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The Structure of the Nucleus of *Odonaticola polyhamatus* (Gregarinea: Actinocephalidae), a Parasite of *Mnais strigata* (Hagen) (Odonata: Calopterygidae)

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Summary. The nucleus of *Odonaticola polyhamatus* was isolated from the body and observed with light, scanning electron and transmission electron microscopy. The nucleus had a thick thread-like structure with which it was tied to the septum. This thread-like structure has not been reported or described previously. The gregarine nuclear surface was covered with a fine fibrous net. This is the first report of the surface structure of a gregarine nucleus as revealed by SEM. Inside the nuclear membrane was a thin honeycomb layer similar to that reported for some other gregarines. Several spherical nucleoli and numerous electron dense small structures were observed inside the nucleus.

Key words: gamont, Gregarinea, nucleolus, nucleus, *Odonaticola polyhamatus*.

INTRODUCTION

The nuclei of several species of gregarines have been observed by transmission electron microscopy (TEM), but none have been observed by scanning electron microscopy (SEM) (Beam *et al.* 1957, 1959; Desportes 1974). The nuclei of gregarines are sturdy and keep their shape when they are isolated from the body. We have taken an interest in the structure of gregarine nuclei and have tried to clarify some of the structural features that provide their sturdiness. The gregarines which parasitize Odonata are very large and their nuclei are correspondingly large and stout. Therefore, gregarines such as *Odonaticola polyhamatus* are excellent material to use in order to investigate the nuclear ultrastructure by SEM. The huge nucleus of *O. polyhamatus* always exists near the cell's septum, so we also studied the mechanism by which this intracellular location was maintained.

Odonaticola polyhamatus (K. Hoshide, 1977) was first described from the damselfly *Mnais strigata* Hagen, under the name *Hoplorhynchus polyhamatus* (Hoshide 1977). Amoji and Kori (1992) shifted the species from *Hoplorhynchus* Carus to *Odonaticola*

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Sarkar and Haldar, 1981 but misspelled the specific epithet as *polyhematus*; the proper spelling is *polyhamatus* (= "many hooks").

MATERIALS AND METHODS

The species used in this study, *Odonaticola polyhamatus*, is a parasite of the damselfly *Mnais strigata* (Hagen). Host damselflies were collected at Inunaki Gorge in the suburbs of Yamaguchi City, Japan, in June, 1994. The intestines of the host were removed, placed in Ephrussi-Beadle Ringer's salt solution (NaCl 7.3g, KCl 3.5g, $CaCl_2$ 2.8g, in 1 l distilled water), and dissected, and the gamonts removed. Gamont nuclei were isolated from the deutomerite by disrupting individual cells with fine needles. Nuclei were then washed with a stream of Ringer's solution from a fine pipette. Specimens for transmission electron microscopy were prefixed in 5% glutaraldehyde in cacodylate buffer for 2 h and post fixed with 1% osmium tetroxide for 2 h. After fixation, the specimens were dehydrated through an ethanol series and embedded in Spurr's resin. Thin sections were made using an LKB ultramicrotome with a diamond knife and examined in a JEM-1200 EX TEM. Specimens for scanning electron microscopy were fixed and dehydrated using the same methods. Following dehydration with ethanol, they were placed in isoamylacetate and critical point dried using CO_2 as the transition liquid. The dried samples were then placed on an aluminum stub, sputter-coated with gold, and examined with a JEOL T-300 scanning electron microscope.

RESULTS

Odonaticola polyhamatus is a large, solitary, gregarine. The average length of the body is about 2600 µm and reaches a maximum size of 3300 µm. The nucleus is spherical or ellipsoidal, about 50-100 µm in diameter, and always located in the center of the deutomerite endoplasm just behind the septum. Ordinarily, observation of the nucleus of mature gamonts *in vivo* is hindered by the density of the endoplasm (Fig. 1). On rare occasions, the nucleus is located on one side and can be observed near the surface (Figs 2, 3). Following dissection of the deutomerite, the isolated nucleus was covered with debris of endoplasm. The isolated nucleus held its spherical or ellipsoidal shape after removal of this debris with a water-jet from a fine pipette (Figs 4, 5). The nucleus was fixed at the center of the septum by a thick, transparent, thread-like structure 70-80 µm long and 5 µm in diameter (Fig. 6). The surface of nucleus was different from that of most other animal cells. In some specimens the nucleus had an irregular rough surface (Fig. 7), and several grooves extended radially from the point where the thread was attached (Figs 7-9). Many spherical or ellipsoidal granules adhered to the anterior one-fifth surface area, and a thin fibrous net covered these granules (Figs 7, 8). The rest of the surface was covered with a homogeneous multi-layered fine fibrous net (Figs 7-11).

By light microscopy the nucleus was transparent except for the many brown nucleoli. Ten to twenty nucleoli were contained in each nucleus (Figs 3-5). By TEM the nucleoli were spherical and highly electron dense, although some nucleoli had a hollow that was less electron dense (Figs 12, 13). The nucleus was bounded by a double membrane and on the outside could be seen many fine electron dense threads. Between the inner and outer membranes there was a thin, space of low electron density. The inner membrane was lined with a relatively thick porous cortical layer (Figs 12, 13). Tangential sections revealed that this layer had a honeycomb pattern.

DISCUSSION

The nucleus of septate gregarines is always located in the deutomerite, but the location within this part of the cell varies depending on the species. In some species the nucleus is located in the anterior part of the deutomerite, in other species it is in the middle, and in some is found closest to the posterior end. The nucleus of *Odonaticola polyhamatus* is always located near the septum but until the present study, we didn't know why it was fixed in this position. The dense endoplasm made it impossible to see the thread-like structure that connected the nucleus so the center of the septum. With successful isolation of the nucleus, this thread-like structure was observed for the first time. This type of connection has not been observed in gregarines until now. It was not be determined in this study whether the thread-like structure functions only to hold the nucleus in place, or whether it plays other roles in the life of the gregarine.

This is also the first observation of an isolated gregarine nucleus using the scanning electron microscope. The whole body of many gregarines has been shown by SEM, but there has been no observation of an isolated nucleus (Heller and Wise1973, Hildebrand and Vinckier 1975, Walker *et al.* 1979). In most species it is technically difficult to make specimens of isolated nuclei using critical point drying because the nucleus is rather small and fragile compared to the whole body. The nucleus of *O. polyhamatus*, however, is large, and is connected to the septum. At the time of isolation, the nucleus remains

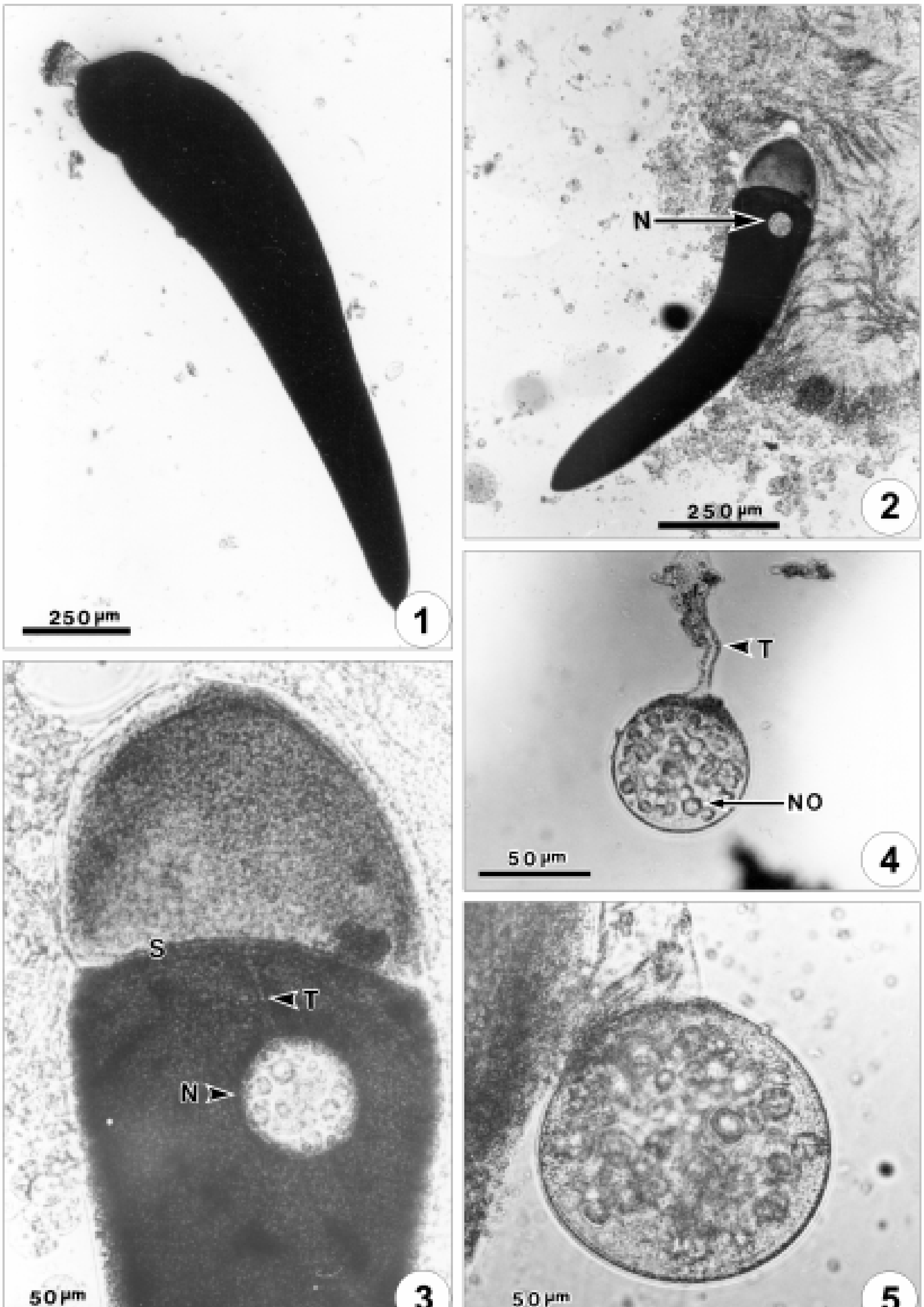

Fig. 1. Mature gamont of *Odonaticola polyhamatus*. Observation of the nucleus is hindered by the dense endoplasm. **Fig. 2.** Another gamont, the nucleus is observed from the surface. N-nucleus. **Fig. 3.** Nucleus in the mature gamont. T-thread-like structure. S-septum. **Fig. 4.** Isolated nucleus with the thread like-structure. Many nucleoli were observed inside the nucleus by light microscope. NO-nucleoli. **Fig. 5.** Another isolated nucleus by light microscope

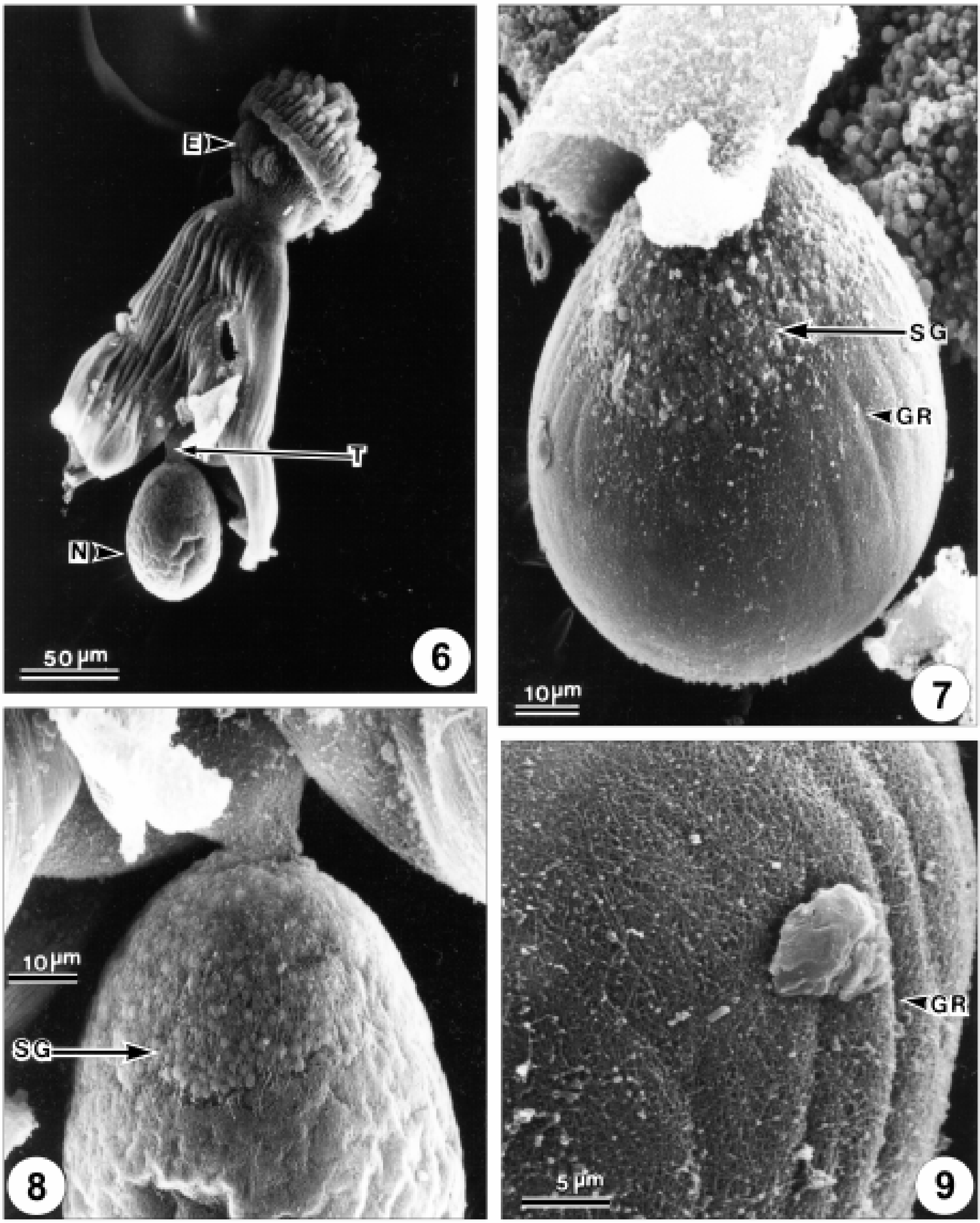

Fig. 6. Dissected gregarine with the nucleus by SEM. E-epimerite. **Fig. 7.** Isolated nucleus connected with a part of the septum. SG-spherical granule, GR-grooves. **Fig. 8.** Anterior part of another isolated nucleus with rough surface. **Fig. 9.** Surface of the nucleus with the fine fibrous net

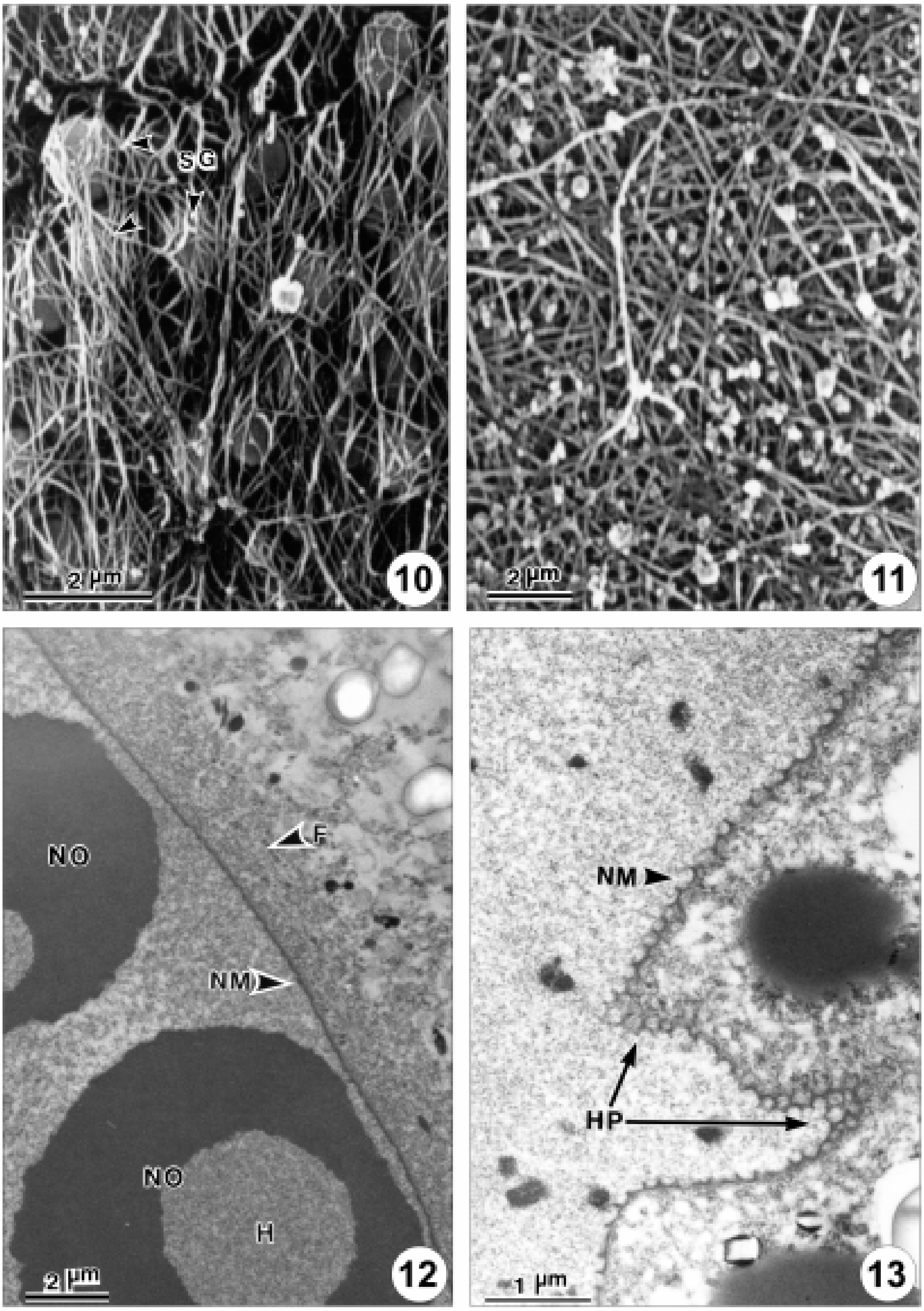

Fig. 10. Magnified fibrous net of the anterior part of nucleus, spherical granules were covered by the fibrous net. **Fig. 11.** Magnified fibrous net of nucleus. **Fig. 12.** Nuclear membrane and nucleolus; fibrous net can be seen outside the nuclear membrane. F-fine fibrous net, NM-nucleus membrane, NO-nucleoli, H-hollow. **Fig. 13.** Nuclear membrane and nucleolus. HP-honeycomb pattern

fastened to a large fragment of the body. Thus we were able to observe the nucleus during processing, and ensure that it was retained during critical point drying.

The nuclear surface of a number of other protozoans has been studied by freeze-fracture techniques. For example, *Trichomonas vaginalis, Tritrichomonas foetus*, and *Blastocystis hominis* have all been shown to have both nuclear pores (Honigberg *et al.* 1984, Yoshikawa *et al.* 1988). No pores were evident on the nucleus of *O. polyhamatus*, but the surface was covered with a fine fibrous network. Thus the *O. polyhamatus* nucleus has a completely different external appearance from the flagellates studied by Yoshikawa *et al.* (1988) and Honigberg *et al.* (1984). In a previous paper, the point was made that comparative ultrastructure could be a key to finding evolutionary relationships among protozoan groups (Hoshide and Todd 1996). Various gregarine species other than *O. polyhamatus* have also been shown to have a honeycomb layer on the nuclear surface (Beam *et al.* 1957, Théodoridès 1959, Hukui 1966, Desportes 1974) and a similar layer was reported earlier for *Amoeba proteus* by Harris and James (1952) and Pitelka (1963). Although the evolutionary significance of this layer is still unknown, the honeycomb and fibrous network are probably the reason why the *O. polyhamatus* nucleus is so stout. Highly electron dense nucleoli have been reported in some gregarines (Hukui 1966, Tronchin and Schrevel 1977) and the nucleoli of *O. polyhamatus* are similarly dense, although some had low density hollows.

In summary, these studies have revealed a new and unique structure in gregarines. The observations explain why nuclear position within the deutomerite is constant in *O. polyhamatus*, and suggest that nuclear position may have a structural basis in other gregarine species. If connections between the nucleus and septum occur in other species, then nuclear position becomes a potential taxonomic character in gregarines.

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REFERENCES

Amoji S. D., Kori S. S. (1992) Revision of the genus *Odonaticola* Sarkar et Haldar, 1981(Apicomplexa: Sporozoasida: Gregarinasina). *Acta Protozool.* **31:**169-171

Beam H. W., Tahmisian T. N., Devine R., Anderson W. (1957) Ultrastructure of the nuclear membrane of gregarine parasite in grasshoppers. *Exp. Cell Res.* **13:** 200-204

Beam H. W.,Tahmisian T. N., Devine R., Anderson W. (1959) Studies on the fine structure of a gregarine parasitic in the gut of the grasshopper, *Melanoplus differentialis. J. Protozool.* **6:** 136-146

Desportes I. (1974) Ultrastructure et evolution nucléarie des trophozoites d'une grégarine d'ephéméroptère; *Enterocystis fungoides* M. Codreanu. *J. Protozool.* **21:** 83-94

Harris P., James T. W. (1952) Electron microscope study of the nuclear membrane of *Amoeba proteus* in thin section. *Experientia* **8:** 384-385

Heller G., Weise R. W. (1973) A scanning electron microscope study of *Gregarina sp.* from *Udeopsylla nigra*. *J. Protozool.* **20:** 61-64

Hildebrand H. F., Vinckier D.(1975) Nouvelles observations sur la grégarine *Didymophyes gigantea* Stein. *J. Protozool.* **22:** 200-213

Honigberg B. M., Volkmann D., Entzeroth R., Scholtyseck E. (1984) A freeze-fracture electron microscope study of *Trichomonas vaginalis* Donne and *Tritrichomonas foetus* (Riedmuller). *J. Protozool.* **31:** 116-131

Hoshide K. (1977) Notes on the gregarines in Japan. 8. Three new species of Eugregarina from Odonata. *Bull. Fac. Educ. Yamaguchi Univ.* **27:** 93-125

Hoshide K., and Todd K. S. (1996) The fine structure of cell surface and hair-like projections of *Filipodium ozakii* Hukui 1939 gamonts. *Acta Protozool.* **35:** 309-315

Hukui T. (1966) The electron microscopical studies on *Stenophora sp.* from *Skleroprotopus ikedai* Takakuwa, 1940. *Bull. Fukuoka Univ. Educ.* **16:** 141-151

Pitelka D. R.(1963) Electron-Microscopic Structure of Protozoa. Pergamon Press, New York

Sarkar N. K., Haldar D. P. (1981) Observations on a new cephaline gregarine (Protozoa: Sporozoa), *Acanthospora bengalensis* n. sp. from an odonate insect. *Arch. Protistenk.* **124:** 378-384

Théodoridès J. (1959) Etude des eugrégarines au microscope électronique. Proc.15th Intern. Congr. Zool. London, 1958**:** 477-478

Tronchin G., Schrevel J. (1977) Chronologie des modifications ultrastructurales au cours de la croissance de *Gregarina blaberae*. *J. Protozool.* **24:** 67-82

Walker M. H., Mackenzie S. P., Bainbridge S. P., Orme C. (1979) A study of the structure and gliding movement of *Gregarina garnhami*. *J. Protozool.* **26:** 566-574

Yoshikawa H., Yamada M., Yoshida Y. (1988) Freeze-fracture study of *Blastocystis hominis*. *J. Protozool.* **35:** 522-528

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