First record of Heterorhabdus papilliger (Calanoida, Heterorhabdidae) from Korean waters based on morphological and molecular features

Article · October 2020

DOI: 10.12651/JSR.2021.10.1.078

4 authors:

Seok-Ju Lee
Marine Biological Resource Center
5 PUBLICATIONS **25** CITATIONS
SEE PROFILE

Jeong Man-Ki
Chonnam National University (Yeosu)
24 PUBLICATIONS **74** CITATIONS
SEE PROFILE

Seo Min Ho
marine ecology research center
36 PUBLICATIONS **205** CITATIONS
SEE PROFILE

Ho Young Soh
Chonnam National University, Yeosu
183 PUBLICATIONS **1,575** CITATIONS
SEE PROFILE

Journal of Species Research 10(1):78-85, 2021

First record of *Heterorhabdus papilliger* (Calanoida, Heterorhabdidae) from Korean waters based on morphological and molecular features

Seok Ju Lee[1], Man-Ki Jeong[2], Min Ho Seo[3], Jang Han Choi[4] and Ho Young Soh[5,*]

[1]*Marine Biological Resource Center, Manseong-ro 19, Yeosu 59697, Republic of Korea*
[2]*Fishery Resource Management Institute based on ICT, Chonnam National University, Daehak-ro 50, Yeosu 59626, Republic of Korea*
[3]*Marine Ecology Research Center, Manseong-ro 19, Yeosu 59697, Republic of Korea*
[4]*Department of Environmental Oceanography, Chonnam National University, Daehak-ro 50, Yeosu 59626, Republic of Korea*
[5]*Department of Ocean Integrated Science, Chonnam National University, Daehak-ro 50, Yeosu 59626, Republic of Korea*

**Correspondent: hysoh@chonnam.ac.kr*

Heterorhabdus papilliger (Claus, 1863) is newly reported from the Tsushima Warm Current realm of the southern Korean waters. Its morphological diagnostic characteristics generally agreed well with the original description and the previous records of *H. papilliger*. The female of *H. papilliger* can be recognized by the genital somite, which in lateral view has a more or less rounded genital prominence and an uninflated posterior ventral margin; the second exopodal segment of male right leg 5 with the medial projection with a large, rounded, plumose proximal lobe, and a poorly developed distal lobe. The genetic difference for the partial mtCOI gene between Korean specimens and *H. papilliger* from Spain and Japan of the same clade is 0.4%, while the difference between Korean specimens is 0.5%. However, the interspecific difference for the mtCOI gene between *H. papilliger* from the Korean waters and the other *Heterorhabdus* species is in the range of 14.7–20.8%, suggesting that the former is a valid species.

Keywords: Copepoda, DNA barcoding, mtCOI gene, Korean waters, Tsushima warm current

DOI:10.12651/JSR.2021.10.1.078

Introduction

The family Heterorhabdidae Sars (1902), a typical mesozooplankton from the epipelagic to the bathypelagic zone, is ecologically important in pelagic marine ecosystems because of its high abundance and the various feeding habits as particle feeders or carnivores (Harding, 1974; Hopkins, 1985; Ohtsuka *et al*., 1997). The members of this family are easily distinguished from other calanoid families owing to their specialized characters in the left caudal ramus (i.e., fused with the anal segment and with greatly elongated marginal seta) and the presence of a large and plumose inner lobe in the basis of male right leg 5 (Park, 2000). This family consists of 67 species in eight genera worldwide (Walter and Boxshall, 2020) and is the first record of the family Heterorhabdidae from Korea.

Heterorhabdus Giesbrecht, 1898 is the most common and species-rich genus in the family. This genus was first established by Claus (1863) based on *Heterochaeta spinifrons* Claus, 1863 and *H. papilligera* Claus, 1863. Giesbrecht and Schmeil (1898) later changed from *Heterochaeta* to *Heterorhabdus* because the name *Heterochaeta* was already occupied. Wolfenden (1911) erected the genus *Alloiorhabdus* for two heterorhabdid species, *Heterorhabdus austrinus* Giesbrecht, 1902 and *Alloiorhabdus medius* Wolfenden, 1911. However, the genus was placed in synonymy with *Heterorhabdus*. Recently, the genus was redefined by Park (2000) based on the most important characters, including the spiny papilla on the sternite of the first pedigerous somite and a saber or falciform spine on the coxa of maxilliped. Park (2000) also divided the genus into four species groups (*spinifrons*, *papilliger*, *fistulosus*, and *abyssalis*) using the morphological similarities in the maxilliped, maxilla, and the fifth leg of male among the recognized species.

Heterorhabdus papilliger has been reported from the epipelagic zone in the equatorial Pacific Ocean, North Atlantic Ocean, Japan, New Zealand, North America, and Indonesia, after the first description from the Mediterranean Sea. Although this species is widely distributed

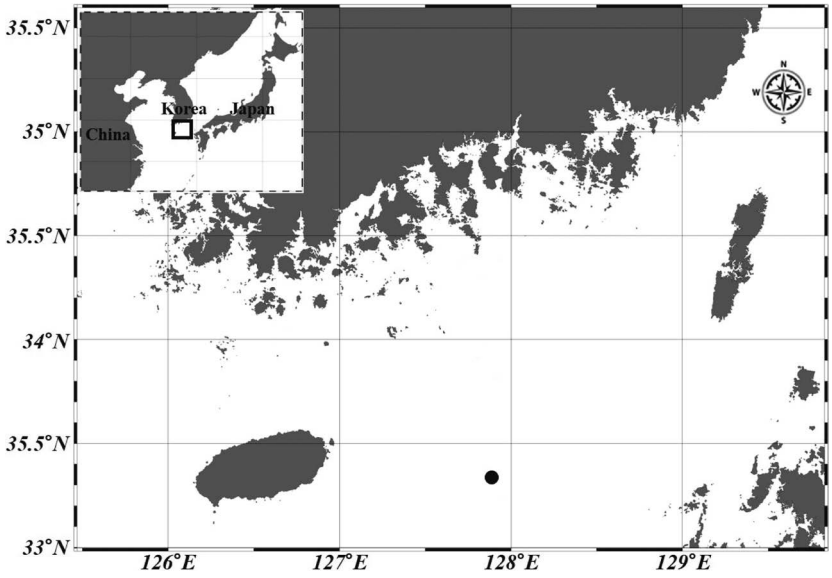

Fig. 1. Map of study area showing sampling location.

worldwide, it had not been taxonomically reported in Korean waters to date (Claus, 1863; Giesbrecht, 1892; Sars, 1925; Ohtsuka *et al*., 1997; Bradford-Grieve, 1999; Park, 2000; Mulyadi, 2004).

This study describes *Heterorhabdus* species from Korean waters and clarifies the taxonomic status by comparing morphological characteristics and the partial mtCOI gene with those of six other *Heterorhabdus* species.

Materials and Methods

Zooplankton samples were collected from the southern offshore of Jeju Island, Korea (Fig. 1) using a Multiple Opening/Closing Net and Environmental Sampling System equipped with 200 μm mesh size (BESS, USA). After dividing the collected samples, one of the samples was fixed in 95% ethanol for DNA analysis and the other final concentration of 5% with neutralized formaldehyde for morphological description. *Heterorhabdus papilliger* sorted in the latter samples was dissected under a dissecting microscope (SMZ745T, Nikon, Tokyo, Japan) in CMC-10 aqueous mounting medium (Masters, Wood Dale, IL, USA), mounted on slides, and then sealed with high-quality nail varnish. Drawings were generated using a differential interference contrast microscope (ECLIPES 80i, Nikon, Tokyo, Japan) equipped with a drawing tube and digital pen display (Cintiq 22HD, Wacom, Kazo, Japan). Morphological terminology follows Huys and Boxshall (1991). Voucher specimens were deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seocheon, South Korea.

To extract genomic DNA from *Heterorhabdus papilliger*, 1.5 mL centrifuge tubes containing 145 μL of 10% Chelex suspension (Bio-Rad Laboratories Inc., Hercules, CA, USA), 5 μL of Proteinase K (25 mg/mL, Bioneer, Daejeon, Korea), and its dissected tissues were incubated at 56°C, for 2 hours. To verify the genetic features of the Korean specimens, partial sequences of mitochondrial cytochorome c oxidase subunit I (mtCOI) genes were amplified using primers made by Folmer *et al*. (1994). The PCR protocol was 94°C for 1 min, 48°C for 1 min, and 72°C for 1 min, for 35 cycles. The sizes of obtained sequences for mtCOI were 592−610 base pairs. The sequences of the Korean specimens were edited using Chromas software version 2.3 (Technelysium Pty Ltd., Brisbane, Australia) and were aligned with the sequences available in the public database (GenBank) using the Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 (Kumar *et al*., 2016). These aligned sequences were used as a dataset to calculate genetic divergences using Kimura 2-parameter (K2P) model and construct neighbor-joining (NJ) phylogenetic trees with 1000 bootstrapping replicates (Kimura, 1980).

Systematic Accounts

Order Calanoida Sars G.O., 1903
Family Heterorhabdidae Sars G.O., 1902
Genus *Heterorhabdus* Giesbrecht, 1898

***Heterorhabdus papilliger* (Claus, 1863) (Figs. 2−4)**
Heterochaeta papilligera Claus, 1863, p. 182, pl. 32, figs.

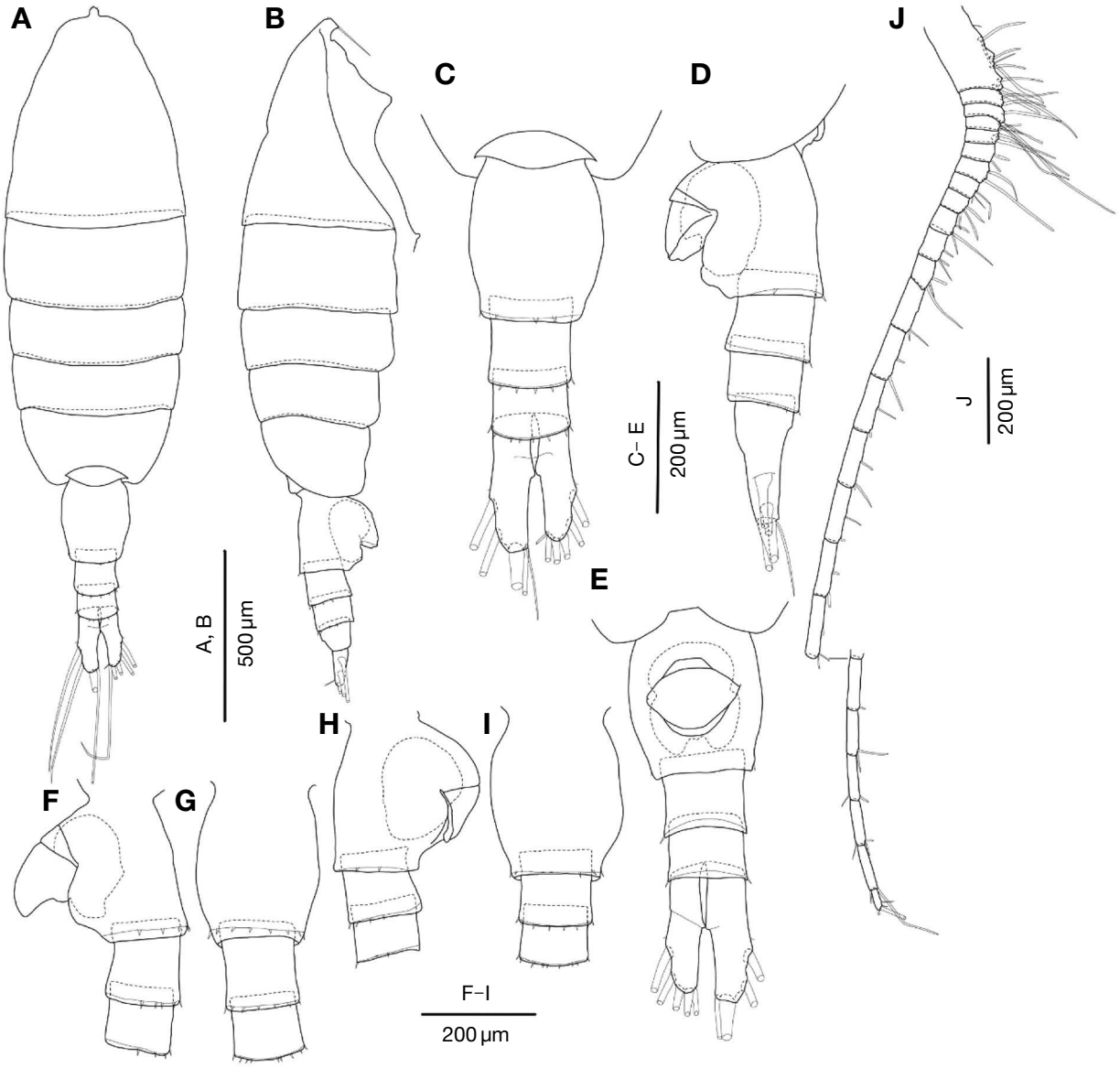

Fig. 2. *Heterorhabdus papilliger*, Female. A. habitus, dorsal; B. habitus, right lateral; C. urosome, dorsal; D. urosome, left lateral; E. urosome, ventral; F. genital somite from different specimen, left lateral; G. genital somite from different specimen, dorsal; H. genital somite from different specimen, right lateral; I. genital somite from different specimen, dorsal; J. antennule.

10–13, 15; Giesbrecht, 1892, p. 372, pl. 20, figs. 4, 7, 8, 10, 15, 17, 22, 23, 34–36, pl. 39, figs. 40, 53.

Heterorhabdus papilliger: Sewell, 1932, p. 300, fig. 97; Bradford-Grieve, 1999, p. 83, fig. 50; Park, 2000, p. 106, fig. 75; Mulyadi, 2004, p. 186, fig. 106.

Materials examined. (MABIK CR00247438) one female dissected and mounted on seven slides, collected from off Jeju Island, Korea (33°25′N, 127°53′E) on 17 May, 2019; (MABIK CR00247439); one male dissected and mounted on four slides, same locality as the above female specimen. Six additional individuals from the same locality were used for molecular analysis and length measurement.

Description. Female. Body length 1.96–2.15 mm (n = 4). Prosome length 1.39–1.50 mm. Body elongate; cephalosome clearly separate from first pedigerous somite, with groove halfway along dorsal margin; anterior margin of cephalosome round in dorsal view, with tubercular rostrum in mid-anterior part; rostrum with a pair of slender filaments; fourth and fifth pedigerous somites fused (Fig 2A, B). Posterior margin of prosome symmetrical and broadly rounded (Fig. 2A, B). Urosome composed of four somites, fourth somite incompletely fused with caudal rami; genital double-somite widest at middle, smoothly inflated

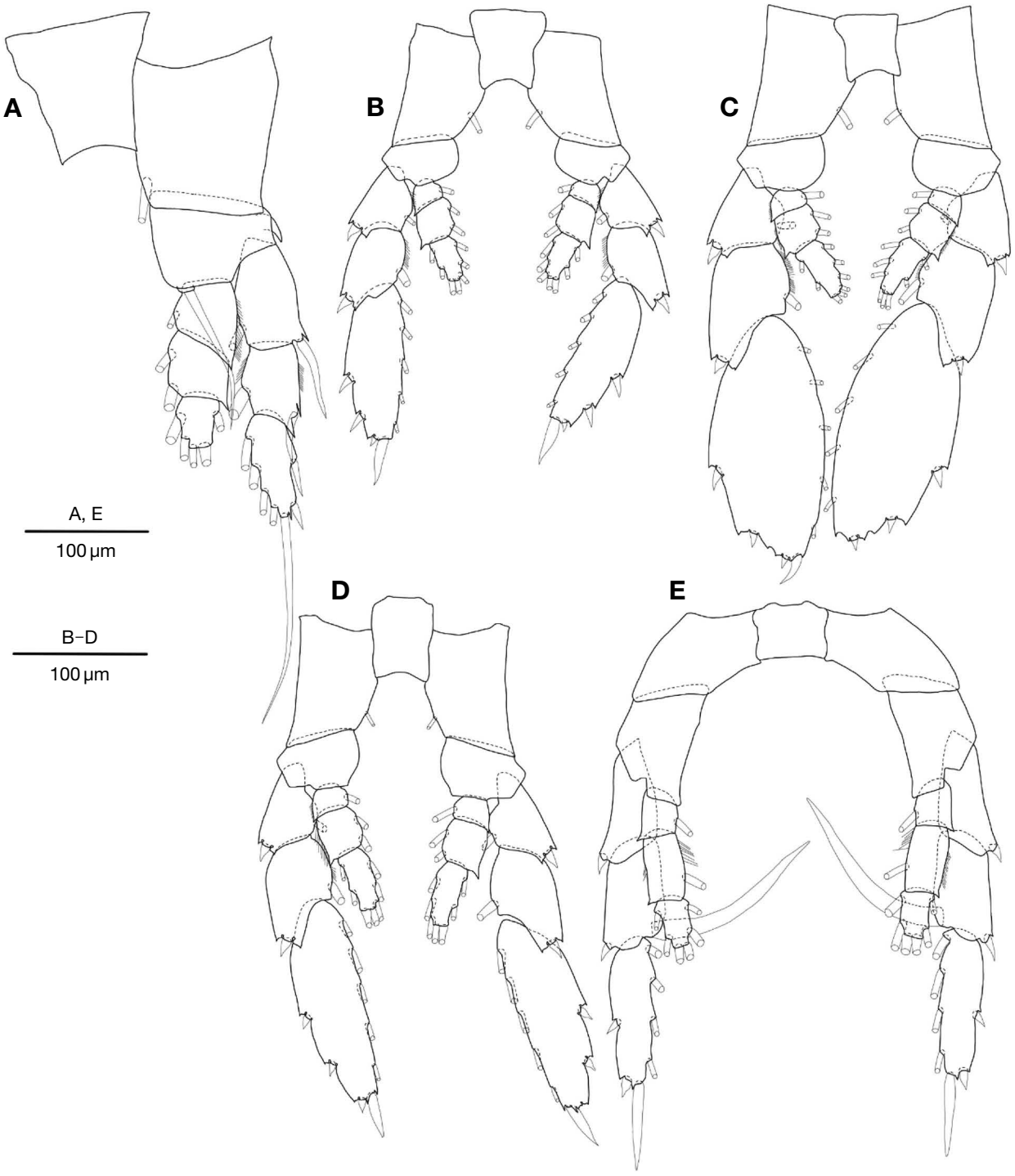

Fig. 3. *Heterorhabdus papilliger*, Female. A. leg 1; B. leg 2; C. leg 3; D. leg 4; E. leg 5.

dorsally, greatly protruded ventrally, almost symmetrical laterally, with ratio of with width-length ratio of 80 : 100; first three urosomites each with row of triangular spinules on dorsoposterior margin (Fig. 2A–E). Proportional lengths of four urosomites and left caudal ramus 38 : 19 : 15 : 85 : 20 (= 100). Caudal rami and anal segment indistinctly separated (Fig. 2C, D). Left caudal ramus extending beyond posterior end of right ramus by about 1/6 its length as measured along medial margin (Fig. 2C). Dorsal appendicular seta of left caudal ramus little longer than

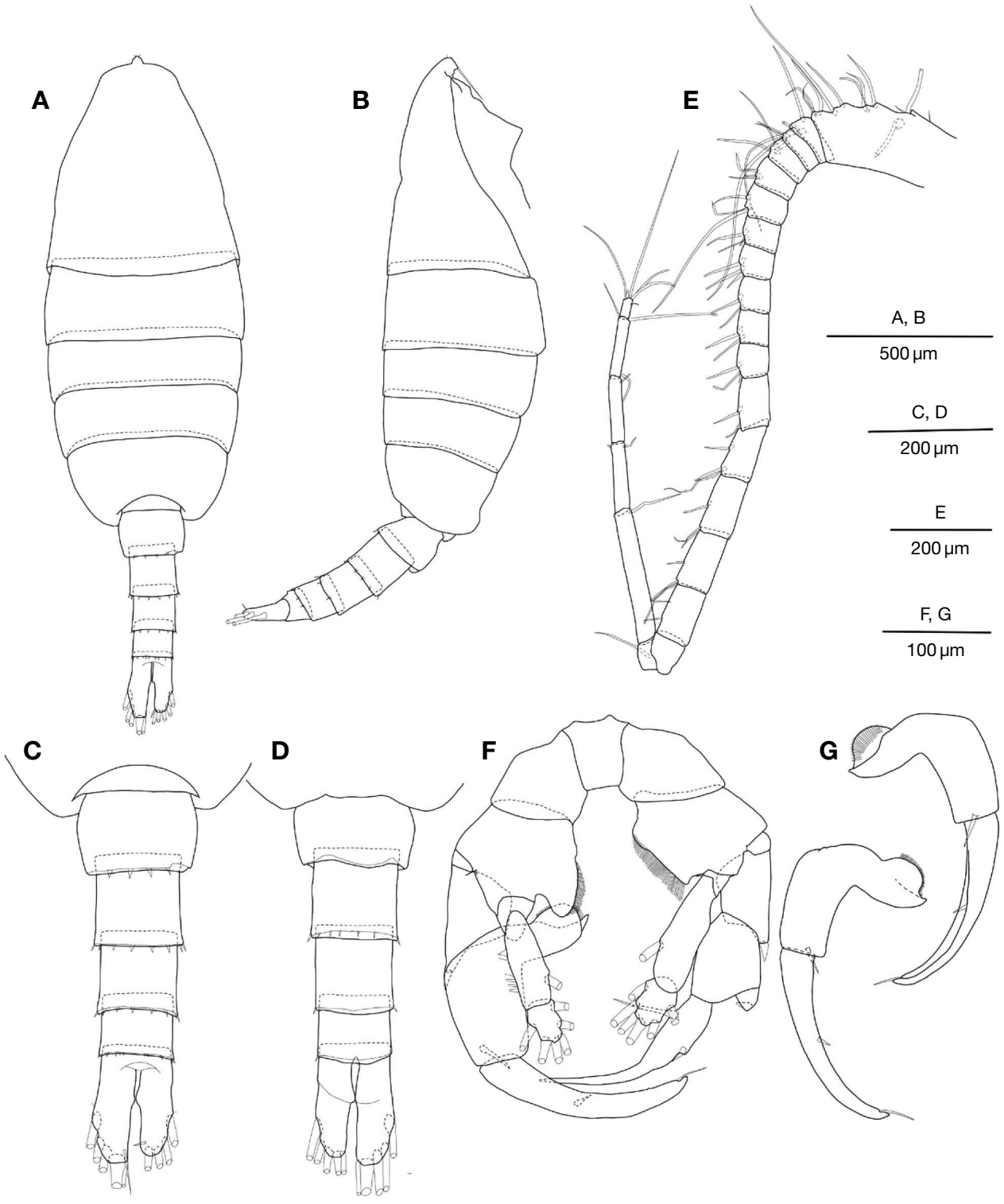

Fig. 4. *Heterorhabdus papilliger*, Male. A. habitus, dorsal; B. habitus, right lateral; C. urosome, dorsal; D. urosome, ventral; E. antennule; F. leg 5 (anterior); G. exopod of left leg 5 (left one: anterior, right one: posterior).

that of right caudal ramus. Fourth marginal seta of left ramus much thicker than other marginal setae and longer than body (Fig. 2C, E).

Antennule reaching about posterior end of third uroso-

mite, 25-segmented; not all aesthetes clearly distinguishable from setae (Fig. 2J). Segments 2–19 each with 1 middle and 2 distal setae/aesthetes (Fig. 2J).

Legs 1 to 4 biramous, each with 3-segmented endopod and 3-segmented exopod; with inner marginal seta at coxa (Fig. 3A–D). Basis of leg 1 with inner marginal seta, have small hooklike process on outer margin (Fig. 3A). Basis of leg 2 to 4 without seta (Fig. 3B–D). Setae and spine formula of leg 1 to 4 as follows (spines, Roman numerals; setae, Arabic numerals):

	Coxa	Basis	Exopodal segment	Endopodal segment
Leg 1	0-1	0-1	I-1; I-1; II,I,4	0-1; 0-2; 1,2,2
Leg 2	0-1	0-0	I-1; I-1; III,I,5	0-1; 0-2; 2,2,3
Leg 3	0-1	0-0	I-1; I-1; III,I,5	0-1; 0-2; 2,2,4
Leg 4	0-1	0-0	I-1; I-1; III,I,5	0-1; 0-2; 2,2,3

Leg 5 symmetrical; basipod, endopod, third exopodal segment, and inner spine of second exopodal segment similar in length (Fig. 3E). Endopod extending beyond distal end of second exopodal segment. Distolateral corners of first and second endopodal segments pointed; inner marginal setae provided with long setules for proximal halves and short setules for distal halves (Fig. 3E). Outer spines of exopod relatively small, all pointing in a distolateral direction.

Male. Body length 1.96–2.00 mm (n = 2). Prosome length 1.38–1.42 mm. Similar in habitus to female except urosome. Urosome 5-segmented; first to fourth urosomites each with row of triangular spinules on dorsoposterior margin; only second urosomite with row of triangular spinules on posterior margin in ventral side (Fig. 4A–D). Leg 1 to 4 similar to female.

Left antennule geniculate, reaching about half of urosome; first two segments fused, segments 19 to 21 fused, segments 22 and 23 fused (Fig. 4E).

Leg 5 asymmetrical (Fig. 4F). Inner lobe of right basis relatively narrow, slightly shorter than 1/2 length segment. Inner lobe of left basis low but clearly distinguishable, and distally produced into short process. In right exopod, medial projection of second segment with large, rounded proximal lobe and without distinguishable distal lobe; whole distal margin of medial projection smoothly curved and merged into relatively large terminal spiniform process (Fig. 4G); outer spine of second segment relatively long and arising close to distal end of segment. Third segment of right exopod smoothly curved, about as long as combined lengths of first 2 segments; its outer spine small, located distal to midpoint of segment; terminal spine about 1/6 length of segment, and terminal lobe about 2/5 length of terminal spine (Fig. 4G). In left exopod, second segment with large lateral conical process terminating with small outer spine; outer spine about 2/3 length of conical process (Fig. 4F). Third segment of left exopod tapering distally into rather spiniform process, with small outer and long inner spine (Fig. 4F).

Distribution. *Heterorhabdus papilliger* from Korean waters was mainly collected at a depth of 75 m of the Tsushima Warm Current off Jeju Island, Korea with water temperature and salinity of about 17.1°C and 34.6 psu, respectively. All specimens obtained were adults.

Remarks. The Korean specimens agree well with the original description and former records of *Heterorhabdus papilliger* by Park (1968, 2000): the genital double somite of

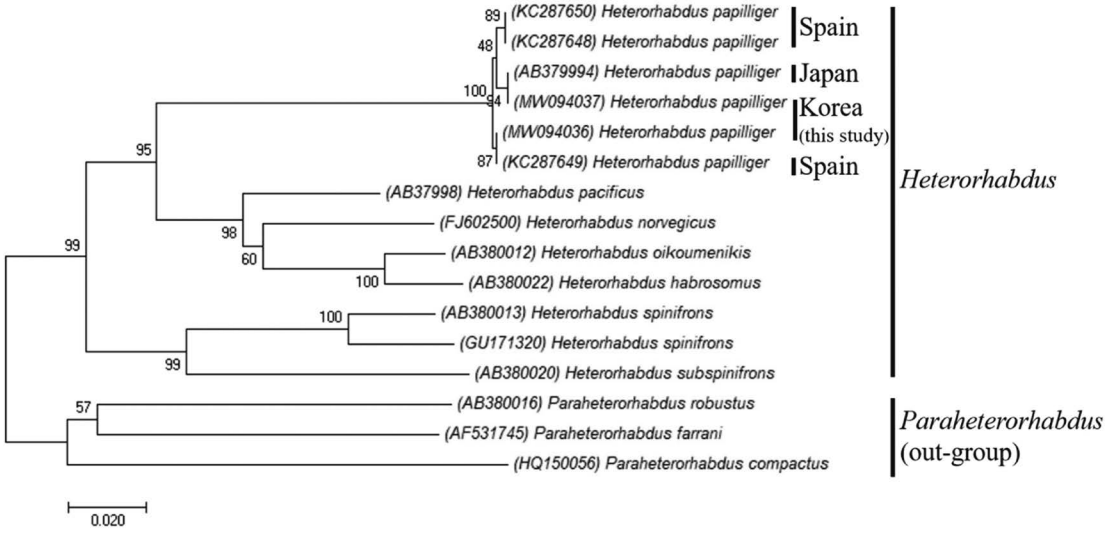

Fig. 5. Molecular phylogenetic analysis by the neighbor-joining method (Kimura 2-parameter model). Numbers in parentheses indicate GenBank accession number. Numbers at branch points indicate bootstrap values (1000 replicates).

Table 1. Mean genetic distances between examined *Heterorhabdus* species based on K2P distance.

		1	2	3	4	5	6	7	8	9
1	*H. papilliger* (Korea) (MW094037, MW094036)									
2	*H. papilliger* (Spain) (KC287650, KC287648, KC287649)	0.003								
3	*H. papilliger* (Japan) (AB379994)	0.005	0.005							
4	*H. pacificus* (AB37998)	0.147	0.151	0.153						
5	*H. oikoumenikis* (AB380012)	0.168	0.173	0.170	0.092					
6	*H. norvegicus* (FJ602500)	0.170	0.175	0.173	0.086	0.096				
7	*H. habrosomus* (AB380022)	0.175	0.179	0.177	0.084	0.033	0.102			
8	*H. spinifrons* (AB380013, GU171320)	0.199	0.199	0.197	0.169	0.162	0.178	0.176		
9	*H. subspinifrons* (AB380020)	0.204	0.208	0.208	0.180	0.177	0.177	0.177	0.132	

H. papilliger females having in lateral view a more or less rounded genital prominence and an uninflated posterior ventral margin; the second exopodal segment of male right leg 5 with the medial projection with a large, rounded, plumose proximal lobe, and a poorly developed distal lobe (Park, 2000). Additionally, we found some minor morphological features in the examined Korean specimens that were not mentioned in previous records of *H. papilliger*. The number of spinules on the posterior margin of each urosomite found from the dorsolateral side varied from 5 to 10 depending on the individual (Fig. 2F–I; Fig. 4C). In all examined male specimens (n = 3), these marginal spinules were also present on the ventral side of the second urosomite (Fig. 4D), but the ventral spinules were not found on any urosomites of the female specimens (Fig. 2E). In spite of these morphological differences, the genetic difference for the partial mtCOI gene between Korean specimens (MW094036 and MW094037) and *H. papilliger* from Spain and Japan is only 0.4%, while the difference between Korean specimens is 0.5% (Table 1; Fig. 5). However, the interspecific difference between *H. papilliger* from the Korean waters and the other six *Heterorhabdus* species was in the range of 14.7–20.8% (Table 1) and similar between calanoid copepods (Soh *et al*., 2012; Jeong *et al*., 2014). Therefore, the morphological and molecular comparison results support the occurrence of *H. papilliger* from Korean waters.

Acknowledgements

This study was supported by grants from the National Marine Biodiversity Institute of Korea (2021M01100).

References

Bradford-Grieve, J.M. 1999. The marine fauna of New Zealand: Pelagic calanoid Copepoda: Bathypontiidae, Arietellidae, Augaptilidae, Heterorhabdidae, Lucicutiidae, Metridinidae, Phyllopodidae, Centropagidae, Pseudodiaptomidae, Temoridae, Candaciidae, Pontellidae, Sulcanidae, Acartiidae, Tortanidae. NIWA Biodiversity Memoirs 111:70-92.

Claus, C. 1863. Die frei lebenden Copepoden mit besonderer Berücksichtigung der Fauna Deutschlands, der Nordsee und des Mittelmeeres. Verlag Von Wilhelm Engelmann. Leipzig.

Folmer, O., M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan inver-tebrates. Molecular Marine Biology and Biotechnology 3(5):294-299.

Giesbrecht, W. 1892. Systematik und Faunistik der pelagischen Copepoden des Golfes von Neapel und der angrenzenden Meeres-Abschnitte: Fauna und Flora des Golfes von Neapel, Berlin: Verlag Von R Friedländer and Shon, Berlin.

Giesbrecht, W. 1902. Zoologie: Copepoden. Resultats du voyage du S.Y. Belgica en 1897-1898-1899. Rapports Scientifiques, Buschmann, Anvers.

Giesbrecht, W. and O. Schmeil. 1898. Copepoda: I. Gymnoplea. Verlag Von R Friedländer and Shon, Berlin.

Harding, G.C.H. 1974. The food of deep-sea copepods. Journal of the Marine Biological Association of the United Kingdom 54(1):141-155.

Hopkins, T.L. 1985. Food web of an Antarctic midwater ecosystem. Marine Biology 89(2):197-212.

Huys, R. and G.A. Boxshall. 1991. Copepod evolution. Ray Society, London.

Jeong, H.G., H.Y. Soh and H.L. Suh. 2014. Morphological and genetic differentiation of heteromorphy in *Labidocera rotunda* (Copepoda, Calanoida, Pontellidae). Zootaxa 3764(2):181-191.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2):111-120.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology Evolution 33(7):1870-1874.

Mulyadi, M.D. 2004. Calanoid Copepods in Indonesian Waters. Research Center for Biology, Indonesia Institute of Sciences, Bogor.

Ohtsuka, S., H.Y. Soh and S. Nishida. 1997. Evolutionary switching from suspension feeding to carnivory in the calanoid family Heterorhabdidae (Copepoda). Journal Crustacean Biology 17(4):577-595.

Park, T. 1968. Calanoid copepods from the central North Pacific Ocean. Fishery Bulletin 66(3):527-572, pls. 1-13.

Park, T. 2000. Taxonomy and distribution of the calanoid copepod Family Heterorhabdidae. Bulletin of the Scripps Institution of Oceanography, University of California 31:1-269.

Sars, G.O. 1902. An Account of the Crustacea of Norway with Short Descriptions and Figures of all the Species. Bergen Museum, Bergen.

Sars, G.O. 1903. An account of the Crustacea of Norway with Short Descriptions and Figures of all the Species. Bergen Museum, Bergen.

Sars, G.O. 1925. Copépodes particulierement bathypélagique provenant des carnpagnes scientifique du Prince Albert 1er de Monaco. Resultats des Campagnes Scientifiques accomplies par le Antarctic Metridia Prince Albert I, Monaco.

Sewell, R.B.S. 1932. The Copepoda of Indian seas. Calanoida. Memoirs of the Indian Museum 10:223-407, text figs. 82-131.

Soh, H.Y., S.W. Kwon, W. Lee and Y.H. Yoon. 2012. A new *Pseudodiaptomus* (Copepoda, Calanoida) from Korea supported by molecular data. Zootaxa 3368(1):229-224.

Walter, T.C. and G.A. Boxshall. 2020. World of Copepods database. Heterorhabdidae Sars G.O., 1902. [http://www.marinespecies.org/aphia.php?p=taxdetails&id=104087/, accessed 05 October 2020].

Wolfenden, R.N. 1911. Die marinen Copepoden der deutschen Südpolar-Expedition 1901-1903. 2. Die pelagischen Copepoden der West-wind drift und des südlichen Eismeers mit Beschreibung mehrerer neuer Arten aus dem Atlantischen Ozean. Deutsche Südpölar-Expedition 12(2):181-380.

Submitted: October 27, 2020
Revised: November 10, 2020
Accepted: November 10, 2020