Appendix II

Polymerase Chain Reactions (PCR) were performed in a 20µl total reaction volume with 10 µl of REDExtract-N-ampl PCR reaction mix (Sigma – Aldrich), 0.8µl of each primer (10µM), 4.4µl of Sigma-water, and 4µl of template DNA. The mitochondrial control region (CR) and the first intron of the nuclear S7 ribosomal protein gene (S7) were amplified using the following pairs of primers: L-pro1 (5' ACTCTCACCCCTAGCTCCCAAAG 3') and H-DL1 (5' CTGAAGTAGGAACCAGATGCCAG 3') [38] for the CR, and S7RPEX1F (5'-TGG CCT CTT CCT TGG CCG TC-3') and S7RPEX2R (5'-AAC TCG TCT GGC TTT TCG CC-3') [39]. An initial denaturation at 94◦C for 7' was followed by 35/30 cycles (denaturation at 94◦C for 30/45'', annealing at 55◦C for 30/45'', and extension at 72◦C for 1'; values CR/S7, respectively) and a final extension at 72◦C for 7' on a Bio-Rad Mycycler thermal cycler. The same primers were used for the sequencing reaction, and the PCR products were purified and sequenced in STABVIDA (http://www.stabvida.net/).