

Strombidinopsis jeokjo n. sp. (Ciliophora: Choreotrichida) from the Coastal Waters off Western Korea: Morphology and Small Subunit Ribosomal DNA Gene Sequence

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ABSTRACT. The planktonic ciliate *Strombidinopsis jeokjo* n. sp. is described from Quantitative Protargol-Stained (QPS) preparations, and the sequence of the small subunit rDNA (SSU rDNA) from cultured cells is reported. This species is ovoid and bluntly tapered towards the posterior. The ranges (and mean \pm standard deviation, $n = 31$) of cell length, cell width, and oral diameter of the QPS-stained specimens were 100–190 μm (149 ± 25), 60–105 μm (79 ± 13), and 55–80 μm (64 ± 5), respectively. Fifteen to seventeen external oral polykinetids had oral membranelle cilia 20–35 μm long. Twenty-six to twenty-eight somatic kineties were equally spaced around the cell body and extended from the oral to the posterior regions with 23–44 dikinetids per kinety. Both kinetosomes of each kinetid bore cilia 3–7 μm long. *Strombidinopsis jeokjo* had two ovoid macronuclei of 25–38 $\mu\text{m} \times 12$ –15 μm . When properly aligned, the sequence of the SSU rDNA of *S. jeokjo* (GenBank Accession No. AJ628250) was approximately 2% different from that of an unidentified *Strombidinopsis* species (GenBank Accession No. AF399132-AF399135), the closest species in the SSU rDNA sequence.

Key Words. Ciliate, DNA, heterotrophic protist, Oligotrichia, plankton, protozoa.

THE species in the genus *Strombidinopsis* are common planktonic ciliates found in coastal and oceanic waters (Buskey and Hyatt 1995; Dale and Lynn 1998; Gifford 1985; Hansen et al. 1993; Jeong et al. 1999; Lynn et al. 1991; Montagnes and Taylor 1994; Putt 1991; Snyder and Ohann 1991). Some species belonging to this genus are known to be voracious grazers, which can have considerable grazing impacts on prey populations, particularly on red-tide dinoflagellates (Jeong et al. 1999; Montagnes and Lessard 1999) and diatoms (Montagnes, Berger, and Taylor 1996). Several new species in the genus *Strombidinopsis* have been described recently (Lynn et al. 1991; Snyder and Ohann 1991; Song and Bradbury 1998). We collected a large new species belonging to the genus *Strombidinopsis* from the coastal waters off western Korea and describe this species in the present study.

MATERIALS AND METHODS

Collection and culturing of *Strombidinopsis* sp. Plankton samples collected with a 40-cm diam., 25- μm mesh plankton net were taken from the coastal waters off Haje, near the mouth of the Mankyong Estuary, Korea (35° 53' N, 126° 37' E), during October 2003. The water temperatures and salinities were 18.8 °C and 29.4 psu, respectively. The 2-L samples were screened gently through a 202- μm Nitex mesh and placed in 1-L polycarbonate (PC) bottles to which were added 50 ml of *f/2* medium, and a mixture of the dinoflagellates *Prorocentrum micans* and *Lingulodinium polyedrum* as food. The bottles were placed on plankton wheels rotating at 0.9 rpm and incubated at 20 °C under the continuous illumination of 20 $\mu\text{E m}^{-2} \text{s}^{-1}$ of cool white fluorescent light. Two days later, aliquots of the enriched water were transferred to 6-well tissue culture plates and a monoclonal culture of *Strombidinopsis* sp. was established by two serial single-cell isolations. Once dense cultures of this *Strombidinopsis* sp. were obtained, they were transferred every two or three days to 500-ml PC bottles of fresh *P. micans* or *L. polyedrum* as prey.

Fixation and staining. Samples for the QPS method (Montagnes and Lynn 1987) were fixed with a modified concentrated Bouin's solution (final concentration 10%, v/v) in which most of the cells were well preserved.

Measurement of *Strombidinopsis* specimens. More than one week after being fixed with Bouin's solution, the samples were stained using QPS. When completely dried, the slides were examined with a compound microscope at a magnification of 400- to 1000 \times . Measurement of the cells followed the recommendations of Lynn et al. (1991), Montagnes and Lynn (1991), and Snyder and Ohann (1991). The following features were measured: cell length (the maximum longitudinal linear distance excluding cilia), cell width (the maximum diam.), number of polykinetids in the external polykinetid zone and in the internal polykinetid zone, number of somatic kineties, number of dikinetids per somatic kinety, and number, shape, size, and position of the macronuclei.

DNA extraction, PCR amplification, sequencing and data analysis. Genomic DNA was extracted from this *Strombidinopsis* sp. with a DNeasy Tissue kit (Qiagen, Stanford, USA) following the manufacturer's instructions. The extracted DNA was divided into two tubes and used to conduct independent PCR reactions. These were performed under the following conditions: one cycle of 3 min at 94 °C; 15 cycles of 30 sec at 94 °C, 40 sec at 56 °C, and 3 min at 72 °C; 25 cycles of 30 sec at 94 °C, 40 sec at 52 °C, and 3 min at 72 °C; and a final cycle of 5 min at 72 °C. The small subunit ribosomal DNA (SSrDNA) was amplified by PCR to a full-length fragment and an internal 600-bp DNA fragment (approximately 600 bp to 1200 bp downstream from the 5' end of the gene). The primers used for PCR were as follows. For the full length fragment, the universal eukaryotic forward primer EukA: 5'-CTG GTT GAT CCT GCC AG-3' and the reverse primer EukB: 5'-TGA TCC TTC YGC AGG TTC-3' were used (Petroni et al. 2002). For the internal 600-bp fragment, forward primer Gas+600: 5'-CGG TAA TKC CAG CTC CAA TAG CG-3' and reverse primer Gas+1220: 5'-CCT GGT GGT GCC CTT CCG TC-3' were used. The PCR fragments were inserted into a pGEM-T vector (Promega, Madison, USA) and at least three colonies for each PCR fragment were selected for DNA sequencing. Sequencing was performed with EukA, EukB, Gas+600, Gas-1220 primers, and two additional primers, Gas+1390 (5'-CTG GTT AAT TCC GAT AAC G-3') and Gas-1540 (5'-GGG CAT CAC AGA CCT GT-3') using an ABI PRISM® 3700 DNA Analyzer (Applied Biosystems, Foster City, USA). All sequences were aligned using the CLUSTALX multiple alignment program (Thompson et al., 1997).

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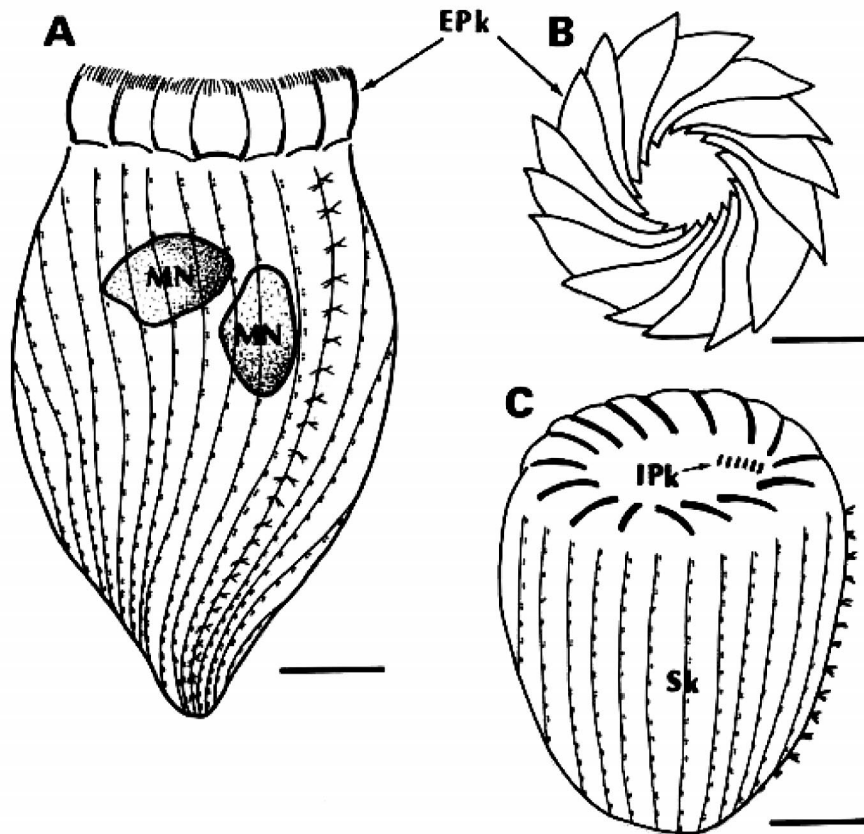


Fig. 1. Schematic diagram of *Strombidinopsis jeokjo* n. sp. (A) Lateral view, indicating the position and shape of external oral polykinetids (EPk), somatic kineties (Sk), and macronuclei (MN). (B) Anterior view, indicating the position and shape of the external oral polykinetids (EPk). (C) Dorsal-lateral view, indicating the position of external oral polykinetids (EPk) and internal oral polykinetids (IPk). Scale bar = 20 μ m.

RESULTS

Strombidinopsis jeokjo n. sp. (Choreotrichida, Strombidinopsidae)

Description. *Strombidinopsis jeokjo* n. sp. is ovoid and bluntly tapered towards the posterior when without prey (Fig. 1–2). The ranges (and mean \pm standard deviation, $n = 31$) of cell length, cell width, and oral diameter of QPS-stained specimens were 100–190 μ m (149 ± 25), 60–105 μ m (79 ± 13), and 55–80 μ m (64 ± 5), respectively (Table 1). Fifteen to seventeen external oral polykinetids (EPk) formed a completely closed circle, and had oral membranelle cilia 20–35 μ m long. There were 2–8 internal oral polykinetids (IPk). Twenty-six to

twenty-eight somatic kineties (Sk) were equally spaced around the cell body and extended from the oral to the posterior regions, with 23–44 dikinetids per kinety. Both kinetosomes of each kinetid bore cilia 3–7 μ m long. There were two ovoid macronuclei of 25–38 μ m \times 12–15 μ m.

Gene sequence of *Strombidinopsis jeokjo* n. sp. The SSU rDNA sequences of *Strombidinopsis jeokjo* n. sp. obtained from two independent PCR reactions were identical (1755 nucleotides; GenBank Accession No. AJ628250).

Time and locality of isolation. *Strombidinopsis jeokjo* n. sp. was found in the coastal waters off Haje, which is located near the mouth of the Mankyeong Estuary, Korea (35° 53' N, 126° 73' E). This species was observed from July to November,

Table 1. Morphometric data of *Strombidinopsis jeokjo* n. sp. All data are based on the protargol-stained specimens.

Character	Range	Average	Standard deviation	Median	Mode	Number of specimens
Length (μ m)	100–190	148.6	24.6	150	163	31
Width (O) * (μ m)	55–80	64.0	4.7	65	68	31
Width (M) * (μ m)	66–105	78.5	12.9	80	68	31
EPk*	15–17	16.2	0.5	16	16	19
IPk*	2–8	5.0	1.9	5	6	9
Sk*	26–28	27.6	0.7	28	28	22
nDk*	23–44	33.9	7.8	34	28	13
MN*	0–2	1.6	0.8	2	2	21
MNL* (μ m)	23–37.5	34.0	2.8	35	32	24
MNW* (μ m)	12.5–16	14.6	1.1	15	15	24

Width (O): Width of the oral part, Width (M): Width of the widest part in body, EPk: number of external oral polykinetids, IPk: number of internal oral polykinetids, Sk: number of somatic kineties, nDk: number of dikinetids per kinety, MN: number of macronuclei, MNL: macronuclear length, MNW: macronuclear width.

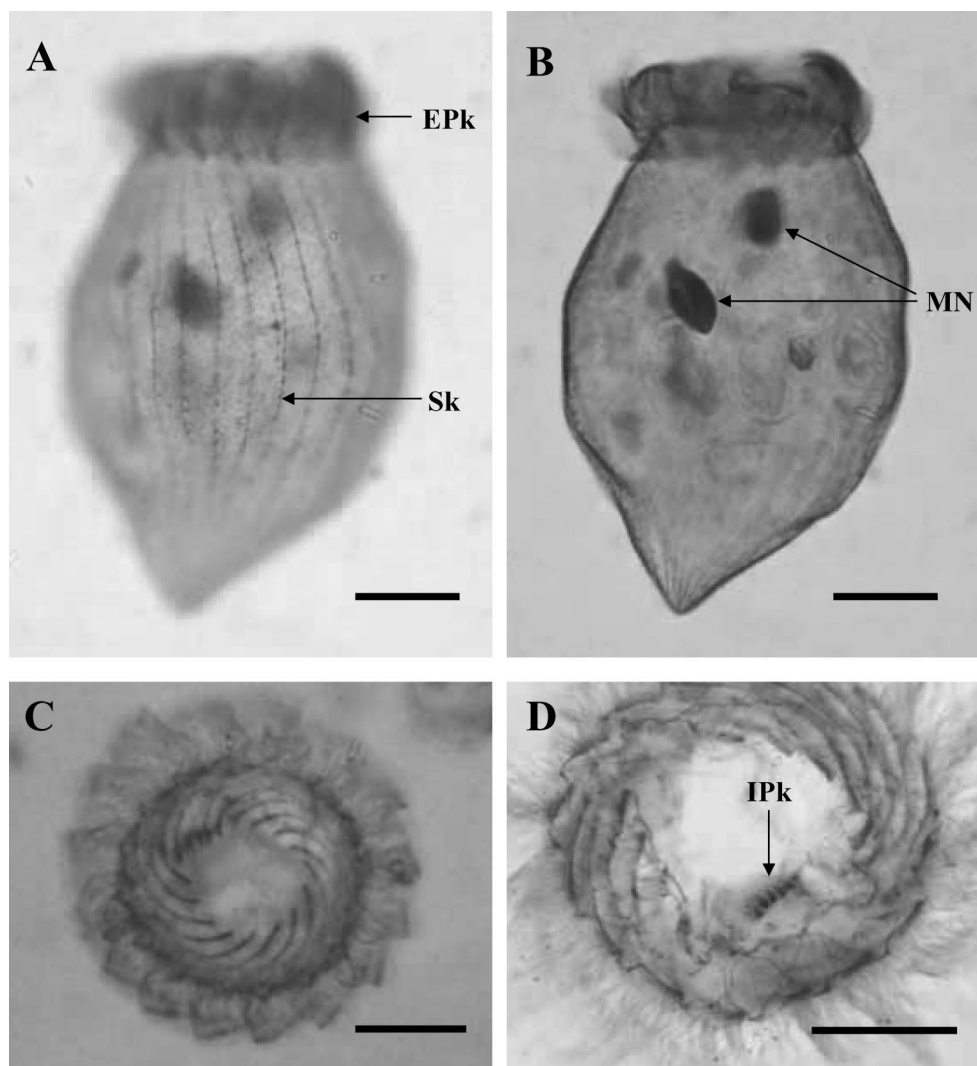


Fig. 2. Micrographs of protargol-stained specimens of *Strombidinopsis jeokjo* n. sp. (A) Lateral view (surface), indicating external polykinetids (EPk) and somatic kineties (Sk). (B) Lateral view (inside), indicating the position and shape of macronuclei (MN). (C) Anterior view, indicating the position and number of external oral polykinetids. (D) Inverted anterior view, indicating the position and number of internal oral polykinetids (IPk) in the internal oral polykinetid zone, separated from the cell body and then inverted. Lateral views of the cell anterior, indicating 2 (E), 3 (F), 6 (G), and 8 (H) internal oral polykinetids (arrows). Scale bar = 20 μ m.

Table 2. Morphological comparison among *Strombidinopsis* species preserved with fixatives. Means followed by ranges in parentheses.

	Length (μ m)	Width (μ m)	EPk*	IPk*	Sk*	nDk*	MN*	MNL* (μ m)	MNW* (μ m)	Reference
<i>S. bates</i>	17 (12–20)	14 (10–17)	16 (14–17)	1	13 (10–16)	~6	2 (1–2)	5 (4–7)	5 (4–6)	(1)
<i>S. sphaira</i>	22 (18–25)	21 (16–28)	14 (13–15)	1	14 (13–15)	~6	2	5 (4–7)	4 (3–5)	(1)
<i>S. chilorhax</i>	29 (24–35)	24 (17–29)	17 (15–18)	1	18 (15–18)	~10	2 (1–2)	9 (7–13)	6 (6–7)	(1)
<i>S. elegans</i>	(30–40)	(30–40)	(26–27)	1	(19–24)	~7–11	2			(4)
<i>S. cercionis</i>	58 (48–65)	30 (24–40)	(13–14)	0	(11–14)	10–14	4 (2–6)	10 (7–31)	6 (4–8)	(1)
<i>S. minima</i>	(40–70)	(30–50)	(26–32)	1	(20–29)	~12–18	2			(4)
<i>S. cheshiri</i>	62 (35–82)	38 (29–49)	(15–16)	4–5	14 (13–19)	20–40	2 (1–6)	~10	~10	(3)
<i>S. spiniferum</i>	76 (62–90)	49 (36–57)	15 (14–15)	3	18 (17–21)	28–58	2 (2–4)	14 (11–18)	11 (9–14)	(1)
<i>S. cheshiri</i>	(34–110)	(32–60)	(14–16)	4	(12–15)	18–32	2			(2)
<i>S. elongata</i>	(80–110)	(40–50)	(19–24)	1–2	(11–15)	~40	2			(4)
<i>S. acuminatum</i>	94 (70–124)	35 (29–50)	15	3	15 (15–16)	20–52	2 (2–4)	14 (8–25)	9 (6–14)	(1)
<i>S. multiauris</i>	95 (42–140)	45 (32–64)	(14–15)	4–5	18 (14–25)	50–60	2 (1–4)	8–12	8–12	(3)
<i>S. jeokjo</i> n. sp.	149 (100–190)	64.0 (55–80)	16 (15–17)	2–8	28 (26–28)	23–44	2 (0–2)	34 (25–38)	15 (13–16)	This study

EPk: number of external oral polykinetids, IPk: number of internal oral polykinetids, Sk: number of somatic kineties, nDk: number of dikinetids per kinety, MN: number of macronuclei, MNL: macronuclear length, MNW: macronuclear width. (1) Lynn et al. 1991; (2) Snyder and Ohman 1991; (3) Montagnes and Taylor 1994; (4) Song and Bradbury 1998.

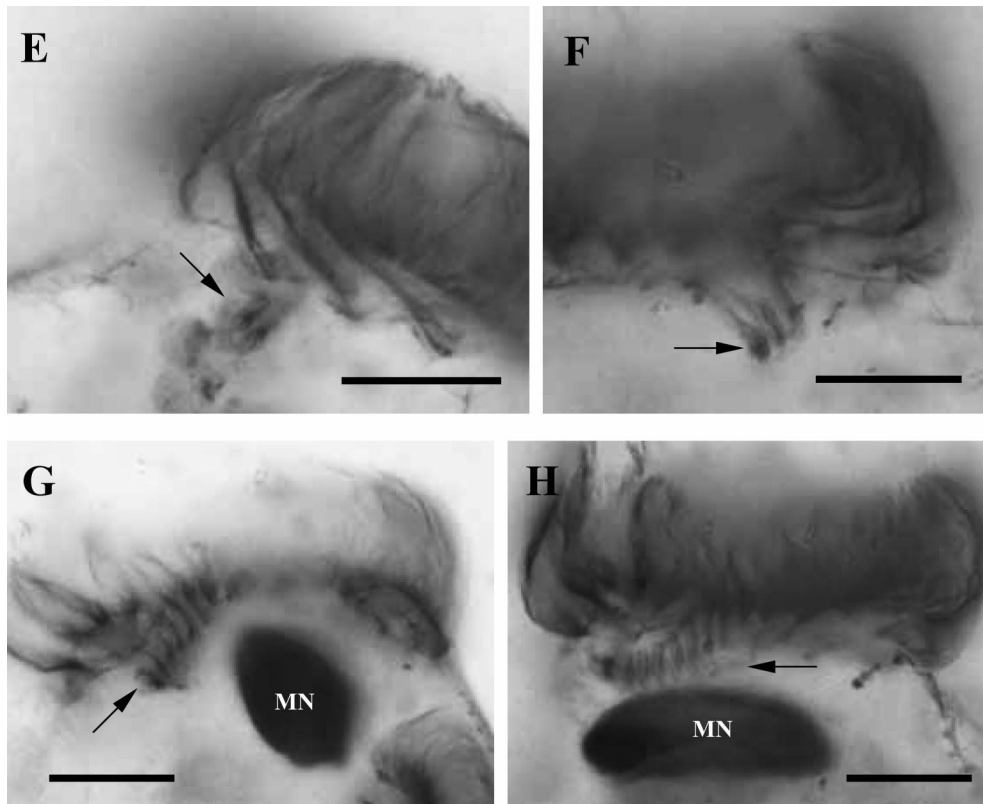


Fig. 2. Continued.

when the ranges of the water temperatures and salinities were 12.9–25.0 °C and 11.4–30.5 psu, respectively.

Remarks on culturing and behavior. *Strombidinopsis jeokjo* n. sp. was initially cultured on the red-tide dinoflagellates *P. micans* and *L. polyedrum*. The ciliate could also be cultured on other red-tide dinoflagellates, such as *Akashiwo sanguinea*, *Cochlodinium polykrikoides*, *Gymnodinium impudicum*, *Heterocapsa triquetra*, *Prorocentrum minimum*, and *Scrippsiella trochoidea*. The ciliate typically swam in a straight line, while rotating itself along this path.

DISCUSSION

Morphological characteristics. The numbers of EPk, IPk, Sk, and macronuclei of the *Strombidinopsis* sp. were similar to those of the *Strombidinopsis* sp., the largest *Strombidinopsis* species so far reported (Jeong et al. 1999). They were collected from the same location, but at different times. However the former ciliate was slightly smaller than the latter after being fixed with modified concentrated Bouin's fixative. The cell length, cell width, and oral diameter (mean) of this *Strombidinopsis* sp. in QPS (mean: 149 μm , 79 μm , and 64 μm , respectively) were much larger than any other *Strombidinopsis* species so far identified, even though the lower boundaries of its cell length and cell width overlapped with the upper boundaries of several other species (Table 2). In cell shape, this species is ovoid and stout, while *Strombidinopsis acuminatum*, which was suggested to be identical to *Strombidinopsis cheshiri* (Dale and Lynn 1998), and *Strombidinopsis multiauris* are elongated and slender. The maximum IPk number of 8 for this species is greater than the maximum of 5 reported for any other *Strombidinopsis* species so far. Its Sk number (26–28) is much greater than that for *S. acuminatum* (15–16), but slightly greater than that for *S. multiauris* (14–25). The number of dikinetids per kinety in this new species (23–44) is less than that for *S. mul-*

tiauris (50–60). However, unlike *S. multiauris* (Montagnes and Taylor 1994), neither large interpolykinetidal ridges nor darkly stained somatic interkinetidal granules were observed.

Gene sequence of *Strombidinopsis*. When aligned properly, the sequence of the SSU rDNA of this *Strombidinopsis* sp. was approximately 2% different from that of an unidentified *Strombidinopsis* (GenBank Accession No. AF399132–AF399135) reported by Snoeyenbos-West et al. (2002). Next to this unidentified *Strombidinopsis* (AF399132–AF399135), an unidentified *Strombidium* sp. SNB99-2, reported by Strüeder-Kypke and Lynn (2003), was the genealogically closest choreotrich (GenBank Accession No. AY129035; 7% difference).

Based on this composite of features, we conclude that this is a new species of *Strombidinopsis*. We name it *Strombidinopsis jeokjo* n. sp. and formally describe it below.

Diagnosis. *Strombidinopsis jeokjo* n. sp. is ovoid and bluntly tapered towards the posterior when without prey. The ranges (and mean) of cell length, cell width, and oral diameter of QPS-stained specimens were 100–190 μm (149), 60–105 μm (79), and 55–80 μm (64), respectively. Fifteen to seventeen external oral polykinetids form a completely closed circle. There are 2–8 internal oral polykinetids and 26–28 somatic kineties. The SSU rDNA sequences of *S. jeokjo* n. sp. (GenBank Accession No. AJ628250) were different from the other *Strombidinopsis* species so far reported.

Etymology. The specific epithet *jeokjo* refers to a red tide in Korean because this species is able to feed on a diverse range of red-tide organisms (Jeong et al. 1999).

Deposition of type material. A slide, USNM # 1023820, of protargol-stained cells representing an hapantotype resides in the International Protozoan Type Slide Collection, National Museum of Natural History, Smithsonian Institution, Washington D.C.

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