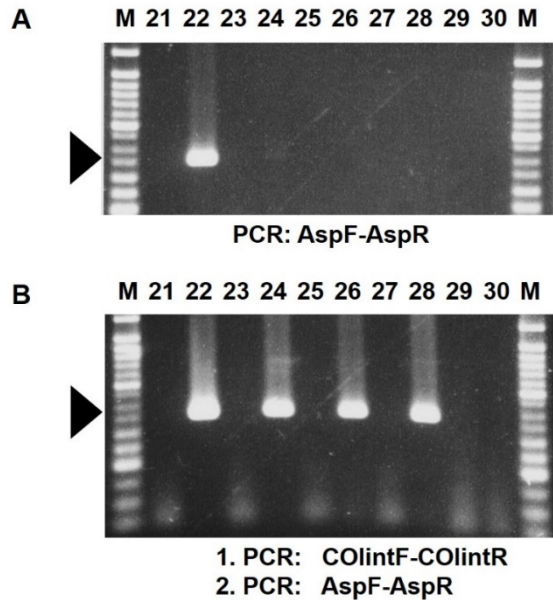


Additional File 3

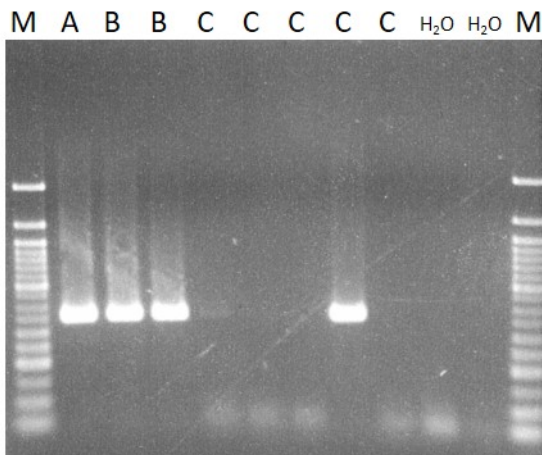
Sensitivity of the *Acanthocheilonema spirocauda* *cox1* nested-PCR



Localisation of diagnostic PCR-primers and alignment of the partial *A. spirocauda* *cox1*-sequence (As) with sequences of *Dirofilaria immitis* (Di, AJ537512) and *Acanthocheilonema viteae* (Av, HQ186249)



Comparison in sensitivity of the *A. spirocauda* *cox1* PCR (A) and the *cox1* nested-PCR (B), lice pools 21-30 from harbour seals, specific 351 bp amplicon (arrow heads), M=molecular weight standard (50 bp ladder)



Microfilariae isolated from adult female *A. spirocauda* were resuspended in PBS and counted under lightmicroscopy. Dilutions of 200 mf/10 μ l (A), 20 mf/10 μ l (B), and 2-4 mf/10 μ l (C) were prepared. Each batch was digested with 1 μ l of Proteinase K (20 mg/ml) for 2 h at 56 $^{\circ}$ C and deactivated 5 min at 95 $^{\circ}$ C. 5 μ l were used as template for the first PCR (COLintF/COLintR) in a 50 μ l reaction mix (conditions: 2' 95 $^{\circ}$ C/ 35x 30'' 94 $^{\circ}$ C, 30'' 50 $^{\circ}$ C, 45'' 72 $^{\circ}$ C/ 5' 72 $^{\circ}$ C). 1 μ l of the first PCR was used as template in a 50 μ l nested PCR (Asp-F/Asp-R; 2'95 $^{\circ}$ C/ 35x 30'' 94 $^{\circ}$ C, 30'' 58 $^{\circ}$ C, 30'' 72 $^{\circ}$ C) and 8 μ l were separated on a 2% agarose gel; M=50 bp molecular weight standard.