Table A. Additional species used in phylogenetic performance. GenBank accession numbers and labels in phylogenetic trees.

|  |  |  |
| --- | --- | --- |
| **Species** | **Locality** | **GenBank accession numbers (labels in phylogenetic trees)** |
| *Petroleuciscus aphipsi* | Aphips River, Russia | *MT-CYB*: HM560177 (1), HM560178 (2)  *S7*: HM560528  *RAG1*: HM560455 |
| *Petroleuciscus borysthenicus* | Fotolivos, Greece | *MT-CYB*: HM560111  *S7*: HM560499  *RAG1*: HM560414 |
| *Petroleuciscus smyrnaeus* | Golcuk Lake, Turkey | *MT-CYB*: HM560114  *S7*: HM560501  *RAG1*: HM560416 |
| *Squalius aradensis* | Algibre R. Arade Basin, Portugal | *MT-CYB*: HM560179 |
| *Squalius carolitertii* | Limia River, Spain | *MT-CYB*: HM560182  *S7*: HM560529  *RAG1*: HM560446 |
| *Squalius castellanus* | Gallo River, Tajo Basin, Spain | *MT-CYB*: DQ521423  *S7*: HM560565  *RAG1*: HM560575 |
| *Squalius malacitanus* | Guadiaro River, Spain | *MT-CYB*: HM560192  *S7*: HM560531  *RAG1*: HM560449 |
| *Squalius pyrenaicus* | Guadalete River, Spain | *MT-CYB*: HM560198  *S7*: HM560568  *RAG1*: HM560579 |
| *Squalius valentinus* | Magro River, Jucar Basin, Spain | *MT-CYB*: HM560212  *S7*: HM560541  *RAG1*: HM560461 |
| *Squalius illyricus* | Cetina Basin, Croatia | *MT-CYB*: HM560183 (1), HM560184 (2)  *S7*: HM560530  *RAG1*: HM560447 |
| *Squalius svallize* | Rama Lake, Neretva Basin (1), Zalomka River, (2). Bosnia-Herzegovina | *MT-CYB*: HM560358 (1), HM560359 (2)  *S7*: HM560538  *RAG1*: HM560458 |
| *Squalius lucumonis* | Arno Basin, Italy | *MT-CYB*: HM560190  *RAG1*: HM560448 |
| *Squalius cephalus* | Elbe R. (1), Oder R. (2), Germany | *MT-CYB*: AJ252807 (1), AY549461 (2) |
| *Squalius laietanus* | Matarraña River, Spain | *MT-CYB*: HM560187, HM560188 |
| *Squalius squalus* | Vipava R. Isonzo Basin. Slovenia (1), Arno River, Italy (2) | *MT-CYB*: HM560204 (1), HM560205 (2)  *S7*: HM560537  *RAG1*: HM560457 |
| *Squalius prespensis* | Prespa Lake, Greece | *MT-CYB*: AF090753 (1), HM560197 (2)  *S7*: HM560564  *RAG1*: HM560457 |
| *Squalius orientalis* | Uluchaj River, Russia | *MT-CYB*: HM560195 (1), HM560196 (2)  *S7*: HM560533  *RAG1*: HM560451 |
| *Squalius moreoticus* | Stymphalia Lake, Greece | *MT-CYB*: KY070375-KY070380 (1-6) |
| *Squalius pamvoticus* | Arachthos River (1-3), Louros River (4-6), Greece | *MT-CYB*: KY070381-KY070383 (1-3), KY070384-KY070386 (4-6)  *S7*: KY070513 (1a), KY070514 (1b) |

Table B. Evolutionary models estimated by jModelTest for the three analysed genes

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **GENE** | **SELECTED MODEL** | **FREQUENCY OF BASES** | **RATE MATRIX** | **GAMMA SHAPE / INVARIABLE SITES** |
| *MT-CYB* | TIM3+I+G  -lnL=5969.3629  k=184 | A = 0.2729  C = 0.2970  G = 0.1513  T = 0.2789 | R(a) [AC] = 1.9146  R(b) [AG] = 66.5160  R(c) [AT] = 1.0000  R(d) [CG] = 1.9146  R(e) [CT] = 13.8820  R(f) [GT] = 1.0000 | Gamma shape = 2.1360  Proportion of invariable sites = 0.5940 |
| *S7* | TPM3uf+G  -lnL=2394.4522  k=116 | A = 0.2943  C = 0.1545  G = 0.1982  T = 0.3530 | R(a) [AC] = 1.5458  R(b) [AG] = 2.8400  R(c) [AT] = 1.0000  R(d) [CG] = 1.5458  R(e) [CT] = 2.8400  R(f) [GT] =1.0000 | Gamma shape = 0.3030 |
| *RAG1* | TIM2ef+G  -lnL=2816.9850  k=116 | Equal | R(a) [AC] =  R(b) [AG] =  R(c) [AT] =  R(d) [CG] =  R(e) [CT] =  R(f) [GT] = | Gamma shape = 0.0720 |

Table C. Autapomorphies in the three analysed genes. \* transversions

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **POSITION *MT-CYB*/**  **POPULATION** | 18 | 42 | 45 | 60 | 66 | 81 | 88 \* | 93 | 105 | 108 | 117 | 144 | 150 | 151 | 156 | 159 | 162 | 174 | 180 \* | 195 \* | 204 | 228 \* | 231 | 237 | 261 \* | 273 | 279 | 285 |
| ALFIOS | **A** | **C** | **T** | **C** | **G** | **C** | **C** | **G** | **G** | **C** | A | **G** | **C** | **C** | **G** | **G** | **C** | **C** | **G** | **G** | **C** | **C** | **G** | **T** | **C** | **T** | **C** | **C** |
| EVROTAS | G | T | C | T | A | T | A | A | C | T | **G** | A | T | T | A | A | T | T | C | C | T | A | A | C | A | C | T | T |
| MIRAS | G | T | C | T | A | T | A | A | C | T | A | A | T | T | A | A | T | T | C | C | T | A | A | C | A | C | T | T |
| PAMISSOS | G | T | C | T | A | T | A | A | C | T | A | A | T | T | A | A | T | T | C | C | T | A | A | C | A | C | T | T |

…. Continuation Table C

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| **POSITION *MT-CYB*/**  **POPULATION** | 291 | 303 \* | 309 | 315 | 327 | 351 | 352 | 366 | 372 | 378 | 384 | 390 | 393 | 396 | 405 | 411 | 426 | 432 | 441 | 444 | 462 | 465 | 468 | 474 | 495 | 510 | 513 | 519 |
| ALFIOS | **C** | **A** | **C** | **T** | **T** | **A** | **A** | **A** | **A** | **A** | **A** | **T** | **C** | **A** | **A** | **A** | **T** | **G** | **A** | **C** | **T** | **C** | **A** | C | **A** | **A** | **T** | **G** |
| EVROTAS | T | C | T | C | C | G | G | G | G | G | G | C | T | G | G | G | C | A | G | T | C | T | G | C | G | G | C | A |
| MIRAS | T | C | T | C | C | A | G | G | G | G | G | C | T | G | G | G | C | A | G | T | C | T | G | C | G | G | C | A |
| PAMISSOS | T | C | T | C | C | A | G | G | G | G | G | C | T | G | G | G | C | A | G | T | C | T | G | **T** | G | G | C | A |

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| **POSITION *MT-CYB*/**  **POPULATION** | 522 | 552 | 573 | 577 | 579 \* | 580 | 582 | 585 \* | 592 | 598 | 606 | 609 | 615 | 630 \* | 639 | 642 | 651 | 675 \* | 693 \* | 696 | 700 | 702 \* | 708 | 709 | 717 \* | 723 | 724 | 748 |
| ALFIOS | **C** | **C** | **T** | A | **C** | **A** | **C** | **A** | **C** | **C** | **G** | **G** | **A** | **C** | **C** | **T** | **A** | **A** | T | **C** | **A** | **C** | **G** | **T** | **T** | **C** | **T** | **C** |
| EVROTAS | T | A | C | **G** | G | G | T | C | T | T | A | A | G | A | T | C | G | C | **C** | T | G | A | A | C | A | T | C | T |
| MIRAS | T | A | C | A | G | G | T | C | T | T | A | A | G | A | T | C | G | C | A | T | G | A | A | C | A | T | C | T |
| PAMISSOS | T | A | C | A | G | G | T | C | T | T | A | A | G | A | T | C | G | C | A | T | G | A | A | C | A | T | C | T |

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| **POSITION *MT-CYB*/**  **POPULATION** | 750 | 759 | 774 \* | 777 | 784 | 786 | 789 | 792 | 798 | 813 | 816 | 819 | 840 | 849 | 852 | 858 | 861 | 888 | 894 | 897 | 900 | 906 | 915 | 918 | 930 |
| ALFIOS | **G** | **G** | **A** | **A** | **C** | **G** | **G** | **T** | **A** | **G** | **G** | **T** | **T** | **T** | **C** | **C** | **A** | **C** | **C** | **A** | **G** | **G** | **A** | **C** | **T** |
| EVROTAS | A | A | T | G | T | A | A | **G** | G | A | A | C | C | C | T | T | G | T | T | G | A | A | C | T | G |
| MIRAS | A | A | T | G | T | A | A | A | G | A | A | C | C | C | T | T | G | T | T | G | A | A | C | T | G |
| PAMISSOS | A | A | T | G | T | A | A | A | G | A | A | C | C | C | T | T | G | T | T | G | A | A | C | T | G |

…. Continuation Table C

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| **POSITION *MT-CYB*/**  **POPULATION** | 1005 | 1017 | 1057 | 1069 | 1074 | 1078 | 1083 | 1089 | 1090 | 1092 | 1095 | 1110 | 1125 | 1129 |
| ALFIOS | **G** | **G** | **G** | **C** | **T** | **G** | **C** | **C** | **A** | **T** | **T** | **A** | **A** | **C** |
| EVROTAS | A | A | A | T | C | A | A | A | G | C | C | C | G | T |
| MIRAS | A | A | A | T | C | A | A | A | G | C | C | C | G | T |
| PAMISSOS | A | A | A | T | C | A | A | A | G | C | C | C | G | T |

…. Continuation Table C

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| **POSITION *S7*/**  **POPULATION** | 68 \* | 69 | 72 | 99 | 130 | 266 \* | 313 | 414-419 | 463-477 | 530 | 565 | 581 \* | 608 \* | 639 |
| ALFIOS | A | C | C | C | T | T | A | ACGTT | - | G | A | A | A | T |
| EVROTAS | **T** | **T** | **T** | **T** | **C** | **A** | **G** | **-** | **TGAGATTAATAATTT** | **A** | **G** | **T** | **C** | **C** |
| MIRAS | A | C | C | C | T | T | A | ACGTT | - | G | A | A | A | T |
| PAMISSOS | A | C | C | C | T | T | A | ACGTT | - | G | A | A | A | T |

…. Continuation Table C

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **POSITION *S7*/**  **POPULATION** | 642-645 | 647 | 653 | 684 \* | 732 \* | 751-752 | 754 | 759 | 802 \* | 819 \* | 829 | 855 \* | 857 | 870 | 876 | 877-892 | 945-946 |
| ALFIOS | - | - | T | A | A | TT | T | T | A | G | A | A | G | A | G | - | TG |
| EVROTAS | **TTA** | **G** | **-** | **T** | **T** | **-** | **C** | **C** | **C** | **T** | **G** | **T** | **A** | **G** | **A** | **ACACTATTTTAAGTG** | **-** |
| MIRAS | - | - | T | A | A | TT | T | T | A | G | A | A | G | A | G | T | TG |
| PAMISSOS | - | - | T | A | A | TT | T | T | A | G | A | A | G | A | G | T | TG |

…. Continuation Table S2

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| **POSITION *RAG1*/**  **POPULATION** | 439 | 768 \* | 773 | 898 | 948 \* | 1002 | 1008 | 1026 | 1032 | 1048 \* | 1161 \* | 1169 | 1173 |
| ALFIOS | G | T | A | T | C | G | C | G | G | C | T | A | G |
| EVROTAS | **A** | **A** | **G** | **C** | **A** | **A** | **T** | **A** | **A** | **A** | **A** | **G** | **A** |
| MIRAS | G | T | A | T | C | G | C | G | G | C | T | A | G |
| PAMISSOS | G | T | A | T | C | G | C | G | G | C | T | A | G |