removed fin size to subsequent morphological analyses.

The principal component analysis divided the populations of *A. arcasii* into three groups corresponding to populations of a) Mediterranean basin (Ebro, Mijares and Turia drainages), b) Western Spain that included Alagón River in the Tajo Drainage and Corneja River in the Duero Drainage and c) Central-Eastern and Northwestern Duero that included all the rivers from Duero Drainage except for the Corneja River. The populations of the Central-Eastern and Northwestern Duero were considered in the same group due to the overlapping of their individuals in the principal component analyses (Fig. 1).

Nevertheless, the population of the Northwestern Duero could be discriminated from the other studied populations by the lesser number of scales on the body. The eigenvalues of the three first principal components, with the Burnaby-corrected matrix, explained most of the variance (Appendix 3). The highest values for eigenvectors and, consequently, the variables that contributed most to the ordination in the PCA were: preorbital length, postorbital length, body depth and anal peduncle length (Appendix 3).

The Mediterranean populations exhibited smaller preorbital length with respect to the other studied populations and values of the postorbital length similar to those from Western Spain populations and therefore the ratio PsOL/PrOL in Mediterranean populations was the highest (Appendix 4; Fig. 2). The body depth was smaller in Western Spain than in the other studied populations, the ratio SL/BD was the highest (Appendix 3; Fig. 2) and the ratio BD/BLD the lowest (Appendix 4; Fig. 2). The Mediterranean populations had a caudal peduncle short and high and had the greater values to body least depth and lesser to the length of the anal peduncle and therefore the ratio APL/BLD, was the smallest (Appendix 4; Fig. 2). The head of the populations from Central-Eastern Duero was smaller and had the highest values in proportion to the standard length (Appendix 4; Fig.2).

The number of scales in the lateral line was lesser in populations of the Mediterranean drainages ($\bar{x}$ = 41.1 37-43 n = 63) and in those of the Northwestern Duero, mainly Esla sub-basin ($\bar{x}$ = 40.9 38-44 n = 36) than in the populatons of Western Spain ($\bar{x}$ = 45.9 43-49 n = 66) and Central-Eastern Duero Drainage ($\bar{x}$ = 45.1 43-48 n = 99) (Fig. 3). Scales number above of lateral line was lesser in Northwestern Duero ($\bar{x}$ = 5.8 5-6 n = 36) than in the other populations from Mediterranean drainages ($\bar{x}$ = 7.1 6-8 n = 63); Western Spain ($\bar{x}$ = 6.8 6-7 n = 66) and Central-Eastern Duero Drainage ($\bar{x}$ = 6.9 6-8 n = 99). Also it was lesser the scales number below of lateral line in Northwestern Duero

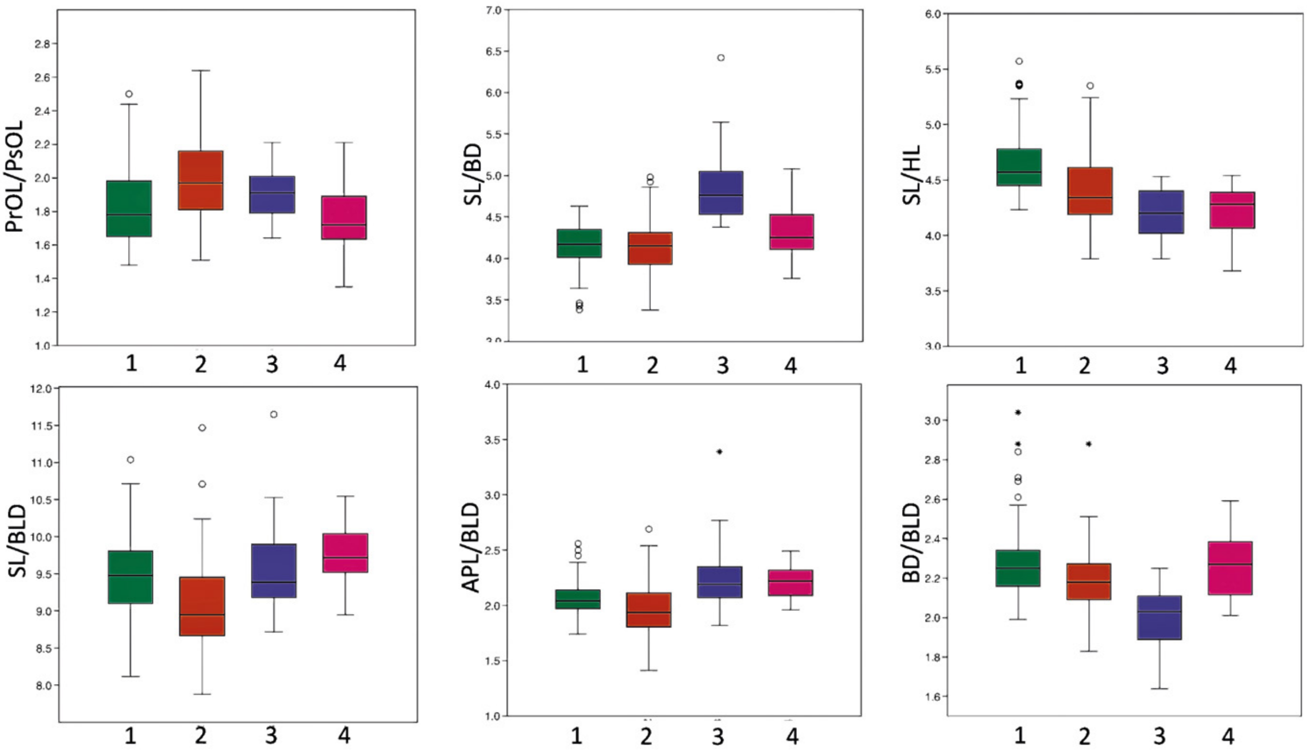

Fig. 2.– Results of the ratios of morphometrics variables that most contributed to differences between populations. Numbers in the X axis correspond to the following populations: 1, Central-Eastern Duero; 2, Mediterranean; 3, Western Spain; and 4, Northwestern Duero. Acronyms are defined in the Material and Method section.

Fig. 2.– Resultados de las proporciones entre las variables morfométricas que más contribuyen a la diferenciación entre las poblaciones: 1, Centro y Este del Duero; 2, Mediterráneo; 3, Oeste de España; y 4, Noroeste del Duero. Los acrónimos de las variables son definidos en el epígrafe de material y métodos.

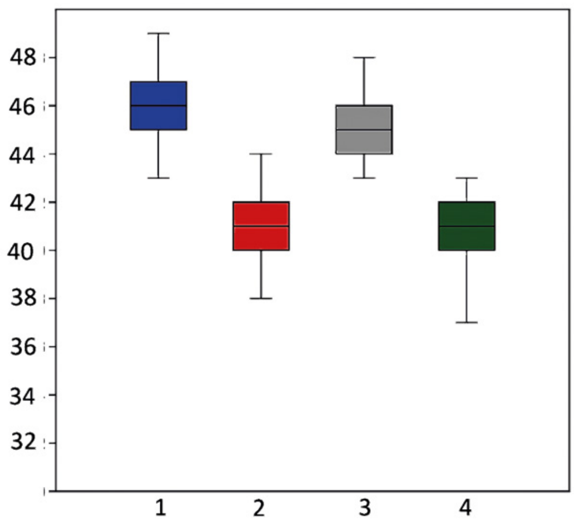

Fig. 3.– Number of caniculate scales in the lateral line in the different populations: 1 Western Spain; 2 Northwestern Duero; 3 Central-Eastern Duero and 4 Mediterranean.

Fig. 3.– Número de escamas caniculadas en la línea lateral para las diferentes poblaciones: 1 Oeste de España; 2 Noroeste del Duero; 3 Centro y Este del Duero y 4 Mediterráneo.

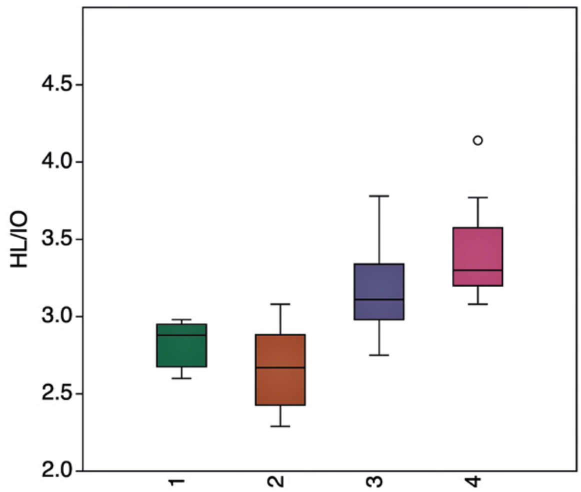

Fig. 4.– Results of the ratio Head Length/Interorbital Distance. Numbers in the X axis correspond to the following populations. 1 Central-Eastern Duero; 2 Mediterranean; 3 Western Spain and 4: Northwestern Duero.

Fig. 4.– Resultados de la proporción entre longitud de la cabeza y la anchura interorbitaria. 1 Centro y Este del Duero; 2 Mediterráneo; 3 Oeste de España y 4 Noroeste del Duero.

($\bar{x}$ = 3.1 3-4 n = 36) than in other populations from Mediterranean drainages ($\bar{x}$ = 4.6 4-5 n = 63); Western Spain ($\bar{x}$ = 4.4 4-5 n = 66) and Central-Eastern Duero Drainage ($\bar{x}$ = 4.2 4-5 n = 99).

Osteology features (Appendix 5)

The smaller snout of Mediterranean populations is more evident in a lateral view of the skull in comparison with other populations (Appendix 5.1)

Table 1.– Diagnostic morphological characters of the four populations studied of *Achondrostoma*.

Tabla 1.– Caracteres morfológicos diagnósticos de las cuatro poblaciones estudiadas de *Achondrostoma*.

	Mediterranean	**Northwestern Duero**	**Central-Eastern Duero**	**Western Spain**
Pharyngeal teeth	5-4	5-5	5-5	5-5
Pharyngeal shape	Hooked teeth	Knife-shaped	Knife-shaped	Knife-shaped
Nº Scales Lateral Line	37-43	38-44	43-48	43-49
Nº Scales above lateral Line	6-8	5-6	6-8	6-7
Nº Scales below Lateral Line	4-5	3-4	4-5	4-5
Skull	Wide	Narrow	Wide	Narrow
Coronoid process	Intermediate	Narrow	Narrow	Wide
Caudal peduncle	short	large	short	large
Head Length	intermediate	large	short	large
Preorbital Length	short	large	large	short
Body Deep	high	low	high	low

Opercular is in Mediterranean populations higher and narrower than in other populations.

An unexpected result of the osteological analyses was the clear differentiation of the population from Northwestern Duero that presented a narrower skull (Appendix 5.2). The results of the index Head Length/ Interorbital Distance shown the Northwestern Duero population with higher values ($\bar{x}$ = 3,4 n = 16) relative to other populations of Duero ($\bar{x}$ = 2.99, n = 5), Mediterranean populations ($\bar{x}$ = 2.7 n = 34) and more similar to the population of Western Spain ($\bar{x}$ = 3.2 n = 23) (Fig. 4). Mediterranean populations had a skull shorter and wider that other populations studied.

Pharyngeal teeth were different in form and number in Mediterranean populations with respect to other populations. The number of teeth in Mediterranean populations was five teeth on the left side and four on the right side, occasionally 5/5 or 4/4 (RPT $\bar{x}$ = 4.1; LPT $\bar{x}$ = 4.9; n = 161). In all the other populations, Western Spain, Central-Eastern Duero and Northwestern Duero the most frequent number was 5/5 occasionally 5/4 or 5/6 (RPT $\bar{x}$ = 4.9; LPT $\bar{x}$ = 5.01; n = 183).

The shape of the teeth was also different between populations. In Mediterranean populations teeth were more robust with a conspicuous hook at the end of the crown and with a more reduced masticatory surface. In the rest of populations, teeth were like *Pseudochondrostoma* and *Parachondrostoma* thinner than the ones of Mediterranean populations and with knife shape (Appendix 5.3).

The posterior process of the basioccipital bone was thinner in Mediterranean populations (Appendix 5.4)

The coronoid process of the dentary was higher and thinner in population of the Northwestern Duero while the anterior process of the dentary was longer in Mediterranean populations (Appendix 5.5).

Table 1 provides a summary of the differences observed among the species.

Genetics

Phylogenetic analyses based on the *MT-CYB* gene were congruent with previous studies and supported two main clades in the phylogenetic tree (Doadrio *et al.*, 2021). Clade I corresponded to *A. occidentale* and populations from Northwestern Duero and Clade II to *A. oligolepis* and the rest of populations (Fig. 5). Within Clade II two sub-clades were well supported, one including populations from Alagón and Cuerpo de Hombre rivers (Tajo Drainage) and Corneja river (Duero Drainage), and a second one grouping populations from Mediterranean drainages clustered together with populations from the Central-Eastern area from Duero Drainage.

Genetic patristic distances based on the *MT-CYB* (Appendix 6) between Northwestern

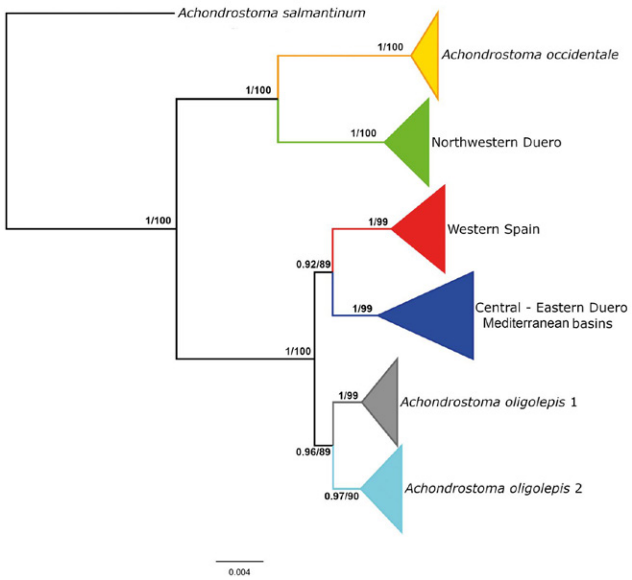

Fig. 5.– Phylogenetic tree rendered by Maximum Likelihood and Bayesian Inference based on the mitochondrial cytochrome b gene. Numbers on branches indicate posterior probability (before dash) and bootstrap (after dash) values.

Fig. 5.– Árbol filogenético del gen mitocondrial citocromo b obtenido a partir de análisis de Máxima Verosimilitud e Inferencia Bayesiana. Los números sobre las ramas indican valores de probabilidad posterior (delante del guión) y los valores de bootstrap (después del guión).

Duero (mainly Esla sub-basin) and the remaining *Achondrostoma* analyzed populations ranged from 4.1% (*A. occidentale*) to 7.9% (*A. salmantinum*). Populations from Alagón, Cuerpo de Hombre and Corneja rivers showed genetic patristic distances ranging from 2.2-2.8 % (*A. oligolepis,* populations of the Mediterranean drainages and Central-Eastern Duero drainages) to 0.6-7.7% (*A. occidentale* and *A. salmantinum* respectively). The genetic distances estimated in the analyzed populations for the *MT-CYB* gene fell within the range found for other leuciscins and cyprinid species (Madeira *et al.*, 2005; Pérez-Rodríguez *et al.*, 2009; Ghanavi *et al.*, 2016; Jouladeh-Roudbar *et al.*, 2017).

Patristic distances between populations from Central-Eastern Duero with respect to Mediterranean drainages were very low and ranged between 0.4 to 0.7% of divergence (Appendix 6).

When we examined the haplotype network none of the haplotypes were shared between groups: Mediterranean (Ebro and Spanish_Levant); Central-Eastern Duero; Western Spain (Corneja, Duero Drainage, Cuerpo de Hombre, Tajo Drainage and Alagón Tajo Drainage and Northwestern Duero. Central-Eastern Duero population had two different groups of haplotypes, one closer to Mediterranean but other more distant to those populations, with divergence based on uncorrected genetic distance around of 1.5% (Fig. 6).

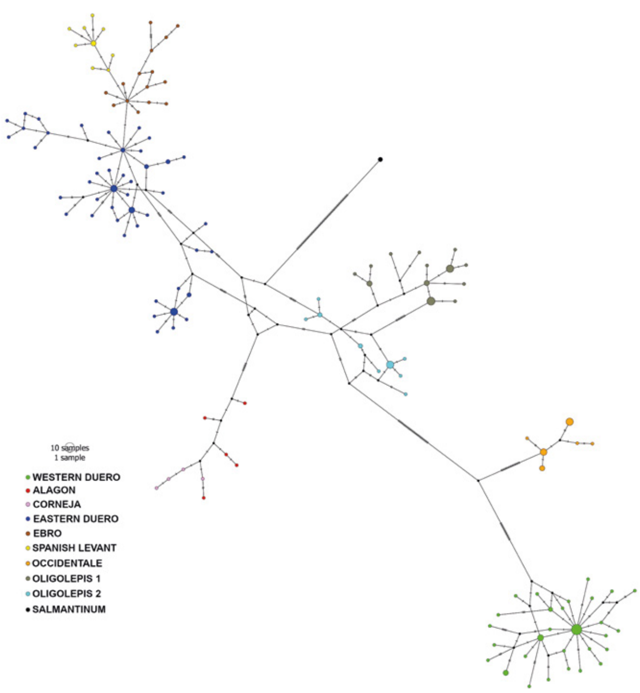

Fig. 6.– Haplotype network of *Achondrostoma* populations based on *MT-CYB* gene. Bars reflect mutational steps between haplotypes. Each circle represents a haplotype, and its size is proportional to its frequency.

Fig. 6.– Red de haplotipos de las poblaciones de *Achondrostoma* basado en el gen *MT-CYB*. Las barras representan los pasos mutacionales entre haplotipos. Cada circulo representa un haplotipo y es proporcional a su frecuencia.

Several processes such as presence of ancient mitochondrial captures, as consequence of hybridization, or retention of ancestral polymorphism could explain the difficulty of mitochondrial DNA to delimitate two different species clearly recognizable by morphological traits (Huber *at al.*, 2008; Perea *et al.*, 2016). Probably, this is the reason of non-correspondence between genetic and morphological differences in Atlantic and Mediterranean populations of the eastern area of Iberian Peninsula. The Mediterranean populations were the most morphologically divergent of all the other populations studied, but presented the smallest genetic distances with respect to the population of the Central-Eastern Duero.

In this study we describe populations corresponding to the populations from the Northwestern Duero, Central-Eastern Duero and Western Spain.

Numerous syntypes of *A. arcasii* are listed in the Naturistorishche Museum of Wien (NMW) from the Duero and Ebro drainages. But the species was only described with individuals from the Ebro Drainage, in the Ebro River near Logroño (La Rioja) and in the Queiles River in Tudela (Navarra) (Steindachner, 1866). Therefore, only individuals from these two localities should be considered as true syntypes. We studied a large series of individuals from the Queiles River near Tudela (see Materials section) and the two syntypes from the same locality (NMW 50646:1.5).

In consequence, *A. arcasii* should be referred only to the populations from Ebro and Levantine drainages (Mijares, Turia and Palancia drainages). We propose the other three differentiated populations (Central-Eastern Duero, Western Spain and Northwestern Duero) to be considered as three new species.

Description of *Achondrostoma* populations

The high degree of morphological and genetic differentiation of *Achondrostoma* populations endemic to Central-Eastern Duero, Western Spain and Northwestern Duero drainages justify the consideration of these population as distinct species. No available names for these populations exist, and therefore these are described as new species in the present study.

Achondrostoma garzonorum sp. nov.

urn:lsid:zoobank.org:act:99188350-392F-4D89-AD8E-E83ECB13E927

Fig. 7, Table 2

Material studied

Holotype: MNCN_ICTIO 293737 69.8 mm SL, 93 mm TL; Alagón River, Tajo Drainage, El Tornadizo, Los Pasiles, Salamanca, Spain, 40°32’34.3”N 5°51’23.1”W, 887 m.a.s.l., Leg. S. Perea, P. Garzón-Heydt, T. Nester and I. Doadrio, 23.III.2019.

Fig. 7.– Holotype of *Achondrostoma garzonorum* sp. nov. from the Alagón River, Tajo drainage. El Tornadizo, Salamanca, Spain. MNCN_ICTIO 293737. SL = 69.8 mm.

Fig. 7.– Holotipo de *Achondrostoma garzonorum* sp. nov. del Río Alagón, Cuenca del Tajo. El Tornadizo, Salamanca, España. MNCN_ICTIO 293737. SL = 69.8 mm.

Table 2.– Morphological variables used to define the morphometric and meristic characters of *A. garzonorum* sp. nov. type series. Variables as described in Methods (SD = standard deviation).

Tabla 2.– Variables morfológicas utilizadas para definir los caracteres morfométricos y merísticos de la serie tipo de *A. garzonorum* sp. nov. Las variables son descritas en la sección de material y métodos (SD = desviación típica).

***Achondrostoma garzonorum* sp. nov.**				
Variable	**Holotype**	**Paratypes (n = 56)**		
		Range	**Mean**	**SD**
TL	93	105.6-33.5	60.5	17.1
SL	69.8	91.3-28.1	51.5	15
HL	19.1	20.3-6.7	12	3.1
PrOL	4.6	5.6-1.5	3.2	0.9
ED	4.2	4.7-2.2	3.1	0.7
PsOL	8.7	10.6-3.1	6.1	1.8
HH	16	17.1-6.1	10.6	2.6
PrDD	41.4	45.8-15.1	27.4	7.8
PrPD	19	20.2-7.2	12.6	3.2
PrVD	39.7	44.4-14.8	26.4	7.3
PrAD	53.6	62.3-20.1	36	10.4
CPL	31.5	37.6-10.9	20.4	6.4
APL	19.9	24.2-7.1	12.1	3.9
PFL	13.6	12.2-3.8	7.3	2.4
VFL	13.4	14-3.6	7	2.6
AFL	7.6	8-2.6	4.8	1.5
AHL	14.4	13.6-3.9	7.6	2.6
DFL	7.6	10.2-2.9	5.4	1.8
DHL	14.1	14.1-4.6	8.3	2.7
CFL	15.8	16.1-5.1	9.9	2.5
BLD	8.2	10.3-2.8	5.5	1.7
BD	19.3	21.4-5.4	10.9	3.8
LLS	47	47-43	44.4	1.2
SRA	7	7-6	6.8	0.5
SRB	5	4-5	4.6	0.2
D	7	7	7	0
A	7	7-8	7.1	0.1

Paratypes: MNCN_ICTIO 293736, MNCN_ICTIO 293738 to ICTIO 293761, 25 individuals, Alagón River, Tajo Drainage, El Tornadizo, Los Pasiles, Salamanca, Spain, 40°32'34.3"N 5°51'23.1"W, 887 m.a.s.l., Leg. S. Perea, P. Garzón-Heydt, T. Nester and I. Doadrio, 23.III.2019. MNCN_ICTIO 273977 to ICTIO 273986, MNCN_ICTIO 273987 to ICTIO 274008, 32 individuals, Corneja River, Duero Drainage, San Bartolomé de Corneja, Ávila, Spain, 40°29'22.9"N 5°23'08.7"W, 999 m.a.s.l., Leg. P. Garzón-Heydt and I. Doadrio, 31.V.2009.

Additional material: MNCN_ICTIO 266554 to ICTIO 266564, 12 individuals, Cuerpo de Hombre River, Tajo Drainage, Montemayor del Río, Salamanca, Spain, 40°20'45.8"N 5°52'30.4"W, 637 m.a.s.l. Leg. P. Garzón-Heydt, J.L. González and I. Doadrio, 21.III.2009. MNCN_ICTIO 283373 to ICTIO 283381, 9 individuals, Cuerpo de Hombre River, Tajo, Drainage,

Table 3.– Autapomorphies in the mitochondrial cytochrome *b* gene detected for all species of the genus *Achondrostoma*. Transversions are indicated with *.

Tabla 3.– Autopomorfías para el gen mitocondrial citocromo b en todas las especies del género *Achondrostoma*. Transversiones son indicadas con *.

Nucleotide position	**21**	**24**	**45**	**81**	**86**	**105**	**114**	**115**	**124**	**126**	**135**	**138**	**150**	**171**	**192**	**199**	**216**	**231**	**243**	**258**	**306**
A. garzonorum sp. nov.	T	C	C	T	T	A	A	T	A	T	T	A	C	C	G	A	C	A	C	A	A
A. asturicense sp. nov.	T	C	C	T	T	A	G	T	A	T	T	A	T	C	A	A	C	A	C	A	A
A. numantinum sp. nov.	T	C	C	T	T	A	A	T	A	T	T	A	C	C	A	A	C	A	C	A	A
A. oligolepis	T	C	C	T	T	A	A	T	A	T	T	A	C	C	A	A	C	G	C	A	A
A. occidentale	T	T	T	T	T	A	A	T	T*	T	T	G	C	C	A	A	C	A	C	A	A
A. arcasii	T	C	C	T	T	A	A	T	A	T	T	A	C	C	A	A	C	A	C	A	A
A. salmantinum	C	C	C	C	C	T*	A	C	A	C	C	A	C	G*	A	G	T	A	T	T*	G

Continuation of previous table…

Nucleotide position	**312**	**318**	**366**	**375**	**393**	**426**	**432**	**441**	**450**	**459**	**471**	**477**	**498**	**504**	**513**	**525**	**568**	**597**	**609**	**615**	**631**
A. garzonorum sp. nov.	T	A	A	A	C	T	T	A	A	C	G	C	T	T	C	C	G	T	C	G	T
A. asturicense sp. nov.	T	A	A	A	C	C	T	A	A	C	G	T	C	T	T	T	G	T	C	A	T
A. numantinum sp. nov.	T	G	A	A	C	T	T	A	A	C	A	C	T	T	C	C	G	T	C	G	T
A. oligolepis	T	A	A	A	C	T	T	A	A	C	A	C	T	T	C	C	G	T	C	G	T
A. occidentale	T	A	A	A	C	T	T	G	A	C	T*	C	C	T	C	C	A	T	C	A	T
A. arcasii	T	A	A	A	C	T	T	A	A	C	A	C	T/C	T	C	C	G	T	C	G	T
A. salmantinum	C	A	T*	G	T	T	C	A	G	T	G	C	G	C	C	C	G	C	G*	T*	C

Continuation of previous table…

Nucleotide position	**672**	**690**	**705**	**709**	**715**	**732**	**738**	**741**	**783**	**786**	**804**	**807**	**813**	**816**	**837**	**852**	**855**	**864**	**867**	**873**	**904**
A. garzonorum sp. nov.	T	T	G	C	C	G	C	G	A	C	C	G	A	A	C	C	G	A	A	C	T
A. asturicense sp. nov.	T	T	G	C	C	A	C	T*	A	A*	C	G	G	G	C	C	A	A	A	C	T
A. numantinum sp. nov.	T	T	G	C	C	A	C	A	A	C	C	G	A	A	C	C	G/A	A	A	C/A	T
A. oligolepis	T	T	G	C	C	A	C	A/G	A	C/T	C	G	A	A	C	C	G/A	A	A	C	T
A. occidentale	T	T	A	T	C	A	T	C*	A	C	T	G	A	A	C	T	C*	G	G	C	T
A. arcasii	T	T	G	C	C	A	C	A	A	C	C	G	A	A	C	C	G/A	A	A	C	T
A. salmantinum	C	C	G	C	G*	A	C	T*	G	C	C	A	A	A	T	C	A	A	A	T	A*

Continuation of previous table…

Nucleotide position	**919**	**939**	**954**	**969**	**990**	**1003**	**1014**	**1020**	**1041**	**1089**	**1096**
A. garzonorum sp. nov.	C	G	C	T	A	C	C	C	C	C	C
A. asturicense sp. nov.	C	A	T	T	A	C	T	C	T	T	C
A. numantinum sp. nov.	C	A	C	T	A	C	C	C	C	C	C
A. oligolepis	C	A	C	T	A	C	C	C	C	C	C
A. occidentale	C	A	C	T	G	C	C	C	C	C	C
A. arcasii	C	A	C	T	A	C	C	C	C	C	C
A. salmantinum	T	A	A*	C	A	T	C	T	C	C	T

Montemayor del Río, Salamanca, Spain, 40°20’46.7”N 5°53’48.8”W, 637 m.a.s.l., Leg. P. Garzón-Heydt, J.L. González and I. Doadrio, 5.V.2010. MNCN_ICTIO 210005 to ICTIO 210010, 6 individuals, Corneja River, Duero Drainage, Villafranca de la Sierra, Ávila, Spain, 40°30’01.5”N 5°14’01.4”W, 1111 m.a.s.l., Leg. I. Doadrio, 9.IX.1999.

Diagnosis

Achondrostoma garzonorum sp. nov. is a member of the *Achondrostoma* genus (Robalo *et al.*, 2007). *Achondrostoma garzonorum* sp. nov. can be differentiated from all other known species of *Achondrostoma* according to the following set of characters: 43-49 ($\bar{x}$ = 45.9; M_d = 46; n = 66) canaliculate scales on the lateral line; 6-7 ($\bar{x}$ = 6.8; M_d = 7; n = 66) scales above the lateral line; 4-5 ($\bar{x}$ = 4.4; M_d = 4; n = 66) scales below the lateral line; 5/5 pharyngeal teeth. Low body depth (SL/BD > 4.4; BD/BLD < 2.2). Dentary with coronoid process high and narrow. Genetic distances from the other species of *Achondrostoma* inferred from the mitochondrial cytochrome *b* gene sequence were: about 7.7% with respect to *A. salmantinum*; about 6.1% with respect to *A. occidentale*; about 2.2% with respect to *A. oligolepis,* 2.7-2.8% *with A. arcasii* (here defined as the Ebro and Mijares, Turia and Palancia drainages); 2.4% with Central-Eastern Duero and 6.1% with Northwestern Duero. The new species has 3 autapomorphies, none of them transversions, in the cytochrome *b* gene (Table 3).

Description

D III (II) = 7, A III = 7, P I 12, V I 8, SLL = 45.9 (43-49), SRA = 6.8 (6-7), SRB = 5 (5-5.6), DPL 5 DPT 5, Vr = 37.4 (36-38). Morphometric and meristic characters of the type material are given in Table 2; measurements used in the morphometric study appear in Appendix 7. A medium-sized species that rarely reaches 150 mm total length. The head is large with the mouth subterminal and SL/HL is 3.8-4.5 ($\bar{x}$ = 4.2). The maximum body is low and the head length is larger than maximum body depth and BD/HL is 0.7-1 ($\bar{x}$ = 0.9). The preorbital distance is short about 0.7-1.2 ($\bar{x}$ = 1) times the eye diameter. Most individuals have a conspicuous depression between the posterior part of the head and start of the trunk. The ventral fins are inserted approximately at the same level of the origin of the dorsal fin. Predorsal length is 0.9-1.2 ($\bar{x}$ = 1 times preventral length. The caudal peduncle is low and minimum body depth is 3.2-5 ($\bar{x}$ = 3.7) times the length of the caudal peduncle and 2.2-3.4 ($\bar{x}$ = 1.8) times the length of the anal peduncle. The minimum body depth is about two times the body depth. Caudal fin is short, and the head length is 1-1.5 ($\bar{x}$ = 1.2) times the length of the caudal fin.

Pigmentation pattern

The upper part of the body is dark grey or dark brown; the ventral side is silvery. Ventral, anal and pectoral fin insertion points are red. Fins rays are black. The scales of the lateral line are black pigmented. The head is clearly lighter below the level of the eye (Fig. 8). The peritoneum is black.

Etymology

The species name *garzonorum* is derived from the Garzón-Heydt family, especially: Dra Paloma Garzón-Heydt, Jesús Garzón-Heydt, and Dra Guillermina

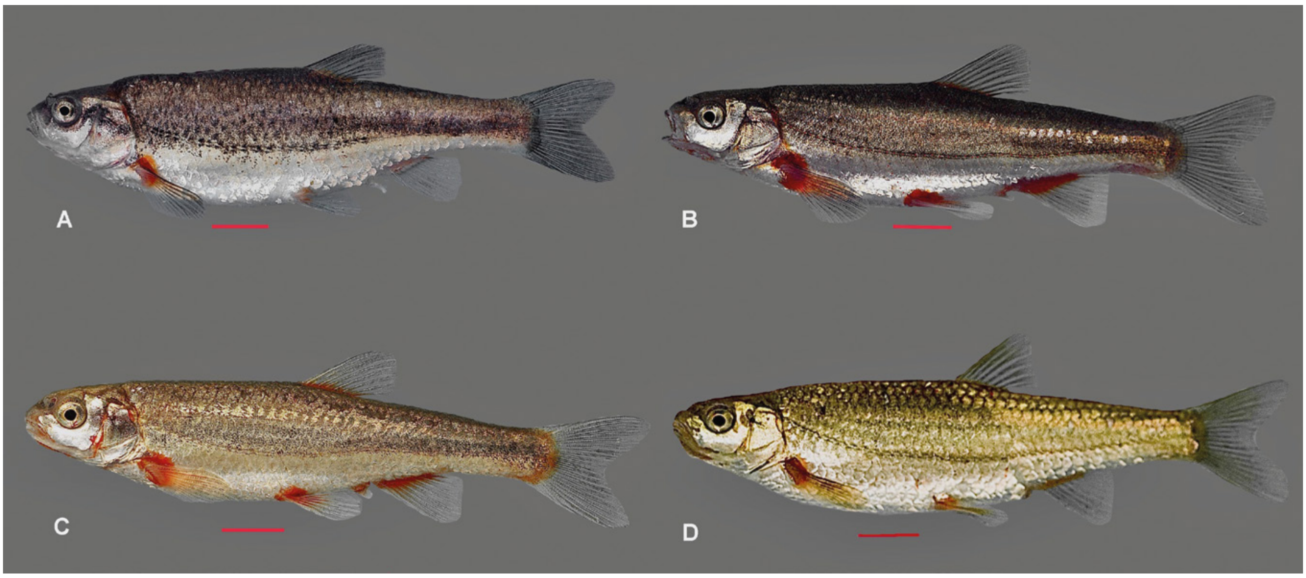

Fig. 8.– Pigmentation pattern in alive individuals of the different species described in this study. A: *A. arcasii*, B: *A. garzonorum* sp. nov. C: *A. numantinum* sp. nov. D: *A. asturicense* sp. nov.

Fig. 8.– Modelo de pigmentación presente en los individuos vivos de las diferentes especies descritas en este trabajo. A: *A. arcasii*, B: *A. garzonorum* sp. nov. C: *A. numantinum* sp. nov. D: *A. asturicense* sp. nov.

Garzón-Heydt for their contribution to the study and conservation of rivers and fauna of the region where *A. garzonorum* inhabits.

Distribution

This new species is endemic to three rivers of the Iberian Peninsula the Alagón and Cuerpo de Hombre rivers in Tajo Drainage and the Corneja River in the Duero Drainage. In Alagón river the species is only present in the head water near of its source. In Cuerpo de Hombre river the range of the species is localized near of its confluence into the Alagón River. In Corneja river the species inhabit a small stretch of the river before its confluence with the Tormes river (Fig. 9).

Common names

Bermeja, Bermejuela salmantina.

Remarks

The species *A. garzonorum* hybridized with *Pseudochondrostoma polylepis* (Steindachner,1864) in Cuerpo de Hombre River and for this reason the individuals of Cuerpo de Hombre were discarded of type series. Not data on the reproduction of the species exist. The species is a typical habitant of rivers with clear water and from moderate to high water flow, in areas with granitic rocks in the riverbed. The species should be considered Critically Endangered according to the IUCN red list criteria due to the extent of its occurrence is lesser than 100 km^2 and its populations are severely fragmented. Only three small populations exist and probably due to human alterations (pollution and dams) the population from the Cuerpo de Hombre river is endangered as a consequence of genetic introgresion with *Pseudochondrostoma polylepis.* Hybridization between species of the genus *Pseudochondrostoma* and *Achondrostoma* has been previously reported when species of these two genera inhabit the same drainage (Collares-Pereira & Coelho, 1983; Elvira, 1986; Doadrio *et al.*, 2011; Vieira-Lanero *et al.*, 2019). However, in Corneja river hybrids with *Pseudochondrostoma duriense,* which inhabit together with *A. garzonorum*, were not detected. No other fish species were collected in the stretch of the Alagón river where this species inhabits.

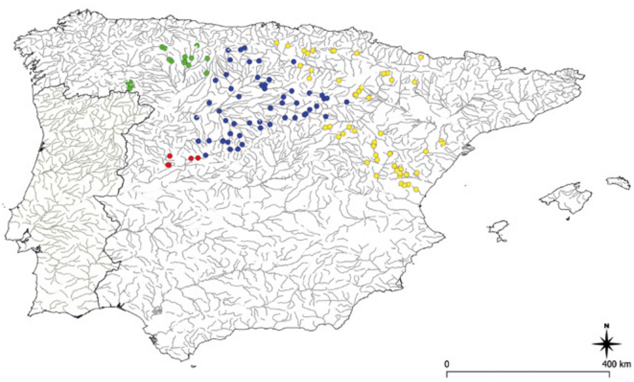

Fig. 9.– Distribution range of *Achondrostoma* species. *A. garzonorum* (red), *A. asturicense* (green), *A. numantinum* (blue), *A. arcasii* (yellow).

Fig. 9.– Área de distribución de las especies de *Achondrostoma*. *A. garzonorum* (rojo), *A. asturicense* (verde), *A. numantinum* (azul), *A. arcasii* (amarillo).

Achondrostoma numantinum sp. nov.

urn:lsid:zoobank.org:act:8616548B-FE9F-469B-81DA-6C18B46AA2DF

Fig. 10, Table 4

Material studied

Holotype: MNCN_ICTIO 293768, SL 77.1 mm, TL 89.4 mm, Voltoya River, Duero Drainage, Labajos, Ávila, Spain, 40°50'54.4"N 4°34'20.3"W, 1.064 m.a.s.l., Leg., P. Garzón-Heydt, S. Perea, T. Nester, and I. Doadrio, 24.III.2019.

Paratypes: MNCN_ICTIO 293767, MNCN_ICTIO 293769 to ICTIO 293772, 5 individuals, Voltoya River, Duero Drainage, Labajos, Ávila, Spain, 40°50'54.4"N 4°34'20.3"W, 1.064 m.a.s.l., Leg. P. Garzón-Heydt, S. Perea, T. Nester and I. Doadrio, 24.III.2019. MNCN_ICTIO 293762 to ICTIO 293766, 5 individuals, Adaja River, Duero Drainage, Blascosancho, Ávila, Spain, 40°52'05.1"N 4°41'00.5"W, 890 m.a.s.l., Leg. P. Garzón-Heydt, S. Perea, T. Nester and I. Doadrio, 24.III.2019. MNCN_ICTIO 273156 to ICTIO 273161, MNCN_ICTIO 273156 to ICTIO 273161, 6 individuals, Adaja River, Duero Drainage, Blascosancho, Ávila, Spain, 40°52'05.1"N 4°41'00.5"W, 904 m.a.s.l., Leg. P. Garzón-Heydt, J.L. González and I. Doadrio, 12.VII.2009. MNCN_ICTIO 296779 to ICTIO 296792, 14 individuals Cega River, Duero Drainage, Lastras de Cuellar, Segovia, Spain, 41°16'27.3"N 4°05'32.9"W, 900 m.a.s.l., Leg. P. Garzón-Heydt, J.L. González and I. Doadrio, 24.VII.2010.

Additional material: MNCNUZA_ICTIO 589 to ICTIO 608, 20 individuals, Pedro River, Duero Drainage, Cuevas de Ayllón, Soria, Spain, 41°23'23.0"N 3°18'29.4"W, Leg. E. Martínez, B. Gutiérrez, and L. Ambrosio, 2.VI.2010. MNCN_ICTIO 30197 to ICTIO 30208, 12 individuals, Adaja River, Duero Drainage, Pradosegar, Ávila, Spain, 40°33'33.6"N 5°03'51.8"W, Leg. I. Doadrio, 18.V.1981. MNCN_ICTIO 33626 to ICTIO 33649, 24 individuals, Adaja River, Duero Drainage, Blascosancho, Burgos, Spain, 40°52'25.7"N 4°40'50.0"W, Leg. I. Doadrio, 18.V1981. MNCN_ICTIO 33853 to ICTIO 336888, 36 individuals, Ucero River, Duero Drainage, Ucero, Soria, Spain, 41°42'47.4"N 3°03'04.1"W, Leg. I. Doadrio, 30. XI.1983. MNCN_ICTIO 45175 to ICTIO 45214, 40 individuals, Moros River, Duero Drainage, Anaya, Segovia, Spain, 40°59'35.6"N 4°18'43.8"W, Leg. P. Rincón, 7.V.1986. MNCN_ICTIO 103403 to ICTIO 103419, 17 individuals, Camesa River, Duero Drainage, Santa Olalla, Cantabria, Spain, 42°55'54.0"N

Fig. 10.– Holotype of *Achondrostoma numantinum* sp. nov. from the Voltoya River, Duero drainage. Labajos, Ávila, Spain. MNCN_ICTIO 293768. SL = 77.1 mm.

Fig. 10.– Holotipo de *Achondrostoma numantinum* sp. nov. del Río Voltoya, Cuenca del Duero. Labajos, Ávila, España. MNCN_ICTIO 293768. SL = 77.1 mm.

4°12’15.0”W, Leg. I. Doadrio, A. Perdices, J.A. G. Carmona, 19.VIII.1991. MNCN_ICTIO 109186 to ICTIO 109219, 34 individuals, Cega River, Duero Drainage, Pajares de Pedraza, Segovia, Spain, 41°09’48.9”N 3°51’03.6”W, Leg. A. Perdices, J. Cubo and J. Dominguez, 13.X.1994. MNCN_ICTIO 109233 to ICTIO 109278, 46 individuals, Duratón River, Duero Drainage, Duruelo, Segovia, Spain, 41°14’18.6”N 3°38’30.8”W, Leg. I. Doadrio, 13.X.1994. MNCN_ICTIO 117473 to ICTIO 117483, 11 individuals, Zapardiel River, Duero Drainage, Los Crespos, Ávila, Spain, 40°52’50.0”N 4°59’26.1”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 13. VI.1995. MNCN_ICTIO 117542 to ICTIO 117555, 14 individuals, Voltoya River, Duero Drainage, Urraca Miguel, Ávila, Spain, 40°41’20.0”N 4°31’08.1”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 15. VI.1995. MNCN_ICTIO 117462 to ICTIO 117465, 4 individuals, Voltoya River, Duero Drainage, El Espinar, Segovia, Spain, 40°41’43.2”N 4°21’01.6”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 4. VII.1995. MNCN_ICTIO 117639 to ICTIO 117642, 4 individuals, Pirón River, Duero Drainage, Samboal, Segovia, Spain, 41°15’19.8”N 4°26’08.3”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 28.VI.1995. MNCN_ICTIO 117666 to ICTIO 117668, 3 individuals, Revinuesa River, Duero Drainage, Vinuesa, Segovia, Spain, 41°55’54.2”N 2°46’11.4”W, Leg. L. Ambrosio, 11.VII.1995. MNCN_ICTIO 117691 to ICTIO 117710, 20 individuals, Moros River, Duero Drainage, Vegas de Matute, Segovia, Spain, 40°47’49.0”N 4°15’27.0”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 4.VII.1995. MNCN_ ICTIO 117828 to ICTIO 117847, 20 individuals, Tielmes River, Duero Drainage, Fresno de Caracena, Soria, Spain, 41°27’09.4”N 3°05’19.1”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 6.VII.1995. MNCN_ICTIO 117848 to ICTIO 117867, 20 individuals, Aranzuelo River, Duero Drainage, Arauzo de Torre, Burgos, Spain, 41°48’03.6”N 3°25’18.8”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 12. VII.1995. MNCN_ICTIO 117921 to ICTIO 117940, 20 individuals, Pilde River, Duero Drainage, Brazacorta, Burgos, Spain, 41°42’48.2”N 3°22’04.4”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 13.VII.1995. MNCN_ICTIO 128732 to ICTIO 128735, 4 individuals, Eresma River, Duero Drainage, Olmedo, Valladolid, Spain, 41°19’31.3”N 4°37’11.6”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 15.VII.1995. MNCN_ICTIO 128891 to ICTIO 128902, 12 individuals, Cega River, Duero Drainage, Viana de Cega, Valladolid, Spain, 41°32’13.3”N 4°45’46.8”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 27.VI.1995. MNCN_ ICTIO 129140 to ICTIO 129182, 43 individuals, Caracena River, Duero Drainage, Caracena, Soria, Spain, 41°23’08.6”N 3°05’16.8”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 6.VII.1995. MNCN_ ICTIO 130068 to ICTIO 130084, 17 individuals, Arandilla River, Duero Drainage, Huerta del Rey, Burgos, Spain, 41°50’53.5”N 3°20’32.6”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 13.VII.1995. MNCN_ICTIO 130476 to ICTIO 130488, 13 individuals, Urbell River, Duero Drainage, Santa Cruz del Tozo, Burgos, Spain, 42°38’41.0”N 3°53’07.0”W, Leg. B. Gutiérrez, E. Martínez and L. Ambrosio, 22. VII.1995. MNCN_ICTIO 161465 to ICTIO 161513, 49 individuals, Rubagón River, Duero Drainage, Porquera de Santullán, Palencia, Spain, 42°53’24.0”N 4°17’26.1”W, Leg. I. Doadrio, 16.VIII.1995. MNCN_ ICTIO 161831 to ICTIO 131865, 35 individuals, Boedo River, Duero Drainage, Báscones de Ojeda, Palencia, Spain, 42°40’03.9”N 4°31’27.6”W, Leg. I. Doadrio, 17.VIII.1995. MNCN_ICTIO 169154 to ICTIO 169156, 3 individuals, Arlanza River, Duero Drainage, Palacios de la Sierra, Burgos, Spain, 41°58’45.9”N 3°09’40.1”W, Leg. I. Doadrio, 20. VII.1995. MNCN_ICTIO 192329 to ICTIO 192331, 3 individuals, Duero River, Duero Drainage, Garray, Soria, Spain, 41°48’36.3”N 2°26’53.0”W, Leg. I. Doadrio and B. Elvira, 31.VII.1981. MNCN_ICTIO 192395 to ICTIO 192399, 5 individuals, Nava River, Duero Drainage, Fuentelcésped, Burgos, Spain, 41°36’07.7”N 3°37’56.8”W, Leg. B. Elvira, 25. VI.1978. MNCN_ICTIO 193004 to ICTIO 193036, 33 individuals, Cubillo River, Duero Drainage, Madrigalejo del Monte, Burgos, Spain, 42°06’55.6”N 3°45’06.8”W, Leg. B. Elvira, 29.IX.1982. MNCN_ICTIO 210419 to ICTIO 210428, 10 individuals, Talegones River, Duero Drainage, Berlanga de Duero, Soria, Spain, 41°27’09.8”N 2°52’38.3”W, Leg. I. Doadrio, 22.X.1999. MNCN_ICTIO 210522 to ICTIO 210524, 3 individuals, Merdancho River, Duero Drainage, Renieblas, Soria, Spain, 41°49’10.0”N 2°23’02.9”W, Leg. I. Doadrio, 29.X.1999. MNCN_ICTIO 210547, ICTIO 210548, 2 individuals, Hornija River, Duero Drainage, Peñaflor de Hornija, Valladolid, Spain, 41°43’07.7”N 4°59’03.7”W, Leg. I. Doadrio, 23.XI.1999. MNCN_ICTIO 211821 to ICTIO 211836, 16 individuals, Ausín River, Duero Drainage, Revillarruz, Burgos, Spain, 42°13’47.3”N 3°39’29.9”W, Leg. I. Doadrio, 6.XI.1999. MNCN_ICTIO 212552 to ICTIO 212587, 36 individuals, Morón River, Duero Drainage, Morón de Almazán, Soria, Spain, 41°24’54.7”N 2°24’30.1”W, Leg. I. Doadrio, 22.X.1999. MNCN_ICTIO 212910 to ICTIO 212931, 22 individuals, Cubillo River, Duero Drainage, Zael, Burgos, Spain, 42°06’26.3”N 3°49’32.8”W, Leg. I.Doadrio, 6.XI.1999. MNCN_ICTIO 213826 to ICTIO 213880, 55 individuals, Hormazuela River, Duero Drainage, Estépar, Burgos, Spain, 42°16’51.3”N 3°54’50.1”W, Leg. I. Doadrio, 7.XI.1999. MNCN_ICTIO 213911 to ICTIO 213942, 32 individuals, Escalote River, Duero Drainage, Berlanga de Duero, Soria, Spain, 41°28’30.6”N 2°51’00.9”W, Leg. I. Doadrio, 22.X.1999. MNCN_ICTIO 192184, 1 individual, Tera River, Duero Drainage, Espejo de Tera, Soria, Spain, 41°53’01.3”N 2°29’24.4”W, Leg. I. Doadrio, 12.II.1974. MNCN_ICTIO 194276, ICTIO 194277, 2 individuals, Duero River, Duero Drainage, Castronuño, Valladolid, Spain, 41°23’31.1”N 5°15’45.6”W, Leg. B. Elvira and I. Doadrio, 13. VII.1981. MNCN_ICTIO 194307 to ICTIO 194322, 16 individuals, Arlanza River, Duero Drainage, Lerma, Burgos, Spain, 42°01’50.5”N 3°45’34.7”W, Leg. B. Elvira and I. Doadrio, 17.VIII.1979. MNCN_ICTIO 210521, 1 individual, Fuentepinilla River, Duero Drainage, Fuentepinilla, Soria, Spain, 41°34’09.4”N 2°45’46.1”W, Leg. F. Morcillo and I. Doadrio, 25.X.1999. MNCN_ICTIO 215228 to ICTIO 215243, 16 individuals, Izana River, Duero Drainage, Quintana Redonda, Soria, Spain, 41°38’35.1”N 2°36’36.0”W, Leg. F. Morcillo and I. Doadrio, 25.X.1999. MNCN_ICTIO 215244 to ICTIO 215282, 39 individuals, Araviana River, Duero Drainage, Ólvega, Soria, Spain, 41°44’10.4”N 1°56’43.8”W, Leg. F. Morcillo and I. Doadrio, 25.X.1999. MNCN_ICTIO 192975 to ICTIO 192990 16 individuals, Duratón River, Duero Drainage, Sebúlcor, Segovia, Spain, 41°17’51.5”N 3°52’05.2”W, Leg. B. Elvira and I. Doadrio, 27.VII.1981. MNCN_ICTIO 29680 to ICTIO 296811, 10 individuals, Oroncillo River, Duero Drainage, Pancorbo, Burgos, Spain, 42°37’40.3”N 3°07’06.4”W, Leg. I. Doadrio, 27.VII.1981. MNCN_ICTIO 30122 to ICTIO 30165 and MNCN_ICTIO 245421 to ICTIO 245447, 71 individuals, Almar River, Duero Drainage, Peñaranda de Bracamonte, Salamanca, Spain, 40°52’20.1”N 5°13’34.6”W, Leg. B. Elvira and I. Doadrio, 13. VIII.1981. MNCN_ICTIO 217598 to ICTIO 217609 12 individuals, Valdeginate River, Duero Drainage, Cisneros, Palencia, Spain, 42°12’50.1”N 4°50’46.1”W, Leg. I. Doadrio, 25.XI.1999. MNCN_ICTIO 217610 to ICTIO 217616, 7 individuals, Maderano River, Duero Drainage, Castrillo de Onielo, Palencia, Spain, 41°51’21.1”N 4°19’43.5”W, Leg. I. Doadrio, 26. XI.1999. MNCN_ICTIO 137076 to ICTIO 137077, 2 individuals, Villalobón River, Duero Drainage, Villalobón, Palencia, Spain, 42°01’45.7”N 4°30’05.2”W, Leg. IFIE, 25.IV.1945. MNCN_ICTIO 193591 to ICTIO 193607, 17 individuals, Rivera River, Duero Drainage, Ventanilla, Palencia, Spain, 42°52’51.3”N 4°33’54.6”W, Leg. Brañosera, 1. VIII.1981. MNCN_ICTIO 140636 to ICTIO 140641 6 individuals, Carrión River, Duero Drainage, Carrión de los Condes, Palencia, Spain, 42°20’28.5”N 4°36’33.9”W, Leg. IFIE, 15.XI.1952. MNCN_ICTIO 296793 to ICTIO 2968019, Voltoya River, Duero Drainage, Juarros de Voltoya, Segovia, Spain, 41°02’11.1”N 4°31’14.1”W, 840 m.a.s.l., Leg. P. Garzón-Heydt, J. L. González and I. Doadrio, 24. VII.2010.

Diagnosis

Achondrostoma numantinum sp. nov. is a member of the *Achondrostoma* genus (Robalo *et al.*, 2007). *Achondrostoma numantinum* sp. nov. can be differentiated from all other known species of its genus according to the following set of characters: 43-48 ($\bar{x}$ = 45, 1, M_d = 45 n = 99) canaliculated scales on the lateral line; 6-8 ($\bar{x}$ = 6.9, M_d = 7 n = 99) scales above the lateral line; 4-5 ($\bar{x}$ = 4.3, M_d = 4 n = 99) scales below the lateral line; small head (SL/HL> 4.2). High body depth in comparison to caudal peduncle depth (BD/BLD > 2). (6)5/5(6) pharyngeal teeth. Dentary with short anterior process and coronoid process high. Genetic distances from the other species of *Achondrostoma* inferred from the mitochondrial cytochrome *b* gene sequence were: about 7.7 % with respect to *A. salmantinum,* about 6.5 % with respect to *A. occidentale*, about 2.2-2.3 % with respect to *A. oligolepis,* 0.6-0.7 % with *A. arcasii* (here defined as the Ebro and Mijares, Palancia and Turia drainages),

Table 4.– Morphological variables used to define the morphometric and meristic characters of *A. numantinum* sp. nov. type series. Variables as described in Methods (SD = standard deviation).

Tabla 4.– Variables morfológicas utilizadas para definir los caracteres morfométricos y merísticos de la serie tipo de *A. numantinum* sp. nov. Las variables son descritas en la sección de material y métodos (SD = desviación típica).

***A. numantinum* sp. nov.**				
Variable	**Holotype**	**Paratypes (n = 31)**		
		Range	**Mean**	**SD**
TL	89.4	90.5-53.5	73.6	8.6
SL	77.1	78.2-47.4	64.1	7.9
HL	17	17.6-9.6	14.7	1.8
PrOL	4.5	4.6-2.1	3.5	0.9
ED	4.1	4.6-2.6	3.9	0.4
PsOL	8.6	9-5.3	7.4	0.9
HH	15.3	15.9-8	11.3	1.9
PrDD	40.7	42.1-25.7	33.9	4.3
PrPD	17.3	18.9-10	15	1.9
PrVD	39	40.4-23	32.2	4.1
PrAD	52.7	58.6-33.1	44	5.3
CPL	31.9	31.9-16	24.7	3.9
APL	18.9	19.6-11.6	15.7	2.1
PFL	12.8	14-7	11	1.9
VFL	11.2	11.5-7.4	9.8	1.2
AFL	7.2	7.9-2.7	5.6	1.2
AHL	11.5	12.7-7	10.4	1.4
DFL	8.2	9.1-5.4	7.3	0.8
DHL	12.3	14.6-6.3	11.7	1.8
CFL	16.2	17.1-9.1	12.2	1.8
BLD	7.6	8.1-4.9	6.7	0.9
BD	16.9	19.1-10.7	14.5	1.9
LLS	44	48-43	45.3	1.5
SRA	8	7-6	6.8	0.4
SRB	4	4-5	4.2	0.4
D	7	7	7	0
A	7	7	7	0

and 6.6 % with Northwestern Duero. The new species has one autapomorphy (non-transversion) in the mitochondrial cytochrome *b* gene (Table 3).

Description

D III = 7, A III = 7, P I 12-13, V I 7-8, SLL = 45.1 (43-48), SRA = 6.9 (6-7), SRB = 5 (4-5), DPL 5 (6) DPT 5 (6), Vr = 37.9 (37-38). Morphometric and meristic characters of the type material are given in Table 4. Measurements used in the morphometric study appear in Appendix 8. A medium sized species that rarely reaches 150 mm standard length. The head is small with the mouth subterminal SL/HL is 4.2-5.6 ($\bar{x}$ = 4.6). Head length is 1-1.4 ($\bar{x}$ = 1.1) times maximum body depth. Head length is similar to posterior head height (HL/HH ratio 1-1.3 $\bar{x}$ = 1.1). The preorbital distance is similar to the eye diameter, measuring 0.7-1.4 ($\bar{x}$ = 1) times the eye diameter at most. The ventral fins are inserted in the origin of the dorsal fin. Predorsal length is 0.9 -1.1 ($\bar{x}$ = 1) times preventral length. Minimum body depth is 3.1-4.2 ($\bar{x}$ = 3.6) times the length of the caudal peduncle and 1.7-2.6 ($\bar{x}$ = 2.1) times the length of the anal peduncle. The caudal fin is short, the same length as the head.

Pigmentation pattern

The general colour pattern in *A. numantinum* sp. nov. is similar to those of *A. garzonorum* sp. nov. and *A. arcasii*. The upper part of the body is dark grey or dark brown; the ventral side being silvery or gold. Ventral, anal and pectoral fin insertion points are red, increasing in intensity and extension in the spawning period. The scales of the lateral line are black pigmented (Fig. 8).

Etymology

The species name *numantinum* is derived from the name given to the pre-Roman population inhabiting an ancient Celtiberian settlement around of the current Garray village in Soria (Central Spain). This population was known for his courage and after 13 months of siege for the romans, the Numantians decided to burn the city before surrendering.

Distribution

The species is endemic to the Duero Drainage but absent in the Northwestern region of this drainage (Esla sub-basin and Portugal rivers in the Duero Drainage) (Fig. 9).

Common name

Bermejuela numantina.

Remarks

Achondrostoma numantinum lives in rivers and streams placed in the central plateau from Spain with heights over 800 m.a.s.l. in oligotrophic waters shady with vegetation. Breeding occurs from April to the end of August (Rincón & Lobón-Cerviá, 1989). The main diet components are detritus, plants and invertebrates (Lobón-Cerviá & Rincón, 1994). The large number of dams and reservoirs for hydroelectric and agricultural use, in Duero Drainage, the introduction of invasive freshwater fish species, and pollution are the main threats to the conservation of *A. numantinum*. However, it is still a common species throughout the Duero Drainage. The trend of its populations is unknown, and projections and models in climate change scenarios have not been made on this species. All this information is necessary to include to *A. numantinum* in the future in any category of threatened of IUCN, so far it is a data deficient species (DD).

Achondrostoma asturicense sp. nov.

urn:lsid:zoobank.org:act:D2A37AB1-816F-42C9-A6B5-EAD4A4F4FF1B

Fig. 11, Table 5

Material studied

Holotype: MNCN_ICTIO 296812, SL 66.8 mm, 80.3 TL mm, Truchas River, Duero Drainage, Sotillo de Sanabria, Zamora, Spain, 42°05'12.0"N 6°42'48.5"W, 1.600 m.a.s.l., Leg., J. Garzón-Heydt, 31.VIII.1974.

Paratypes: MNCN_ICTIO 296813, 1 individual, Truchas River, Duero Drainage, Sotillo de Sanabria, Zamora, Spain, 42°05'12.0"N 6°42'48.5"W, 1.600 m.a.s.l., Leg., J. Garzón-Heydt, 31.VIII.1974. MNCN_ICTIO 192192-192197, 6 individuals, Peces Lagoon, Duero Drainage, Galende, Zamora, Spain, 42°10'34.0"N 6°43'46.2"W, 1700 m.a.s.l., Leg. J. Garzón-Heydt, 15.V.1974. MNCN_ICTIO 296.814-296.821, 8 individuals, Peces Lagoon, Duero Drainage, Galende, Zamora, Spain, 42°10'34.0"N 6°43'46.2"W, 1700 m.a.s.l., Leg. J. Garzón-Heydt, 17.V.1974. MNCN_ICTIO 192324-192328, 5 individuals, Garandones Lagoon, Duero Drainage, Galende, Zamora, Spain, 42°08'17.9"N 6°47'11.8"W, 1614 m.a.s.l., Leg. J. Garzón-Heydt, 18.V.1974. MNCN_ICTIO 257371-257383, MNCN_ICTIO 257385-86, 15 individuals, Tera River, Duero Drainage, Ribadelago, Zamora, Spain, 42°07'22.1"N 6°44'53.3"W, 1007 m.a.s.l., P. Garzón-Heydt and I. Doadrio, 5.VI.2003.

Additional material: MNCN_ICTIO 30518, 1 individual, Luna River, Duero Drainage, Villasecino, León, Spain, 42°57'04.0"N 6°01'32.7"W, Leg. J. Muñoz Cobos, 8.VII.1975. MNCN_ICTIO 163649, 1 individual, Torío River, Duero Drainage, Garrafe de Torío, León, Spain, 42°43'54.2"N 5°31'12.3"W, Leg. I. Doadrio, 15.VIII.1995. MNCN_ICTIO 192482-192493, 12 individuals Cea River, Duero Drainage, Sahagún, León, Spain, 42°22'36.5"N 5°02'26.0"W, Leg. B. Elvira, 20.VIII.1978. MNCN_ICTIO 193458-193479, 22 individuals Órbigo River, Duero Drainage, Azadón, León, Spain, 42°38'13.2"N 5°49'06.7"W, Leg. I. Doadrio, 27.IX.1982. MNCN_ICTIO 296822-296852, 31 individuals Cea River, Duero Drainage, Mondreganes, León, Spain, 42°41'47.9"N

Fig. 11.– Holotype of *Achondrostoma asturicense* sp. nov. from the Truchas River, Duero drainage. Sotillo de Sanabria, Zamora, Spain. MNCN_ICTIO 296812. SL = 66.8 mm.

Fig. 11.– Holotipo de *Achondrostoma asturicense* sp. nov. del Río Truchas, Cuenca del Duero. Sotillo de Sanabria, Zamora, España. MNCN_ICTIO 296812. SL = 66.8 mm.

Table 5.– Morphological variables used to define the morphometric and meristic characters of *A. asturicense* sp. nov. type series. Variables as described in Methods (SD = standard deviation).

Tabla 5.– Variables morfológicas utilizadas para definir los caracteres morfométricos y merísticos de la serie tipo de *A. asturicense* sp. nov.. Las variables son descritas en la sección de material y métodos (SD = desviación típica).

***Achondrostoma asturicense* sp. nov.**				
Variable	**Holotype**	**Paratypes (n = 35)**		
		Range	**Mean**	**SD**
TL	80.3	96.1-49.4	68.1	11
SL	66.8	82.8-42.8	57.4	9.6
HL	13.4	21.4-9.8	13.9	2.2
PrOL	3.1	6-1.6	3.6	0.7
ED	3.4	4.6-3	3.7	0.3
PsOL	7	11.4-4.5	6.8	1.4
HH	13.3	17.2-9.4	12.2	1.7
PrDD	36	45.5-21.7	31.6	5.4
PrPD	14.5	22.2-11.7	15	2.1
PrVD	34.6	43.7-21.5	30.1	5
PrAD	46.8	57.4-29.4	40.4	6.9
CPL	25.4	32.1-16.3	21.7	3.6
APL	14.3	18.2-8.9	12.8	2.1
PFL	10.8	14.1-7.4	10.4	1.7
VFL	10.1	12.4-4.3	8.1	2.2
AFL	7.1	8.9-3.5	5.6	1.2
AHL	10.9	13.5-5.9	9	1.7
DFL	7.4	8.8-3.5	5.6	1.2
DHL	11.5	14.4-6.6	9.8	2
CFL	14.9	17.2-8.5	12.8	2
BLD	6.9	8.1-4.3	5.7	0.9
BD	14.5	17.8-8.6	12.3	2
LLS	41	38-44	40.9	1.2
SRA	6	5-6	5.8	0.4
SRB	3	3-4	3.1	0.3
D	7	7	7	0
A	7	7	7	0.1

5°01’38.1”W, Leg. P. Garzón-Heydt and I. Doadrio, 15.VII.2009. MNCN_ICTIO 296.853-296.854, 2 individuals Esla River, Duero Drainage, Villarroañe, León, Spain, 42°41’47.9”N 5°01’38.1”W, Leg. P. Garzón-Heydt and I. Doadrio, 4.VI.2010. MNCN_ ICTIO 296.855-296.857, 3 individuals Porma River, Duero Drainage, Puente de Villarente, León, Spain, 42°33’07.1”N 5°26’39.5”W, Leg. P. Garzón-Heydt and I. Doadrio, 4.VI.2010. MNCN_ICTIO 296.858-296.869, 12 individuals Cea River, Duero Drainage, Puente Almuhey, León, Spain, 42°33’07.1”N 5°26’39.5”W, Leg. P. Garzón-Heydt and I. Doadrio, 4.VI.2010. MNCN_ICTIO 296.870-296.879, 10 individuals Curueño River, Duero Drainage, Ambasaguas de Curueño, León, Spain, 42°42’32.0”N 5°22’39.8”W, Leg. P. Garzón-Heydt and I. Doadrio, 4.VI.2010. MNCN_ICTIO 296.880-296.903, 24 individuals Omaña River, Duero Drainage, Las Omañas, León, Spain, 42°40’42.4”N 5°52’16.8”W, Leg. P. Garzón-Heydt and I. Doadrio, 30.VI.2010. MNCNAT_ICTIO 2882-2891, 10 individuals Torío River, Duero Drainage, Villarrodrigo de las Regueras, León, Spain, 42°37’49.8”N 5°31’31.2”W, Leg. P. Garzón-Heydt and I. Doadrio, 9.VI.2005. MNCNAT_ICTIO 2924-2963, 70 individuals Torío River, Duero Drainage, Villaobispo de las Regueras, León, Spain, 42°36’37.6”N 5°32’22.2”W, Leg. S. Perea and I. Doadrio, 9.VI.2005. MNCNAT_ICTIO 3994-4003, 10 individuals Porma River, Duero Drainage, Camposolillo, León, Spain, 42°58’51.8”N 5°15’23.3”W, Leg. I. Doadrio, 6.X.2006. MNCNAT_ ICTIO 4004-4033 25 individuals Torío River, Duero Drainage, Palazuelo de Torío, León, Spain, 42°42’11.9”N 5°31’48.0”W, Leg. S. Perea and I. Doadrio, 29.IX.2006.

Diagnosis

Achondrostoma asturicense sp. nov. is a member of the *Achondrostoma* genus (Robalo *et al.*, 2007). *Achondrostoma asturicense* sp. nov. can be differentiated from all other known species of its genus according to the following set of characters: 38-

44 ($\bar{x}$ = 40.9, M_d = 41 n = 36) canaliculate scales on the lateral line; 5-6 ($\bar{x}$ = 5.8, M_d = 6 n = 36) scales above the lateral line; 3-4 ($\bar{x}$ = 3.1, M_d = 3 n = 36) scales below the lateral line. Caudal peduncle low (SL/BLD<10). High body depth in comparison to caudal peduncle depth (BD/BLD > 2). 5/5 pharyngeal teeth. Dentary with coronoid process wide. Narrow skull with HL/ID>3. Genetic distances from the other species of *Achondrostoma* inferred from the *MT-CYTB* gene sequence were: about 7.7 % with respect to *A. salmantinum*, about 4.1 % with respect to *A. occidentale*, about 6-6.3 % with respect to *A. oligolepis,* 6.8-6.9 % with *A. arcasii* (here defined as the Ebro and adjacent Mediterranean rivers), and 6.6 % with Central-Eastern Duero. The new species has 13 autapomorphies in the mitochondrial cytochrome *b* gene, one of which is a transversion (Table 3).

Description

D III (II) = 7, A III = 7, P I 13, V I 7-8, SLL = 40.9 (38-44), SRA = 5.8 (5-6), SRB = 3.1 (3-4), DPL 5 DPT 5, Vr = 37.9 (37-38). Morphometric and meristic characters of the type material are given in Table 5. Measurements used in the morphometric study appear in Appendix 9. A medium sized species that rarely reaches 120 mm standard length. The head is large as in *A. garzonorum* with the mouth subterminal SL/HL is 3.7-4.5 ($\bar{x}$ = 4.2). Head length is similar to maximum body depth ($\bar{x}$ = 1, 0.8-1.2). Head height is lesser that the head length (HL/HH ratio 1-1.3 $\bar{x}$ = 1.2). The preorbital distance is lesser to the eye diameter, measuring 0.7-1.1 ($\bar{x}$ = 0.9) times the eye diameter. The ventral fins are inserted in the origin of the dorsal fin, the proportion (ratio PrD/PrV 1 -1.1; $\bar{x}$ = 1). The caudal peduncle is large and low minimum body depth is 3.4-4.2 ($\bar{x}$ = 3.7) times the length of the caudal peduncle and 2-2.5 ($\bar{x}$ = 2.2) times the length of the anal peduncle. The caudal fin is short, the same length as the head.

Pigmentation pattern

The general colour pattern in *A. asturicense* sp. nov. is similar to those of *A. garzonorum* sp. nov. and *A. arcasii*. The upper part of the body is dark grey or dark brown; the ventral side being silvery or gold. Ventral, anal and pectoral fin insertion points are red, increasing in intensity and extension in the spawning period. The scales of the lateral line are black pigmented (Fig.8).

Etymology

The species name *asturicense* is derived from the name Astura given to the Esla river during the Roman empire.

Distribution

The species is endemic to the Esla sub-basin within of the Duero Drainage and it is probably present also in Duero Drainage in Portugal. (Fig. 9).

Common name

Bermejuela del Esla.

Remarks

Achondrostoma asturicense lives in rivers and lakes placed in the western of the Cantabrian mountains in oligotrophic waters shady with vegetation. Breeding occurs during summer and spring. As occurs in *A. numantinum* the large number of dams and reservoirs for hydroelectric and agricultural use, in Duero Drainage, the introduction of invasive freshwater fish species, and pollution are the main threats to the conservation of the species. *Achondrostoma asturicense* is still a common species but with more restricted area of distribution than *A. numantinum*. The trend of their populations is unknown, and projections and models in climate change scenarios have not been made on this species. Identical to *A. numantinum*, this information is necessary to include this species in the future in any category of threatened of IUCN, so far it is a data deficient species (DD).

The distribution of *Achondrostoma* species, including the new species described in this work, is shown in Fig. 12. The populations from Galicia and Tajo-Júcar were named *A. asturicense*? and *A. numantinum*? attending to the genetically closest populations (Robalo *et al.,* 2006) but they may be different species and a morphological study must be carried out.

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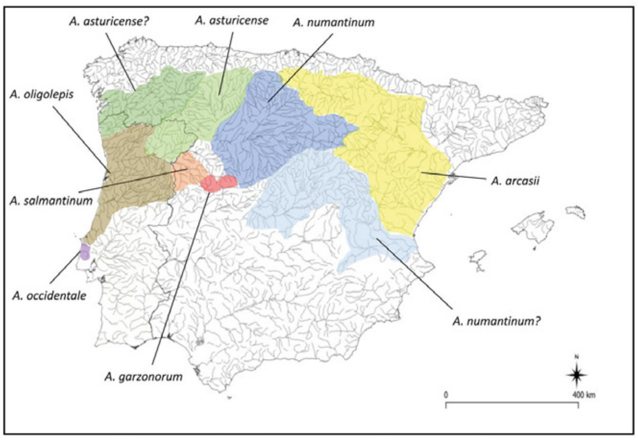

Fig. 12.– Distribution of the species of the Iberian endemic genus *Achondrostoma*.

Fig. 12.– Distribución geográfica de las especies pertenecientes al género *Achondrostoma*, endémico de la Península Ibérica.

scan at the MNCN-CSIC. This research study was funded by the Spanish Ministry of Science and Innovation and the State Agency of Investigation (MCIN/ AEI/ 10.13039/501100011033) as a part of the Project Aphanius (PID2019-103936GB-C22).

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Appendices

Appendix 1.– Osteological material studied. Populations names and collection sites (River and Drainages). SD: Dry Skeletons. CS: Cleaned and stained specimens. CT: Computer tomography. PT: Pharyngeal teeth.

Apéndice 1.– Material estudiado para el análisis osteológico. Nombre de las poblaciones y de lugares de colecta (ríos y cuencas). SD: esqueletos completos guardados en seco. CS: Transparentados y teñidos. CT: tomografía computarizada. PT: dientes faríngeos.

Population	Drainage	River	SD	CS	CT	PT
Northwestern					4	61
	Duero	Tera			4	
		Torío				61
Central-Eastern			8	7	8	90
	Duero	Eresma				30
		Adaja	1	7	4	30
		Voltoya			4	
		Lobos				30
		Abion	3			
		Pedro	3			
		Sequillo	1			
Mediterranean			18	11	4	161
	Ebro	Jiloca				30
		Queiles	18	10	4	63
	Turia	Alfambra				20
	Mijares	Mijares				30
	Palancia	Palancia		1		18
Western			2		4	32
	Tajo	Cuerpo de Hombre	2			32
		Alagón			4	

Appendix 2.– Species and populations names, collection sites (locality, river and drainage). GenBank accession numbers with an * indicate new sequences obtained in this study

Apéndice 2.– Nombre de las especies y de las poblaciones, localidades de colecta (lugar, río y cuenca). Número de acceso en la base de datos de GenBank, un asterisco significa secuencias obtenidas en este estudio.

Species (population designation)	Locality	River	Drainage	GenBank Accession
A. salmantinum	Puebla de Yeltes/Salamanca	Yeltes	Duero	OP945765-66*
A. occidentale	Mafra/Portugal	Safarujo	Safarujo	AY254660-65
A. occidentale	Torres Vedras/Portugal	Sizandro	Sizandro	AY254669-73
A. occidentale	Gosundeira/Portugal	Sizandro	Sizandro	HM560057-58
A. occidentale	Lourinha/Portugal	Alcabrichel	Alcabrichel	AY254585-588
A. oligolepis	Barcelos/Portugal	Cávado	Cávado	AY254595-99
A. oligolepis	Gois/Portugal	Ceira	Mondego	AY254648-53
A. oligolepis	Porto de Mós/Portugal	Lis	Lis	AY254628-31
A. oligolepis	Ponte de Lima/Portugal	Limia	Limia	AY254624-27
A. oligolepis	San Pedro de Moel/Portugal	San Pedro	San Pedro	AY254666-68
A. oligolepis	Vouzela/Portugal	Zela	Vouga	AY254682-84
A. oligolepis	Óbidos/Portugal	Real	Real	AT254656-59
A. oligolepis	Alcobaça/Portugal	Alcoa	Alcoa	AY254691-94
A. oligolepis	Tornada/Portugal	Tornada	Tornada	AY254679
A. oligolepis	Portugal	Minho	Minho	AY254635-37, AY254644
A. oligolepis	Portugal	Paiva	Duero	AY254601-05
A. oligolepis	Portugal	Tavora	Duero	AY254600
***A. arcasii* Mediterranean**	Tulebras/Navarra	Queiles	Ebro	OP945873*
***A. arcasii* Mediterranean**	Medinaceli/Soria	Jalón	Ebro	AY254621
***A. arcasii* Mediterranean**	Valderrobres/Teruel	Matarraña	Ebro	OP945874-77*
***A. arcasii* Mediterranean**	Huesca/Huesca	Isuela	Ebro	OQ389210*

Species (population designation)	Locality	River	Drainage	GenBank Accession
***A. arcasii* Mediterranean**	Egino/Álava	Zirauntza	Ebro	OP945878-85*
***A. arcasii* Mediterranean**	Formiche Alto/Teruel	Mijares	Mijares	OP945886-88*
***A. arcasii* Mediterranean**	Olba/Castellón	Mijares	Mijares	AY254632
***A. arcasii* Mediterranean**	Valbona/Teruel	Valbona	Mijares	AY254633-34
***A. arcasii* Mediterranean**	Bejis/ Castellón	Palancia	Palancia	AY254654-55
***A. arcasii* Mediterranean**	Aguilar de Alfambra/Teruel	Alfambra	Turia	OP945889-91*
***A. arcasii*, NW Duero**	Torre de Moncorvo/Portugal	Sabor	Duero	AY254614-18
***A. arcasii* NW Duero**	Portugal	Manzanas	Duero	X99424, AY254619
***A. arcasii* NW Duero**	Las Omañas/León	Omaña	Duero	OP945767-778*
***A. arcasii* NW Duero**	Ambasaguas del Curueño/León	Curueño	Duero	OP945779-794*
***A. arcasii* NW Duero**	Mondreganes/León	Cea	Duero	OP945795*
***A. arcasii* NW Duero**	Ribadelago/Zamora	Tera	Duero	OP945798-805*
***A. arcasii* NW Duero**	Nuez/Zamora	Neu	Duero	OP945796-97*
***A. arcasii* W Spain**	El Tornadizo/Salamanca	Alagón	Tajo	OP945868-71*
***A. arcasii* W Spain**	San Bartolomé de Corneja /Ávila	Corneja	Duero	OP945864-67*
***A. arcasii* W Spain**	Montemayor del Río/Salamanca	Cuerpo de Hombre	Tajo	OP945872*
***A. arcasii* CE Spain**	Sotillo/Segovia	Duratón	Duero	OP945816-22*, AY254612
***A. arcasii* CE Spain**	Villasandin/Burgos	Odra	Duero	OP945823-32*
***A. arcasii* CE Spain**	Tardesillas /Soria	Tera	Duero	OP945806-15*
***A. arcasii* CE Spain**	Peñalba de San Estebán/Soria	Pedro	Duero	OP945833-42*
***A. arcasii* CE Spain**	Rebollo/Segovia	Cega	Duero	OP945843-51*
***A. arcasii* CE Spain**	Juarros de Voltoya/Segovia	Voltoya	Duero	OP945852-58*
***A. arcasii* CE Spain**	Navas de Oro/Segovia	Eresma	Duero	OP945859-63*
***A. arcasii* CE Spain**	Niharra/Segovia	Adaja	Duero	AY254611
***A. arcasii* CE Spain**	Castillo de la Reina/Segovia	Arlanza	Duero	AY254607
***A. arcasii* CE Spain**	Salinas de Pisuerga/Segovia	Pisuerga	Duero	AY254609

Appendix 3.– Eigenvalues and eigenvectors for the first three principal components (PC1-PC3) of 15 morphometric variables for all *Achondrostoma arcasii* populations. Acronyms are defined in the Material and Methods section. In bold, variables with the highest eigenvectors for each PC.

Apéndice 3.– Eigenvalores y eigenvectores para los tres primeros componentes principales (PC1-PC3) de 15 variables morfométricas para todas las poblaciones de *Achondrostoma arcasii*. Las abreviaturas están descritas en el epígrafe de Material y Métodos. En negrita, variables con los eigenvectores más altos para cada CP.

Variables	PCI	PCII	PCIII
Eigenvalue	0.0626	0.0103	0.0033
% variance	76,822	12,672	4.007
Eigenvectors			
SL	0.0310	0.2199	0.0531
HL	-0.1514	0.1879	0.2456
PrOL	**-0.5535**	0.0100	**0.6340**
ED	0.0819	0.2671	0.1037
PsOL	0.0008	**0.4373**	0.0779
HH	-0.1620	0.2242	0.2589
PrDD	0.0265	0.3421	-0.0008
PrPD	-0.1183	0.1885	-0.0510
PrVD	0.1272	0.3676	0.0024
PrAD	0.2146	0.3725	0.0237
CPL	0.0111	0.1783	0.0529
APL	-0.2132	0.2425	**-0.4261**
CFL	-0.1265	-0.2811	0.1764
BLD	0.3952	-0.0277	0.2385
BD	**0.5849**	-0.0805	**0.4190**

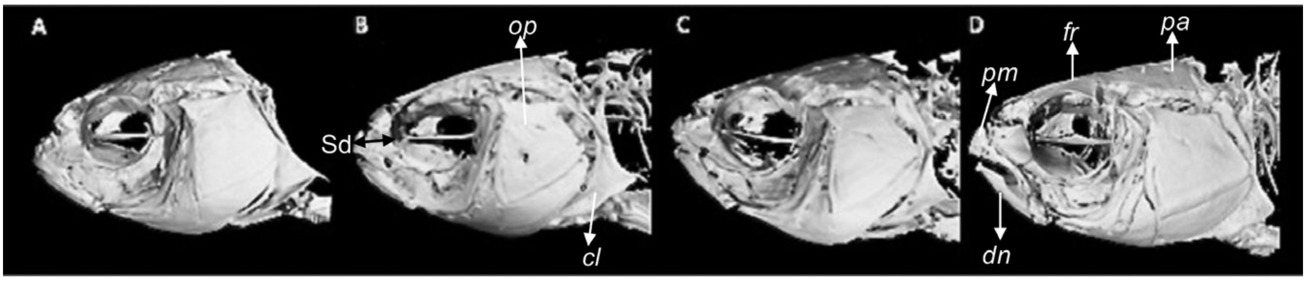
Appendix 4.– Ratios of morphometric variables and scale count. Values are mean (minimum and maximum). Acronyms are defined in the Material and Methods section. In bold indixes more different in the populations studied

Apéndice 4.– Valores de la media, y entre paréntesis valores mínimos y máximos, para diferentes proporciones de las variables morfométricas. Las abreviaturas están descritas en el epígrafe de Material y Métodos. En negrita se señalan los índices más diferentes entre poblaciones.

Measurements	Central-Eastern Duero	Mediterranean basins	Western Spain	Northwestern Duero
SL/HL	**4.64 (4.3-5.6**)	4.43 (3.8-5.3)	4.26 (3.8-4,6)	4.22 (3.7-4.5)
SL/PrOL	17.51 (13.9-26.2)	17.88 (12.7-25.5)	16,16 (13.4-19.8)	16.13 (12.6-20.8)
HL/PrOL	3.78 (3.3-4.9)	**4.02 (3.2-4.9)**	3.79 (3.4-4.5)	3.81 (3.3-4.6))
SL/PsOL	9.53 (8.4 -10.8)	8.98 (7.6-10.5)	8.56 (7.5-9.6)	9.27 (7.9-10.7)
HL/PsOL	2.07 (1.8-2.2)	2.03 (1.6-2.3)	2,01 (1.9-2,2)	**2.19 (2-2.4)**
PsOL/PrOL	1.84 (1.5-2.5)	**1.99 (1.5-2.6)**	1.89 (1.6-2.2)	**1.74 (1.3-2.2)**
HL/HH	1.13 (1-1.3)	1.15 (1-1.26)	1.11 (1-1.2)	1.15 (1_1.3)
SL/APL	4.61 (4.1-5.4)	**4.65 (4-5.6)**	4.36 (3.9-4.9)	4.42 (4-5)
SL/CPL	2.65 (2.4-2.9)	2.7 (2.4-3.1)	2.57 (2-2.8)	2.6 (2.3-2.9)
SL/BLD	9.48 (8.1-10.7)	**9.04 (7.9-11.5)**	**9.57 (8.7-13.1)**	**9.73 (8.9-10.5)**
APL/BLD	2.06 (1.7-2.6)	**1.96 (1.4-2.7)**	2.21 (1.8-3.4)	2.21 (2-2.5)
SL/BD	4.2 (3.38-5.47)	4.14 (3.4-5)	**4.96 (4.4-6.4)**	4.33 (3.8-5.1)
BD/BLD	**2.27 (2-3)**	2.19 (1.8-2.9)	**1.9 (1.6-2.2)**	2.26 (2-2.6)
N. Scales	**45.1, 43-48**	**41.1, 37-43**	**45.9, 43-49**	**40.9, 38-44**

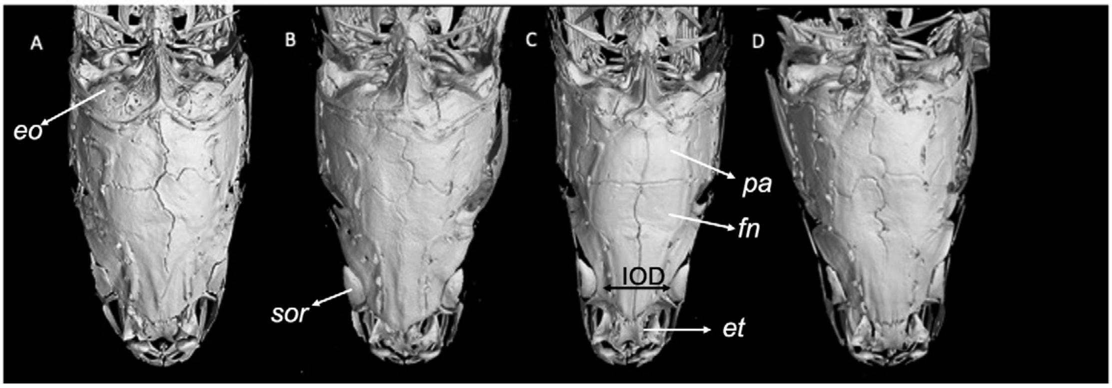
Appendix 5.– Osteological features.

Apéndice 5.– Características osteológicas.



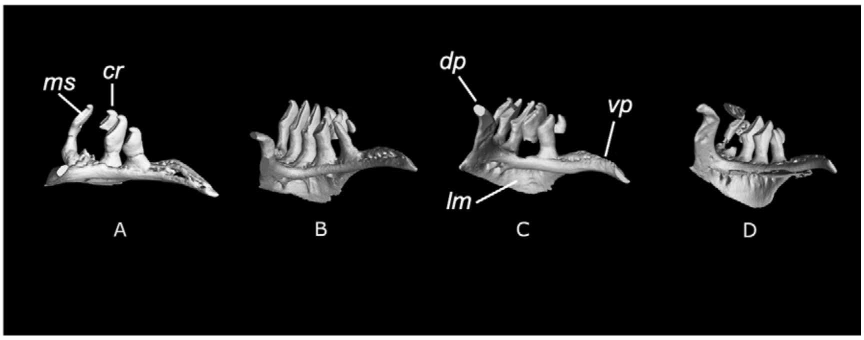
Appendix 5.1.– Lateral view of the skulls of *Achondrostoma* populations under study: A Mediterranean; B Central-Eastern Duero; C Northwestern Duero and D Western Spain. Sd snout distance. *op* opercle. *cl* cleithrum. *pm* premaxilla. *dn* dentary. *fn* frontal. *pa* parietal.

Apéndice 5.1.– Vista lateral del cráneo de las poblaciones estudiadas de *Achondrostoma*. A Mediterráneas; B Centro y Este del Duero; C Noroeste del Duero y D Oeste de España. Sd distancia del hocico. *op* opercular. *cl* cleitro. *pm* premaxilar. *dn* dentario. *fn* frontal. *pa* parietal.



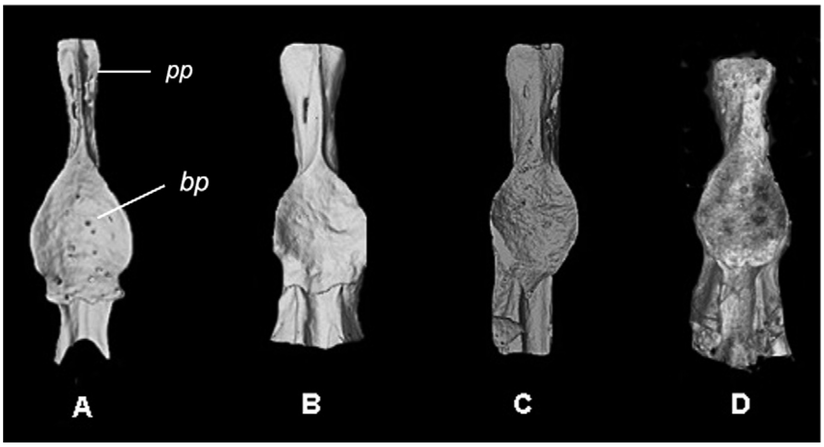
Appendix 5.2.– Dorsal view of the skull of *Achondrostoma* populations under study: A Mediterranean; B Central-Eastern Duero; C Northwestern Duero and D Western Spain. IOD Interorbital distance. *et* ethmoid. *fn* frontal. *pa* parietal. *eo* exoccipital. *sor* supraorbital.

Apéndice 5.2.– Vista dorsal del cráneo de las poblaciones estudiadas de *Achondrostoma*. A Mediterráneas; B Centro y Este del Duero; C Noroeste del Duero y D Oeste de España. IOD Distancia Interorbitria. *et* etmoides. *fn* frontal. *pa* parietal. *eo* exoccipital. *sor* supraorbitario.



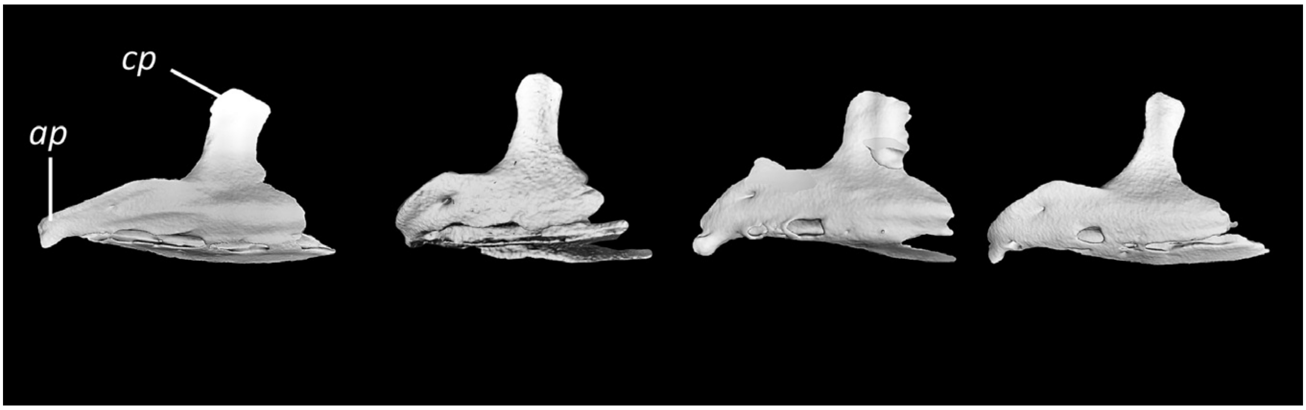
Appendix 5.3.– Pharyngeal teeth of the population studied. A Mediterranean; B Central-Eastern Duero; C Northwestern Duero and D Western Spain. *ms* masticatory surface. *cr* crown. *dp* dorsal process.vp ventral process.

Apéndice 5.3.– Dientes faríngeos de las poblaciones estudiadas. A Mediterráneas; B Centro y Este del Duero; C Noroeste del Duero y D Oeste de España. *ms* superficie masticatoria. *cr* corona. *dp* proceso dorsal.vp proceso ventral.



Appendix 5.4.– Basioccipital bone of the population studied. A Mediterranean; B Central-Eastern Duero; C Northwestern Duero and D Western Spain. *pp* posterior process. *bp* basioccipital plate.

Apéndice 5.4.– Hueso basioccipital de las poblaciones estudiadas. A Mediterráneas; B Centro y Este del Duero; C Noroeste del Duero y D Oeste de España. *pp* proceso posterior. *bp* placa del basioccipital.



Appendix 5.5.– Dentary bone of the *Achondrostoma* population under study. A Mediterranean; B Central-Eastern Duero; C Northwestern Duero; y D Western Spain. *cp* coronoid process. *ap* anterior process.

Apéndice 5.5.– Hueso dentario de las poblaciones estudiadas de *Achondrostoma*. A Mediterráneas; B Centro y Este del Duero; C Noroeste del Duero y D Oeste de España. *cp* proceso coronoideo. *ap* proceso anterior.

Appendix 6.– Genetic uncorrected distances among the analyzed populations based on the *MT-CYB* gene.

Apéndice 6.– Distancias genéticas no corregidas entre las poblaciones analizadas basadas en el gen *MT-CYB*.

	Western Spain	**Northwestern Duero**	**Central-Eastern Duero**	**Ebro**	**Spanish Levant**	***A. occidentale***	***A. oligolepis* 1**	***A. oligolepis* 2**	***A. salmantimun***
Western Spain	-	0.061	0.024	0.027	0.028	0.061	0.022	0.022	0.077
Northwestern Duero		-	0.066	0.068	0.069	0.041	0.063	0.06	0.079
Central-Eastern Duero			-	0.006	0.007	0.065	0.022	0.023	0.077
Ebro				-	0.004	0.066	0.025	0.026	0.079
Spanish Levant					-	0.068	0.023	0.026	0.08
A. occidentale						-	0.061	0.058	0.087
A. oligolepis 1							-	0.013	0.08
A. oligolepis 2								-	0.075
A. salmantinum									-

Appendix 7.– Statistical variables used to define the morphometric characters of 56 individual specimens *A. garzonorum* sp. nov. Each variable is divided by the standard length and multiplied by ten. The variables and material examined are described in Materials and Methods section (SD = standard deviation).

Apéndice 7.– Variables estadísticas utilizadas para definir los caracteres morfométricos de 56 individuos de *A. garzonorum* sp. nov. Las variables y el material examinado son descritos en el apartado de material y métodos (SD = Desviación típica).

***Achondrostoma garzonorum* sp. nov.**			
Variable	**n = 56**		
	Range	**Mean**	**SD**
SL	33.5-105.6	64.2	20.2
HL/SL	2-2.7	2.4	0.02
HH/SL	1.8-2.4	2.1	0.01
PrOL/SL	0.4-0.7	0.6	0.01
ED/SL	0.5-0.8	0.5	0.01
PsOL/SL	0.9-1.4	1.2	0.01
PrPD/SL	2.1-2.9	2.5	0.02
PrDD/SL	4.8-5.6	5.3	0.02
PrVD/SL	4.1-5.5	5.1	0.03
PrAD/SL	5.8-7.3	7	0.03
CPL/SL	3.5-4.2	3.9	0.02
APL/SL	2-2.7	2.3	0.02
PFL/SL	0.8-2.3	1.4	0.03
VFL/SL	1-1.3	1.1	0.03
DFL/SL	0.8-1.3	1	0.01
DFH/SL	1.2-2.1	1.6	0.02
AFL/SL	0.7-1.2	0.9	0.01
AFH/SL	1-1.7	1.5	0.02
CFL/SL	1.5-2.3	2	0.02
BD/SL	1.4.-2.4	2.1	0.02
BLD/SL	0.7-1.1	1	0.01

Appendix 8.– Statistical variables used to define the morphometric characters of 99 individual specimens *A. numantinum* sp. nov. Each variable is divided by the standard length and multiplied by ten. The variables and material examined are described in Materials and Methods section (SD = standard deviation).

Apéndice 8.– Variables estadísticas utilizadas para definir los caracteres morfométricos de 99 individuos de *A. numantinum* sp. nov. Las variables y el material examinado son descritos en el apartado de material y métodos (SD = Desviación típica).

***A. numantinum* sp. nov.**			
Variable	**n = 99**		
	Range	**Mean**	**SD**
SL	42.5-77.3	56.6	9.1
HL/SL	1.8-2.4	2.2	0.01
HH/SL	1.7-2.2	1.9	0.01
PrOL/SL	0.4-0.7	0.6	0.01
ED/SL	0.5-0.7	0.6	0.01
PsOL/SL	0.9-1.2	1.1	0.01
PrPD/SL	1.9-2.8	2.4	0.02
PrDD/SL	5-5.7	5.4	0.01
PrVD/SL	4.9-5.5	5.2	0.01
PrAD/SL	6.7-7.5	7.1	0.02
CPL/SL	3.5-4.1	3.8	0.01
APL/SL	2.4-2.8	2.2	0.01
PFL/SL	1.5-2.3	1.8	0.01
VFL/SL	1.1-1.9	1.4	0.01
DFL/SL	0.9-1.3	1.1	0.01
DFH/SL	1.4-2	1.7	0.01
AFL/SL	0.7-1.2	1	0.01
AFH/SL	1.2-1.9	1.5	0.01
CFL/SL	1.7-2.6	2.3	0.01
BD/SL	1.8.-3	2.4	0.02
BLD/SL	0.9-1.2	1.1	0.01

Appendix 9.– Statistical variables used to define the morphometric characters of 36 individual specimens *A. asturicense* sp. nov. Each variable is divided by the standard length and multiplied by ten. The variables and material examined are described in Materials and Methods section (SD = standard deviation).
Apéndice 9.– Variables estadísticas utilizadas para definir los caracteres morfométricos de 36 individuos de *A. asturicense* sp. nov. Las variables y el material examinado son descritos en el apartado de material y métodos (SD = Desviación típica).

***Achondrostoma asturicense* sp. nov.**			
Variable	**n = 36**		
	Range	**Mean**	**SD**
SL	42.8 -82.8	57.6	9.6
HL/SL	2-2.8	2.4	0.02
HH/SL	1.8-2.4	2.1	0.02
PrOL/SL	0.4-0.8	0.6	0.01
ED/SL	0.5-0.8	0.7	0.01
PsOL/SL	1-1.4	1.2	0.01
PrPD/SL	2-3.1	2.6	0.02
PrDD/SL	5.1-5.9	5.5	0.02
PrVD/SL	4.7-5.6	5.3	0.02
PrAD/SL	6.7-7.4	7.1	0.02
CPL/SL	3.3-4.3	3.8	0.02
APL/SL	1.9-2.5	2.2	0.02
PFL/SL	1.4-2.2	1.8	0.02
VFL/SL	1-1.8	1.4	0.01
DFL/SL	0.7-1.2	1	0.01
DFH/SL	1.4-2.1	1.8	0.02
AFL/SL	0.7-1.1	1	0.01
AFH/SL	1.3-1.8	1.6	0.01
CFL/SL	1.5-2.6	2.3	0.02
BD/SL	1.8.-2.6	2.1	0.02
BLD/SL	0.8-1.1	1	0.01