

Figure S1. Gel image showing results on the two-step molecular identification protocol. A) Gel showing the digestion pattern of the marker RAG1 with the restriction enzyme EcoO109I. The first group (*P. perezii*, *P. lessonae* and *P. bergeri*) have no cleavage site for the restriction enzyme, resulting in a band of 1,000 bp, which represents the whole marker sequence. The second group (*P. ridibundus* and *P. kurtmuelleri*) has one cleavage site and produces two bands of around 400 bp and 600 bp. The species of the third group (*P. kl. grafi*, *P. kl. esculentus*, *P. kl. hispanicus* and klepton PK) have two different genomes, one from the first group which is represented by a band of 1,000 bp; the other belonging to a species from the second group, which produces two faint bands of 400 bp and 600 bp. B) Gel image showing size differences of the SAI-1 PCR product for *P. perezii* (853 pb), *P. lessonae* (306 pb), *P. bergeri* (298 pb), *P. ridibundus* (839-843 pb) and *P. kurtmuelleri* (717 pb). Kleptons contain two bands, corresponding to the sizes of their respective parental species. Kleptons PK, *P. kl. hispanicus* and *P. kl. esculentus* were obtained in silico by mixing DNA from the parental species.

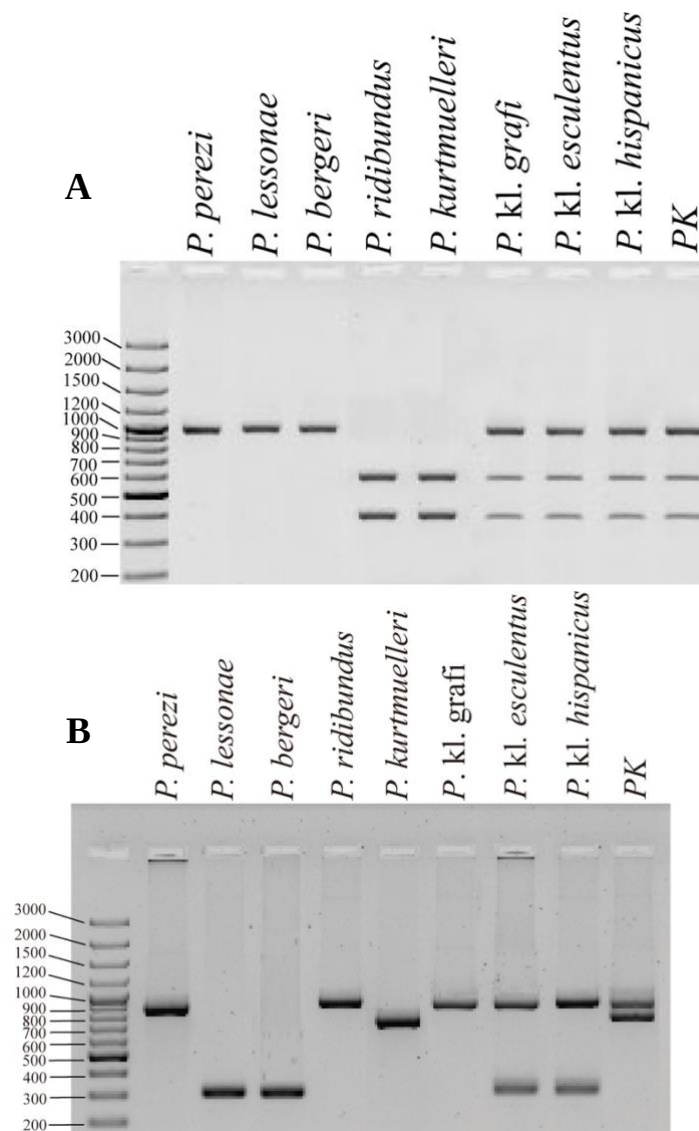
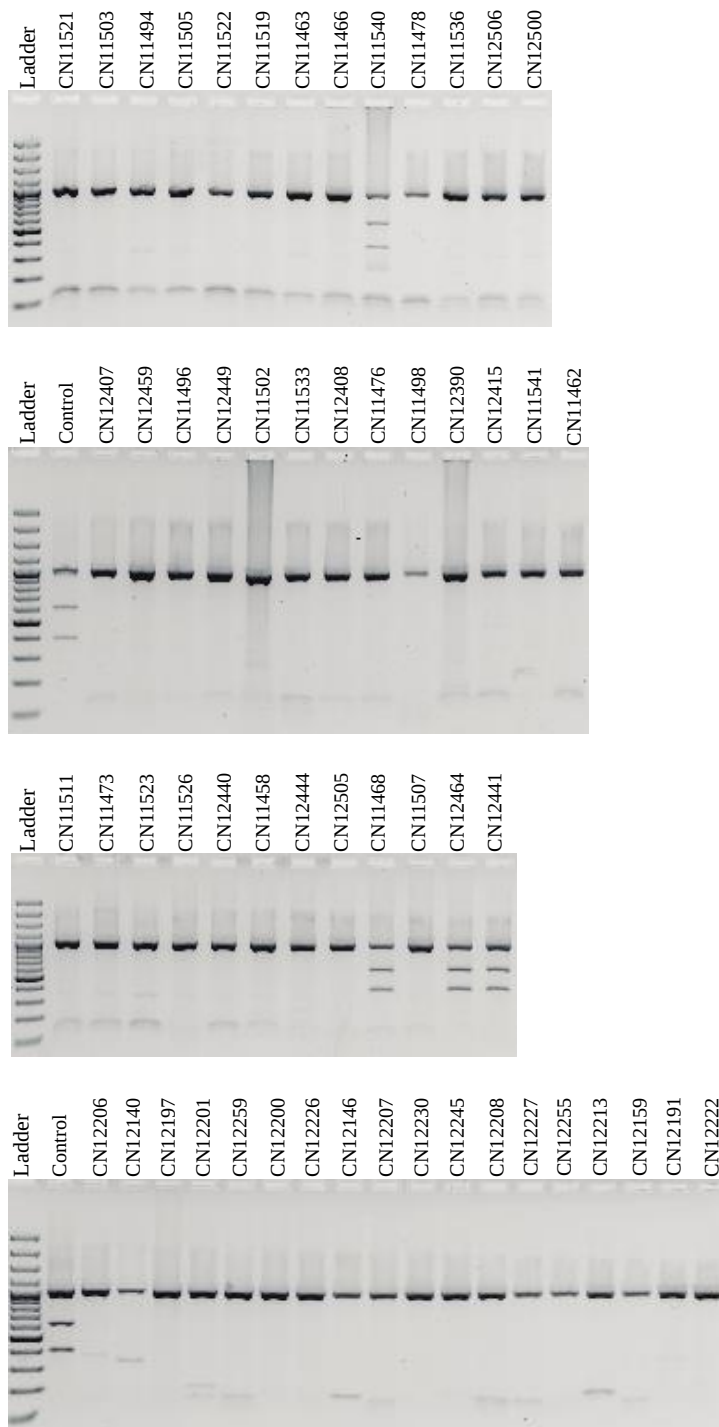
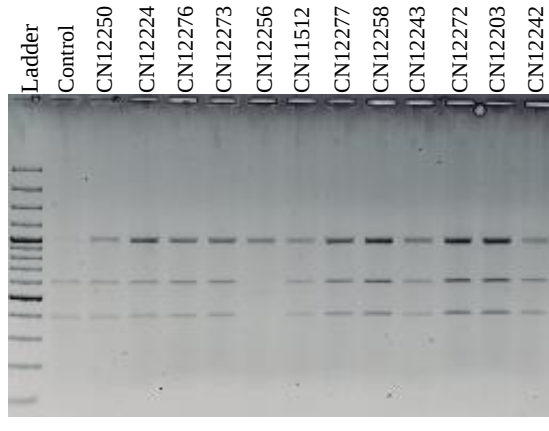
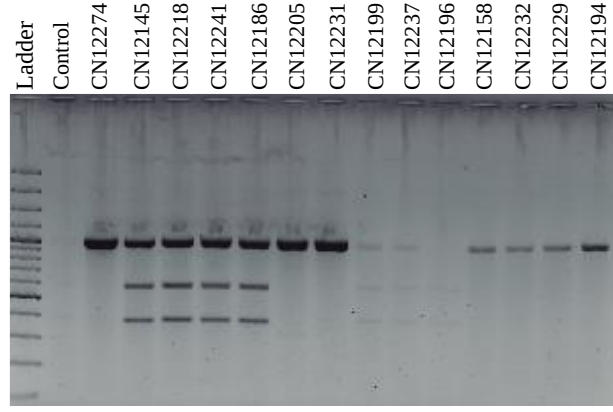
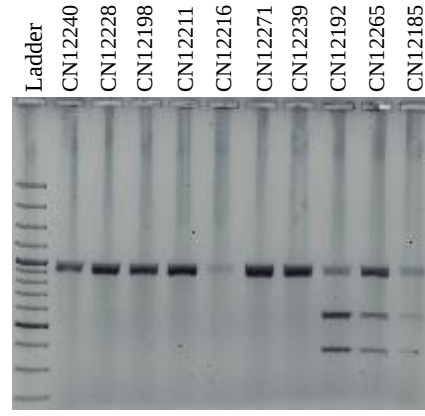
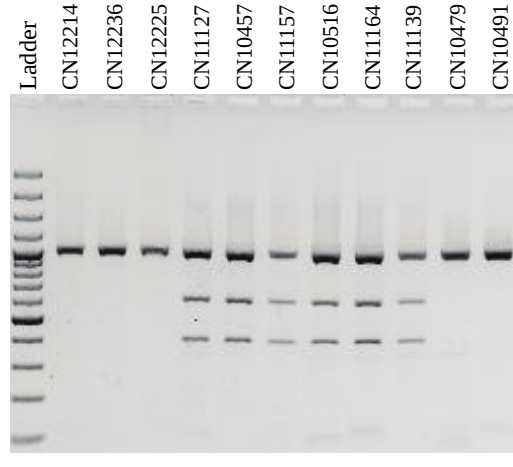
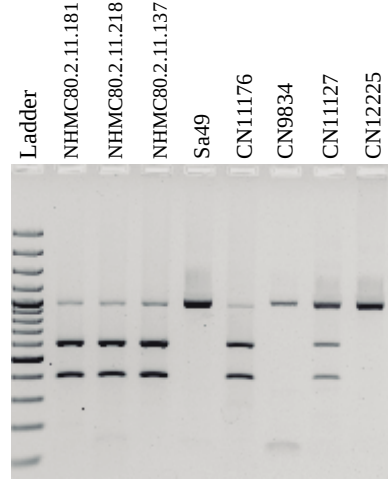
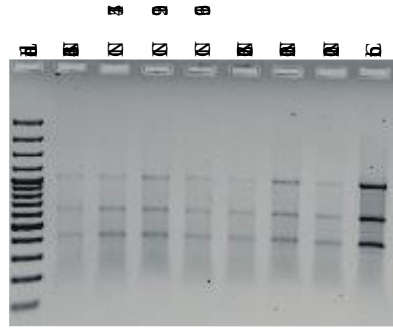
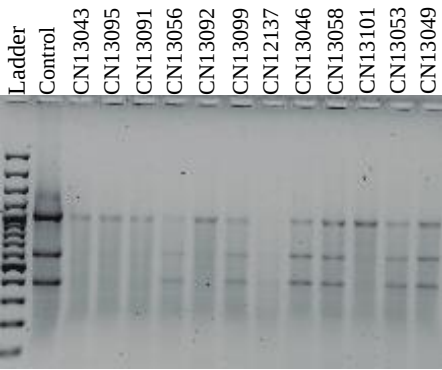
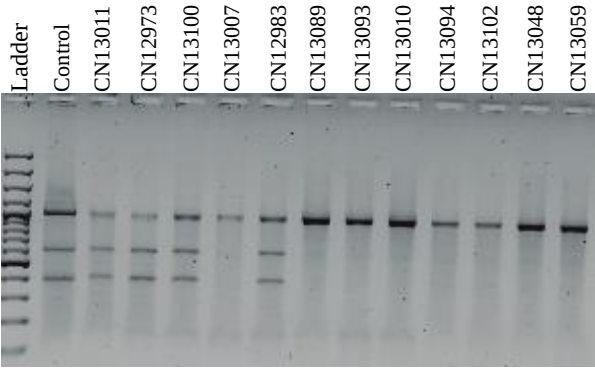
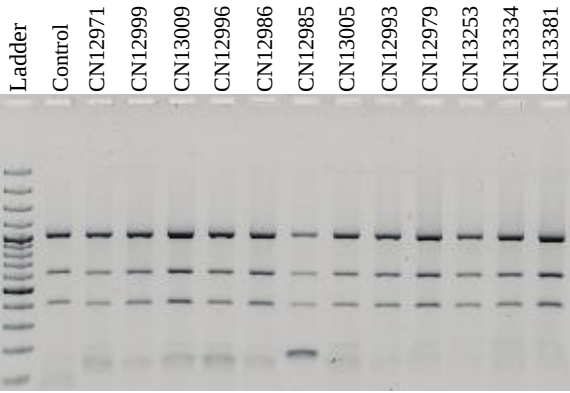
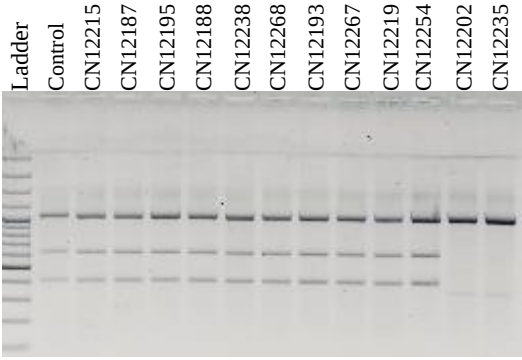
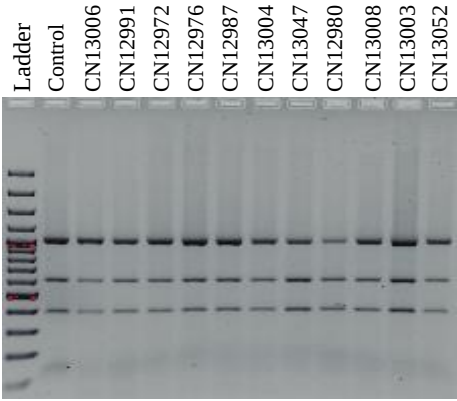
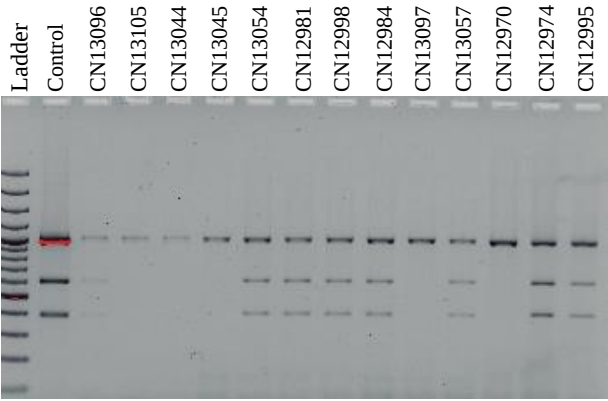
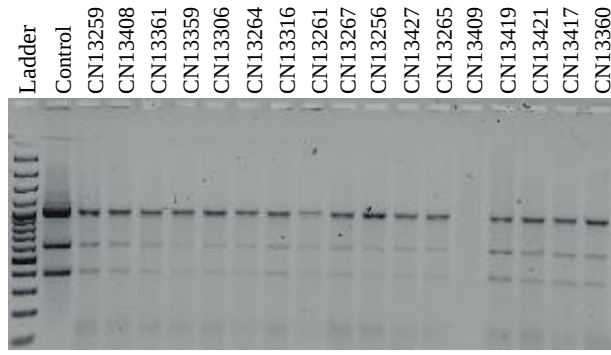
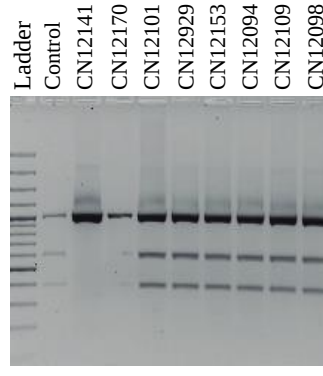
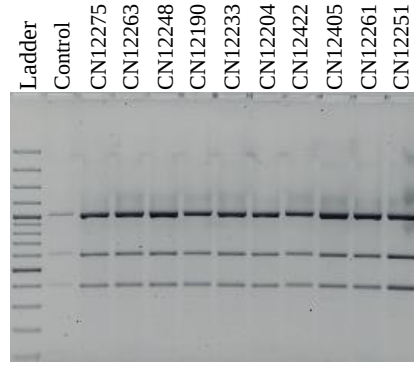
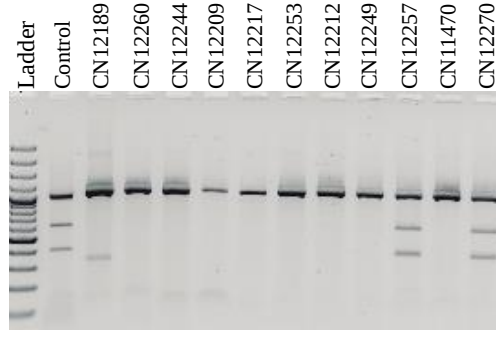
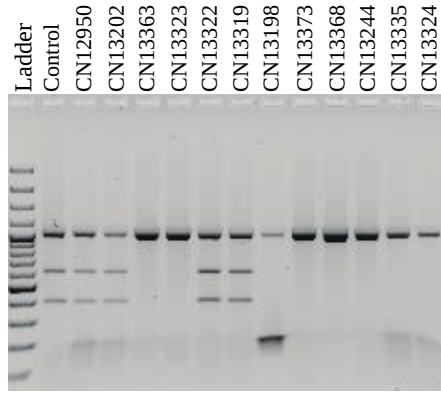


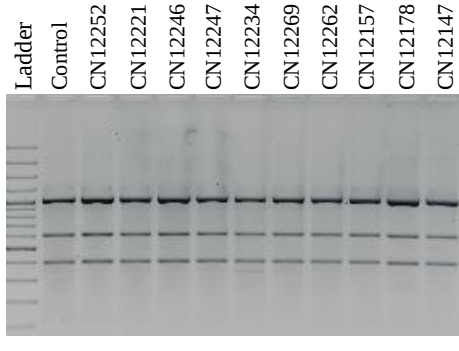
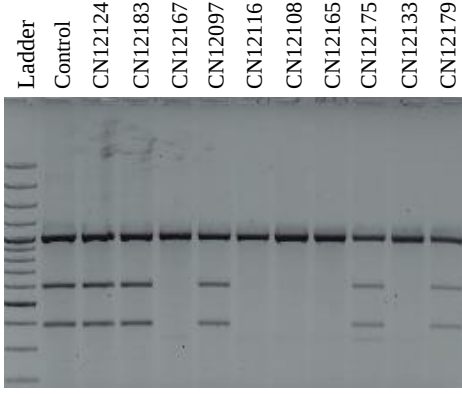
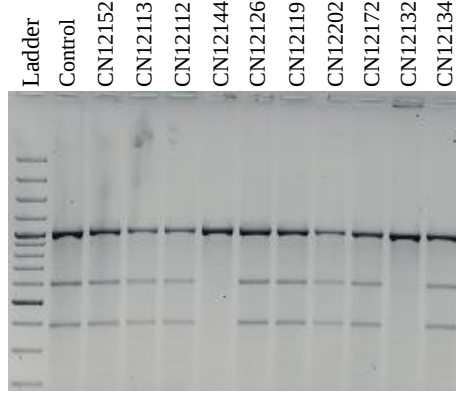
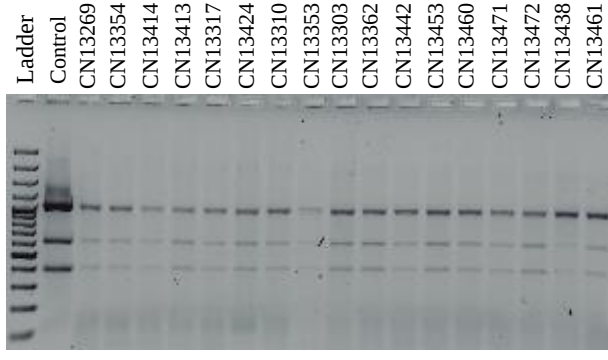
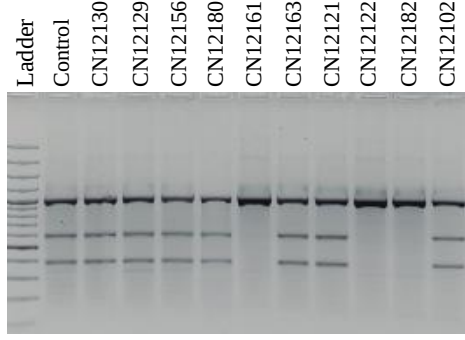
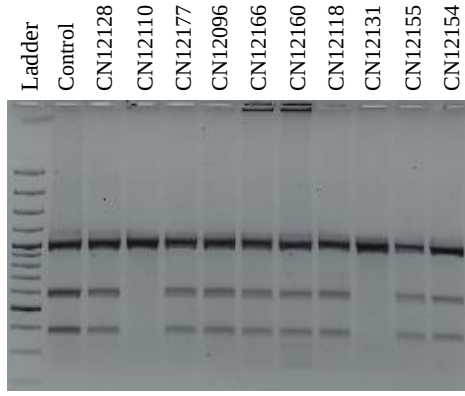
Figure S2. Results of the first step of the two-step molecular identification protocol for European water frogs. Agarose gels display the product of RAG1 molecular marker digestion using the EcoO109I restriction enzyme.

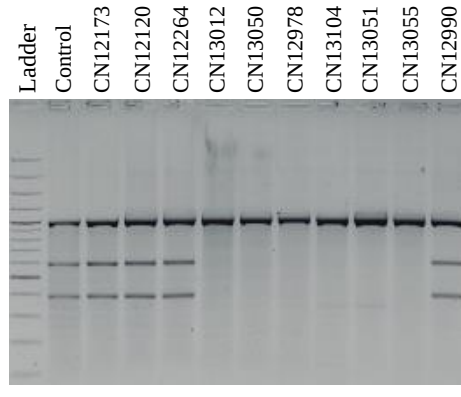
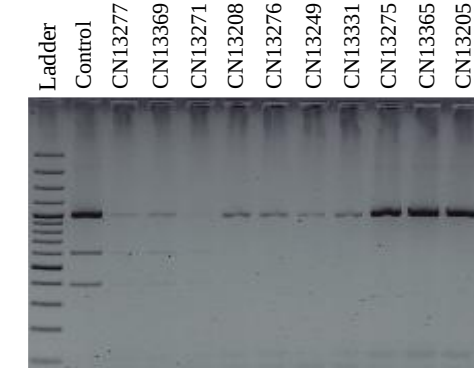
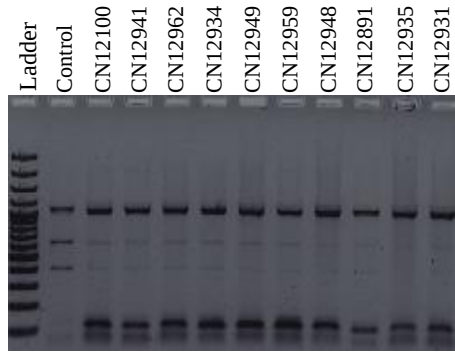
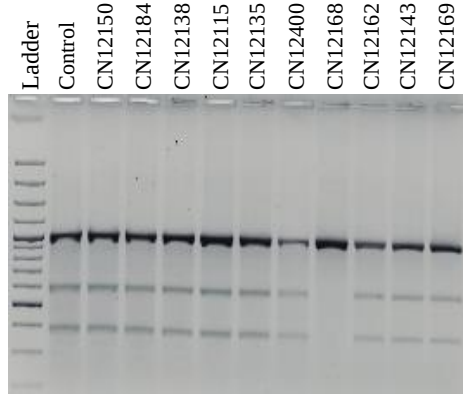
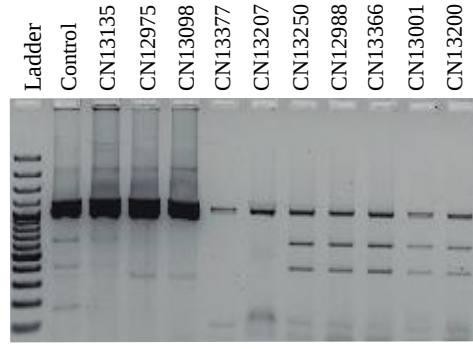
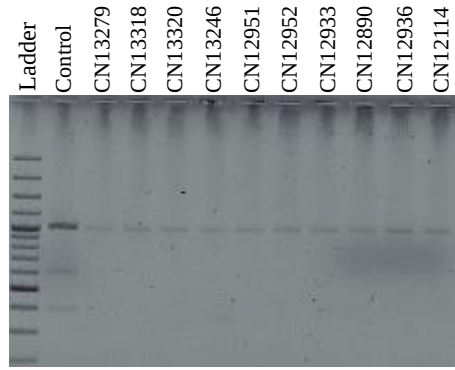


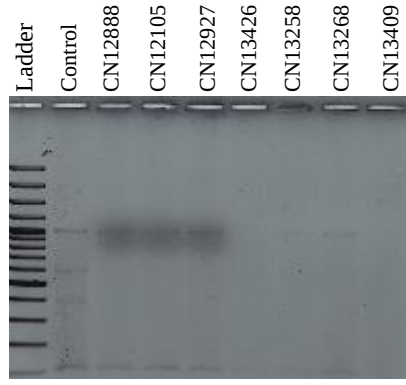
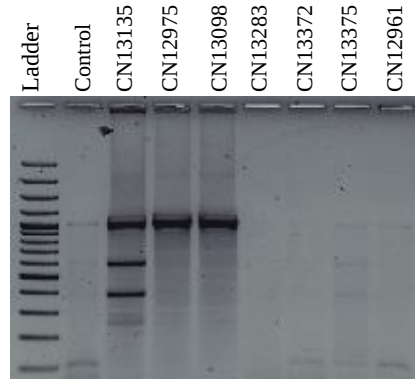
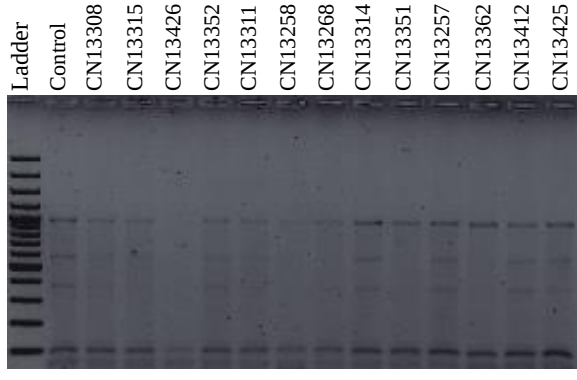
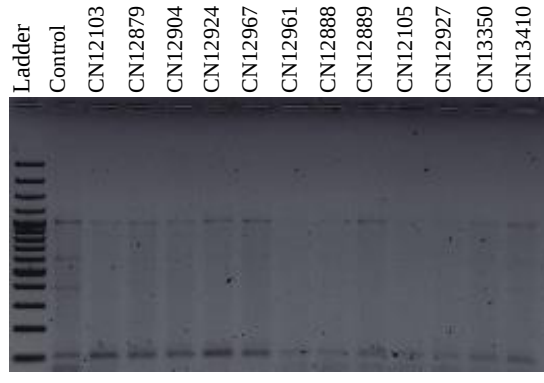
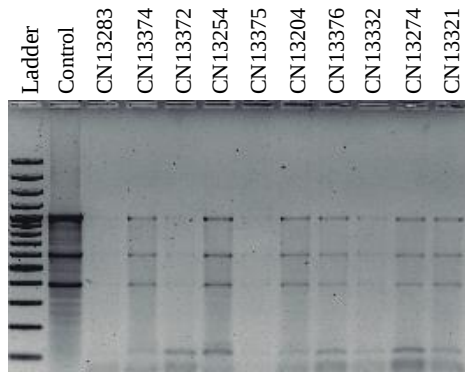












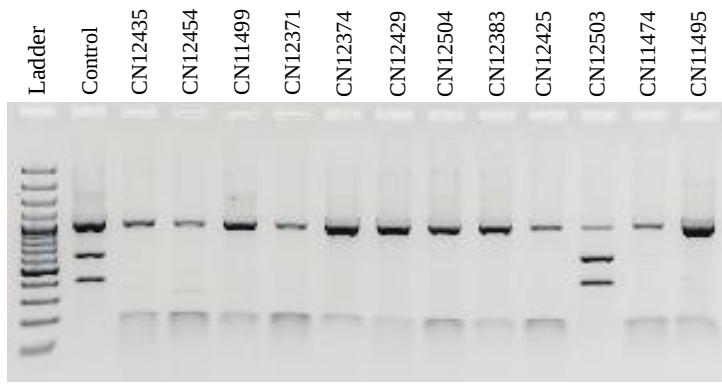
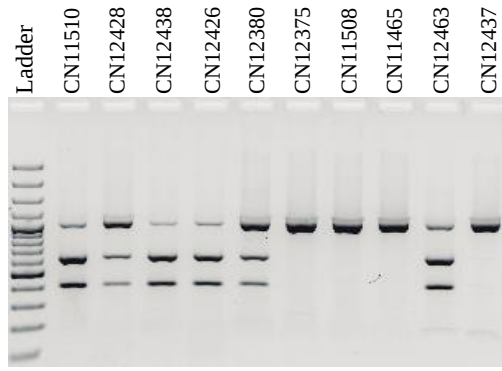
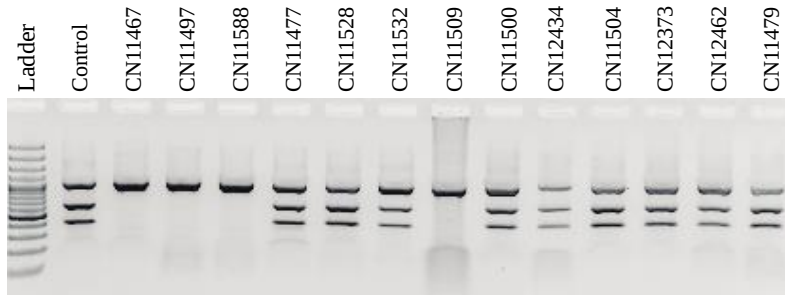
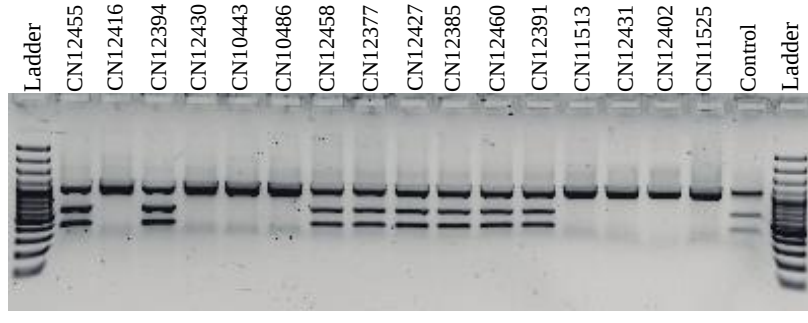


Figure S3. Results of the second step of the two-step molecular identification protocol for European water frogs. Agarose gels display the SAI-1 PCR product amplified for all the samples collected in this study.

