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New record of two marine synchaetid rotifers (Monogononta: *Synchaeta*) from Korea

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In this study, we identified two marine synchaetid rotifers, *Synchaeta grimpei* Remane, 1929 and *S. vorax* Rousselet, 1902, in Korea, which are the first synchaetid rotifers collected from a marine environment in the country. Prior to this study, all six synchaetids recorded in Korea were collected from freshwater environments. The morphological characteristics of both species are consistent with those recorded in previous studies of each species. *Synchaeta grimpei* is distinguished from other synchaetid rotifers by its cone-shaped body, wide and flat apical field, indistinct auricles, and long foot with two separated small toes. The morphological characteristics of Korean *S. vorax* specimens were most similar to the original description of Rousselet (1902), with its slender and cylindrical trunk shape, strongly convex apical field, and short foot with two small, separated toes. The rami of the Korean *S. vorax* specimen contained one frontal hook and several distinct and large teeth. Here, we provide the morphological diagnoses of the two synchaetid rotifers and the sequences of the partial mitochondrial cytochrome *c* oxidase subunit I (COI) of the two species.

Keywords: COI, marine rotifers, SEM, *Synchaeta grimpei*, *Synchaeta vorax,* trophi

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Introduction

The genus *Synchaeta* Ehrenberg, 1832 comprises 37 species that inhabit diverse environments in fresh-, brackish, and marine waters (Segers, 2007; Jersabek and Leitner, 2013; Wilke *et al*., 2019). This genus is characterized by its transparent and soft body, large head with auricles, one-segmented short foot with two toes, and virgate-type trophi (Hollowday, 2002). All species belonging to this genus are planktonic. Unlike other taxa in the subclass Monogononta Plate, 1889, this genus has a high proportion of species, with over 20 out of 37 species, inhabiting brackish water or marine environments (Hollowday, 2002; Wallace *et al*., 2006; Wilke *et al*., 2019).

Yamamoto (1953) recorded three synchaetid rotifers in Korea for the first time: *S. longipes* Gosse, 1887; *S. oblonga* Ehrenberg, 1832; and *S. tremula* (Müller, 1786). Since then, three additional synchaetid species were recorded in Korea: *S. lakowitziana* Lucks, 1930; *S. pectinata* Ehrenberg, 1832; and *S. stylata* Wierzejski, 1893 (Cho, 1979; Turner, 1986). These six synchaetid rotifers currently recorded in Korea are all freshwater species (National Institute of Biological Resources, 2021). The majority of the monogonont rotifers recorded in Korea, including the genus *Synchaeta*, were collected from fresh or brackish water, and recently, few investigations on rotifers in pure marine environments had been conducted.

Recent studies have shown that the phylum Rotifera Cuvier, 1817 contains approximately 455 species- and subspecies-level taxa that have been recorded in saltwater ecosystems (Fontaneto *et al*., 2006; Leasi and De Smet, 2020). However, the number of rotifers inhabiting saltwater is still underestimated, and numerous species may potentially be discovered in saltwater environments of many countries because of the following reasons: (1) most of the research has been European-biased, (2) numerous species that were considered to live exclusively in freshwater habitats have also been discovered in saltwater in many studies, and (3) accumulated data from rotifer distribution studies reveal that the endemism of rotifer was much higher than expected (Fontaneto *et al*., 2006; Wallace *et al*., 2006).

In this study, we conducted a field survey from several marine environments, and collected two synchaetid rotifers from Incheon Port, Korea: *Synchaeta grimpei* Remane, 1929 and *S. vorax* Rousselet, 1902. Here, we

provide morphological diagnoses of two new Korean records, in addition to partial mitochondrial cytochrome *c* oxidase subunit I (COI) sequences.

Materials and Methods

Two synchaetid rotifers were collected from Incheon Port using a 100-μm mesh plankton net. The rotifers were transferred to the laboratory alive and stored at 4°C. Before observation and preservation, the rotifers were anesthetized with a few drops of either carbonated water or 1% bupivacaine solution. Live and preserved specimens were observed under an optical microscope (DM2500, Leica, Germany). Trophi samples for scanning electron microscopy (SEM) were prepared following the methods of De Smet (1998) and observed using SU8010 and S-4300SE (Hitachi, Japan) at 10−15 V accelerating voltage. The morphological identification of synchaetid rotifers was based on Hollowday (2002), Koste and Voigt (1978), and Wilke *et al*. (2019). Specimens were deposited at the National Institute of Biological Resources, Korea (NIBR).

Total DNA was extracted from five specimens of each species, one specimen per tube, using a LaboPass™ Tissue Genomic DNA Isolation Kit Mini (Cosmo Genetech, Korea). Partial COI sequences were amplified using the LCO1490/HCO2198 primers (Folmer *et al*., 1994) and Takara Ex Taq® (Takara Bio Inc., Japan). The PCR conditions were as follows: 2 min at 95°C for the initial denaturation; followed by 40 cycles of 95°C for 15 s, 42°C for 30 s, 72°C for 1 min; and a final extension at 72°C for 5 min. PCR products were sequenced using the same primers at Macrogen (Korea), and the sequences were trimmed and aligned using Geneious 8.1.9 (https://www.geneious.com). To calculate the intraspecific genetic data, we obtained additional sequences of the two species from GenBank. The GenBank accession number of the sequences is presented in the molecular analysis section for each species. MEGA11 was used to calculate genetic distance (p-distance) (Tamura *et al*., 2021).

Systematic Accounts

Phylum Rotifera Cuvier, 1817 윤형동물문
Class Eurotatoria De Ridder, 1957 진윤충강
Subclass Monogononta Plate, 1889 단소아강
Order Ploima Hudson & Gosse, 1886 유영목
Family Synchaetidae Hudson & Gosse, 1886
털혹윤충과
Genus *Synchaeta* Ehrenberg, 1832 털혹윤충속

***Synchaeta grimpei* Remane, 1929 (Figs. 1A, 2A, 3)**
나팔털혹윤충 (신칭)
Synchaeta grimpei Remane, 1929: 122.
Synchaeta procera Galliford, 1946: 15−16.

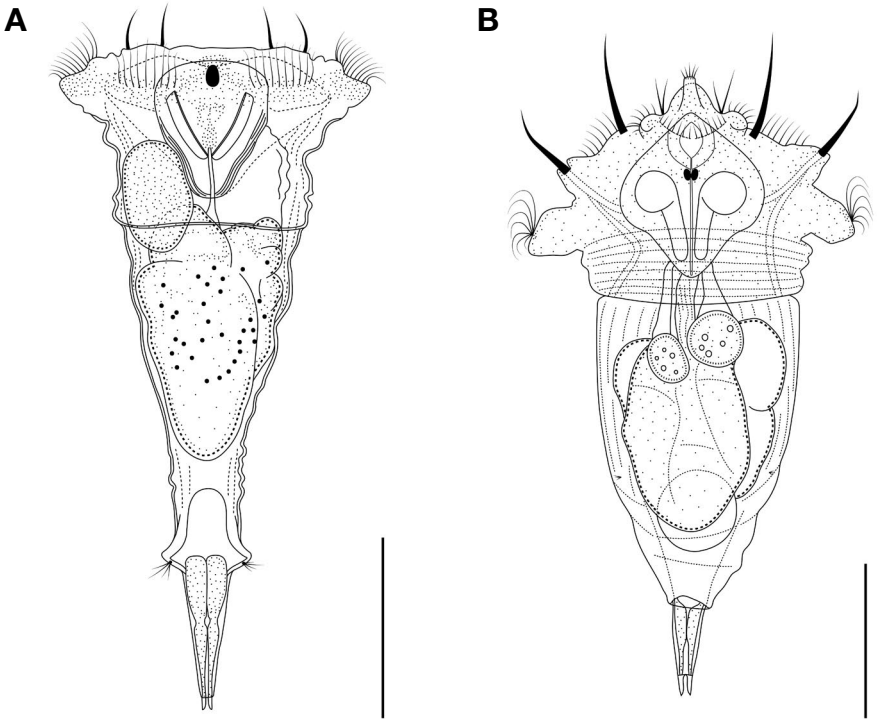

Fig. 1. Line drawing of rotifers. A. *Synchaeta grimpei*. B. *Synchaeta vorax*. Scale bars: 100 μm.

Fig. 2. Optical microscopic images of rotifers. A. *Synchaeta grimpei*. B. *Synchaeta vorax*. Scale bars: 100 μm.

Material examined. Korea, Incheon, Incheon Port (37° 27′00″N, 126°39′22″E), 24 Oct 2019, Hee-Min Yang. Voucher Number: NIBRIV0000879588.

Diagnosis. Body 285–366 μm in length, cone-shaped, and gradually tapering to foot. Apical field wide and flat, with four lateral and dorsolateral styles. Color of auricles and rotatory organ orange. Red eyespot located near mastax. One pair of lateral antennae at the end of the trunk. Foot long, one-fourth of body length, 81–85 μm. Pedal glands symmetrical and elongated, each gland with two swollen parts. Length of pedal glands approximately equal to length of foot. Two small toes on the tip of the foot. Toe tips completely separated. Trophi virgate type. Apical rami with frontal hook, one distinct tooth, and serrated inner margin. Fine needle structure on the inner margin of mid-rami. Hypopharynx located in front of needle structure. Manubrium straight with broad lamella. Tip of manubrium knob-shaped. Fulcrum thick and dagger-like. Fulcrum consisted of thick rod on dorsal side with broad lamella on ventral side.

Distribution. Korea (this study), Baltic Sea (Remane, 1929), British Atlantic coast (Galliford, 1946), Gulf of Mexico (Koste, 1981), Peter the Great Bay (Pacific Ocean) (Chernyshev, 2005).

Remarks. *Synchaeta grimpei* is easily distinguished from other synchaetid species based on the following characteristics: (1) cone-shaped body, (2) wide and flat apical field, (3) indistinct auricles, and (4) long foot with two separated small toes. This species has been recorded from brackish water or marine environments (Hollowday, 2002; Wilke *et al*., 2019). The Korean specimens were collected in September and October, at a water temperature of 20–24°C and salinity of 28.5–29.6‰. The morphological characteristics of the Korean specimens were generally consistent with those observed in previous studies (Hollowday, 2002; Wilke *et al*., 2019), except for the number of rami teeth. The descriptions by Koste (1981) and Hollowday (2002) documented the presence of multiple distinct teeth on rami; however, Korean specimens did not have distinct teeth in rami as described by Wilke *et al*. (2019). This is the second record of *S. grimpei* from the Pacific Ocean after Chernyshev (2005).

Molecular analysis. Partial COI sequences were obtained from five specimens. The intra-specific genetic distances were 0.0–1.7% within the Korean population (627 bp) (GenBank accession numbers: ON038411–ON038415). The genetic distances of Korean and German *S. grimpei* specimen were 0.4–1.2% (483 bp, Table 1) (GenBank accession numbers: MK905783–MK905785) (Wilke *et al*., 2020).

***Synchaeta vorax* Rousselet, 1902 (Figs. 1B, 2B, 4)**
먹보털혹윤충(신칭)
Synchaeta vorax: Rousselet, 1902: 408–410.

Material examined. Korea, Incheon, Incheon Port (37° 27′00″N, 126°39′22″E), 31 Mar 2021, Hee-Min Yang. Voucher Number: NIBRIV0000895436.

Diagnosis. Head large and wide. Apical field strongly convex. One tubular antenna on the center of apical field, tip of antenna with tuft. Four long styles present on apical field. Lateral auricles large, directed semi-caudally. Two small eyespots located near mastax. Head and trunk distinctly separated by wrinkled neck. Trunk cylindrical, tapered to posterior end. Longitudinal fold present on trunk. Lateral antennae at posterior third of trunk. Foot short, 60–65 μm in length. Pedal glands symmetrical and approximately the same length as the foot. Two small, separated toes on the foot. Total length 410–460 μm. Trophi virgate. Rami with frontal hook and several distinct teeth. One spine on middle of frontal hook. Each side of ramus teeth separated into two groups: three to four teeth on apical group and two teeth

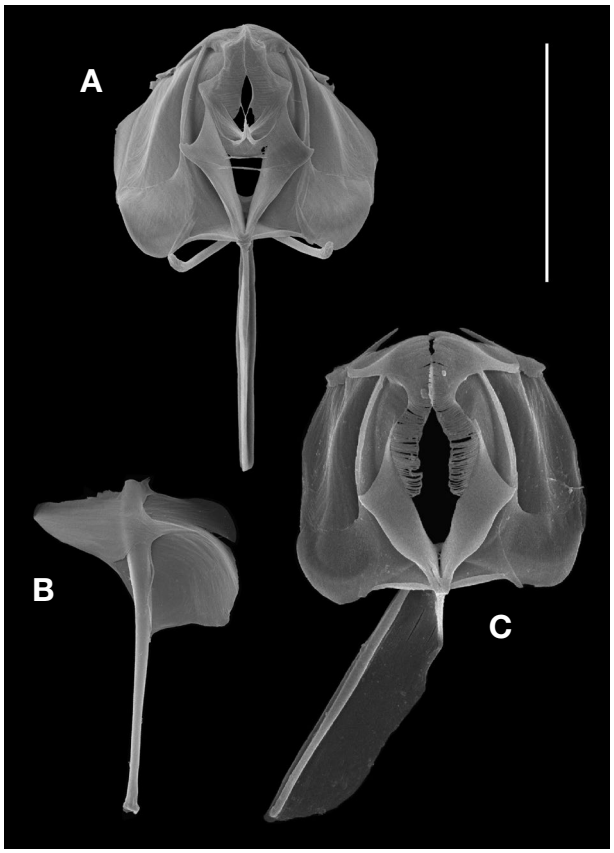

Fig. 3. SEM image of the trophi of *Synchaeta grimpei*. A. ventral view. B. manubrium, lateral view. C. incus, ventral view. Scale bar: 50 μm.

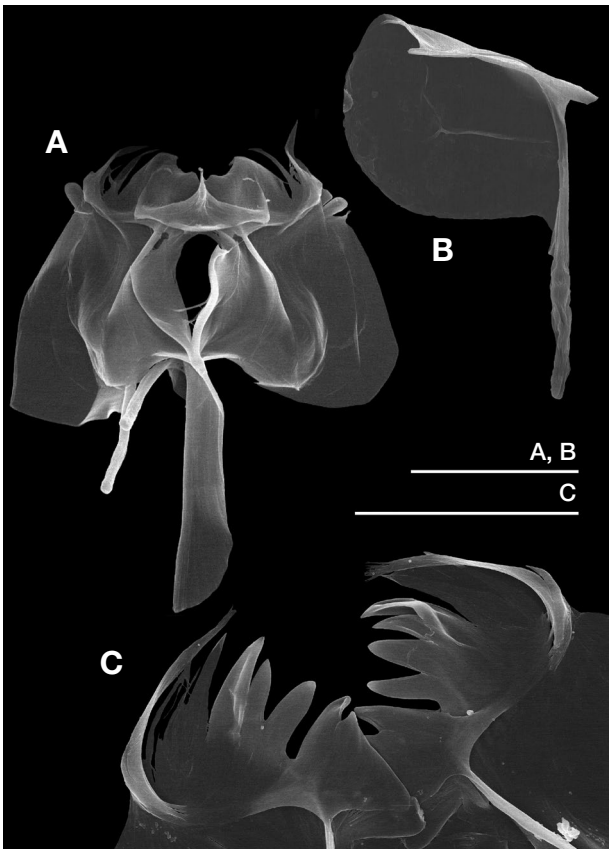

Fig. 4. SEM image of the trophi of *Synchaeta vorax*. A. ventral view. B. manubrium, lateral view. C. rami teeth, dorsal view. Scale bars: A, B: 40 μm, C: 30 μm.

Table 1. Genetic distance of *Synchaeta grimpei* from Korea and Germany (p-distance).

No.	Species	GenBank No.	1	2	3	4	5	6	7	Reference
1	*Synchaeta grimpei*	ON038411								This study
2	*Synchaeta grimpei*	ON038412	0.6							This study
3	*Synchaeta grimpei*	ON038413	0.6	0.0						This study
4	*Synchaeta grimpei*	ON038414	0.8	1.4	1.4					This study
5	*Synchaeta grimpei*	ON038415	0.8	1.4	1.4	1.7				This study
6	*Synchaeta grimpei*	MK905783	0.4	1.0	1.0	1.2	0.8			Wilke *et al.* (2020)
7	*Synchaeta grimpei*	MK905784	0.4	1.0	1.0	0.8	0.8	0.4		Wilke *et al.* (2020)
8	*Synchaeta grimpei*	MK905785	0.4	1.0	1.0	0.8	0.8	0.4	0.0	Wilke *et al.* (2020)

Table 2. Genetic distance of *Synchaeta vorax* from Korea and Germany (p-distance).

No.	Species	GenBank No.	1	2	3	4	5	6	7	8	9	10	11	Reference
1	*Synchaeta vorax*	ON038419												This study
2	*Synchaeta vorax*	ON038420	0.6											This study
3	*Synchaeta vorax*	ON038421	0.6	0.0										This study
4	*Synchaeta vorax*	ON038422	0.8	1.1	1.1									This study
5	*Synchaeta vorax*	ON038423	0.8	1.1	1.1	0.0								This study
6	*Synchaeta vorax*	MK905832,33,35	11.9	12.2	12.2	12.1	12.1							Wilke *et al.* (2020)
7	*Synchaeta vorax*	MK905834	12.1	12.4	12.4	12.2	12.2	0.2						Wilke *et al.* (2020)
8	*Synchaeta vorax*	MK905836,39	11.3	11.6	11.6	11.4	11.4	1.0	1.1					Wilke *et al.* (2020)
9	*Synchaeta vorax*	MK905837	11.3	11.6	11.6	11.4	11.4	0.6	0.8	0.3				Wilke *et al.* (2020)
10	*Synchaeta vorax*	MK905838	11.3	11.6	11.6	11.4	11.4	1.0	1.1	0.3	0.3			Wilke *et al.* (2020)
11	*Synchaeta vorax*	MK905840,41	11.1	11.4	11.4	11.3	11.3	1.1	1.3	0.2	0.5	0.5		Wilke *et al.* (2020)
12	*Synchaeta vorax*	MK905842	11.6	11.9	11.9	11.8	11.8	1.0	1.1	0.6	0.3	0.6	0.8	Wilke *et al.* (2020)

on basal group. Hypopharynx wide and crown-shaped. Manubrium straight and simple, with broad lamella. Fulcrum thick, machete-shaped.

Distribution. Cosmopolitan.

Remarks. The external morphological characteristics of Korean *S. vorax* specimens were most similar to the original description by Rousselet (1902). The trunk shape of the Korean specimens and the original description were slender and cylindrical, whereas those of Lie-Pettersen (1905) and Wilke *et al.* (2019) were plump and wineglass-shaped. In terms of trophi morphology, several variations were recorded in rami teeth. Wilke *et al.* (2019) reported that *S. vorax* has no distinct teeth and only a serrated plate. However, descriptions from Rousselet (1902), Lie-Pettersen (1905), and Arndt *et al.* (1990) indicated that the rami teeth of *S. vorax* possess several distinct teeth. The rami of the Korean specimen contained one frontal hook and distinctly large teeth. The teeth are divided into two groups as indicated in the 'Diagnosis' section, with each ramus containing three to four teeth on the apical group and two teeth on the basal group. The rami teeth formula of the Korean specimen was similar to that of *S. curvata* Lie-Pettersen, 1905, described by Arndt *et al.* (1990). However, the two species are clearly distinguished by morphological characteristics such as the tubular apical antenna and the number of eyespots. *Synchaeta vorax* was recorded as a eurythermal species in a previous study (Hollowday, 2002), and the Korean specimen was collected from November to March, at a water temperature of 5−10°C and a salinity of 26.0−29.3‰.

Molecular analysis. Partial COI sequences were obtained from five specimens. The intra-specific genetic distances were 0.0−1.1% within the Korean population (657 bp) (GenBank accession numbers: ON038419−ON038423). The genetic distances between the Korean and German *S. vorax* specimens were 11.1−12.4% (621 bp, Table 2) (GenBank accession numbers: MK905832−MK905842) (Wilke *et al.*, 2020). These genetic distances were unusually large within the same species, even if they were in different population. Recently, as molecular analysis has been introduced to the rotifer research, cryptic species have been identified in various species (García-Morales and Elías-Gutiérrez, 2013; Obertegger *et al.*, 2014; Papakostas *et al.*, 2016; Kordbacheh *et al.*, 2017; 2018). Most of the cryptic species are morphologically indistinguishable and have large genetic distance. In this regard, we reconfirmed the external and trophi characteristics of the Korean *S. vorax* specimens, and found no significant difference from the previously known morphological characteristics. For the accurate assessment of biodiversity, the problem of cryptic species must be solved, and for this, it is considered that additional studies on biogeographical, ecological, and reproductive are needed in addition to morphological analysis.

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