

A New Species of Gregarine *Stylocephalus arabica* (Protozoa: Apicomplexa) from the Mid Gut of a Coleopteran Insect *Adesmia cancellata* (Klug)

Eglal A. Koura and Afaf A. Al-Jubair

Department of Zoology, Girls College of Education,
Riyadh, Saudi Arabia

ABSTRACT. A new species of septate gregarine was discovered in the mid gut region of the coleopteran insect *Adesmia cancellata* (Klug) during a general survey of protozoans. All the insects were collected from six cities in the Central Region of Saudi Arabia.

An investigation of the biology of this species revealed unique characteristic features different from all other described species of the genus *Stylocephalus* (Ellis). The most pronounced distinguishing features shown by the new species relate to its length, its maximum length being 2483 μm . The epimerite consists of two parts: a proximal neck which is hyaline and retractile and a distal disc provided with a large number of small papillae reaching a length of 99 μm at the longest specimens. The protomerite is variable in shape, wider than long with a constriction at the septum with dense cytoplasm. The deutomerite is elongated, and carrot-shaped. The nucleus is circular to elongated 31-83 μm with 5 karyosomes. Syzygy is side-by-side with individuals aligned in opposite directions. The name suggested for this new species is *Stylocephalus arabica*.

Protozoan parasite of insects are very well known and considerable attention has been given to studies by workers during the 20th century. Ellis in 1912 found a new species of gregarine in the mid gut of *Adesmia cancellata* and named it *Stylocephalus giganteus*, Watson (1916) found *S. oblongatus* in the mid gut of two species of insects *Blaps kollari* (Seidlitz) and *Opatroides vicinus* (Fairmaire) and Tuzet et Theodorides (1951) recorded *S. variabilis* in the mid gut of *Pimelia arabica* (Klug).

No records of gregarine parasites by Saudi workers have been published to date. For this reason, a preliminary survey on the protozoan parasites of insects in the central region of Saudi Arabia was initiated. During this investigation ciliates, amoebas, flagellates and gregarines were found in a number of insects examined.

Of particular interests was the discovery of a previously unknown gregarine which occurred in the mid gut region of the coleopteran *Adesmia cancellata* (Klug). A study of this newly discovered gregarine, latter named *Stylocephalus arabica* nov. sp. in *A. cancellata* (Klug), revealed the parasite to have unique characteristic features which have never been recorded in any of the existing species of the genus *Stylocephalus*. The arrangement of taxa within the phylum was based on that of Levine *et al.* (1980).

Materials and Methods

Four species of insects were examined, these were *Adesmia cancellata* (Klug); *Pimelia arabica* (Klug); *Blaps Kollari* (Seidlitz) and *Opateriodes vicinus* (Fairmaire). They were collected during the period between January 1985 to June 1986, larvae, nymphs and adult insects were collected from six cities of Saudi Arabia namely Riyadh, Diriyah, Durma, Al-Majma'ah and Buraidah. The majority of the specimens coming from Riaydh.

After removing the elytra and the clipping off the head and the posterior end of the abdomen, the entire gut was removed from the posterior end. The parasites can sometimes be seen even without opening the gut, particularly in starval specimens. The gametocysts are also easily detected within the intestine and the rectum.

For making smears the guts were teased in drops of normal saline solution, the parasites were taken out, dried in the air for a couple of minutes and fixed in Schaudinn's fluid for an hour; Carnoy's mixture for five to ten minutes; sublimatic acetic for seven to eleven minutes or Bouin's fluid for two to three hours. Fixed smears were stained in Ehrlich's haematoxylin, Delafield's haematoxylin, and Mann's methyl blue-eosin.

Gametocysts were fixed in the same fixatives and stained in Heidenhain's iron alum haematoxylin. For histological studies, the infected part of the alimentary tracts were fixed in Bouin's Doboscq Brazil, 10% neutral formalin, at 4-10°C, Carnoy's for 2-8 hours then washed and dehydrated in a graded series of ethanol solutions. Tissues were routinely embedded in paraffin wax (60°C) and then sectioned at 3-8 µm on a rotary microtome and stained in haematoxylin and eosin; Mallory's triple stain; Heidenhain's iron haematoxylin; silver impregnation techniques and Delafield's haematoxylin. It was noted that staining with Heidenhain's iron haematoxylin, following overnight mordanting in 3% iron alum, gave excellent iron alum, gave excellent results.

The distribution of carbohydrates was investigated with Best carmine, Alcian

blue, PAS and Methylene blue azur II and Basic fuchsin. Nucleic acid was investigated by a Feulgen technique. Lipid was demonstrated by Oil Red O-isopropanol method.

Results

Like many other gregarines, *S. arabica* (Plate 1) has two major phases in its life cycle, intracellular and extracellular.

The Intracellular Stages

The young stages of *S. arabica* was first observed within the epithelium of

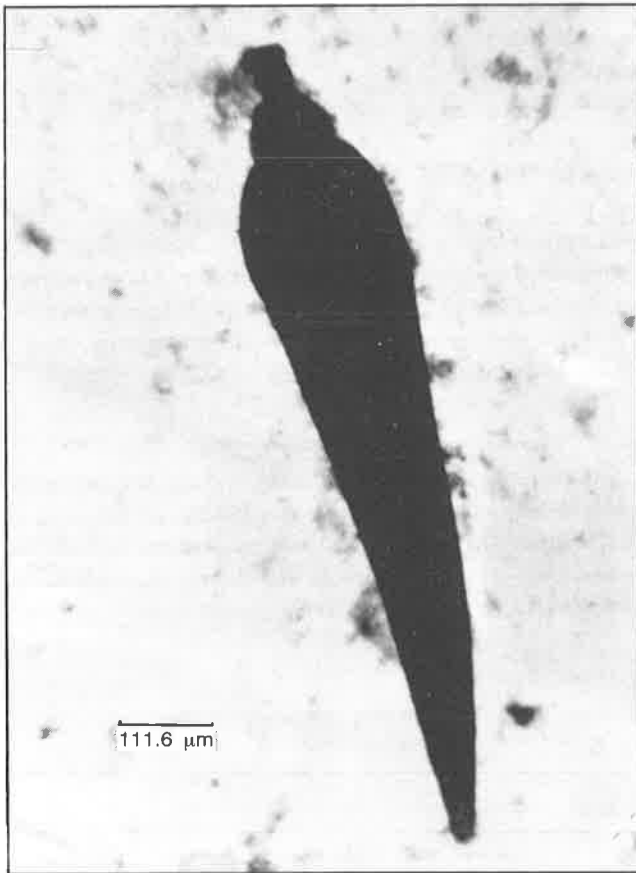


Plate 1. *Stylocephalus arabica* (nov. sp.) in the mid gut of *Adesmia cancellata* (Klug).
(Light micrograph, live specimen).

alimentary canal. Each individual is embedded completely in the cytoplasm of an epithelial cell generally rounded at one end tapering gradually at the other end (as shown in Plate 2), its measures $6.2 - 21.7 \times 3.1 - 15.5 \mu\text{m}$. As it grows further, the body divides into two compartments by an ectoplasmic septum. The trophozoite continues to grow within the epithelial cells of the intestine and finally pushes outwards into the lumen to become the extracellular trophozoite but attached to the ciliated epithelial cell with the epimerite.

The Extracellular Stages

The extracellular stages of *S. arabica* are as follows:

1. The adult trophozoites
2. Sporadin
3. Syzygy
4. The gametocyst
5. The sporocyst
6. The sporozoite

1. The adult trophozoite:

Detached trophozoites are seen free in the lumen (Plate 3). The trophozoite is elongated, tapering slightly forming a carrot shape, and composed of two compartments separated by septum. The protomerite posses an attaching process called epimerite. It consists of two parts: a proximal neck which is hyaline and retractile, and a distal disc provided with large numbers of small papillae which may reach $99 \mu\text{m}$ in the largest specimens.

2. Sporadin:

The sporadins of *S. arabica* are characteristically solitary and measure $180 - 2483 \mu\text{m}$ in length (Plate 4). The nucleus is variable in size and shape (circular, semi-circular or elongated). It has a distinct nuclear membrane surrounding the nucleoplasm in which several karyosomes are clearly discernible in section (Plate 5) their number varies from one to five.

3. Syzygy:

The associating pair apparently come together side-by-side in opposite direction (Plate 6).

4. The gametocyst:

The gametocyst encloses two equal-sized gametocytes and appears almost ovoid (Plate 7a). The cyst is covers externally by an enveloping wall which is uniform around. The cyst measures $428 - 503 \mu\text{m}$ long and $319 - 347 \mu\text{m}$ wide including the enveloping wall. The envelope is about $6 \mu\text{m}$ thick.

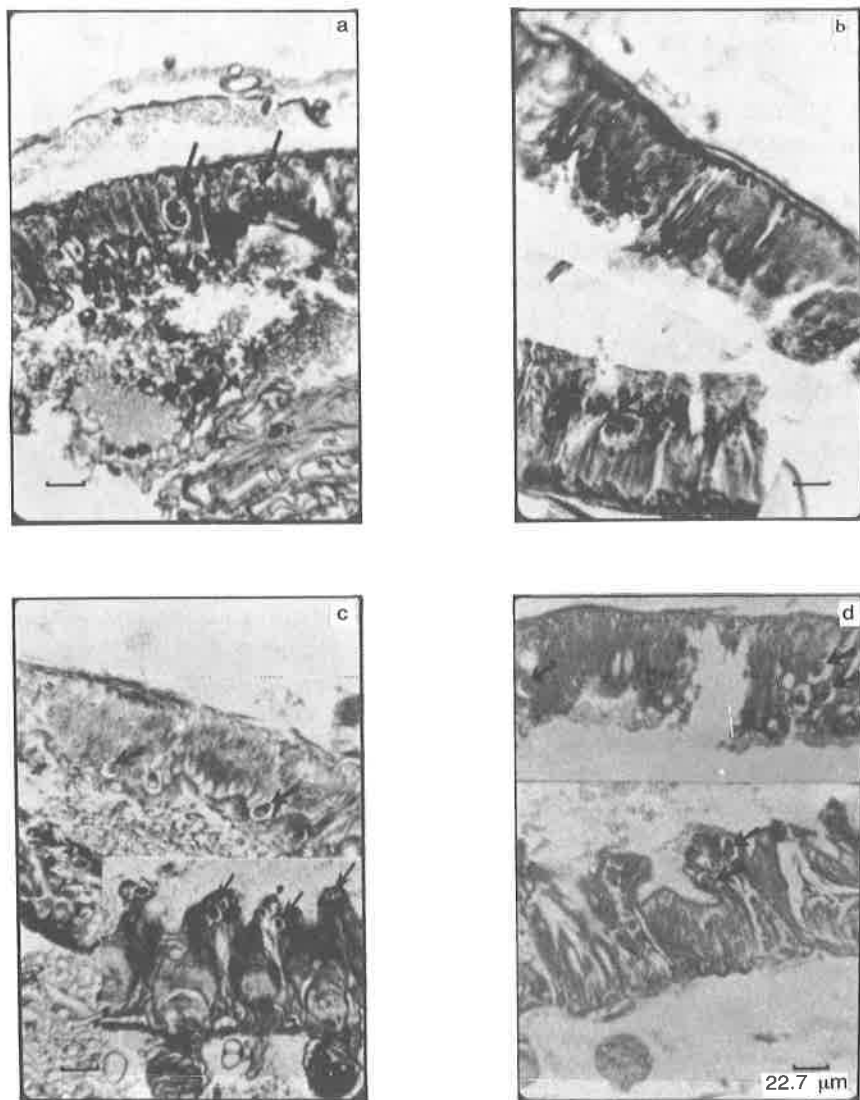


Plate 2. Transverse sections of the intestine of *Adesmia cancellata* (Klug) showing intracellular stages (arrowed) of *Stylocephalus arabica* (note the pear-shaped appearance, cavities occur around the intracellular trophozoites, arrowed).

a: Heidenhain's iron haematoxylin (H.H.).

b: Delafield haematoxylin.

c: Giemsa.

d: Feulgen reaction.

Light micrographs.

All scales equal 22.7 µm.

Further stages of development of gametes and zygotes within the gametocyst were not observed.

A mature gametocyst contains a large number of sporocysts clustering together in a form of chain as observed from a ruptured gametocyst (Plate 7b).

5. *The sporocysts:*

The sporocysts are ovoidal (12.4 - 15.5 μm) long are dark brown and contain an unknown number of sporozoites.

6. *The sporozoites:*

The sporozoites were rarely seen.

The possible developmental stages of life cycle of *S. arabica* is shown in Fig 1.

Discussion

S. arabica is similar in number of characters to other septate gregarines. These characters are: 1. presence of septum; 2. presence of syzygy, gametocysts and sporocysts; 3. body structure composed of an outer epicyte and an inner entocyte enclosing the single nucleus and many cytoplasmic inclusions.

S. arabica is solitary, elongated with a maximum length is up to 2483 μm . while the maximum length attain by other species is 2 mm. The epimerite consists of two parts: 1. a proximal neck which is hyaline and retractile, in this respect it resembles *S. gladiator* (Blanchard) and *S. bahli* (Misra), but it differs from them and from other species of *Stylocephalus* in the distal region which is a disc provided with a large number of small papillae and the epimerite may reach 99 μm in the largest specimens. The protomerite is variable in shape, wider than long, with a constriction at the septum and the cytoplasm is very dense, in other species of *Stylocephalus*, it has a definite shape. The deutomerite is elongated and carrot-shaped. While the other species of *Stylocephalus* previously described are either cylindrical, elongated or filiform. (Misra 1941 and Lipa 1967).

It is seen that despite the differences of size of the trophozoite and sporadins of *S. arabica*, the gametocysts are very small in relation to body length agreeing with the other observations of the different species of *Stylocephalus*.

During this investigation, sporocysts have been seen in the living state to contain several sporozoites, although it was not possible to determine the exact number. In most other gregarines, each sporocyst contains eight sporozoites and the maximum number recorded was 16 in blasto-gregarines (Levine *et al.* 1980).

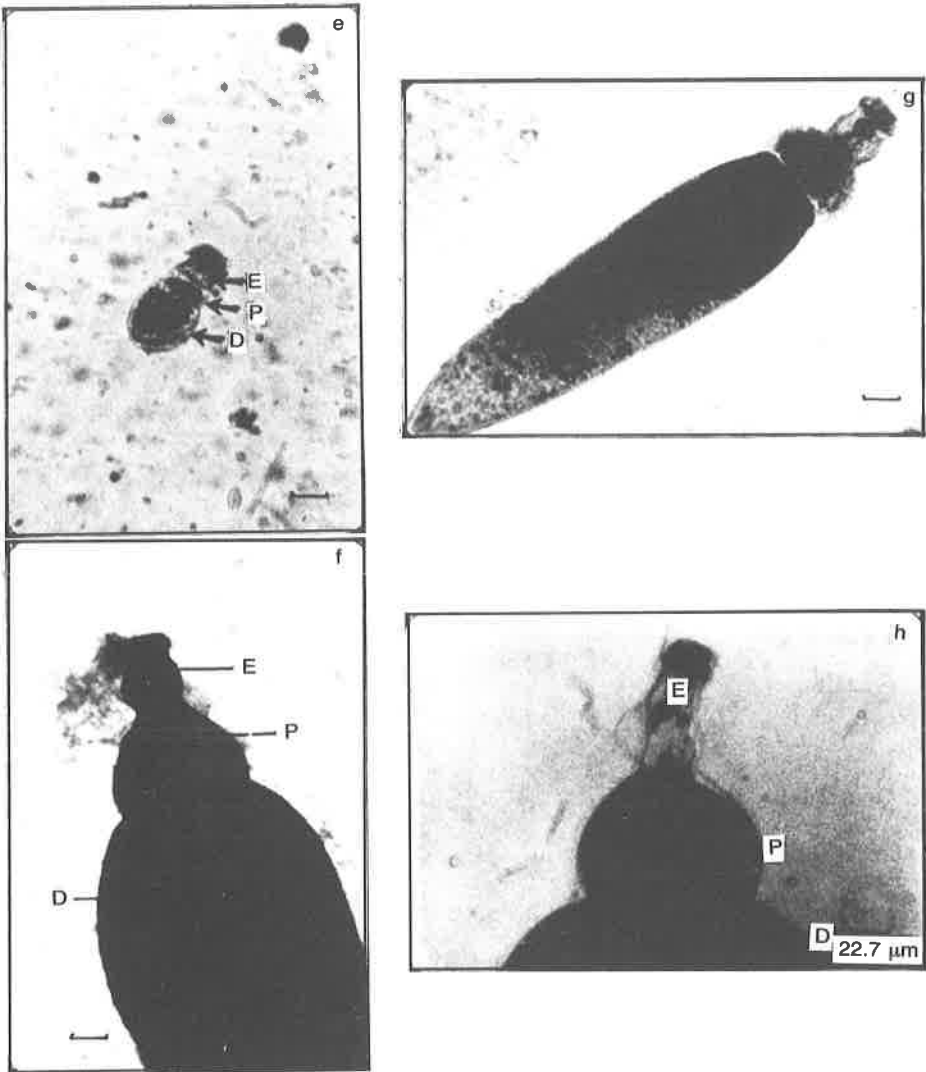


Plate 3. Live trophozoites of *Stylocephalus arabica* as seen by light microscopy.

e: Young trophozoite.

d: Adult trophozoite.

f & h: Fully grown trophozoites (note the two parts of the epimerite E).

All scales equal 22.7 μm.

E— Epimerite.

P— Protomerite.

D— Deutomerite.

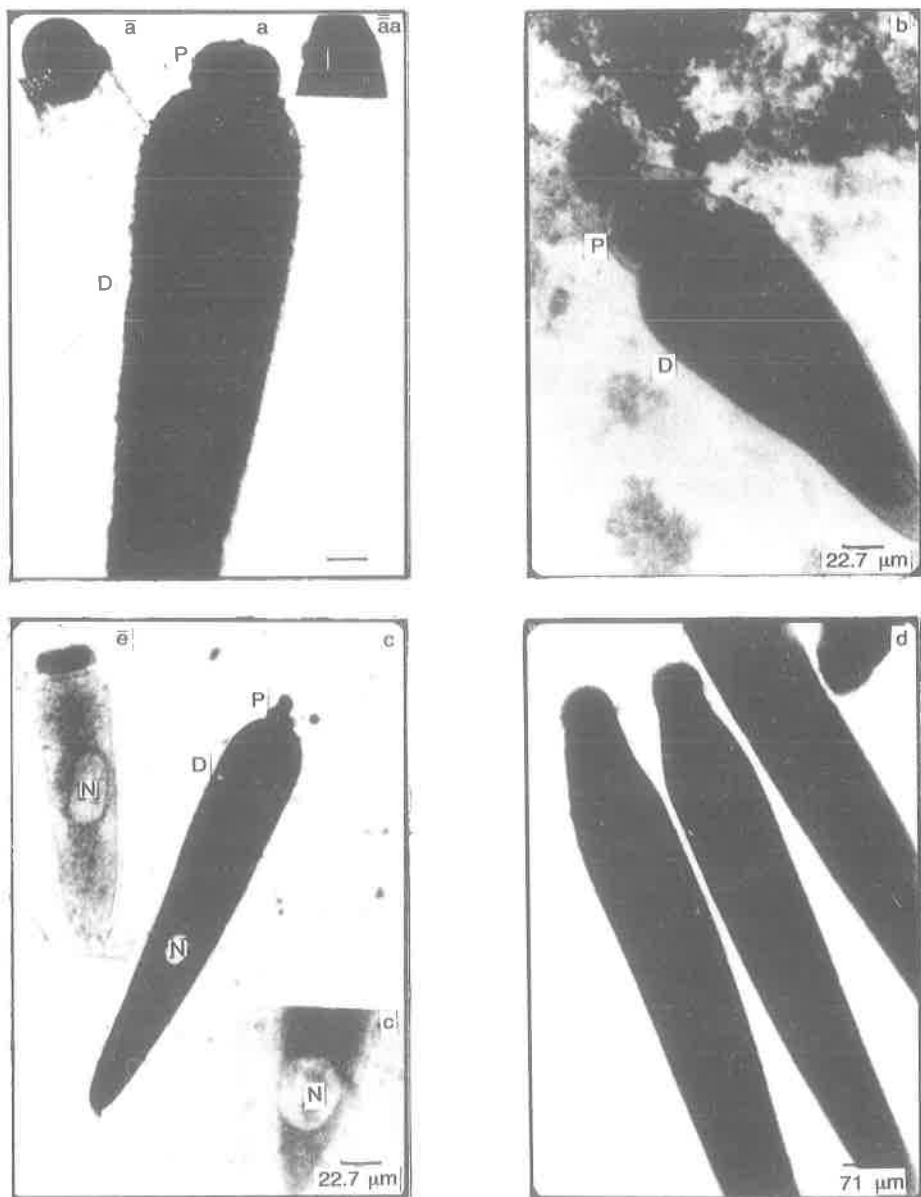


Plate 4. Live sporadins of *Stylocephalus arabica* as seen by light microscopy.

- a, a' & a'': Fully grown sporadins (note the different shapes of protomerite).
- b: A sporadin with dome-shaped protomerite (note the carrot-shaped body).
- c, c' & c'': Different positions of the nucleus (N) (note the epicyte contains two layers in c, c'').
- d: Sporadins showing the density of cytoplasm.

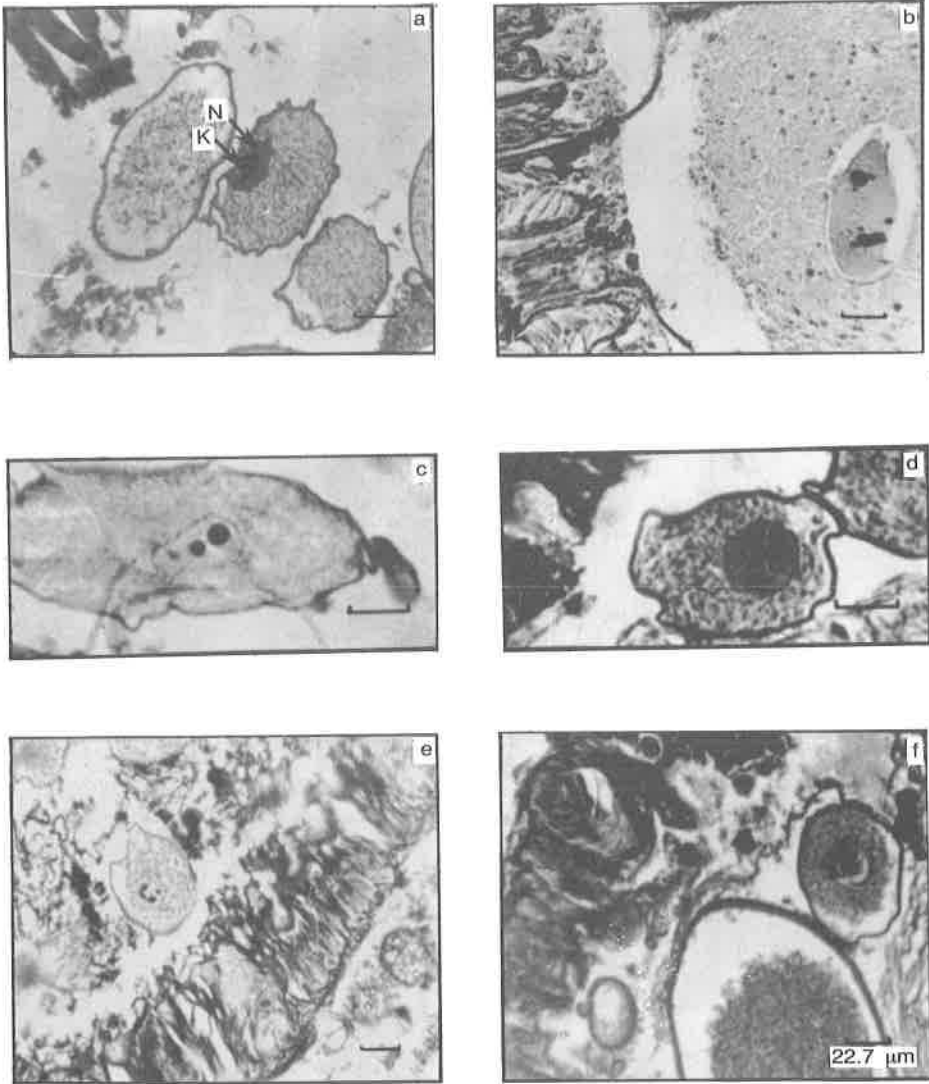


Plate 5. Section of *Stylocephalus arabica* showing nucleus and karyosomes.

- a: One karyosomes (stained with Feulgen reaction).
- b: Two karyosomes (stained with Mallory triple).
- c & d: Three karyosomes (stained with H.H., note in (d) nuclear membrane is very clear).
- e: Four karyosomes (stained with Giemsa).
- f: Five karyosomes (stained with H.H.)

Light micrographs.

All scales equal 22.7 µm.

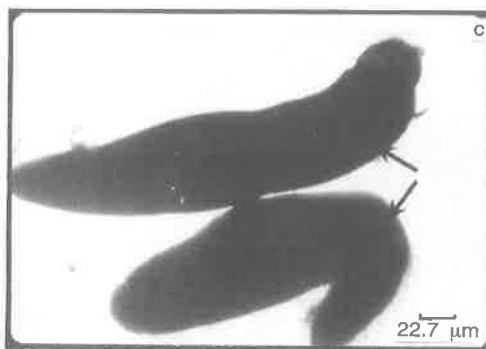
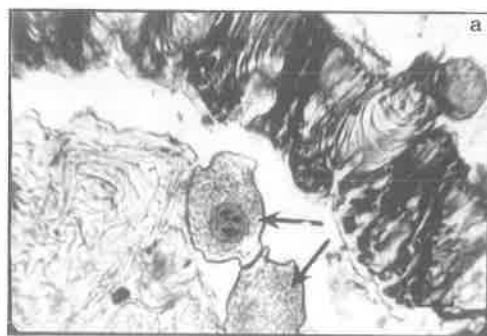


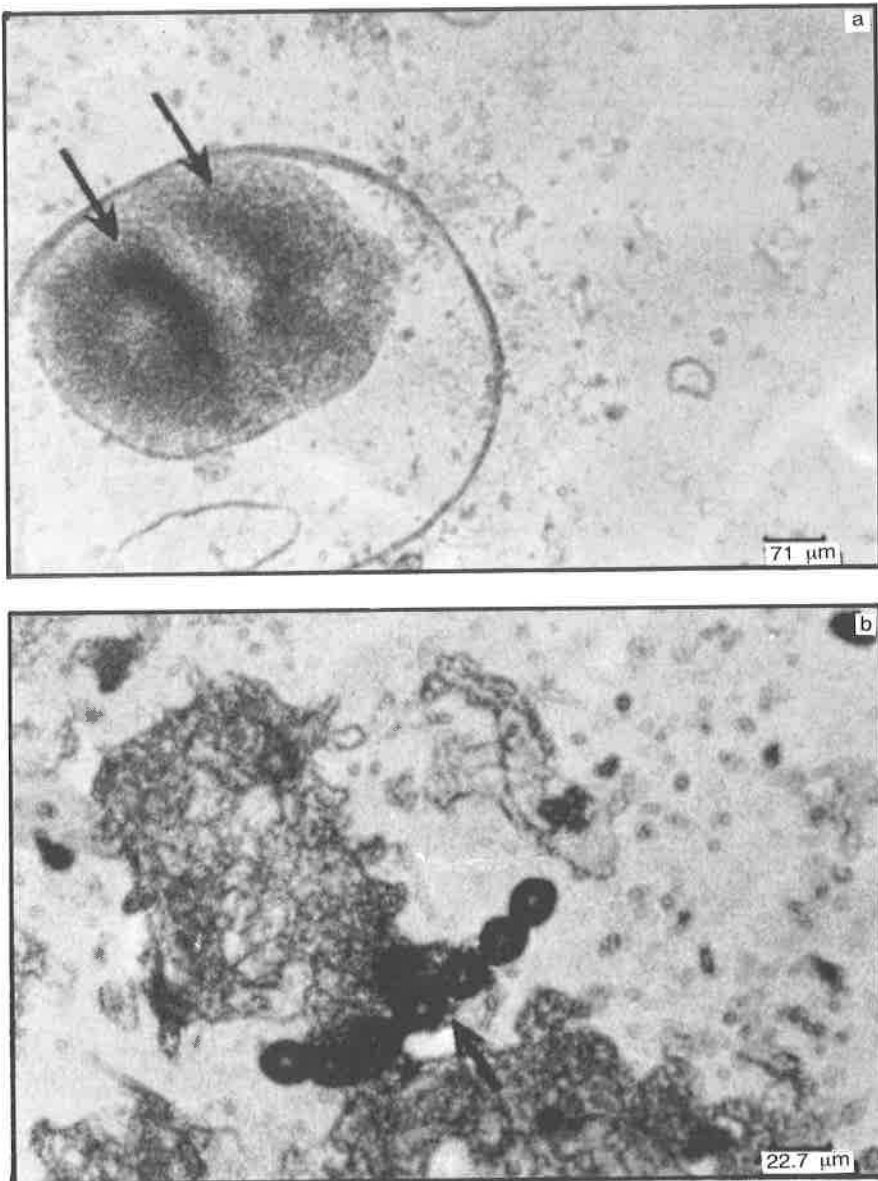
Plate 6. Cases of syzygy of *Stylocephalus arabica*.

a: Section of host gut (stained with H.H.)

b & c: side-by-side association (observed live).

Light micrographs.

All scales equal 22.7 μm.

**Plate 7.**

a: *Stylocephalus arabica* gametocyst.

Note the two gametocytes and the enveloping cyst wall (ruptured).

b: Chain of individual sporocysts obtained from a ruptured gametocyst.

Light micrographs. Live specimens.

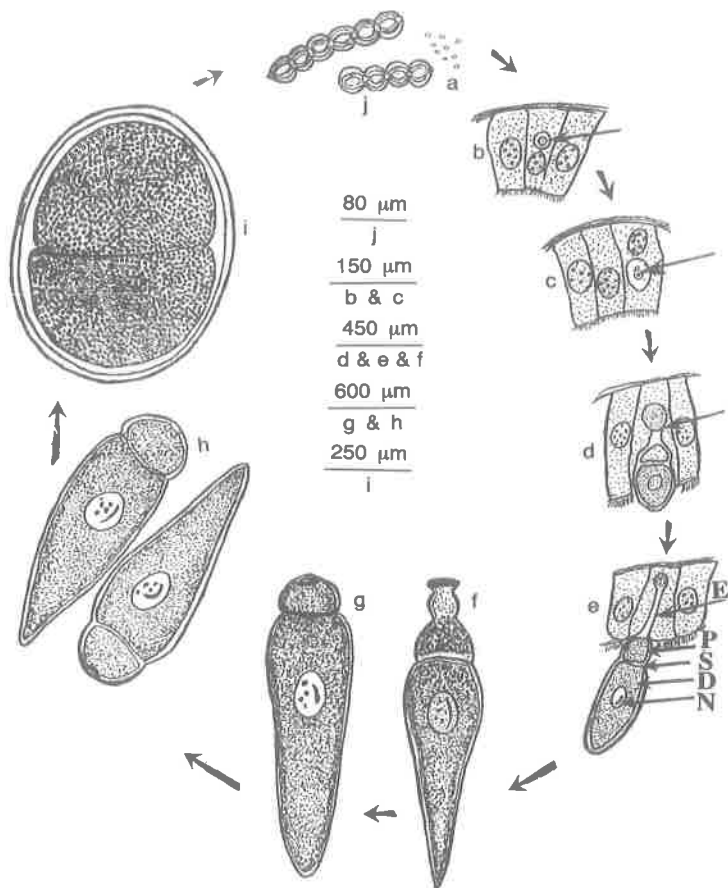


Fig. 1. Diagram illustrating the possible developmental cycle of *Stylocephalus arabica* of *Adesmia cancellata* (Klug). Figures have been drawn with the help of Camera lucida.

- Sporozoites.
- Earliest intracellular stages from a section of host mid gut (arrowed).
- Young trophozoite showing protomerite and deutomerite (arrowed).
- Advanced intracellular trophozoite pushing into the gut lumen (arrowed).
- Adult extracellular trophozoite attached with the epimerite (note the body of the trophozoite is divided by septum (s) into three parts: an epimerite (E) used for attachment; a protomerite mid section (P) and a posterior deutomerite (D) contains a single nucleus (N).
- A fully grown trophozoite.
- A fully grown sporadin.
- Sporadins in syzygy (side-by-side).
- A freshly formed gametocyst with two equal gametocytes.
- Spores liberated in a chain.

The sporocyst is ovoidal in shape (12.5 - 15 μm) in length, this feature again differs from the sporocysts of the other *Stylocephalus*.

Slides bearing the holotype the paratype and the hapantotype specimens have been deposited at the Zoology Section of the British Museum, (Natural History). The holotype is on slide no. 1986: 10:17:1 prepared from the sections of the infected mid gut of *Adesmia cancellata*, collected from Riyadh and stained using H.H. The paratype is on slide on. 1986: 10:17:2, prepared from sections of the gut of the same insect, stained also with H.H. The hapantotype is on slide no. 1986: 10:17:3, stained with PAS.

References

- Blanchard, L.F. (1905) Deux gregarines nouvelles parasites de tenbrionides des maures. *Ass. Franc. Plavace t sei Comptes Renous* **33**: 923-928.
- Ellis, M.M. (1912) A new species of polycystid gregarine from the United States. *Zool Anz.* **39**: 25-27.
- Levine, N.D., Corliss, J.O., Cox, F.E.G., Deroux, G., Grain, J., Honigberg, B.M., Leedale, G.F., Loebich, A.R., Lom, J., Lynn, D., Merifield, E.G., Page, F.C., Poljansky, G., Sprague, V., Vavra, J. and Wallace, F.G. (1980) A newly revised classification of the protozoa. *J. Protozool.* **27**: 37-58.
- Lipa, J.J. (1967) Studies on gregarines (Gregarinomorpha) of arthropods in Poland. *Acta Protozool.* **5**: 97-179.
- Misra, P.L. (1941) Observations of a new gregarine, *Stylocephalus bahli* sp. nov., from the alimentary canal of an Indian beetle, *Gonocephalum helopioids*, *From Rec. Industrial Mus.* **XL III**: 43-72.
- Tuzet, O., and Theodorides, T. (1951) Gregarines nouvelles le *Coleopteres tenebroinides*. *Ann. parasit. Hum. Comp.* **26**: 394-406.
- Watson, M.W. (1916) Studies on gregarines including description of twenty one species and a synopsis of the eugregarine records from the Myriapoda, Coleoptera and Orthoptera of the world. *Illinois Biol. Mong.* **2**(3): 211-468.

(Received 31/10/1987;
in revised form 14/12/1988)

نوع جديد من الجريجارينات سمى استيلوكيفلس العربي من المعى المتوسط لاحدى الحشرات غمدية الاجنحة ادميا كانسلاتا (كلاج)

اجلال أبو السعود قورة و عفاف عبدالله الجبير

قسم علم الحيوان - كلية التربية للبنات بالرياض - المملكة العربية السعودية

تم اكتشاف نوع جديد من الجريجارينات المجزأة سمى استيلوكيفلس العربي في امعاء إحدى الحشرات غمدية الأجنحة ادميا كانسلاتا (كلاج) اثناء عمل مسح شامل للأوالي المصاحبة لبعض الحشرات في مراحل التطور (يرقه - حورية - حشرة كاملة) التي جمعت من ست مدن من المنطقة الوسطى بالمملكة العربية السعودية - وبالدراسة البيولوجية بالمجهر الضوئي لهذا النوع، وجد انه يختلف في بعض مواصفاته التفصيلية عن الأنواع الأخرى التابعة لنفس الجنس وهذه الاختلافات هي :

(أ) طويل يصل طوله إلى $2483 \mu m$

(ب) الزائدة الأمامية أنبوبية مستطيلة مكونة من جزئين: الأول القريب (proximal) من القطعة الأمامية اسطوانى طويل شفاف يشاهد به ألياف طويلة لها القدرة على الانقباض والانبساط وله جدار سميك والجزء البعيد (distal) عبارة عن قرص سميك مزود بعدد كبير من الحلقات الصغيرة ويصل طول الزائدة الأمامية $(99 \mu m)$.

(ج) القطعة الأمامية تأخذ اشكالاً مختلفة (شكل القبة - شكل شبه المنحرف الشكل الدائري والمفلطح).

(د) القطعة الخلفية طويلة تستدق في اتجاه الخلف معطية شكل الجزيرة .

(هـ) النواه دائرية أو شبه دائرية أو متطاولة .

(و) الكريوسومات من واحد إلى خمسة .

(ز) يتحد اسبورادين جانب إلى جانب في وضع متعاكس .

ونظراً لوجود كل هذه الاختلافات بين هذا النوع والأنواع الأخرى المعروفة من جنس *Stylocephalus* (Ellis) فقد اعتبر نوعها جديداً، أطلق عليه اسم استيلوكيفلس العربي . وقد تم تسجيله في المتحف البريطاني (التاريخ الطبيعي) قسم الأوليات .

وقد أمكن تتبع دورة الحياة في مراحل داخل الخلايا وتشمل الطور الجرثومي والتروفوزويت الصغير ومراحل خارج الخلايا وتشمل التروفوزويت التام النمو، اسبورادين، اتحاد اسبورادين، حوصلة الأمشاج والحوصلة الجرثومية وبالرغم من كثافة الإصابة إلا أن بعض أطوار دورة الحياة شوهدت بقلّة مثل اتحاد اسبورادين، حوصلة الأمشاج والحوصلة الجرثومية في تجويف امعاء الحشرة . ويتكون جدار الجسم في المراحل خارج الخلايا من طبقتين مع وجود ثنيات طولية منتظمة على السطح الخارجي، وشوهد نوعين من الحركة - انزلاق وانحناء .

وقد بينت قطاعات الأمعاء حدوث بعض التغيرات النسيجية في طلائية الأمعاء حيث تقل كثافة السيتوبلازم في الخلية المصابة بالمقارنة بالخلايا غير المصابة، كما يتغير موضع النواة . كما بينت القطاعات أيضاً وجود فجوات تحيط بالتروفوزويت وهو داخل الخلايا تحتوي في الحالة الحية على سائل شفاف يحتمل أنه يمد التروفوزويت النامي بالغذاء .

كما تم تعيين المواد الكربوهيدراتية (الباراجلايكوجين وعديدات السكر المخاطية)، الدهون، الحمض النووي الرايبوزي منقوص الاوكسجين والميتوكوندريا وأجسام جولجي في كل من الجريجارين ونسيج العائل وأعطت نتائج مشابهة للأنواع الأخرى من الجريجارين .