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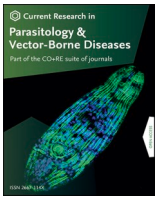

Helminth fauna of the black goby *Gobius niger* L. (Gobiiformes: Gobiidae) from the Finnish Archipelago, Baltic Sea: Molecular and morphological data

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ABSTRACT

Black gobies (*Gobius niger*) from the Finnish Archipelago, Baltic Sea, were screened for helminth infections in summer 2020. Helminths were identified morphologically and/or molecularly. Altogether 26 novel sequences were generated and analysed using maximum likelihood estimation. Morphological and phylogenetic analyses based on mitochondrial genes revealed the presence of 8 species belonging to the Digenea (*Diplostomum mergi* Lineage 3), Cestoda (*Bothriocephalus scorpii*), Nematoda (*Contracaecum rudolphii* A, *Cucullanus* sp. and *Hysterothylacium aduncum*), and Acanthocephala (a putative new species of *Corynosoma*, *Corynosoma semerme* and *Neoechinorhynchus* sp.). Phylogenetic and comparative sequence analyses revealed that the putative new acanthocephalan species is closely related to *C. neostrumosum* described from the Caspian seal, *Pusa caspica*, in the Caspian Sea. The black goby represents a new host record for four parasite species (*Diplostomum mergi* Lineage 3, *Contracaecum rudolphii* A, *Corynosoma semerme* and *Corynosoma* sp.). The Finnish Archipelago is a novel locality record for three species (*Corynosoma* sp., *Diplostomum mergi* Lineage 3 and *Bothriocephalus scorpii*).

1. Introduction

The black goby, *Gobius niger* L. (Gobiiformes: Gobiidae), is a demersal fish living in shallow marine to brackish habitats. This species is generally common throughout its range which is limited in the north by the Arctic circle in Iceland and Norway, runs along the North-East Atlantic coasts, the Mediterranean and Black Seas, and throughout the Atlantic coasts of Africa from Morocco to Mauritania (Froese and Pauly, 2000). As a predator of benthic and demersal invertebrates as well as smaller fish, and prey to larger fish, birds, and seals, *G. niger* is a key species in trophic interactions and as such a valuable host for many parasite species (Zander et al., 1993). The parasite fauna of *G. niger* has been studied in detail in the North-East (NE) Atlantic off Spain (Sanmartín et al., 2001), the Black Sea (Kvach, 2005), the Sea of Marmara (off Turkey) (Öktener, 2005), and the southwestern Baltic Sea (Zander, 2004; Zander et al., 1993; Zander and Kesting, 1998).

The Baltic Sea is a peripheral sea of the NE Atlantic Ocean, connected to the North Sea *via* the rather narrow and shallow Skagerrak, Kattegat, Belts and Sound. Between these consecutive connections, salinity drops rapidly from about 30 in the North Sea to about 10 at the entrance of the Baltic and drops further across the Baltic Sea to below 3 in the Gulf of Bothnia, the northernmost arm of the Baltic Sea (Fig. 1) (salinity values are in PSU taken from Ojaveer et al., 2010, and given without units in accordance with the Practical Salinity Scale 1978 (PSS-78)). Due to this steep gradient and a rather young ecosystem, estimated to be only 8000 years old (Ojaveer et al., 2010), the overall biodiversity of the Baltic Sea is impoverished relative to that of the North Sea or the NE Atlantic (Johannesson and André, 2006), a situation being exacerbated in more recent times by, e.g. eutrophication, pollution, overfishing and the negative impact of invasive species (e.g. Ojaveer et al., 2010).

The Finnish Archipelago Sea marks the border between the North-West Baltic Proper and the Bothnian Sea (Fig. 1). It is a dense congregation of islands, islets, and skerries reaching westward from Finland towards the Swedish coast, from which it is separated by the narrow (*c.*30 km) but deep (*c.*300 m) Sea of Åland. This region, with a salinity between 5.6 and 6.2 (Suominen et al., 2010) also marks the shift from an

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ecosystem dominated by marine species in the Baltic Proper to a predominantly freshwater biodiversity in the Bothnian Sea (Ojaveer and Kalejs, 2008). The Bothnian Sea with its lower salinity (< 5.5) also marks the northern limit of the distribution of the black goby in the Baltic Sea (Urho and Lehtonen, 2008); as far as we are aware, there is no report of this fish species from the Bothnian Bay. This habitat shift makes the ecosystem of the Archipelago Sea particularly interesting. We expected to find both marine and freshwater parasites, and thus new host-parasite combinations.

Faunistic reports are important for recording ecological changes over time. To fully understand the ecological context and diversity of any taxa, it is important to support morphological identifications of specimens with molecular methods. Many taxa contain separate lineages readily distinguished using DNA sequencing. Furthermore, larval parasites are difficult to identify morphologically, and we expected to find many larval forms in *G. niger* because due to its intermediate trophic level, this fish species is acting as both, intermediate and definitive host for parasites.

Here, we report the helminth diversity in *G. niger*, from a brackish ecosystem in the Baltic Sea by integrating morphological and molecular approaches for the first time. DNA sequences obtained in our study were used to assess the taxonomic affiliation of most of the parasites collected and connect morphology and genotype. We also provide vouchers for the morphologically and/or molecularly identified species and brief descriptions of the acanthocephalans. Our study provides new host records, new parasite localities, and comments on the natural history of some of the parasites collected. Finally, we compare the parasite diversity of black gobies from the Finnish Archipelago with that of other regions.

2. Materials and methods

2.1. Specimen collection

Altogether 38 *G. niger* (total body length 5.7–10.8 cm) were collected between July and August 2020 from 8 localities in the Finnish Archipelago Sea (Fig. 1; Supplementary Table S1). Fish were caught using bottom and gill nets and examined for helminths within hours after their capture at the Archipelago Research Institute of the University of Turku. Helminths were recovered from the eyes, stomach, intestine, liver, gonads, and mesenteries using a stereomicroscope (magnifications of up to 40×). No ectoparasites were found on the body surface, fins, and gills. Trematodes, cestodes, and nematodes were fixed in 10% formalin for morphological study or in 96% ethanol for molecular analyses. Acanthocephalans were placed in tap water, left in a refrigerator for 12 h, and then fixed in 80% ethanol for morphological study and in 96% ethanol for molecular analyses. Infection parameters were estimated following Bush et al. (1997).

2.2. Morphological data

For parasite identification based on morphology, nematodes were cleared in glycerine. Trematodes, cestodes, and acanthocephalans were stained with Mayer's carmine, dehydrated through an ethanol series, cleared in methyl salicylate, and mounted as permanent slides in Canada balsam. Cleared and mounted specimens were examined with an Olympus BX51 compound microscope. Helminths were identified according to Van Cleave (1953), Khalil (1994), Nickol et al. (2002), and Anderson et al. (2009). A voucher specimen of *Neoechinorhynchus* sp. is

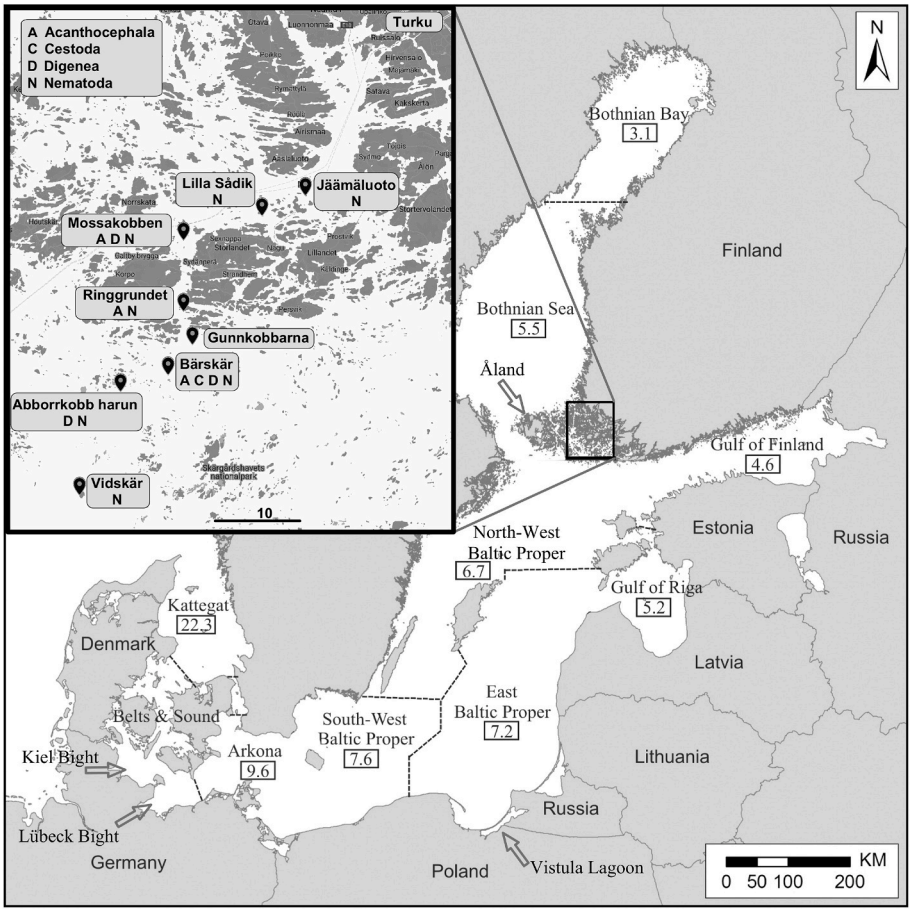

Fig. 1. Map of the Baltic Sea and its basins with salinity (in PSU; amended from Ojaveer et al., 2010). The inset shows the Finnish Archipelago Sea with sampling sites and recorded parasite groups indicated.

deposited in the Parasitic Worms collection of the Natural History Museum (NHMUK), London, UK (accession number NHM 2023.11.21.1). Voucher specimens for diplostomid metacercariae and raphidascaridid third-stage larvae are deposited in the Helminthological Collection of the Institute of Parasitology (IPCAS), Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic (accession numbers IPCAS D-880 and IPCAS N-860, respectively). Cystacanths of *Corynosoma* spp. were prepared for scanning electron microscopy (SEM) following Hernández-Orts et al. (2022) and examined with a JEOL JSM7401-F scanning electron microscope (JEOL Ltd., Tokyo, Japan) at 4 kV at the Laboratory of Electron Microscopy, IPCAS.

2.3. Molecular data and phylogenetic analyses

For nematodes, a small piece from the mid-body level, and for acanthocephalans the dorsal or lateral surface of the body was excised and used for DNA isolation and sequencing. The remaining parts of the individuals were kept and prepared as hologenophores *sensu* Pleijel et al. (2008). DNA was isolated from 12 individual metacercariae of *Diplostomum* spp.; the remaining metacercariae collected from the same host individual were kept and fixed as paragenophores *sensu* Pleijel et al. (2008). Parasites/parasite tissues fixed in 96% ethanol were air-dried and then added to TNES (Asahida et al., 1996) prior to DNA extraction using phenol-chloroform.

Different mitochondrial genes were amplified for each taxonomic group, based on the best reference dataset, using standard protocols for molecular identification (Table 1); *cox*1 for diplostomids and acanthocephalans, and *cox*2 for nematodes. A subsample of the PCR product was visualized on a 1% agarose gel, and positive samples were cleaned using ExoSAP-IT™ (GE Healthcare Life Sciences, Buckinghamshire, UK). Sanger sequencing was performed by SEQme s. r.o. (seqme.eu, Dobříš, Czech Republic) using the PCR primers (Table 1). The newly generated sequences were deposited in the GenBank database under the accession numbers OR831215-OR831226, OR832779-OR832784, OR832864, OR837770 and OR854803-OR854808.

Sequences were analysed using the Geneious Prime® 2022.2.2 software (https://www.geneious.com). For each taxonomic group, sequences of representative species and lineages were downloaded from GenBank to build a reference dataset (Supplementary Tables S2–S6). The newly generated sequences were added, and the datasets aligned using MAFFT v7.450 (Katoh and Standley, 2013); the resulting five alignments were checked by eye for mismatches. The best-fit nucleotide substitution models were estimated using ModelFinder (Kalyaanamoorthy et al., 2017) implemented in IQ-TREE v1.6.12 (Chernomor et al., 2016) according to the corrected Akaike information criterion (AICc). Maximum likelihood analyses were run independently in IQ-TREE, with 1000 bootstrap replicates to assess nodal support using the following models: TPM2u+F+I+G4 for *Diplostomum* spp.; TPM3u+F+G4 for anisakid nematodes; HKY+F+I+G4 for raphidascaridid nematodes; TVM+F+I+G4 for neoechinorhynchid acanthocephalans; and K3Pu+F+I+G4 for polymorphid acanthocephalans. Trees were visualized using FigTree version 1.4.4. (Rambaut, 2009).

3. Results

3.1. Molecular identification of helminths

A total of 26 sequences for helminths were generated in this study; 12 for *cox*1 (728–831 nt) from eye lens metacercarial isolates of *Diplostomum* (Diplostomida: Diplostomidae), 3 for *cox*2 (597 nt) from isolates of *Contracaecum rudolphii* Hartwich, 1964 (Rhabditida: Anisakidae), 3 for *cox*2 (525–590 nt) from isolates of *Hysterothylacium aduncum* (Rudolphi, 1802) (Rhabditida: Raphidascarididae), 6 for *cox*1 (671–1474 nt) from isolates of *Corynosoma semerme* (Forssell, 1904) (Polymorphida: Polymorphidae), 1 for *cox*1 (671 nt) from the isolate of *Corynosoma* sp. and 1 for *cox*1 (1053 nt) from the non-gravid isolate of *Neoechinorhynchus* sp. (Neoechinorhynchida: Neoechinorhynchidae). Representative sequences for isolates identified based on morphology as *Bothriocephalus scorpii* (Müller, 1776) (Bothriocephalidea: Bothriocephalidae) and *Cucullanus* sp. (Rhabditida: Cucullanidae) could not be obtained.

The phylogenetic analysis of the *cox*1 dataset for *Diplostomum* spp. (18 taxa; 44 sequences; 354 nt; Supplementary Table S2) resulted in a tree (Fig. 2) in which the 12 novel sequences clustered with the sequences of the isolates classified as *Diplostomum mergi* Lineage 3 *sensu* Georgieva et al. (2013) collected from the first intermediate host, *Radix auricularia* (L.) (Lymnaeidae) (JX986880) and the second intermediate host, *Salmo trutta fario* L. (Salmonidae) (KR149525) in Germany. The genetic divergence within this clade was 0–2.0% (0–7 nt), which corresponds to the intraspecific variation previously reported for members of the genus *Diplostomum* (Schwelm et al., 2021; Faltýnková et al., 2022). This result indicates that metacercariae we collected belong to *Diplostomum mergi* Lineage 3 *sensu* Georgieva et al. (2013).

The analysis of the *cox*2 dataset for anisakid nematodes (18 taxa; 27 sequences; 483 nt; Supplementary Table S3) placed the 3 novel sequences derived from third-stage larval (L3) isolates of *Contracaecum* in a clade with *Contracaecum rudolphii* A *sensu* Bullini et al. (1986) from the definitive host *Phalacrocorax carbo sinensis* (Staunton) (Phalacrocoracidae) (GenBank: EF122201 and EF513501) in Italy (Fig. 3A). The novel *cox*2 sequences diverged by 0.6–1.7% from the sequences for *C. rudolphii* A retrieved from GenBank.

The phylogenetic analysis of the *cox*2 dataset for *Hysterothylacium* spp. (14 taxa; 25 sequences; 489 nt; Supplementary Table S4) clustered the new sequences of L3 and adult isolates identified as *H. aduncum* together with published sequences of larval and adult isolates of *H. aduncum* from marine fishes from Europe (Fig. 3B). The sequence divergence within this clade was 0–4.7% (0–22 nt). Sequence divergence between isolates of *H. aduncum* from Europe and South Korea was 9.6–10.9% (42–47 nt).

In the phylogenetic analysis of the *cox*1 dataset for neoechinorhynchid acanthocephalans (32 taxa; 33 sequences; 434 nt; Supplementary Table S5), the novel sequence of the isolate identified as *Neoechinorhynchus* sp. clustered with a sequence of an unidentified neoechinorhynchid from *S. trutta* in Austria (GenBank: MN780975). Genetic divergence between these two sequences was 1.4% (6 nt),

Table 1
Primers used for PCR amplification and sequencing.

Target taxon	Marker	Primer name (orientation)	Primer sequence (5′-3′)	Reference
Digenea	*cox*1	Dice1F (F)	ATTAACCCTCACTAAATTWCNTTRGATCATAAG	van Steenkiste et al. (2015)
		Dice14R (R)	TAATACGACTCACTATACCHACMRTAAACATATGATG	
Nematoda	*cox*2	211 (F)	TTTTCTAGTTATATAGATTGRTTTYAT	Nadler and Hudspeth (2000)
		210 (R)	CACCAACTCTTAAAATTATC	Folmer et al. (1994)
Acanthocephala	*cox*1	#507 (F)	AGTTCTAATCATAA(R)GATAT(Y)GG	Nadler et al. (2006)
		HC02198 (R)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
		Cory-CO1/F (F)[a]	GCTTCGTTGGTTTATGTCTTTGA	Sasaki et al. (2019)
		Cory-CO1/R2 (R)[a]	ACCTGACAATTGGAAATTGCCTGT	

Abbreviations: F, forward; R, reverse.
[a] Primers only used for cystacanths of *Corynosoma* spp.

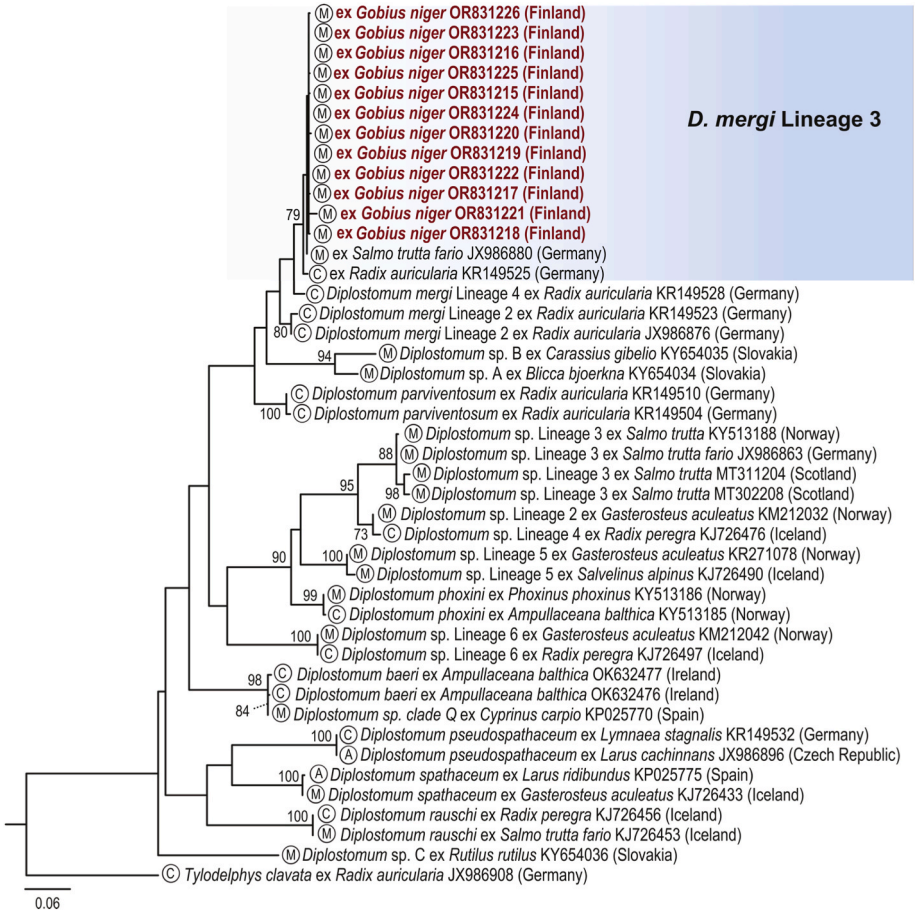

Fig. 2. Maximum likelihood estimates of the phylogenetic relationships of *Diplostomum* spp. based on the *cox*1 dataset (Supplementary Table S2) employing the TPM2u+F+I+G4 substitution model. Numbers indicate the maximum likelihood bootstrap support (values > 65% shown only). The newly generated sequences are indicated in red and bold. Developmental stage for each taxon is indicated in a circle in terminal taxon labels. The scale-bar indicates the number of substitutions per site. *Abbreviations*: A, adult; C, cercaria; M, metacercaria.

suggesting conspecificity. They appeared as sister to *Neoechinorhynchus* (*Neoechinorhynchus*) *simansularis* Roitman, 1961 from *Salvelinus alpinus* (L.) (Salmonidae) in Russia, but with negligible bootstrap support (Fig. 4A).

The analysis of the *cox*1 data for polymorphid acanthocephalans (22 taxa; 47 sequences; 640 nt; Supplementary Table S6) resulted in a tree (Fig. 4B) consistent with previous phylogenetic assessments of *Corynosoma* (Hernández-Orts et al., 2022). Novel sequences generated for cystacanth isolates of *Corynosoma semerme* collected from black gobies in Finland formed a clade with published sequences of adult isolates of *C. semerme* from the grey seal *Halichoerus grypus* (Fabricius) (Phocidae) (GenBank: MF001277) from the Baltic Sea, the northern fur seal *Callorhinus ursinus* L. (Otariidae) (GenBank: MK119253 and JX442192) from Alaska, and the spotted seal *Phoca largha* Pallas (Phocidae) (GenBank: LC465312, LC465313 and LC465392) from Japan. Intraspecific divergence range within the *Corynosoma semerme* clade was 0–1.4% (0–8 nt). The newly generated sequence for the cystacanth of *Corynosoma* sp. clustered with the sequence of a recently described species, *Corynosoma neostrumosum* Amin, Chaudhary, Sharifdini & Singh, 2023 (GenBank: OQ745812) from the Caspian seal *Pusa caspica* (Gmelin) (Phocidae) in the Caspian Sea (Amin et al., 2023). These two sequences showed a divergence of 3.84% (20 nt) and were the sister clade (87% bootstrap support) to a clade containing all specimens of *C. strumosum* (Rudolphi, 1802) and *C. magdaleni* Montreuil, 1958. The observed divergence is slightly larger than expected for the genus *Corynosoma* (Hernández-Orts et al., 2022) and indicates that the newly generated sequence may belong to a new species.

3.2. Morphological characterization of acanthocephalans

Cystacanths of *Corynosoma semerme* and *Corynosoma* sp. possess a pipe-shaped body (Fig. 5A, C). The foretrunk in both species is inflated, forming a spiny disk for attachment, while the hindtrunk is cylindrical and is covered with spines on its ventral surface. The trunk of *C. semerme* is 2.1–2.3 mm long and 0.9–1.0 mm wide (disc diameter) in males (n = 2), and 2.0–2.3 mm long and 0.8–1.1 mm wide in females (n = 2). Somatic spines extend between 75% and 100% of hindtrunk length in males (Figs. 5A) and 75% in females. The proboscis is subcylindrical, slightly markedly at the posterior third (Fig. 5B), armed with 23–24 longitudinal rows (Fig. 5E) of 12–13 hooks (Fig. 5B) (each row with 8–9 anterior large hooks and 4–5 spiniform hooks) in males, and 24–25 longitudinal rows of 12–14 hooks (each row with 8–9 anterior large hooks and 4–5 spiniform hooks) in females. Genital spines surround the genital pore in males (Fig. 5A) and females.

The trunk of the female cystacanth of *Corynosoma* sp. is 3.5 mm long and 1.3 mm wide. Somatic spines in this female extend to almost the mid-level of the hindtrunk (Fig. 5C). The proboscis is subcylindrical, widening markedly at the posterior third (Fig. 5D), armed with 20 longitudinal rows (Fig. 5F) of 9–10 hooks (Fig. 5D) (each with 6–7 anterior large hooks and 3 spiniform hooks, distal spiniform hooks not observed in Fig. 5D). Genital spines could not be observed due to invagination of the posterior end.

The immature female of *Neoechinorhynchus* sp. has numerous ovarian balls (Fig. 6A). The cylindrical trunk is 4.3 mm long and 0.6 mm wide at anterior third. The body wall is thick, with four dorsal nuclei and one

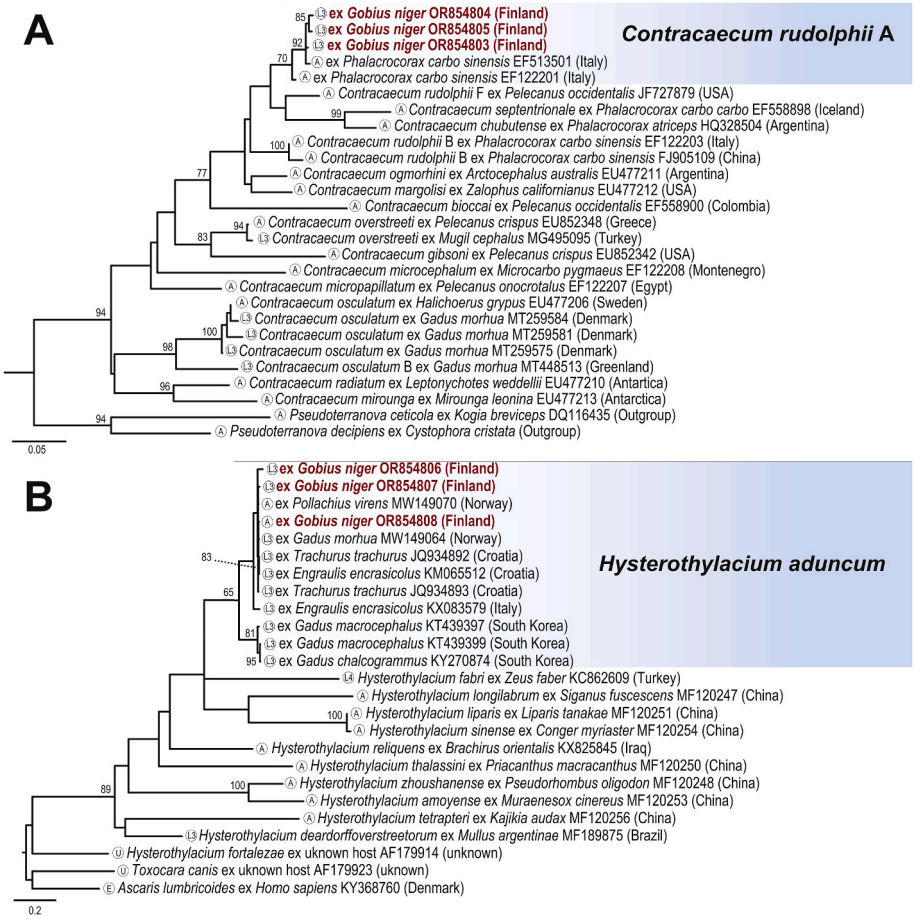

Fig. 3. Maximum likelihood estimates of the phylogenetic relationships of anisakid (**A**) and raphidascaridid (**B**) nematodes based on the *cox*2 datasets (Supplementary Tables S3 and S4, respectively) employing the TPM3u+F+G4 and HKY+F+I+G4 substitution models, respectively. Numbers indicate the maximum likelihood bootstrap support (values > 65% shown only). The newly generated sequences are indicated in red and bold. Developmental stage for each taxon is indicated in a circle in terminal taxon labels. The scale-bar indicates the number of substitutions per site. *Abbreviations:* A, adult; E, egg; L3, third-stage larva; L4, fourth-stage larva; U, unknown stage.

ventral giant hypodermal nucleus (Fig. 6A). The cylindrical proboscis is small, 125 μm long and 102 μm wide, with a well-developed apical organ (Fig. 6B) and armed with three circles of six hooks each. The apical hooks are massive, 55 μm long, and are considerably longer than the middle and posterior hooks (Fig. 6C). Roots of these hooks are simple and well-developed, 26 μm long. The middle and posterior hooks are small and almost equal in size, 24 μm and 22 μm long, respectively. The roots of the middle and posterior hooks are small and simple, 12 μm and 10 μm long, respectively. The neck is not visible. The proboscis receptacle is single-walled, 328 μm long and 103 μm wide, with an ovoid cephalic ganglion at its posterior end (Fig. 6B). The lemnisci are digitiform, about the same length, 772 μm and 749 μm long, respectively. The left lemniscus has one elongated giant nucleus, while the right lemniscus has two giant nuclei (Fig. 6B). The gonopore is terminal. This morphology is somewhat similar to that of *Neoechinorhynchus* (*Neoechinorhynchus*) *rutili* (Müller, 1780) but with a single non-gravid specimen a reliable identification is not possible.

3.3. Overview of helminths infecting G. niger in the Archipelago Sea

Twenty-seven of the 38 black gobies from the Archipelago Sea off Finland were infected with helminths (overall prevalence of 71%). Eight taxa were identified based on morphological and/or molecular data (see above): the digenean *Diplostomum mergi* Lineage 3; the cestode *B. scorpii*; the nematodes *Contracaecum rudolphii* A, *Cucullanus* sp. and *Hysterothylacium aduncum*, and the acanthocephalans *Corynosoma semerme*, *Corynosoma* sp. and *Neoechinorhynchus* sp.

Four species were represented by larval stages, one by a non-gravid form, two by adults, and one species was found as both larval and adult stages (Table 2). Overall, the helminth fauna of *G. niger* in the Archipelago Sea was characterised by a high representation of larval forms (five species, 63%; Tables 2 and 3) and a low representation of species with freshwater life-cycles (two species, *D. mergi* Lineage 3 and *Neoechinorhynchus* sp.; 25%). Infection parameters of the helminths found in *G. niger* are shown in Table 2. Five helminth taxa, i.e. *D. mergi* Lineage 3, *Contracaecum rudolphii* A, *Cucullanus* sp., *H. aduncum* and *Corynosoma semerme* were more prevalent, with larval stages of *D. mergi* Lineage 3 being the most prevalent and abundant (representing 67.4% of all helminth specimens collected). Species richness in individual fish ranged from 1 to 4 helminth species (mean species richness of 1.1). A single host (2.6%) was infected with four helminth species; three (7.9%) with three species; six (15.8%) with two species; and 17 (44.7%) with one species. Altogether, we report four helminth species found for the first time in the black goby (i.e. *Diplostomum mergi* Lineage 3, *Contracaecum rudolphii* A, *Corynosoma semerme* and a putative new species of *Corynosoma*), and three new geographical records in the Finnish Archipelago Sea (for *Diplostomum mergi* Lineage 3, *B. scorpii* and *Corynosoma* sp.).

4. Discussion

To the best of our knowledge, this is the first survey of black gobies using molecular methods to support the morphological identification of

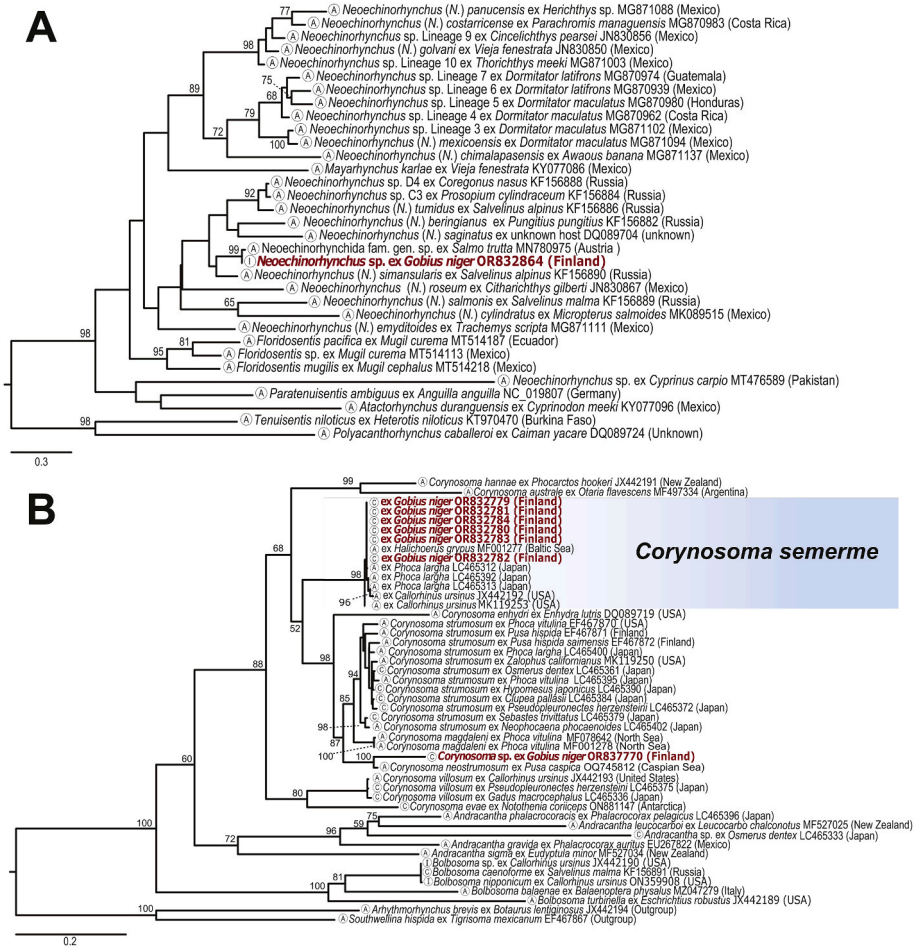

Fig. 4. Maximum likelihood estimates of the phylogenetic relationships of neoechinorhynchid (**A**) and polymorphid (**B**) acanthocephalans based on the *cox*1 datasets (Supplementary Tables S5 and S6, respectively) employing the TVM+F+I+G4 and K3Pu+F+I+G4 substitution models, respectively. Numbers indicate the maximum likelihood bootstrap support (values > 65% shown only). The newly generated sequences are indicated in red and bold. Developmental stage for each taxon is indicated in a circle in terminal taxon labels. The scale-bar indicates the number of substitutions per site. *Abbreviations:* A, adult; C, cystacanth; I, immature.

parasites, allowing the distinction of parasite lineages and unambiguous identification of larvae. Overall, the observed helminth diversity in terms of species richness (8 species) is comparable to that found in previous studies. Sanmartín et al. (2001) examined 894 specimens in northern Spain in two different rias (coastal inlets) and reported 13 helminth taxa, including cestodes, nematodes, and acanthocephalans but no digeneans. Nine of these were identified only to the genus or family level. Taking this into account, there is a potential overlap of up to five taxa (*Bothriocephalus* sp., *Hysterothylacium* sp., *Contracaecum* sp., *Cucullanus* sp. and "Acanthocephala") between the faunas of *G. niger* from Archipelago Sea and the Spanish Atlantic coast. Zander and colleagues monitored the parasite fauna of gobiid fishes, including *G. niger* in the Kiel and Lübeck Bights (Fig. 1) in the Belts and Sound area of the Baltic Sea (salinity 8–32) between 1983 and 2000 (Zander et al., 1993; Zander and Kesting, 1998; Zander, 2003). For each dataset containing 18 to 103 host specimens, a maximum of eight helminth species were reported with an overall prevalence between 77% and 83%, comparable to our finding of 71% (Table 3). Considering that some species were only identified to the genus level, up to four taxa (*B. scorpii*, *Contracaecum* sp., *Corynosoma* sp. and *Hysterothylacium* sp.) are shared between the faunas of *G. niger* from Archipelago Sea and the Belts and Sound (2–3 taxa in common in the individual samples; Table 3). The proportion of freshwater species varied greatly (0–38%) in the Belts and Sound, even within the same locality (13–38%) (Table 3). Our initial expectation to see a general shift towards more freshwater parasites in this host was not supported, such a shift was only observed in the case of diplostomids. Despite the low salinity of the study area (5.5–6.2) only 25% of the helminths in our sample are freshwater forms, comparable to the number in the Belts and Sound area. A noticeable difference, however, is the absence of marine digeneans in our samples. Zander and colleagues reported two (Zander et al., 1993; Zander and Kesting, 1998) and three (Zander, 2003) marine digenean species, but no freshwater species. Two of these, *Cryptocotyle lingua* (Creplin, 1825) and *Cryptocotyle concavum* (Creplin, 1825) (both Opisthorchiidae), have also been reported from *G. niger* in the Black Sea (Kvach, 2005). The helminth fauna of *G. niger* in the Archipelago Sea is characterised by a lower proportion of larval forms (63% *vs* 88–100%). This may be due to the specific position of this host in the food webs in our study region.

Diplostomum mergi is a complex of freshwater species of four distinct lineages (Schwelm et al., 2021) distinguishable by cercarial morphology and molecular markers (Georgieva et al., 2013; Selbach et al., 2015). Metacercariae of *D. mergi* Linage 3 *sensu* Georgieva et al. (2013) were first detected using molecular markers in *Salmo trutta fario* and *Gobio gobio* (L.) (Gobionidae) in the River Ruhr (Georgieva et al., 2013), while cercariae were characterised morphologically and molecularly from *R. auricularia* in Hengsteysee (River Ruhr catchment) (Selbach et al., 2015). The presence of *D. mergi* Linage 3 in black gobies from the Finnish Archipelago is the first record of this species from a marine fish host and the first record in a brackish environment. This species was the most prevalent and abundant parasite in our sample. At nearly 40%, the prevalence was much higher in our sample than that reported from *S. trutta fario* in Germany (prevalence range of 9.5–11.1% in trout from

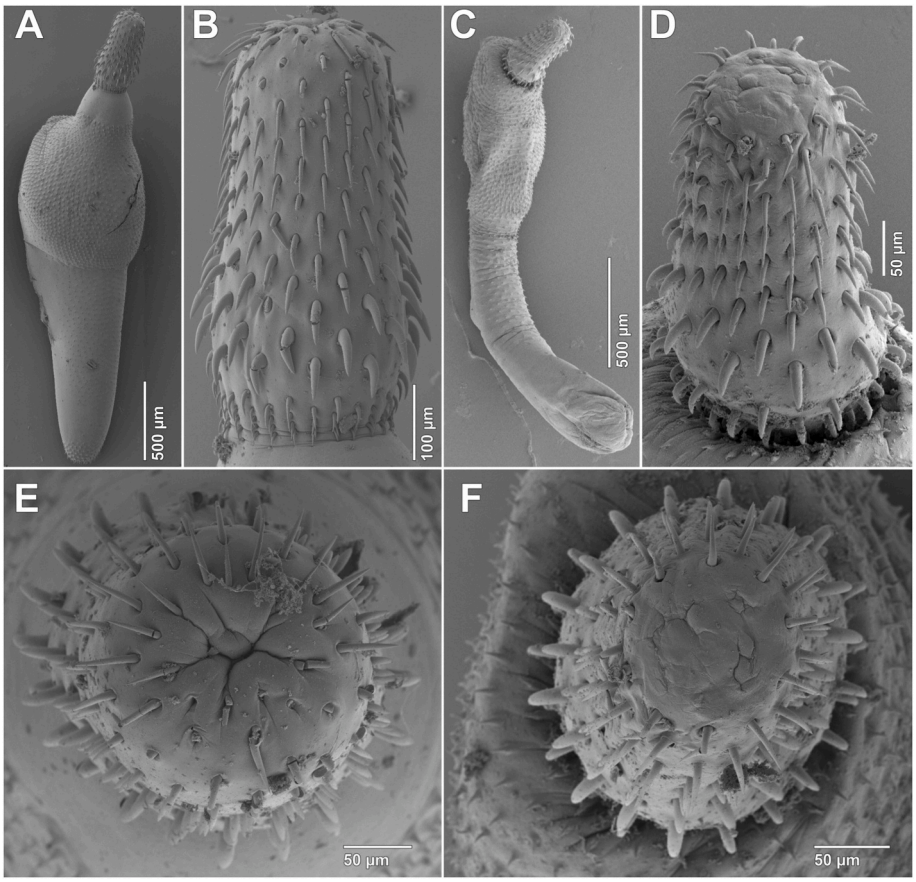

Fig. 5. Scanning electron photomicrographs of excysted cystacanths of *Corynosoma* spp. from *Gobius niger* from the Archipelago Sea, Finland. **A**, **B**, **E** Male *Corynosoma semerme* (Forssell, 1904) (hologenophore; GenBank sequence OR832781). **A** Whole worm, ventrolateral view. **B** Proboscis, ventral view. **E** Proboscis, apical view. **C**, **D**, **F** Female *Corynosoma* sp. (hologenophore; GenBank sequence OR837770). **C** Whole worm, ventrolateral view. **D** Proboscis, ventral view. **F** Proboscis, apical view.

the rivers Ruhr and Lenne; Georgieva et al., 2013). *Diplostomum* spp. are freshwater species but have been recorded from several marine fish hosts in the north of the Bay of Bothnia (Valtonen and Gibson, 1997). Their intermediate snail host, *R. auricularia*, is a strict freshwater inhabitant, which to our knowledge has never been recorded in the Archipelago Sea, or even the Bay of Bothnia with its very low salinity. Therefore, it would be interesting to study which snail host acts as the first intermediate host for *D. mergi* Linage 3 in the Finnish Archipelago.

Bothriocephalus scorpii is a tapeworm of marine fishes known from around the world, including *G. gobius* in the Southwest Baltic (Zander, 2003) and the round goby *Neogobius melanostomus* (Pallas) (Gobiidae) in the brackish Vistula Lagoon, Southern Baltic (Rolbiecki, 2006). An unidentified specimen of *Bothriocephalus* was also reported from *G. gobius* in the NE Atlantic, off Spain (Sanmartín et al., 2001). Our study provides the second record from a brackish environment to date.

Hysterothylacium aduncum is a parasitic nematode in marine and brackish fishes and crustaceans in cold and temperate waters of the northern and southern hemispheres. It has been recorded in *G. gobius* from the Sea of Marmara (Öktener, 2005) and *N. melanostomus* from the Baltic Sea off the Polish coast as well as in *Platichthys flesus* (L.) (Pleuronectidae) from the Gulf of Finland (Køie, 1999) and several other non-gobiid species in the southwestern Baltic Sea (Zander and Reimer, 2002).

Species of the genus *Cucullanus* parasitize fishes (definitive hosts) and polychaetes (intermediate hosts) from freshwater, brackish, and marine environments. In the Gulf of Finland only *C. heterochrous* Rudolphi, 1802 has been reported from *P. flesus* (Køie, 1999). The same species has been recorded in *G. niger* along the Atlantic coast of Spain together with an unidentified species of *Cucullanus* (Sanmartín et al., 2001). We observed non-encapsulated adults in the lumen of the gut with a prevalence of 13.2% indicating that *G. niger* is the definitive host for at least some cucullanid nematodes in brackish waters.

Contracaecum rudolphii (*sensu lato*) is a species complex consisting of five lineages that can be distinguished based on their distribution, ecology, including host preference and life-cycle (Mattiucci et al., 2020) as well as molecular markers (Zhu et al., 2007). The complex life-cycle of these nematodes includes fish-eating birds (definitive host), a crustacean (first intermediate host), and up to two fish hosts (second intermediate hosts). *Contracaecum rudolphii* A *sensu* Bullini et al. (1986) is most prevalent in birds and fish from brackish environments as opposed to *C. rudolphii* B *sensu* Bullini et al. (1986), which is found in freshwater habitats. The definitive hosts of *C. rudolphii* A and B are mostly cormorants (Carmeno et al., 2022; Mattiucci et al., 2020). Great cormorants were first recorded to breed in Finland as recently as 1996 (Lehikoinen, 2006) but have since greatly increased in abundance (van Eerden et al., 2021). Most colonies are located along the shoreline of the mainland and a few colonies are distributed throughout the Archipelago Sea, but currently none in the Åland Archipelago (van Eerden et al., 2021). In 2012, *C. rudolphii* was reported from cormorants from the Åland Archipelago but, interestingly, most individuals belonged to lineage B (Szostakowska and Fagerholm, 2012), while we observed only specimens of lineage A.

Three acanthocephalan species were identified in this study based on morphological and molecular data, two belonging to the genus *Corynosoma* Lühe, 1904, and one to the genus *Neoechinorhynchus* Stiles & Hassall, 1905. *Corynosoma* is a large genus combining species using

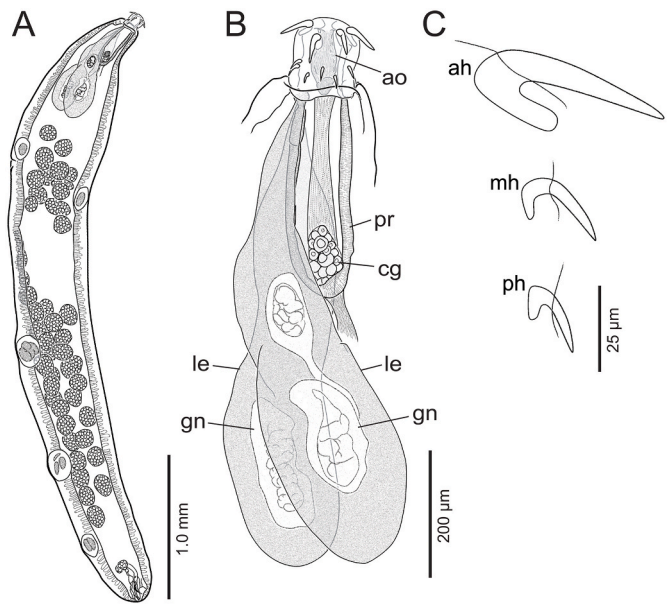

Fig. 6. Line drawings of *Neoechinorhynchus* sp. (hologenophore; GenBank sequence OR832864; morphological voucher number NHM 2023.11.21.1) from *Gobius niger* from the Archipelago Sea, Finland. **A** Whole worm, lateral view. **B** Anterior body end, with detail of the proboscis receptacle and lemnisci, lateral view. **C** Hooks of proboscis, lateral view. *Abbreviations*: ah, anterior hook; ao, apical organ; cg, cephalic ganglion; gn, giant nucleus; le, lemniscus; mh, middle hook; ph, posterior hook; pr, proboscis receptacle.

mainly pinnipeds as definitive hosts, fishes as paratenic hosts and amphipods as intermediate hosts. In the Baltic, three species are well documented, *C. semerme*, *C. magdaleni* and *C. strumosum* (Nickol et al., 2002; Leidenberger et al., 2020). Both morphological and molecular data helped identify several specimens unambiguously as *C. semerme*. One specimen was morphologically similar to *C. magdaleni* in trunk shape and proboscis hook formula, but the *cox*1 sequence showed a large (≥ 9.7%) genetic divergence when compared to GenBank sequences for *C. magdaleni* from North Sea harbour seals (*Phoca vitulina* L.). In the phylogenetic analysis based on *cox*1, this specimen represents a putative new species more closely related to *C. neostrumosum* described from *P. caspica* in the Caspian Sea. Species of the family Phocidae are closely related (Arnason et al., 2006) and *Corynosoma* spp. frequently infect many species in this group (Leidenberger et al., 2020). Our findings support the notion that the diversity of *Corynosoma* in the Baltic is greater than previously known.

Neoechinorhynchus is a large genus with a global distribution, but we found only one non-gravid specimen in our sampling. The low sequence divergence (1.4%) suggests that our specimen is conspecific with another unidentified neoechinorhynchid from brown trout in Austria (GenBank: MN780975). An unambiguous species identification based on morphological characters is not possible for the non-gravid specimens of *Neoechinorhynchus* (Amin, 2002). However, the host and distribution of these unidentified neoechinorhynchids are consistent with that of *N.* (*N.*) *rutili*, one of the oldest, most common, and most widely distributed neoechinorhynchid species in Europe (de Jong et al., 2014), which is also common in the Gulf of Bothnia (Valtonen, 1979). However, no sequence data are available for this species in GenBank. Molecular data associated with proper morphological vouchers, and accompanied

Table 2
Taxonomic composition, life-cycle stage, microhabitat and population descriptors of helminths recovered in *Gobius niger* from the Archipelago Sea, Finland.

Species	Microhabitat	Life-cycle stage	*N*	P (%)	MA ± SD	GenBank ID
Digenea						
Diplostomum mergi Lineage 3 *sensu* Georgieva et al. (2013)	Eye lens	M	38	39.5	2.50 ± 5.1	OR831215-OR831226
Cestoda						
Bothriocephalus scorpii (Müller, 1776)	Intestine	A	38	2.6	0.03 ± 0.2	–
Nematoda						
Contracaecum rudolphii A *sensu* Bullini et al. (1986)	Stomach, intestine	L3	38	15.8	0.20 ± 0.5	OR854803-OR854805
Cucullanus sp.	Stomach	A	38	13.2	0.30 ± 0.8	–
Hysterothylacium aduncum (Rudolphi, 1802)	Intestine, liver, mesenteries	L3, L4, A	38	23.7	0.50 ± 1.6	OR854806-OR854808
Acanthocephala						
Corynosoma semerme (Forssell, 1904)	Mesenteries	C	38	13.2	0.16 ± 0.4	OR832779-OR832784
Corynosoma sp.[a]	Mesenteries	C	38	2.6	0.03 ± 0.2	OR837770
Neoechinorhynchus sp.[b]	Intestine	A	38	2.6	0.03 ± 0.2	OR832864

Abbreviations: A, adult; C, cystacanth; L3, third-stage larvae; L4, fourth-stage larvae; M, metacercariae; U, unknown; *N*, number of fish dissected; P, prevalence; MA, mean abundance; SD, standard deviation.
[a] Putative new species closely related to *Corynosoma neostrumosum* Amin et al. (2023) (Fig. 4).
[b] Non-gravid female.

Table 3
Comparative data for helminth faunas in *Gobius niger* studied in the Baltic Sea.

Locality	Archipelago Sea	Dahmeshöved, Lubeck Bight	Dahmeshöved, Lubeck Bight	Blank Eck, Kiel Bight	Dahmeshöved, Lubeck Bight	Dahmeshöved, Lubeck Bight
Source	Present study	Zander et al. (1993)	Zander and Kesting (1998)		Zander (2003, 2004)	
Sample size	38	103[a]	38	70	26	38
Total no. of species	8	8	5	5	8	5
Overall prevalence (%)	71	80	–	–	77	83
No. of taxa in common[c]		3[b]	2[b]	3[b]	2[b]	2[b]
Freshwater forms (%)	25	25	0	0	13	38
Larval forms (%)	63	63	100	100	88	88

[a] Composite sample collected during 1983–1990.
[b] Conditionally considered taxa in common - taxa identified only to the genus level by Zander et al. (1993), Zander and Kesting (1998), and Zander (2003, 2004).
[c] Taxa in common with the present study.

by ecological and geographical data are needed to understand the diversity of this large and non-monophyletic genus, particularly in Europe (Pinacho-Pinacho et al., 2017).

5. Conclusions

This first study of black goby parasites in the Finnish Archipelago Sea revealed four new host records (*D. mergi* Lineage 3, *C. rudolphii* A., *C. semerme* and *Corynosoma* sp.) and three species (*D. mergi* Lineage 3, *B. scorpii* and *Corynosoma* sp.) first recorded in this region of the Baltic Sea. Furthermore, we indicate the possible presence of a not yet formally described species of *Corynosoma*. The observed parasites constitute a mixture of freshwater and marine species, as could be expected in an area equally affected by marine and freshwater influences, but *G. niger* appears to mostly retain its marine parasite fauna. In one instance, that of *D. mergi* Lineage 3, we report a habitat shift from freshwater to a brackish environment and a marine host species for the first time. It would be interesting to know whether *D. mergi* Lineage 3 made this shift in other parts of its range and how far its presence extends into the marine realm.

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Ethical approval

Fishing permits were held through the Archipelago Research Institute of the University of Turku.

CRediT authorship contribution statement

Inga Martinek: Conceptualization, Project administration, Funding acquisition, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Jesús S. Hernández-Orts:** Conceptualization, Formal analysis, Investigation, Visualization, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data collected and analysed during the study are provided in the article and its supplementary files. The newly generated sequences are deposited in the GenBank database under the accession numbers OR831215-OR831226, OR832779-OR832784, OR832864, OR837770 and OR854803-OR854808. The voucher specimens are deposited at the Natural History Museum, London, under the accession numbers NHM2023.11.21.1. and the Institute of Parasitology, České Budějovice, under the accession numbers IPCAS N-860 and IPCAS D-880.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2023.100169.

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