Basic local alignment search tool (BLAST) was employed using for each retrieved Nanopore sequence against the non-redundant GenBank database to identify homologous nucleotide sequences (Altschul et al., 1997). The sequences from the top 50 BLASTn hits based on the lowest e-value were downloaded for each queried sequence. For each individual Nanopore sequence, the corresponding 50 nucleotide hit sequences were aligned using MAFFT v7 (Katoh & Standley, 2013) before being trimmed using BMGE (Criscuolo & Gribaldo, 2010). For Gregarine Nanopore SSU sequences, we used performed these set with the addition of the set of 100 SSU sequences from Boisard et al. (xxxxxxxx), and LSU sequences were added to the LSU datasets. Maximum likelihood analyses were performed using fasttree version 2.1.11 with the -gtr and –gamma options (Price et al., 2009), and phylogenetic trees were visualised using PRESTO in the NGPhylogeny.fr online workflow (Lemoine et al., 2019).

Subsequently, hit sequences that were phylogenetically divergent were manually removed from the dataset for each tree. Datasets were then merged according to parasite phyla and duplicate sequences were removed. The workflow above was re-run using the selected and combined sequences to check and manually prune the phylogenetic trees by phyla. The final dataset for each parasite phyla were aligned in MAFFT version 7 (Katoh & Standley, 2013), trimmed with BGME (Criscuolo & Gribaldo 2010), and rooted maximum likelihood trees with 1,000 bootstraps were constructed in fasttree version 2.1.11 using the -gtr option (Price et al., 2009). The phylogenetic trees were visualised and produced using the programme FigTree version 1.4.4 (Rambaut, 2018).

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