

## **Two Newly Recorded Epistylid Ciliates, *Epistylis chrysemydis* and *E. entzii* (Ciliophora: Oligohymenophora: Peritrichida) in Korea**

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### **ABSTRACT**

Two epistylid ciliates collected from the sewage treatment plant in Ulsan, Korea were identified as *Epistylis chrysemydis* Bishop and Jahn, 1941 and *E. entzii* Stiller, 1935. They were examined *in vivo* using DIC microscope. Protargol impregnation used to reveal the infraciliature and cytological details. The improved diagnoses for these species are as followings. *E. chrysemydis*: peristomial lip has two bulges encircling the oral region and ciliary rows wound about one and half around peristome; one contractile vacuole located ventrally; stalk ramified hollow; membranelles 1 and 3 are longer than membranelle 2. *E. entzii*: peristomial lip has one bulge encircling the oral region and ciliary rows wound about 1 and 1/4 to 1 and 1/3 around peristome; one contractile vacuole located dorsally; stalk ramified, not hollow; membranelles 1 and 2 are longer than membranelle 3. They are new to Korean fauna.

Key words: Peritrichida, Epistylidae, freshwater, taxonomy, Korea

### **INTRODUCTION**

The fresh water contains diverse assemblage of ciliates. The peritrichous ciliates are one of the conspicuous groups among them. They are usually encountered in benthic habitats of aquatic

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environments. Up to now, more than 50 species of the *Epistylis* were known to science but only two species of *Epistylis* have been reported from Korea so far (Bick, 1972; Cho and Shin, 2003). We redescribe two species of epistylid peritrichs which are new to Korean fauna.

## MATERIALS AND METHODS

The present study was based on the specimens which collected at 9 June, 2004 from the aeration tank of Hoeya sewage treatment plant in Ulsan, Korea. The specimens were cultured at room temperature in the laboratory with commercial mineral water, enriched with dried wheat grains for supplying fungal and bacterial nutrients. The shapes and movements of the living specimens were observed by DIC microscope and captured by CCD camera. The infraciliature was observed by using the modified protargol method (Wilbert, 1975; Shin and Kim, 1993). The illustrations of the impregnated specimens and the living specimens were made with the aid of a camera. Biometrical analysis was performed using the method described in Sokal and Rohlf (1973). We adopted the classification schemes established by Small and Lynn (1985) and Lynn and Corliss (1991).

## RESULTS

Two epistylid ciliates were characterized cytomorphologically, analyzed biometrically and redescribed with illustrations of ciliatures and distributions. They are compared with related species and arranged as a systematic schemes.

Phylum Ciliophora Doflein, 1901

Class Oligohymenophora Jankowski, 1967

Order Peritrichida Stein, 1859

Family Epistylidae Jankowski, 1979

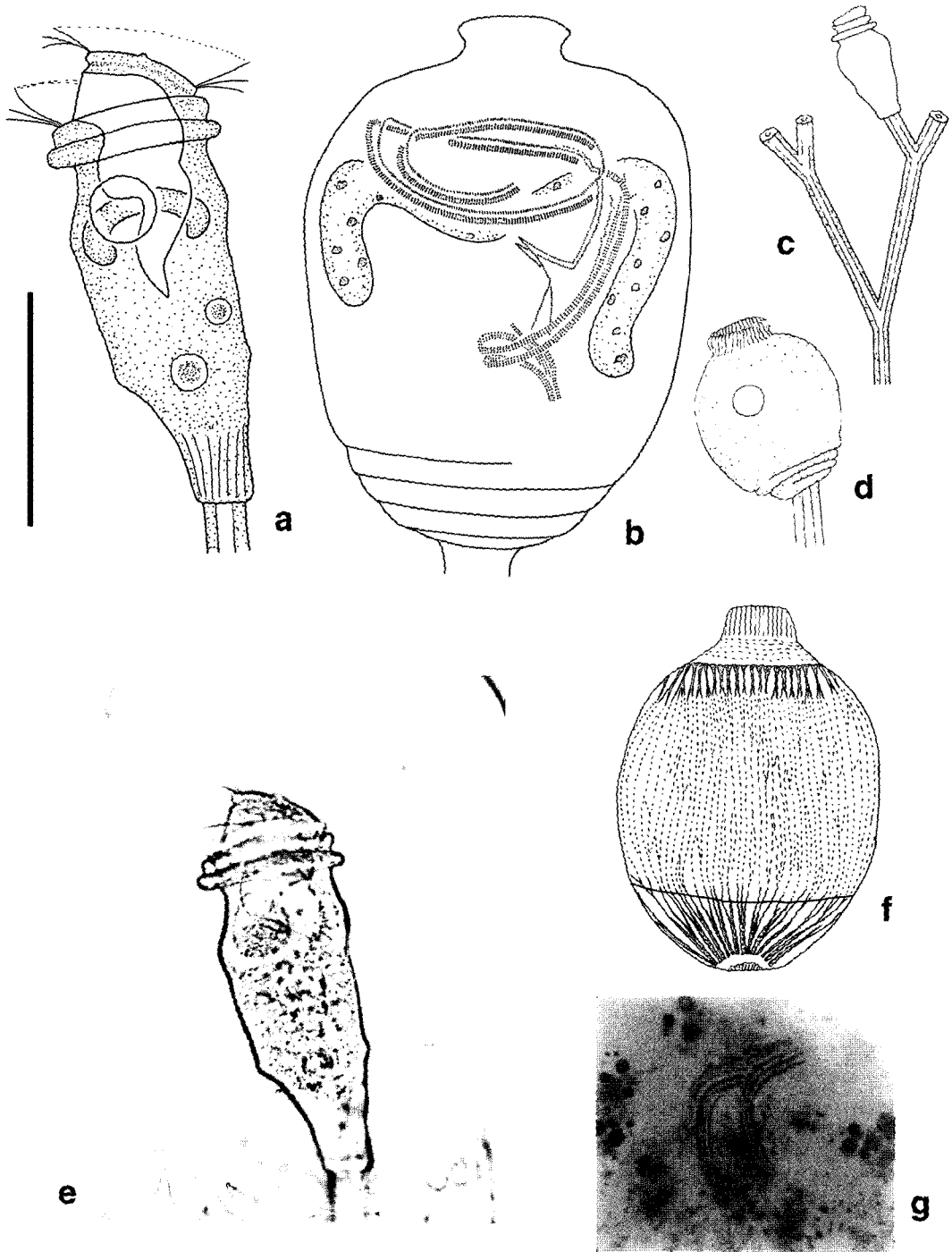
Genus *Epistylis* Ehrenberg, 1830

### \**Epistylis chrysemydis* Bishop and Jahn, 1941 (Fig. 1, Table 1)

*Epistylis chrysemydis* Bishop and Jahn, 1941 (cited from Foissner et al., 1992); Foissner et al., 1992, p. 182.

*Epistylis balatonica*: Stiller, 1971, p. 43.

**Description.** General morphology and behavior: Colonial and sessile. Elongated zooids shaped inverted cylindrical-ellipsoid form rather than bell-shaped, borne upon ends of branched stalk. Stalk ramified dichotomously, about 16-20  $\mu\text{m}$  in diameter, surface smooth without transverse stripes or foldings, inner part hollow without muscle (Fig. 1c). Body of extended zooid ranging about 160-215  $\mu\text{m}$  long and about 60-70  $\mu\text{m}$  wide *in vivo*. The diameter of peristome about 60-70  $\mu\text{m}$  *in vivo*. Peristomial lip around peristome when expanded having two definite bulges encircling oral region and ciliary rows wound about one and half around peristome. Peristomial disc



**Fig. 1.** *Epistylis chrysemydis*. a, e, typical extended zooids (*in vivo*); b, pattern of three membranelles and undulating membrane, and macronucleus; c, dichotomously branched hollow stalk; d, contracted zooid; f, somatic myoneme; g, proximal end of membranelles. Scale bar = 100  $\mu$ m.

**Table 1.** Biometric characterization of *Epistylis chrysemydis*. The abbreviations in the table are as follows: L = live specimen; S = protargol stained specimen; Med. = median; Min. = minimum; Max. = maximum; SD = standard deviation; SE = standard error; CV = coefficient of variation in %; n = population size.

Characteristics	Method	Mean	Med.	Min.	Max.	SD	SE	CV	n
Body length	L	190	180	160	215	22.85	7.23	12.03	10
Body width	L	62	60	60	70	3.50	1.11	5.64	10
Number of macronucleus	S	1	1	1	1	0	0	0	10
Number of contractile vacuole	L	1	1	1	1	0	0	0	10
Diameter of peristome	L	65.5	65	60	70	3.69	1.17	5.63	10
Buccal cavity length	L	86	87.5	75	95	7.0	2.21	8.13	10
Body length/Buccal cavity length	L	2.23	2	1.89	2.87	0.36	0.11	16.17	10

elevated obliquely, convex and pointed when zooid completely extended. Cytopharynx large and deep. Buccal cavity long about the half of zooid length (Fig. 1a and 1e). Pellicle smooth with horizontal striations. Cytoplasm colourless, usually containing brownish small and large granules and containing several large food vacuoles in freshly mounted specimens. One large, contractile vacuole lying ventrally and beside funnel-shaped vestibulum, contracting every 20-30 seconds. One macronucleus lying horizontally, oriented in anterior half of zooid, 3/4 circle-shaped, thick and band-like (Fig. 1a).

**Infraciliature:** Undulating membrane (Um) and oral membranelles turning around peristomial disc, about 1 and 1/2 turns, before entering vestibulum. Three membranelles (M1, M2 and M3) in proximal ends consisting of three kineties, respectively; M1 and M2 paralleling upward side by side within vestibulum; M3 very short about 1/2 length of M2, comparing with long M1. Um passing around the other side of vestibulum to membranelles. Germinal membrane extending from end of Um. Adoral ciliary wreath (ACW) composed of close-set fragments of kineties (Fig. 1b).

Cortex of cell having longitudinal contractile fibrils of myoneme starting from around scopula (Sa). Myoneme in middle part of zooid thin, like dotted line (Fig. 1f).

**Distribution.** Europe, Japan and Korea

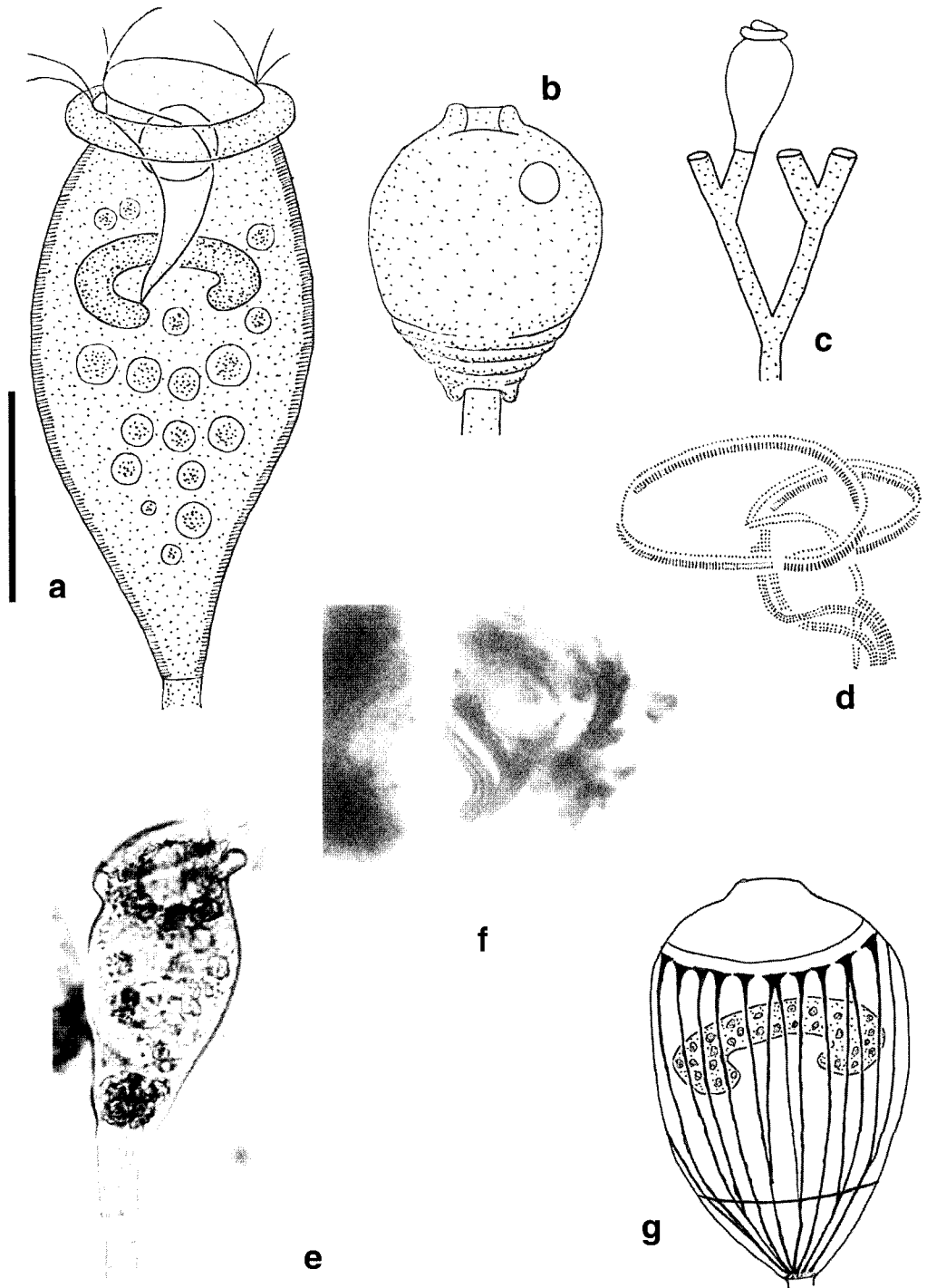
**Remarks.** As a part of the biometrical data, coefficients of variation (CV) were calculated. The number of macronucleus and contractile vacuole showed 0.00 of CV. Thus these characters are found to be very constant and considered as the important diagnostic features of this genus. Comparatively low CV ranging from 5.63 to 12.03 were shown in the following characters: The length of body, the width of body, the diameter of peristome, the length of buccal cavity. These characters are very important for identification of species because of their low variability (Table 1).

**\**Epistylis entzii* Stiller, 1935 (Fig. 2, Table 2)**

*Epistylis entzii* Stiller, 1935, p. 151; Foissner et al., 1992, p. 190.

*Epistylis urceolata*: Kahl, 1935, p. 686; Stiller, 1971, p. 44.

**Description.** General morphology and behavior: Colonial and sessile. Elongated zooids shaped



**Fig. 2.** *Epistylis entzii*, a, e, typical elongated zooids; b, contracted cell; c, dichotomously compact stalk; d, three membranelles and undulating membrane pattern; f, the end of membrane; g, myoneme and macronucleus. Scale bar = 50  $\mu$ m.

inverted long club form, borne upon ends of branched stalk. Stalk ramified dichotomously, about 11–13  $\mu\text{m}$  in diameter, surface smooth without transverse stripes or foldings, inner part compact and not hollow without muscle (Fig. 2c). Body of extended zooid ranging about 107–145  $\mu\text{m}$  long and about 27–53  $\mu\text{m}$  wide *in vivo*. Peristome about 1/3 zooid length (Fig. 2a). The diameter of peristome about 29–53  $\mu\text{m}$  *in vivo*. Peristomial lip around peristome, when zooid elongated, having one definite bulge encircling oral region, and its ciliary rows wound about 1 and 1/4 to 1 and 1/3 around the peristome. Peristomial disc obliquely elevated, convex and round when zooid completely extended (Fig. 2a and 2e). Cytopharynx funnel-shaped and deep. Pellicle smooth with horizontal striations. Cytoplasm colourless, usually containing several large food vacuoles in freshly mounted specimens. One large contractile vacuole lying horizontally, beside peristomial lip, and dorsally near peristome. One macronucleus lying horizontally oriented in anterior half of cell, 3/4 circle-shaped, thick and band-like (Fig. 1a).

**Infraciliature:** Undulating membrane (Um) and oral membranelles turning around peristomial disc, about 1 and 1/4–1/3 turns, before entering vestibulum. Three membranelles (M1, M2 and M3) in proximal ends consisting of three kineties, respectively. The proximal end of M1 clearly adjacent to that of M2. M1 and M2 longer than membranelle 3 (M3) in proximal ends. M3 very short, about 1/4 length of M2, compared with long M1. Um passing around vestibulum on opposite wall to membranelles. Germinal membrane extending from end of Um. Adoral ciliary wreath composed of close-set fragments of kineties (Fig. 2d).

Cortex of cell having longitudinal contractile fibrils of myoneme starting from around scopula. Somatic myonemes in middle part of zooid thick relatively (Fig. 2g).

**Distribution.** Europe, Japan and Korea

**Remarks.** As a part of the biometrical data, coefficients of variation (CV) were calculated. The number of macronucleus and contractile vacuole showed 0.00 of CV. Thus these characters are found to be very constant and considered as the important diagnostic features of this genus. Comparatively low CV ranging from 9.4 to 10.94 were shown in the following characters: The length of body, buccal cavity. These characters are very important for the identification of species because of their low variability (Table 2).

**Table 2.** Biometric characterization of *Epistylis entzii*. The abbreviations in the table are as follows: L = live specimen; S = protargol stained specimen; Med. = median; Min. = minimum; Max. = maximum; SD = standard deviation; SE = standard error; CV = coefficient of variation in %; n = population size.

Characteristics	Method	Mean	Med.	Min.	Max.	SD	SE	CV	n
Body length	L	127.73	120	107	145	13.98	4.21	10.94	11
Body width	L	41.82	40	27	53	9.50	2.86	22.71	11
Diameter of peristome	L	43.64	43	29	53	9.97	3.01	22.85	11
Buccal cavity length	L	42	42	35	49	3.95	1.19	9.40	11
Body length/Buccal cavity length	L	3.03	3.08	2.34	3.38	0.28	0.09	9.36	11
Number of macronucleus	S	1	1	1	1	0	0	0	11
Number of contractile vacuole	L	1	1	1	1	0	0	0	11

## DISCUSSION

*Epistylis chrysemydis* is very similar to *E. hentscheli* Kahl, 1935 for the following two characteristics: adoral ciliary spiral on peristomial disc turns less than two times, and stalk is hollow. The former is different from the latter for the followings: *E. chrysemydis* has two bulges while *E. hentscheli* has one. *E. chrysemydis* is different from *E. galea* Ehrenberg, 1831, *E. coronata* Nusch, 1970, *E. plicatilis* Ehrenberg, 1831 and *E. entzii* Stiller, 1935 because the former has a hollow stalk and the others have a compact stalk. *E. chrysemydis* could be mistaken for species of *Campanella* because the former has the ciliary rows wind about 1 and 1/2 around the peristome while the latter has 4-6 windings of ciliary rows. The characteristic of Korean population of *E. chrysemydis* are mostly coincident with those of European population (Stiller, 1971; Bick, 1972; Foissner et al., 1992; Cho and Shin, 2003).

*Epistylis entzii* is very similar to *E. corona* Nusch, 1970 and *Epistylis plicatilis* Ehrenberg, 1831 for the following two characteristics: They have a compact stalk and one bulge in peristomial lip. *E. entzii* is different from *E. corona* and *E. plicatilis* in body shape: *E. entzii* is club-shaped, while *E. corona* cylindroid and *E. plicatilis* funnel-shaped. The shape of peristomial disc of *E. entzii* is convex and round while that of *E. corona* is convex and pointed. The length of membranelle 3 (M3) in *E. entzii* is about 1/4 of M2 while that of *E. plicatilis* is about 1/2. The germinal membrane is extending from the end of undulating membrane in *E. entzii*, however in *E. plicatilis*, it is extending from the half of undulating membrane. *E. entzii* is different from *E. chrysemydis* for the followings: the former has a compact stalk, one bulge, dorsal contractile vacuole and rounded convex peristomial disc, while the latter a hollow stalk, two bulges, ventral contractile vacuole and pointed convex peristomial disc. The size of Korean population of *E. entzii* is a little smaller than that of European population (Kahl, 1935; Stiller, 1935, 1971; Bick, 1972; Foissner et al., 1992; Cho and Shin, 2003).

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(섬모충문: 소막충강: 주모목)

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요 약

울산 인근의 하수처리장에서 채집된 섬모충류가 겹가지섬모충과 (Epistylidae)에 속하는 원반겹가지섬모충 (*Epistylis chrysemydis*)과 겹가지섬모충 (*Epistylis entzii*)으로 각각 동정되었다. 이 종들은 DIC 현미경을 이용하여 생체관찰하였으며, Protacol로 염색하여 세부형질을 관찰하였다. *E. chrysemydis*는 위구부의 위구순에는 2개의 bulge가 있으며, 섬모열은 위구부에서 1½바퀴를 돌고, 1개의 수축포는 배쪽에 위치하며, 줄기가 텅 비어 있는 형태이다. 구부막판 1과 3의 끝이 구부막판 2보다 길다. 반면에 *E. entzii*는 위구부의 위구순에는 1개의 bulge가 있으며 섬모열은 위구부에서 1¼-1½바퀴를 돈다. 수축포는 등쪽에 위치하며 줄기는 팽 찬 형태이다. 구부막판 1과 2의 끝이 구부막판 3보다 길다. 이종들은 한국에서 처음 보고된다.