

A Taxonomic Reappraisal of *Trachelomonas hispida* (Euglenophyceae) from Korean Inland Waters

Jun Tae Kim, Woongghi Shin and Sung Min Boo*

Department of Biology, Chungnam National University, Daejeon 305-764, Korea

A total of 47 samples of *Trachelomonas hispida* from 34 inland waters, Korea were observed in detail for cells and their lorica using LM and SEM. The species is ellipsoid with small spines and dense perforations on the cell surface. Under the SEM, the shape of the collar is simply thickened and erected without encircled spines, crown with encircled spines, and crenulated with toothed spines. However, cell size, shape, lorica and collar are variable among Korean populations. These results indicate that infraspecific taxa of *T. hispida* should be reappraised.

Key Words: Euglenophyceae, inland waters, Korea, morphology, taxonomy, *Trachelomonas hispida*

INTRODUCTION

A freshwater euglenoid species, *Trachelomonas hispida*, was described by Deflandre (1926) on the features of cell shape, flagellum aperture and lorica ornaments. The morphology of the species is very variable in the field and includes about 13 infraspecific taxa (Deflandre 1926; Huber-Pestalozzi 1955; Bourrelly 1970; Philipose 1988; Coute and Therezien 1994). The infraspecific taxa have been described based on cell size and lorica characteristics. However, the features known to be diagnostic for some infraspecific taxa has been variable within a single specimen (West 1977; West and Walne 1980).

As *T. hispida* is common and wide-spread in the world (i.e. Huber-Pestalozzi 1955; Tell and Domitrovic 1985; Conforti and Joo 1994), the species has been listed in many floral and limnological reports in Korea (Chung *et al.* 1991; Kim *et al.* 1991; Kim *et al.* 1993; Choi *et al.* 1994; Park *et al.* 1995; Kim *et al.* 1996; Kim and Boo 1996; Boo *et al.* 1997; Lee and Boo 1998). Although Skvortzov (1932), Chung (1970), and Wui and Kim (1987) gave the taxonomic notes on the species using a few local samples, the descriptions and illustrations of the species from Korea require more intensive work.

The present study was aimed to appraise the diagnostic features of *Trachelomonas hispida* and to contribute its

taxonomy. We in details described the cell shape, loricate, and cytological features of the species based on collections from the whole country using the light and scanning electron microscope.

MATERIALS AND METHODS

A total of 48 *Trachelomonas hispida* samples were collected in 34 waters in Korea (Table 1). The samples were concentrated with plankton net of 20 μm in mesh size at a depth of 10 cm and brought back in 100 ml bottles to the laboratory. The live samples were kept at 4°C for observing cytological features. The others were fixed with Lugol's solution for scrutinizing the intracellular details. All the specimens were observed under X1000 using a light microscope (LM, Olympus VANOX AHBT3). The number of cells was counted on the Sedgewick Rafter counting cell under an inverted microscope (Olympus IX70) (Kim and Boo 1998).

For the scanning electron microscopy (SEM), the cells were isolated from the bloomed populations under an Olympus IX70 inverted light microscope and fixed in 2.5% glutaraldehyde solution (final concentration) at 4°C for 1.5 hr. The samples were dehydrated in a graded ethanol series, in hexamethyldisilazane (HMDS) for 10 min, and then allowed to settle glass coverslips coated with 0.1% (w/v) poly-L-Lysine. The specimens on coverslips were mounted on aluminum stubs with silver adhesives and coated with a thin layer of gold palladium

*Corresponding author (smb00@hanbat.chungnam.ac.kr)

Fig. 1. Cell features of *Trachelomonas hispida* observed by light microscope. A-C. Cell shapes. D. Cell extruding cell from lorica. E and F. Empty envelopes without the naked cell. G and H. Naked cells showing nucleus, paramylon bodies and paramylon-sheathed chloroplasts. (Scale bars: A-H = 10 μm)

using a Pelco SC-7 vacuum evaporator. The treated cells were observed under field emission of a JSM5410 SEM operating at 10-15 kV.

The voucher specimens have been deposited as liquid samples, slides, iconographs, and photographs in the herbarium of Chungnam National University, Daejeon, Korea.

RESULTS

Morphology under LM

The cells were usually elliptical and conically protruded at the posterior end in *Trachelomonas hispida* (Fig. 1). They were 22-38 μm long and 19-28 μm broad. The shape of lorica was broadly ellipsoid and slightly

swelled on the lateral side. (Fig. 1A-E).

The naked cell (Fig. 1 F) was used for observing cytological features. Pellicle was distinctly observed on the released or naked cells and was elastically covered with right-handed striae. The pellicular strips were very faint and thin with 19-25 striae per 10 μm . Under the pellicle, the tiny and mucous grains were spirally arranged along the striae (figures not shown).

Chloroplasts were parietal discoids and 5-8 μm broad. The margin of chloroplast was round or slightly undulated. The number of chloroplast ranged from 6 in small cells and 15 in large cells. Pyrenoids were located in the center of each discoid chloroplast, which are doubly sheathed by paramylon caps (Fig. 1H). Paramylon bodies were oval with a depressed center on the apical view

Fig. 2. Cell surface of *Trachelomonas hispida* observed by scanning electron microscope. The cells in a single collection from Yongwha, Iksan show considerable variations in the ornamentation of lorica surface and collar. A-D. Flagellum aperture and lorica surface of *Trachelomonas hispida*. A and B. Whole cells showing the short and fine spines on the lorica surface and collar. C. Small and short spines. D. Smooth surface of the lorica. E and F. Collar rim of a spiny form. G. Collar rim of crenulate form. H and I. Perforation of lorica and spiny ornamentation. J. Smooth surface of the lorica. (Scale bars: A-D = 5 μm ; E-G = 1 μm)

and rod on the side view (Fig. 1G). They were very numerous and were small with less than 3 μm in length.

Nucleus was spherical to elliptical and positioned in the posterior portion of cell (Fig. 1H). The position was same in both the loricate and naked cells. The nucleus was 10-17 μm broad. The stigma was singly situated near the reservoir (Fig. 1A, G, H). The color of stigma was pale yellowish red to bright crimson according to collections. The cytoplasmic granules were coarse or scattered.

Morphology under SEM

The surface of lorica was porous and covered with shapely pointed spines (Fig. 2). The pores were fine and less than 0.3 μm in diameter. The porosity were irregular and dense along the lorica surface (Fig. 2A-D, H-J), but partly distributed in the anterior and posterior portion (Fig. 2C).

The spines were fine and short with less than 0.6 μm in length. They were amorously decorated like sharp protuberances (Fig. 2A-C, H, I) and distributed throughout the lorica surface. The spines were limited in the

Table 1. Collection sites and dates of *Trachelomonas hispida* and the water types

| Sites | Collection dates | Water types |
|-------------------------|--|----------------------|
| Jiahm, Wanju | 29 Jan. 1997 | agricultural swamp |
| Nambangje, Asan | 9 Mar. 1997, 4 Apr. 1997 | agricultural swamp |
| Yeosan, Yeosan | 26 May 1997 | agricultural swamp |
| Yesan, Yesan | 9 Mar. 1997 | agricultural swamp |
| Yonghwa, Iksan | 26 May 1997 | agricultural swamp |
| Bomunho, Kyeongju | 22 Apr. 1997 | artificial reservoir |
| Seoho, Suwon | 15 Feb. 1997, 20 Apr. 1997 | artificial reservoir |
| Dohwadam, Boryeong | 16 Sep. 1995 | mountainous stream |
| Pyeongna, Boryeong | 8 Sep. 1996 | mountainous stream |
| Upo, Changnyeong | 5 Jan. 1997 | natural wetland |
| Daepyeong, Hamahn | 6 Jun. 1997 | natural wetland |
| Ilweol, Suweon | 20 Apr. 1997 | natural wetland |
| Sajipo, Changnyeong | 5 Jun. 1997 | natural wetland |
| Sidong, Hamahn | 6 Jun. 1997 | natural wetland |
| Duckjinmot, Cheonju | 15 Sep. 1996 | artificial pond |
| Jungwoimot, Kwangju | 5 Apr. 1997 | artificial pond |
| Susungmot, Daegu | 25 Dec. 1996 | artificial pond |
| Yeongtapji, Daejeon | 16 Aug. 1996 | artificial pond |
| Andongho, Andong | 25 Dec. 1996 | artificial reservoir |
| Haenam, Haenam | 10 Nov. 1996 | agricultural pond |
| Nonsan, Nonsan | 26 May 1997 | artificial reservoir |
| Kumgang, Buyeo | 10 Sep. 1994, 28 May, 29 Oct. 1995 | river |
| Kumgang, Kongju | 29 Jul. 1995 | river |
| Kumgang, Napo | 4 Apr. 1996, 23 Jun. 1996, 25 May 1997 | river |
| Nakdongang, Gupo | 19 Jan. 1997 | river |
| Namgang, Jinju | 19 Jan. 1997 | river |
| Guryongpo, Pohang | 22 Apr. 1997, 15 Dec. 1996 | roadside ditch |
| Daejoncheon, Daejeon | 21 Dec. 1996 | urban ditch |
| Kumhogang, Daegu | 25 Dec. 1996, 28 Aug. 1997 | urban ditch |
| Mushimcheon, Cheongju | 6 Oct. 1996, 4 Jan. 1997 | urban ditch |
| Samcheoncheon, Cheonju | 15 Sep. - 29 Dec. 1996, 27 Apr. - 29 Jun. 1997 | urban ditch |
| Kyeongjucheon, Kyeongju | 19 Jan. 1997 | urban ditch |
| Uncheonmot, Kwangju | 29 Dec. 1996 | urban swamp |

anterior, posterior portion on a single specimen (Fig. 2D), or occasionally absent (Fig. 2J).

The flagellum aperture was characterized by the presence of collar (Fig. 2A-G). The collar was short and distinct with 3-5 μm in diameter and 1-2 μm in height. The shape of the collar was simply thickened and erected without encircled spines (Fig. 2D, G), crown with encircled spines (Fig. 2C), and crenulated with toothed spines (Fig. 2A, B, E). The morphological variations of the collar rim were frequently observed on cells of a single collection.

Field data

Trachelomonas hispida commonly occurred throughout a year in inland waters of Korea. Although the population size was low with less than 100 cells ml^{-1} in most

collections, more than 500 cells ml^{-1} were counted at agricultural swamps in Yonghwa, Iksan and Nambangje, Asan and at natural wetland in Sidong, Hamahn.

DISCUSSION

Trachelomonas hispida from Korean inland waters is characterized by its being ellipsoid with small spines and dense perforations on the cell surface under LM and the shape of the collar is simply thickened and erected without encircled spines, crown with encircled spines, and crenulated with toothed spines. The above-mentioned features of the Korean populations accord with the original description of the species (Deflandre 1926), belonging to the section *Rotundatae* (Huber-Pestalozzi 1955). The oval to elliptical shape with narrowed and

Table 2. Morphological comparison among infraspecific taxa of *Trachelomonas hispida*

| | <i>T. hispida</i> var. <i>hispida</i> (Perty) Stein emend. Deflandre | var. <i>acuminata</i> Deflandre | var. <i>caudata</i> Lemmermann | var. <i>coronata</i> (Lemm.) Deflandre | var. <i>crenulato-collis</i> (Maskell) Lemmermann | |
|-----------------------------|---|--|---|--|---|------------------------------------|
| Demension (μm) | 26-32 \times 19-23 | 27-30 \times 20-25 | similar to type | 29-32 \times 20 | 22 \times 18 | |
| Shape | ovate, narrowed anteriorly | slightly protrude at posterior | oval | oblong-oval | oval | |
| Flagellar aperture | slightly raised | without collar | short collar | short collar with a circle of spines | short collar, coarsely toothed margin | |
| Lorica surface | beset with minute, sharp-pointed warts | punctate, with short and sharp spines | with unique short caudae in posterior portion | uniformly beset with short sharp spines | with minute and sharply pointed warts | |
| Reference | Deflandre (1926) | Huber-Pestalozzi (1955) | Huber-Pestalozzi (1955) | Prescott (1962), Coute and Therezien (1994) | Huber-Pestalozzi (1955), Akiyama <i>et al.</i> (1981) | |
| (continued) | | | | | | |
| | f. <i>glabra</i> Philipose | f. <i>patula</i> Deflandre | f. <i>recta</i> Deflandre | var. <i>duplex</i> Deflandre | var. <i>papillata</i> Skvortzov | var. <i>punctata</i> Lemmermann |
| 31.7 \times 14.4 | similar to type variety | similar to type variety | 33-34 \times 25-26 | 30-33 \times 23-25 | 34-40 \times 28-30 | 32-34 \times 29 |
| similar to type | similar to type variety | with a vase-like collar | ovoid | without a collar | oval | broadly oval |
| similar to type | similar to type variety | short collar with a coarsely toothed margin | ovoid | without a collar | without a collar | with slightly raised rim |
| smooth | similar to type variety | punctate, unevenly beset with short sharp spines | without a collar | with short spines only in anterior and posterior | smooth, but a few minute spines | densely punctate |
| Philipose (1988) | Deflandre (1926) Huber-Pestalozzi (1955) | Deflandre (1926) Philipose (1988) | Huber-Pestalozzi (1955), Akiyama <i>et al.</i> (1981) | Huber-Pestalozzi (1955), Prescott (1962) | Huber-Pestalozzi (1955), Prescott (1962) | Huber-Pestalozzi (1955) |
| (continued) | | | | | | |
| | var. <i>rugosa</i> Coute et Therezien | f. <i>minima</i> Kufferath | f. <i>minor</i> Bourrelly | Korean specimens | | |
| 26 \times 20 | | 19 \times 12 | 18-20 \times 15-16 | 22-38 (19-28 | | |
| similar to type | | broadly ellipsoid | similar to type | ellipsoid | | |
| similar to type | | similar to type | similar to type | short collar; with or without spine, toothed or crown-shaped | | |
| rugose | | similar to type | similar to type | punctate, with short spines | | |
| Coute and Therezien (1994) | | Huber-Pestalozzi (1955) | Conforti and Joo (1994) | this study (1999) | | |

rounded ends preferentially distinguishes this species from other related species such as *T. australica* (Playf.) Defl. and *T. robusta*. Swir emend Defl. A certain degree of shape or size variations has been used for description of the varieties or forms (Huber-Pestalozzi 1955). For

example, var. *acuminata* is based on the conically protruded cone of the posterior end, f. *minor* and *minima* are based on the small size. However, these-like variations of the cell size and occur sometimes appear in a single population in Korean inland waters.

Lorica ornamentation is the only character for classifying species and infraspecific taxa in *Trachelomonas* (Castillo and Alamos 1993; Couté and Thérézien 1994). In *T. hispida*, many infraspecies, var. *duplex*, var. *papillata*, var. *punctata*, and f. *patula*, have been described on the basis of the distribution of the pore and spines.

Light and scanning electron microscopy have shown that the pores and spines are different depending on the sampling locations and developmental stages of the cells (Pringsheim 1953; West 1977; West *et al.* 1980a, b; Walne 1980). The porosity and ornamentation of lorica surface under scanning electron microscope is variable in populations from Korea. This indicates that lorica characters should be carefully used for description of species or infraspecific taxa.

The collar has been regarded as a criterion of the infraspecific criteria, especially based on the features of rim (Huber-Pestalozzi 1955; Tell and Domitrovic 1985; Dunlap *et al.* 1986; Philipose 1988; Wolowski 1992). According to the protologue (Deflandre 1926), *T. hispida* has a simply raised collar without any ornamentation. Thus, var. *crenulato-collis* and var. *coronata*, var. *coronata-punctata*, f. *recta*, and f. *glabra* have been described on the basis of the collar character.

The cytological features have been poorly remarked in *Trachelomonas hispida* taxonomy, owing to difficulty of observation induced by the envelope. These characters are important for species and generic circumscription of euglenoid algae (Pringsheim 1953; Singh 1956; Leedale 1975). It may be achieved by the observation of young and naked cells, which were released from the lorica in blooming populations.

The pellicular features indicate that *Trachelomonas hispida* may be related with *Euglena viridis* and *E. gracilis*. Moreover, the arrangement of cytoplasmic granules under the pellicle, which is observed in this study and the above-mentioned *Euglena* species (Kim *et al.* 1998), suggests that *Trachelomonas* is closely related to *Euglena*. The striation pattern of *T. hispida* is similar to that of *Euglena* members having flexible creeping motion.

The chloroplasts with double sheathed pyrenoids distinguish *Trachelomonas hispida* species from other *Trachelomonas* species (Pringsheim 1953; Bourrelly 1970; Philipose 1988). The oval to rod shaped paramylon bodies commonly occur in *Trachelomonas* species (Singh 1956).

Morphological variations of cell size, shape, and surface characteristics observed in Korean populations of *Trachelomonas hispida* require that *T. hispida* var. *coronata* and var. *crenulato-collis*, which have been reported in

Korea (Chung 1970; Kim 1998), should be reduced to *T. hispida* var. *hispida*. Other infraspecific taxa in Table 2 may be reduced to a few taxa, but this needs more intensive taxonomic studies using their specimens.

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