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18S rRNA gene metabarcoding for investigation of gastrointestinal parasite diversity in great cormorants

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The great cormorant (*Phalacrocorax carbo*) is a migratory, fish-eating bird that may act as a carrier for various pathogens, raising potential ecological and public health concerns. This study investigated the diversity of gastrointestinal parasites in great cormorants from the Republic of Korea using 18S rRNA gene metabarcoding and conventional diagnostic methods. Fecal samples were collected from 10 great cormorants in Korea, and DNA was extracted for metabarcoding targeting the V4 and V9 regions of the 18S rRNA gene using the MiSeq platform. Conventional PCR and microscopic examination were used for validation. The V4 region of the 18S rRNA gene revealed the presence of *Baruscapillaria spiculata*, *Contracaecum* sp., and *Isospora lugensae*. The V9 region analysis identified the following parasites: *Tetratrichomonas* sp., *Histomonas meleagridis*, *Trichomitus* sp., *Tetratrichomonas prowazekii*, *B. obsignata*, *Monosiga ovata*, and *Fasciola gigantica*. Furthermore, conventional PCR confirmed the presence of *Contracaecum* sp., *Isospora* sp., and unspecified trichomonads, and microscopic examination identified eggs of capillarid, *Contracaecum*, and *Eustrongylides* and trophozoites of flagellated protozoa in the collected cormorant fecal samples. The findings underscored the potential ecological role of great cormorants as hosts for diverse gastrointestinal parasites and the utility of 18S rRNA gene metabarcoding for screening the diversity of gastrointestinal parasites in great cormorants.

Keywords Metabarcoding, Next-generation sequencing, *Phalacrocorax carbo*, Gastrointestinal parasite, Piscivorous birds

Great cormorants (*Phalacrocorax carbo*) are piscivorous and migratory birds that consume a wide variety of fish species[1]. The global population of great cormorants has increased significantly in recent years, which has led to concerns about their potential role in the spread of diseases across regions[2,3]. As these birds migrate between different ecosystems, they may act as carriers of pathogens, thereby introducing and spreading infectious diseases to new areas[4]. Research conducted previously has documented their role in the transmission of pathogens, including *Escherichia coli*, avian influenza virus, and *Vibrio cholerae*[1,5–8]. Furthermore, various gastrointestinal parasites have been identified in great cormorants, including *Clinostomum, Contracaecum, Eustrongylides, Baruscapillaria, Sarcocystis, Blastocystis, Giardia*, and *Cryptosporidium*[2,3,9–15]. Given their diverse diet, which encompasses a broad range of fish, they are susceptible to exposure to a variety of parasite species. Notably, a paucity of comprehensive research on gastrointestinal parasites in great cormorants persists, particularly in the Republic of Korea, underscoring the necessity for further investigation[1,9,16]. Addressing this research gap is imperative to develop effective management strategies that will protect both ecological and public health.

Next-generation sequencing (NGS) for metabarcoding expands the capabilities of traditional methods like PCR, microscopy, and culture-based screening by enabling high-throughput, comprehensive, and detailed analyses of diverse biological samples[17]. This approach substantially reduces the labor, cost, and time required for

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comprehensive identification of microbial or eukaryotic communities by enabling the simultaneous detection of a broad range of organisms[18,19]. However, the technology also has limitations, such as PCR errors during the metabarcoding process, short read length compared to Sanger sequencing, high computational requirements for analyzing large amounts of data[20,21].

Moreover, metabarcoding has the potential to identify rare or novel pathogens that are often challenging or impossible to identify using traditional methods, thereby enhancing the depth and accuracy of ecological studies[22,23]. While metabarcoding targeting the bacterial 16S rRNA gene has been established and is widely used for bacterial community studies, the development of metabarcoding for eukaryotes based on the 18S rRNA gene is still in its preliminary stages[24]. The V4 and V9 regions of the 18S rRNA gene are of particular interest in eukaryote metataxonomic studies[25]. However, the results of metabarcoding can vary depending on the primer sets, target regions, PCR conditions, and reference databases used. Therefore, it is necessary to confirm the results through a traditional method such as conventional PCR or real-time PCR[26].

The objective of this study was to evaluate the diversity of gastrointestinal parasites in great cormorants in the Republic of Korea (Korea) through the use of metabarcoding of the V4 and V9 regions of the 18S rRNA gene. Furthermore, the findings were compared with those obtained through the use of conventional PCR and microscopy.

Methods

Collection of great cormorant fecal samples and DNA extraction

A total of 10 great cormorants (without sex information) hunted by professional hunters in Paroho Lake, Yanggu, Gangwon Province, Korea were used in the study. The hunted great cormorants were individually wrapped in plastic bags and transported to the Department of Parasitology, School of Medicine, Chungbuk National University, Cheongju, Korea.

The great cormorants in Korea are classified as noxious wild animals by the Korean government and can be hunted by authorized hunters. The animals in this study were hunted for regular hunting by the authorized hunters, not for the experiment.

For the purpose of DNA extraction, fecal samples were collected from each great cormorant cloaca using a cotton swab. The DNA was extracted from all samples on the day of collection using the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The extracted DNA was stored at – 20 °C until further use.

Microscopic examination

The collected fecal samples were examined by the direct smear method and the saturated saline flotation method. The samples were observed using a BX53 light microscope (Olympus, Tokyo, Japan). The parasite species were classified based on their morphological characteristics, and the overall parasitic composition within the population was described and compared with the NGS results.

Library construction and 18S rRNA gene amplicon sequencing

The 10 DNA samples extracted from the feces were pooled as a single sample and the pooled DNA was sent to Macrogen (Daejeon, Korea) for NGS running. For the metataxonomic studies, the V4 and V9 hypervariable regions of the 18S rRNA gene was amplified by PCR using the universal primer sets: 18S V4F (5'-CCAGCAGCCGCGG TAATTCC-3') and 18S V4R (5'-ACTTTCGTTCTTGATTAA-3') targeting the V4 variable region of 18S rRNA gene, and 1380F (5'-CCCTGCCHTTTGTACACAC-3') and 1510R (5'-CCTTCYGCAGGTTCACCTAC-3') targeting the V9 variable region of 18S rRNA gene. The PCR conditions for both regions were as follows: an initial denaturation at 95 °C for 3 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. DNA purification was performed using AMPure beads (Agencourt Bioscience, Beverly, MA, USA). The PCR products were amplified for final library construction containing the index using NexteraXT Indexed primer. Library quantification was then performed using qPCR according to the qPCR Quantification Protocol Guide (KAPA Library Quantification kits for Illumina Sequencing platforms) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). Paired-end sequencing was carried out on the Illumina Miseq™ platform (Illumina, San Diego, USA).

Bioinformatic analysis

Paired-end raw reads of V4 and V9 regions obtained from Illumina sequencing were processed using the DADA2 pipeline (v1.18.0) for metataxonomic analysis[27]. Adapter and primer sequences were removed, with forward and reverse reads truncated to 250 bp and 200 bp, respectively, using Cutadapt (v3.2)[28]. The reads were performed error correction, merging, denoising, and removal of chimeric sequences to generate amplicon sequence variants (ASVs)[27]. Chimeric sequences were identified and removed using the consensus method implemented in the remove BimeraDenovo function within DADA2. ASVs with lengths shorter than 350 bp were filtered out using R (v4.0.3). Taxonomic classification was conducted using QIIME (v1.9.0). The reference database employed was the 18S rRNA dataset from the National Center for Biotechnology Information (NCBI_NT_20231204). Each ASV was aligned to the closest matching organism in the reference database utilizing the BLAST + algorithm (v2.9.0), with alignment criteria set to query coverage > 85% and identity > 85%[29].

Conventional PCR and phylogenetic analysis

To corroborate the identification of parasites derived from the metabarcoding analysis and microscopic examination, a conventional PCR was conducted. To identify the genera *Histomonas, Isospora, Tetratrichomonas*, and *Trichomitus*, the 18S rRNA of each parasite was amplified using PCR with the AccuPower HotStart PCR Premix Kit (Bioneer, Daejeon, Korea) (Table 1)[30–33]. Furthermore, the ITS-1 region was amplified for the purpose

Targets	Primer	Sequences (5'–3')	PCR condition	References
Histomomas	18S-F	GCAGTTAAAACGCTCGTAGTC	95 °C/15 m; 40 cycles: 95 °C/30 s, 53 °C/30 s, 72 °C/60 s; 72 °C/10 m	[30]
	18S-R	AACGCTAGACAGGTCAACCC		
Fasciola	FascF	ACCGGTGCTGAGAAGACG	95 °C/5 m; 30 cycles: 95 °C/45 s, 60 °C/45 s, 72 °C/60 s; 72 °C/7 m	[34]
	FascR	CGACGTACGTGCAGTCCA		
Isospora	ExCycF	AATGTAAAACCCTTCCAGAGTAAC	94 °C/15 m; 35 cycles: 94 °C/45 s, 55 °C/45 s, 72 °C/60 s; 72 °C/10 m	[31]
	ExCycR	GCAATAATCTATCCCCATCACG		
	NesCycF	AATTCCAGCTCCAATAGTGTAT	94 °C/15 m; 35 cycles: 94 °C/45 s, 55 °C/45 s, 72 °C/60 s; 72 °C/10 m	
	NesCycR	CAGGAGAAGCCAAGGTAGGCRTTT		
Tetratrichomonas	Tgf	GCAATTGTTTCTCCAGAAGTG	95 °C/15 m; 40 cycles: 94 °C/30 s, 60 °C/30 s, 72 °C/60 s; 72 °C/10 m	[32]
	Tgr	GATGGCTCTCTTTGAGCTTG		
Trichomitus	T18SF	GGAAGCACACTTCGGTCATAG	94 °C/5 m; 30 cycles: 94 °C/60 s, 56 °C/60 s, 72 °C/120 s; 72 °C/7 m	[33]
	T18SRi	CCTTCCGTCAATTCCTTCAA		
	T18SFi	AGGGTTTCTGTCGATCAAGG	94 °C/5 m; 30 cycles: 94 °C/60 s, 56 °C/60 s, 72 °C/120 s; 72 °C/7 m	
	T18SR	CGTTACCTTGTTACGACTTCTCC		
Nematode common	N18SF	CGCGAAYRGCTCATTAYAACAGC	95 °C/5 m; 35 cycles: 95 °C/30 s, 54 °C/30 s; 72 °C/60 s; 72 °C/5 m	[75]
	N18SR	GGGCGGTATCTGATCGCC		
V4 region of 18S rRNA	18S V4F	CCAGCAGCCGCGGTAATTCC	95 °C/3 m; 25 cycles: 95 °C/30 s, 55 °C/30 s, 72 °C/30 s; 72 °C/5 m	[76]
	18S V4R	ACTTTCGTTCTTGATTAA		
V9 region of 18S rRNA	1380F	CCCTGCCHTTTGTACACAC	95 °C/3 m; 25 cycles: 95 °C/30 s, 55 °C/30 s, 72 °C/30 s; 72 °C/5 m	[77]
	1510R	CCTTCYGCAGGTTCACCTAC		

Table 1. Primers used to identify eukaryotes and parasites in great cormorants' feces.

of identifying the genus *Fasciola*[34]. To identify the genera *Baruscapillaria*, *Contracaecum*, and *Eustrongylides*, a nematode-specific primer set targeting the 18S rRNA gene was employed. All positive PCR products were submitted to Macrogen for direct sequencing.

The resulting sequences were aligned using MEGA 11 and compared with sequences deposited in the NCBI GenBank database. Additionally, phylogenetic trees were constructed using the maximum likelihood method with 500 bootstrap replications in MEGA 11 to analyze the molecular characteristics of the obtained sequences.

Results

Microscopic examination

On the basis of the microscopic examination using the direct smear method and the fecal flotation method, a total of three helminths and trophozoites of protozoa were identified (Fig. 1A–D). The direct smear method identified eggs of capillarid and *Contracaecum* sp. as well as a large number of trophozoites of flagellated protozoa with different sizes and morphological characteristics. The flotation method did not detect any protozoa, but revealed the presence of eggs of capillarid, *Contracaecum* sp., and *Eustrongylides* sp. However, while the morphological characteristics of the eggs and trophozoites allowed classification of the parasites by type, identification to species level was not possible.

18S rRNA gene sequencing data

A total of 227,100 and 254,640 reads were generated in the V4 and V9 regions, respectively, through the NGS. Following filtering, 103,104 read pairs for the V4 region and 123,773 read pairs for the V9 region were retained. The mean length of the V4 sequence fragments was 388 base pairs (ranging from 375 to 401 bp), while the mean length of the V9 sequence fragments was 130 base pairs (ranging from 110 to 147 bp).

Taxonomic compositions of the V4 and V9 regions

A bioinformatics analysis identified 20 and 18 distinct amplicon sequence variants (ASVs) in the V4 and V9 regions, respectively. These ASVs were assigned to 12 and 16 species, respectively. The assigned species included eukaryotes belonging to diverse taxonomic groups, including parasites, birds, and fishes. Furthermore, the V4 region analysis revealed the presence of fungal and algal species, whereas the V9 region analysis identified a water mold.

At the phylum level, the V4 region identified Chordata, Nematoda, and Apicomplexa, with relative abundances of 98.394%, 1.538%, and 0.006%, respectively. Additionally, an unclassified phylum was identified with a relative abundance of 0.062%. In the V9 region analysis, the following phyla were identified: Parabasalia, Chordata, Nematoda, Oomycota, and Platyhelminthes, with relative abundances of 82.642%, 9.890%, 0.152%, 0.002%, and 0.002%, respectively. The unclassified phylum constituted 7.312% of the total.

At the species level, the V4 region analysis identified *Baruscapillaria spiculata*, *Contracaecum* sp., and *Isospora lugensae* as parasites, *Melopsittacus undulatus* as a bird, *Hypomesus transpacificus*, *Gobio gobio*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*, and *Tachysurus fulvidraco* as fishes, and *Cryptomonas rostratiformis* as algae. The V9 region analysis revealed the presence of several parasites, including *Tetratrichomonas* sp., *Histomonas meleagridis*, *Trichomitus* sp., *Tetratrichomonas prowazekii*, *Baruscapillaria obsignata*, *Monosiga ovata*, and

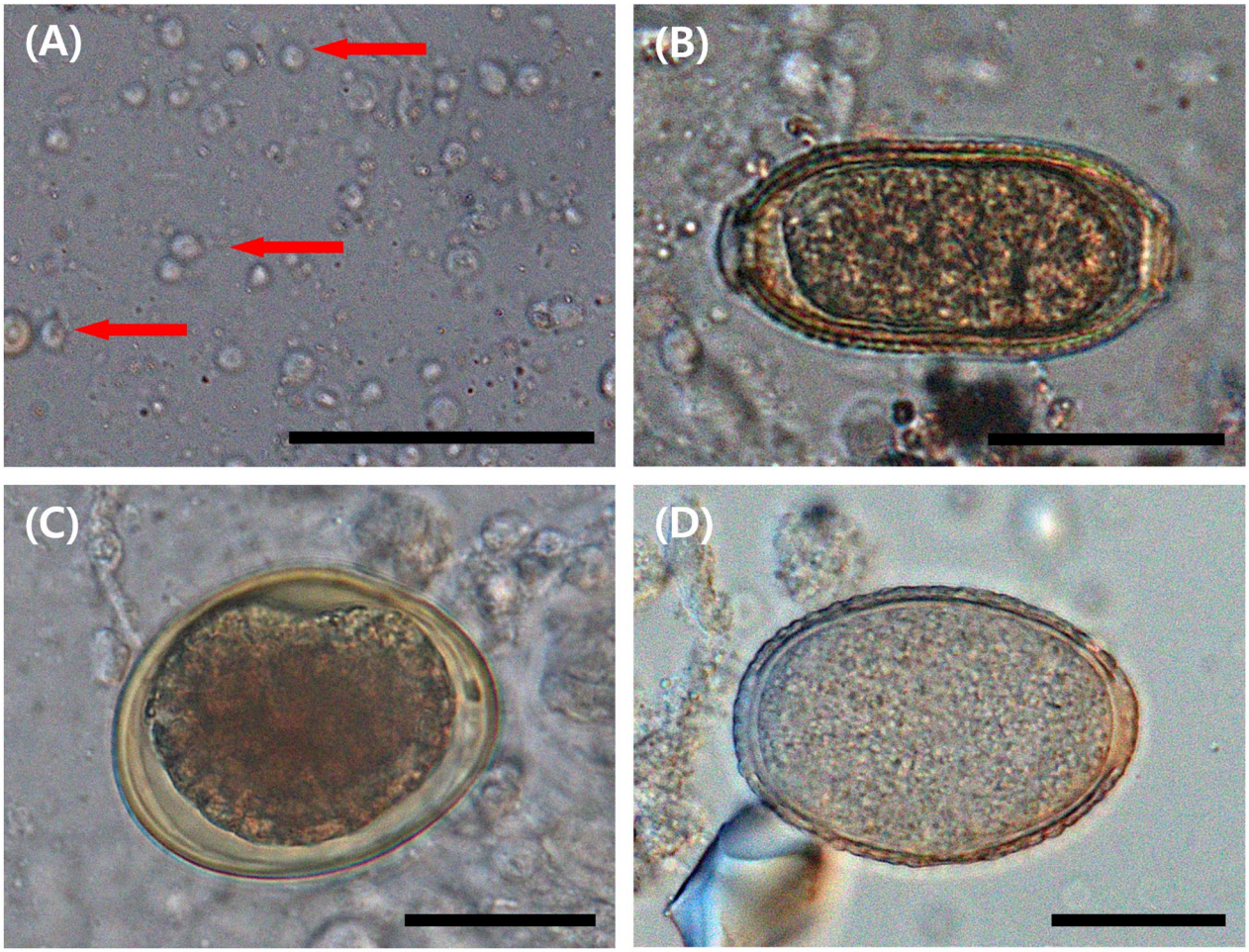

Fig. 1. Parasites detected in the feces of great cormorants (*Phalacrocorax carbo*) captured in Yanggu, Gangwon Province, Republic of Korea. (**A**) Trophozoites of flagellate protozoans (red arrows), (**B**) Egg of a capillarid parasite, (**C**) Egg of *Contracaecum* sp., and (**D**) Egg of *Eustrongylides* sp. Scale bar = 25 μm.

Fasciola gigantica. Additionally, the following organisms were identified: *Calypte anna* (bird), *Osmerus mordax*, *Carassius auratus*, *Ctenopharyngodon idella*, *Hemibarbus labeo*, and *Culter alburnus* (fish), and *Saprolegnia parasitica* (water mold).

In the V4 region analysis, *Melopsittacus undulatus* (98.155%), a bird, exhibited the highest proportion of abundance, followed by *Baruscapillaria spiculata* (1.402%), which is a parasite. In the V9 region analysis, *Tetratrichomonas* sp. exhibited the highest abundance (42.405%), followed by *Histomonas meleagridis* (14.829%) (Table S1). The relative abundance of each species is presented in Fig. 2A,B.

Upon comparison of the identified ASVs with sequences from the NCBI_NT database, it was determined that the percentage of identity for some parasite sequences was insufficient to ascertain the exact species. For instance, in the V4 region, the ASV assigned to *I. lugensae* exhibited a sequence with only 95.607% identity. In the V9 region, ASVs assigned to *Tetratrichomonas* sp., *Histomonas meleagridis*, and *Baruscapillaria obsignata* exhibited 85.586–90.826%, 85.965%, and 94.000%, respectively (Table S1).

Identification of parasites by conventional PCR

To confirm the presence of parasites identified in metabarcoding results, conventional PCR was performed. The results indicated the presence of *Contracaecum* sp., *Isospora* sp., and unspecified trichomonads, with the highest prevalence observed in unspecified trichomonads in great cormorants (Table 2). However, attempts to identify *Baruscapillaria* spp., *Histomonas meleagridis*, and *Fasciola gigantica* by conventional PCR were not successful.

Molecular characteristics and phylogenetic analysis

The 18S rRNA gene fragment of *Contracaecum* sp. obtained by conventional PCR was compared with the sequences in the GenBank database using the BLAST algorithm. The results revealed that *Contracaecum* in this study exhibited 100% identity with *C. eudyptulae* (EF180072) from *Eudyptula minor* in Australia, as well as with *C. rudolphii* (OP467579) from *Diomedea sanfordi* in New Zealand, and 99.89% identity with *C.*

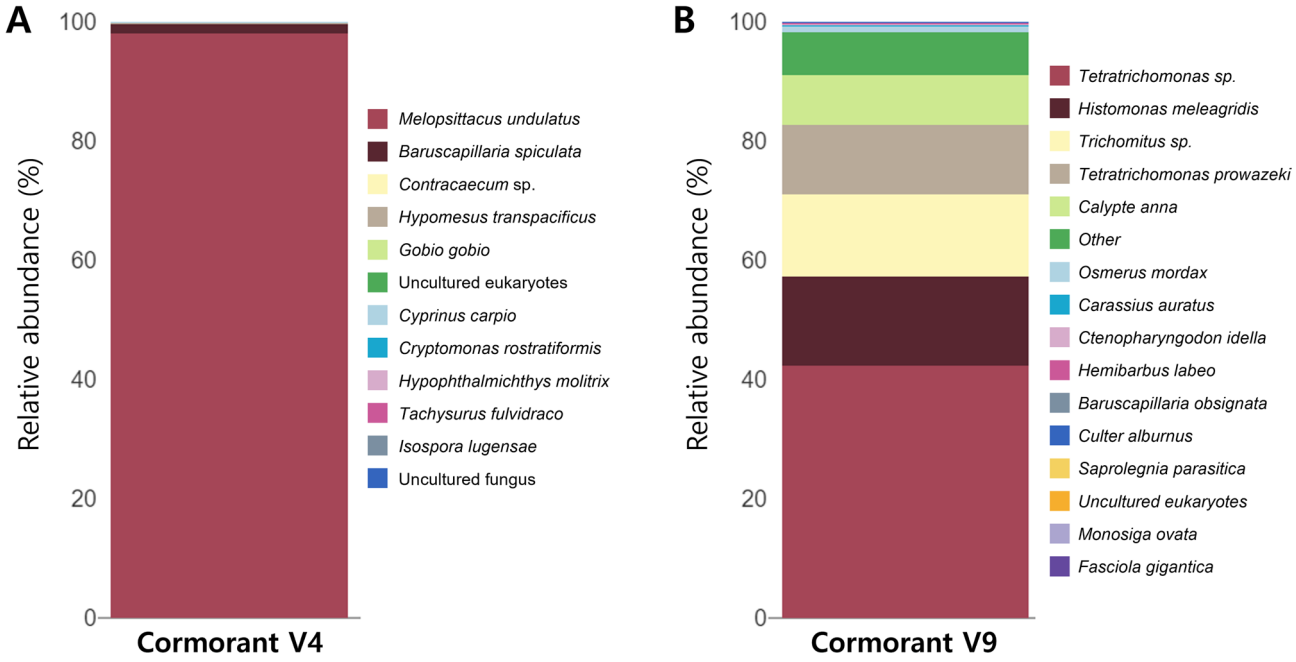

Fig. 2. Taxonomic composition of eukaryotes in great cormorants' feces by (**A**) V4 and (**B**) V9 regions of the 18S rRNA gene. Detailed species and relative abundances are described in Table S1.

Sample ID	Parasites		
	Contracaecum	*Isospora*	Trichomonads*
Cormorant-1	–	–	+
Cormorant-2	–	–	+
Cormorant-3	–	–	+
Cormorant-4	–	+	+
Cormorant-5	–	–	+
Cormorant-6	–	+	+
Cormorant-7	–	–	+
Cormorant-8	–	–	–
Cormorant-9	+	–	+
Cormorant-10	–	–	+
Total (n)	1	2	9

Table 2. Conventional PCR results for parasites in great cormorants. *If one of the PCRs for *Tetratrichomonas*, *Trichomitus*, and *Histomomas* was positive, the results are reported as positive. The PCR results were confirmed by sequencing.

australe (PP739191) from *Phalacrocorax brasilianus* in Brazil. A phylogenetic analysis also demonstrated a close relationship between *Contracaecum* sp. in this study and previously reported *Contracaecum* spp. (Fig. 3).

With regard to *Isospora*, two samples were identified as positive by conventional PCR. The BLAST results demonstrated that the two sequences exhibited the highest degree of identity with *I. lugensae* (MW287753) from the *Kerguelen petrel* in Australia, with a percentage identity range of 95.82–95.92%. The phylogenetic analysis revealed that the sequences in this study exhibited the closest relationship with *I. lugensae*, yet they were clearly diverged (Fig. 4). In light of the identity and phylogenetic relationships, the *Isospora* identified in this study were designated as *Isospora* sp.

Of the 10 great cormorant samples, a total of nine samples tested positive for trichomonads by conventional PCR (Table 2). The utilization of different primer sets in conventional PCR yielded varying results, with distinct trichomonads being identified upon analysis of the PCR products, as opposed to the intended target pathogen for which the primers were originally designed. For example, primer sets for *Tetratrichomonas, Trichomitus*, and *Histomonas* yielded DNA fragments of unspecified trichomonads (Fig. 5 and S1, Table S2). Furthermore, the majority of the trichomonads identified in this study exhibited low identity with any of *Tetratrichomonas, Trichomitus*, and *Histomonas* in the GenBank database. However, the phylogenetic analysis demonstrated that

Fig. 3. Phylogenetic analysis of 18S rRNA of *Contracaecum*. To construct the phylogenetic tree, the maximum-likelihood method was used with 500 bootstrap replications. The sequences obtained in this study are indicated with bold and arrows.

the identified trichomonads belonged to the family Trichomonadidae. Consequently, the authors designated the sequences as unspecified trichomonads.

Discussion

This study employed metabarcoding to analyze the V4 and V9 regions of the 18S rRNA gene, with the objective of characterizing the intestinal parasites of great cormorants and elucidating their role as a host for various parasites. This advanced approach has been demonstrated to be highly sensitive in identifying a wide range of parasites in comparison to conventional methods such as PCR and microscopy[24]. While the metabarcoding analysis yielded the most diverse array of parasite species in this study, some of the results did not align with those obtained through conventional PCR or microscopic examination. Nevertheless, the findings indicated the presence of comparable parasites at the family or genus level.

The genus *Contracaecum* is a nematode belonging to the family Anisakidae, which is distributed globally, including Japan, Australia, China, and New Zealand[35–39]. *Contracaecum* has been documented in both freshwater and marine ecosystems, utilizing fish as intermediate and/or paratenic hosts, while aquatic mammals and piscivorous birds, including great cormorants, serve as definitive hosts[40]. Furthermore, in humans, ingestion of the L3 stage larvae of *Contracaecum* sp. with fish can result in penetration of the gastric and intestinal walls,

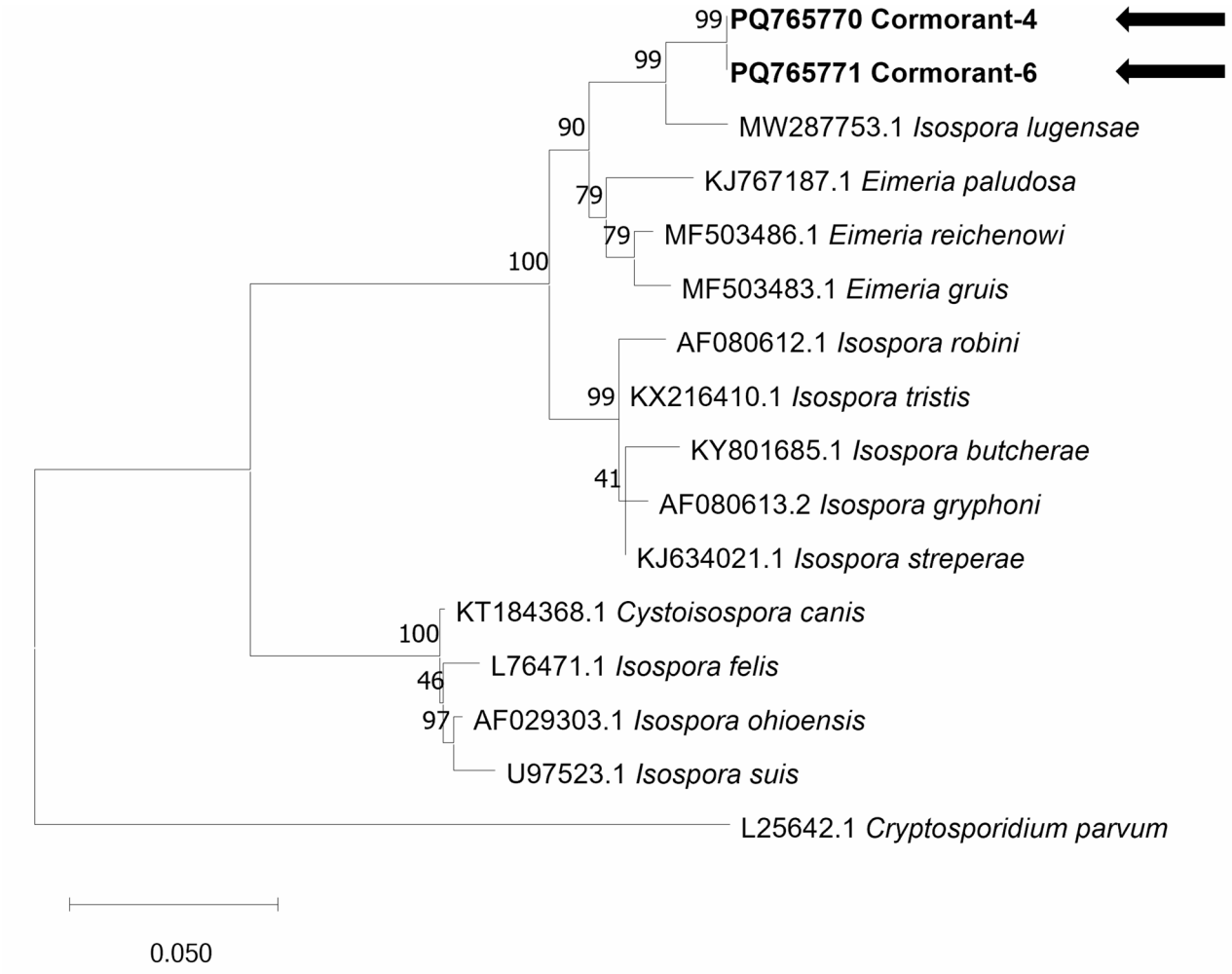

Fig. 4. Phylogenetic analysis of 18S ribosomal RNA of *Isospora*. To construct the phylogenetic tree, the maximum-likelihood method was used with 500 bootstrap replications. The sequences obtained in this study are indicated with bold and arrows.

leading to abdominal pain, fever, diarrhea, and vomiting[41]. Among the species within the genus *Contracaecum*, *C. rudolphii* sensu lato is a cosmopolitan nematode that is commonly found in great cormorants and is occasionally associated with severe gastric lesions[2]. Recent molecular analyses have identified six sibling species within the *C. rudolphii* complex (*C. rudolphii* A-F)[39,42–45]. The BLAST result and phylogenetic analysis demonstrated that the sequence obtained in the present study is closely related to the *C. rudolphii* s.l. that has been deposited in GenBank (OP467579). However, the precise species could not be identified due to the 18S region being highly conserved among all ascarid nematodes, including *Contracaecum* spp[46]. To address this issue, additional molecular markers (ITS-1, ITS-2, and *cox1*) were amplified as previously described[47–49]. However, all results were negative for the genus (data not shown). Nevertheless, this finding lends support to the hypothesis that great cormorants may acquire *Contracaecum* through the consumption of fish, consistent with the findings of previous studies on the feeding habits of great cormorants[1]. This novel identification contributes to our understanding of host-parasite interactions within aquatic ecosystems and the role of piscivorous birds in the transmission of parasites.

Isospora is a genus of apicomplexan protozoa in the family Eimeriidae, known for causing diarrhea in various vertebrates[50]. In contrast with the results obtained by metabarcoding, the conventional PCR results revealed that the *Isospora* sp. identified in this study shared only 95.82–95.92% identity with *I. lugensae* from the *Kerguelen petrel* in Australia (MW287753)[51]. The study of *Isospora* in birds is a relatively underexplored field, and the available reference databases are limited. As a result, the authors were unable to definitively identify the species. These findings indicate that metabarcoding is a sensitive and effective method for detecting novel eukaryotes, including parasites.

Members of the protozoan family Trichomonadidae, which includes genera such as *Tritrichomonas, Trichomonas, Tetratrichomonas, Trichomitus*, and *Pentatrichomonas*, are called trichomonads[52]. Trichomonads are characterized by the presence of three to five flagella and mainly are found in the cecum and colon of a wide range of vertebrates and invertebrates, including mammals and birds[53,54]. Among trichomonads, *Tritrichomonas foetus*, which causes abortion in cattle, and *Trichomonas vaginalis*, a sexually transmitted disease in humans,

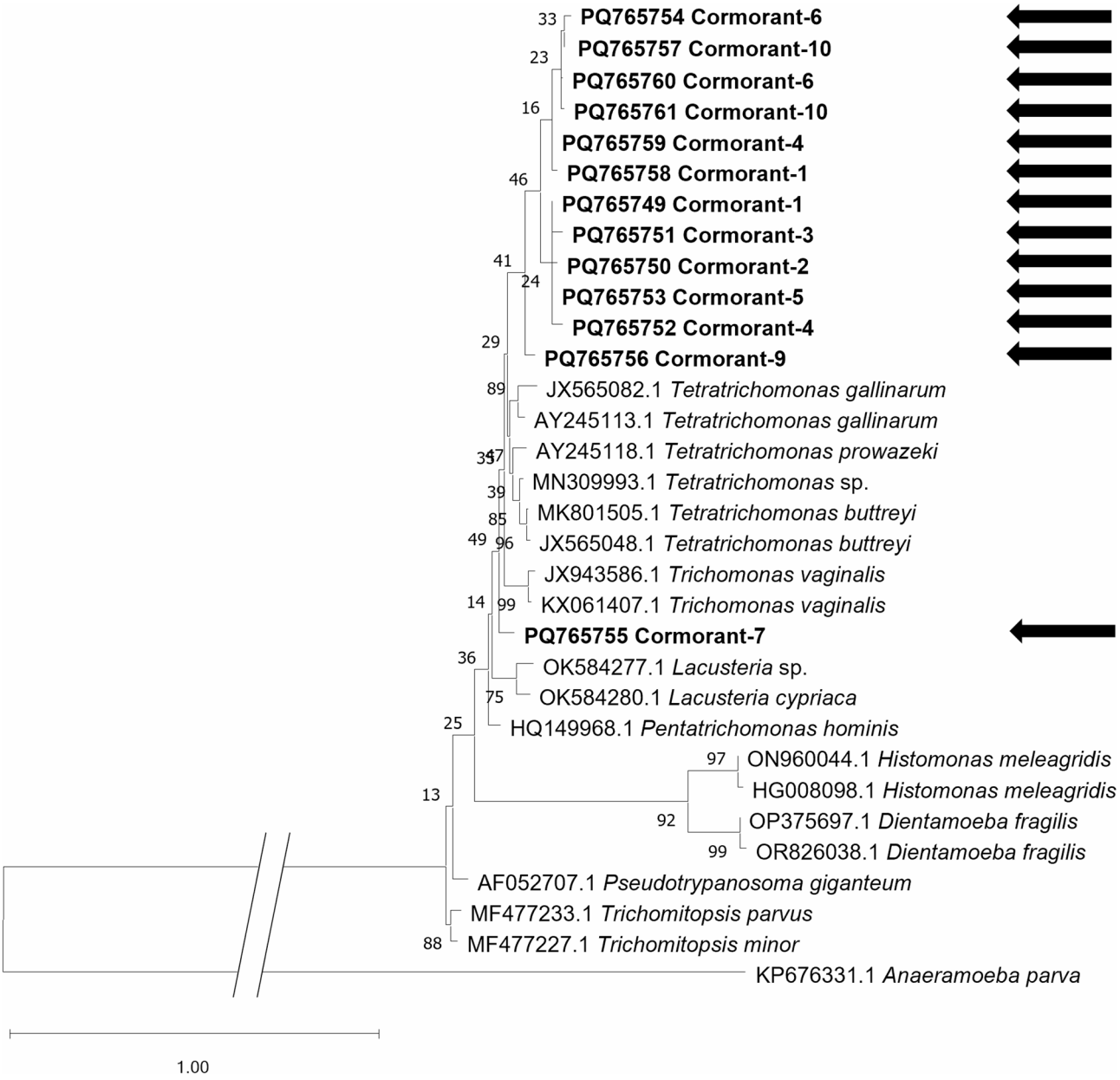

Fig. 5. Phylogenetic analysis of 18S ribosomal RNA of trichomonads. To construct the phylogenetic tree, the maximum-likelihood method was used with 500 bootstrap replications. The sequences obtained in this study are indicated with bold and arrows.

are well-studied. However, the pathogenicity of many other trichomonads remains poorly understood[55,56]. The family Dientamoebidae, genus *Histomonas*, is closely related to the family Trichomonadidae. The latter is known to cause blackhead disease in birds, with *H. meleagridis* representing a particularly well-studied example[57].

In this study, the results of the metabarcoding identified the presence of *Tetratrichomonas, Trichomitus*, and *Histomonas*, albeit with a low identity of 85.586–95.495%. To validate the results, conventional PCR targeting each genus was performed; however, all were identified as unspecified trichomonads. The primer sets employed targeted different genera, yet due to genetic relatedness, the authors hypothesize that all primer sets amplified unspecified trichomonads. Furthermore, BLAST results demonstrated a low identity of 85.71–98.24% with sequences affiliated with the genera *Tetratrichomonas, Trichomitus*, and *Histomonas* within the GenBank database. Phylogenetic analysis indicated that the unspecified trichomonads belong to the family Trichomonadidae, yet are distinct from the known genus. This represents one of the most significant advantages of metataxonomic analysis, namely its capacity to reveal previously uncharacterized biodiversity.

The genus *Baruscapillaria* is a parasitic nematode belonging to the family Capillariidae. This nematode has been documented to infect the intestines and stomachs of birds and mammals[58]. The genus *Baruscapillaria* is distinguished by a well-developed membranous bursa with small lobes exhibiting minute projections and

a long, sclerotized spicule with a smooth spicular sheath[58]. *B. obsignata* has been documented in a variety of avian species, including chickens in Japan, turkeys in India and Brazil, and rock partridges in Korea[59–63]. Furthermore, a previous study indicated that this parasite is associated with high mortality rates and severe enteritis in turkeys[59]. In this study, *Baruscapillaria obsignata* and *B. spiculata* were identified through 18S rRNA gene metabarcoding. It is noteworthy that this study represents the first detection of both *B. obsignata* and *B. spiculata* in great cormorants using metabarcoding. Furthermore, although the eggs of *Baruscapillaria* were identified through light microscopy, they were not corroborated by conventional PCR. For more accurate species identification, adult worms need to be collected and morphologically analyzed, and their role as natural hosts of great cormorants needs to be further investigated.

The genus *Eustrongylides* (family Dioctophymatidae) is a nematode that infects various fish species and piscivorous birds in freshwater ecosystems, causing a disease that is commonly referred to as big red worm disease[64]. Mortality due to infection has been documented in both fish and bird hosts, and *Eustrongylides* is also of zoonotic importance. A previous study reported the mortality of an Australian darter (*Anhinga novaehollandiae*) associated with severe diffuse verminous coelomitis resulting from *Eustrongylides* infection[65]. In humans, the consumption of raw or undercooked fish harboring the parasite's larval stages can lead to gastritis, enteritis, intestinal perforation, and cutaneous lesions[66–68]. Currently, three species of *Eustrongylides* are recognized: *E. tubifex, E. ignotus*, and *E. excisus*[64,66]. These parasites are distributed globally, with reports from Korea, Australia, Japan, India, and China[69–71]. In the present study, the presence of *Eustrongylides* sp. eggs was identified using microscopic analysis. Conventionally, species identification of *Eustrongylides* has been primarily based on the morphology of eggs and adult parasites[64,66,72], though the utility of this approach in distinguishing among different species based solely on egg morphology was not optimal in the present study.

It was unexpected that one ASV was assigned to *F. gigantica*, which is typically found in ruminants in tropical regions[73]. The ASV was observed only in the V9 region, with a relative abundance of 0.002% (Table S1). Additionally, the evidence for *F. gigantica* was not supported by either light microscopy or conventional PCR. This result is probably due to two reasons. First, a parasite from the family Echinostomatidae, which belongs to the same superfamily Echinostomatoidea as *F. gigantica*, was detected but may have been misassigned due to lack of reference databases. In fact, parasites such as the genus *Pestasiger*, which belongs to the family Echinostomatidae, have been found in great cormorants[74]. Second, the observed *F. gigantica* DNA fragments in the cormorant may have been incidental, indicating transient materials rather than evidence of an actual infection. However, it is difficult to draw definitive conclusions from the current results.

The 18S rRNA gene metabarcoding approach proved effective in monitoring the dietary habits of great cormorants. The results differed according to the target region, as evidenced by the analysis of parasites. Metabarcoding revealed the presence of distinct ASVs, which are suspected to be the genes of fish ingested by great cormorants. The identification of fish in fecal samples provides valuable insights into the feeding behavior and ecological interactions of these animals within their natural habitats. This non-invasive method offers insights into their role in aquatic ecosystems and facilitates the development of strategies to mitigate their impact on fish populations. This approach facilitates a deeper comprehension of the dietary habits of great cormorants and furnishes crucial data for the management of their impact on local ecosystems and fisheries.

In this study, an ASV assigned to birds was identified in each of the V4 and V9 regions. Each ASV was subsequently assigned to *Melopsittacus undulatus* and *Calypte anna*, respectively, rather than to great cormorants. Nevertheless, subsequent investigation via BLAST analysis demonstrated that both ASVs exhibited 99.74–100% identity with the sequence of great cormorants (XR_010370890) present within the GenBank database. This outcome is likely attributable to the absence of a reference database for the 18S rRNA gene utilized for taxonomic assignment and the limited sequence length obtained through the V4 and V9 region metabarcoding, which may impede the resolution and accuracy of taxonomic identification. The unexpected detection of avian sequences in each region underscores the necessity for more comprehensive reference databases and longer sequence reads to enhance the reliability of eukaryotic diversity studies using metabarcoding.

This study employed conventional PCR to validate the metabarcoding results; however, certain limitations remained. Even after PCR, sequencing, BLAST, and phylogenetic analyses, the exact species of identified parasites were not determined due to the high degree of genetic or absence of reference sequences in GenBank database. Further studies on different molecular markers, including 18S rRNA, ITS-1, ITS-2, *cox1*, and others are needed with longer and more comprehensive sequence data. These efforts will enhance the taxonomic resolution and diagnostic accuracy of parasitic organisms.

The present study investigated gastrointestinal eukaryotes, with a focus on parasites, in great cormorants. While this study identified some pathogenic parasites, including *Baruscapillaria*, the authors are unable to draw conclusions about the pathogenicity of other parasites, including *Isospora* sp. and unspecified trichomonads, identified in this study due to the lack of research on parasites in wild birds, including great cormorants. Further studies are necessary to determine the pathogenicity, natural hosts, and transmission routes of individual pathogens.

Conclusion

In conclusion, the use of metabarcoding of the 18S rRNA gene represents an effective approach for the investigation of gastrointestinal parasites in great cormorants. This study identified a diverse range of taxa, including *Contracaecum*, *Isospora*, *Baruscapillaria*, *Eustronglyides*, unspecified trichomonads, and other eukaryotes. However, the limited availability of comprehensive reference databases indicates the necessity for further research to enhance the accuracy and reliability of metabarcoding-based assessments of eukaryotic diversity.

Data availability

The raw sequence data were deposited in the NCBI GenBank database (Unspecified trichomoands PQ765749-PQ765768; *Contrcaecum* sp., PQ765769; *Isospora* sp., PQ765770-PQ765771) or Sequence Read Archive (SRA) under BioProject PRJNA1198058.

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References

1. Laviad-Shitrit, S. et al. Great cormorants (*Phalacrocorax carbo*) as potential vectors for the dispersal of *Vibrio cholerae*. *Sci. Rep.* **7**, 7973 (2017).
2. Rokicki, J., Sołtysiak, Z., Dziekońska-Rynko, J. & Borucińska, J. Pathology associated with *Contracaecum rudolphii* (Nematoda: Anisakidae) infection in the great cormorant *Phalacrocorax carbo* (L. 1758). *Helminthologia* **48**, 29–35 (2011).
3. Moravec, F. & Scholz, T. Helminth parasites of the lesser great cormorant *Phalacrocorax carbo sinensis* from two nesting regions in the Czech Republic. *Folia Parasitol.* **63**, 1 (2016).
4. Víchová, B. et al. Molecular screening for bacteria and protozoa in great cormorants (*Phalacrocorax carbo sinensis*) nesting in Slovakia, central Europe. *Acta Parasit.* **61**, 585–589 (2016).
5. Klimaszyk, P. & Rzymski, P. Complexity of ecological impacts induced by great cormorants. *Hydrobiologia* **771**, 13–30 (2016).
6. Klimaszyk, P. & Rzymski, P. Impact of cormorant (*Phalacrocorax carbo sinensis* L.) colonies on microbial pollution in lakes. *Limnol. Rev.* **13**, 139–145 (2013).
7. Klimaszyk, P. May a cormorant colony be a source of coliform and chemical pollution in a lake?. *Ocean Hydrol.* **41**, 67–73 (2012).
8. Albini, S. et al. Shedding of zoonotic pathogens and analysis of stomach contents in great cormorants (*Phalacrocorax carbo sinensis*) from Switzerland between 2007 and 2012. *Schweiz Arch. Tierheikd.* **156**, 389–394 (2014).
9. Rzymski, P., Słodkowicz-Kowalska, A., Klimaszyk, P., Solarczyk, P. & Poniedziałek, B. Screening of protozoan and microsporidian parasites in feces of great cormorant (*Phalacrocorax carbo*). *Environ. Sci. Pollut. Res. Int.* **24**, 9813–9819 (2017).
10. Cammilleri, G. et al. Identification of *Contracaecum rudolphii* (Nematoda: Anisakidae) in great cormorants *Phalacrocorax carbo sinensis* (Blumenbach, 1978) from southern Italy. *Vet. Sci.* **10**, 194 (2023).
11. Medema, G. J. *Cryptosporidium* and *Giardia*; new challenges to the water industry. *Thesis University of Utrecht.*, 85–98 (1999).
12. Plutzer, J. & Tomor, B. The role of aquatic birds in the environmental dissemination of human pathogenic *Giardia duodenalis* cysts and *Cryptosporidium* oocysts in Hungary. *Parasitol. Int.* **58**, 227–231 (2009).
13. Iwaki, T., Waki, T., Arakawa, J. & Ogawa, K. The digenean *Clinostomum complanatum* found from great cormorant *Phalacrocorax carbo* in Japan. *Fish Pathol.* **53**, 132–135 (2018).
14. Rusconi, A. et al. Occurrence of *Eustrongylides excisus* (Nematoda:Dioctophymatidae) in european perch (*Perca fluviatilis*) and great cormorant (*Phalacrocorax carbo*) in Lake Annone, northern Italy. *J. Parasitol.* **108**, 209–216 (2022).
15. Prakas, P., Butkauskas, D., Švažas, S. & Stanevičius, V. Morphological and genetic characterisation of *Sarcocystis halieti* from the great cormorant (*Phalacrocorax carbo*). *Parasitol. Res.* **117**, 3663–3667 (2018).
16. Butkauskas, D. et al. Genetic diversity of great cormorants *Phalacrocorax carbo* in the eastern Baltic region. *Biologija* **62** (2016).
17. Alkathiri, B. et al. 16S rRNA metabarcoding for the identification of tick-borne bacteria in ticks in the Republic of Korea. *Sci. Rep.* **14**, 19708–19711 (2024).
18. Trzebny, A., Liberska, J., Slodkowicz-Kowalska, A. & Dabert, M. Metabarcoding reveals low prevalence of microsporidian infections in castor bean tick (*Ixodes ricinus*). *Parasite Vectors* **15**, 26 (2022).
19. Choi, J. H. et al. Metabarcoding of pathogenic parasites based on copro-DNA analysis of wild animals in South Korea. *Heliyon* **10**, e30059 (2024).
20. Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C. & Willerslev, E. Towards next-generation biodiversity assessment using DNA metabarcoding. *Mol. Ecol.* **21**, 2045–2050 (2012).
21. Pawluczyk, M. et al. Quantitative evaluation of bias in PCR amplification and next-generation sequencing derived from metabarcoding samples. *Anal. Bioanal. Chem.* **407**, 1841–1848 (2015).
22. Rodino, K. G. & Pritt, B. S. Novel applications of metagenomics for detection of tickborne pathogens. *Clin. Chem.* **68**, 69–74 (2022).
23. Lewis, E., Hudson, J. A., Cook, N., Barnes, J. D. & Haynes, E. Next-generation sequencing as a screening tool for foodborne pathogens in fresh produce. *J. Microbiol. Methods* **171**, 105840 (2020).
24. Kim, S. L. et al. Metabarcoding of bacteria and parasites in the gut of *Apodemus agrarius*. *Parasite Vectors* **15**, 486 (2022).
25. Choi, J. & Park J. S. Comparative analyses of the V4 and V9 regions of 18S rDNA for the extant eukaryotic community using the Illumina platform. *Sci. Rep.* **10** (2020).
26. Kounosu, A., Murase, K., Yoshida, A., Maruyama, H. & Kikuchi, T. Improved 18S and 28S rDNA primer sets for NGS-based parasite detection. *Sci. Rep.* **9**, 15789–15812 (2019).
27. Callahan, B. J. et al. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581–583 (2016).
28. Martin. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* **17**, 10 (2011).
29. Camacho, C. et al. BLAST: Architecture and applications. *BMC Bioinf.* **10**, 1–9 (2009).
30. Bilic, I., Jaskulska, B., Souillard, R., Liebhart, D. & Hess, M. Multi-locus typing of *Histomonas meleagridis* isolates demonstrates the existence of two different genotypes. *PLoS ONE* **9**, e92438 (2014).
31. Lee, S. et al. Identification of *Cystoisospora ohioensis* in a Diarrheal Dog in Korea. *Korean J. Parasitol.* **56**, 371 (2018).
32. Grabensteiner, E. & Hess, M. PCR for the identification and differentiation of *Histomonas meleagridis*, *Tetratrichomonas gallinarum* and *Blastocystis* spp. *Vet. Parasitol.* **142**, 223–230 (2006).
33. Dimasuay, K. G. B. & Rivera, W. L. Molecular characterization of trichomonads isolated from animal hosts in the Philippines. *Vet. Parasitol.* **196**, 289–295 (2013).
34. Rokni, M. B. et al. Identification and differentiation of *Fasciola hepatica* and *Fasciola gigantica* using a simple PCR-restriction enzyme method. *Exp. Parasitol.* **124**, 209–213 (2010).
35. Mattiucci, S. et al. Allozyme and morphological identification of shape *Anisakis*, *Contracaecum* and *Pseudoterranova* from Japanese waters (Nematoda, Ascaridoidea). *Syst. Parasitol.* **40**, 81–92 (1998).
36. Nadler, S. A. et al. Molecular phylogeny of clade III nematodes reveals multiple origins of tissue parasitism. *Parasitology* **134**, 1421–1442 (2007).
37. Bennett, J., Poulin, R. & Presswell, B. Large-scale genetic investigation of nematode diversity and their phylogenetic patterns in New Zealand's marine animals. *Parasitology* **149**, 1794–1809 (2022).
38. Garbin, L. E. et al. Redescription and molecular characterization of *Baruscapillaria spiculata* (Nematoda: Capillariidae) parasitizing the neotropic cormorant *Phalacrocorax brasilianus* from two Argentinian lagoons. *Parasitol. Res.* **120**, 1637–1648 (2021).
39. Shamsi, S., Norman, R., Gasser, R. & Beveridge, I. Genetic and morphological evidences for the existence of sibling species within *Contracaecum rudolphii* (Hartwich, 1964) (Nematoda: Anisakidae) in Australia. *Parasitol. Res.* **105**, 529–538 (2009).
40. Mattiucci, S. & Nascetti, G. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host—Parasite co-evolutionary processes. *Adv. Parasitol.* **66**, 47–148 (2008).

41. Shamsi, S. & Barton, D. P. A critical review of anisakidosis cases occurring globally. *Parasitol. Res.* **122**, 1733–1745 (2023).
42. Szostakowska, B. & Fagerholm, H. Coexistence and genetic variability of *Contracaecum rudolphii* A and *Contracaecum rudolphii* B (Nematoda: Anisakidae) in cormorants, *Phalacrocorax carbo sinensis*, in the Baltic region. *J. Parasitol.* **98**, 472–478 (2012).
43. Roca-Geronès, X. et al. Genetic diversity of *Contracaecum rudolphii* sp. A (Nematoda: Anisakidae) parasitizing the European Shag Phalacrocorax aristotelis desmarestii from the Spanish Mediterranean coast. *Front. Vet. Sci.* **10**, 1122291 (2023).
44. D'Amelio, S., Cavallero, S., Dronen, N. O., Barros, N. B. & Paggi, L. Two new species of *Contracaecum* Railliet & Henry, 1912 (Nematoda: Anisakidae), *C. fagerholmi* n. sp. and *C. rudolphii* F from the brown pelican *Pelecanus occidentalis* in the northern Gulf of Mexico. *Syst Parasitol.* **81**, 1–16 (2012).
45. D'Amelio, S. et al. Genetic characterization of members of the genus *Contracaecum* (Nematoda: Anisakidae) from fish-eating birds from west-central Florida, USA, with evidence of new species. *Parasitology* **134**, 1041–1051 (2007).
46. Zhu, X., Gasser, R. B., Jacobs, D. E., Hung, G. C. & Chilton, N. B. Relationships among some ascaridoid nematodes based on ribosomal DNA sequence data. *Parasitol. Res.* **86**, 738–744 (2000).
47. Shamsi, S., Gasser, R., Beveridge, I. & Alizadeh Shabani, A. *Contracaecum pyripapillatum* n. sp. (Nematoda: Anisakidae) and a description of *C. multipapillatum* (von Drasche, 1882) from the Australian pelican, *Pelecanus conspicillatus*. *Parasitol Res.* **103**, 1031–1039 (2008).
48. Amor, N. et al. New insights into the coexistence of *Contracaecum rudolphii* A and *Contracaecum rudolphii* B (Nematoda: Anisakidae) in *Phalacrocorax carbo sinensis* from Sardinia: genetic variability and phylogenetic analysis. *Parasitology* **147**, 1538–1551 (2020).
49. Garbin, L., Mattiucci, S., Paoletti, M., González-Acuña, D. & Nascetti, G. Genetic and morphological evidences for the existence of a new species of *Contracaecum* (Nematoda: Anisakidae) parasite of *Phalacrocorax brasilianus* (Gmelin) from Chile and its genetic relationships with congeners from fish-eating birds. *J. Parasitol.* **97**, 476–492 (2011).
50. Curry, A. & Smith, H. V. Emerging pathogens: *Isospora*, *Cyclospora* and microsporidia. *Parasitology* **117**, 143–159 (1999).
51. Yang, R. et al. Morphological and genetic characterization of the first *Isospora* species (*I. lugensae* n. sp.) from a kerguelen petrel (*Lugensa brevirostris*). *Parasitol. Res.* **120**, 1037–1047 (2021).
52. Kamaruddin, M. et al. Molecular characterization of various trichomonad species isolated from humans and related mammals in Indonesia. *Korean J. Parasitol.* **52**, 471–478 (2014).
53. Amin, A., Bilic, I., Liebhart, D. & Hess, M. Trichomonads in birds—A review. *Parasitology* **141**, 733–747 (2014).
54. Stabler, R. M. *Trichomonas gallinae*: A review. *Exp. Parasitol.* **3**, 368–402 (1954).
55. Dąbrowska, J. et al. *Tritrichomonas foetus*: A study of prevalence in animal hosts in Poland. *Pathogens* **9**, 203 (2020).
56. Mielczarek, E. & Blaszkowska, J. *Trichomonas vaginalis*: pathogenicity and potential role in human reproductive failure. *Infection* **44**, 447–458 (2016).
57. McDougald, L. R. Blackhead disease (histomoniasis) in poultry: A critical review. *Avian Dis.* **49**, 462–476 (2005).
58. Carvalho, E. L. d. et al. Systematic and parasite-host relationship by *Baruscapillaria appendiculata* in *Phalacrocorax brasilianus* collected from Marajó island, state of Pará, Brazil. *Rev. Bras. Parasitol. Vet.* **32**, e007423 (2023).
59. Palanivelu, M. et al. *Baruscapillaria obsignata*: a serious cause of enteropathy and high mortality in turkeys (*Meleagris gallopavo*). *Vet. Q.* **36**, 145–149 (2016).
60. Tamaru, M., Yamaki, S., Jimenez, L. A. & Sato, H. Morphological and molecular genetic characterization of three *Capillaria* spp. (*Capillaria anatis*, *Capillaria pudendotecta*, and *Capillaria madseni*) and *Baruscapillaria obsignata* (Nematoda: Trichuridae: Capillariinae) in avians. *Parasitol. Res.* **114**, 4011–4022 (2015).
61. Jortner, B. S., Helmboldt, C. F. & Pirozok, R. P. Small intestinal histopathology of spontaneous capillariasis in the domestic fowl. *Avian Dis.* **11**, 154–169 (1967).
62. Pinto, R. M., Brener, B., Tortelly, R., Menezes, R. C. & Muniz-Pereira, L. C. Capillariid nematodes in Brazilian turkeys, *Meleagris gallopavo* (Galliformes, Phasianidae): Pathology induced by *Baruscapillaria obsignata* and *Eucoleus annulatus* (Trichinelloidea, Capillariidae). *Mem. Inst. Oswaldo Cruz.* **103**, 295–297 (2008).
63. Park, S. & Shin, S. Concurrent *Capillaria* and *Heterakis* infections in zoo rock partridges, *Alectoris graeca*. *Korean J. Parasitol.* **48**, 253–257 (2010).
64. Mazzone, A. et al. Morphological and molecular characterization of larval and adult stages of *Eustrongylides excisus* (Nematoda: Dioctophymatoidea) with histopathological observations. *J. Parasitol.* **105**, 882–889 (2019).
65. Sutherland, M., Shamsi, S., Phalen, D. N. & Macwhirter, P. J. *Eustrongylides* induced verminous coelomitis in an Australian darter (Anhinga novaehollandiae). *Eolophus.* **2**, 33–36 (2018).
66. Shamsi, S. et al. Occurrence and characterisation of *Eustrongylides* species in Australian native birds and fish. *Food Waterb Parasit.* **30**, e00189 (2023).
67. Eberhard, M. L. & Ruiz-Tiben, E. Cutaneous emergence of *Eustrongylides* in two persons from South Sudan. *Am. J. Trop. Med. Hyg.* **90**, 315–317 (2014).
68. Eberhard, M. L., Hurwitz, H., Sun, A. M. & Coletta, D. Intestinal perforation caused by larval *Eustrongylides* (Nematoda: Dioctophymatoidae) in New Jersey. *Am. J. Trop. Med. Hyg.* **40**, 648–650 (1989).
69. Honcharov, S. L. et al. Distribution of the nematodes of the genus *Eustrongylides* (Nematoda, Dioctophymatidae) in the world. *Regul. Mech. Biosyst.* **13**, 73–79 (2022).
70. Zhang, S. et al. Morphological and phylogenetic analysis of *Eustrongylides* sp. and *Gnathostoma spinigerum* parasitizing the Asian swamp eel *Monopterus albus* in China. *Pathogens.* **10**, 711 (2021).
71. Choe, S. et al. Two echinostome species, *Pegosomum bubulcum* and *Nephrostomum ramosum* (Digenea: Echinostomatidae), from an eastern cattle egret, *Bubulcus ibis coromandus* Republic of Korea. *Parasites Hosts Dis.* **54**, 485–496 (2016).
72. Measures, L. N. The development of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in oligochaetes. *J. Parasitol.* **74**, 294–304 (1988).
73. Lalor, R. et al. Pathogenicity and virulence of the liver flukes *Fasciola hepatica* and *Fasciola gigantica* that cause the zoonosis fasciolosis. *Virulence* **12**, 2839–2867 (2021).
74. Tkach, V. V., Kudlai, O. & Kostadinova, A. Molecular phylogeny and systematics of the Echinostomatoidea Looss, 1899 (Platyhelminthes: Digenea). *Int. J. Parasitol.* **46**, 171–185 (2016).
75. Floyd, R. M., Rogers, A. D., Lambshead, P. J. D. & Smith, C. R. Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Mol. Ecol. Notes* **5**, 611–612 (2005).
76. Stoeck, T. et al. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol. Ecol.* **19**, 21–31 (2010).
77. Amaral-Zettler, L. A., McCliment, E. A., Ducklow, H. W. & Huse, S. M. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. *PLoS ONE* **4**, e6372–e6372 (2009).

Author contributions

Seongjun Choe and Seung-Hun Lee conceptualized the study. Chang Hyeon Lee, Heon Woo Lee and Seongjun Choe collected the fecal samples of great cormorants. Chang Hyeon Lee and Heon Woo Lee conducted microscopic examination. Subin Lee, Badriah Alkathiri, Dong-Hyuk Jeong and Ju Yeong Kim performed the experiments. Subin Lee drafted the manuscript. Subin Lee performed bioinformatics analysis. Subin Lee contributed to

the revision of the manuscript. Dong-Hyuk Jeong, Ju Yeong Kim, Seongjun Choe and Seung-Hun Lee reviewed and contributed to the manuscript. Seung-Hun Lee supervised the study. All the authors have read and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval
All animal experiments were conducted in accordance with the guidelines and regulations and the study was approved by the Institutional Animal Care and Use Committee (IACUC) of Chungbuk National University (Approval Number: CBNUA-24-0001-01). In addition, all the procedures in this study followed the ARRIVE guidelines.

Additional information
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