

Supplementary Figure S1. Schematic illustration of the allelic frequencies at six microsatellite loci studied for de C, P and A genomes in nine populations of *S. alburnoides* and in *A. hispanica*. Circles represent distinct alleles, and their surfaces are directly proportional to their frequencies.

Supplementary Figure S2. Neighbour-Joining tree from the DCE genetic distance estimated with microsatellite data: a) all the species that participate in the *S. alburnoides* complex including *A. hispanica*, the closest species to the paternal ancestor, and the reconstituted paternal ancestor (AA); b) and c) populations of *S. alburnoides* from Douro, Mondego, Tagus and Guadiana River basins with the same ploidy level (2n: a; 3n:b). Branch lengths are proportional to the genetic distances between populations. Numbers indicate nodes with bootstrap support higher than 50% in 1000 replications.

Supplementary Figure S3. Putative reproductive modes and relationships between the *S. alburnoides* complex biotypes observed in nature, in the Mondego River basin adapted from Pala & Coelho (2005) (a) and the Douro River basin (b) C is the genome of the parental host (*S. carolitertii*) and A is the genome of the 'unknown' ancestor. (*), biotype very rare in natural populations; dotted forms have never been observed in natural populations, (—) pathways previously described (Carmona et al. 1997; Pala & Coelho 2005; Cunha et al. 2008); (---), hypothetical pathway; (CC'), indicates the reconstituted *S. carolitertii*. Spermatozoid symbol with C indicates fertilization by C sperm; spermatozoid symbol with CA indicates fertilization by CA sperm produced by diploid and tetraploid males; (G), gynogenesis; (1), hybridogenesis; (2), production of unreduced eggs with high levels of syngamy (3), meiotic hybridogenesis; (4) normal meiosis.

Supplementary Figure S4. Scatter plots of pairwise $F_{ST} / (1 - F_{ST})$ (a) and $R_{ST} / (1 - R_{ST})$ (b) against geographical distances in 9 *Squalius alburnoides* populations. Mantel tests did not indicate a significant relationship between genetic and geographical distances in *S. alburnoides* regardless of whether $F_{ST} / (1 - F_{ST})$ or $R_{ST} / (1 - R_{ST})$ was used.