The clonal dynamics and molecular epidemiology of Amoebic Gill Disease in *Salmo salar* via multiplex amplicon sequencing.

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Amoebic gill disease (AGD), caused by various strains of *Neoparamoeba perurans*, is as a worldwide disease of salmonids aquaculture associated with sustained and intensive year-on-year losses (≥20%). *N. perurans* hosts in the vicinity of its nucleus an endosymbiotic aflagelate Kinetoplastid called *Perkinsela spp known as Perkinsela-like organism* (PLO) or *ihctyobodo necator* related organism (IRO), similar to kinetoplastid pathogens of human and domestic livestock (Trypanosoma brucei sp., *T. cruzi*, *Leishmania sp.*). As well as a Kinetoplastid, the *N. perurans* cytoplasm is also home to many intracellular prokaryotes. Genome sequencing efforts have been delayed and direct sequencing of parasite genomic material is frustrated by microbial contamination. Rapid, low cost- characterization of *N. perurans* genetic polymorphisms strains at once would be a powerful epidemiological surveillance management for AGD. To quantify the genetic diversity associated with AGD, we developed a simple, cost-effective, multiplexamplicon sequencing protocol tool based on massive parallel amplification of >400 information hotspots throughout the target genomes of *N.* perurans and its endosymbionts. These markers will be applied to daily samples across four sites of Salmon farms, two sites in west Scotland, and two sites in western Ireland over two summers seasons, 2019 in Ireland and 2021 in Scotland to establish the colonisation and re-infection dynamics of this pathogen. To sequence the AGD agent, more than one Terra bp of genomic and transcriptomic data isolated for pure *N. perurans* culture treated with antibiotics were generated with Nanopore and Novaseq Illumina. The data were processed, curated, and assembled to characterize the functional associations between the host and its endosymbiont. A candidate list of housekeeping, metabolic and accessories genes will be amplified with the Genome-wide locus sequence typing (GLST) method and will provide a unique level of genomics, spatial and temporal resolution to understand the dynamics of parasite.

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