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Ultrastructure of *Cryptoglena pigra* from Korea

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Cryptoglena pigra Ehrenberg from Korea was a photosynthetic euglenoid alga, which had typical characteristics of the Euglenales. The ultrastructure examination of *C. pigra* revealed certain features which were distinctly photosynthetic euglenoid: one U-shaped chloroplast with thylakoid membranes; two paramylon grains appressed to both sides of the chloroplast; eyespot associated with the chloroplast but not part of it. Three flagellar roots were associated with the two basal bodies. The four-membered dorsal root arose from the dorsal body and extended anteriorly following the reservoir membrane. At the base of the reservoir the dorsal band was nucleated by the dorsal root and it ran anteriorly between the reservoir membrane and eyespot. The dorsal band was continued with the microtubules of the canal and the pellicle. The singlet dorsal microtubules at the transition level arranged into doublets by a successive linkage of the existing adjacent microtubules, and the doublets rearranged into the cytoskeletal microtubules that were continuous with four microtubules in pellicles. Finally, the sixteen ridges gave rise to the pellicular ridges. The five to six-membered ventral root extended anteriorly into a cytoplasmic pocket through the reservoir and lined a cytoplasmic pocket.

Key Words: basal body, *Cryptoglena pigra*, cytoplasmic pocket, Euglenophyceae, microtubule arrangement, microtubule root, ultrastructure

INTRODUCTION

The genus *Cryptoglena* was first described by Ehrenberg (1831). Although several species have been mentioned, only *C. pigra* was considered valid by Stein (1878), Klebs (1892) and Lemmermann (1913) and recently *Phacus agilis* was transferred to this genus as *C. skujae* by Marin *et al.* (2003). *C. pigra* is the best known species, but there have been some doubt as to which class this entity belongs. For example, Leedale (1967) described it as an uncertain euglenoid among photosynthetic genera because paramylon is absent and the eyespot is a part of one of two chloroplasts.

More recently ultrastructural investigations have demonstrated its euglenalian features (Rosowski and Lee 1978). They described *C. pigra* as having pellicular strip, canal and reservoir with microtubular cytoskeleton, two flagella, single large U-shaped chloroplast, eyespot independent of the chloroplast, a nucleus with permanently condensed chromatin, and paramylon grain. Since this report on general ultrastructural information Owens *et al.* (1988) described the flagellar apparatus and reservoir/canal cytoskeleton of *C. pigra*. The ultrastructural studies of the flagellar roots system and the reservoir/canal cytoskeleton have recently used to provide clues to the phylogeny of phototrophic euglenoids (Willey and Wibel 1985a, b, 1987; Surek and Melkonian 1986; Shin and Boo 2001; Shin *et al.* 2000, 2001, 2002). In this study we first describe ultrastructural details of *Cryptoglena pigra* from Korean freshwater.

MATERIALS AND METHODS

Culture. Water samples were collected with a plankton net (mesh size, 20 μm) on July 2004 in a small pond of Hongsung, Korea, from which *Cryptoglena pigra* cells were isolated by Pasteur capillary pipette under the inverted microscope (Olympus IX70, Japan). Unialgal cultures were grown in an modified AF-6 medium (Watanabe and Hiroki 1997) and maintained in test tubes at 20°C with conditions of 14:10 L:D period and 30 μmol photons$\cdot m^{-2}\cdot s^{-1}$ irradiance from white fluorescent tubes.

Light microscopy. Culture strains from Korea were observed and identified under a Nikon Eclipse 80i (Nikon Co., Japan) equipped with differential interference contrast (DIC) optics. Images were captured with a DS-5M (Nikon Co., Japan) photomicrographic system

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attached to the microscope.

Transmission electron microscopy. Harvested cells were fixed in 2.5% glutaraldehyde with the culture medium (pH 6.6) at 4°C for 1.5 h. They were rinsed with the medium and postfixed in 1% OsO_4 in distilled water at 4°C for 1h. The fixed cells were embedded in 1% agar and dehydrated in a graded ethanol series. They were dehydrated by three changes of absolute ethanol at room temperature, by a mixture of absolute ethanol and propylene oxide for 15 min, and finally by two changes of propylene oxide for 15 min. The dehydrated cells were embedded in Spurr's epoxy resin (Spurr 1969) and polymerized at 70°C for 48 h. Thin serial sections (60-80 nm) were cut using a diamond knife on a RMC MT-XL ultramicrotome (Boeckeler Instruments Inc., Tucson, Arizona) and mounted on slot grids with formvar film. The sections were sequentially stained with uranyl acetate and lead citrate (Reynolds 1963), and examined in a transmission electron microscope (JEM 1010, JEOL, Tokyo, Japan) operating at 80 KeV.

RESULTS

Cryptoglena pigra had an average length of 15.2 ± 0.78 μm (range 13.59-16.69 μm) and an average width of 9.73 ± 1.41 μm (range 6.77-13.55 μm) (n = 25). Cells were almost symmetrical and oval (Fig. 1). The emergent flagellum was almost body length. The stigma was ruby-red in color, placed in right side from ventral view. The sulcus extends longitudinally from the apex to the end of cell.

C. pigra was a typical euglenoid in most ultrastructural features. The nucleus, including condensed chromosomes, was subcentrally located in the cell (Figs 2A-C). Only one chloroplast was located under the pellicular strips, U-shape, and had no pyrenoids (Figs 2A-C). The mitochondria were distributed internally in the cell and had plate-like cristae. Each cell contained two large paramylon grains between the chloroplast and pellicle and lateral to the sulcus (Figs 2A-B).

The cell body was striated with 16 longitudinally oriented ridges, which were alternatively high and low (Fig. 2B). The pellicle consisted of plasmalemma, epiplasmic layer, microtubules, and endoplasmic reticulum (Figs 2D-E). The plasmalemma overlay the ridge-groove articulation of the overlapping pellicular strips (Figs 2D-E). The epiplasmic layer was slightly electron-opaque and semi-continuous under the plasmalemma. Microtubules were distributed under the epiplasmic layer and their

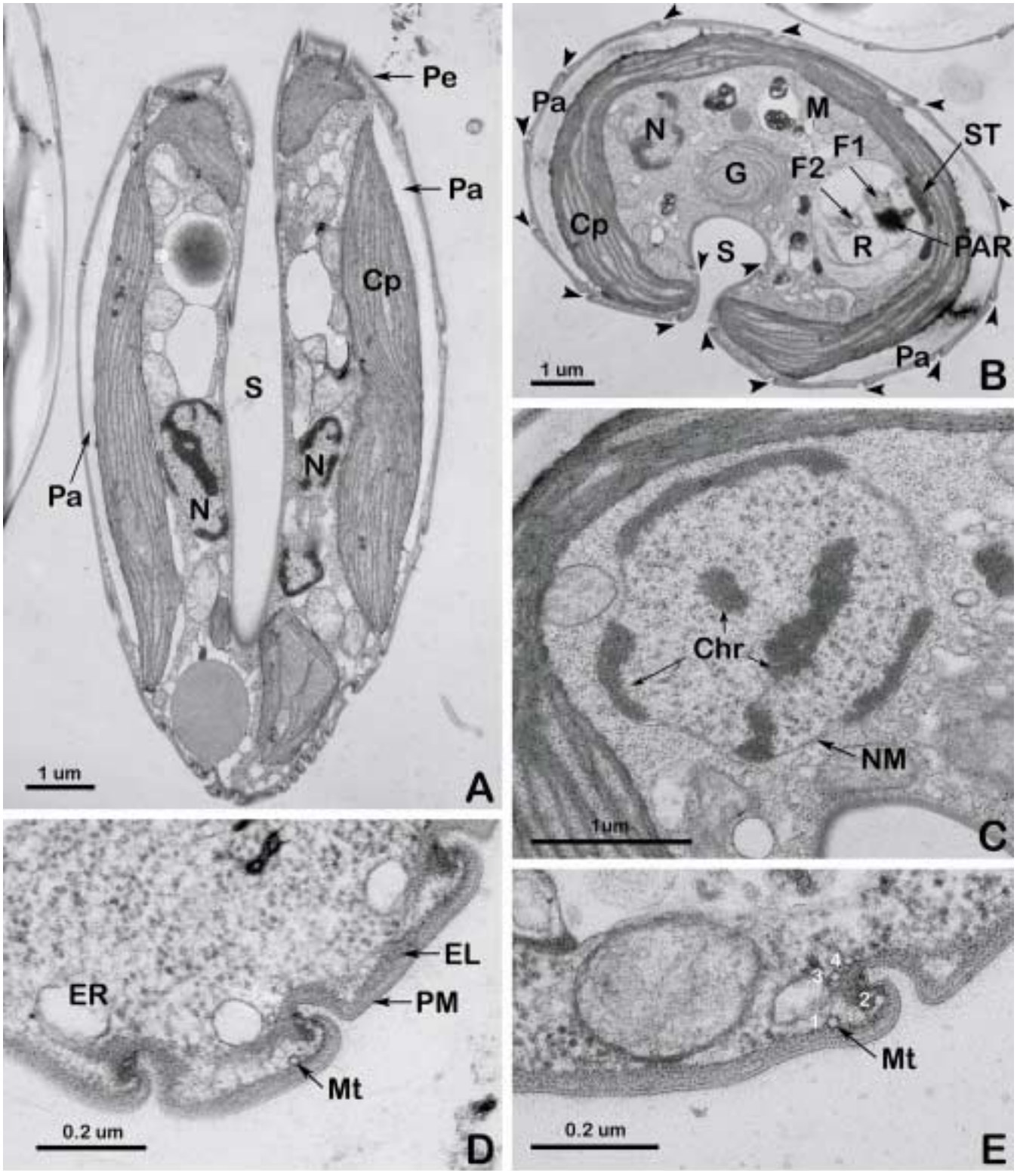
Fig. 1. Light microscope photograph of *Cryptoglena pigra* showing the stigma (ST), flagellum (F), and sulcus in the center of the cell (arrow).

number was four in the ridge. The endoplasmic reticulum was tubular, lay between pellicular strips, and ran parallel to each ridge (Figs 2D-E).

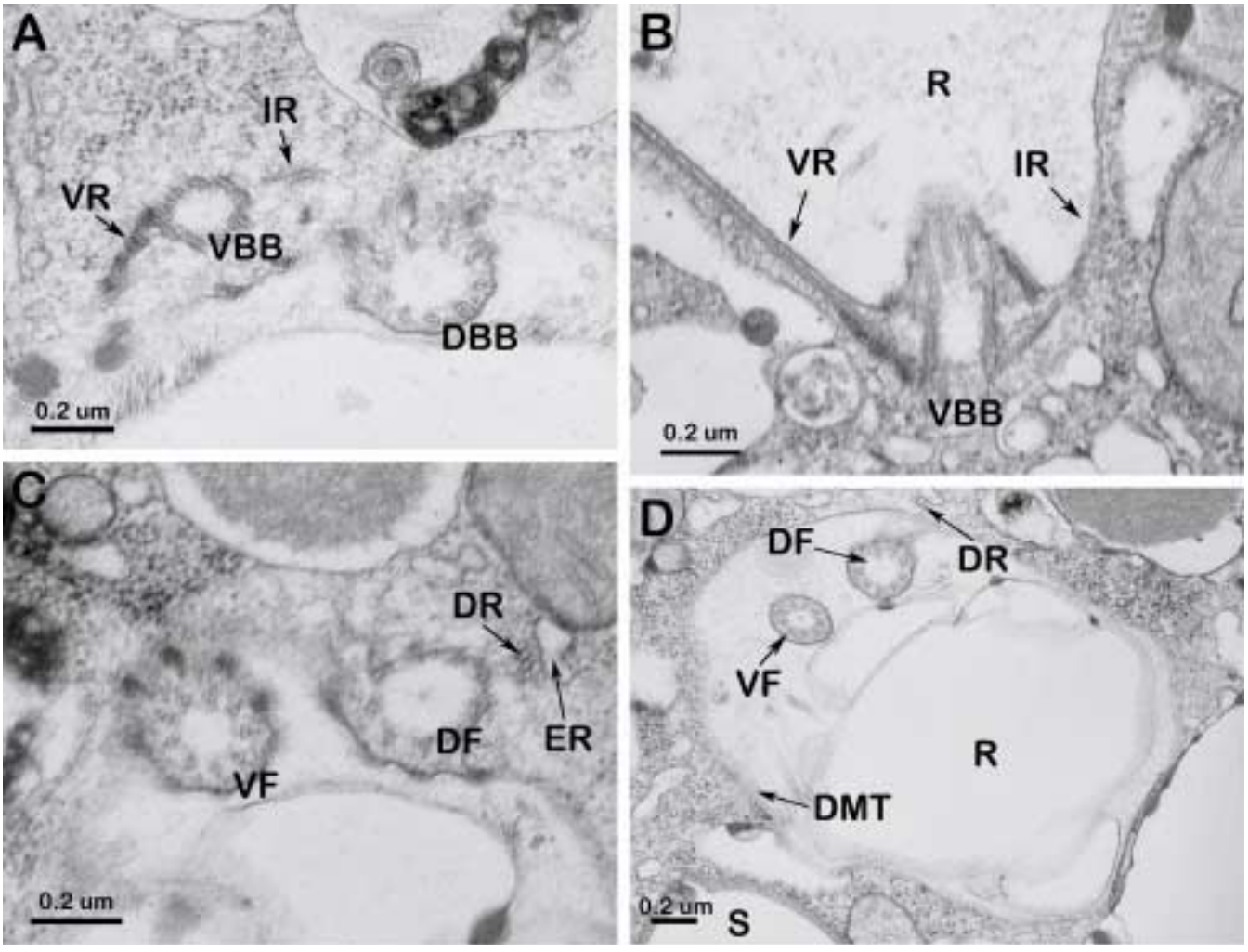
One emergent flagellum, designated as the dorsal flagellum, had paraxonemal rod (Fig. 2B). The other flagellum, designated as the ventral flagellum, remained in the reservoir due to its short length (Fig. 2B). The base of each flagellum had the hollow transition zone that lacked internal elements (Figs 3A-C). The basal body complex consisted of two basal bodies, three microtubular roots, and fibers (Figs 3A-C). The ventral basal body was located on the left side near the contractile vacuole and anchored to the ventral root. The intermediate root was located to the right side of the ventral basal body (Figs 3A-B). Dorsal and ventral basal bodies were located posterior to the reservoir and showed a relatively constant orientation to each other (Figs 3A, C). The dorsal basal body was located on the right side and anchored to the dorsal root (Fig. 3C).

The ventral root consisted of 5 microtubules in the reservoir (Figs 4A-B). The microtubules formed a part of the cytoplasmic pocket at the reservoir/canal transition level (Figs 4A-D), which referred to as the reinforcing microtubular band (MTR). The MTR was characteristically related with the pocket.

The dorsal root was composed of 4 microtubules at the basal body level and was associated with the ER (Figs 3C-D). A group of microtubules lined the reservoir but did not appear to be connected with either of the basal bodies (Fig. 3D). For this reason it referred to as dorsal band of the microtubules (DMT). At the eyespot level, the DMT microtubules increased to more than 60 micro-



Figs 2A-E. General ultrastructure of *C. pigra.* **A.** Longitudinal section through the sulcus (S) showing the nucleus (N), chloroplast (Cp), pellicle (Pe), and big paramylon (Pa) beneath the pellicle. **B.** Cross section showing small sulcus, large paramylon, U-shaped chloroplast (Cp), Golgi body (G), mitochondria (M), pellicular strip, and reservoir (R) containing two flagella. The dorsal flagellum (F1), emergent flagellum, has the paraflagellar rod (PAR) and the ventral flagellum (F2), non-emergent, is placed in the reservoir. Arrowheads indicate to the ridges of pellicular strips. **C.** Cross section of the nucleus showing electron dense chromosomes (Chr) attached to the nuclear membrane (NM). **D-E.** Cross sections of pellicle showing plasmalemma (PM), epiplasmic layer (EL), microtubules (Mt), and ER. Microtubule 1 is located in ridge region beneath epiplasmic layer, microtubule 2 in crest of the heel, microtubule 3, and 4 in the lower heel region beneath the groove.



Figs 3A-D. Basal body complex and cytoskeleton of reservoir base. **A.** The ventral root (VR) is located near the left side of the ventral basal body (VBB) and the intermediate root (IR) between dorsal basal body (DBB) and ventral basal body. **B.** The ventral root and intermediate root originate at the ventral basal body. **C.** Cross section through the bottom of the reservoir (R) shows four-membered dorsal root (DR) and ER. **D.** Cross section through the reservoir show the dorsal root, dorsal microtubules (DMT), and two flagella; dorsal (DF) and ventral flagellum (VF).

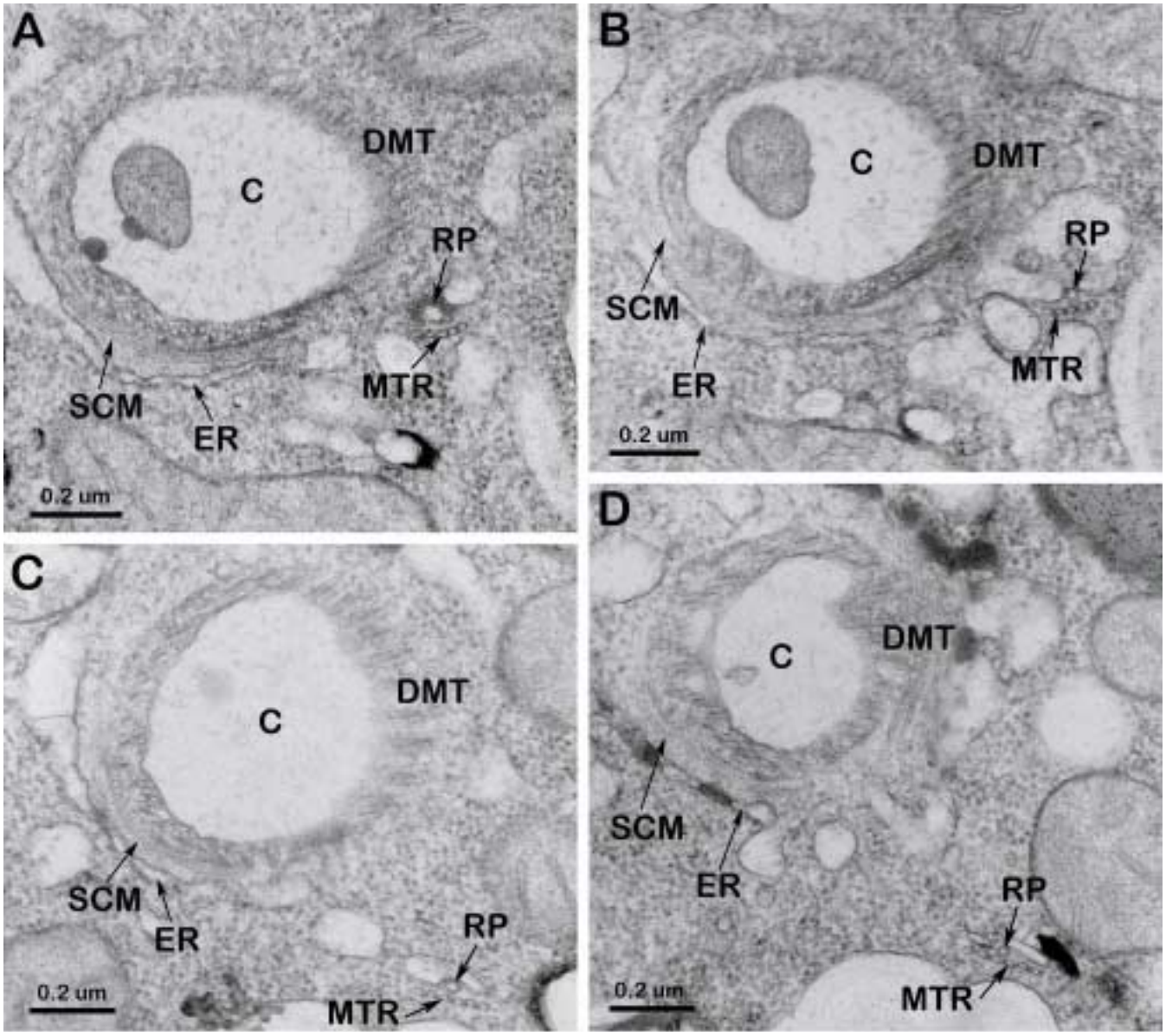
tubules. Most of microtubules occurred as the singlet microtubules below the reservoir membrane (Figs 4A-C). The singlet microtubules began to pair at the reservoir/canal transition level while the semi-circular microtubules (SCM) began to occur (Figs 4A-D).

Most of DMT microtubules appeared doublets at the reservoir/canal transition level (Fig. 5A). The reservoir membrane and doublet microtubules were surrounded in turn by transversely oriented microtubules (Figs 5A-B). In the more upper level, doublets of the microtubules occurred (Figs 4A-B). Electron-opaque material accumulated in the plasmalemma and among microtubules (Fig. 5B). The membrane of the reservoir/canal transition region became convoluted due to the pairing of DMT and encircling of SCM microtubules (Figs 5B-D). The reservoir gradually decreased in diameter and formed the canal that was supported by ER, the microtubules of the DMT, and SCM (Figs 5B-C).

At the canal level, the membrane was more convoluted and became pellicular ridge (Figs 5C-D). Finally 16 ridges were formed, and each ridge had 4 microtubules (Fig. 4D). Thus, the DMT singlets were continuous with the pellicular cytoskeleton, forming the notch microtubules.

DISCUSSION

The general ultrastructures of *Cryptoglena pigra* from Korea are similar to those of *C. pigra* (Rosowski and Lee 1978; Owens *et al.* 1988) and the other euglenalian members (Buetow 1968; Surek and Melkonian 1986; Farmer and Triemer 1988) by having large endosome and con-

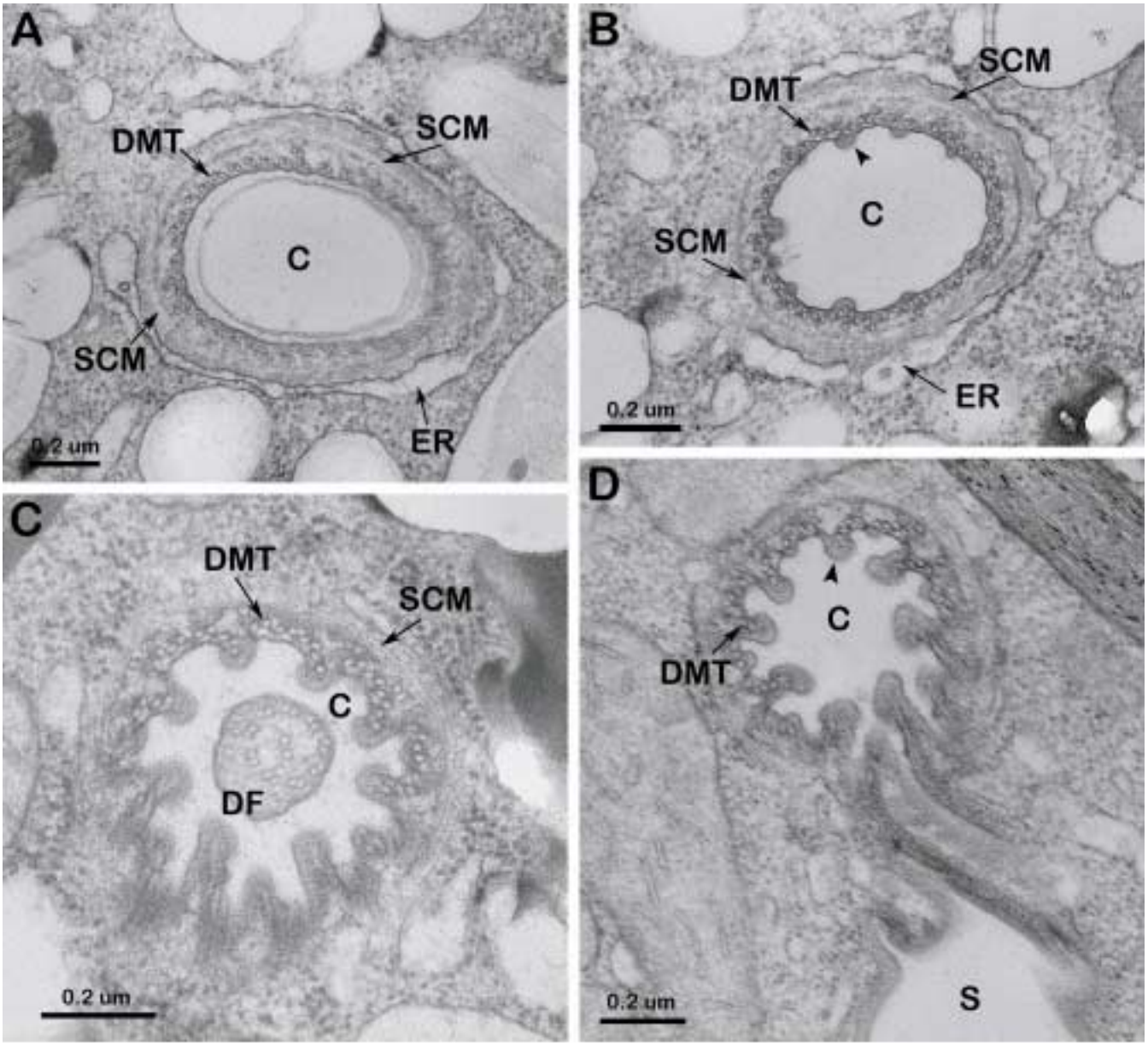


Figs 4A-D. A-B and **C-D.** Serial section through reservoir / canal transition region showing the MTR / pocket (RP), dorsal microtubule, and semicircular microtubule (SCM). The pocket is lined with the reinforcing microtubules (MTR). The semi-circular microtubules surround the singlet dorsal microtubules.

densed chromosomes, longitudinally arranged pellicle, two unequal flagella, and asymmetrical roots in basal body complex. Almost identical features of these general ultrastructures suggest the taxonomic stability of *C. pigra* in the Euglenales.

Since Willey and Wibel (1985a, b) observed a MTR / pocket in Colacium libellae Rosowski *et* Willey, referred to as a presumptive vestigial cytopharynx or vestigial feeding apparatus, Shin *et al.* (2002) reported the existence of a cytoplasmic pocket in the photosynthetic euglenoid genera. The MTR / pocket, consisting of a cytoplasmic pocket and reinforcing microtubules (MTR), has the least structural complexity among four feeding apparatus types in euglenoid. For example, *Cryptoglena*, *Colacium*, *Strombomonas*, *Trachelomonas*, and *Euglena* do not have rods or fibers around the cytoplasmic pocket. However, the feeding apparatus of *Eutreptia pertyi*, *Phacus trypanon*, *P. pleuronectes*, and *Lepocinclis fusiformis* is associated with rod-like structure or fibers. In this study, we confirmed that *C. pigra* has the simplest type of cytoplasmic pocket as known by Owens *et al.* (1988).

The cytoskeletal microtubules of *C. pigra* arranged from singlets at the reservoir level into doublets at the canal level. The doublet microtubules finally rearranged into two-over-two quadruplets at the canal level, which in turn become microtubules of the pellicular ridges.



Figs 5A-D. Cross section of the canal (C) region and development of pellicular strip. **A.** Cross section showing the doublet of the dorsal microtubules. The semi-circular microtubules occur above the ER. **B.** Cross section of the canal region showing the rearranged dorsal microtubules. The canal membrane is convoluted (arrowhead), and doublets of microtubules occur. The ER surrounds the semi-circular microtubules. **C.** Glancing section showing convoluted pellicular ridges surrounded by the semi-circular microtubules. **D.** Glancing section showing pellicular ridges with four microtubules.

Although three-over-two pentaplet microtubules at the canal level are common in the other euglenalian members; *Euglena* (Surek and Melkonian 1986; Shin *et al.* 2000), *Colacium* (Willey and Wibel 1987), and *Cryptoglena* (Owens *et al.* 1988), two-over-two quadruplets have found only in *Trachelomonas volvocina* (Shin *et al.* 2002) and *Cryptoglena pigra*. However, a two-over-one triplet pattern of microtubules in the canal region is characteristic in *Eutreptia* (Dawson and Walne 1991) and *Tetreutreptia* (Triemer and Lewandowski 1994) of the Eutreptiales, and *Rhabdomonas* Fresenius and *Menoidium* Perty (Leedale and Hibberd 1974) of the Rhabdomonadales. Therefore, the number and arrangement of cytoskeleton may be of the importance for understanding phylogenetic relationships in euglenoids.

According to previous publications, the number of pellicular strips differed from fourteen to sixteen. Rosowski and Lee (1978) reported that *C. pigra* (LB1212/1) from the Culture Collection of Algae and Protozoa at Scotland (CCAP) has about fourteen strips interconnecting to

form the pellicle. Later, Owens *et al.* (1988) described that *C. pigra* (LB571) from the University of Texas Culture Collection (UTEX) has fifteen canal ridges in the majority of cells examined. However, our result showed that *C. pigra* from Korea has sixteen pellicular strips.

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