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Morphological and preliminary molecular characterization of two indigenous *Brachionus* species (Rotifera: Monogononta) from a brackish coastal lagoon in Korea

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Abstract

Brackish water ecosystems form unique ecological structures in which aquatic organisms of both freshwater and marine origin coexist, resulting in high levels of biodiversity distinct from those of typical freshwater or marine systems. However, the biodiversity and accurate taxonomic identification of microfauna within these ecosystems remain poorly understood. In this study, two rotifer strains collected from Hwajinpo Lagoon, a coastal lagoon along the eastern coast of Korea, were investigated using a single isolation-based culture approach for species identification. Based on morphological analyses, the collected rotifers were assigned to the class Monogononta and the genus *Brachionus*. Notably, these strains exhibited morphological characteristics distinct from those of *Brachionus plicatilis* and *Brachionus rotundiformis*, which are commonly reported inhabitants of brackish environments, suggesting potential taxonomic differentiation from previously described species. Furthermore, polymerase chain reaction (PCR)-based molecular evidence provided additional support for the observed taxonomic differentiation. These findings suggest that the rotifer strains examined in this study may represent potentially distinct indigenous lineages and demonstrate their potential as indigenous biological resources for aquaculture and research applications.

Keywords: Brackish Lagoon, Rotifers, *Brachionus*, Identification, Bioresource

Introduction

Coastal lagoons are dynamic transitional ecosystems that are subject to substantial physicochemical fluctuations due to seawater–freshwater exchange (Lill et al., 2013; Montagna et al., 2023). In such environments, microfaunal communities, including planktonic and protozoan groups, often exhibit distinctive community structures and life-history traits shaped by species-specific physiological characteristics (Sanvicente-Añorve et al., 2022). However, in highly dynamic lagoon

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ecosystems, the adaptive responses of microfauna to rapid environmental changes remain poorly understood (Danovaro & Pusceddu, 2007; Marshall & Duggan, 2024). In this context, accurate identification of microfaunal communities in aquatic ecosystems provides essential reference data for biodiversity assessments. It also facilitates the identification of previously unrecorded or potentially novel biological resources, enhancing biodiversity assessments (Leasi & Cline, 2022). In particular, studying microfauna in lagoon ecosystems along the eastern coast of Korea may provide valuable insights into regional biodiversity and serve as a foundation for future assessments of trophic structure and food-web dynamics (Mieczan et al., 2015).

Rotifers of the subclass Monogononta (Rotifera: Eurotatoria) are widespread microfauna inhabiting a broad range of aquatic environments, from freshwater to marine ecosystems (Dumont, 1983). Due to their short life cycles, rapid growth rates, and high reproductive capacities, rotifers function as important primary consumers in aquatic food webs, facilitating the transfer of energy and nutrients from phytoplankton to higher trophic levels (Segers, 2004; Yoshimatsu & Hossain, 2014). In addition to their ecological roles, rotifers are widely used as model organisms in aquaculture, physiology, toxicology, and molecular ecology due to their ease of laboratory culture and suitability for mass production (Park et al., 2022; Snell & Janssen, 1995). In particular, *Brachionus plicatilis* and *Brachionus rotundiformis* are among the most widely used rotifer species, serving as model organisms in fields such as aquaculture, ecotoxicology, and molecular ecology (Dahms et al., 2011; Lee et al., 2022; Lubzens et al., 2001). Their suitability for mass culture and ease of handling makes them indispensable in hatchery systems, where they are used as live feed for larval fish. Due to their efficiency in nutrient transfer to early-stage larvae, they are often referred to as "live food capsules" (Das et al., 2012; Kandathil Radhakrishnan et al., 2020). However, despite their widespread application, the majority of rotifer strains employed in research and aquaculture are of non-native origin, thereby emphasizing the need for identification and utilization of indigenous Korean species to support sustainable domestic seed production.

Rotifers are known to exhibit a high degree of phenotypic plasticity, with body size and morphological traits varying substantially in response to environmental factors such as temperature (Ge et al., 2025), pH (Lee et al., 2020), and microplastic (Kim et al., 2024). Although such plasticity can lead to taxonomic ambiguity and potential misidentification, morphological traits remain a fundamental basis for species identification, particularly when applied with ecological and biogeographical context in mind (Fontaneto et al., 2009). This is especially critical in morphologically similar or environmentally plastic species groups, where distinct evolutionary lineages may be conflated or conspecific individuals may be mistakenly split into multiple taxa (Xiao et al., 2026). These challenges are magnified in highly variable ecosystems such as coastal lagoons; nevertheless, when guided by detailed trait evaluation and site-specific environmental data, morphology-based approaches remain effective for detecting both recognized and cryptic species.

In this study, we combined morphology-based identification through visual observation with laboratory isolation and cultivation to investigate two rotifer species collected from a natural lagoon environment. Furthermore, by examining morphological traits expressed under standardized culture conditions, we assessed whether these strains exhibit consistent characteristics indicative of indigenous taxa, potentially distinct from previously described *Brachionus* species.

Materials and Methods

Sampling and cultivation

Monogonont rotifers were collected and isolated using a 45 μm mesh plankton net (CL-NP4045A with a mouth diameter of 40 cm and a net length of 80 cm, DAIHAN CHEMLAB, Incheon, Korea) from Hwajinpo Lagoon in Goseong, Korea (38°28'22.60"N, 128°26'17.68"E; Fig. 1). Rotifers were morphologically identified under a stereomicroscope (S8APO, Leica, Wetzlar, Germany) using petri dishes. Ovigerous amictic females were individually isolated, transferred into 12-well plates, and cultured under controlled conditions (24℃ and 15 psu salinity). Cultures were fed daily with *Chlorella vulgaris* at approximately 2×10^5 cells mL^{-1} to establish and maintain single clonal lineages. *B. plicatilis* and *B. rotundiformis* strains used for comparative experiments were maintained in parallel under identical conditions in an aquarium at the Department of Marine Convergence Science, College of Life Sciences, Kangwon National University, Gangneung, Korea.

Morphological measurements

Morphological analyses were conducted using wild-caught rotifer individuals that were immediately fixed in 1% neutral formalin following field sampling. Morphometric analysis of the lorica was conducted using individuals of identical age. Neonates were selected within 30 min immediately after hatching, and adult females were selected within 30 min after laying their first egg according to the biological minimum size criterion. All

Fig. 1. Sampling sites in the brackish lagoon of Hwajinpo.

specimens were rinsed with sterile seawater (15 psu) and fixed in 1% formalin. To minimize inter-image positional errors, only rotifers positioned with their dorsal sides facing downward were imaged, and all individuals were carefully oriented and centered within the microscopic field prior to imaging to prevent distortion. All stereomicroscopic images were acquired using a stereomicroscope (BX50, Olympus, Tokyo, Japan) equipped with a digital camera (DP73, Olympus).

Scanning electron microscopy (SEM)

Whole-body specimens for scanning electron microscopy (SEM) observation were prepared as follows: (1) rotifers were fixed in a 2.5% glutaraldehyde solution at 4℃ for 4 h and then rinsed with 1% PBS buffer; (2) specimens were dehydrated through an ascending ethanol series (25%, 50%, 70%, 90%, 95%, and 100%), with each step lasting 10 min; (3) 100% ethanol was replaced twice, for 10 min each; and (4) specimens were subsequently processed using a critical point dryer (EM CPD300, Leica) to replace the remaining alcohol prior to drying. Furthermore, for trophi analysis, trophi were isolated using 4%–5% sodium hypochlorite (Yuhan-Chlorox, Seoul, Korea) for approximately 3 min and prepared for SEM (IT-700, JEOL, Tokyo, Japan) according to De Smet (1998). SEM observations were performed using a scanning electron microscope operated at an accelerating voltage of 10 kV.

Genomic DNA preparation and polymerase chain reaction (PCR)

For DNA extraction, rotifers were maintained under controlled laboratory conditions to obtain sufficient biomass. Healthy amictic females were cultured for multiple generations under standardized conditions to ensure consistency. The culture conditions included a salinity of 15 psu, a 12:12 h light:dark photoperiod, and a diet of *C. vulgaris* at 2×10^5 cells individual^{-1} day^{-1}. To determine the genetic identity of two rotifer species collected from Hwajinpo, approximately 5,000 amictic females were starved before DNA extraction to avoid potential contamination from culture media. Rotifers were homogenized in extraction buffer (100 mM NaCl, 10 mM Tris-Cl pH 8.0, 25 mM ethylenediaminetetraacetic acid [EDTA], 0.5% sodium dodecyl sulfate [SDS], 20 μg/mL proteinase K, 1 μg/mL ribonuclease [RNase]) using a Teflon homogenizer, followed by overnight incubation at room temperature. DNA was extracted using the phenol–chloroform–isopropanol method, and precipitated with 0.2 M ammonium acetate by centrifugation at 10,000×g for 10 min. The pellet was washed with 70% ethanol, air-dried, and resuspended in tris-EDTA (TE) buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0). DNA quality and quantity were assessed using a Nanodrop spectrophotometer and 1.2% agarose gel electrophoresis.

To amplify partial mitochondrial cytochrome c oxidase subunit I (mtDNA COI) sequences, species-specific forward and reverse primers were designed based on three representative euryhaline *Brachionus* sp. (*B. plicatilis, B. rotundiformis, and Brachionus koreanus*) (Table 1). These primers were adapted from previously validated species-specific protocols, with each

Table 1. Polymerase chain reaction (PCR) and sequencing primers

Species	Designation	
	Forward (5′ to 3′)	Reverse (5′ to 3′)
Brachionus koreanus	ATTATCTTCTGCTATTGATGCTG	CCGTAATAACCGAAAATCTCTTT
Brachionus plicatilis	GGTGTCTCTGTTGATTTAGCGAT	CTACATCCAACCCTACAGTAAA
Brachionus rotundiformis	TTAGATCGTTTACCTCTAATGCT	CACTGAACTGCTGATATTCTTAG

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pair producing a diagnostic amplicon of known size under standardized polymerase chain reaction (PCR) conditions. In this approach, PCR functions as a diagnostic tool: successful amplification of a specific-sized band indicates identity with the corresponding reference species, whereas absence of amplification suggests mitochondrial sequence divergence or the presence of a distinct lineage. The mitochondrial COI gene has been widely used as a DNA barcode marker due to its high resolution for species identification across diverse animal taxa, achieving over 95% species-level resolution in previous large-scale studies (Ratnasingham & Hebert, 2007).

PCR amplifications were carried out in a total reaction volume of 20 μL, containing 3 μL of template DNA, 1 μL each of forward and reverse primers, 2 μL of 10 × PCR reaction buffer, 2 μL of 10 mM dNTP mixture, 1.5 μL of 20 mM $MgCl_2$, and 0.2 μL of Taq DNA polymerase, with nuclease-free water added to adjust the final volume. Thermal cycling was performed with an initial denaturation at 95℃ for 5 min, followed by 35 cycles of denaturation at 95℃ for 40 s, annealing at 58℃ for 30 s, and extension at 72℃ for 90 s. A final extension step was conducted at 72℃ for 10 min, and the reactions were subsequently held at 4℃. Amplification products were confirmed by electrophoresis on a 1.2% agarose gel.

Statistical analysis

Statistical analyses were performed using SPSS v29.0 (IBM, Armonk, NY, USA). Data from all experiments are presented as mean ± SE. To compare rotifer strains, data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p < 0.05$. Significant differences among rotifer strains are indicated by different letters.

Results and Discussion

Morphological characteristics of collected rotifers

Our data showed that both collected strains are loricate rotifers characterized by the presence of a rigid lorica and belonging to the class Monogononta (Wallace et al., 2006). The large-type strain exhibited a mean lorica length of 214 ± 8.3 μm and a width of 184 ± 12.1 μm, while the small-type strain displayed a mean lorica length of 184 ± 12.1 μm and a width of 163 ± 9.3 μm. Based on comparisons with previously reported size ranges, the large-type corresponds to the average size range reported for *B. ibericus*, *B. plicatilis*, and *B. rotundiformis,* whereas the small-type closely matches the size range of *B. ibericus* and *B. koreanus* (Table 2). Both strains exhibited a dorsal lorica bearing six anterior spines, with the median pair being the longest and separated by a deep U-shaped sulcus. SEM revealed distinct variations in anterior spine morphology. The large-type exhibited relatively blunt, less sharply pointed spines, whereas the small-type had distinctly sharper and more pointed spines (Fig. 2). This difference in anterior spine morphology is consistent with previous findings that highlight anterior spine sharpness as a key morphological trait for differentiating rotifer types in aquaculture (Han et al., 2025). Such morphological differentiation may reflect underlying species-level divergence, especially considering that microfaunal communities, including rotifers, often comprise multiple co-occurring species within the same habitat. This pattern of spatiotemporal coexistence is particularly well documented in

Table 2. Morphometric data for species of the genus *Brachionus* (Monogononta)

Species	Body size (μm)		References
	Length	Width	
Brachionus angularis	86 ± 3.1	75 ± 3.6	Ogello et al. (2016)
Brachionus asplanchnoidis	295 ± 8.1	205 ± 4.8	Guerrero-Jiménez et al. (2019)
Brachionus calyciflorus	235–256	155–161	Xue et al. (2017)
Brachionus ibericus	176–220	126–163	Ciros-Pérez et al. (2001)
Brachionus koreanus	172 ± 11	–	Kang et al. (2019)
Brachionus manjavacas	254	202	Johnston et al. (2018)
Brachionus paranguensis	217 ± 13.8	160 ± 10.9	Guerrero-Jiménez et al. (2019)
Brachionus plicatilis	123–292	114–119	Snell & Carrillo (1984)
Brachionus quadridentatus	170	120	Saksena & Kulkarni (1986)
Brachionus rotundiformis	140 ± 6	124 ± 7.8	Song et al. (1999)
Brachionus rubens	200–250	–	Mohr & Adrian (2002)

*WoRMS (World Register of Marine Species) accepted species.

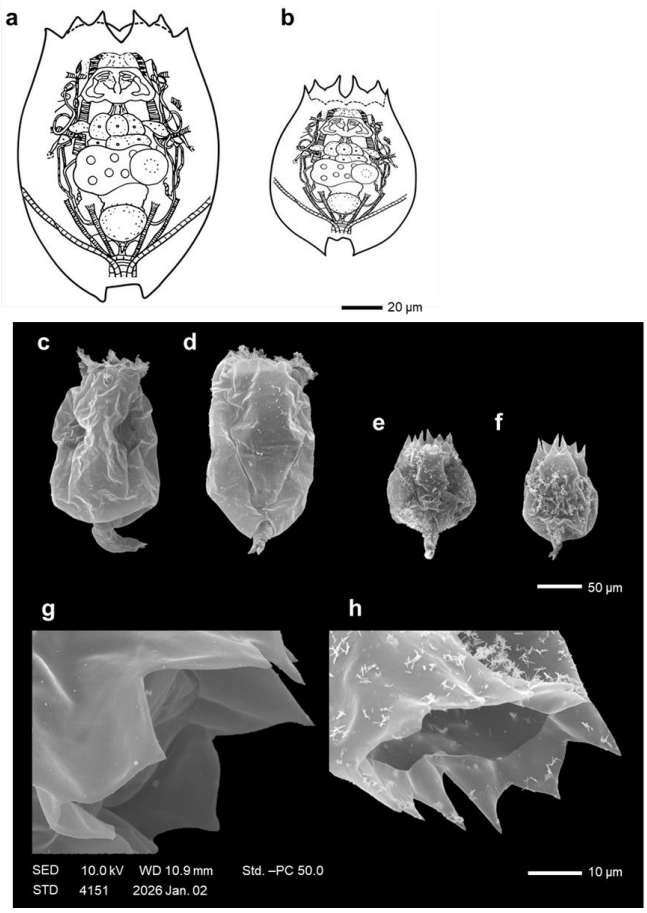

Fig. 2. Line drawing of (a) large-type; (b) small-type and scanning electron microscopy (SEM) image of the habitus of *Brachionus* sp. (c) Large-type; (d) *Brachionus plicatilis*; (e) small-type, (f) *Brachionus rotundiformis*, (g) large-type anterior spine view, and (h) small-type anterior spine view. SED, secondary electron detector; WD, working distance; STD, standard observation mode.

the genus *Brachionus* (Gilbert, 2022; Montero-Pau et al., 2011). Accordingly, although the two strains in this study were collected from the same locality, they likely represent distinct species rather than intraspecific morphological variants.

Morphological assessment of rotifers under controlled experimental conditions

To accurately compare the Hwajinpo strains with previously reported species (*B. plicatilis* and *B. rotundiformis*), we measured lorica size under standardized and controlled culture conditions. Morphological traits, including body size, are known to be strongly influenced by environmental factors such as temperature, salinity, and food availability (Ge et al., 2025; Walczyńska & Serra, 2022). As a result, species identification based solely on field-fixed specimens may lead to misinterpretation. Phenotypic plasticity, in particular, is widely recognized as a major limitation in the taxonomic identification of aquatic invertebrates, including rotifers. To minimize this issue and enable reliable morphological discrimination among closely related taxa, all comparisons were conducted under identical laboratory conditions. Size differences between taxa were observed among neonate individuals hatched synchronously within a 30-minute interval at the end of the culture experiment. The Hwajinpo-collected strains exhibited significantly smaller hatchling sizes than *B. plicatilis*, while the small-type strain showed sizes comparable to *B. rotundiformis*. These results suggest that, at the neonate stage, the Hwajinpo strains are morphologically distinct from *B. plicatilis*, indicating potential species-level divergence. A similar pattern was observed in adults at their minimum developmental size. The size difference detected at the neonate stage became even more evident in adults. In comparison with the Hwajinpo strains, *B. plicatilis* consistently exhibited a larger body size, indicating clear morphological separation. The large-type strain also displayed a substantially greater body size than both the small-type and *B. rotundiformis*, further supporting their classification as distinct morphotypes despite being collected from the same habitat. At the egg stage, the large-type strain produced eggs similar in size to those of *B. plicatilis*, whereas the small-type strain produced eggs comparable to *B. rotundiformis* (Fig. 3). This pattern aligns with previous observations that, within the genus *Brachionus*, large-bodied strains generally produce larger eggs, while smaller strains produce correspondingly smaller eggs (Snell et al., 2019).

Observation of rotifer trophi using scanning electron microscopy (SEM)

To allow a detailed morphological comparison among the examined rotifer strains, the trophi within the mastax were examined and compared. Comparative analyses of trophi size in both ventral and dorsal views revealed significant differences among the examined strains. In the ventral view, measurements of manubrium length (A) and width (B) clearly differentiated the strains, while in the dorsal view, ramus width (G) and length (H) also showed significant size variation ($p < 0.05$). Overall, *B. rotundiformis* consistently exhibited the smallest trophi dimensions. Manubrium length (A) was greatest in *B. plicatilis* (38.3 ± 2.41 μm) and smallest in *B. rotundiformis* (20.6 ± 1.57 μm), with the difference being statistically significant. Similarly,

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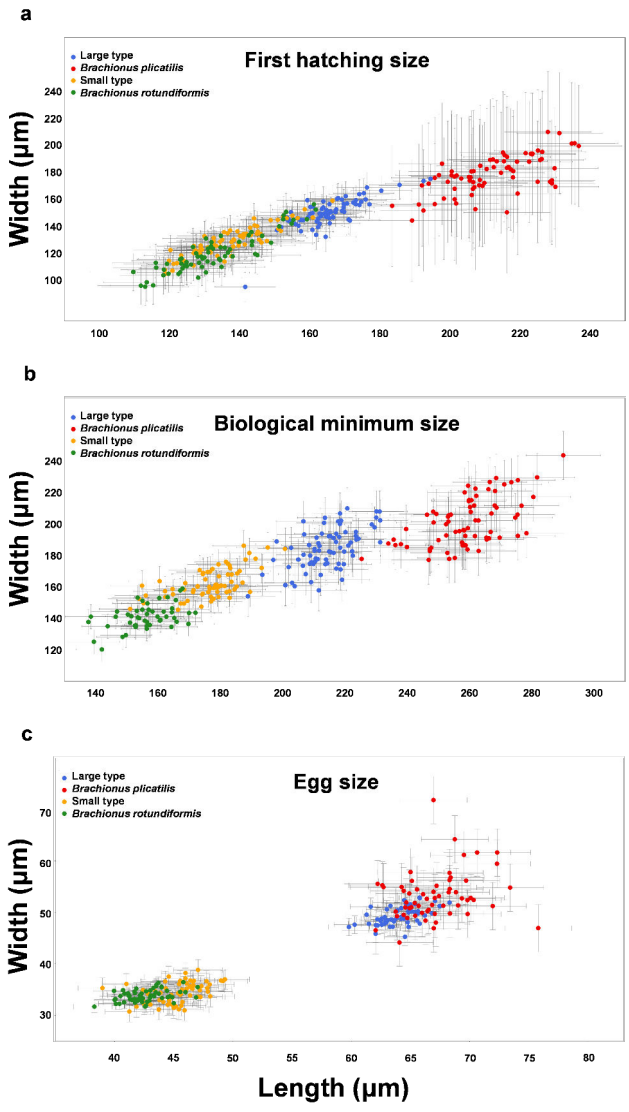


Fig. 3. Scatter plot showing the comparison of body size for growth level stage. (a) Neonate; (b) biological minimum size (stage of possible reproduction); (c) egg size.

manubrium width (B) was largest in large-type (17.4 ± 1.87 μm) and smallest in *B. rotundiformis* (9.1 ± 0.66 μm; $p < 0.05$). Uncus length (C) was relatively large in large-type and *B. plicatilis* (28.0 ± 2.04 μm and 27.8 ± 1.90 μm, respectively), whereas significantly smaller values were observed in small-type and *B. rotundiformis* (17.5 ± 1.47 μm and 17.2 ± 1.61 μm, respectively; $p < 0.05$). In contrast, basifenestra length (D), fulcrum width (E), and fulcrum length (F) exhibited patterns similar to uncus length, with comparable sizes between large-type and *B. plicatilis*, as well as between small-type and *B. rotundiformis*. Ramus width (G) differed significantly among strains, with *B. plicatilis* showing the largest value (39.5 ± 1.76 μm), followed by large-type (35.1 ± 2.84 μm), small-type (23.9 ± 1.55 μm), and *B. rotundiformis* (21.5 ± 2.35 μm; $p < 0.05$). Ramus length (H) exhibited a similar pattern; however, no significant difference was detected between small-type and *B. rotundiformis* ($p > 0.05$; Fig. 4 and Table 3). Overall morphology of individual trophi components in the two collected rotifer strains revealed that both exhibited malleate type. This trophi type is widely recognized as characteristic of the genus *Brachionus* (Wallace et al., 2006). The presence of malleate trophi in both strains therefore provides additional morphological evidence supporting their assignment to the genus *Brachionus*. Furthermore, subtle but consistent differences in trophi morphology were observed between the two collected rotifer strains. These differences provide further support for the possibility that the two rotifers, despite being collected from the same habitat, represent distinct strains (Fontaneto & Melone, 2006).

Molecular identification of collected rotifer strains

We performed PCR amplification of the mitochondrial COI marker under standardized conditions across all experimental

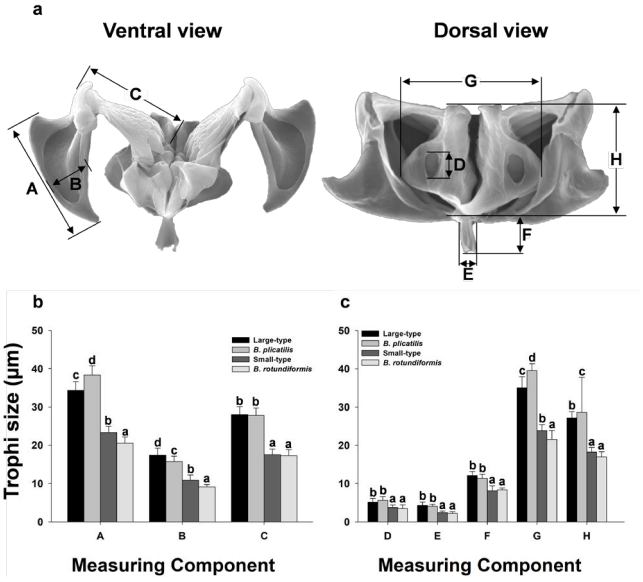


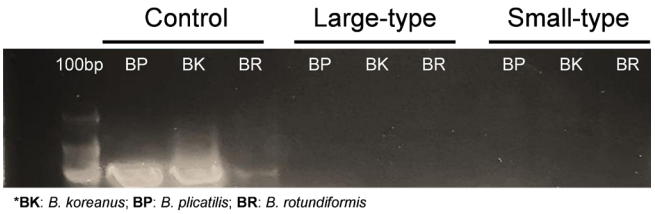
Fig. 4. Comparison of trophi dimensions. (a) Specimen showing the measurement locations: A, manubrium length; B, manubrium width; C, uncus length; D, basifenestra length; E, fulcrum width; F, fulcrum length; G, ramus width; and H, ramus length. (b) Measurements from the ventral view. (c) Measurements from the dorsal view. Different letters above the bars indicate significant differences ($p < 0.05$).

Table 3. Comparison of trophi size for each species

Species	Ventral view				Dorsal view			
	Manubrium		Uncus	Basifenestra	Fulcrum		Ramus	
	Length (μm)	Width (μm)	Length (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)
	A	B	C	D	E	F	G	H
Large-type	34.3 ± 2.21[c]	17.4 ± 1.87[c]	28.0 ± 2.04[b]	5.1 ± 1.00[b]	4.3 ± 0.85[b]	12.1 ± 1.01[b]	35.1 ± 2.84[c]	27.1 ± 1.66[b]
Small-type	23.3 ± 1.64[b]	10.9 ± 1.36[b]	17.5 ± 1.47[a]	3.8 ± 0.65[a]	2.4 ± 0.40[a]	8.1 ± 1.33[a]	23.9 ± 1.55[b]	18.3 ± 1.15[a]
Brachionus plicatilis	38.3 ± 2.41[d]	15.8 ± 1.36[d]	27.8 ± 1.90[b]	5.7 ± 0.95[b]	4.0 ± 0.55[b]	11.3 ± 1.06[b]	39.5 ± 1.76[d]	28.6 ± 9.11[c]
Brachionus rotundiformis	20.6 ± 1.57[a]	9.1 ± 0.66[a]	17.2 ± 1.61[a]	3.5 ± 0.96[a]	2.2 ± 0.44[a]	8.4 ± 0.50[a]	21.5 ± 2.35[a]	17.0 ± 1.35[a]

[a–d] Different letter above numbers indicate significant differences ($p < 0.05$).

groups. In the control group (*B. plicatilis* and *B. rotundiformis*), clear amplicons of the expected size were successfully generated, confirming the validity of the primer sets and the reliability of the PCR protocol (Fig. 5). In contrast, no PCR products were obtained from either the large-type or small-type Hwajinpo strains, despite multiple attempts using high-quality genomic DNA and optimized reaction conditions. The COI primers used in this study were not universal primers, but species-specific primers previously validated against multiple *Brachionus* taxa commonly used in aquaculture and phylogenetic studies. Therefore, this consistent lack of amplification is not indicative of technical failure, but rather suggests that the mitochondrial COI regions in the Hwajinpo strains differ significantly from known reference sequences. This finding provides preliminary molecular evidence that the two collected strains may represent distinct genetic lineages not currently represented in existing COI databases for the genus *Brachionus*. Considering that the primers successfully amplified COI sequences in closely related *Brachionus* species under identical conditions, it is likely that the absence of amplification in the Hwajinpo strains reflects biologically meaningful mitochondrial sequence divergence.



*BK: *B. koreanus*; BP: *B. plicatilis*; BR: *B. rotundiformis*

Fig. 5. Visualization of agarose gel electrophoresis results following polymerase chain reaction (PCR) amplification with mtDNA cytochrome oxidase subunit I (COI) primers in experimental species, showing the band of amplification of the COI gene region. The leftmost lane indicates a 100 bp DNA ladder.

However, we only performed COI-based PCR analysis using species-specific primers, and further studies incorporating complete mitochondrial genome sequencing and nuclear markers such as 18S ribosomal RNA (rRNA) or internal transcribed spacer (ITS) are needed to clarify the phylogenetic placement and confirm the taxonomic status of the Hwajinpo strains.

Conclusion

In this study, we examined two rotifer strains collected from the Hwajinpo coastal lagoon on the eastern coast of Korea using detailed morphological comparisons and a preliminary molecular approach. Morphological assessments performed under standardized culture conditions revealed that the large-type and small-type strains exhibited clear differences in body size, spine structure, egg size, and trophi morphology, while both were consistent with diagnostic features of the genus *Brachionus*. When compared with reference species, the large-type strain showed partial morphological similarity to *B. plicatilis*, and the small-type strain resembled *B. rotundiformis*. However, amplification of the mitochondrial COI region was not detected in either Hwajinpo strain, although the primers yielded expected products in the control group under identical conditions. This result suggests that the possibility of mitochondrial sequence divergence relative to commonly used *Brachionus* taxa and highlights the need for further sequencing to clarify their phylogenetic identity. Taken together, the observed morphological distinctiveness and preliminary molecular findings suggest that the two Hwajinpo rotifer strains may represent potentially distinct indigenous lineages within the genus *Brachionus*. In light of the dynamic environmental conditions characteristic of coastal lagoons, these strains may serve as ecologically compatible and valuable indigenous resources for domestic aquaculture. Furthermore, they can be

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considered promising model organisms for future studies in ecotoxicology and molecular ecology in Korea.

Competing interests

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

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Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

Not applicable.

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