

Experimental evaluation of *Haplosporidium pinnae* DNA detection and degradation under semi-controlled conditions

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Electrophoresis

Gels were prepared with 100 mL of TBE buffer, 2g of GRS Agarose LE, GA110.0500 (Grisp, Portugal) and 5 µL of Xpert Green DNA Stain (20.000X), GS01.0001 (Grisp, Portugal). The buffer was prepared by diluting TBE Buffer (10X), BG12.0110 (Grisp, Portugal) with ultrapure water produced using the Halios system (Neptec, Germany). The same buffer was used to run the electrophoresis. Prior to loading on the gel, PCR products were mixed with GRS DNA Loading Buffer Blue (6X), GLB01.0001 (Grisp, Portugal), by adding 9 µL of the PCR product to 1 µL of the loading buffer. The size marker used was GRS Ladder 50bp, GL031.0050 (Grisp, Portugal). The casting system used was the Biometra Compact M system (Analytik Jena, Germany). The power supply used was Biometra PS 300TP (Analytik Jena, Germany). The electrophoresis was performed at 100 V for 70 minutes, and the gel was visualised on the BXT-26.MX UVIpure transilluminator (UVITEC, England). Results of PCR reactions are presented in the figures below (Figures S1-S6).

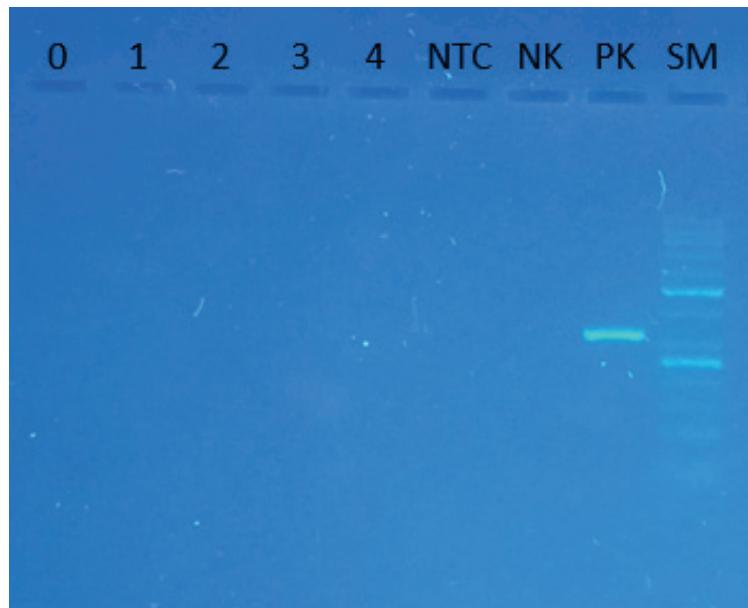


Fig. S1: Results of PCR with HAP-F1/HAP-R2 primers from samples obtained with GF filters. 0–4 – day of the experiment, NTC – no template control, NK – negative control, PK – positive control, SM – size marker.

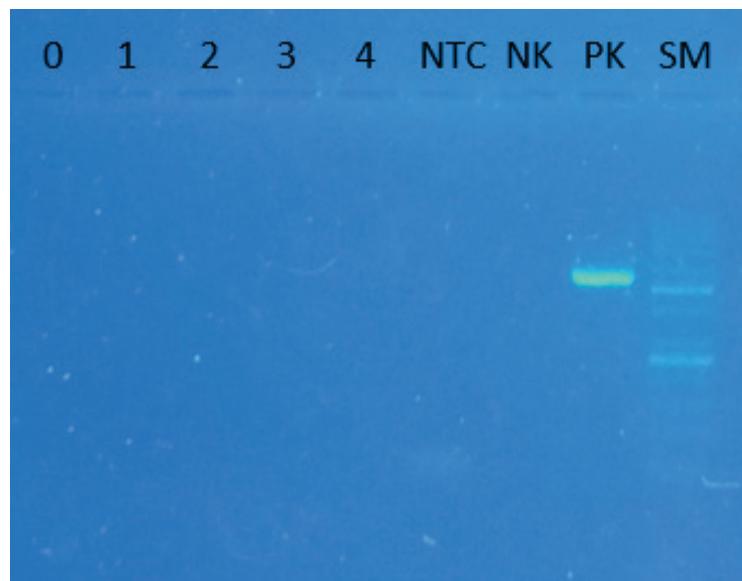


Fig. S2: Results of PCR with HPNF3/HPNR3 primers from samples obtained with GF filters. 0–4 – day of experiment, NTC – no template control, NK – negative control, PK – positive control, SM – size marker.

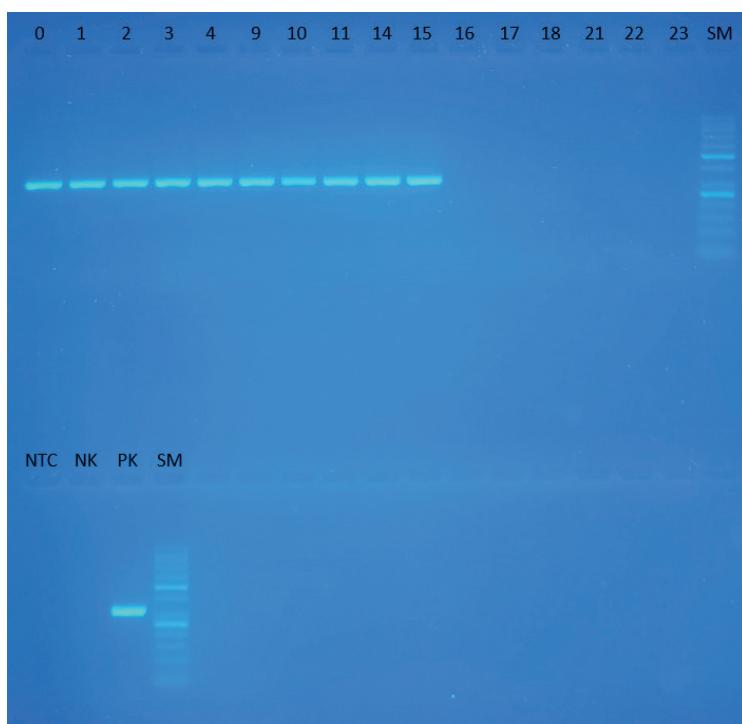


Fig. S3: Results of PCR with HAP-F1/HAP-R2 primers from samples obtained with MCE filters in the live-infected (first) experiment. 0–23 – day of experiment, SM – size marker, NTC – no template control, NK – negative control, PK – positive control.

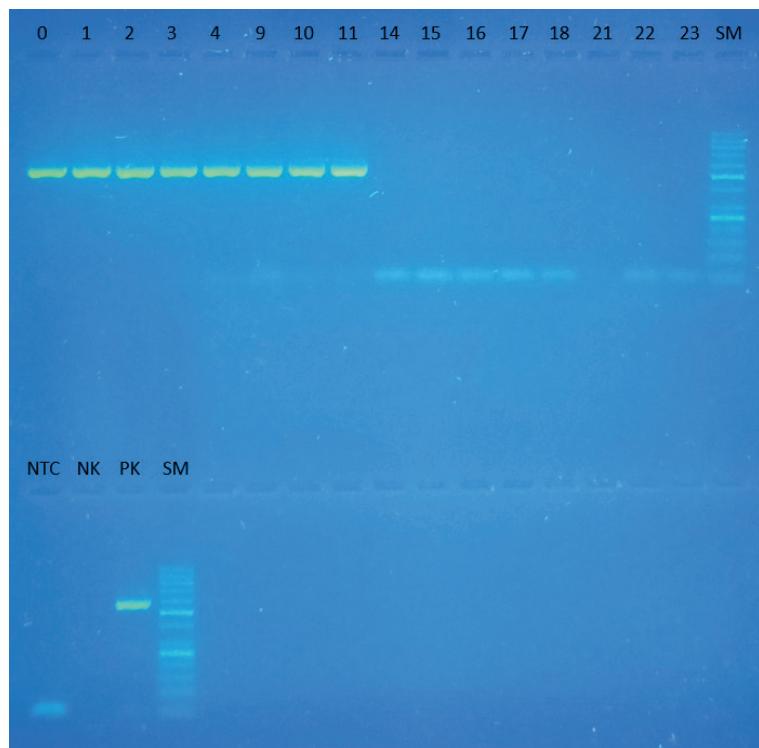


Fig. S4: Results of PCR with HPNF3/HPNR3 primers from samples obtained with MCE filters in the live-infected (first) experiment. 0–23 – day of experiment, SM – size marker, NTC – no template control, NK – negative control, PK – positive control.

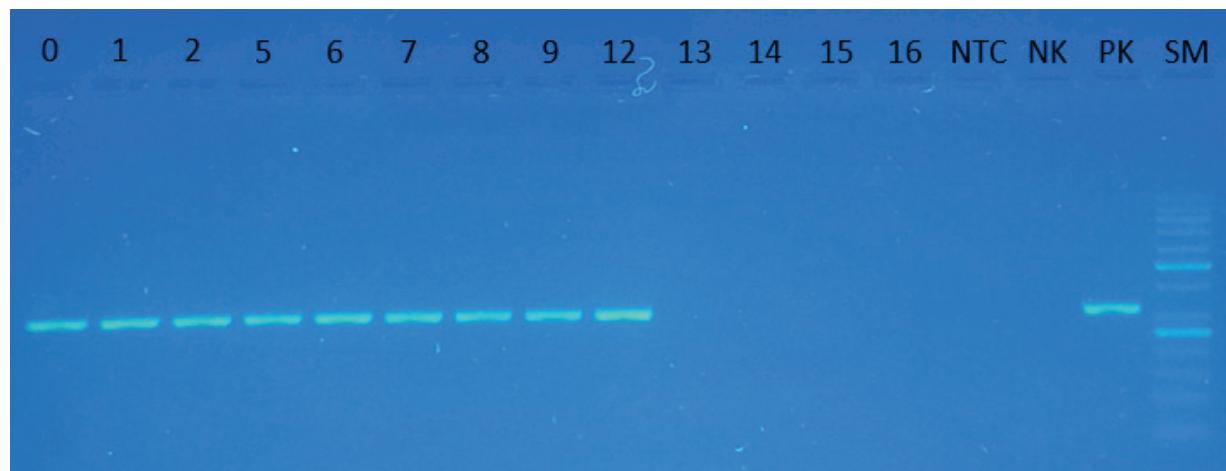


Fig. S5: Results of PCR with HAP-F1/HAP-R2 primers from samples obtained in the post-mortem (second) experiment. 0–16 – day of experiment, NTC – no template control, NK – negative control, PK – positive control, SM – size marker.

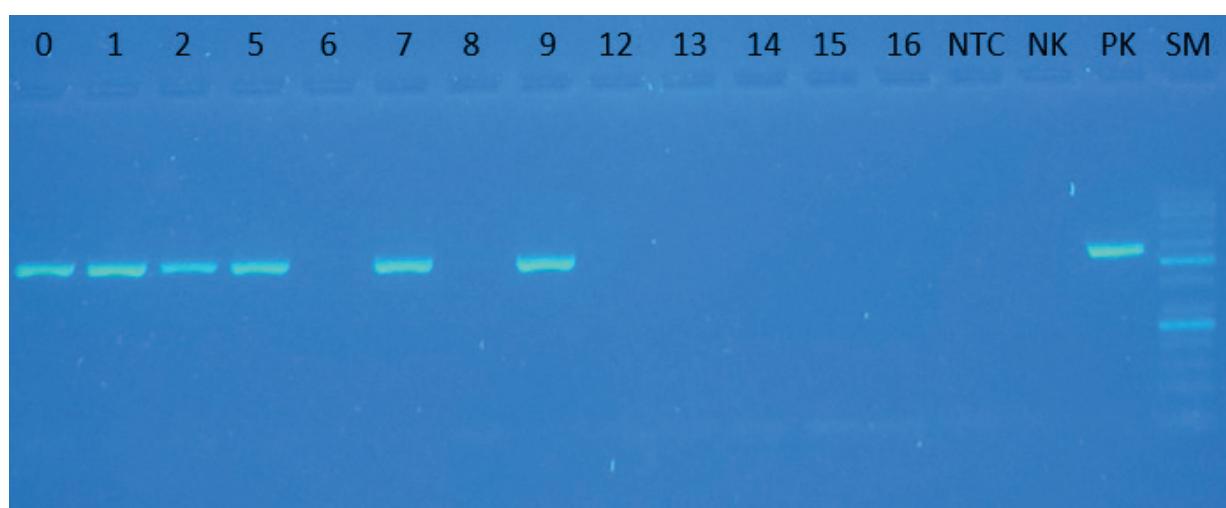


Fig. S6: Results of PCR with HPNF3/HPNR3 primers from samples obtained in the post-mortem (second) experiment. 0–16 – day of experiment, NTC – no template control, NK – negative control, PK – positive control, SM – size marker.