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DESCRIPTION OF TWO NEW SPECIES OF THE GENUS *SQUALIUS* BONAPARTE, 1837 (ACTINOPTERYGII, LEUCISCIDAE) IN THE IBERIAN PENINSULA

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ABSTRACT

Two new species, *Squalius gaditanus* sp. nov. and *Squalius tartessicus* sp. nov. are described on the basis of morphological and genetic traits. *Squalius gaditanus* is restricted to the Barbate, Jara and Miel drainages in the province of Cádiz (Southern Spain). *Squalius gaditanus* sp. nov. can be distinguished from other *Squalius* species from the Iberian Peninsula through a combination of morphometric, meristic and genetic characters: 36-40 ($\bar{\chi}$=38) pored scales on the lateral line; 6-7 ($\bar{\chi}$=6.7) scales above the lateral line; 2-3 ($\bar{\chi}$=2.8) scales below the lateral line; vertebrae 37-39 ($\bar{\chi}$=38); second infraorbital bone narrower than the third in adults; maxilla with reduced pointed anterior process; posterior process of the maxilla long and thin; lower branch of the pharyngeal bone short and robust; pharyngeal plate of basioccipital rounded and two autapomorphies in the mitochondrial cytochrome *b* gene. *Squalius tartessicus* sp. nov. inhabits the Almargem, Gilão, Odiel, Guadiana, Guadalquivir, Guadalete, Guadalhorce, Velez, Guadalfeo and Segura drainages in the southern part of the Iberian Peninsula. *Squalius tartessicus* sp. nov. can be differentiated from other *Squalius* species from the Iberian Peninsula through a set of morphometric, meristic and genetic traits: 37-41($\bar{\chi}$=38.8) pored scales on the lateral line; 6-7 ($\bar{\chi}$=7) scales above the lateral line; 2-3 ($\bar{\chi}$=2.9) scales below the lateral line; 37-39 ($\bar{\chi}$=38) number of vertebrae; infraorbital bones unusually wide in adults; maxilla with discernable pointed anterior process; posterior process of the maxilla long and thin; lower branch of the pharyngeal bone short and robust; pharyngeal plate of basioccipital triangular in shape; posterior lamina of cleithrum expanding posteriorly.

Keywords. Taxonomy, Iberian Peninsula, *Squalius*, Cypriniformes, Leuciscidae, genetics, morphology.

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RESUMEN

Descripción de dos especies nuevas del género *Squalius* Bonaparte, 1837 (Actinopterygii, Leuciscidae) en la Península Ibérica

Se describen dos nuevas especies, *Squalius gaditanus* sp. nov. y *Squalius tartessicus* sp. nov. sobre la base de caracteres morfológicos y genéticos. *Squalius gaditanus* está restringida a las cuencas de Barbate, Jara y Miel en la provincia de Cádiz (sur de España). *Squalius gaditanus* sp. nov. se puede distinguir de otras especies del género *Squalius*, de la Península Ibérica, a través de una combinación de caracteres morfométricos, merísticos y genéticos: 36-40 ($\bar{\chi}$=38) escamas canaliculadas en la línea lateral; 6-7 ($\bar{\chi}$=6.7) escamas por encima de la línea lateral; 2-3 ($\bar{\chi}$=2,8) escamas debajo de la línea lateral; vértebras 37-39 ($\bar{\chi}$=38); en los ejemplares adultos el segundo infraorbitario es más estrecho que el tercero; maxilar con su proceso anterior poco puntiagudo; proceso posterior del maxilar largo y delgado; la rama inferior del hueso faríngeo es corta y robusta; placa faríngea del basioccipital redondeada y dos autapomorfias en el gen mitocondrial citocromo *b*. *Squalius tartessicus* sp. nov. vive en las cuencas de los ríos Almargem, Gilao, Odiel, Guadiana, Guadalquivir, Guadalete, Guadalhorce, Vélez, Guadalfeo y Segura en el sur de la Península Ibérica. *Squalius tartessicus* sp. nov. se puede distinguir de otras especies del género *Squalius*, de la Península Ibérica, a través de una combinación de caracteres morfométricos, merísticos y genéticos; 37-41 ($\bar{\chi}$=38.8), escamas canaliculadas en la línea lateral; 6-7 ($\bar{\chi}$=7) escamas por encima de la línea lateral; 2-3 ($\bar{\chi}$=2.9) escamas por debajo de la línea lateral; 37-39 ($\bar{\chi}$=38) número de vértebras; en los adultos infraorbitarios excepcionalmente anchos; maxilar con la apófisis anterior puntiaguda; apófisis posterior del maxilar larga y delgada; la apófisis inferior del hueso faríngeo es corta y robusta; placa faríngea del basioccipital de forma triangular; lámina posterior de cleitro extendida.

Palabras clave. Taxonomía, Península Ibérica, *Squalius*, Cypriniformes, Leuciscidae, genética, morfología.

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Introduction

The Iberian Peninsula presents an insular freshwater ichthyofauna as a consequence of its hydrological isolation since the formation of the unsurmountable barrier represented by the Pyrenees during the Oligocene and the opening of the Strait of Gibraltar in the Miocene 5.33 million years ago (Krijgsmann *et al*., 2018). This is especially noticeable for primary freshwater fish families, such as: Nemacheilidae, Cobitidae, Leuciscidae and Cyprinidae. That is, those fishes whose ancestors entered inland waters much earlier and cannot survive in seawater, being strictly confined to freshwater systems (Myers, 1938; Darlington, 1948).

The insularity of the Iberian freshwater ichthyofauna is manifested in its faunistic composition with few genera, due to the difficulty of colonizing the Peninsula by different lineages and by a high number of endemic species as a consequence of the several hydrological reorganizations that have occurred since the Oligocene within the Iberian Peninsula (Perea *et al*., 2016).

The genus *Squalius* Bonaparte, 1837 is one of the genera with a large number of endemic species in the Iberian Peninsula (Doadrio *et al*., 2011). This genus is currently represented in the Iberian Peninsula by two different lineages, one formed by ancient Mediterranean endemisms and the other by a lineage from Central and East Europe and the northern areas of the Mediterranean basin towards Minor Asia, described as Euroasiatic (Sanjur *et al*., 2003). Divergence between both Euroasiatic and Mediterranean lineages should have occurred in the Late Miocene, approximately 7 Ma (Sanjur *et al*., 2003). The Euroasiatic lineage is represented in Spain by an autochthonous single species, *Squalius laietanus* Doadrio, Kottelat & Sostoa, 2007, distributed next to the Pyrenees, in the northeast of the Iberian Peninsula and in the southwest of France.

The Mediterranean lineage is much more diverse in the Iberian Peninsula and some species have aroused the interest of evolutionary biologists in recent years. Currently, nine species are recognized, all of them endemic to the peninsula: *Squalius alburnoides* (Steindachner, 1866), *Squalius aradensis* (Coelho, Bogutskaya, Rodrigues & Collares-Pereira, 1998), *Squalius carolitertii* (Doadrio, 1988), *Squalius castellanus* Doadrio, Perea & Alonso, 2007, *Squalius malacitanus* Doadrio & Carmona, 2006, *Squalius palaciosi* (Doadrio, 1980), *Squalius pyrenaicus* (Günther, 1868), *Squalius torgalensis* (Coelho, Bogutskaya, Rodrigues & Collares-Pereira, 1998) and *Squalius valentinus* Doadrio & Carmona, 2006. One of these species, *S. alburnoides* is unique within the European leuciscid fauna due to its hybrid origin, with specimens of different ploidies and reproduction by hybridogenesis (Carmona *et al*., 1997; Cunha *et al*., 2004, 2011). Probably *S. palaciosi*, of which specimens with different ploidies have also been described, was a species of hybrid origin but seems to have been extirpated since the end of the 20th century (Doadrio *et al*., 2011). The nomenclature of these two hybrid-origin species is controversial, as they result from ancient hybridizations between one species of the *Squalius* genus and species belonging to different lineages than *Squalius*. In this context, we follow the latest nomenclature revision of the group (Collares-Pereira & Coelho, 2010) to provide nomenclatural stability, but it requires further review.

A genetic study based on nuclear and mitochondrial gene sequences on the populations from southern drainages in Spain found two well different lineages within *S. malacitanus* (Perea *et al*., 2016). These two lineages, one inhabiting the Atlantic slope and the other the Mediterranean slope, were geographically separated with the reorganization of the Iberian southern drainages which occurred during the opening of the Gibraltar Strait 5.33 Ma (Krijgsmann *et al*., 2018). Only the population from the Miel River, belonging to the Atlantic lineage, drains to the Mediterranean Sea as a consequence of secondary contacts during the Quaternary (Perea *et al*., 2016). Divergence between these two lineages of *S. malacitanus* occurred in the Mio-Pliocene period around 4.9 Ma (2.1–8.5 Ma) (Perea *et al*., 2016). A posterior work extended the genetic study of the genus *Squalius* to all populations from the Iberian Peninsula and, by analyzing six nuclear genes, corroborated the existence of these two Atlantic and Mediterranean lineages within *S. malacitanus* (Perea *et al*., 2020, 2021). In addition, populations of *S. pyrenaicus* were not monophyletic based on nuclear markers and three independent lineages were recognized: a Northern clade distributed throughout the Tajo Drainage and ascribed to the nominal species *S. pyrenaicus*; a Sado clade inhabiting the Sado drainage in Portugal; and a Southern clade distributed in the remaining Iberian drainages (Perea *et al*. 2021). Genomic data also corroborated the existence of these three independent lineages (Mendes *et al*. 2021). Phylogenetic relationships among *Squalius* species, based on nuclear genes, have shown that *S. pyrenaicus* is not monophyletic and the Northern clade is a sister group of *S. carolitertii* and *S. castellanus* with respect to one group formed by the Southern clade, the Sado clade, *S. valentinus* and *S. malacitanus* (Perea *et al*. 2020, 2021). To determine whether the lineages of *S. pyrenaicus* from the southern Iberian Peninsula and Sado, and the two lineages of *S. malacitanus* (Atlantic and Mediterranean), could be identified as distinct species, Perea *et al*. (2020) conducted a Bayesian nuclear multilocus species delimitation analysis following the MSC framework (Yang & Rannala, 2010; Yang, 2015). They utilized 4871 base pairs obtained from six nuclear loci plus 1140 base pairs of one mitochondrial locus. The analysis yielded robust support, with a posterior probability of 1.00 for these lineages, endorsing the hypothesis that these lineages should indeed be considered as separate species.

In this paper we present a formal description for the two independent lineages inhabiting Spain: the Southern lineage of *S. pyrenaicus* and the Atlantic lineage of *S. malacitanus*.

Material and methods

Morphology

The morphometric and meristic study of the genus *Squalius* was based on the analysis of 58 specimens belonging to the Mediterranean lineage of *S. malacitanus,* 42 specimens from the type locality (Table 1). Ninety-eight specimens of the *Squalius* population previously assigned to the Atlantic lineage of *S. malacitanus* (Perea *et al*. 2020, 2021). Eighty-one specimens belonging to the Northern lineage of *S. pyrenaicus.* One hundred twenty-five specimens of the *Squalius* population previously assigned to the Southern lineage of *S. pyrenaicus* (Perea *et al*. 2020, 2021) (Table 1.).

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

Twenty-four morphometric measurements (in mm) and eleven meristic variables were recorded from digital photographs using TpsDig ver. 1.4 (Rohlf, 2003). The following abbreviations were used for morphometric and meristic characteristics: TL, total length; SL, standard length; PrDD, predorsal distance; PrPD, prepectoral distance; PrVD, preventral distance; PrAD, preanal distance; APL, anal peduncle length; CPL, caudal peduncle length; HL, head length to opercular; PrOL, preorbital length; ED, eye diameter; PsOL, postorbital length; NL, head length to nape; HH, head high; PmxL, premaxilla length; PFL, pectoral fin length; VFL, ventral fin length; DFL, dorsal fin length; DHL, dorsal fin height; 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) was performed using the covariance matrix for morphometric characters. Row-wise bootstrapping was carried out to 100.000 replicates and 95% bootstrap confidence intervals are given to the eigenvalues. Statistical analyses were carried out using Past ver. 4.12 (Hammer *et al*., 2001), we used the option scree plot to indicate the number of significant components.

Osteology

Osteological features were investigated through computer tomography (CT) scan and digital dissection using VGStudio MAX ver. 2.2 (Volume Graphics, http://www.volumegraphics.com) of the following localities:

- <u>*Squalius pyrenaicus* of the Northern Lineage</u>: MNCN_ICTIO 213943-52, 10 specimens from Almonte River, Jaraicejo (Cáceres); MNCN_ ICTIO 267007-16, 10 specimens from Jerte River, Navaconcejo (Cáceres).
- <u>*Squalius pyrenaicus* of the Southern Lineage</u>: MNCN_ICTIO 272254-63, 10 specimens from Ciudadeja River, Las Navas de la Concepción (Sevilla); MNCN_ICTIO 272324-33, 10 specimens from Guadalete River, Puerto Serrano (Cádiz); MNCN_ICTIO 289456, 1 specimen from Estena River, Navas de Estena (Ciudad Real).
- <u>*Squalius malacitanus* of the Mediterranean Lineage</u>: MNCN_ICTIO 212280-89, 10 specimens (paratypes) from Guadalmina River, Benahavis (Málaga); MNCN_ICTIO 63647-56 10 specimens from Hozgarganta River, Jimena de la Frontera (Cádiz).
- <u>*Squalius malacitanus* of the Atlantic Lineage</u>: MNCN_ICTIO 29700-09, 10 specimens from Jara River, Tarifa (Cádiz); MNCN_ICTIO 296956-65, 10 specimens from Vega River, Tarifa (Cádiz).

Additionally dry skeletons preserved in the MNCN collections were studied from the following localities:

Table 1.– Material studied for morphometric analyses. Population Names, Collection Numbers, Sites (River, Drainages, and Coordinates), and Number of Individuals (before slash morphometry, after slash CT-Scan). * *Material from the same locality as the holotype and paratypes*

Tabla 1.– Material estudiado para el análisis morfométrico. Nombre de las poblaciones, número de colección, lugares de colecta (ríos y cuencas) y número de individuos (antes de la barra morfometría, después de la barra CT-Scan). * *Material de la misma localidad que el holotipo y los paratipos.*

Species name	After Perea *et al.* (2020)	Current Study	Collection Number Morphometry	Collection Number. CT-Scan	Drainage	River/N° ind	Coordinates
S. malacitanus	*S. malacitanus* Mediterranean pop	*S. malacitanus*	MNCN_ICTIO 212280-311 MNCN_ICTIO 243699-703 MNCN_ICTIO AT17800-04	MNCN_ICTIO 212280-89	Guadalmina	Guadalmina/42*/10	36.517502, -5.040016
			MNCN_ICTIO AT9702-07		Guadalmina	Guadaiza/6/-	36.525778, -4.992991
				MNCN_ICTIO 63647-56	Guadiaro	Hozgarganta/-/10	36.441565, -5.469525
			MNCN_ICTIO AT9487-96		Guadiaro	Guadiaro/10/-	36.657761, -5.283549
	S. malacitanus Atlantic pop	*S. gaditanus* sp. nov.	MNCN_ICTIO 297000-23 MNCN_ICTIO 243727-55	MNCN_ICTIO 297000-09	Jara	Jara/53*/10	36.103309, -5.632100
			MNCN_ICTIO 296955-99	MNCN_ICTIO 296956-65	Jara	Vega/45*/10	36.028230, -5.610120
S. pyrenaicus	*S. pyrenaicus* Northern pop	*S. pyrenaicus*	MNCN_ICTIO 213943-72 MNCN_ICTIO 215791-816	MNCN_ICTIO 213943-52	Tajo	Almonte/56/10	39.647739, -5.831628
			MNCN_ICTIO 196609- 23 MNCN_ICTIO 266495-504	MNCN_ICTIO 267007-16	Tajo	Jerte/25/10	40.227672, -5.741362
	S. pyrenaicus Southern pop.	*S. tartessicus* sp. nov.	MNCN_ICTIO AT17308-35		Guadalhorce	Grande/28/-	36.701739, -4.881667
			MNCN_ICTIO AT17877-92		Vélez	Sabar/16/-	36.966244, -4.259209
			MNCN_ICTIO 280674-89		Vélez	Cuevas/17/-	36.966244, -4.259209
			MNCN_ICTIO 27265-310		Guadalquivir	Cala/46*/-	37.959673, -6.222448
			MNCN_ICTIO 27225-242	MNCN_ICTIO 271254-63	Guadalquivir	Ciudadeja/18*/10	37.917956, -5.480368
				MNCN_ICTIO 272324-33	Guadalete	Guadalete/-/10	36.945710, -5.474864

- Squalius pyrenaicus of the Northern Lineage: MNCN_ICTIO 69458-62, 69463-68, 20671-73, 20680, 14 specimens from Alburrel River, Valencia de Alcántara (Cáceres); MNCN_ICTIO 69484-85, 2 specimens from Jarama River, Talamanca del Jarama (Madrid); MNCN_ICTIO 69470, 1 specimen from Tajo River, Villarreal de San Carlos (Cáceres); MNCN_ICTIO 69471, 1 specimen from Salor River, Membrio (Cáceres); MNCN_ICTIO 69477, 20674, 2 specimens from Guadalix River, San Agustín de Guadalix (Madrid); MNCN_ICTIO 20277, 20280, 20675, 20676, 4 specimens from Pinilla Reservoir, Pinilla (Madrid); MNCN_ICTIO 20278, 1 specimen, from Aurela River, Santiago de Alcántara (Cáceres).
- Squalius pyrenaicus of the Southern Lineage: MNCN_ICTIO 69469, 1 specimen from Gévora River, Alburquerque (Badajoz); MNCN_ICTIO 69472-74, 3 specimens from Záncara River, Zafra de Záncara (Cuenca). MNCN_ICTIO 69475, 69476, 69478, 3 specimens from Robledillo River, Solana del Pino (Ciudad Real).

Institutional acronyms: MNCN_ICTIO, Ichthyological Collection, Museo Nacional de Ciencias Naturales (Spain).

Genetic analyses

Genetic analyses of Iberian *Squalius* have been the focus of previous studies to resolve their phylogenetic relationships and biogeography with mitochondrial and nuclear genes (Sanjur *et al*., 2003; Perea *et al*., 2016, 2020, 2021). For this reason, the Iberian populations of the genus *Squalius* are well studied group from the phylogenetic point of view. In this study we reanalyse a data set of 268 sequences for the mitochondrial cytochrome *b* gene (*MT-CYB*, 924 pb) obtained from previously published sequences (see Appendix 1 for GenBank accession numbers). Two different phylogenetic analyses were performed using Bayesian inference (BI), implemented in MrBayes ver. 3.2 (Ronquist *et al*., 2012), and Maximum Likelihood, carried out 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 TNF+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). For nuclear phylogeny, we derived a concatenated tree topology from the phylogenetic tree presented by Perea *et al*. (2020), based on the analysis of six nuclear genes and 4871 bp including gaps (Bayesian Inference and Maximum Likelihood analysis; GenBank Accession Numbers: *MT-CYB*: MT008486–MT008603; *RAG1*: MT008604–MT008704, *S7*: MT00855–MT008805, *EFA1α*: MT008910-MT009018, *EGR2b*: MT051740–MT051843, *RHO*: MT008806–MT008909, *ACTB*: MT051635–MT051739). We also calculated the uncorrected p-distances and verify the presence of autapomorphies among *Squalius* populations studied for the *MT-CYB* gene using Mega X (Kumar *et al*., 2018) to sequences download from the GeneBank data base (Appendix1).

Results and discussion

The principal component analysis to all populations and species divided the studied species of *Squalius* into four groups corresponding to populations of: a) *S. pyrenaicus* from the Northern Lineage (Tajo Drainage); b) *S. pyrenaicus* from the Southern Lineage (Guadalquivir, Guadalhorce and Vélez Drainages); c) *S. malacitanus* from the Mediterranean Lineage (Guadalmina and Guadiaro Drainages); and d) *S. malacitanus* from Atlantic Lineage (Jara drainage). All populations of *Squalius pyrenaicus* and all populations of *Squalius malacitanus* were considered in different groups without overlapping and the same occurred with the Northern and Southern lineages of *S. pyrenaicus* (Fig. 1). On the contrary, Atlantic and Mediterranean lineages of *S. malacitanus* showed a wide overlapping (Fig. 1). This overlapping between the Mediterranean and Atlantic lineages of *S. malacitanus* was similar to that found between populations from different rivers within the same species. A certain arrangement can be observed between the populations from the Almonte and Jerte Rivers, within the Northern Lineage, which live in rivers with different typologies. The Almonte River is a Mediterranean-like river influenced by severe water stress during the summer, with specimens of *S. pyrenaicus* surviving in disconnected pools. On the contrary, the Jerte is a mountain river with a permanent flow throughout the year. It can also be observed in the Southern Lineage a certain arrangement separating the populations from Vélez and Guadalquivir drainages.

The eigenvalues of the two first principal components, with the Burnaby-corrected matrix, explained most of the variance (Table 2).

The highest values for eigenvectors, and consequently the variables that contributed most to the ordination in the PCA, were the measurements related

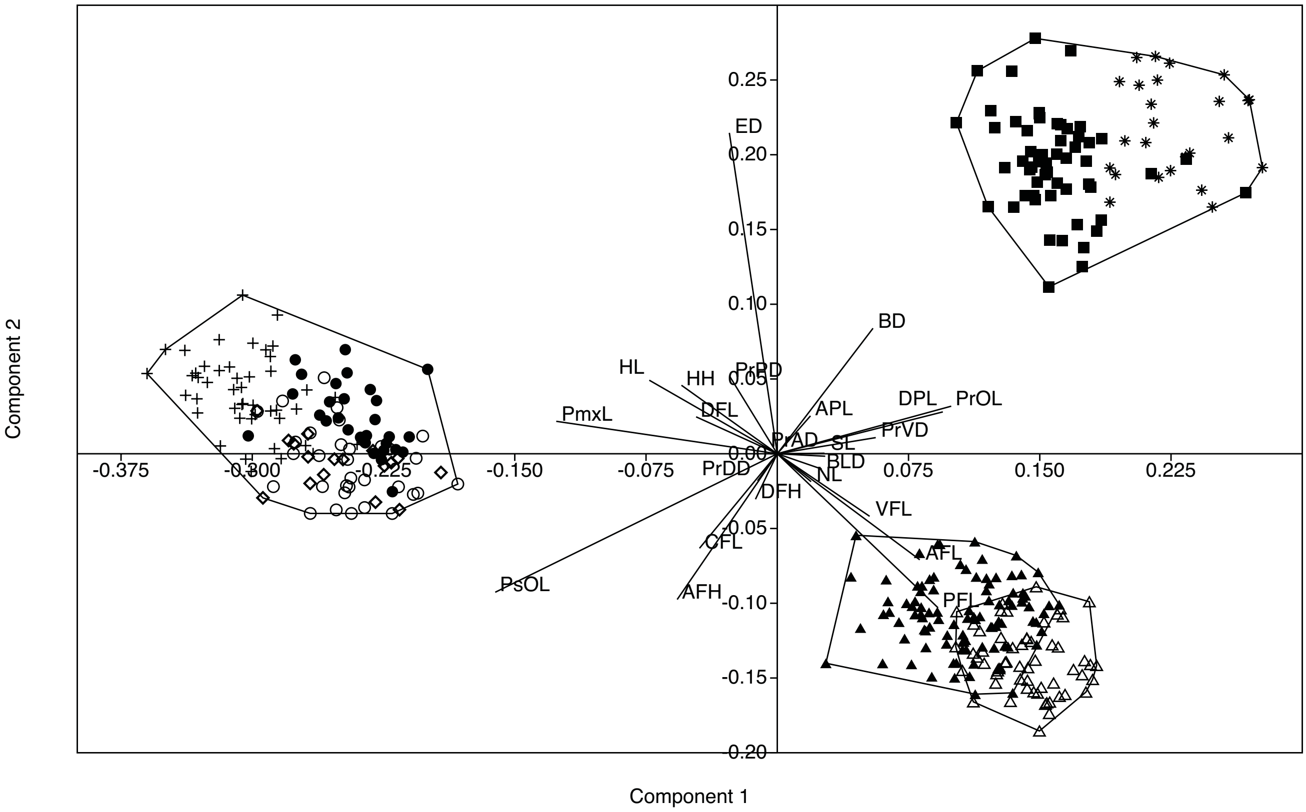

Fig. 1.– Variables that most contributed to the PCA to all populations of the genus *Squalius*. Symbols: Fill Squares, Almonte River (Tajo Drainage), Northern Lineage of *S. pyrenaicus*. Stars, Jerte River (Tajo Drainage), Northern Lineage of *S. pyrenaicus.* Triangles, Mediterranean Lineage of *S. malacitanus*. Fill Triangles, Atlantic Lineage of *S. malacitanus*. Dots, Grande River (Guadalhorce drainage), Southern Lineage of *S. pyrenaicus*. Circles, Sabar and Cuevas Rivers (Vélez Drainage), Southern Lineage of *S. pyrenaicus.* Plus, Cala River (Guadalquivir Drainage), Southern Lineage of *S. pyrenaicus.* Diamonds, Ciudadeja River (Guadalquivir Drainage), Southern Lineage of *S. pyrenaicus.* Abbreviations are defined in the Material and methods section.

Fig. 1.– Variables que más contribuyen al ordenamiento en el PCA para todas las poblaciones del género *Squalius*. Símbolos: Cuadrados, en negro río Almonte (Cuenca del Tajo), Linaje del Norte de *S. pyrenaicus*. Estrellas, río Jerte (Cuenca del Tajo), Linaje del Norte de *S. pyrenaicus*. Triángulos, Linaje Mediterráneo de *S. malacitanus*. Triángulos en negro, Linaje Atlántico de *S. malacitanus*. Puntos negros, río Grande (Cuenca del Guadalhorce), Linaje del Sur de *S. pyrenaicus*. Círculos, ríos Sabar y Cuevas (Cuenca del Vélez), Linaje del Sur de *S. pyrenaicus.* Más, río Cala (Cuenca del Guadalquivir), Linaje del Sur de *S. pyrenaicus.* Diamantes, río Ciudadeja (Cuenca del Guadalquivir), Linaje del Sur de *S. pyrenaicus.* Las abreviaturas están descritas en el epígrafe de Material y métodos.

with the different proportions of the head (Table 2) as occur in other Iberian species of the genus *Squalius* (Doadrio, 1988; Doadrio & Carmona, 2006; Doadrio *et al*., 2007a, 2007b). The length and height of the caudal peduncle was not as decisive in the ordination of the populations as in other morphological studies of *Squalius* species (Doadrio & Carmona, 2006).

To clarify the variables that in each species contribute most to the ordination of the PCA we conducted a separated PCA for the populations of each species. Populations of *S. pyrenaicus* were divided in Northern and Southern lineages (Appendix 2) and the eigenvalues of the two first principal components, with the Burnaby-corrected matrix, explained most of the variance (Appendix 3).

The eigenvectors and consequently the variables that most contributed to the ordination of the PCA were preorbital, postorbital and premaxilla length (Appendix 3). This was a result of the different position of the eye on the head between the specimens of both lineages. In the southern lineage, the eye was displaced toward the snout resulting in a shorter preorbital lengths and a longer postorbital length. The PCA also shown a small differentiation between the populations from the Tajo drainage, as was explained previously. This was not so evident in the Southern lineage.

Regarding *Squalius malacitanus,* the first two PCs arranged the specimens in Atlantic and Mediterranean lineages (Appendix 4). However, the variance was spread among most of the PCs. The four first PCA were significant and eigenvalues to the first two PCs only explained 38.99 % of the variance (Appendix 5). For these reasons, we interpret that the PCA was not successful and our morphometric variables do not adequately separate the two lineages of *S. malacitanus*.

Table 2.– Eigenvalues and eigenvectors for the first two principal components (PCI-PCII) of 23 morphometric variables for all the *Squalius* populations. Acronyms are defined in the Material and Methods section. In bold variables with the highest eigenvectors for each PC.

Tabla 2.– Eigenvalores y eigenvectores para los dos primeros componentes principales (PCI-PCII) de 23 variables morfométricas para todas las poblaciones del género *Squalius*. Las abreviaturas están descritas en el epígrafe de Material y Métodos. En negrita variables con los eigenvectores más altos para cada CP.

	PCI	PCII
Eigenvalue	0.039	0.016
% Variance	60.3	24.98
Eig. 2.5%	57.37	22.29
Eig. 97%	63.2	27.64
Eigenvectors		
SL	0.085	-0.005
PrDD	-0.035	-0.056
PrPD	-0.084	0.160
PrVD	0.174	0.033
PrAD	0.083	0.001
APL	0.059	0.078
DPL	0.292	0.086
HL	-0.226	0.152
PrOL	**0.307**	0.098
ED	-0.084	**0.662**
PsOL	**-0.497**	-0.286
NL	0.060	-0.058
HH	-0.169	0.141
PmxL	**-0.389**	0.067
PFL	0.283	-0.318
VFL	0.163	-0.129
DFL	-0.143	0.076
DFH	-0.038	-0.094
AFL	0.251	-0.219
AFH	-0.176	-0.301
CFL	-0.137	-0.195
BD	0.169	0.259
BLD	0.078	-0.032

The number of scales in the lateral line is very similar in all Mediterranean species of the genus *Squalius*, although some differences were observed in the studied populations (Fig. 2).

The number of scales in the lateral line was smaller in populations of the Atlantic lineage of *S. malacitanus* ($\tilde{X}$=38, 36-40, n=98) and in the Southern populations of *S. pyrenaicus* ($\tilde{X}$=39, 37-41, n=125). Populations of the Northern Lineage of *S. pyrenaicus* ($\tilde{X}$=41, 39-43, n=81) and of Mediterranean Lineage of *S. malacitanus* ($\tilde{X}$=40, 39-43, n=58) had highest number of scales in the lateral line.

The number of vertebras was also smaller in the Atlantic Lineage of *S. malacitanus* ($\tilde{X}$=37, 36-38, n=20). The values greater were to the Northern Lineage of *S. pyrenaicus* ($\tilde{X}$=39, 39-41, n=20) and intermediate values were to the Mediterranean Lineage of

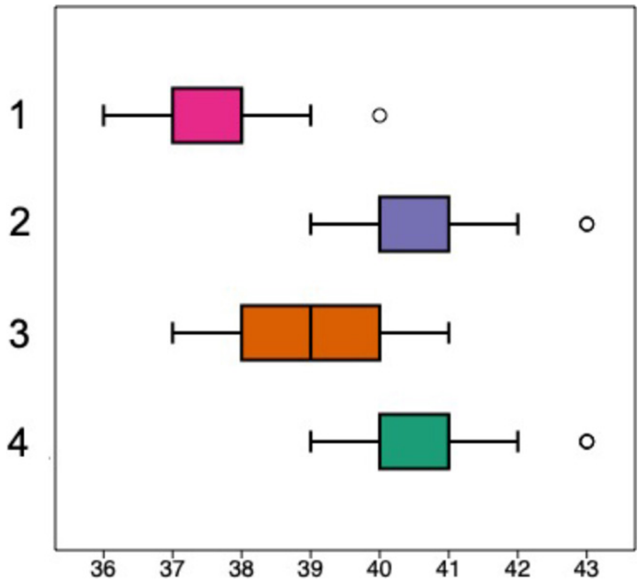

Fig. 2.– Number of pored scales in the lateral line in the different populations: 1, Atlantic Lineage of *S. malacitanus*; 2, Mediterranean Lineage of *S. malacitanus*; 3, Southern Lineage of *S. pyrenaicus* and 4, Northern Lineage of *S. pyrenaicus*.

Fig. 2.– Número de escamas caniculadas en la línea lateral para las diferentes poblaciones: 1, Linaje Atlántico de *S. malacitanus*; 2, Linaje Mediterráneo de *S. malacitanus*; 3, Linaje del sur de *S. pyrenaicus* y 4, Linaje del Norte de *S. pyrenaicus*.

S. malacitanus ($\tilde{X}$=38, 37-39, n=20) and Southern Lineage of *S. pyrenaicus* ($\tilde{X}$=38, 37-39, n=20) (Appendix 6).

In the case of *S. malacitanus* these differences cannot be explained by differences in environmental variables since both lineages inhabit adjacent rivers with identical typology.

Osteology features

Infraorbital bones were large in all populations of *Squalius* except in the Mediterranean population of *S. malacitanus,* which had narrower infraorbital bones. This was more conspicuous on 2nd and 3rd infraorbitals (Fig. 3). All the examined adult specimens from the Southern population of the *S. pyrenaicus* had exceptionally wide infraorbital bones (Fig. 3).

The skull of the Southern population of *S. pyrenaicus* was wider than in other populations of the genus and with a wide ethmoids bone (Appendix 7).

The maxilla of the Northern populations of *S. pyrenaicus* was very robust, with a posterior process stronger than in any other studied population (Appendix 8). The anterior process of the maxilla was more pointed in *S. pyrenaicus* populations, both Northern and Southern lineages, than in *S. malacitanus*, as was previously described (Doadrio & Carmona, 2006). The coronoid process was variable depending on the size of the specimen, but always was more perpendicular to the skull axis in *S. pyrenaicus* (northern and southern populations), whereas in *S. malacitanus* (Atlantic and Mediterranean populations)

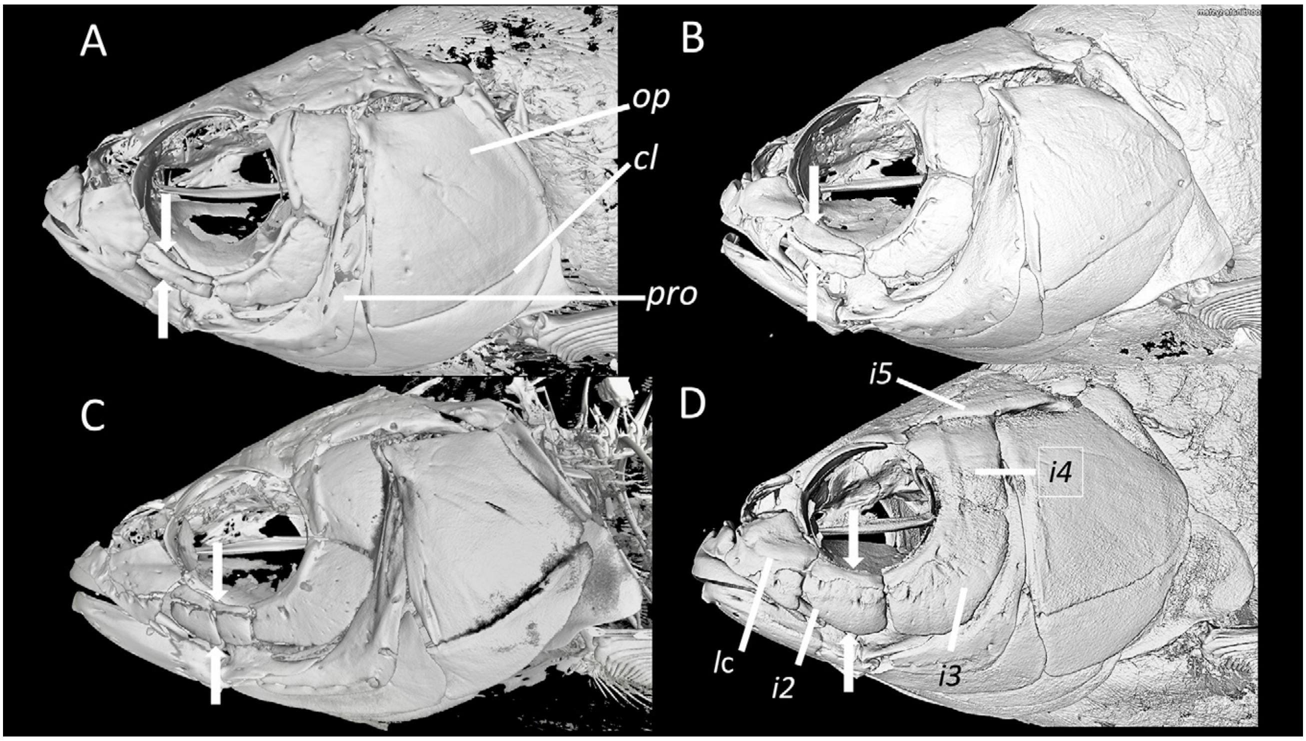

Fig. 3.– Infraorbital bones of *Squalius* populations under study: A, Mediterranean population of *S. malacitanus* from type locality; B, Atlantic population of *S. malacitanus*; C, Northern population of *S. pyrenaicus* and D, Southern population of *S. pyrenaicus*. Arrows indicate the height of the second infraorbital. Abbreviations: *i2* = 2th infraorbital, *i3* = 3th infraorbital, *i4* = 4th infraorbital, *i5* = 5th infraorbital, *lc* = lachrymal, *op* = opercle, *pro* = preoperculum.

Fig. 3.– Huesos infraorbitarios de las poblaciones estudiadas del género *Squalius*. A, población mediterránea de *S. malacitanus* proveniente de la localidad típica. B, población atlántica de *S. malacitanus*. C, población meridional de *S. pyrenaicus*. D, población septentrional de *S. pyrenaicus*. Las flechas indican la altura del segundo infraorbitario. Abreviaturas: *i2* = segundo infraorbitario, *i3* = tercer infraorbitario, *i4* = cuarto infraorbitario, *i5* = quinto infraorbitario *lc* = lagrimal, *op* = opérculo, *pro* = preopérculo.

it was generally more inclined towards the back of the skull.

Basioccipital shape was also distinguishable between *S. pyrenaicus* and *S. malacitanus*. All populations of *S. pyrenaicus* had a clear triangular shape while populations of *S. malacitanus* had a more rounded and laterally expanded pharyngeal plate short (Appendix 9).

The pharyngeal teeth in the populations of *S. pyrenaicus* had strong and very conspicuous denticulations. On the contrary, in the populations of *S. malacitanus* the denticulations were less evident (Appendix 10). The pharyngeal teeth were thinner in the Mediterranean populations of *S. malacitanus* than in remaining populations, which was very evident in the two small pharyngeal teeth of the second row (Appendix 9). Considering specimens of the same size, the Mediterranean populations of *S. malacitanus* had the lower branch of the pharyngeal bone longer and less robust.

The cleithrum shape was very variable with the size of the individuals but usually the Southern population of *S. pyrenaicus* had a posterior lamina more expanding, as was previously described (Doadrio & Carmona, 2006) (Appendix 11).

Genetics

The most divergent species based on the *MT-CYB* gene were the Portuguese *Squalius aradensis* and *S. torgalensis*, sister to the remaining Iberian *Squalius* species. Previous studies on the phylogenetic relationships of the Iberian *Squalius* showed three highly divergent nuclear and mitochondrial clades in the species *S. pyrenaicus*, as is showed in the phylogenetic tree of the Fig. 4A. These three mitochondrial lineages are constituted by Northern populations (Tagus Drainage), Southern populations (Guadiana and Guadalquivir Drainage) and Sado Drainage, and they were the sister group of *Squalius valentinus*. Nevertheless, the nuclear monophyly of these three lineages was not recovered (Fig. 4B): indeed, Northern populations of *S. pyrenaicus* were clustered in a polytomy together with *S. carolitertii* and *S. castellanus*, and they were not closely related with the other two *S. pyrenaicus* clades (Southern and Sado). The species *Squalius malacitanus* also exhibited highly mitochondrial and nuclear divergent clades (Fig. 4A and 4B), which encompassed the Atlantic and the Mediterranean populations separately. The species *Squalius carolitertii* was closely related with *Squalius castellanus*. The mitochondrial and

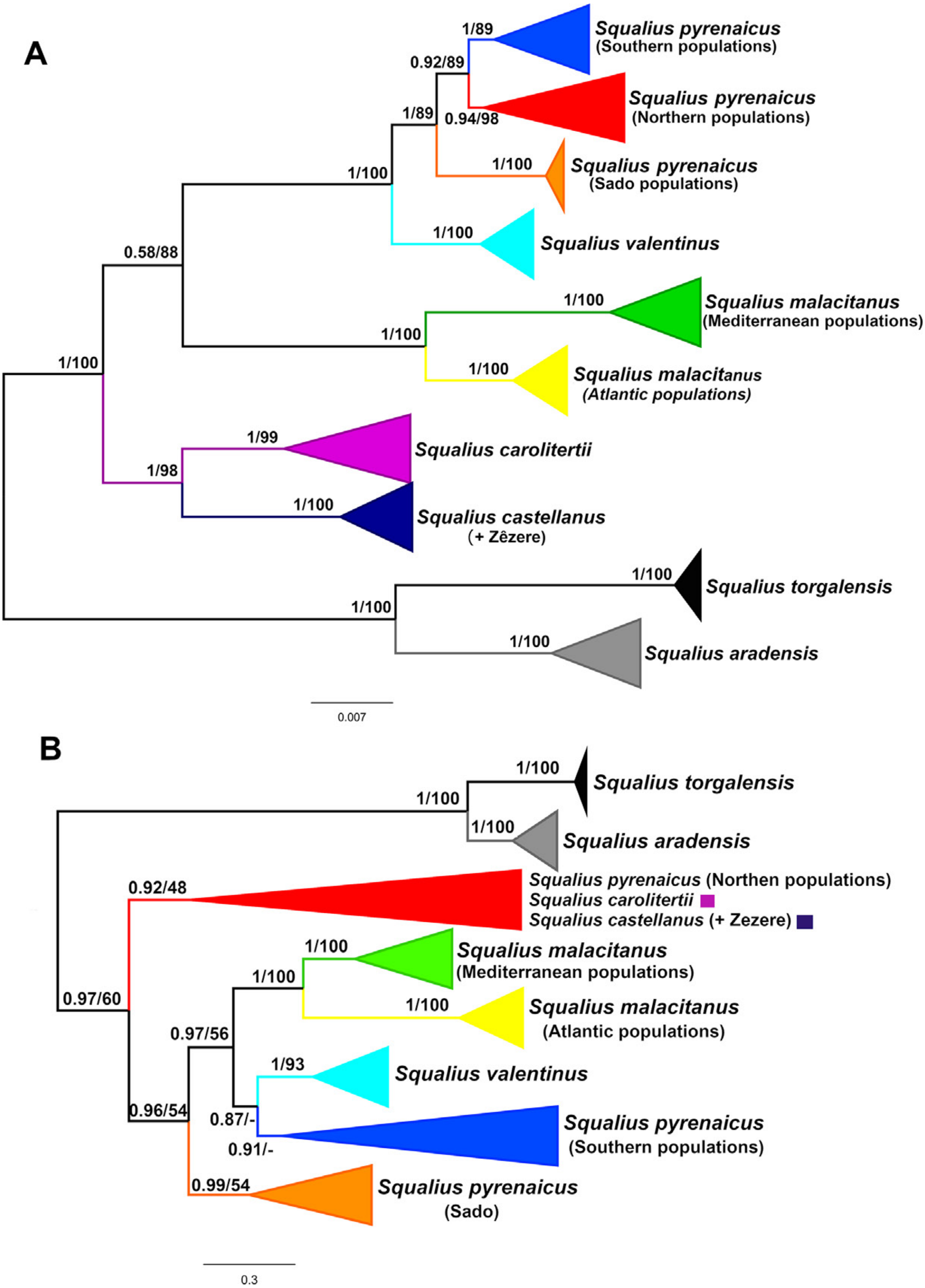

Fig. 4.– A. Phylogenetic tree rendered by Maximum Likelihood and Bayesian Inference based on *MT-CYB* gene. B. Phylogenetic tree topology obtained from Perea *et al*., 2020, based on six nuclear genes. In both tree topologies terminal nodes are collapsed. Numbers on branches indicate posterior probability (before slash) and bootstrap (after slash) values.

Fig. 4.– A. Árbol filogenético generado por Máxima Verosimilitud e Inferencia Bayesiana a partir del gen *MT-CYB.* B. Árbol filogenético obtenido de Perea *et al*., 2020 a partir de seis genes nucleares. En ambas topologías los nodos terminales han sido colapsados. Los números en las ramas indican valores de probabilidad posterior (antes de la barra) y de bootstrap (después de la barra).

nuclear phylogenetic relationships inferred in this study were in concordance with previous studies of the genus (Doadrio & Carmona, 2003, 2006; Sanjur *et al*., 2003; Almada & Sousa-Santos, 2010; Perea *et al*., 2020, 2021; Mendes *et al*. 2021).

Uncorrected-p genetic distances based on *MT-CYB* between all Iberian *Squalius* species ranged from 1.6%, between Northern and Southern populations of *S. pyrenaicus* and 12.1%, between *S. valentinus* and *S. torgalensis* (Appendix 12). Uncorrected-p genetic distances of Southern populations of *S. pyrenaicus* relative to the remaining species were 2.7% with *S. valentinus*, 6, and 6.4% with *S. carolitertii* and *S. castellanus*, 7.6 and 8.6% with Mediterranean and Atlantic populations of *S. malacitanus*, and finally 10.6 and 11.6% with *S. aradensis* and *S. torgalensis*. In turn, uncorrected-p distances of Atlantic populations of *S. malacitanus* relative to the other analyzed species were 4.2% with Mediterranean populations of *S. malacitanus*, from 7.9 to 8.7% with the clade formed by *S. valentinus* and the three divergent lineages of *S. pyrenaicus*, and, finally, 10.9 and 11.4% with *S. aradensis* and *S. torgalensis*.

Atlantic population of *S. malacitanus* had two autapomorphies in the mitochondrial *MT-CYB* gene, none of them were transversions (Appendix 13).

TAXONOMY

Description of the *Squalius* populations

The high degree of morphological and genetic differentiation of *Squalius malacitanus* populations endemic to the Atlantic drainages and to the Miel drainage in the Mediterranean slope, and of the *Squalius pyrenaicus* populations from Southern Iberian drainages justifies 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.

Squalius gaditanus Doadrio & Perea sp. nov.

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Figs. 5–6, Table 3

Holotype: MNCN_ICTIO 296955 89.3 mm SL, 104.2 mm TL; Vega River, Jara Drainage, Tarifa, Cádiz, Spain, 36.028230, -5.610120, 7 m.a.s.l., Leg. P. Garzón-Heydt, T. Nester, A. López Solano and I. Doadrio, 13.V.2022.

Paratypes: MNCN_ICTIO 296956-70, 15 specimens, Vega River, Jara Drainage, Tarifa, Cádiz, Spain, 36.028230, -5.610120, 7 m.a.s.l., Leg. P. Garzón-Heydt, T. Nester, A. López Solano and I. Doadrio, 17.V.2022. MNCN_ICTIO 296971-99 29 specimens, Vega River, Jara Drainage, Tarifa, Cádiz, Spain, 36.028230, -5.610120, 7 m.a.s.l., Leg. P. Garzón-Heydt, T. Nester, A. López Solano and I. Doadrio, 30.IV.2022. MNCN_ICTIO 297000-23, 24 specimens, Jara River, Jara Drainage, Tarifa, Cádiz, Spain, 36.103309, -5.632100, 8 m.a.s.l., Leg. P. Garzón-Heydt and I. Doadrio, 29.X.2022.

Additional material

Barbate Drainage: MNCN_ICTIO 196716-20, 5 specimens, Almodovar River, Facinas, Cádiz, Spain, 36.175707, -5.718908, 130 m.a.s.l., Leg. B. Elvira, 28.X.1986. MNCN_ICTIO 197661, 197671-74, 25348, 34272, 7 specimens, Barbate River, Casas Viejas-Benalup, Cádiz. Spain. 36.333889. -5.791512. 112 m.a.s.l.. Leg. P. Garzón-Heydt and I. Doadrio. 25.X.1978. MNCN_ICTIO 297024-34, 11 specimens,

Fig. 5.– Holotype of *Squalius gaditanus* sp. nov. from the Vega River, Jara Drainage, Tarifa, Cádiz, Spain. MNCN_ICTIO 296955 SL=89.3 mm. Scale bar = 5 mm.

Fig. 5.– Holotipo de *Squalius gaditanus* sp. nov. del río Vega, cuenca del río Jara, Tarifa, Cádiz, España. MNCN_ICTIO 296955. SL=89,3 mm. Escala = 5 mm.

Celemín River, Casas Viejas-Benalup, Cádiz, Spain, 36.299330, -5.781360, 112 m.a.s.l., Leg. P. Garzón-Heydt and I. Doadrio. MNCN_ICTIO 297035-65, 31 specimens, Celemín River, Casas Viejas-Benalup, Cádiz, Spain, 36.304878, -5.720132, 25 m.a.s.l., Leg. J. L. González, S. Perea, P. Garzón-Heydt and I. Doadrio, 25.VI.2010. MNCN_ICTIO 196546-196547, 2 specimens, Rocinejo River, Alcalá de los Gazules, Cádiz, Spain, 36.456306, -5.660076, 165 m.a.s.l., Leg. P. Garzón-Heydt and I. Doadrio, 21. IX.1979.

Jara Drainage: MNCN_ICTIO 196203-07, 5 specimens, Jara River, Tarifa, Cádiz, Spain, 36.058995, -5.637340, 7 m.a.s.l., Leg. L. Domínguez-Nevado and B. Elvira, 15.V.1981. MNCN_ICTIO 208205 – 08, 4 specimens, Jara River, Tarifa, Cádiz, Spain, 36.057973, -5.637296, 7 m.a.s.l., Leg. P. Garzón-Heydt and I. Doadrio, 30.IV.2000. MNCN_ICTIO 243727-55, 29 specimens, Jara River, Tarifa, Cádiz, Spain, 36.076393, -5.633670, 7 m.a.s.l., Leg. I. Doadrio, 11.V.2002. MNCN_ICTIO 272136-157, 22 specimens, Jara River, Tarifa, Cádiz, Spain, 36.075378, -5.632994, 7 m.a.s.l., Leg. B. Prieto, J. L. González and I. Doadrio, 27.V.2009. MNCN_ICTIO 297066-69, 4 specimens, Jara River, Tarifa, Cádiz, Spain, 36.078611, -5.632257, 53 m.a.s.l., Leg. J. L. González, S. Perea, P. Garzón-Heydt and I. Doadrio, 25.VI.2010. MNCN_ICTIO 243780-86, 7 specimens, Vega River, Tarifa, Cádiz, Spain, 36.028230, -5.610120, 7 m.a.s.l., Leg. I. Doadrio, 1.VI.2001. MNCN_ICTIO 297070-99, 30 specimens, Vega River, Tarifa, Cádiz, Spain, 36.028230, -5.610120, 5 m.a.s.l., Leg. J. L. González, S. Perea, P. Garzón-Heydt and I. Doadrio, 25.VI.2010. MNCN_ICTIO 297100-01, 2 specimens, Vega River, Tarifa, Cádiz, Spain, 36.028230, -5.610120, 7 m.a.s.l., Leg. P. Garzón-Heydt, T. Nester, A. López-Solano and I. Doadrio, 13.V.2022. MNCN_ICTIO 297102-17, 16 specimens, Vega River, Tarifa, Cádiz, Spain, 36.103309, -5.632100, 8 m.a.s.l., Leg. P. Garzón-Heydt. and I. Doadrio, 29.X.2022.

Miel Drainage: MNCN_ICTIO 286853-286868, 16 specimens, Miel River, Algeciras, Cádiz, Spain, 36.116310, -5.485037, 20 m.a.s.l., Leg. P. Garzón-Heydt and I. Doadrio, 10.V.2002. MNCN_ICTIO 272271-272323, 52 specimens, Miel River, Algeciras, Cádiz, Spain, 36.116951, -5.483589, 20 m.a.s.l., Leg. M. Casal, S. Perea and I. Doadrio, 27.V.2009. MNCN_ICTIO 297118-21, 4 specimens, Miel River, Algeciras, Cádiz, Spain, 36.118285, -5.481130, 58 m.a.s.l., Leg. J. L. González, S. Perea, P. Garzón-Heydt and I. Doadrio, 26.VI.2010.

Diagnosis. *Squalius gaditanus* sp. nov. is a member of the Mediterranean clade of the Iberian species of the genus *Squalius* (Sanjur *et al*., 2003; Perea *et al*., 2020). *Squalius gaditanus* sp. nov. can be differentiated from all other known species of *Squalius* from the Iberian peninsula according to the following set of characters: 36-40 ($\bar{x}$=38; $\tilde{X}$=38; n=98) pored scales on the lateral line; 6-7 ($\bar{x}$=6.7; $\tilde{X}$=7; n=98) scales above the lateral line; 2-3 ($\bar{x}$=2.8; $\tilde{X}$=3; n=98) scales below the lateral line; 36-38 ($\bar{x}$=37; $\tilde{X}$=37; n=10) number of vertebrae. Second infraorbital bone narrower than the third in adults. Maxilla with reduced pointed anterior process. Dentary short with inclined coronoid process. Posterior process of the maxilla long and thin. The lower branch of the pharyngeal bone is short and robust. Pharyngeal plate of basioccipital rounded. *Squalius gaditanus* sp. nov. is distinguishable from *S. malacitanus,* the morphological and phylogenetically most related species by lesser number of pored scales on the lateral line $\bar{x}$=38 36-40 *vs* $\bar{x}$=41, 39-43; lesser number of vertebrae 36-38 ($\bar{x}$=37) *vs* 37-39 ($\bar{x}$=38); second infraorbital bone wide *vs* narrow; lower branch of the pharyngeal bone short and robust *vs* long and thin and pharyngeal teeth robust *vs* thin. Genetic distances from the other species of *Squalius* inferred from the mitochondrial *MT-CYB* gene were: 4.2% with respect to *S. malacitanus*; 8.5% with respect to *S. pyrenaicus* of Northern population; 8.6% with respect to *S. pyrenaicus* of Southern population; 8.6% with respect to *S. valentinus*; 8.7% with respect to *S. castellanus*; about 7.9% with respect to *S. carolitertii*; 11.4% with respect to *S. torgalensis* and 10.9% with respect to *S. aradensis*. The new species has two autapomorphies none of them transversions in the *MT-CYB* gene (positions 714 and 870; Appendix 9).

Description. D III (II) 8; A III (II) 8; P I 14; V I 8; C 17; LLS 38 (36-40); SRA 6-7; SRB= 2-3; RPT 5.2; LPT 5.2; Vr = 37 (36-38). Morphometric and meristic characters of the type material are given in Table 3; measurements used in the morphometric study appear in Appendix 11. A medium-sized species that rarely reaches 130 mm of standard length. The head is short with the mouth terminal and SL/HL is 3.6-4.3 ($\bar{x}$=4). The head length is similar to the height maxima of the body and BD/HL is 0.9-1.2 ($\bar{x}$=1). The preorbital distance is short and HL/PrOL is 3.8-4.8 ($\bar{x}$=4.3). The caudal peduncle is high and CPL/BLD is 3-3.8 ($\bar{x}$=3,4). The minimum body depth is 2-2.5 ($\bar{x}$=2.3) times lesser than the maximum body depth. The ventral fins are inserted approximately at the same level of the origin of the dorsal fin and PrDD/PrVD is 1-1.1 ($\bar{x}$=1.1). Fins are short. Without or very small nuptial tubercles in males.

Pigmentation pattern. The body is silver with the dorsal portion dark grey, which is clearly visible in all specimens. The scales have one big black spot on the base and a series of small black spots on the distal border. The basis of pectoral fins is brown or orange. Fins rays dark grey.

Etymology. The species name gaditanus is derived from the Phoenician name of the current Cádiz province where the species is distributed.

Table 3.– Morphological variables used to define the morphometric and meristic characters of *S. gaditanus* sp. nov. type series. Variables as described in the Material and methods section (SD = standard deviation).

Tabla 3.– Variables morfológicas utilizadas para definir los caracteres morfométricos y merísticos de la serie tipo de *S. gaditanus* sp. nov. Las variables son descritas en la sección de Material y métodos (SD = desviación típica).

***Squalius gaditanus* sp. nov.**				
Variable	**Holotype**	**Paratypes (n = 68)**		
		Range	**Mean**	**SD**
TL	104.2	39.5-139.9	85.9	19
SL	89.3	33.3-120	73	16.5
PrDD	50	17.9-62.7	39.4	8.4
PrPD	22.9	8-27.9	23.1	4
PrVD	45.7	16.1-59.5	44.9	8.3
PrAD	64.8	22.6-83.9	61.4	11.7
APL	21.9	7.9-29.4	20.2	4.1
CPL	35	12.5-44.7	32.6	6.3
HL	23.3	7.8-28.5	22.7	4.1
PrOL	5.6	1.7-6.4	5.6	1.1
ED	5.9	2.4-7.6	6.3	1
PsOL	11.3	3.9-14.3	11.1	2
NL	17.7	6.3-21.5	17.2	2.9
HH	17.5	6.1-21.6	17.2	3.1
PmxL	7.8	2.5-8.6	7.3	1.3
PFL	14.9	5.9-19.9	15.5	2.8
VFL	13.7	5.1-18.8	13.6	2.4
DFL	11.7	3.5-15.5	10.6	2.2
DHL	16	6.2-21.6	16.4	2.9
AFL	9.8	3.7-12.7	8.3	1.8
AHL	13.7	4.7-17.7	13.3	2.3
CFL	18.2	7-23	17.6	3.1
BLD	10.5	3.9-13.7	9.8	1.9
BD	23.7	8.9-31.9	21	4.5
LLS	38	36-39	37.5	0.7
SRA	7	6-7	6.8	0.5
SRB	3	2-3	2.8	0.4
D	8	8	8	0
A	8	8	8	0

Distribution. This new species is endemic to three small drainages of southern Spain: Jara, Barbate that drain on the Atlantic slope and Miel on the Mediterranean slope around of Gibraltar Strait. Probably *S. gaditanus* was widely distributed in the ancient Janda lagoon but this was dried up in the 20th century (Perea *et al.*, 2016) (Fig. 6).

Common name. Cachuelo gaditano.

Remarks. The species typically inhabits rivers with a Mediterranean typology conditioned by severe water stress during the summer, with specimens of *S. gaditanus* surviving in disconnected pools. During the autumn these rivers can have large discharges that considerably increase the flow of the river, sometimes causing disasters in human infrastructures. The drying up of the Janda Lagoon (Finlayson *et al.*, 1997), with its 50 km^2, eliminated an important refuge for *S. gaditanus* in the face of the great discharges of autumn and the summer droughts. Reservoirs have drastically transformed the habitat of *S. gaditanus* in the Barbate drainage and have been a source of introduction and proliferation of invasive species. In this basin, the species is distributed

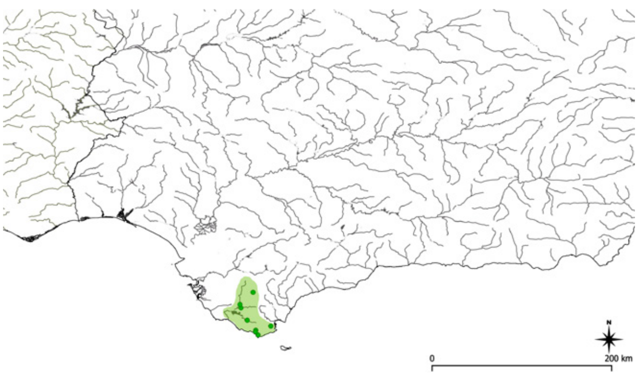

Fig. 6.– Distribution range of *S. gaditanus*. Dots: localities with *S. gaditanus* specimens in the MNCN_ICTIO collection

Fig. 6.– Área de distribución de *S. gaditanus*. Puntos localidades con ejemplares de *S. gaditanus* en la colección del MNCN_ICTIO.

almost exclusively in the headwaters of the rivers. The species should be considered Critically Endangered according to the IUCN red list criteria due to the extent of its occurrence being less than 100 km^2 and to the fragmentation of its populations.

Squalius tartessicus sp. nov.

urn:lsid:zoobank.org:act:E78C1F26-5FAC-4976-8148-CC89B9597E1D
Figs. 7–8, Table 4

Holotype: MNCN_ICTIO 272254 112.2 mm SL. 128 mm TL; Ciudadeja River, Guadalquivir Drainage, Las Navas de la Concepción, Sevilla, Spain, 37.917956, -5.480368, 434 m.a.s.l., Leg. P. Garzón-Heydt, J. L González, B. Prieto and E. Herrero, 05.V.2007.

Paratypes: MNCN_ICTIO 272255-272271, 17 specimens Ciudadeja River, Guadalquivir Drainage, Las Navas de la Concepción, Sevilla, Spain, 37.917956, -5.480368, 434 m.a.s.l., Leg. P. Garzón-Heydt. J. L. González, B. Prieto and E. Herrero, 05.V.2007. MNCN_ICTIO 272656-272701, 46 specimens Cala River, Guadalquivir Drainage, Santa Olalla de Cala, Huelva, Spain, 37.959673, -6.222448, 518 m.a.s.l., Leg. P. Garzón-Heydt & I. Doadrio, 19.IV.2009.

Additional material. See Appendix 14.

Diagnosis. *Squalius tartessicus* sp. nov. is a member of the Mediterranean clade of the genus *Squalius* (Sanjur *et al*., 2003; Perea *et al*., 2020). *Squalius tartessicus* sp. nov. can be differentiated from all other known species of *Squalius* from Iberian peninsula according to the following set of characters: 37-41 ($\bar{\chi}$=38.8, $\tilde{X}$=39, n=125) pored scales on the lateral line; 6-7 ($\bar{\chi}$=7, $\tilde{X}$=7, n=125) scales above the lateral line; 2-3 ($\bar{\chi}$=2.9; $\tilde{X}$=3; n=125) scales below the lateral line; 37-39 ($\bar{\chi}$=38; $\tilde{X}$=38; n=20) number of vertebrae. Infraorbital bones unusually wide in adults. Maxilla with discernable pointed anterior process. Dentary short, not inclined. Posterior process of the maxilla long and thin. The lower branch of the pharyngeal bone is short and robust. Pharyngeal plate of basioccipital triangular in shape. Posterior lamina of cleithrum expanding posteriorly. *Squalius tartessicus* sp. nov. is distinguishable from *S. pyrenaicus,* by lesser number of pored scales on the lateral line 37-41 ($\bar{\chi}$=38.8) *vs* 39-43 ($\bar{\chi}$=40); short preorbital length *vs* long preorbital length; mouth subterminal *vs* terminal mouth; in adults specimens 2nd infraorbital bone as wide as 3rd *vs* 2nd infraorbital narrower than 3rd; ethmoid bone wide *vs* narrow; in adults lamina of cleithrum expanding posteriorly *vs* scarcely expanding posteriorly.

Genetic distances from the other species of *Squalius,* inferred from the mitochondrial *MT-CYB* gene sequences, were: 7.6% with respect to *S. malacitanus*; 1.6% with respect to *S. pyrenaicus* of Northern population; 8.6% with respect to *S. gaditanus*; 2.7% with respect to *S. valentinus*; 6.4% with respect to *S. castellanus*; 6% with respect to *S. carolitertii*; 11.6% with respect to *S. torgalensis* and 10,6% with respect to *S. aradensis*.

Description. D III 8; A III 8; P I 114-15; V I 8; C 17; LLS 39 (37-41); SRA 6-7; SRB 2-3; RPT 5.2 LPT 5.2; Vr= 38 (37-39). Morphometric and meristic characters of the type material are presented in Table 4; measurements used in the morphometric study are listed in Appendix 12. *Squalius tartessicus* sp. nov. is a medium-sized species that rarely reaches 200 mm of standard length. The head is short with the mouth terminal and SL/HL is 3.8-4.7 ($\bar{\chi}$=4.2). The head length is shorter than the height maxima of the body

Fig. 7.– Holotype of *Squalius tartessicus* sp. nov. from the River Ciudadeja, Guadalquivir Drainage, Sevilla, Spain. MNCN_ICTIO 272254. SL= 112.2 mm. Scale bar = 10 mm.

Fig. 7.– Holotipo de *Squalius tartessicus* sp. nov. del río Ciudadeja, cuenca del río Guadalquivir, Sevilla, Spain. MNCN_ICTIO 272254. SL= 112.2 mm. Escala = 10 mm.

Table 4.– Morphological variables used to define the morphometric and meristic characters of *S. tartessicus* sp. nov. type series. Variables as described in the Material and methods section (SD = standard deviation).

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

***Squalius tartessicus* sp. nov.**				
Variable	**Holotype**	**Paratypes (n = 63)**		
		Range	**Mean**	**SD**
TL	128	58.1-140.3	82.4	20.5
SL	112.2	49.3-122	71	18.2
PrDD	59.8	26.4-64.3	38.4	9.5
PrPD	25.4	11.8-29.6	17.6	4.1
PrVD	55.1	24.3-58.9	35.4	8.6
PrAD	77.1	34.3-82.6	48.9	12.4
APL	26.1	10.9-29.6	17	4.5
CPL	42.3	18.2-47	26.7	7.1
HL	25.6	11.3-30.1	17.4	4.1
PrOL	6.6	2.7-7.5	4	1.2
ED	6.5	2.9-7.8	4.7	1
PsOL	12.5	5.7-15.6	8.8	2.1
NL	19.9	9.1-22.4	13.3	3.1
HH	19,9	9.1-22.4	13.5	3.1
PmxL	7.6	3.5-9.5	5.4	1.3
PFL	17.1	8-20.3	11.7	2.8
VFL	15.8	6.7-19	10.4	2.8
DFL	13.8	5.6-15.3	8.3	2.3
DHL	17.5	7.7-19.9	11.7	2.7
AFL	11.8	4.8-13.1	7.2	2.1
AHL	13.4	6.3-15.5	9.5	2
CFL	19.7	9.6-22.3	13.3	2.9
BLD	13.6	5.6-14.1	8.2	2.2
BD	28.8	11.6-32	18.8	4.9
LLS	40	37-41	38.6	1
SRA	7	6-7	6.8	0.4
SRB	3	2-3	2.9	0.4
D	8	8	8	0
A	8	8	8	0

and BD/HL is 1-1.4 (χ=1.2) and head length to nape; is similar to the head high and NH/NL is 0.9-1.2 (χ=1). The preorbital distance is usually shorter than in other *Squalius* species but was very variable in the different populations and HL/PrOL is 3.4-5.2 (χ=4.1). Maxilla is shorter than in *S. malacitanus* and the length is slightly greater than eye diameter and MxL/ED is 1-1.3 (χ=1.1). The caudal peduncle is high and CPL/BLD is 2.9-3.7 (χ=3,3). The minimum body depth is deeper than in other *Squalius* species and is 2-2.7 (χ=2) times lesser than the maximum body depth. The ventral fins are inserted approximately at the same level of the origin of the dorsal fin and PrDD/PrVD is 1-1.1 (χ=1.1). With small nuptial tubercles in males distributed throughout the body. Summary of diagnostic traits of *S. malacitanus*, *S. gaditanus*, *S. pyrenaicus* and *S. tartessicus* is presented in Table 5.

Pigmentation pattern. The body is silver to brownish but without the characteristic dorsal portion dark grey of *S. malacitanus* and *S. gaditanus*. The scales have one big black spot on the base and a series of small black spots on the distal border. The basis of pectoral fins is brown or orange. With small nuptial tubercles in males.

Etymology. The species name tartessicus is derived from Tartessos, a culture that for about 400 years (8^{th}-5^{th} centuries BC) was present in the southwest of the Iberian Peninsula where *S. tartessicus* sp. nov. is currently distributed.

Distribution. This new species is distributed throughout the Atlantic drainages of the southern Iberian Peninsula from Almargem in Portugal to Guadalete in Spain, including the main drainages of Guadiana and Guadalquivir. In the Mediterranean

Table 5.– Diagnostic morphological characters of the four populations studied.

Table 5.– Caracteres morfológicos diagnósticos de las cuatro poblaciones estudiadas.

	S. malacitanus	***S. gaditanus***	***S. pyrenaicus***	***S. tartessicus***
LLS	$\bar{x}$=40 39-43	$\bar{x}$=38 36-40	$\bar{x}$=41 39-43	$\bar{x}$=39 37-41
Vr	$\bar{x}$=38, 37-39	$\bar{x}$=37, 38-36	$\bar{x}$=39, 41-39	$\bar{x}$=38, 37-39
Infraorbitals 2th, 3th	Narrow	wide	wide	Unusually wide
Maxilla Anterior process	Scarce pointed	Scarce pointed	Very pointed	Very pointed
Maxilla Posterior process	Short, Robust	Long, Thin	Long, Thin	Long, Thin
Pharyngeal Plate	Rounded	Rounded	Triangular	Triangular
Pharyngeal teeth	Denticulated	Denticulated	Strongly Denticulated	Strongly Denticulated
Pharyngeal Bone	Long, Thin	Short, Robust	Short, Robust	Short, Robust
Cleithrum lamina posterior	Not extended	Not extended	Not extended	Extended
Pigmentation	Dorsally contrasted	Dorsally contrasted	Dorsally not contrasted	Dorsally not contrasted

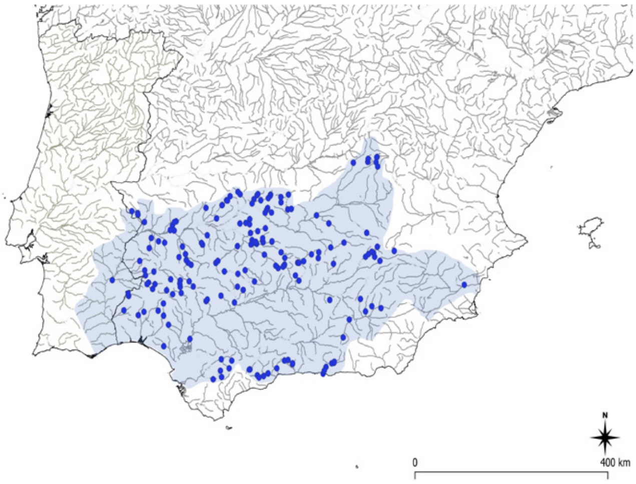

Fig. 8.– Distribution range of *S. tartessicus*. Dots: localities with *S. tartessicus* specimens in the MNCN_ICTIO collection.

Fig. 8.– Área de distribución de *S. tartessicus*. Puntos localidades con ejemplares de *S. tartessicus* en la colección del MNCN_ICTIO.

slope the species is distributed in Guadalhorce Vélez, Guadalfeo and Segura drainages (Fig. 6).

Common name. Cachuelo meridional.

Remarks. The species lives in very different types of habitats from mountain rivers with a permanent flow throughout the year to Mediterranean-like rivers conditioned by severe water stress during the summer, where surviving specimens are found in disconnected pools. This species prefers deep pools within rivers and it forms a well-studied hybrid complex in many of the basins with *S. alburnoides* (Cunha *et al*., 2004). The species should be considered Vulnerable according to the IUCN red list criteria due to the decreasing of its distribution area and population number mainly by increasing of reservoirs, presence of invasive species and pollution by agriculture.

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References

Almada, V.C. & Sousa-Santos, C., 2010. Comparisons of the genetic structure of *Squalius* populations (Teleostei, Cyprinidae) from rivers with contrasting histories, drainage areas and climatic conditions based on two molecular markers. *Molecular Phylogenetics and Evolution*, 57 (2): 924-931. https://doi.org/10.1016/j.ympev.2010.08.015

Burnaby, T.P., 1966. Growth-invariant discriminant functions and generalized distances. *Biometrics*, 22: 96-110. https://doi.org/10.2307/2528217

Carmona, J.A., Sanjur, O.I., Doadrio, I., Machordom, A. & Vrijenhoek, R.C. 1997. Hybridogenetic reproduction and maternal ancestry of polyploid Iberian fish: the *Tropidophoxinellus alburnoides* complex. *Genetics* 146 (3): 983-993. https://doi.org/10.1093/genetics/146.3.983

Collares-Pereira, M.J., & Coelho, M.M. 2010. Reconfirming the hybrid origin and generic status of the Iberian cyprinid complex *Squalius alburnoides*. *Journal of Fish Biology*, 76 (3): 707-715. https://doi.org/10.1111/j.1095-8649.2009.02460.x

Cunha, C., Coelho, M.M., Carmona, J.A. & Doadrio, I., 2004. Phylogeographical insights into the origins of the *Squalius alburnoides* complex via multiple hybridization events. *Molecular Ecology*, 13 (9): 2807-2817. https://doi.org/10.1111/j.1365-294X.2004.02283.x

Cunha, C., Doadrio, I., Abrantes, J. & Coelho, M.M., 2011. The evolutionary history of the allopolyploid *Squalius alburnoides* (Cyprinidae) complex in the northern Iberian Peninsula. *Heredity* 106 (1): 100-112. https://doi.org/10.1038/hdy.2010.70

Darlington, P.J., 1948. The geographical distribution of cold-blooded vertebrates. *The Quarterly Review of Biology* 23 (1): 1–26. https://doi.org/10.1136/bmj.2.614.377)

Doadrio, I., 1988. *Leuciscus carolitertii* n. sp. from the Iberian Peninsula (Pisces; Cyprinidae). *Senckenbergiana Biologica* 68 (4/6): 301-309.

Doadrio, I. & Carmona, J.A., 2003. Testing freshwater Lago Mare dispersal theory on the phylogeny relationships of Iberian cyprinid genera *Chondrostoma* and *Squalius* (Cypriniformes, Cyprinidae). *Graellsia*, 59 (2-3): 457-473. https://doi.org/10.3989/graellsia.2003.v59.i2-3.260

Doadrio, I. & Carmona, J.A., 2006. Phylogenetic overview of the genus *Squalius* (Actinopterygii. Cyprinidae) in the Iberian Peninsula. with description of two new species. *Cybium* 30 (3): 199-214.

Doadrio, I., Kottelat, M. & de Sostoa, A., 2007a. *Squalius laietanus*. a new species of cyprinid fish from north-eastern Spain and southern France (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters* 18 (3): 247-256.

Doadrio, I., Perea, S. & Alonso, F., 2007b. A new species of the genus *Squalius* Bonaparte, 1837 (Actinopterygii. Cyprinidae) from the Tagus River basin (central Spain). *Graellsia* 63 (1): 89-100. https://doi.org/10.3989/graellsia.2007.v63.i1.83

Doadrio, I., Perea, S., Garzón-Heydt, P. & González, J.L., 2011. *Ictiofauna continental española: bases para su seguimiento*. D.G. Medio Natural y Política Forestal. MARM. Madrid. 616 pp.

Finlayson, J.C., Pacheco, F.G., Recio-Espejo, J.M., Mas-Cornella, M., Castro-Román, J.C., Dueñas-López, M.A., Finlayson, G. & Mosquera, M.A.J., 1997. Integrative multi-scale analysis of the impact of the drainage of the La Janda lake (Cádiz Province, Spain) and a model for its sustainable regeneration. *Transactions on Ecology and the Environment*, 16: 203-212. https://doi.org/10.2495/ECOSUD970211

Hammer, O., Harper, D.A.T. & Ryan P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaentologia Electronica*, 4 (1): 9pp.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. & Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14 (6): 587-589. https://doi.org/10.1038/nmeth.4285

Krijgsmann, W., Capellaa, W., Simona, D., Hilgena, F.J., Kouwenhovena, T. J. Meijera, P.T., Sierrob, F.J., Tulburea, M.A., van den Bergb, B. C. J. van der Scheeb, M. & Flecker, R., 2018. The Gibraltar corridor: watergate of the messinian salinity crisis. *Marine Biology* 403: 238–246. https://doi.org/10.1016/j.margeo.2018.06.008.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547-1549. https://doi.org/10.1093/molbev/msy096.

Mendes, S.L., Machado, M.P., Coelho, M.M. & Sousa, V.C., 2021. Genomic data and multi-species demographic modelling uncover past hybridization between currently allopatric freshwater species. *Heredity* 127: 401-412. https://doi.org/10.1038/s41437-021-00466-1

Minh, B.Q., Nguyen, M.A.T. & von Haeseler, A., 2013. Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, 30 (5): 1188-1195. https://doi.org/10.1093/molbev/mst024

Myers, G.S., 1938. Fresh-water fishes and west Indian zoogeography. *Annual Report of the Board of Regents of the Smithsonian Institution,* 1937: 339–364.

Perea, S., Cobo-Simon, M. & Doadrio, I., 2016. Cenozoic tectonic and climatic events in southern Iberian Peninsula: Implications for the evolutionary history of freshwater fish of the genus *Squalius* (Actinopterygii. Cyprinidae). *Molecular Phylogenetics and Evolution* 97: 155-169. https://doi.org/10.1016/j.ympev.2016.01.007

Perea, S., Sousa-Santos, C., Robalo, J. & Doadrio, I. 2020. Multilocus phylogeny and systematics of Iberian endemic *Squalius* (Actinopterygii. Leuciscidae). *Zoologica Scripta*, 49 (4): 440-457. https://doi.org/10.1111/zsc.12420

Perea, S., Sousa-Santos, C., Robalo, J. & Doadrio, I., 2021. Historical biogeography of the Iberian Peninsula: Multilocus phylogeny and ancestral area reconstruction for the freshwater fish genus *Squalius* (Actinopterygii. Leuciscidae). *Journal of Zoological Systematics and Evolutionary Research*, 59 (4): 858-886. https://doi.org/10.1111/jzs.12464

Rohlf, F.J., 2003. Bias and error in estimates of mean shape in geometric morphometrics. *Journal of Human Heredity*, 44 (6): 665-683. https://doi.org/10.1016/S0047-2484(03)00047-2

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61: 539-542. https://doi.org/10.1093/sysbio/sys029

Sanjur, O.I., Carmona, J.A. & Doadrio, I., 2003. Evolutionary and biogeographical patterns within Iberian populations of the genus *Squalius* inferred from molecular data. *Molecular Phylogenetics and Evolution* 29 (1): 20-30. https://doi.org/10.1016/S1055-7903(03)00088-5

Schwarz, G., 1978. Estimating the dimension of a model. *Annals of Statistics*, 6 (2): 461–464. https://doi.org/10.1214/aos/1176344136

Trifinopoulos, J., Nguyen, L-T., von Haeseler, A. & Minh, B. Q., 2013. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acid Research*, 44 (1): 232-235. https://doi.org/10.1093/nar/gkw256

Yang, Z. 2015. The BPP program for species tree estimation and species delimitation. *Current Zoology*. 61 (5): 854-865. https://doi.org/10.1093/czoolo/61.5.854

Yang, Z. & Rannala, B. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences*, 107 (20): 9264-9269. https://www.pnas.org/doi/full/10.1073/pnas.0913022107

APPENDICES

Appendix 1.– GenBank samples used in the *Squalius MT-CYB* phylogenetic analyses

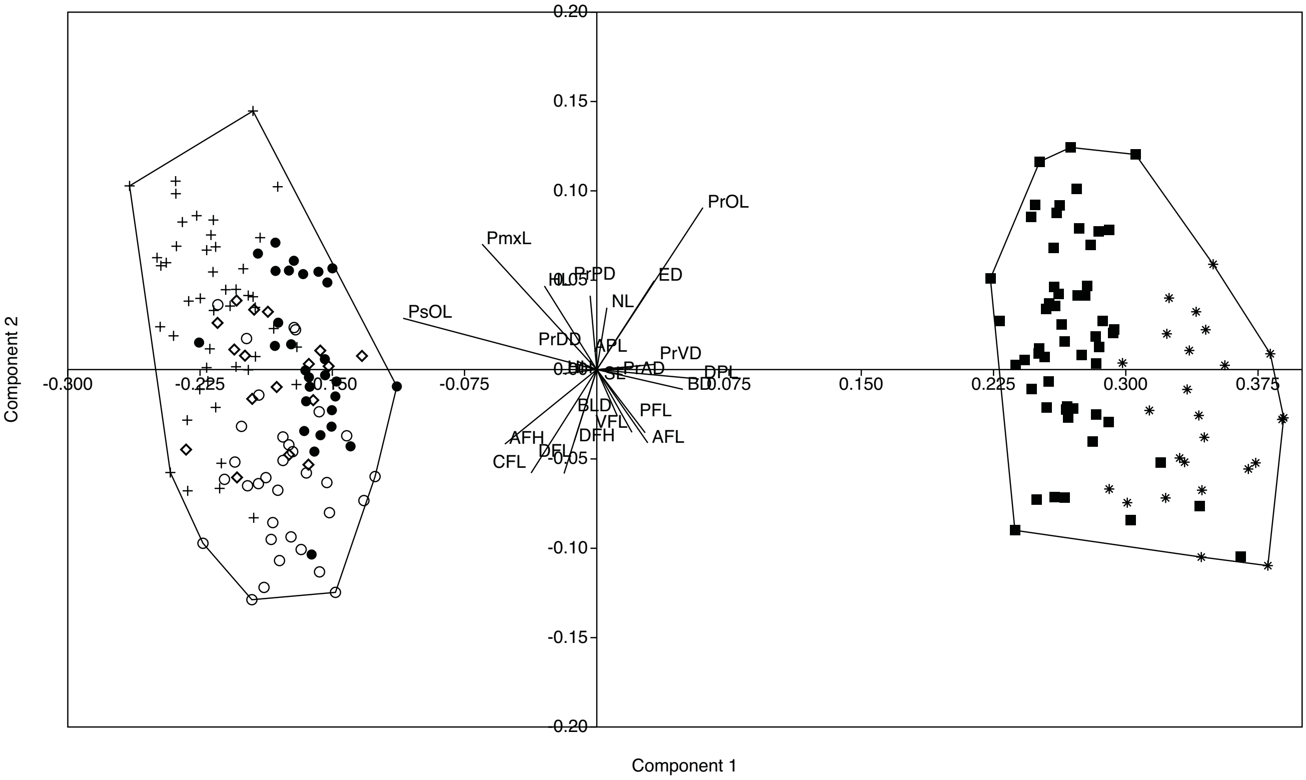
Apéndice 1.– Muestras provenientes del GenBank estudiadas en el análisis filogénetico del *MT-CYB* para el género *Squalius*.

Spacies Name	**After Perea *et al.* (2020)**	**Current Study**	**River. Drainage. Country**	**GenBank Numbers**
Squalius aradensis	*Squalius aradensis*	*Squalius aradensis*	Bensafrim. Portugal	AJ698711
Squalius aradensis	*Squalius aradensis*	*Squalius aradensis*	Bordeira. Portugal	AJ698451
Squalius aradensis	*Squalius aradensis*	*Squalius aradensis*	Quarteira. Quarteira. Portugal	AJ852503, AJ852497, DQ003258
Squalius aradensis	*Squalius aradensis*	*Squalius aradensis*	Arade. Arade. Portugal	AJ852490, AJ852482, AJ852481, AJ852480, AJ583084
Squalius aradensis	*Squalius aradensis*	*Squalius aradensis*	Alvor. Porugal	AJ852466, AJ852465, AJ583074
Squalius aradensis	*Squalius aradensis*	*Squalius aradensis*	Aljezur. Portugal	AJ852458, AJ852456, AJ852453, AJ852452, AJ852449
Squalius aradensis	*Squalius aradensis*	*Squalius aradensis*	Seixe. Portugal	AJ852433, AJ852429
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Agueda. Vouga. Portugal	MT008596
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Vouga. Vouga. Portugal	AJ698455
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Cávado. Cávado. Portugal	MT008594
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Corvo. Mondego. Portugal	MT008589
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Arunca. Mondego. Portugal	MT008587
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Alva. Mondego. Portugal	MT008585
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Neiva. Neiva. Portugal	MT008583
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Alcoa. Alcoa. Portugal	MT008582
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Ceira. Mondego. Portugal	AJ698456
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Ave. Ave. Portugal	AJ698453
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Limia. Limia. Portugal	MT008576, HM560182, HM560181
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Támega. Duero. Spain	MT008569
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Mayas. Duero. Spain	MT008567
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Alberche. Tajo. Spain	MT008565, MT008564
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Huebra. Duero. Spain	MT008558
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Adaja. Duero. Spain	MT008556
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Hormazuelas. Duero. Spain	DQ521430
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Cega. Duero. Spain	DQ521429
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Valdavia. Duero. Spain	DQ521426
Squalius carolitertii	*Squalius carolitertii*	*Squalius carolitertii*	Boedo. Duero. Spain	DQ521425
Squalius castellanus	*Squalius castellanus*	*Squalius castellanus*	Bullones. Tajo. Spain	MT008487
Squalius malacitanus	*Squalius malacitanus* (Atlantic populations)	*Squalius gaditanus* sp. nov.	Guadalmina. Guadalmina. Spain	KU571594, KU571593, KU571592, KU571591
Squalius malacitanus	*Squalius malacitanus* (Atlantic populations)	*Squalius gaditanus* sp. nov.	Genal. Guadiaro. Spain	KU571566, KU571565, KU571564, KU571562, KU571561
Squalius malacitanus	*Squalius malacitanus* (Atlantic populations)	*Squalius gaditanus* sp. nov.	Guadiaro. Guadiaro. Spain	KU571560, KU571559
Squalius malacitanus	*Squalius malacitanus* (Atlantic populations)	*Squalius gaditanus* sp. nov.	Guadalevín. Guadiaro. Spain	KU571558, KU571556, KU571554, KU571553
Squalius malacitanus	*Squalius malacitanus* (Mediterranean populations)	*Squalius malacitanus*	Celemin. Barbate. Spain	KU571590, KU571589, KU571588, KU571587, KU571586, KU571584, KU571583, KU571582, KU571581, KU571580

Spacies Name	After Perea *et al.* (2020)	Current Study	River. Drainage. Country	GenBank Numbers
Squalius malacitanus	*Squalius malacitanus* (Mediterranean populations)	*Squalius malacitanus*	Vega. Vega. Spain	KU571579
Squalius malacitanus	*Squalius malacitanus* (Mediterranean populations)	*Squalius malacitanus*	Jara. Jara. Spain	KU571578, KU571577, KU571576, KU571574, KU571573, KU571572
Squalius malacitanus	*Squalius malacitanus* (Mediterranean populations)	*Squalius malacitanus*	Miel. Miel. Spain	KU571571, KU571569, KU571568, KU571567
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Zêzere. Tajo. Portugal	MT008492 (mitochondrial closely to *S. castellanus*), MT008491
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Rivera de Trevijana. Tajo. Spain	AJ627329
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Tietar. Tajo. Spain	AJ627328
Squalius pyrenaicus	*Squalius pyrenaicus (Northern populations)*	*Squalius pyrenaicus*	Sorraia. Tajo. Spain	AJ627327
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Sever. Tajo. Spain	AJ627326
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Serta. Tajo. Portugal	AJ627325
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Pesquero. Tajo. Spain	AJ627324, AJ627323
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Huso. Tajo. Spain	AJ627322
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Gévalo. Tajo. Spain	AJ627321, MT008500
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Arroyo de la Vid. Tajo. Spain	AJ627320
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Cofio. Tajo. Spain	AJ627319
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Cedena. Tajo. Spain	AJ6273178, AJ627317
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Aurela. Tajo. Spain	AJ627316
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Almonte. Tajo. Spain	AJ627315
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Alburrel. Tajo. Spain	AJ627314
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Jerte. Tajo. Spain	AJ627313
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Caparro. Tajo. Spain	AJ627312
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Arrago. Tajo. Spain	AJ627311, MT008498, MT008497
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Acebo. Tajo. Spain	AJ627310
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Alagón. Tajo. Spain	MT008553, MT008552
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Batuecas. Tajo. Spain	MT008495
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Erjas. Tajo. Spain	MT008493
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Tajuña. Tajo. Spain	MT008508
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Ompolveda. Tajo. Spain	MT008506 (mitochondrial of *S. castellanus*), MT008505
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Lage. Lage. Portugal	MT008541, MT008540
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Lis. Lis. Portugal	MT008533

Spacies Name	After Perea *et al.* (2020)	Current Study	River. Drainage. Country	GenBank Numbers
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Colares. Colares. Portugal	MT008531, MT008530
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Samarra. Samarra. Portugal	MT008529
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Lizandro. Lizandro. Portugal	MT008526
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Piedra. Ebro. Spain	MT008511 (mitochondrial of *S. castellanus*), MT008509
Squalius pyrenaicus	*Squalius pyrenaicus* (Northern populations)	*Squalius pyrenaicus*	Baias. Ebro. Spain	DQ521436, DQ521435, DQ521434
Squalius pyrenaicus	*Squalius pyrenaicus* (Sado)	*Squalius sp.*	Odivelas. Sado. Portugal	AJ627309, AJ627308, AJ627307, MT008534
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Zújar. Guadiana. Spain	AJ627306, OP728011
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Gévora. Guadiana. Spain	AJ627305
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Sillo. Guadiana. Spain	AJ627304, KU571657
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Ruidera. Guadiana. Spain	AJ627303, AJ627302
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Matachel. Guadiana. Spain	AJ627301
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Estena. Guadiana. Spain	AJ627300, AJ6273299, AJ6273297, MT008543
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Azuer. Guadiana. Spain	AJ627296, AJ627295
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Arronches. Guadiana. Portugal	AJ627294
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Robledillo. Guadalquivir. Spain	AJ627293, KU571624, KU571620, KU571619
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Montemayor. Guadalquivir. Spain	AJ627292, AJ627291, AJ627290
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Molinos. Guadalquivir. Spain	AJ627289
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Manzano. Guadalquivir. Spain	AJ627288
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Villar. Odiel. Spain	MT008551, MT008550, KU571654
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Guadarranque. Guadiana. Spain	MT008547, MT008546
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Esteras. Guadiana. Spain	MT008544
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Guadalfeo. Guadalfeo. Spain	MT008525, KU571613, KU571612, KU571611
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Cuevas. Velez. Spain	MT008523, KU571596
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Sabar. Velez. Spain	KU571598, KU571597
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Fresneda. Guadalquivir. Spain	MT008519, KU571616, KU571614
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Cala. Guadalquivir. Spain	MT008517, KU571639, KU571638, KU571637, KU571636, KU571635, KU571634, KU571633, KU571632, KU571631, KU571630
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Segura. Segura. Spain	MT008515, MT008514
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Guadiana. Spain	DQ263239, DQ263236
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Calaboza. Guadiana. Spain	KU571656

Spacies Name	**After Perea *et al.* (2020)**	**Current Study**	**River. Drainage. Country**	**GenBank Numbers**
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Montoro. Guadiana. Spain	KU571652, KU571651
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Yeguas. Guadiana. Spain	KU571649, KU571647, KU571646
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Cabrillas. Guadalquivir. Spain	KU571645, KU571643
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Hueznar. Guadalquivir. Spain	KU571644, KU571629, KU571628, KU571627
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Viar. Guadalquivir. Spain	KU571642, KU571641
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Rivera de Ciudadeja. Guadalquivir. Spain	KU571625, KU571622, KU571621
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Guadalete. Guadalete. Spain	KU571610, KU571609, KU571608, HM560199, HM560198
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Grande. Guadalhorce. Spain	KU571605, KU571604
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Turón. Guadalhorce. Spain	KU571600
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Pereilas. Guadalhorce. Spain	KU571599
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Almargem. Almargem. Portugal	AJ698717
Squalius pyrenaicus	*Squalius pyrenaicus* (Southern populations)	*Squalius tartessicus* sp. nov.	Queimado. Queimado. Portugal	AJ698715
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Micena. Júcar. Spain	MT008490
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Algar. Algar. Spain	MT008489, MT008488, KR871755, KR871753, KR871752, KR871751
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Bullent. Serpis. Spain	KR871767, KR871728, KR871726, KR871725
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Albufera de Valencia. Spain	KR871766, KR871765, KR871764, KR871763, KR871762, KR871761, KR871724, KR871723
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Tuejar. Turia. Spain	KR871759
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Grande. Júcar. Spain	KR871757
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Magro. Júcar. Spain	KR871747
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Júcar. Júcar. Spain	KR871742, KR871741, KR871732, KR871730
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Sellent. Júcar. Spain	KR871735, KR871734
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Cabriel. Júcar. Spain	KR871720
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Vinalopo. Vinalopó. Spain	KR871756
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Carbo. Mijares. Spain	KR81739, KR871737
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Turia. Turia Spain	KR871721
Squalius valentinus	*Squalius valentinus*	*Squalius valentinus*	Serpis. Serpis. Spain	KR871750, KR871743
Squalius torgalensis	*Squalius torgalensis*	*Squalius torgalensis*	Mira. Torgal. Portugal	DQ061934, GU938832, GU938830, GU938825, GU938822, GU938815, GU938810, GU938790, GU938783, GU938775, GU938763
Squalius torgalensis	*Squalius torgalensis*	*Squalius torgalensis*	Torgal. Torgal. Portugal	OP728007



Appendix 2.– Variables that most contributed to the PCA to *S. pyrenaicus* populations. Symbols: Fill Squares, Almonte River (Tajo Drainage), Northern Lineage of *S. pyrenaicus*. Stars, Jerte River (Tajo Drainage), Northern Lineage. Dots, Grande River (Guadalhorce drainage), Southern Lineage. Circles, Sabar and Cuevas Rivers (Vélez Drainage), Southern Lineage. Plus, Cala River (Guadalquivir Drainage), Southern Lineage. Diamonds Ciudadeja River (Guadalquivir Drainage), Southern Lineage. Abbreviations are defined in Materials and Methods.

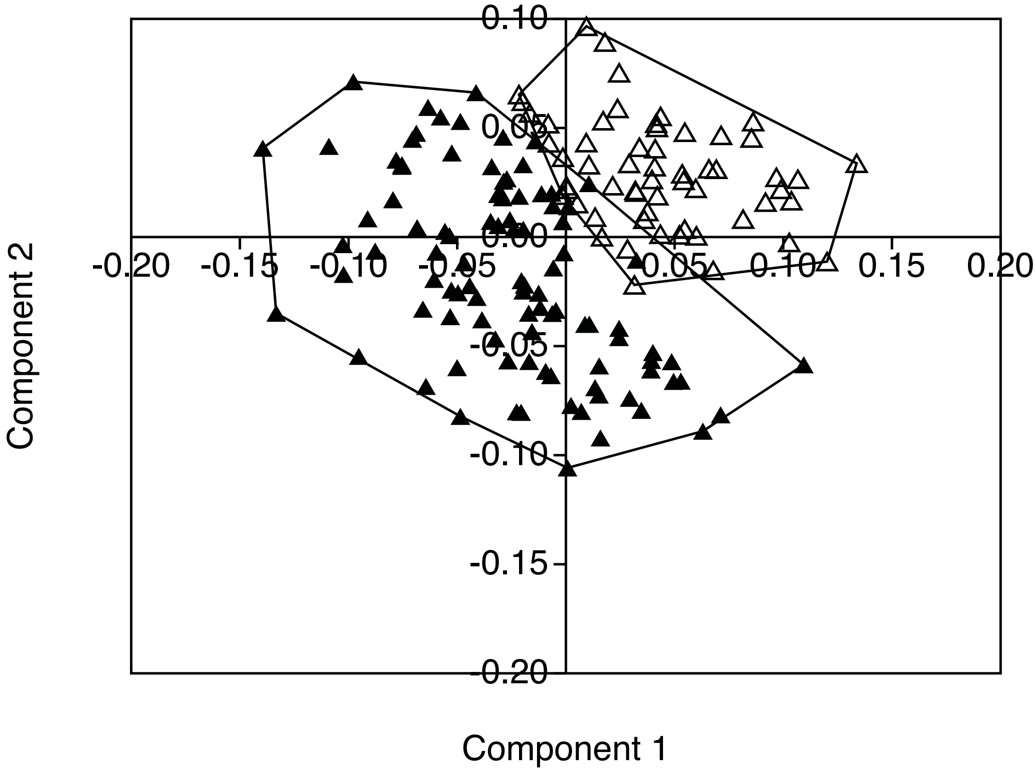
Apéndice 2.– Variables que más contribuyen al ordenamiento en el PCA para todas las poblaciones de *S. pyrenaicus*. Símbolos: Cuadrados en negro, río Almonte (Cuenca del Tajo), Linaje del Norte. Estrellas, río Jerte (Cuenca del Tajo), Linaje del Norte. Puntos negros, río Grande (Cuenca del Guadalhorce), Linaje del Sur. Círculos, ríos Sabar y Cuevas (Cuenca del Vélez), Linaje del Sur. Más, río Cala (Cuenca del Guadalquivir), Linaje del Sur. Diamantes, río Ciudadeja (Cuenca del Guadalquivir), Linaje del Sur. Las abreviaturas están descritas en el epígrafe de Material y Métodos.

Appendix 3.– Eigenvalues and eigenvectors for the first two principal components (PCI-PCII) of 23 morphometric variables for all the *Squalius pyrenaicus* 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 dos primeros componentes principales (PCI-PCII) de 23 variables morfométricas para las poblaciones de *Squalius pyrenaicus*. Las abreviaturas están descritas en el epígrafe de Material y Métodos. En negrita variables con los eigenvectores más altos para cada CP.

	PCI	**PCII**
Eigenvalue	0.057	0.003
% Variance	84.72	4.82
Eig. 2.5%	83.48	4.08
Eig. 97.5%	85.87	5.71
Eigenvectors		
SL	0.076	-0.015
PrDD	-0.055	0.023
PrPD	-0.020	0.216
PrVD	0.174	0.015
PrAD	0.078	0.002
APL	0.082	0.005
DPL	0.302	-0.028
HL	-0.155	0.244
PrOL	**0.314**	0.473

	PCI	**PCII**
ED	0.171	0.260
PsOL	**-0.574**	0.150
NL	0.030	0.181
HH	-0.101	-0.012
PmxL	-0.340	**0.367**
PFL	0.143	-0.185
VFL	0.104	-0.183
DFL	-0.100	-0.304
DFH	-0.067	-0.210
AFL	0.151	-0.214
AFH	-0.272	-0.218
CFL	-0.195	-0.302
BD	0.255	-0.058
BLD	0.061	-0.136



Appendix 4.– Variables that most contributed to the PCA to populations of *Squalius malacitanus*. Triangles, Mediterranean Lineage. Fill Triangles, Atlantic Lineage. Abbreviations are defined in Material and methods.

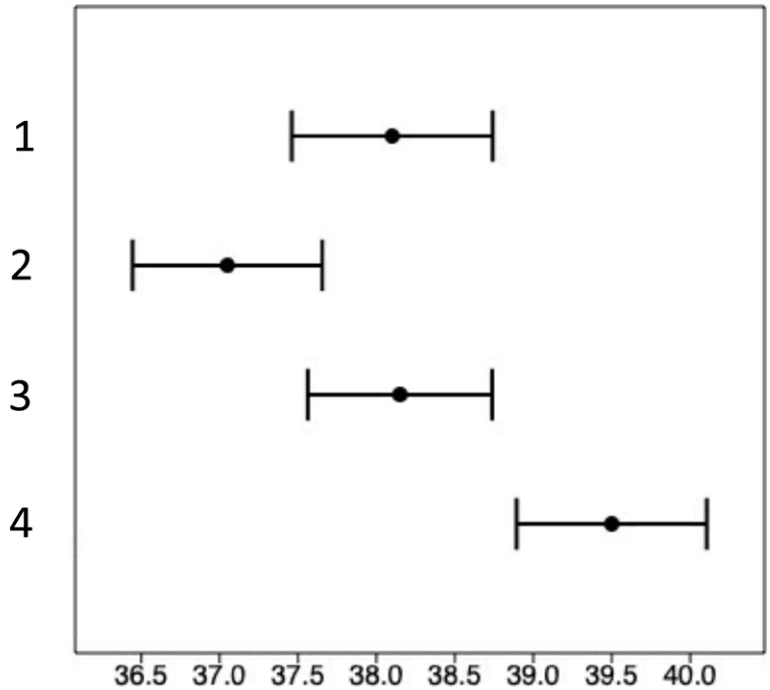
Apéndice 4.– Variables que más contribuyen al ordenamiento en el PCA para las poblaciones de *Squalius malacitanus*. Símbolos: Triángulos, Linaje Mediterráneo. Triángulos en negro, Linaje Atlántico de *S. malacitanus*. Las abreviaturas están descritas en el epígrafe de Material y métodos.

Appendix 5.– Eigenvalues and eigenvectors for the first two principal components (PCI-PCII) of 23 morphometric variables for *Squalius malacitanus*. Acronyms are defined in the Material and methods section. In bold variables with the highest eigenvectors for each PC.

Apéndice 5.– Eigenvalores y eigenvectores para los dos primeros componentes principales (PCI-PCII) de 23 variables morfométricas para las poblaciones de *Squalius malacitanus*. Las abreviaturas están descritas en el epígrafe de Material y métodos. En negrita variables con los eigenvectores más altos para cada CP.

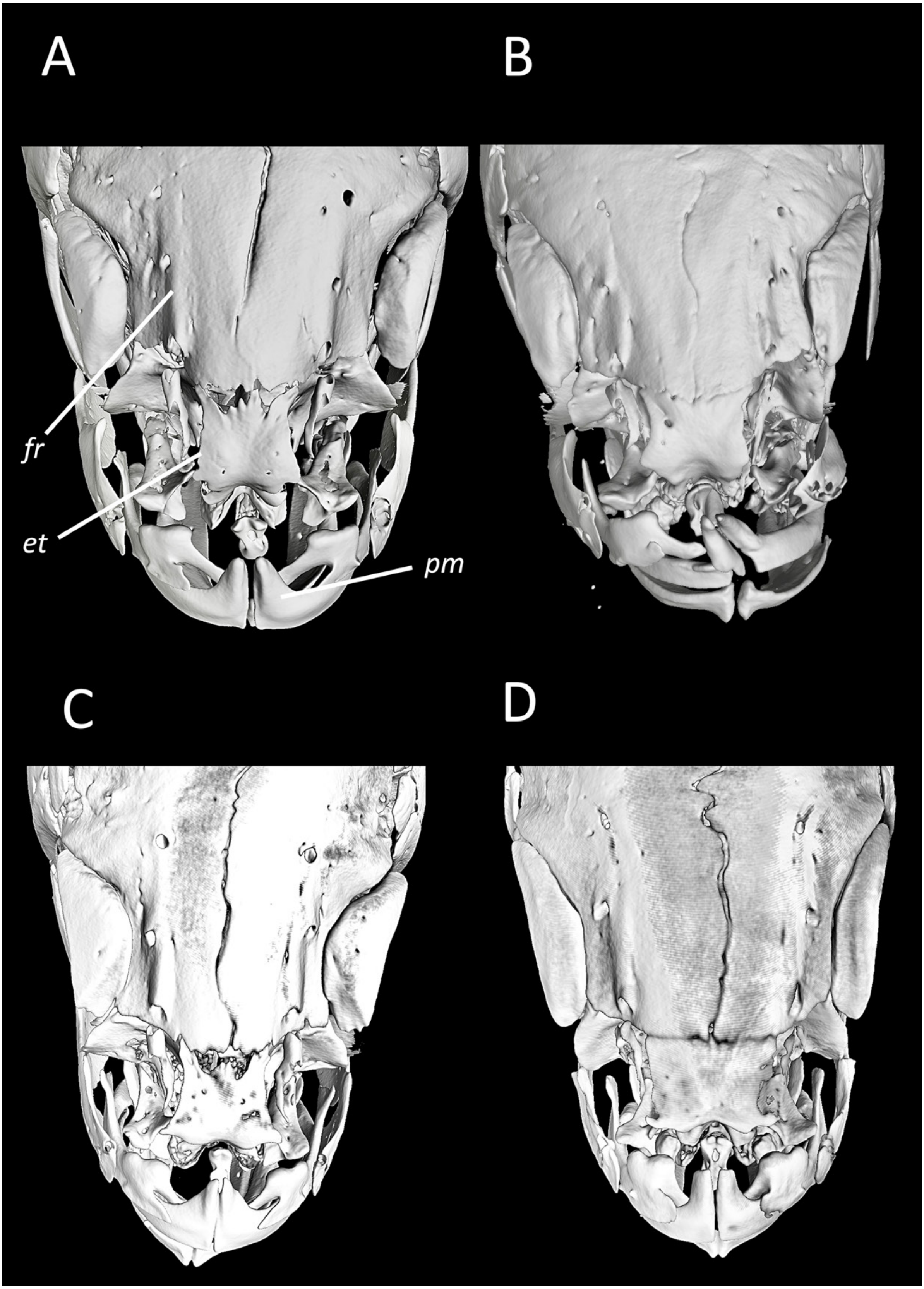
	PCI	**PCII**	**PCIII**	**PCIV**
Eigenvalue	0.003	0.002	0.001	0.001
% Variance	23.18	15.81	12.08	8.10
Eig. 2.5%	20.02	13.68	8.04	6.14
Eig. 97.5%	28.08	19.02	14.03	10.29
Eigenvectors				
SL	0.169	0.054	-0.086	0.023
PrDD	0.105	0.059	-0.045	0.130
PrPD	-0.259	0.031	0.052	-0.029
PrVD	0.035	0.039	-0.020	-0.004
PrAD	0.104	-0.002	-0.070	0.006
APL	**0.422**	0.234	-0.158	0.010
CPL	0.208	0.152	-0.221	0.122
HL	-0.168	0.106	-0.039	-0.050
PrOL	**-0.470**	0.336	-0.219	-0.106
ED	-0.254	0.085	0.177	0.187
PsOL	-0.041	0.081	-0.029	-0.146
NL	-0.100	0.158	0.088	0.004
HH	-0.097	-0.141	0.132	0.006
PmxL	**-0.431**	-0.082	-0.071	-0.143

	PCI	PCII	PCIII	PCIV
PFL	0.208	**0.493**	0.269	-0.040
VFL	0.179	0.115	0.166	-0.036
DFL	0.189	-0.328	0.178	**-0.767**
DFH	0.044	-0.127	0.280	-0.001
AFL	0.099	-0.211	**-0.404**	-0.001
AFH	0.075	-0.086	**0.447**	0.364
CFL	-0.062	**-0.409**	0.249	0.239
BD	0.038	-0.314	-0.310	0.307
BLD	0.078	-0.160	-0.257	0.037



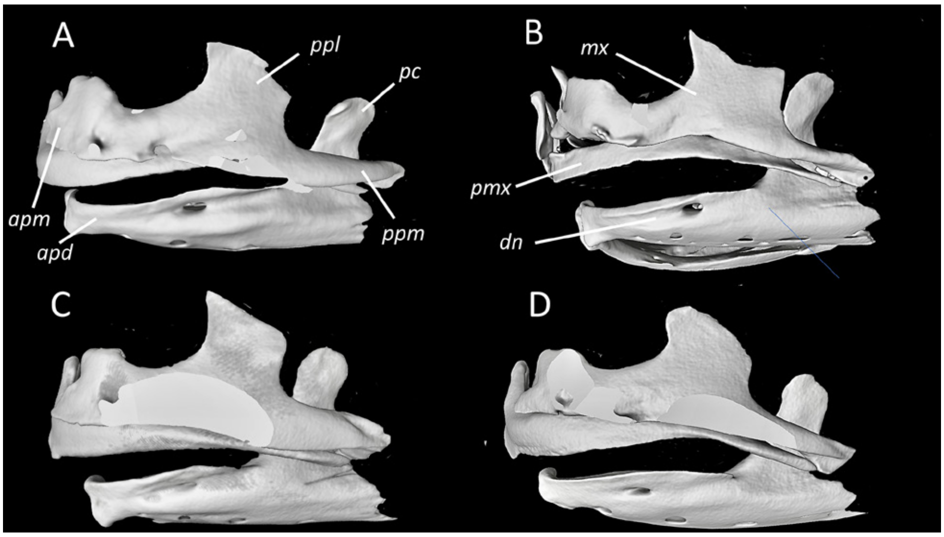
Appendix 6.– Number of vertebrae in the different populations: 1, Mediterranean Lineage of *S. malacitanus*; 2, Atlantic Lineage of *S. malacitanus*; 3, Southern Lineage of *S. pyrenaicus* and 4, Northern lineage of *S. pyrenaicus*.

Apéndice 6.– Número de vertebras para las diferentes poblaciones: 1, Linaje Mediterráneo de *S. malacitanus*; 2, Linaje Atlántico de *S. malacitanus*; 3, Linaje del sur de *S. pyrenaicus* y 4, Linaje del Norte de *S. pyrenaicus*.



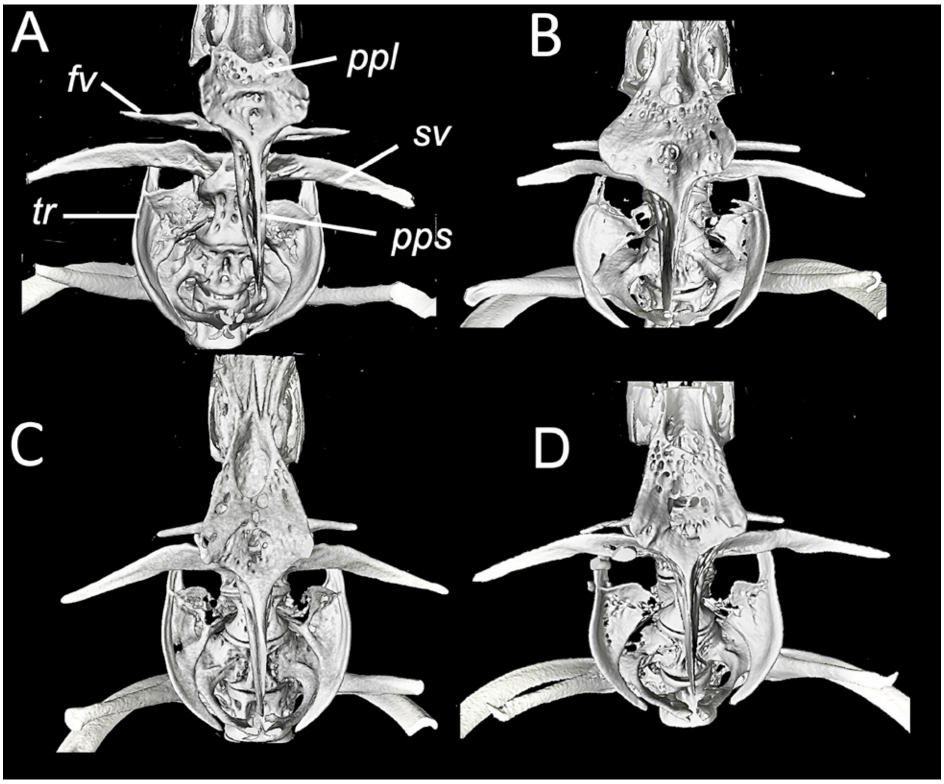
Appendix 7.– Ethmoid bone of *Squalius* populations under study: A, Mediterranean population of *S. malacitanus* from type locality; B, Atlantic population of *S. malacitanus*; C, Northern population of *S. pyrenaicus* and D, Southern population of *S. pyrenaicus*. Abbreviations: *fr* = frontal, *et* = ethmoid, *pm* = premaxilla

Apéndice 7.– Etmoides de las poblaciones estudiadas del género *Squalius*. A, población mediterránea de *S. malacitanus* proveniente de la localidad típica. B, población atlántica de *S. malacitanus*. C, población meridional de *S. pyrenaicus*. D, población septentrional de *S. pyrenaicus*. Abreviaturas: *fr* = frontal, *et* = etmoides, *pm* = premaxilar.



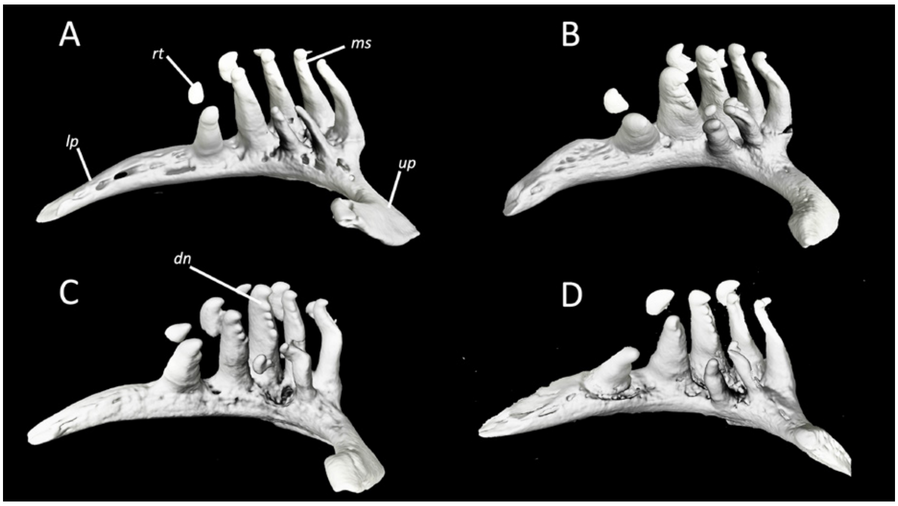
Appendix 8.– Oral jaws of *Squalius* populations under study. A, Mediterranean population of *S. malacitanus* from type locality; B, Atlantic population of *S. malacitanus*; C, Northern population of *S. pyrenaicus* and D, Southern population of *S. pyrenaicus*. Abbreviations: *dn* = dentary, *mx* = maxilla, *pc* = coronoid process, *pmx* = premaxilla, *ppl* = palatine process, *ppm* = posterior process of the dentary.

Apéndice 8.– Aparato mandibular anterior de las poblaciones estudiadas del género *Squalius*. A, población mediterránea de *S. malacitanus* proveniente de la localidad tipo. B, población atlántica de *S. malacitanus*. C, población meridional de *S. pyrenaicus*. D, población septentrional de *S. pyrenaicus*. Abreviaturas: *dn* = dentario, *mx* = maxilar, *pc* = apófisis coronoidea, *pmx* = premaxilar, *ppl* = proceso palatino, *ppm* = apófisis posterior del dentario.



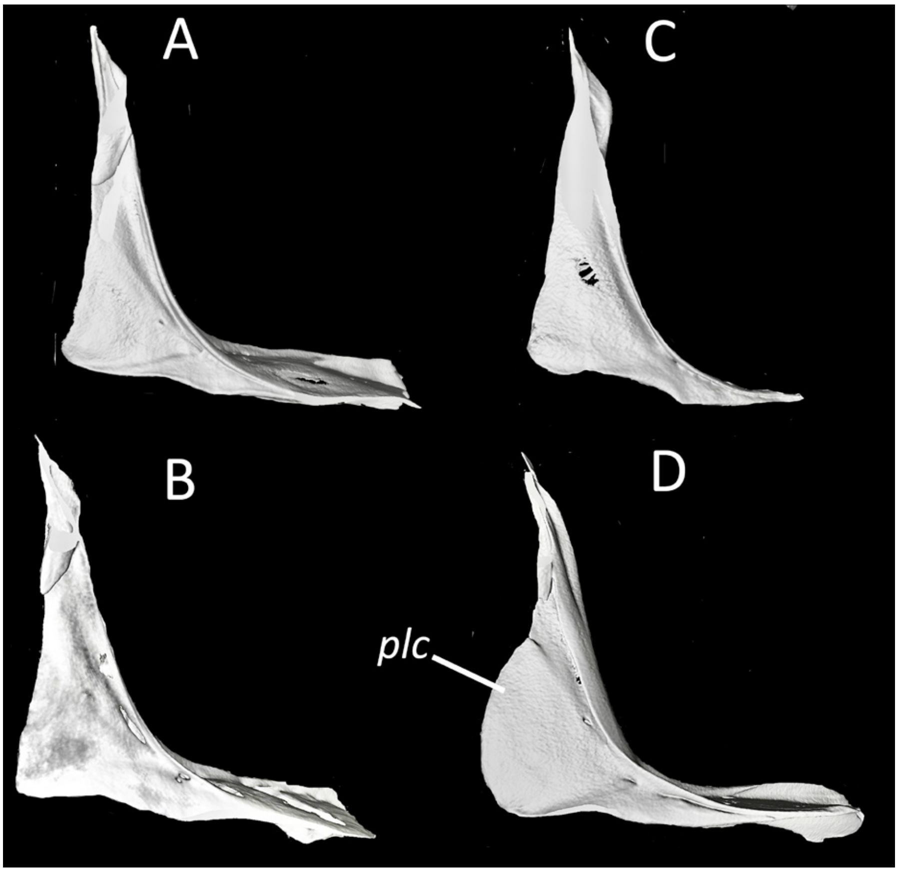
Appendix 9.– Basioccipital of *Squalius* populations under study. A, Mediterranean populations of *S. malacitanus* from type locality; B, Atlantic populations of *S. malacitanus*; C, Northern populations of *S. pyrenaicus* and D, Southern populations of *S. pyrenaicus*. Abbreviations: *fv* = lateral processes of the first vertebra, *mx* = maxilla, *ppl* = pharyngeal plate, *pps* = posterior process of the basioccipital, *sv* = lateral processes of the second vertebra, *tr* = tripus.

Apéndice 9.– Basioccipital de las poblaciones estudiadas del género *Squalius*. A, población mediterránea de *S. malacitanus* proveniente de la localidad tipo. B, población atlántica de *S. malacitanus*. C, población meridional de *S. pyrenaicus*. D, población septentrional de *S. pyrenaicus*. Abreviaturas: *fv* = apófisis lateral de la primera vertebra, *ppl* = placa faríngea, *pps* = apófisis posterior del basioccipital, *sv* = apófisis lateral de la segunda vertebra, *tr* = trípode.



Appendix 10.– Pharyngeal teeth of *Squalius* populations under study. A, Mediterranean populations of *S. malacitanus* from type locality; B, Atlantic populations of *S. malacitanus*; C, Northern populations of *S. pyrenaicus* and D, Southern populations of *S. pyrenaicus*. Abbreviations: *dn* = denticulations, *lp* = lower process of pharyngeal bone, *ms* = masticatory surface, *rt* = replacement teeth, *up* = upper process of pharyngeal bone.

Apéndice 10.– Dientes faríngeos de las poblaciones estudiadas del género *Squalius*. A, población mediterránea de *S. malacitanus* proveniente de la localidad tipo. B, población atlántica de *S. malacitanus*. C, población meridional de *S. pyrenaicus*. D, población septentrional de *S. pyrenaicus*. Abreviaturas: *dn* = denticulaciones, *lp* = apófisis inferior del hueso faríngeo, *ms* = superficie masticatoria, *rt* = dientes de reemplazo, *up* = apófisis superior del hueso faríngeo.



Appendix 11.– Cleithrum of *Squalius* populations under study. A, Mediterranean population of *S. malacitanus* from type locality; B, Atlantic population of *S. malacitanus*; C, Northern population of *S. pyrenaicus* and D, Southern population of *S. pyrenaicus*. Abbreviation: *plc*, posterior lamina of the cleithrum.

Apéndice 11.– Cleitro de las poblaciones estudiadas del género *Squalius*. A, población mediterránea de *S. malacitanus* proveniente de la localidad típica. B, población atlántica de *S. malacitanus*. C, población meridional de *S. pyrenaicus*. D, población septentrional de *S. pyrenaicus*. Abreviatura: *plc*, posterior lamina del cleitro.

Appendix 12.– Uncorrected-p genetic distances between species and populations for the genus *Squalius* from the Iberian Peninsula.

Apéndice 12.– Distancias genética no corregidas entre las diferentes especies y poblaciones del género *Squalius* en la Penínula Ibérica.

	S. aradensis	***S. pyrenaicus* Northen populations**	***S. pyrenaicus* Sado**	***S. pyrenaicus* Southern populations**	***S. carolitertii***	***S. castellanus***	***S. malacitanus* Atlantic populations**	***S. malacitanus* Mediterranean populations**	***S. valentnius***	***S. torgalensis***
S. aradensis	0.6									
S. pyrenaicus Northern populations	10.2	0.1								
S. pyrenaicus Sado	10.8	2.2	0.12							
S. pyrenaicus Southern populations	10.6	1.6	2.2	0.9						
S. carolitertii	10.2	5.9	6.3	6	0.7					
S. castellanus	11	6.5	6.9	6.4	4.2	0.8				
S. malacitanus Atlantic populations	10.9	8.5	8.4	8.6	7.9	8.7	0.7			
S. malacitanus Mediterranean populations	10.4	7.6	7.8	7.6	7.1	7.3	4.2	0.5		
S. valentinus	11.1	2.9	3.0	2.7	6.3	6.9	8.6	7.4	0.4	
S. torgalensis	6	11.5	11.9	11.6	10.9	11.8	11.4	11.9	12.1	0.3

Appendix 13.– Autapomorphies in the mitochondrial cytochrome *b* gene detected for the genus *Squalius*. Transversions are indicated with *.

Apéndice 13.– Autapomorfías para el gen mitocondrial citocromo b en el género *Squalius*. Transversiones son indicadas con *.

Species	**141**	**145**	**180**	**207**	**264**	**282**	**288**	**309**	**312**
S. aradensis	G	C	A	C	C	T	A	T	T
S. pyrenaicus Northern populations	C	C	A	C	C	T	A	T	T
S. pyrenaicus Sado	C	C	A	C	C	T	A	T	T
S. pyrenaicus Southern populations	C	C	A	C	C	T	A	T	T
S. carolitertii	C	C	A/G	C	C	T	A	T	T
S. castellanus	C	C	T	C	C	T	A	T	T
S. malacitanus Atlantic populations	C	C	A	C	C	T	A	T	T
S. malacitanus Mediterranean populations	C	C	A	C	C	T	G	T	T
S. valentinus	C	T	A	C	C	T	A	C	T
S. torgalensis	A	C	A	T	A	C	A	T	C

…. Continued from Appendix 13

Species	**327**	**333**	**354**	**423**	**429**	**457**	**465**	**507**	**513**
S. aradensis	T	A	A	A	C	G	C	C	C
S. pyrenaicus Northern populations	T	A	C	A	C	G	C	C	C
S. pyrenaicus Sado	T	A	C	A	C	G	C	C	C
S. pyrenaicus Southern populations	T	A	C	A	C	G	C	C	C
S. carolitertii	T	A	C	A	C	G	C	T	C
S. castellanus	T	G	C	A	C	G	T	C	C
S. malacitanus Atlantic populations	C	A	C	G	C	G	C	C	C
S. malacitanus Mediterranean populations	T	A	C/T	A	C	T	C	C	C
S. valentinus	T	A	C	A	C	G	C	C	T
S. torgalensis	T	A	C	A	T	G	C	C	C

…. Continued from Appendix 13

Species	546	558	630	633	634	675	696	718	748
S. aradensis	C	A	C	A	A	A	A	A	C
S. pyrenaicus Northern populations	C	A	A/G	C/T	A	A	A	A	C
S. pyrenaicus Sado	C	A	A	C	A	A	A	A	C
S. pyrenaicus Southern populations	C	A	A	C	A	A	A	A	C
S. carolitertii	C	A	G	G	A	A	A	A	C
S. castellanus	C	A	G	C	A/G	A	A	A	T
S. malacitanus Atlantic populations	C	A	G	A	A	A	A	A	C
S. malacitanus Mediterranean populations	C	A	G	A	A	A	A	A	C
S. valentinus	T	A	A	C	A	A	A	A	C
S. torgalensis	C	G	A/G	A	T	T	G	G	C

Appendix 14.– Localities of *Squalius tartessicus* in MNCN_ICTIO Collection.

Appendix 14.– Localidades de *Squalius tartessicus* en la Colección de Ictiología del MNCN.

Guadalquivir Drainage

MNCN_ICTIO 267479-267480, Albardado River, Bélmez, Córdoba, Spain, 38.253096, -5.164937, Leg., González, J.A., I. Doadrio, 20.3.2000. MNCN_ICTIO 272848-272849, Cabrillas River, Villaviciosa de Córdoba, Códoba, Spain, 38.007210, -5.067791, Leg., González, J.L; Prieto, B; Herrera, J., 26.5.2009. MNCN_ICTIO 272656-272714, Cala River, Santa Olalla de Cala, Huelva, Spain, 37.959673, -6.222448, Leg., Doadrio, I.; Garzón-Heydt, P., 19.4.2009. MNCN_ICTIO 53479-53496, Castril River, Cortes de Baza, Granada, Spain, 37.678218, -2.786500, Leg., Bernat, Y.; Cubo, J., 14.6.1989. MNCN_ICTIO 267207-267214, Corumbel River, Paterna del Campo, Sevilla, Spain, 37.473507, -6.458322, Leg., Doadrio, I.; González, J.L.; Garzón-Heydt, P.; Prieto, B., 18.5.2009. MNCN_ICTIO 272254-272270, de Ciudadeja River, Navas de la Concepción, Sevilla, Spain, 37.917956, -5.480368, Leg., González, J.L; Prieto, B; Herrera, J, 26.5.2009. MNCN_ICTIO 59099-59104, de Garcíez River, Jimena, Jaén, Spain, 37.854004, -3.452911, Leg., Bernat, Y.; Cubo, J., 22.9.1989. MNCN_ICTIO 157067-157078, de la Mesta River, Villapalacios, Albacete, Spain, 38.570836, -2.634844, Leg., Doadrio, I.; González, J.A.; Ambrosio, L., 30.10.1997. MNCN_ICTIO 43420-43421, de la Rocina River, El Rocío, Huelva, Spain, 37.145579, -6.547194, Leg., Domínguez, L., 24.2.1985. MNCN_ICTIO 286685-286690, de las Buenas Hierbas River, Azuel, Córdoba, Spain, 38.340363, -4.414474, Leg., 18.3.2000. MNCN_ICTIO 49416-49424, de las Yeguas River, Fuencaliente, Ciudad Real, Spain, 38.394627, -4.295620, Leg., Doadrio, I.; Cubo, J., 13.3.1989. MNCN_ICTIO 57873-57875, de las Yeguas River, Venta de Azuel, Jaén, Spain, 38.363134, -4.322248, Leg., Doadrio, I.; Cubo, J., 4.9.1987. MNCN_ICTIO 42727-42728, de San Marcos River, Fontanarejo, Ciudad Real, Spain, 39.191816, -4.539761, Leg., Barrachina, P., 17.7.1984. MNCN_ICTIO 158176-158180, del Buey River, Pozo de la Serna, Ciudad Real, Spain, 38.731251, -3.187420, Leg., Gutiérrez, B.; Ambrosio, L., 19.11.1995. MNCN_ICTIO 53499-53504, Fardes River, Villanueva de las Torres, Granada, Spain, 37.554051, -3.088891, Leg., Bernat, Y.; Cubo, J., 13.6.1989. MNCN_ICTIO 242284, Fresneda River, El Viso del Marqués, Ciudad Real, Spain, 38.585604, -3.657199, Leg., Doadrio, I. & cols, 26.2.2000. MNCN_ICTIO 158151-158161, Fresneda River, Frinca la Freneda. Viso del Marqués, Ciudad Real, Spain, 38.585604, -3.657199, Leg., Gutiérrez, B.; Ambrosio, L., 20.11.1995. MNCN_ICTIO 248239, Fresneda River, Huertezuelas, Ciudad Real, Spain, 38.494465, -3.667806, Leg., Doadrio, I.; Ornelas, P.; Perea, S., 19.5.2004. MNCN_ICTIO 114021-114024, Grande River, El Centenillo, Jaén, Spain, 38.346026, -3.711763, Leg., Doadrio, I.; Garzón-Heydt, P., 6.7.1994. MNCN_ICTIO 152919, Grande River, El Centenillo, Jaén, Spain, 38.346026, -3.711763, Leg., Doadrio, I.; Garzón-Heydt, P., 4.12.1992. MNCN_ICTIO 54480-54489, Guadalbarbo River, Obejo, Córdoba, Spain, 38.135581, -4.855818, Leg., Doadrio, I.; Cubo, J., 19.4.1989. MNCN_ICTIO 138456, Guadalentin River, Peal del Becerro, Jaén, Spain, 37.869390, -2.880686, Leg., IFIE, 13.10.1924. MNCN_ICTIO 69793-69794, Guadalmar River, Puebla de Alcocer, Badajoz, Spain, 39.024035, -5.126504, Leg., Doadrio, I.; Cubo, J., 22.4.1988. MNCN_ICTIO 271901, Guadalmena River, Albadalejo, Ciudad Real, Spain, 38.514549, -2.785715, Leg., González, J.L.; Prieto, B.; Herrera, J., 5.7.2009. MNCN_ICTIO 126470-126477, Guadalmena River, Alcaraz, Albacete, Spain, 38.667702, -2.566078, Leg., Doadrio, I.; Ambrosio, L., 23.10.1996. MNCN_ICTIO 157490-157540, Guadalmena River, Bienservida (Puente de la carretera Bienservida-Albadejo), Albacete, Spain, 38.555159, -2.731737, Leg., González, J.A.; Ambrosio, L., 31.10.1996. MNCN_ICTIO 157914-157998, Guadalmena River, Villapalacios, Albacete, Spain, 38.590866, -2.669143, Leg., González, J.A.; Ambrosio, L., 30.10.1996. MNCN_ICTIO 126254-126273, Guadalimar River, Villaverde de Guadalimar, Albacete, Spain, 38.451992, -2.517728, Leg., Alonso, F., 28.3.1996. MNCN_ICTIO 24916-24923, Guadalquivir River, El Puntal, Sevilla, Spain, 37.257238, -6.060055, Leg., Lozano, L., 30.4.1948. MNCN_ICTIO 53548, Guardal River, San Clemente (Sierra Moncayo), Granada, Spain, 37.757611, -2.668137, Leg., Bernat, Y.; Cubo, J., 15.6.1989. MNCN_ICTIO 197669, Guarrizas River, Aldequemada, Jaén, Spain, 38.408030, -3.384138, Leg., Doadrio, I., 7.5.1978. MNCN_ICTIO 127621-197622, Jándula River, Andújar, Jaén, Spain, 38.149855, -4.015852, Leg., Doadrio, I., 4.10.1980. MNCN_ICTIO 18760, Majavacas River, Fuente Obejuna, Córdoba, Spain, 38.290298, -5.416990, Leg., Doadrio, I., 26.5.1999.

MNCN_ICTIO 54675-54676, Montoro River, La Solanilla, Ciudad Real, Spain, 38.446805, -3.940565, Leg., Doadrio, I.; Cubo, J., 20.4.1989. MNCN_ICTIO 53584-53589, Orce River, Llanos de Orce, Granada, Spain, 37.728568, -2.497680, Leg., Bernat, Y.; Cubo, J., 15.6.1989. MNCN_ ICTIO 240495-240507, Robledillo River, Solana del Pino, Ciudad Real, Spain, 38.422477, -4.026718, Leg., Doadrio, I., 19.11.1994. MNCN_ICTIO 24982, Rumblar River, Baños de la Encina, Jaén, Spain, 38.424256, -4.038561, Leg., Cobo, J.M., 10.4.1974. MNCN_ICTIO 157737-157746, Turruchel River, Bienservida, Albacete, Spain, 38.512802, -2.615983, Leg., González, J.A.; Ambrosio, L., 30.10.1996. MNCN_ICTIO 24433-24440, Viar River, Pallares, Badajoz, Spain, 38.059507, -5.997013, Leg., Barrachina, P.; Sunyer, C., 28.12.1984. MNCN_ICTIO 266128, Yeguas River, Azuel, Córdoba, Spain, 38.362725, -4.321812, Leg., Doadrio, I.; González, J.L.; Garzón-Heydt, P.; Prieto, B., 19.5.2009. MNCN_ICTIO AT 17090 - 17092, Hueznar, River, El Pedroso. Sevilla, Spain, 30S 259132, 4193764, Leg., I. Doadrio, J.L.González, Gema, P.Garzón-Heydt. MNCN_ICTIO AT 17181, Montoro River, Ventillas. Ciudad Real, Spain, 30s379879, 4262773, Leg., I. Doadrio, J.L.González, Gema, P.Garzón-Heydt. MNCN_ICTIO AT 17206-17223, Cabrera River, Casas de Montealegre.Andujar. Jaén, Spain, 30S 404430, 4231849, Leg., I. Doadrio, J.L.González, P.Garzón-Heydt. MNCN_ICTIO AT 21560 - 21563, Cabrera, River, Lugar Nuevo. Andujar. Jaén, Spain, 38.193206, -4.094899.

Guadiana Drainage

MNCN_ICTIO 137187-137195, Albarregas River, Mérida, Badajoz, Spain, 8.564224, -71.191819, Leg., IFIE, 21.6.1947. MNCN_ICTIO 153012-153043, Alcarrache River, Higuera de Vargas, Badajoz, Spain, 38.450813, -6.996069, Leg., Doadrio, I.; Cubo, J., 21.4.1988. MNCN_ ICTIO 24821, Alcollarín River, Alcollarín, Badajoz, Spain, 39.262935, -5.760773, Leg., Barrachina, P.; Sunyer, C., 29.12.1984. MNCN_ICTIO 158127, Alcudia River, Alamillo, Ciudad Real, Spain, 38.713097, -4.815668, Leg., Doadrio, I., 22.11.1995. MNCN_ICTIO 25013-25067, Aljucén River, Aljucén , Badajoz, Spain, 39.045322, -6.348508, Leg., Doadrio, I., 21.7.1984. MNCN_ICTIO 24341-24360, Ardila River, Ardila, Badajoz, Spain, 38.169787, -6.417826, Leg., Barrachina, P.; Sunyer, C., 27.12.1984. MNCN_ICTIO 272949, Ardila River, Oliva de la Frontera, Badajoz, Spain, 38.230073, -6.887153, Leg., Doadrio, I.; González, J.L; Garzón-Heydt, P., 19.4.2009. MNCN_ICTIO 25333, Ardila River, Valuengo, Badajoz, Spain, 38.286945, -6.731510, Leg., Barrachina, P.; Sunyer, C., 27.11.1984. MNCN_ICTIO 105782-105788, Brezoso River, Cabañeros, Ciudad Real, Spain, 39.347835, -4.361869, Leg., Doadrio, I.; González, J.A.; Perdices, A., 4.3.1992. MNCN_ICTIO 268965, Bullaque River, Retuerta de Bullaque, Ciudad Real, Spain, 39.428346, -4.365585, Leg., Doadrio, I.; González, J.L.; Garzón-Heydt, P.; Prieto, B., 16.5.2009. MNCN_ICTIO 240511-240512, Cigüela River, Horcajada de la Torre, Cuenca, Spain, 40.041476, -2.571649, Leg., Doadrio, I., 8.10.2001. MNCN_ICTIO 270667-270668, de Calaboza River, Rosal de la Frontera, Huelva, Spain, 37.914358, -7.200447, Leg., Doadrio,I Garzón-Heydt, P. Jl. González, 18.4.2009. MNCN_ICTIO 187529-187538, del Madroño River, Peraleda de Zaucejo, Badajoz, Spain, 38.420431, -5.577042, Leg., Doadrio, I., 28.5.1999. MNCN_ICTIO 187734-187756, Del Moral River, Ribera del Fresno, Badajoz, Spain, 38.509247, -6.263701, Leg., Doadrio, I., 27.5.1999. MNCN_ICTIO 39776-39792, del Sillo River, Cumbres de San Bartolomé, Huelva, Spain, 38.095049, -6.711542, Leg., Barrachina, P. I. Doadrio, J. Cubo, 9.8.1984. MNCN_ICTIO 283449-283456, del Sillo River, Encinasola, Huelva, Spain, 38.119189, -6.831598, Leg., J.L. González; I. Doadrio; P. Garzón-Heydt, 28.5.2010. MNCN_ICTIO 69802-69803, Estena River, Bohonal, Ciudad Real, Spain, 39.458641, -4.808416, Leg., Doadrio, I.; Cubo, J., 23.4.1988. MNCN_ICTIO 264613, Estena River, Navas de Estena, Ciudad Real, Spain, 39.496530, -4.541259, Leg., Doadrio, I.; Perea, S., 21.7.2006. MNCN_ ICTIO 126161-126162, Estenilla River, Anchuras, Ciudad Real, Spain, 39.458641, -4.808416, Leg., Doadrio, I., 5.10.1995. MNCN_ICTIO 24720-24723, Estenilla River, Valdeazores, Badajoz, Spain, 39.474254, -4.792097, Leg., Sunyer, C., 3.5.1985. MNCN_ICTIO 253961-253967, Esteras River, Baterno, Badajoz, Spain, 38.878116, -4.930531, Leg., Doadrio, I.; Ornelas, P.; Perea, S., 28.4.2004. MNCN_ICTIO 248084-248085, *Esteras* River, Saceruela, Ciudad Real, Spain, 38.950527, -4.651952, Leg., Doadrio, I.; Ornelas, P.; Perea, S., 22.5.2004. MNCN_ICTIO 268912-268922, Esteras River, Valdemanco de Esteras, Ciudad Real, Spain, 38.906068, -4.795259, Leg., Doadrio, I.; González, J.L.; Garzón-Heydt, P.; Prieto, B., 16.5.2009. MNCN_ICTIO AT 16961, Esteras, River, Siruela. Badajoz, Spain, 30S 332593, 4305245, Leg., I. Doadrio, J.L.González, G. Solis, P. Garzón-Heydt. MNCN_ICTIO 44414, Gévora River, Alburquerque. Ermita de Nuestra Señora de Carrión, Badajoz, Spain, 39.182827, -7.033341, Leg., Doadrio, I.; Elvira, B., 8.5.1987. MNCN_ICTIO 24730-24384, Gévora River, La Codosera, Badajoz, Spain, 39.211948, -7.141414, Leg., Barrachina, P.; Sunyer, C., 3.5.1985. MNCN_ICTIO 218261, Gébalo River, Alcaudete de la Jara, Toledo, Spain. 39.794379, -4.868787, Leg., Doadrio. I.; Ambrosio, L., 15.3.2000. MNCN_ICTIO 24316-24330, Guadajira River, Solana de Barros, Badajoz, Spain, 38.730777, -6.531945, Leg., Barrachina, P.; Sunyer, C., 2.5.1985. MNCN_ICTIO 253610-253617, Guadalemar River, Garbayuela, Badajoz, Spain, 39.032746, -5.018206, Leg., Doadrio, I.; Ornelas, P.; Perea, S., 24.4.2004. MNCN_ICTIO 24331-24340, Guadalemar River, Fuenlabrada de los Montes, Badajoz, Spain, 39.090419, -4.944551, Leg., Barrachina, P.; Sunyer, C., 16.7.1984. MNCN_ICTIO 25315, Guadalupejo River, Guadalupe, Cáceres, Spain, 39.441082, -5.312282, Leg., Sunyer, C., 1.5.1985. MNCN_ICTIO 54499-54506, Guadámez River, Valle de la Serena, Badajoz, Spain, 38.699075, -5.823548, Leg., Doadrio, I.; Cubo, J., 18.4.1989. MNCN_ICTIO 269976-270014, Guadarranque River, Alia, Cáceres, Spain, 39.507865, -5.164098, Leg., Doadrio, I.; González, J.L.; Garzón-Heydt, P.; Prieto, B., 15.5.2009. MNCN_ICTIO 24945-24975, Guadiana River, Daimiel, Ciudad Real, Spain, 39.149038, -3.699871, Leg., Sánchez Bermejo, G., 27.10.1913. MNCN_ICTIO 24407, Guadiana River, Mérida, Badajoz, Spain, 38.923038, -6.427833, Leg., Barrachina, P.; Sunyer, C., 24.7.1984. MNCN_ICTIO 25279-25280, Guadiana River, Helechosa, Badajoz, Spain, 39.330040, -4.901371, Leg., Doadrio, I., 29.6.1981. MNCN_ICTIO 267468, Jabalón River, Bazán, Ciudad Real, Spain, 38.657495, -3.435319, Leg., Perea, S., 23.9.2004. MNCN_ICTIO 215384, Jualón River, Palomares del Campo, Cuenca, Spain, 39.961477, -2.589338, Leg., Doadrio, I., 11.6.1996. MNCN_ICTIO 24701-24702, Matachel River, Alange, Badajoz, Spain, 38.670796, -6.199076, Leg., Barrachina, P.; Sunyer, C., 1.5.1985. MNCN_ICTIO 24600-24610, Matachel River, Hornachos, Badajoz, Spain, 38.058660, -5.173617, Leg. Barrachina, P.; Sunyer, C., 28.12.1984. MNCN_ICTIO 19671-24702, Molinillo River, El Molinillo, Ciudad Real, Spain, 39.466056, -4.222432, Leg., I. Doadrio, 25.7.1976. MNCN_ICTIO 24704-24707, Ortigas River, Magacela, Badajoz, Spain, 38.843604, -5.736454, Leg., Barrachina, P.; Sunyer, C., 29.12.1984. MNCN_ICTIO 126132-126133, Piedrala River, Porzuna, Ciudad Real, Spain, 39.247090, -4.170298, Leg., Doadrio, I., 6.10.1995. MNCN_ICTIO 24489-24498, Pijotilla River, Retamal, Badajoz, Spain, 38.752292, -6.644393, Leg., Barrachina, P.; Sunyer, C., 28.12.1984. MNCN_ICTIO 248057-248058, Quejigares River, Fontanosa, Ciudad Real, Spain, 38.745863, -4.531601, Leg., Doadrio, I.; Garzón-Heydt, P.; Ornelas, P.; Perea, S., 22.5.2001. MNCN_ICTIO 24489-24498, Retín River, Llera, Badajoz, Spain, 38.424136, -6.092681, Leg., Doadrio, I.; Cubo, J., 19.4.1989. MNCN_ICTIO 248057-248058, Riansares River, Los Huelves, Cuenca, Spain, 39.954101, -3.012588, Leg., Doadrio, I., 12.6.1996. MNCN_ICTIO 211214-211225, Ruecas River, Cañamero, Cáceres, Spain, 39.379431, -5.377607, Leg., Doadrio, I.; González, J.L.; Garzón-Heydt, P.; Prieto, B., 15.5.2009. MNCN_ICTIO 240507, Ruecas River, Logrosán (Villanueva de la Serena), Cáceres, Spain, 39.305479, -5.458731, Leg., Doadrio, I.; Martínez, E.; Corcuera, A., 20.3.2003. MNCN_ICTIO 253685, Siruela River, Tamurejo, Badajoz, Spain, 39.012147, -4.947007, Leg., Doadrio, I.; Ornelas, P.; Perea, S., 28.4.2004. MNCN_ICTIO 25650-25654, Tablillas River, Veredilla, Ciudad Real, Spain, 38.582982, -4.360105, Leg., Doadrio, I.; Cubo, J., 13.3.1989. MNCN_ICTIO 40057-40061, Tamujar River, Almadén, Ciudad Real, Spain, 38.770204, -4.901411, Leg., Barrachina, P. I. Doadrio, J. Cubo, 5.7.1984. MNCN_ICTIO 190164-190174, Usagre River, Hinojosa del Valle, Badajoz, Spain, 38.462778, -6.128719, Leg., Doadrio, I., 27.5.1999. MNCN_ICTIO 40072-40076, Valdeazogues River, Almadén, Ciudad Real, Spain, 38.743215, -4.834095, Leg., Barrachina, P., I. Doadrio, 5.7.1984. MNCN_ICTIO 253534-253535, Valdeazogues River, Almadenejos, Ciudad Real, Spain, 38.755547, -4.704267, Leg., Doadrio, I.; Ornelas, P.; Perea, S., 28.4.2004. MNCN_ICTIO 158497-158502, Valdeazogues River, Chillón, Ciudad Real, Spain, 38.727068, -4.869323, Leg., Gutiérrez, B.; Blazquez, Luis Ambrosio, 7.11.1995. MNCN_ICTIO 126530-126540, Valdehornos River, Navalpino, Ciudad Real, Spain, 39.260746, -4.610820, Leg., Doadrio, I., 5.10.1995. MNCN_ICTIO 211435-211443, Valdejudíos River, Carrascosa del Campo, Cuenca, Spain, 40.012791, -2.738227, Leg., Doadrio, I., 12.6.1996. MNCN_ICTIO 114946, Valdejudíos River, Saelices, Cuenca, Spain, 39.967090, -2.744416, Leg., Doadrio, I., 18.2.1996. MNCN_ICTIO 79313-79329, Zancara River, Zafra de Záncara, Cuenca, Spain, 39.895067, -2.560379, Leg., Doadrio, I.; González, J.A.; Perdices, A., 3.3.1992. MNCN_ICTIO 45272, Zapatón River, Botoa, Puente Albarragena, Badajoz, Spain, 39.045590, -6.906453, Leg., Doadrio, I.; Elvira, B., 8.5.1987. MNCN_ICTIO 212588-212592, Zujar River, Cabeza del Buey, Badajoz, Spain, 38.676974, -5.172985, Leg., Doadrio, I., 2.6.1999. MNCN_ICTIO 187881-187886, Zujar River, Peraleda del Zaucejo, Badajoz, Spain, 38.451395, -5.539105, Leg., Doadrio, I., 28.5.1999. MNCN_ICTIO 7276-212592, de la Cagurria Spring, Ossa de Montiel, Albacete, Spain, 38.881602, -2.764065, Leg., Doadrio, I.; González, J.A.; Perdices, A., 7.4.1992. MNCN_ICTIO AT 16643, Murtigas River, Valles de Carrasco. Huelva, Spain, 29s695261, 4211596, Leg., I.Doadrio, J.L.González, P.Garzón-Heydt. MNCN_ICTIO 268534-268536, Guadalmez River, Guadalmez, Cuidad Real, Spain, 38.702859, -4.920903, Leg., Doadrio, I.; González, J.L.; Garzón-Heydt, P.; Prieto, B., 17.5.2009. MNCN_ICTIO 267879-267880, Guadalmez River, San Benito, Badajoz, Spain, 38.547056, -4.672129, Leg., Doadrio, I.; González, J.L.; Garzón-Heydt, P.; Prieto, B., 17.5.2009. MNCN_ICTIO AT 21610 - 21620, El Chorro, River, Navas de Estena. Ciudad Real, Spain, 39.488724, -4.532027.

Odiel Drainage

MNCN_ICTIO 24927-24937, Cascabelero River, Villanueva de las Cruces, Huelva, Spain, 37.620888, -7.022039, Leg., Doadrio, I., 12.4.1979. MNCN_ICTIO 243711-243718, del Villar River, Zalamea la Real, Huelva, Spain, 37.689514, -6.652147, Leg., Doadrio, I., 24.3.2002. MNCN_ICTIO 196712-196715, Odiel River, Campofrío, Huelva, Spain, 37.800374, -6.552634, Leg., Doadrio, I., 9.12.1979. MNCN_ICTIO 253684-253685, Tamuja River, Calañas, Huelva, Spain, 37.672534, -6.917234, Leg., Doadrio, I., 12.4.1979. MNCN_ICTIO 25282-25297, Tinto River, Cerca de Berrocal, Huelva, Spain, 37.613033, -6.550242, Leg., Domínguez Nevado, L., 26.1.1991.

Segura Drainage

MNCN_ICTIO 157696-157704, de Bogarra River, Las Mohedas, Albacete, Spain, 38.603804, -2.252087, Leg., González, J.A.; Ambrosio, L., 31.10.1996. MNCN_ICTIO 25650-25654, Segura River, Orihuela, Alicante, Spain, 38.085164, -0.946064.

Vélez Drainage

MNCN_ICTIO 280678-280689, de la Cueva River, Riogordo, Málaga, Spain, 36.927500, -4.296446, Leg., I. Doadrio, J.L. González, P. Garzón-Heydt, B. Prieto, 14.6.2009. MNCN_ICTIO 211435-211443, Vélez River, Viñuelas, Málaga, Spain, 36.884624, -4.146318, Leg., Doadrio, I.; Garzón-Heydt, P., 22.10.1978. MNCN_ICTIO AT 17877- 17892, Sabar River, Sabar. Málaga, Spain, 30s388774, 4089165, Leg., I. Doadrio, J.L.González, P. Garzón-Heydt.

Guadalhorce Drainage

MNCN_ICTIO 25586, del Burgo River, El Burgo, Málaga, Spain, 36.790234, -4.941212, Leg., Doadrio, I.; Garzón-Heydt, P., 24.10.1978. MNCN_ICTIO 212312, Fahala River, Alhaurín el Grande, Málaga, Spain, 36.689047, -4.685710, Leg., Doadrio, I.; Garzón-Heydt, P., 6.3.2000. MNCN_ICTIO 69911, Guadalhorce River, Cártama, Málaga, Spain, 36.729350, -4.602957, Leg., Doadrio, I.; Garzón-Heydt, P., 10.7.1984. MNCN_ICTIO 263947-263954, Turón River, El Burgo, Málaga, Spain, 36.787862, -4.952219, Leg., Doadrio, I.; Perea, S., 14.3.2006. MNCN_ICTIO AT17308-

17329, Grande River, Alozaina, Málaga, Spain, 36.701739, -4.881667, Le**g.** Doadrio, I.; Garzón-Heydt, P., G. Solis, J.L. Gonález, 24.VI.2010.

GUADALETE DRAINAGE

MNCN_ICTIO 264042-264046, Guadalete River, Zahara, Cádiz, Spain, 36.807310, -5.328511, Leg., Doadrio, I.; Perea, S., 18.2.2006. MNCN_ICTIO AT 17442 - 17457, Guadalete River, Puerto Serrano. Cádiz, Spain, 30s272604, 4089355, Leg., I. Doadrio, J.L.González, Gema, P.Garzón-Heydt. MNCN_ICTIO 25071-25078, Guadalporcún River, Olvera, Cádiz, Spain, 36.924825, -5.280355, Leg., Doadrio, I.; Garzón-Heydt, P., 27.3.1983. MNCN_ICTIO 197621-197622, Majaceite River, El Bosque, Cádiz, Spain, 36.771175, -5.491703, Leg., Garzón-Heydt, P. Doadrio,I, 26.10.1978.

GUADALFEO DRAINAGE

MNCN_ICTIO 24765, Guadalfeo River, Motril, Granada, Spain, 36.884244, -3.416690, Leg., Lozano, L., 13.8.1930. MNCN_ICTIO 19219-192191, Guadalfeo River, Órgiva, Granada, Spain, 36.890440, -3.377251, Leg., Doadrio, I.; Garzón-Heydt, P., 3.10.1978. MNCN_ICTIO 195961, Guadalfeo River, Vélez de Benaudalla, Granada, Spain, 36.827457, -3.522552, Leg., Doadrio, I.; Garzón-Heydt, P., 21.10.1978.