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The First Record of the *Marphysa victori* (Polychaeta, Eunicida, Eunicidae) from Korea, with DNA Barcode Data

Hana Kim[1,2], Keun-Yong Kim[3], War War Phoo[4], Chang-Hoon Kim[4,*]

[1]Department of Taxonomy and Systematics, National Marine Biodiversity Institute of Korea, Seocheon 33662, Korea
[2]Department of Biological Sciences, Inha University, Incheon 22212, Korea
[3]Department of Genetic Analysis, AquaGenTech Co., Ltd., Busan 48300, Korea
[4]Department of Marine Bio-materials and Aquaculture, Pukyong National University, Busan 48513, Korea

ABSTRACT

A eunicid polychaete, *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 is described for the first time from the intertidal zones of the Korean coasts. It is characterized by having three types of pectinate chaetae (INS, isodont-narrow-slender; AWS, anodont-wide-slender; and AWT, anodont-wide-thick), appearance of pectinate chaetae from chaetiger 2, the chaetae consisted of pectinate and compound spinigers, and pygidium with one pair of pygidial cirri. In genetic analysis based on cytochrome *c* oxidase subunit I (*COI*), intra-specific genetic distance between the specimens of *M. victori* from its type locality, France and Korea are in the range of 0.000−0.013. This paper includes the morphological description and photographs of *M. victori* new to Korean fauna, with partial sequences of the mitochondrial *COI* as DNA barcode data on this species.

Keywords: DNA barcode, Korea, *Marphysa victori*, Polychaeta, taxonomy

INTRODUCTION

The genus *Marphysa* is one of the species-rich groups of the eunicid genera including 72 described species (Lavesque et al., 2020; Read and Fauchald, 2020). The members of *Marphysa* generally inhabit intertidal shores, and are widely used as bait in recreational fishing (Hutchings and Karageorgopoulos, 2003; Glasby and Hutchings, 2010; Lavesque et al., 2017; Cole et al., 2018). This genus is characterized by the following diagnostics: the presence of three antennae and two lateral palps, notopodial branchiae, and the presence of distinct pectinate chaetae on the median and posterior parapodia (Glasby and Hutchings, 2010; Zanol et al., 2017; Wang et al., 2018).

The type species of the genus, *Marphysa sanguinea* (Montagu, 1813) had been recognized as a cosmopolitan species (Hutchings and Kupriyanova, 2018) with a very brief original description. Hutchings and Karageorgopoulos (2003) re-described this species with neotype specimens and suggested that all records of this species from outside the type locality should be checked. Thereafter, this species has been re-evaluated as another species, instead of *M. sanguinea*, *M. mullawa* (Hutchings and Karageorgopoulos, 2003), *M. elityeni* (Lewis and Karageorgopoulos, 2008), *M. fauchaldi* (Glasby and Hutchings, 2010), *M. kristina* (Zanol et al., 2016), *M. multipectinata*, *M. tribranchiata*, *M. tripectinata* (Liu et al., 2017), *M. pseudosessiloa* (Zanol et al., 2017), *M. victori* (Lavesque et al., 2017), *M. aegypti* (Elgetany et al., 2018), *M. hongkongensa* (Wang et al., 2018), *M. maxidenticulata* (Liu et al., 2018), *M. iloiloensis* (Glasby et al., 2019), *M. baileybrockae*, *M. birgeri* (Molina-Acevedo and Idris, 2020), *M. gaditana* and *M. chirigota* (Martin et al., 2020) based on the taxonomic work of Hutchings and Karageorgopoulos (2003). They pointed out that the structure and distribution of pectinate chaetae are important specific characteristics of the *Marphysa* group (Liu et al., 2017).

In Korean waters, *M. sanguinea* was recorded as a cosmopolitan species (Rho and Song, 1975; Paik, 1975, 1989; Rho and Lee, 1987, 1988; Lee, 1998) without detailed taxonomic consideration on the structure and distribution of pectinate

*To whom correspondence should be addressed
Tel: 82-51-629-5910, Fax: 82-51-629-5908
E-mail: chkpknu@hanmail.net

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chaetae. Hence, we carefully examined the *Marphysa* specimens collected from some Korean waters in accordance with modern taxonomy.

In this study, we found a new to Korean fauna, *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017, and provide its detailed description and images with DNA barcode data on this species.

MATERIALS AND METHODS

Sampling and morphological observations

Specimens were collected from muddy sand of intertidal zones of Taean-gun, Chungcheongnam-do, Geoje-si, Gyeongsangnam-do and Hallim-eup, Jeju-do, fixed with 95% ethyl alcohol for both morphological and genetic analyses. Morphological observation was carried out with appendages dissected in a petri-dish using a pair of dissection forceps and surgical knives under a stereomicroscope (Carl Zeiss Axioskop II, Göttingen, Germany). The dissected materials were mounted onto temporary slides using glycerol or permanent slides using polyvinyl lactophenol solution. Photographs were captured by an image system, LAS V4.7 (Leica Microsystems, Heerbrugg, Switzerland). For scanning electron microscopy the materials were dehydrated by t-butyl alcohol freeze dryer, VFD-21S (Vacuum Device, Ibaraki, Japan). They were mounted on stubs and coated with gold-palladium.

Observation was carried out using a scanning electron microscope, SU3500 (Hitachi, Tokyo, Japan). The voucher specimens were deposited in the National Marine Biodiversity Institute of Korea under specimen numbers, MABIKNA 00156461–00156467.

Molecular analysis

Genomic DNA (gDNA) of five specimens were extracted from the tissue of muscle in posterior segments using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacture's protocol. Amplifications of partial sequences of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) were carried out by polymerase chain reaction (PCR) method using a set of primers, Poly-MT-00001f (5′-ATGCGMTGAYTTTACTCWAC-3′) and Poly-MT-01105r (5′-TGGAARTGGGCTACTACATA-3′), newly designed in this study. PCR amplification was conducted in a total volume of 20 μL: 10 μL of 2× DyeMIX-Tenuto (Enzynomics, Daejeon, Korea), 0.5 μL of each primer, 1 μL of gDNA, and 8 μL of

Table 1. A list of *Marphysa* species, type localities, collection localities, Genbank accession numbers, voucher numbers and references

Species	Type locality	Collection locality	GenBank accession No.	Voucher No.	Reference
M. aegypti	Suez Canal, Egypt	Suez Canal, Egypt	MF196968	–	Elgetany et al. (2018)
M. bifurcata	WA, Australia	Qld, Australia	KX172177	–	Zanol et al. (2016)
M. brevitentaculata	Tobago	Quintana Roo, Mexico	GQ497548	–	Zanol et al. (2010)
M. californica	California, USA	California, USA	GQ497552	–	Zanol et al. (2010)
M. disjuncta	California, USA	California, USA	GQ497549	–	Zanol et al. (2010)
M. fauchaldi	NT, Australia	NT, Australia	KX172165	–	Zanol et al. (2016)
M. hongkonensa	Hong Kong	Hong Kong	MH598526	–	Wang et al. (2018)
M. iloiloensis	Iloilo, Philippines	Tigbauan, Philippines	MN106279	–	Glasby et al. (2019)
M. kristiani	NSW, Australia	NSW, Australia	KX172152	–	Zanol et al. (2016)
M. mossambica	Mozambique	Iloilo, Philippines	KX172164	–	Zanol et al. (2016)
M. mullawa	Qld, Australia	NSW, Australia	KX172166	–	Zanol et al. (2016)
M. pseudosessiloa	NSW, Australia	NSW, Australia	KY605405	–	Zanol et al. (2010)
M. sanguinea	Devon, UK	Callot Island, France	GQ497547	–	Zanol et al. (2010)
M. tripectinata	Beihai, China	Beihai, China	MN106271	–	Glasby et al. (2019)
M. victori	Arcachon Bay, France	Arcachon Bay, France	MG384996	AM W.49047	Lavesque et al. (2017)
M. victori	Arcachon Bay, France	Arcachon Bay, France	MG384997	MNHN-IA-TYPE 1803	Lavesque et al. (2017)
M. victori	Arcachon Bay, France	Arcachon Bay, France	MG384998	MNHN-IA-TYPE 1804	Lavesque et al. (2017)
M. victori	Arcachon Bay, France	Arcachon Bay, France	MG384999	MNHN-IA-TYPE 1806	Lavesque et al. (2017)
M. victori	Arcachon Bay, France	Jeju-si, South Korea	MT396174	MABIKNA00156467	Present study
M. victori	Arcachon Bay, France	Taean-gun, South Korea	MT396176	MABIKNA00156465	Present study
M. victori	Arcachon Bay, France	Taean-gun, South Korea	MT396175	MABIKNA00156464	Present study
M. victori	Arcachon Bay, France	Geoje-si, South Korea	MT396173	MABIKNA00156462	Present study
M. victori	Arcachon Bay, France	Geoje-si, South Korea	MT396172	MABIKNA00156461	Present study
M. viridis	Florida, USA	Ceara, Brazil	GQ497553		Zanol et al. (2010)

sterile water. Hotstart-PCR was carried out according to the following cycling program: 94°C for 5 min, 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, followed by 35 cycles, and final extension at 72°C for 7 min. The PCR products were purified with a QIAquick PCR Purification Kit (Qiagen, Chatsworth, CA, USA). Sequences for the PCR products were obtained by an Applied Biosystems 3730*xl* DNA sequencer and deposited in National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) under GenBank accession numbers, MT396172−MT396176. The newly obtained *COI* sequences of *M. victori* were aligned with those of other *Marphysa* species (Table 1) using Geneious Pro v.9.1.8 (Biomatters, Auckland, New Zealand). The genetic distances were calculated using the Kimura-2-parameter model in MEGA X software (Kumar et al., 2018).

SYSTEMATIC ACCOUNTS

[1]*Order Eunicida Fauchald, 1977

[2]*Family Eunicidae Berthold, 1827

[3]*Genus *Marphysa* Quatrefages, 1866

[4]***Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 (Figs. 1, 2)**

Marphysa victori Lavesque, Daffe, Bonifácio & Hutchings, 2017: 6, figs. 2−4.

Marphysa bulla Liu, Hutchings & Kupriyanova, 2018: 6, figs. 2−4 (subjective synonym).

Material examined. South Korea: three specimens (MABIKNA00156464−00156466), Chungcheongnam-do: Taeangun, Iwonmyeon, 36°54′04.5″N, 126°16′53.7″E, 4 Jul 2015, Kim CH; three specimens (MABIKNA00156461−00156463), Gyeongsangnam-do: Geoje-si, Sadeung-myeon, 34°52′29.0″N, 128°28′23.6″E, 9 Mar 2016, Kim CH; one specimen (MABIKNA00156467), Jeju-do: Jeju-si, Hallim-eup, 33°24′17.0″N 126°13′43.1″E, 28 Mar 2018, Kim CH. All specimens were collected from muddy sand of intertidal zones.

Description. Body, complete, up to 20.9 cm long (9.1−20.9 cm) and 0.9 cm wide (0.65−0.9 cm). Prostomium shorter than peristomium, as wide as peristomium, bilobed with upper lips separated by deep ventral and dorsal sulcus with each lobe semi-rounded anteriorly. Eyespots present, positioned between palps and lateral antennae. Prostomial appendages smooth, slightly tapering; median antenna longest, and then lateral antenna, palps shorter than antennae. Antennophores and palpophores smooth with surface slightly wrinkled. First ring of peristomium longer than second ring of peristomium (2 to 3 times) (Fig. 1A, B).

Maxillary apparatus with four pairs and one single of maxillae. Maxillary formula: I = 1 + 1, II = 4 + 4, III = 5 − 6 + 0, IV = 3 − 4 + 6 − 7, V = 1 + 1 (Fig. 1E).

Parapodia with inconspicuous pre-chaetal neuropodial lobe. Post-chaetal neuropodial lobe conical in anterior parapodia, thereafter gradually getting wider and rounded; longer than chaetal lobe in anterior chaetigers, shorter in median and posterior chaetigers (Fig. 1C). Dorsal cirri triangular, longer than chaetal lobe in anterior chaetigers, shorter than chaetal lobe in median chaetigers and as long as chaetal lobe in posterior chaetigers. Ventral cirri tapering, approximately as long as dorsal cirri in anterior chaetigers, but inflated base of round shape with round tip, shorter than dorsal cirri in medium chaetigers, gradually decreasing in size toward the posterior chaetigers. Ventral cirri with small globular papillae. Branchiae pectinate (Fig. 1D), beginning on chaetiger 32 (29−36), extending posteriorly by last few chaetigers; number of branchial filaments 1−3 in first chaetigers, maximum number of branchial filaments 6 in mid-body (Fig. 1D); filaments longer than dorsal cirri, gradually increasing in size, filaments slightly annulated.

Chaetae arranged in two bundles: supra-acicular and subacicular, separated by a row of aciculae. Aciculae dark, with lighter blunt tips, very protruding, ranging from 3−6 per parapodum (Fig. 1C). Subacicular hooks present, transparent/pale yellow, bidentate (Fig. 2C). Supra-acicular bundle with limbate and pectinate chaetae; subacicular with compound spiniger chaetae and subacicular hooks (Fig. 2D). Limbate chaetae of different lengths with similar hirsute blades to one another. Compound spinigers present, along entire body except the last few chaetigers. Compound falcigers absent. Pectinate chaetae present from chaetiger 2. Pectinate chaetae of three types restricted to supra-acicular fascicle of chaetae (Fig. 2A, D): isodont, narrow, slender (INS) with 12−28 short teeth present from anterior to posterior chaetigers; anodont, wide, slender (AWS) with 9−14 short teeth from median to posterior chaetigers; anodont, wide, thick (AWT) with 2−4 large teeth present from median to posterior chaetigers. Subacicular bundle comprising compound spiniger chaetae, with surface of blade hirsute (Fig. 2D).

Pygidium with one pair of long pygidial cirri on ventral margin, anus slightly crenulated with 12 small indentations (Fig. 1F).

Remarks. *Marphysa victori* was originally described by Lavesque et al. (2017) based on specimens collected from Arcachon Bay in western France. According to Lavesque et al. (2017, 2020) and Abe et al. (2019), the *M. victori* population in France was considered to be introduced from East Asia

Korean name: [1]*털갯지렁이목, [2]*털갯지렁이과, [3]*바위털갯지렁이속, [4]*빗가시바위털갯지렁이

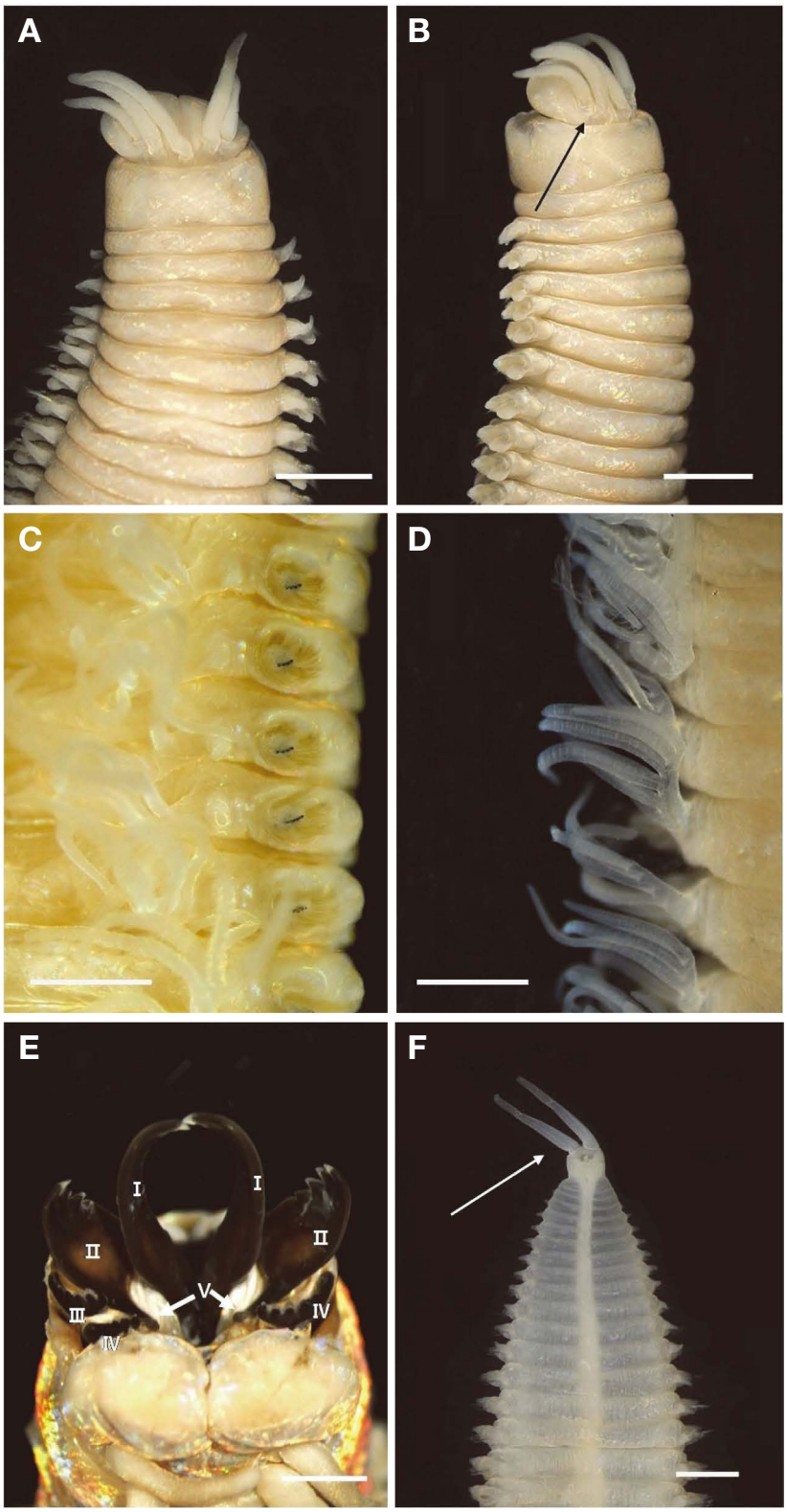

Fig. 1. Light microscopy images of *Marphysa victori*: A, Anterior end, dorsal view; B, Anterior end, lateral view (left); C, Anterior parapodia, lateral view (right); D, Structure of branchiae from median region, dorsal view; E, Jaws, anterior dorsal view; F, Pygidium from posterior region. Black arrow indicates eye. White arrow indicates pygidium. A–D, MABIKNA00156466, E, MABIKNA00156467, F, MABIKNA00156461. I, II, III, IV, V, components of maxillae. Scale bars: A, B = 2.0 mm, C–F = 1.0 mm.

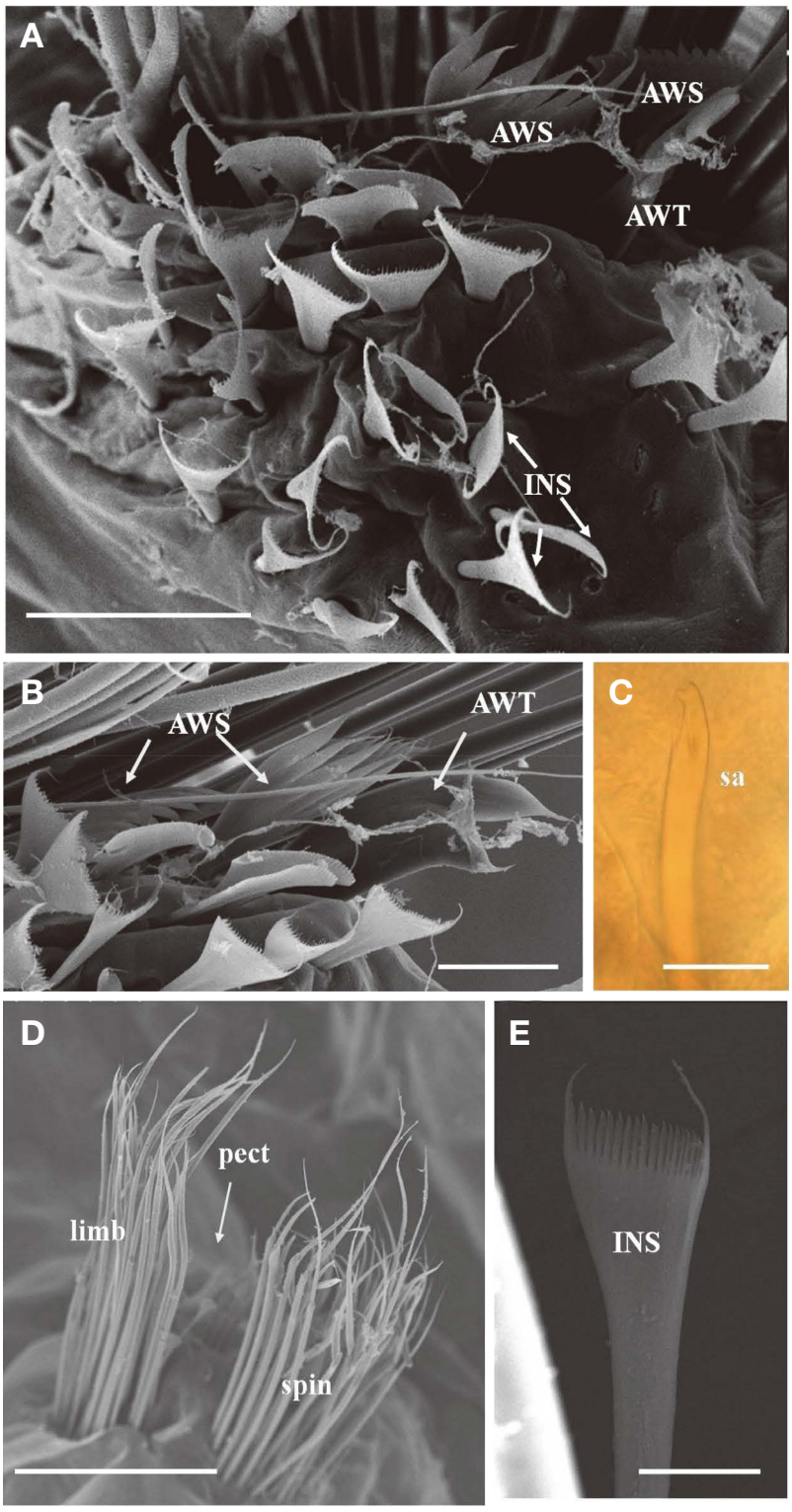

Fig. 2. Scanning electron microscopy (A, B, D, E) and light microscopy (C) images of *Marphysa victori*: A, B, Different types of pectinate chaetae (medium parapoida); C, Bidentate subacicular hook (medium parapoida); D, Limbate and pectinate chaetae, Compound spinigers chaetae (medium parapodia); E, Isodont, narrow, slender chaetae with many teeth (anterior parapodia). A, B, D, MABIKNA00156461; C, MABIK NA00156467; E, MABIKNA00156462. AWS, anodont-wideslender; AWT, anodont-wide-thick; INS, isodont-narrow-slender; limb, limbate chaetae; pect, pectinate chaetae; sa, subacicular hook; spin, spiniger chaetae. Scale bars: A = 50 μm, B = 30 μm, C = 100 μm, D = 300 μm, E = 15 μm.

Table 2. Genetic distance calculated by Kimura-2-parameter model based on mitochondrial cytochrome *c* oxidase subunit I (*COI*) sequence among five *Marphysa* species

	Species	Accession No. (Voucher No.)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	*Marphysa victori*	MT396172 (MABIKNA00156461)																								
2	*Marphysa victori*	MT396173 (MABIKNA00156462)	0.000																							
3	*Marphysa victori*	MT396175 (MABIKNA00156464)	0.001	0.001																						
4	*Marphysa victori*	MT396176 (MABIKNA00156465)	0.001	0.001	0.000																					
5	*Marphysa victori*	MT396174 (MABIKNA00156467)	0.008	0.008	0.007	0.007																				
6	*Marphysa victori*	MG384996 (AM W.49047)	0.007	0.007	0.005	0.005	0.012																			
7	*Marphysa victori*	MG384997	0.006	0.006	0.005	0.005	0.013	0.000																		
8	*Marphysa victori*	MG384998	0.002	0.002	0.000	0.000	0.006	0.000	0.000																	
9	*Marphysa victori*	MG384999	0.002	0.002	0.000	0.000	0.007	0.000	0.000	0.000																
10	*Marphysa aegypti*	MF196968	0.204	0.204	0.204	0.204	0.214	0.204	0.202	0.204	0.202															
11	*Marphysa bifurcata*	KX172177	0.178	0.178	0.178	0.178	0.184	0.178	0.178	0.178	0.178	0.190														
12	*Marphysa brevitentaculata*	GQ497548	0.184	0.184	0.184	0.184	0.188	0.182	0.182	0.190	0.182	0.194	0.201													
13	*Marphysa californica*	GQ497552	0.176	0.176	0.176	0.176	0.180	0.182	0.181	0.195	0.186	0.164	0.184	0.193												
14	*Marphysa disjuncta*	GQ497549	0.229	0.229	0.229	0.229	0.238	0.234	0.238	0.245	0.244	0.224	0.230	0.221	0.232											
15	*Marphysa fauchaldi*	KX172165	0.190	0.190	0.190	0.190	0.196	0.190	0.190	0.190	0.190	0.195	0.184	0.192	0.170	0.201										
16	*Marphysa hongkongensa*	MH598526	0.221	0.221	0.221	0.221	0.224	0.221	0.221	0.234	0.219	0.197	0.199	0.232	0.208	0.263	0.206									
17	*Marphysa iloiloensis*	MN106279	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.172	0.207	0.211	0.198	0.202	0.242	0.206	0.157								
18	*Marphysa kristiani*	KX172152	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.191	0.172	0.208	0.203	0.187	0.223	0.154	0.189	0.199							
19	*Marphysa mossambica*	KX172164	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.213	0.220	0.220	0.225	0.238	0.238	0.172	0.213	0.221	0.189						
20	*Marphysa mullawa*	KX172166	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.209	0.197	0.161	0.193	0.224	0.187	0.178	0.166	0.161	0.195					
21	*Marphysa pseudosessiloa*	KY605405	0.243	0.243	0.243	0.243	0.236	0.243	0.243	0.243	0.243	0.213	0.166	0.220	0.209	0.251	0.192	0.218	0.219	0.204	0.215	0.183				
22	*Marphysa sanguinea*	GQ497547	0.196	0.196	0.198	0.198	0.199	0.189	0.196	0.198	0.196	0.167	0.177	0.198	0.185	0.242	0.193	0.188	0.214	0.205	0.238	0.201	0.211			
23	*Marphysa tripectinata*	MN106271	0.193	0.193	0.193	0.193	0.193	0.193	0.193	0.193	0.193	0.162	0.189	0.208	0.205	0.235	0.164	0.188	0.187	0.184	0.189	0.191	0.180	0.175		
24	*Marphysa viridis*	GQ497553	0.181	0.181	0.182	0.182	0.176	0.199	0.205	0.212	0.208	0.224	0.203	0.198	0.190	0.228	0.200	0.186	0.192	0.216	0.236	0.168	0.227	0.196	0.191	

(China or Japan) by the oyster trade.

We newly found *M. victori* specimens in Korean waters based on morphological and molecular data of its type locality. *M. victori* is characterized by the following characteristics: (1) three types of pectinate chaetae (INS, AWS, and AWT); (2) the appearance of pectinate chaetae from the chaetiger 2; (3) chaetae consisting of pectinate and compound spinigers; and (4) a pygidium with one pair of pygidial cirri. These features appeared in the Korean specimens.

However, the Korean *M. victori* materials showed a minor difference from the original description of the France materials by the presence of subacicular hooks. Bidentate subacicular hooks were present in the Korean specimens but were absent in the France materials (Lavesque et al., 2017, 2020). Lavesque et al. (2020) reported that the absence of hook was found on the large specimens of the holotype from France (300 mm long), but hooks are clearly shown on a small specimen from Japan (30 mm long). In the case of the Korean specimens (91−209 mm length) that were smaller than the holotype (300 mm length) from France, all showed the presence of bidentate subacicular hooks on the posterior.

Marphysa victori closely resembles *M. sanguinea* in having compound spinigers and branchiae present over most of the body. However, *M. victori* is distinguished from *M. sanguinea* by having three types of pectinate chaetae instead of two types (INS and AWS, instead of INS, AWS, and AWT for *M. victori*) (Hutchings and Karageorgopoulos, 2003; Lavesque et al., 2017). This species is also similar to *M. tripectinata* which was recently described from the south coast of China (Liu et al., 2017), with three types of pectinate chaetae (INS, AWS, and AWT). However, *M. victori* has both asymmetrical and symmetrical pectinate chaetae, whereas *M. tripectinata* only has an asymmetrical one. This study also determined the DNA barcode sequences of the mitochondrial *COI* gene from five *M. victori* specimens (GenBank accession numbers MT396172−MT396176). Their lengths were 964 bp in total. We compared the *M. victori* specimens from France (type locality) and other *Marphysa* species based on *COI* obtained in this study (Table 1). The intra-specific genetic distance among the *M. victori* specimens from France (type locality) and Korea (this study) was in the range of 0.000−0.013 (Table 2). The inter-specific distances between *M. victori* and other *Marphysa* species ranged from 0.154 to 0.244. The mean of the *COI* sequence divergence between the species pairs in southern European Atlantic polychaetes was 23.7% (range 20.0−31.1%). Therefore, the genetic analysis results could be considered distinct enough to distinguish the species (Lobo et al., 2016).

Habitat. Intertidal muddy sand flats.

Distribution. Korea, Japan, China, Arcachon Bay (Bay of Biscay, western France).

ORCID

Hana Kim: https://orcid.org/0000-0002-8994-2162
Keun-Yong Kim: https://orcid.org/0000-0002-7647-3766
War War Phoo: https://orcid.org/0000-0001-6294-8188
Chang-Hoon Kim: https://orcid.org/0000-0002-6247-4733

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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