

Mendes *et al.*, 2025. A new species of the genus *Squalius* (Leuciscidae, Actinopterygii) from the Sado River basin in Portugal. *Limnetica* 44(2), 2025.

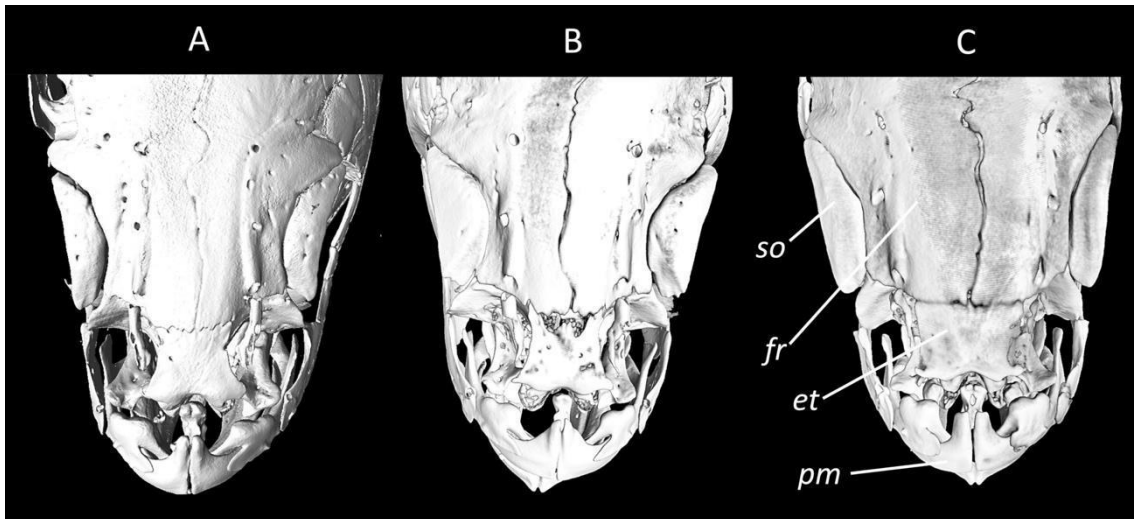
SUPPLEMENTARY INFORMATION

Appendix 1. - Species, Sample IDs, collection sites (river basin and river) and Genbank accession numbers of the mitochondrial *MT-CYB* gene sequences used in this study. New sequences obtained for this study are indicated with an asterisk (*). All sequences were used to construct the phylogenetic tree. Sequences used for calculating genetic distances and determining autapomorphies are indicated in the last column. *Especies, ID de las muestras, lugares de captura (cuenca y río) y números de acceso al Genbank de las secuencias del gen mitocondrial MT-CYB utilizadas en este estudio. Las nuevas secuencias obtenidas para este estudio se indican con un asterisco (*). Todas las secuencias fueran utilizadas para el análisis filogenético. Las secuencias utilizadas para calcular las distancias genéticas y determinar las autapomorfías se indican en la última columna.*

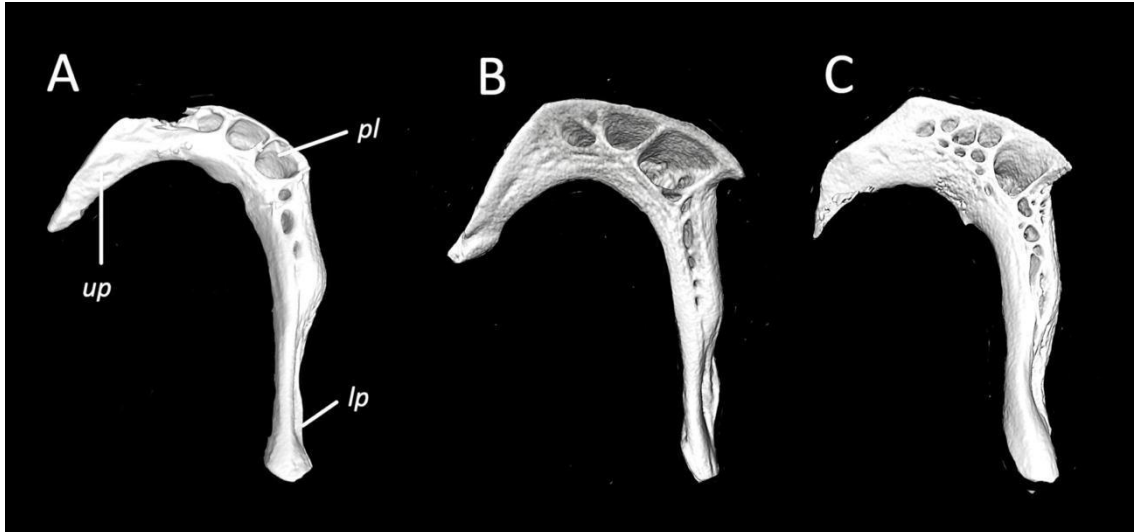
| Species | Sample ID | River basin | River | GenBank accession number | Used for autapomorphies and genetic distances |
|--------------------------------------|-----------|-------------|--------------|--------------------------|---|
| <i>Squalius caetobrigus sp. nov.</i> | LSAD1 | Sado | Grândola | EF459077.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | LSAD10 | Sado | Grândola | EF459078.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | LSAD21 | Sado | Grândola | PP489329* | yes |
| <i>Squalius caetobrigus sp. nov.</i> | LSAD23 | Sado | Grândola | EF459076.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | LSAD30 | Sado | Grândola | PP489330* | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCO9 | Sado | Corona | PP489331* | yes |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCO11 | Sado | Corona | PP489332* | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCO12 | Sado | Corona | PP489333* | yes |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCO14 | Sado | Corona | PP489334* | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCO16 | Sado | Corona | PP489335* | yes |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCA2 | Sado | Campilhas | KU370318.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCA3 | Sado | Campilhas | KU370319.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCA11 | Sado | Campilhas | KU370316.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCA14 | Sado | Campilhas | KU370315.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSCA15 | Sado | Campilhas | KU370317.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSM13 | Sado | Marateca | PP489336* | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSM14 | Sado | Marateca | PP489337* | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSM18 | Sado | Marateca | PP489338* | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSM21 | Sado | Marateca | PP489339* | yes |
| <i>Squalius caetobrigus sp. nov.</i> | SPSM22 | Sado | Marateca | PP489340* | yes |
| <i>Squalius caetobrigus sp. nov.</i> | SPSMT13 | Sado | São Martinho | KU370360.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSMT16 | Sado | São Martinho | KU370362.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSMT18 | Sado | São Martinho | KU370364.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSMT19 | Sado | São Martinho | KU370365.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSMT20 | Sado | São Martinho | KU370367.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSOD10 | Sado | Odivelas | KU370334.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSOD11 | Sado | Odivelas | KU370335.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSOD12 | Sado | Odivelas | KU370336.1 | no |
| <i>Squalius caetobrigus sp. nov.</i> | SPSOD13 | Sado | Odivelas | KU370337.1 | no |

| | | | | | |
|--------------------------------------|----------------|--------------|------------------------------|------------|-----|
| <i>Squalius caetobrigus sp. nov.</i> | SPSOD14 | Sado | Odivelas | KU370338.1 | no |
| <i>Squalius pyrenaicus</i> | MNCNLp426alava | Ebro | Baias | DQ521436 | yes |
| <i>Squalius pyrenaicus</i> | AT2478 | Ebro | Piedra | MT008509 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr29 | Tagus | Almonte | AJ627315 | yes |
| <i>Squalius pyrenaicus</i> | Leutajo71at | Tagus | Almonte | AF421791 | yes |
| <i>Squalius pyrenaicus</i> | AT6710 | Tagus | Jerte | MT816918 | no |
| <i>Squalius pyrenaicus</i> | AT6711 | Tagus | Jerte | MT816919 | no |
| <i>Squalius pyrenaicus</i> | AT6712 | Tagus | Jerte | MT816920 | no |
| <i>Squalius pyrenaicus</i> | AT6713 | Tagus | Jerte | MT816921 | no |
| <i>Squalius pyrenaicus</i> | AT6714 | Tagus | Jerte | MT816922 | no |
| <i>Squalius pyrenaicus</i> | MNCNpyr24 | Tagus | Acebo | AJ627310 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr25 | Tagus | Arrago | AJ627311 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr26 | Tagus | Caparro | AJ627312 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr27 | Tagus | Jerte | AJ627313 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr28 | Tagus | Abulrrel | AJ627314 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr30 | Tagus | Aurela | AJ627316 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr31 | Tagus | Cedena | AJ627317 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr33 | Tagus | Cofio | AJ627319 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr34 | Tagus | Arroyo de la Vid | AJ627320 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr35 | Tagus | Gévalo | AJ627321 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr36 | Tagus | Huso | AJ627322 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr38 | Tagus | Pesquero | AJ627324 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr39 | Tagus | Sertã stream, Zêzere river | AJ627325 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr40 | Tagus | Sever | AJ627326 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr41 | Tagus | Sorraia | AJ627327 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr42 | Tagus | Tiétar | AJ627328 | yes |
| <i>Squalius pyrenaicus</i> | MNCNpyr43 | Tagus | Trevijana | AJ627329 | yes |
| <i>Squalius pyrenaicus</i> | AT6668 | Tagus | Erjas | MT008493 | yes |
| <i>Squalius pyrenaicus</i> | AT6783 | Tagus | Batuecas | MT008495 | yes |
| <i>Squalius pyrenaicus</i> | AT25294 | Tagus | Ompolveda | MT008505 | yes |
| <i>Squalius pyrenaicus</i> | AT16317 | Tagus | Tajuna | MT008508 | yes |
| <i>Squalius pyrenaicus</i> | AT27275 | Tagus | Alagón | MT008553 | yes |
| <i>Squalius pyrenaicus</i> | SCLIS3 | Lis | Lis | MT008533 | yes |
| <i>Squalius pyrenaicus</i> | LLZD11 | Lizandro | Lizandro | MT008526 | yes |
| <i>Squalius pyrenaicus</i> | LSMRX22 | Samarra | Samarra | MT008529 | yes |
| <i>Squalius pyrenaicus</i> | LC34 | Colares | Colares | MT008531 | yes |
| <i>Squalius pyrenaicus</i> | SPLAG3 | Lage | Lage | MT008541 | yes |
| <i>Squalius tartessicus</i> | AT3164 | Guadiana | Estena | MT816884 | no |
| <i>Squalius tartessicus</i> | AT3165 | Guadiana | Estena | MT008542 | yes |
| <i>Squalius tartessicus</i> | AT3166 | Guadiana | Estena | MT008543 | yes |
| <i>Squalius tartessicus</i> | AT3167 | Guadiana | Estena | MT816885 | no |
| <i>Squalius tartessicus</i> | AT3168 | Guadiana | Estena | MT816886 | no |
| <i>Squalius tartessicus</i> | MNCNpyr14 | Guadiana | Estena | AJ627300 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr17 | Guadiana | Ruidera | AJ627303 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr10 | Guadiana | Azuer | AJ627296 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr15 | Guadiana | Matachel | AJ627301 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr18 | Guadiana | Sillo | AJ627304 | yes |
| <i>Squalius tartessicus</i> | AT8116 | Guadiana | Guadarranque | MT008547 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr20 | Guadiana | Zújar | AJ627306 | yes |
| <i>Squalius tartessicus</i> | AT1955 | Guadiana | Esteras | MT008544 | yes |
| <i>Squalius tartessicus</i> | CG2 | Guadiana | Calaboza | KU571656 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr19 | Guadiana | Xévoira | AJ627305 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr8 | Guadiana | Arronches stream, Caia river | AJ627294 | yes |
| <i>Squalius tartessicus</i> | LGV6 | Guadiana | Oeiras | DQ263239 | yes |
| <i>Squalius tartessicus</i> | AT2491 | Guadalquivir | Rivera de Ciudadeja | MT816874 | no |
| <i>Squalius tartessicus</i> | AT2492 | Guadalquivir | Rivera de Ciudadeja | KU571621 | yes |
| <i>Squalius tartessicus</i> | AT2495 | Guadalquivir | Rivera de Ciudadeja | KU571622 | yes |

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|------------------------------|-------------------------|----------------------------|---------------------|----------|-----|
| <i>Squalius tartessicus</i> | AT2496 | Guadalquivir | Rivera de Ciudadeja | KU571623 | yes |
| <i>Squalius tartessicus</i> | AT2569 | Guadalquivir | Rivera de Ciudadeja | KU571625 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr2 | Guadalquivir | Manzano | AJ627288 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr3 | Guadalquivir | Molinos | AJ627289 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr6 | Guadalquivir | Montemayor | AJ627292 | yes |
| <i>Squalius tartessicus</i> | MNCNpyr7 | Guadalquivir | Robledillo | AJ627293 | yes |
| <i>Squalius tartessicus</i> | AT7425 | Guadalquivir | Viar | KU571642 | yes |
| <i>Squalius tartessicus</i> | AT17190 | Guadalquivir | Montoro | KU571652 | yes |
| <i>Squalius tartessicus</i> | AT17174 | Guadalquivir | Yeguas | KU571649 | yes |
| <i>Squalius tartessicus</i> | AT17167 | Guadalquivir | Cabrillas | KU571645 | yes |
| <i>Squalius tartessicus</i> | AT17092 | Guadalquivir | Huéznar | KU571644 | yes |
| <i>Squalius tartessicus</i> | AT7376 | Guadalquivir | Cala | MT008517 | yes |
| <i>Squalius tartessicus</i> | AT2086 | Guadalquivir | Fresneda | MT008519 | yes |
| <i>Squalius tartessicus</i> | BA997 | Almargem | Almargem | AJ698717 | yes |
| <i>Squalius tartessicus</i> | AT8768 | Guadalete | Guadalete | KU571610 | yes |
| <i>Squalius tartessicus</i> | AT824 | Guadalhorce | Pereilas | KU571599 | yes |
| <i>Squalius tartessicus</i> | AT3289 | Guadalhorce | Turón | KU571600 | yes |
| <i>Squalius tartessicus</i> | AT17317 | Guadalhorce | Grande | KU571605 | yes |
| <i>Squalius tartessicus</i> | AT9779 | Velez | Sabar | KU571598 | yes |
| <i>Squalius tartessicus</i> | AT17872 | Velez | Cuevas | MT008523 | yes |
| <i>Squalius tartessicus</i> | AT696 | Guadalfeo | Guadalfeo | MT008525 | yes |
| <i>Squalius tartessicus</i> | EA9G | Segura | Segura | MT008515 | yes |
| <i>Squalius tartessicus</i> | AT8622 | Odiel | Villar | MT008551 | yes |
| <i>Squalius carolitertii</i> | AT18088 | Duero (ES) / Douro (PT) | Tâmega | MT008569 | yes |
| <i>Squalius carolitertii</i> | AT8878 | Duero (ES) / Douro (PT) | Adaja | MT008556 | yes |
| <i>Squalius carolitertii</i> | SCNEI10 | Neiva | Neiva | MT008583 | yes |
| <i>Squalius carolitertii</i> | SCVAG11 | Vouga | Águeda | MT008596 | yes |
| <i>Squalius castellanus</i> | AT21126 MNCNLPGALLO6 | Tagus | Bullones | MT008487 | yes |
| <i>Squalius castellanus</i> | 24 | Tagus | Gallo | DQ521423 | yes |
| <i>Squalius valentinus</i> | AT4376 | Júcar | Magro | HM560212 | yes |
| <i>Squalius valentinus</i> | MNCN85581 | Júcar | Magro | KR871747 | yes |
| <i>Squalius valentinus</i> | MNCN85597 | Júcar | Grande | KR871757 | yes |
| <i>Squalius valentinus</i> | MNCN85594 | Vinalopó | Vinalopó | KR871756 | yes |
| <i>Squalius malacitanus</i> | AT17801 | Guadalmina | Guadalmina | KU571594 | yes |
| <i>Squalius malacitanus</i> | AT17800 | Guadalmina | Guadalmina | KU571593 | yes |
| <i>Squalius malacitanus</i> | AT774 | Guadalmina | Guadalmina | KU571592 | yes |
| <i>Squalius malacitanus</i> | AT733 | Guadalmina | Guadalmina | KU571591 | yes |
| <i>Squalius gaditanus</i> | AT17556 | Jara | Jara | KU571578 | yes |
| <i>Squalius gaditanus</i> | AT17544 | Jara | Jara | KU571576 | yes |
| <i>Squalius gaditanus</i> | AT17541 | Jara | Jara | KU571574 | yes |
| <i>Squalius gaditanus</i> | AT17597 | Vega | Vega | KU571579 | yes |
| <i>Squalius torgalensis</i> | MNCNL1260torgal | Mira | Torgal | DQ521438 | yes |
| <i>Squalius torgalensis</i> | SQTO1 | Mira | Torgal | OP728007 | yes |
| <i>Squalius torgalensis</i> | AT1260 | Mira | Mira | HM560210 | yes |
| <i>Squalius aradensis</i> | BA732 | Arade | Arade | AJ852482 | yes |
| <i>Squalius aradensis</i> | BA756 | Alvor | Alvor | AJ583074 | yes |
| <i>Squalius aradensis</i> | BA317 | Seixe | Seixe | AJ852429 | yes |



Appendix 2.- Ethmoid bone of *Squalius* populations under study: A, Sado population; B, *S. pyrenaicus* from Tagus River basin and C, *S. tartessicus* from Type locality. *fr*, frontal. *et*, ethmoid. *pm*, premaxilla. *so*, supraorbital. *Etmoides de las poblaciones estudiadas del género Squalius*. A, población del Sado. B, población de la cuenca del Tajo de *S. pyrenaicus* y C, población de *S. tartessicus* de la localidad tipo. *fr*, frontal. *et*, etmoides. *pm*, premaxilar. *so*, supraorbital.



Appendix 3.- Pharyngeal teeth of *Squalius* populations under study. A, Sado population; B, *S. pyrenaicus* from Tagus River basin and C, *S. tartessicus* from Type locality. *lp*, lower process of pharyngeal bone. *up*, upper process of pharyngeal bone. *pl*, pharyngeal plate. *Dientes faríngeos de las poblaciones estudiadas del género Squalius*. A, población del Sado. B, población de la cuenca del Tajo de *S. pyrenaicus* y C, población de *S. tartessicus* de la localidad tipo. *lp*, apófisis inferior del hueso faríngeo. *up*, apófisis superior del hueso faríngeo. *pl*, lámina faríngea.

Appendix 4. – Uncorrected p-distances calculated for the mitochondrial *MT-CYB* gene between the different Iberian *Squalius* species using MEGA 11.
Distancias genéticas no corregidas para el gen mitocondrial MT-CYB entre las diferentes especies ibéricas de Squalius utilizando MEGA 11.

| | Sado lineage - <i>Squalius caetobrigus</i> <i>sp. nov.</i> | <i>Squalius</i> <i>pyrenaicus</i> | <i>Squalius</i> <i>tartessicus</i> | <i>Squalius</i> <i>carolitertii</i> | <i>Squalius</i> <i>castellanus</i> | <i>Squalius</i> <i>valentinus</i> | <i>Squalius</i> <i>malacitanus</i> | <i>Squalius</i> <i>gaditanus</i> | <i>Squalius</i> <i>torgalensis</i> | <i>Squalius</i> <i>aradensis</i> |
|--|---|--------------------------------------|---------------------------------------|--|---------------------------------------|--------------------------------------|---------------------------------------|-------------------------------------|---------------------------------------|-------------------------------------|
| Sado lineage - <i>Squalius caetobrigus sp. nov.</i> | — | — | — | — | — | — | — | — | — | — |
| <i>Squalius pyrenaicus</i> | 0,025 | — | — | — | — | — | — | — | — | — |
| <i>Squalius tartessicus</i> | 0,024 | 0,013 | — | — | — | — | — | — | — | — |
| <i>Squalius carolitertii</i> | 0,059 | 0,053 | 0,053 | — | — | — | — | — | — | — |
| <i>Squalius castellanus</i> | 0,066 | 0,058 | 0,057 | 0,037 | — | — | — | — | — | — |
| <i>Squalius valentinus</i> | 0,032 | 0,028 | 0,027 | 0,056 | 0,065 | — | — | — | — | — |
| <i>Squalius malacitanus</i> | 0,082 | 0,080 | 0,081 | 0,075 | 0,084 | 0,084 | — | — | — | — |
| <i>Squalius gaditanus</i> | 0,079 | 0,075 | 0,075 | 0,070 | 0,074 | 0,076 | 0,044 | — | — | — |
| <i>Squalius torgalensis</i> | 0,119 | 0,111 | 0,112 | 0,107 | 0,114 | 0,118 | 0,118 | 0,123 | — | — |
| <i>Squalius aradensis</i> | 0,106 | 0,097 | 0,100 | 0,100 | 0,103 | 0,109 | 0,107 | 0,104 | 0,061 | — |

Appendix 5. – Autapomorphies detected in the mitochondrial *MT-CYB* gene of the Iberian *Squalius* species. Autapomorphies in the Sado lineage are indicated in bold and transversions with an asterisk. *Autapomorfías detectadas en el gen mitocondrial MT-CYB de las especies ibéricas de Squalius. Autapomorfías en la población del Sado son indicadas en negrita y las transversiones con un asterisco.*

| Species | 24 | 47 | 60 | 88 | 90 | 123 | 141 | 145 | 180 | 195 | 207 | 264 | 282 | 285 | 288 | 309 | 312 |
|--|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Sado lineage - <i>Squalius caetobrigus sp. nov.</i> | C | G | C | A | G | A | C | C | A | T | C | C | T | C | A | T | T |
| <i>Squalius pyrenaicus</i> | C | G | C | C | A | A | C | C | A | T | C | C | T | T | A | T | T |
| <i>Squalius tartessicus</i> | C | G | C | C | A | A | C | C | A | T | C | C | T | T | A | T | T |
| <i>Squalius carolitertii</i> | C | G | C | C | A | A | C | C | A/G | T | C | C | T | T | A | T | T |
| <i>Squalius castellanus</i> | C | G | C | C | A | A | C | C | T | T | C | C | T | T | A | T | T |
| <i>Squalius valentinus</i> | C | G | T | C | A | A | C | T | A | T | C | C | T | T | A | C | T |
| <i>Squalius malacitanus</i> | C | G | C | C | A | A | C | C | A | C | C | C | T | T | A | T | T |
| <i>Squalius gaditanus</i> | T | G | C | C | A | G | C | C | A | T | C | C | T | T | G | T | T |
| <i>Squalius torgalensis</i> | C | A | C | C | A | A | A | C | A | T | T | A | C | T | A | T | C |
| <i>Squalius aradensis</i> | C | G | C | C | A | A | G | C | A | T | C | C | T | T | A | T | T |

Appendix 5 (cont). – Autapomorphies detected in the mitochondrial *MT-CYB* gene of the Iberian *Squalius* species. Autapomorphies in the Sado lineage are indicated in bold and transversions with an asterisk. *Autapomorfías detectadas en el gen mitocondrial MT-CYB de las especies ibéricas de Squalius. Autapomorfías en la población del Sado son indicadas en negrita y las transversiones con un asterisco.*

| Species | 327 | 333 | 336 | 341 | 352 | 354 | 423 | 429 | 465 | 507 | 513 | 522 | 531 | 546 | 558 | 580 | 627 |
|--|-----|-----|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----------|-----|-----|-----|-----|-----|
| Sado lineage - <i>Squalius caetobrigus sp. nov.</i> | T | A | G* | A | G | C | A | C | C | C | C | C* | A | C | A | A | C |
| <i>Squalius pyrenaicus</i> | T | A | C | A | G | C | A | C | C | C | C | G | A | C | A | A | C |
| <i>Squalius tartessicus</i> | T | A | C | A | G | C | A | C | C | C | C | G/A | A | C | A | A | C |
| <i>Squalius carolitertii</i> | T | A | C | A | G | C | A | C | C | T | C | G | A | C | A | A | C |
| <i>Squalius castellanus</i> | T | G | C | A | G | C | A | C | T | C | C | G | A | C | A | A | C |
| <i>Squalius valentinus</i> | T | A | C | A | G | C | A | C | C | C | T | G | A | T | A | A | C |
| <i>Squalius malacitanus</i> | C | A | C | G | G | C | G | C | C | C | C | A | G | C | A | A | C |
| <i>Squalius gaditanus</i> | T | A | C | A | G | T | A | C | C | C | C | G | A | C | A | A | C |
| <i>Squalius torgalensis</i> | T | A | C | A | A | C | A | T | C | C | C | A | A | C | G | G | T |
| <i>Squalius aradensis</i> | T | A | C | A | G | A | A | C | C | C | C | A | A | C | A | A | C |

| Species | 630 | 633 | 675 | 696 | 718 | 748 | 756 | 810 | 939 | 951 | 978 | 990 | 1056 | 1071 | 1083 | 1090 | 1113 |
|--|-----|-----|-----|-----|-----|-----|-----|-----------|-----|-----|-----|-----|------|------|------|------|------|
| Sado lineage - <i>Squalius caetobrigus sp. nov.</i> | A | C | A | T | A | C | C | C* | A | C | A | A | A | A | A | A | A |
| <i>Squalius pyrenaicus</i> | A/G | C/T | A | T | A | C | C | A | A | C | A | A | A | A | A | A | A |
| <i>Squalius tartessicus</i> | A | C | A | T | A | C | C | A | A | C | A | A | A | A | A | A | A |
| <i>Squalius carolitertii</i> | G | G | A | T | A | C | C | A | A | C | A | A | A | A | A | A | A |
| <i>Squalius castellanus</i> | G | C | A | T | A | T | T | A | G | C | A | A | A | A | A | A | A |
| <i>Squalius valentinus</i> | A | C | A | T | A | C | C | A | A | C | A | A | A | A | A | A | A |
| <i>Squalius malacitanus</i> | G | A | A | T | A | C | C | A | A | C | A | A | A | A | C | A | A |
| <i>Squalius gaditanus</i> | G | A | A | T | A | C | C | A | A | C | A | G | G | G | G | A | A |
| <i>Squalius torgalensis</i> | G | A | T | C | G | C | C | A | A | T | A | A | A | A | A | A | G |
| <i>Squalius aradensis</i> | C | A | A | T | A | C | C | A | A | C | G | A | A | A | A | G | A |