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The first record of Rhabdocoela, *Alcha sinensis*, Wang & Hu, 2019 (Platyhelminthes: Polycystididae) from Korea

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In this study, a species of Rhabdocoela is reported from Korea for the first time. The kalyptorhynch *Alcha sinensis* Wang & Hu, 2019, was collected from the intertidal zone of Anmyeon Island. Compared to the original study on *A. sinensis* and previous studies on *A. evelinae*, we provide additional detail of these two species through pictures of the stylet and a improved description of the prostate stylet type III, which is composed of a bowtie-shaped base and two pairs of heteromorphic plates with fringed or serrated edges. Two plates are also provided with a rounded "blade". Also, we discuss the importance of the prostate stylet type III as a diagnostic character. After a thorough comparison of the descriptions of *A. evelinae* Marcus, 1949, and *A. sinensis*, we conclude that the Korean specimens belong to the latter species. Finally, we discuss the difficulties of interpreting diagnostic characters of the small and complex prostate stylet type III within the genus *Alcha*.

Keywords: *Alcha*, flatworm, Kalyptorhynchia, morphology, taxonomy

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Introduction

The order Rhabdocoela is a species-rich group of microturbellarian flatworms occurring in marine and freshwater habitats around the world. Although more than 1,500 species have been recorded globally, there are no records of rhabdocoels from Korean waters. Moreover, only 18 species are known from adjacent areas, most of which are described in a few studies focusing on the East Sea in Russia and Japan (Nasonov, 1932; Evdonin, 1968; 1970a; 1970b; 1971; 1977; Ax, 1992; 2008; Takeda and Kajihara, 2018). Undoubtedly, this is but a small fraction of the actual number of species in this area as the diverse coastal areas of the marginal seas surrounding the Korean Peninsula represent an important, yet mostly underexplored part of the Northwest Pacific Ocean.

Rhabdocoels fall within two clades: the Kalyptorhynchia with a proboscis (Tessens *et al*., 2014), and the Dalytyphloplanida without a proboscis (Van Steenkiste *et al*., 2013). Most kalyptorhynchs are eukalyptorhynchs (with an undivided proboscis) as opposed to the less taxon-rich schizorhynchs (with a split proboscis). Polycystididae is by far the most species-rich eukalyptorhynch family and representatives are characterized by very complex and variable male and female atrial organs (Artois and Schockaert, 2003; 2005). Many taxa of polycystidids are commonly found in marine sediments and on seaweeds. Here we report on the polycystidid *Alcha sinensis* Wang & Hu, 2019 from Korea, which is also the first record of a rhabdocoel from the Yellow Sea and the Korean Peninsula. We provide new details on the morphology of its prostate stylet type III and discuss its importance as a diagnostic character. Finally, we provide a thorough comparison with the original specimens of *A. sinensis* from southern China and with its only other congeneric species *Alcha evelinae* Marcus, 1949.

Materials and Methods

Specimens were collected in the intertidal zone of Anmyeon Island, Chungcheongnam-do, Korea (36°24′43.5″N, 126°22′34.3″E) by Kimi Kim on 13 July 2019 and 3 November 2019. Brown algae were handpicked from the sampling site and transferred to the lab. Live animals were extracted from the algae using the

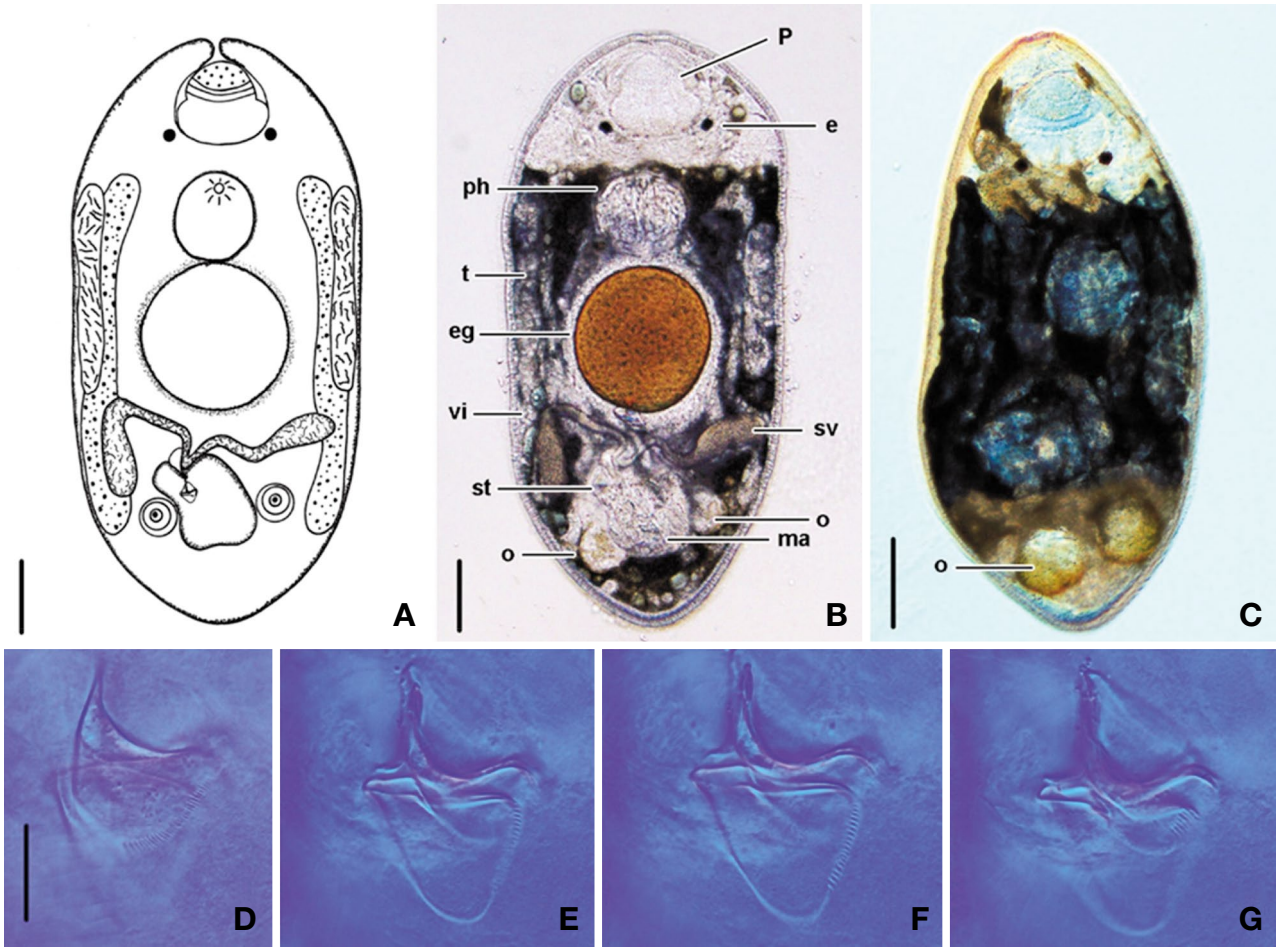

Fig. 1. *Alcha sinensis* Wang & Hu, 2019: (A) habitus from a live animal; (B, C) squeezed live specimen showing the characteristic bands of blue and yellow pigment; (D) ventral plate of stylet; (E & F) dorsal plate of stylet; (G) lamellar plate covering (D) and (F). Abbreviations used in the figures: e: eye; eg: (fertilized) egg; ma: male atrium; o: ovary; p: proboscis; ph: pharynx; st: stylet; sv: seminal vesicle; t: testis; vi: vitellarium. Scale bars; A–C: 100 μm, D–G: 20 μm).

$MgCl_2$ decantation method (Schockaert, 1996). Individual worms were isolated under a stereo-microscope EZ4 (Leica, Germany) or SZ61 (Olympus, Japan) and studied with a differential interference contrast microscope BX53 (Olympus, Japan). The animals were subsequently whole mounted with lactophenol for permanent preservation. Live animals and whole-mounted specimens were photographed under different magnifications using a digital camera DP22 (Olympus, Japan) with CellSens standard software (Olympus, Japan). Measurements of the live animals and the prostate stylet type III (terminology of Artois *et al*., 2003) in the whole mounts were performed with the same software. Figures were produced in Photoshop CS6 (Adobe, USA). All whole-mounted specimens are deposited in the National Institute of Biological Resource in Korea (NIBR).

Results

Order Rhabdocoela Ehrenberg, 1931
Family Polycystididae Graff, 1905
Genus *Alcha* Marcus, 1949

Alcha sinensis Wang & Hu, 2019 (Fig. 1)

New locality. Anmyeon Island, Chungcheongnam-do, Korea (36°24′43.5″N, 126°22′34.3″E), on brown algae in the intertidal zone (13 July 2019, 3 November 2019).
Known distribution. South China Sea, Eastern Shenzhen City, Guangdong Province, China (Hu *et al*., 2019).
Material examined. Observations on two live animals. KOR-UW1-WM5 (salinity 31‰, July 13, 2019): whole-mounted specimen. KOR-UW3-WM1 (salinity 34‰, November 3, 2019): whole-mounted specimen.

Description. Animals 0.8–0.9 mm long (Fig. 1A, B). General morphology as in Hu *et al*. (2019). Central two quarters of its body completely dark-blue because of dorsal subepidermal pigment granules, anterior and posterior quarter with brown-yellow parenchymatous pigment granules (Fig. 1B, C), particularly around paired ovaries (o, Fig. 1B, C). Cone-shaped proboscis about 140 μm long (p, Fig. 1B). Two black eyes situated posterior to proboscis (e, Fig. 1B). Pharynx about 120 μm in diam. and at 30–40% of body length (ph, Fig. 1B). Vitellaria poorly visible, stretching from pharynx to posterior end of animals (vi, Fig. 1B). One of the specimens carries a 200 μm-long fertilized egg (eg, Fig. 1B).

Male system consists of paired testes, situated midbody beside and behind pharynx (t, Fig. 1B), paired seminal vesicles (sv, Fig. 1B), and prostate stylet type III located inside male atrium (st, Fig. 1B). Stylet is about 43 μm long and has a complex morphology. Proximal base ("skirt" in Karling & Schockaert, 1977) is asymmetrically bowtie-shaped and carries two pairs of overlapping plates. Two plates of each pair differ in size and shape and are attached to each other along full length of fortified axis ("tongue" in Karling & Schockaert, 1977) arising from base. Largest plate of first pair (p1, Fig. 1D) is more or less triangular with smooth straight edge on one side, smooth rounded corner ("blade" in Karling & Schockaert, 1977), and one fringed edge on the other side. Much smaller plate of first pair (p2, Fig. 1E) has one more pronounced serrated edge over short distance followed by protruding rounded corner ("blade"). Largest plate of second pair (p3, Fig. 1F) is similar to p1, but somewhat smaller and without rounded corner. Smallest plate of second pair (p4, Fig. 1G) also has one more pronounced serrated edge, but over longer length than p2 and lacking protruding rounded corner.

Discussion

The polycystidid genus *Alcha* currently contains two species: *A. evelinae* Marcus, 1949, and *A. sinensis*. The type species, *A. evelinae*, was originally described from the Atlantic coast of Brazil (Marcus, 1949), but has subsequently been found in several locations around the world including California (Karling & Schockaert, 1977), Kenya (Jouk & De Vocht, 1989; Artois & Tessens, 2008), Mexico (Artois & Tessens, 2008), Hawaii (Tessens *et al*., 2014), Cuba (Diez *et al*., 2018), Curaçao (Van Steenkiste & Leander, 2018), and Canada (Van Steenkiste & Leander, 2018). The second species, *A. sinensis*, was recently described from Southern China (Hu *et al*., 2019).

Both species of *Alcha* are nearly identical, but—according to Hu *et al*. (2019)—differ from each other based on the morphology of the prostate stylet type III and the genetic distance evidenced in a phylogenetic tree. In *A. sinensis*, the stylet seems to have a more or less asymmetrical bowtie-shaped base and large triangular plates (Hu *et al*., 2019), while in *A. evelinae* the stylet has a more triangular base with an obvious lateral projection, and shorter plates with more deeply serrated edges, hook-like ridges, and more pronounced rounded "blades" (Karling & Schockaert, 1977; Diez *et al*., 2018). Existing descriptions of the prostate stylet type III in *A. evelinae* and *A. sinensis* mention "three" (Marcus, 1949; Karling & Schockaert, 1977; Hu *et al*., 2019) to "several" plates (Van Steenkiste & Leander, 2018), thus expressing a degree of uncertainty regarding the number of plates. Indeed, the very small size of the stylet and overlapping plates makes it difficult to assess the correct number in both species. Measurements for the total length of the stylet vary between 31–53 μm for *A. evelinae*. For the Chinese specimens of *A. sinensis*, no measurements for the total length are given; however, the stylet measures to 44 μm based on Fig. 1G–J in Hu *et al*. (2019), which is about the same length as in the Korean specimens. Based on the above-mentioned differences of the prostate stylet type III between *A. evelinae* and *A. sinensis*, and with the uncertainty of the number of plates in mind, the two specimens from Korea are provisionally placed within *A. sinensis*.

Moreover, in Van Steenkiste & Leander (2018) the prostate stylet type III in specimens of *A. evelinae* from British Columbia clearly differs from that in the specimens from Curaçao and other areas around the world. In fact, it strongly resembles those of the Korean specimens and *A. sinensis*. As such, we are confident that the specimens from Canada also belong to *A. sinensis* and should be transferred to this species, thereby supporting the suggestion of Van Steenkiste & Leander (2018) that the nominal species *A. evelinae* potentially consists of multiple species. With this knowledge, we would also like to reiterate Van Steenkiste & Leander (2018)'s call for an integrative taxonomic revision of the different populations of *A. evelinae* and *A. sinensis* based on molecular data and a detailed revision of the stylet morphology.

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