Novel morphological and molecular data on the species of *Gonocerca* Manter, 1925 from off Korea

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INTRODUCTION

Gonocerca Manter, 1925 is a genus of hemiuroid trematodes parasitizing in the digestive tract or ovary of gadiid fishes occurring in deep-sea marine waters. The genus *Gonocerca* is characterized by non-filamentous eggs and testes posterior to ovary and vitellaria. *Gonocerca trematomi* Byrd, 1963 was synonymized with *G. phycidis* Manter, 1925 by Gibson (1976). *Derogenes kobayashii* Layman, 1930 and *Gigantogorgoderina caelorinchi* Machida & Kuramochi, 1994 were transferred to the genus *Gonocerca*. There are currently 15 valid species in the genus. The morphological features that distinguish species in the genus are i.e., body shape, position of ventral sucker, sucker size, lobation of ovary and vitellaria, and testes arrangement.

Gadus macrocephalus Tilesius, 1810 is a major capture fishery source in Korea. However, parasitological surveys of the Pacific cod off Korea have been mainly focused on helminths with medical importance such as larvae of *Anisakis*, and virtually nothing is known on the real helminth diversity of this important to fisheries species. Several parasites such as *Lepidapedon gadi* (Yamaguti, 1934) Acena, 1947, *Derogenes gadi* Shen, 1995, *Eubothrium rugosum* (Batsch, 1786) Nybelin, 1922 , *Grillotia (Grillotia) erinaceus* (van Beneden, 1858) Guiart, 1927, *Nybelinia* sp. plerocercoid-I, scolex polymorphus-II, *Echinorhynchus gadi* Zoega in Müller, 1776, have already been reported in neighboring countries, present study was conducted to examine the diversity of parasites in Korean Pacific cod.

MATERIALS & METHODS

Specimen collection and morphological observation

Twelve Pacific cod fish (*Gadus macrocephalus* Tilesius, 1810 caught off Guryongpo, Pohang were purchased in June, July and October 2021. Trematodes were collected from host stomach and washed in 0.85% saline, fixed with 70% ethanol or AFA solution under coverglass pressure. Ten specimens were stained in Semichon's acetocarmine, dehydrated through a graded ethanol series, cleared in Xylene and mounted in Permount. Morphological observation and measurement were made using Olympus BX53.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted using 5% Chelex extraction method. The partial 28S nuclear ribosomal DNA (D1-D3) was amplified using LSU-5 (5'-TAG GTC GAC CCG CTG AAY TTA AGC A-3') and 1500R (5' -GCT ATC CTG AGG GAA ACT TCG-3'). The PCR reaction was conducted with a total volume of 40 μl consisting of 25 μl of distilled water, 8 μl of ExPCR XO 5x Master Mix (Elpis-biotech), 2 μl of each primer (10 pM), 1 μl of 5X Band Doctor (Solgent) and 2 μl of DNA template. The Mastercycler nexus (Eppendorf) was used with a cycling profile as follows: an initial denaturation step (94°C for 3 min), 40 cycles of amplification (94 °C for 30 s, 56°C for 30 s, 72°C for 2 min) and a final extension step (72°C for 7 min). The PCR products were directly sequenced (CosmoGenetech).

Alignments and the phylogenetic analysis

Newly generated sequences were assembled and trimmed with Geneious 2021.2.2. The published 28S sequences belonging to superfamily Hemiuroidea Looss, 1899 were added using MEGAX and aligned by MAFFT online. Ambiguously aligned positions were excluded by Gblock built in Seaview 5. Genetic distance was calculated using MEGAX. To estimate the best nucleotide substitution model for the dataset, jModelTest 2.1.4 was used. The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) calculated and General Time Reversible (GTR)+ gamma distribution (G) was the most suitable nucleotide substitution model for the dataset. Bayesian inference analyses was performed using MrBayes version 3.2.6 via CIPRES portal (parametes: nchains = 4, nst = 6, rates = invgamma, ngmmacat = 4, sumt burnin = 2,500, sample freq = 1,000; 10,000,000 generations). Maximum Likelihood analyses was carried out using PhyML online (bootstrap = 1000 replicates). Phylogenetic tree was visualized in FigTree v.1.4.4. and Adobe Illustrator 2020.

RESULTS & CONCLUSION

Phylum Platyhelminthes Claus, 1887
Class Trematoda Rudolphi, 1808
Order Plagiorchiida La Rue, 1957
Superfamily Hemiuroidea Looss, 1899
Family Gonocercidae Skrjabin & Guschanskaja, 1955
Genus *Gonocerca* Manter, 1925

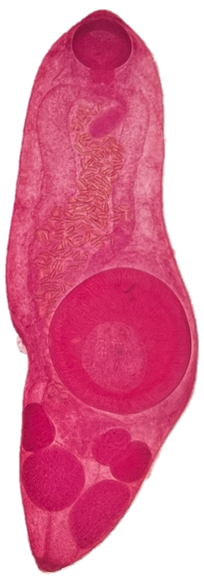

Fig. 1.
Photomicrograph of mature specimen of *Gonocerca* sp. Scale bar = 500 ㎛.

Host: *Gadus macrocephalus*, Pacific cod (Gadiformes: Gadiidae).
Locality: Guryongpo, Pohang, Republic of Korea.
Site of infection: stomach.
Prevalence: 5/12 (41.7%).

Description (Fig. 1).

[Based on nine flattened specimens; seven measured]
Body fusiform, tapering both anterior and posterior extremity, aspinose, widest at ventral sucker region, 2.68-5.31 × 0.84-1.42 ㎜. Pre-oral lip conspicuous. Oral sucker subterminal, globular, 0.24-0.46 × 0.29-0.49 ㎜. Pharynx directly lies after the oral sucker, spherical, 0.11-0.18 × 0.11-0.22 ㎜. Oesophagus very short. Ventral sucker globular, post-equatorial, bigger than oral sucker, 0.52-0.88 × 0.48-0.87 ㎜. Sucker width ratio 1:1.7-1:2.3. Forebody 1.28-2.96 ㎜, 45.4-58.3% to the body. Caeca blind, reaching posterior end of body. Testes two, subglobular, diagonal, lies at end of body. The right testis 0.29-0.54 × 0.25-0.48 ㎜. The left testis 0.30-0.51 × 0.21-0.41 ㎜. Ovary globular, unlobed, some distance behind the acetabulum, 0.20-0.38 × 0.15-0.38 ㎜. Seminal vesicle sacculate, undivided, somewhat curved, 0.16-0.50 × 0.10-0.22 ㎜. Pars prostatica short, opposite cecal bifurcation, surrounded by profuse prostate gland cell forming transverse oval. Vitellaria compact, unlobed, two globular masses, lateral to ovarian level. Seminal receptacle unvisible. Uterus interccaecal, transversally coiled, pre-ovarian. Eggs without filaments, yellow, 47-54 × 22-31 (50 × 26) ㎛ (n = 30).

Table 1
Genetic differentiation (n) of *Gonocerca* spp. by partial 28S sequences (1,064 bp).

No.	Species		1	2	3	4	5	6
1	***Gonocerca* sp. (P220-2)**	**In this study**						
2	***Gonocerca* sp. (P220-1)**		1					
3	*G. oshoro* (KY197013)		5	4				
4	*G. muraenolepisi* (HF543941)		9	8	8			
5	*G. crassa* (KY197012)		17	16	16	18		
6	*G. phycidis* (KY197009)		11	10	10	14	16	

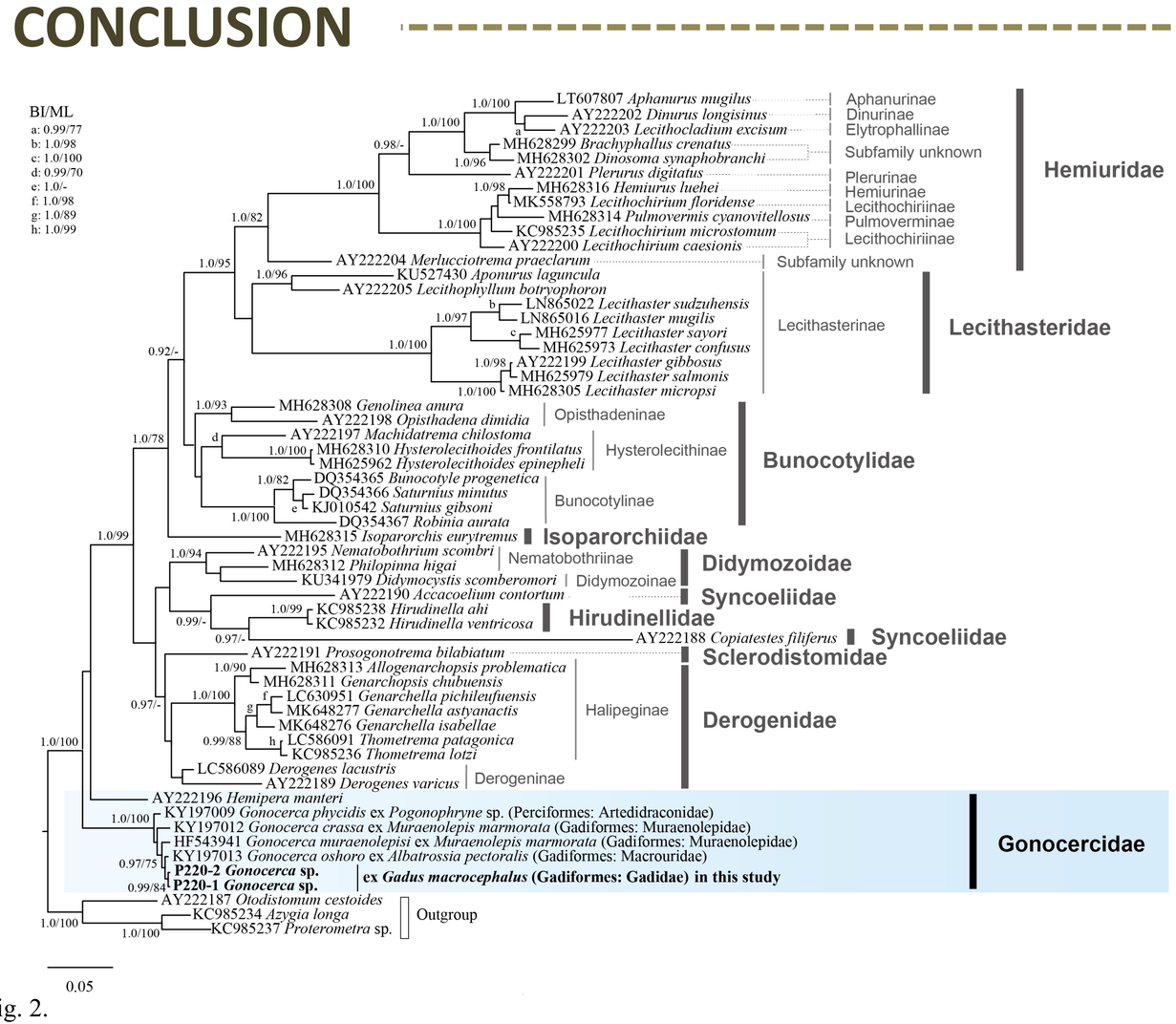

Fig. 2.
Phylogenetic relationships of the superfamily Hemiuroidea Looss, 1899 obtained with Bayesian algorithm and Bayesian inference analysis of 28S rRNA gene sequence. Posterior probabilities less than 0.90 and bootstrap value less than 70 are not indicated. Newly generated sequences in this study are marked in bold.

Conclusion

The present specimen was observed having fusiform body, post-equatorial acetabulum, unlobed ovary and vitellaria, diagonal testes. This specimen is most similar to *Gonocerca crassa* Manter, 1934 in general morphology. However, our specimens differs from *G. crassa* by bigger seminal vesicle and short pars prostatica with prostate gland cell.

As a result of phylogenetic analysis using partial 28S by comparing 877 bases of 57 taxa, we were able to genetically confirm that the newly-generated sequence belongs to the genus *Gonocerca*, and the closest taxon was *G. oshoro* Shimazu, 1970 (Fig. 2). Intra-type variation in species of *Gonocerca* was 0-1 bases and inter-type variation was 4-18 bases (Table 1).

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