# ON THE LIFE-HISTORY OF A NEW GREGARINE, *GREBNECKIELLA*[1] *PIXELLAE*, SP. NOV., FROM THE CENTIPEDE, *SCOLOPENDRA MORSITANS* LINN., WITH A NOTE ON THE FAMILY DACTYLOPHORIDAE LÉGER, 1892.

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CONTENTS.



## INTRODUCTORY AND HISTORICAL.

Early in 1939 Prof. K. N. Bahl very kindly called my attention to the excellent contribution made by Pixell-Goodrich on "*Nina*, a remarkable gregarine", which she found in the gut of *Scolopendra cingulata* Latreille and *S. subspinipes* Leach, and suggested that I should work out the life-history of the gregarines occurring in *Scolopendra morsitans* Linn.,[2] which is the commonest centipede found at Lucknow (India). I may mention at once that *Scolopendra morsitans* harbours only one species of gregarine, *i.e. Grebneckiella pixellae*, sp. nov.; I have not been able to find any other gregarine during my examination extending over three years of the intestinal parasites of this centipede.

In 1873 Grebnecki described *Nina gracilis* (=*Pterocephalus nobilis* Sokolow, 1911) from *Scolopendra cingulata* Latr. (*S. cingulata* var.

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[1] Synonyms: *Nina* Grebnecki, 1873 and *Pterocephalus* Aimé Schneider, 1887. *Nina* was used as the generic name for a mollusc (Gray, 1850), while *Pterocephalus* had been used as the generic name for an elasmobranch fish (Swainson, 1838), a Nematode (Linstow, 1899), and a Trilobite (Raw, 1907), hence these names are inadmissible. But the generic name *Grebneckiella*, after Grebnecki, recently introduced by Bhatia (1938) is available and I have therefore adopted it in my paper.

[2] Identification of this centipede was made by Prof. K. N. Bahl and confirmed at the Indian Museum through kind courtesy of Dr. Baini Prashad, Director, Zoological Survey of India, Calcutta.

*hispanica* Newp., *vide* Watson, 1916). Schneider (1887) recorded *Pterocephalus nobilis* from *Scolopendra morsitans* collected from Banyuls, but, according to Léger and Duboscq (1909) *Scolopendra cingulata* alone is found at Banyuls and not *Scolopendra morsitans*; Pixell-Goodrich (1938) also mentions that Schneider had wrongly named his *Scolopendra*. Kölliker (1848) described *Gregarina scolopendrae* from *Scolopendra morsitans* collected from Trieste, but Pixell-Goodrich has pointed out that he also was wrong in naming his centipede. According to her, Kölliker's centipede " may have been *Scolopendra cingulata* " but not *S. morsitans*, as this latter species has never been recorded from that locality. Labbé (1899) suggested that Kölliker's gregarine probably belonged to the genus *Pterocephalus* and not " *Gregarina* " Watson (1916), however, has rejected Labbé's suggestion and has asserted that from Köiliker's fig. 30 of *Gregarina scolopendrae* it appears that the protomerite is very different from that of *Nina*, and that since Kölliker had given no account of the epimerite of his gregarine it is impossible to say in which genus his specimen should be placed. In my opinion Kölliker's fig. 30 represents really a specimen of *Grebneckiella* with a contracted knob-like protomerite, a fact which has also been suggested by Pixell-Goodrich.

Since 1873 the following species of this genus have been recorded up to date : (1) *Pterocephalus giardi* Léger, 1899, (=*Nina giardi* Sokolow, 1900) from *Scolopendra oraniensis* Verh. (2) *P. giardi corsicum* Léger and Duboscq, 1903, (=*N. giardi corsicum* Sokolow, 1911) from *Scolopendra oraniensis lusitanica* Verh., (3) *Nina indica* Merton, 1911 from *Scolopendra subspinipes* Leach., and (4) *Nina navillae* Mitra and Chakravarty, 1937 from *Scolopendra* sp.

It would appear, therefore, that the gregarines described by various authors from *Scolopendra morsitans* are really not from this species but from other species of *Scolopendra*, and that *Scolopendra morsitans sensu stricto* has not been examined at all for gregarines. My observations on the only gregarine of *Scolopendra morsitans* have convinced me that it is specifically different from the hitherto described species of *Grebneckiella*, and therefore, I propose for it the name *Grebneckiella pixellae*, sp. nov., associating it with the name of Dr. Helen Pixell-Goodrich, M.A. (Oxon.), D.Sc. (Lond.), as a token of my appreciation for her remarkable observations on *Nina*(=*Grebneckiella*).

## Material and methods.

Specimens of *Scolopendra morsitans* were collected from beneath stones, bricks, etc., of old and neglected buildings in and around Lucknow, or flower pots in the University gardens. They were kept singly in wide glass jars with a fine wire-gauze cover. In summer they were kept in shade on moist earth under laboratory conditions, but in winter they were covered with straw and rags. Milk was the best diet to keep them alive for months together. At times they were fed on apples, carrots, etc. It was surprising to note that if this centipede was provided with tea it extruded several (many times more than the usual number of) gametocyts along with its faeces.

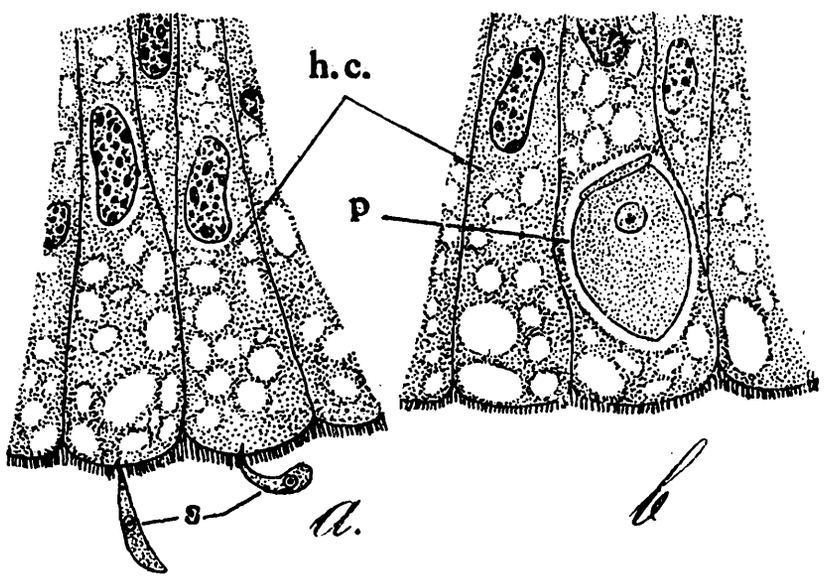
Pieces of the gut of *Scolopendra morsitans* were fixed in alcoholic Bouin, Schaudinn's fluid, and Gilson's mixture, sectioned at 4μ to 6μ and stained with iron-alum haematoxylin, Delafield's haematoxylin and eosin, and Mallory's triple stain. Gametocysts were fixed at various stages of development in warm Dobell's modification of Bouin (with a few drops of chloroform just before use) for 24 hours, sectioned at 2μ to 4μ and stained with iron-alum haematoxylin and orange-G or chromotrop 2 R. Total preparations and smears were fixed in warm Schaudinn's fluid, the former were stained with Delafield's haematoxylin and also borax carmine, while the latter were stained with iron-alum haematoxylin and at times counter-stained with chromotrop 2 R.

All drawings were made with the aid of a *Camera lucida* and magnification of the text-figures are given.

## Life-history of *Grebneckiella pixellae*, sp. nov.

### (*a*) Sporozoites and their development.

Fresh smears of live sporozoites obtained by rupturing mature spores in Ringer's solution under a coverglass, when examined under an oil-immersion lens, revealed that the sporozoites perform flexional movements followed by passive gliding movements when they become less energetic. Fixed and stained preparations showed that the sporozoites are spindle-shaped bodies measuring 5μ to 7μ in length. The cytoplasm of the sporozoites is homogeneous and the centrally located nucleus in each is of a vesicular type (Text-fig. 1*a*).



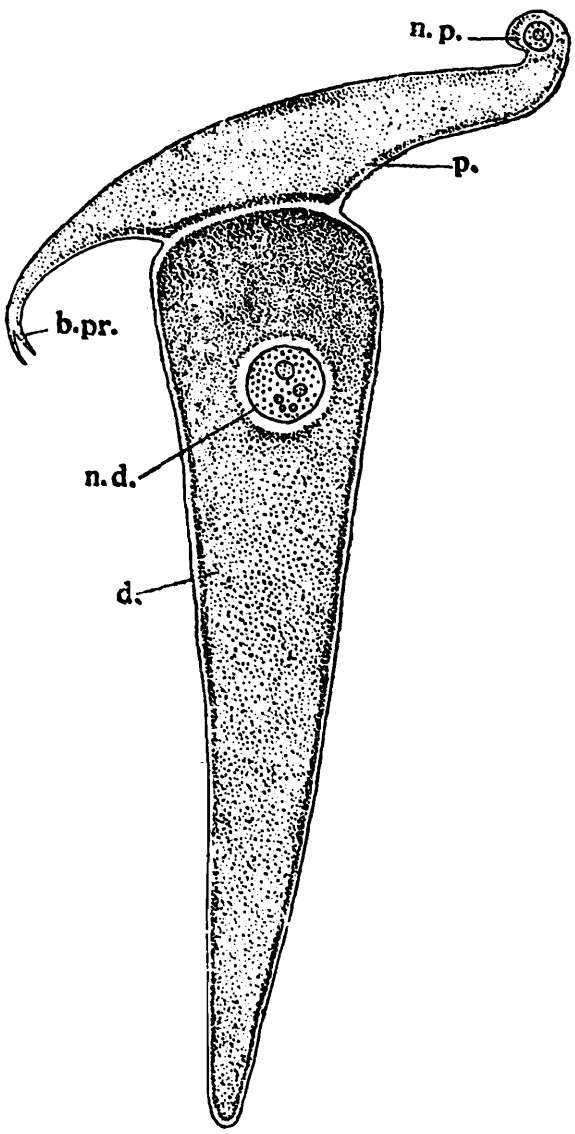

Text-fig. 1.—*a*. Two sporozoites (s) attached to the epithelial cells (h. c.) of the host's gut: ×2,386. *b*. A trophozoite (p) lying within the epithelial cell: ×1,636.

The walls of the ingested spores are probably dissolved by the action of the gutfluid of *Scolopendra morsitans* and the sporozoites are liberated into the lumen of its gut and make their way towards the epithelial cells of the intestine to which they attach themselves. After penetrating these cells they are found to undergo an intra-cellular development (Text-fig. 1*b*). Later on, due to increased growth, the trophozoites, as they are now called, break through the intestinal cells and hang themselves into the lumen of the intestine while still remaining

attached by their epimerites to the epithelial lining. They grow in this situation till they attain maturity.

Pixell-Goodrich and all other previous workers have held that *Grebneckiella* leads an entirely extracellular existence, and they make no mention of an intracellular stage at all. My studies of the sections of fixed and stained material of the gut of *Scolopendra morsitans* have, however, convinced me that *Grebneckiella pixellae* passes through an intracellular phase during its development before it comes to the adult stage.

Pixell-Goodrich states, " Some of the young vegetative stages attain a considerable size before satisfactorily attaching themselves. . .Presumably, therefore, they can absorb food and grow while free in the lumen" In support of her statement she has sketched fig. 11c, Pl. 7 in her paper, but her figure represents, as far as I think, a contracted sporozoite rather than a " very young trophozoite". I have found several such instances



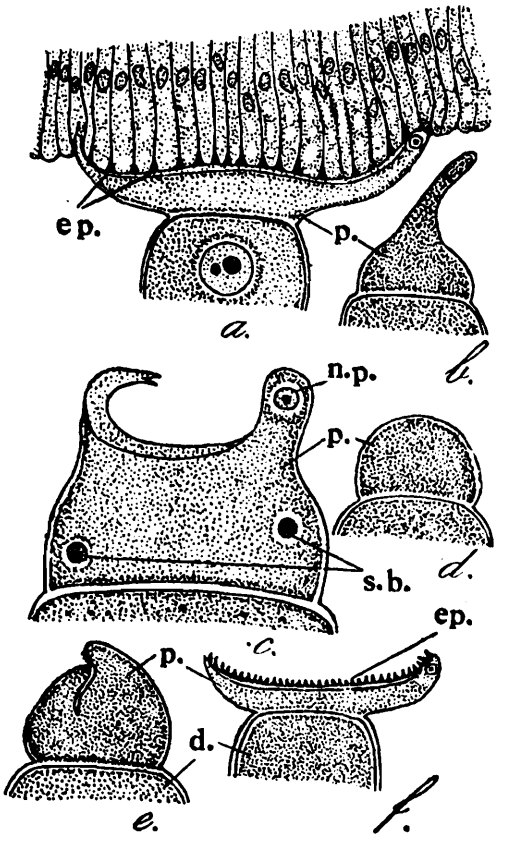

TEXT-FIG. 2.—A freshly detached adult specimen of *G. pixellae* : b. pr., bifid tip of the anucleated arm of the protomerite; n. d., deutomeritic nucleus; n. p., protomeritic nucleus; p., protomerite : ×323.

of contracted sporozoites in the living condition. Further, the specimen represented by her fig. 1, Pl. 7 appears to me to have been previously attached, but having lost its epimerite in the epithelial cells had dropped

free into the lumen of the gut and due to the contraction of its protomerite looked as if it had never found an attachment. In fact, sporozoites of gregarines always at first attach themselves and then grow further. Pixell-Goodrich herself describes smaller individuals than the one shown in her fig. 1, Pl. 7 " firmly fixed to the gut wall with epimerites complete " It would appear, therefore, that the specimen which she regards as having " showed no signs of ever having been attached " to the gut-epithelium is really a later stage of *Grebneckiella* after its detachment.

(*b*) Trophozoites.

The youngest trophozoite that I have come across measures 10·2μ × 6·6μ in size (Text-fig. 1*b*). The protomerite is not very conspicuous inside the cell, and it is only after the parasite has come out of the cell that the protomerite expands and attaches itself to the free borders of several cells of the gut-epithelium (Text-fig. 3*a*). However, after




Text-fig. 3.—*a-f*, Protomerite of *G. pixellae* in various shapes, d., deutomerite; ep., epimerites with filaments; n. p., protomeritic nucleus; p., protomerite; *s. b.* siderophilic bodies in the protomerite: × 323.

this preliminary attachment the digitiform epimerite grows from the edge of the protomerite thereby affording a firm hold to the parasite.

A fresh smear of the gut of *Scolopendra morsitans* in Ringer's solution showed the parasites moving actively and the active movements performed by the protomerites, specially of the young and freshly detached cephalonts, being interesting to note. The protomerite shows

lateral contractions and expansions, as well as forward and backward movements. Due to its mobile nature it can assume various shapes, and at times the contractions are so strong that the protomerite is reduced to a mere knob-like elevation at the top of the deutomerite (Text-fig. 3 *a-e*). When the protomerite faces upwards, *i.e.* against the coverglass, its sucker-like appearance becomes very evident. The high degree of contractility of the protomerite is due to the presence of myonemes set along the free margin of the sucker. Pixell-Goodrich has mentioned that the protomerites of *Grebneckilella* could be "used as mobile suckers for attachment." As in *Echinomera* this is an exceptional instance of marked contractility of the protomerite amongst gregarines, and I agree with her remark that such an instance has never been "definitely stated before"

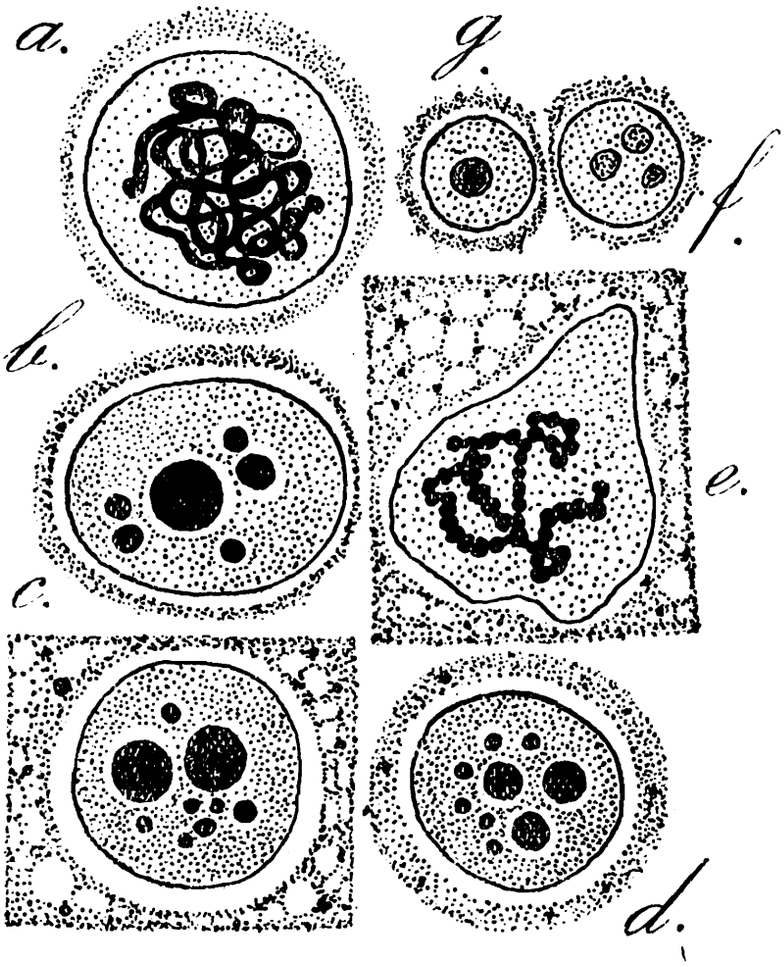
At times, however, it was also noticed that the protomerite of *Grebneckiella pixellae* pressed itself against the surface of the slide and that the deutomerite contracted postero-anteriorly resulting in the formation of convolutions on its surface. The deutomerite itself helps in movement and particularly comes into action when there is an impediment in front of the protomerite.

Fixed and stained preparations reveal that the epimerite of *Grebneckiella pixellae* is formed of several digitiform protuberances bearing thread-like filaments at their distal extremities (Text-fig. 3*a*). These protuberances stain black with iron-alum haematoxylin and, when deprived of their thread-like processes, appear as denticles beset on the edge of the protomerite (Text-fig. 3*f*). The epimerites are caducous, *i.e.* they are torn-off from the protomerite and are left behind in the epithelium when the trophozoites attain maturity and drop into the lumen of the gut.

In an extended condition the parasite presents a T-shaped appearance, the protomerite forming the cross-bar, one arm of which is definitely longer than the other, and the deutomerite forming the vertical limb (Text-fig. 2). In a detached individual the longer arm, which contains a small nucleus at its distal end is usually upturned, while the shorter arm, which is characterized by its bifid distal extremity, either curves posteriorly or is reduced to a knob. The cytoplasm of the protomerite is comparatively less dense than that of the deutomerite. The nucleus of the protomerite is vesicular and contains one to three chromatic bodies (Text-fig. 4 *f, g*). This nucleus seems to have only a vegetative function and takes no part in the reproductive processes. At times I have noted, besides the nucleus, one or two siderophilous bodies in the protomerite of *Grebneckiella pixellae* (Text-fig. 3*c*). Their origin and function could not be determined.

The deutomerite is elongated; it is widest immediately behind the septum and gradually tapers posteriorly to a blunt end. But in young cephalonts the posterior end of the deutomerite is pointed. In a full grown individual it measures 3,050μ in length and 90·6μ in width at its maximum diameter. The pellicle is about 3μ in thickness and the myocyte is very conspicuous. The cytoplasm of the deutomerite is very dense and highly granular, being replete with prominent granules which stain deep black with iron-alum haematoxylin.

The nucleus of the deutomerite is spherical or slightly ovoid in shape (Text-fig. 4 *b-d*) and, on an average, measures 44·8μ in diameter. It



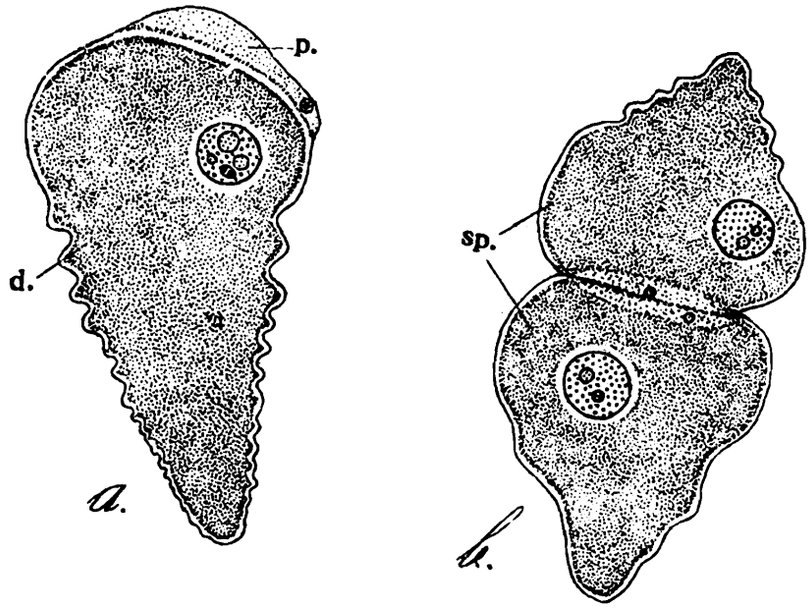

Text-fig. 4.—*a-c*, Deutomeritic nucleus; *b*, *c* and *d* from whole mounts, and *a* and *b* from sections; *f* and *g*, protomeritic nucleus, from whole mounts: ×600.

contains one to three big nucleoli and several small deeply staining granules; the nuclear membrane is distinct. Usually the nucleus is located anteriorly though it may be found in any region of the deutomerite. Merton (1911) has described and sketched the deutomerite nucleus of *Grebneckiella indica* as having a spireme of chromatin material —a statement not borne out by the description given by Léger and Duboscq (1909) for that of *G. gracilis*. Chakravarty (1938) has described the nucleus of *Grebneckiella navillae* as being spherical and having one karyosome and several small chromatin granules. Pixell-Goodrich has mentioned that the nucleus of *Grebneckiella* studied by her agreed with that of Léger and Duboscq's gregarine and certainly not with that of Merton's gregarine. The deutomerite nucleus of *Grebneckiella pixellae*, no doubt, resembles most that of *Grebneckiella gracilis*, but in some sporonts and various sections passing through the nucleus of *G. piexellae* the chromatin net-work (Text-fig. 4 *a*, *e*.) was very apparent, indicating that the nucleus was ready for division. I think Merton has sketched the nucleus of one such sporont.

The body of *Grebneckiella pixellae* shows an apparent bilateral symmetry—the plane of symmetry passing between the bifids tips of the one arm and the distal extremity of the other arm along the long axis of the deutomerite. Ratio of the length of the protomerite to the total length L. P. : L. T. : : 1 : 15-23 ; width of the protomerite to that of the deutomerite W. P. : W. D. : : 1·2-2·5 : 1.

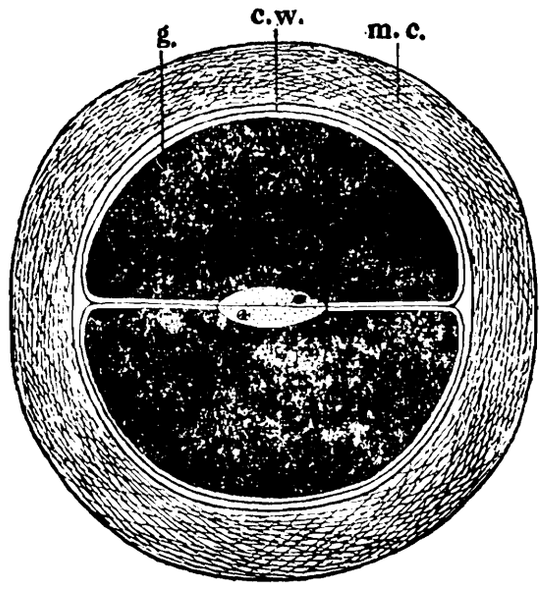
(*c*) Sporonts and association.

Each sporont is characterized by having a reduced and laterally flexed protomerite (Text-fig. 5*a*) and by the absence of an epimerite. The




Text-fig. 5.—*a*. A contracting sporont, from a fresh smear: p., protomerite; d. deutomerite: ×190; *b*. Two sporonts (sp.) in association: ×190 (Livespecimens).

cytoplasm is very dense and appears blue by reflected light and the deutomerite nucleus very often becomes masked in its substance. The sporonts show a passive gliding movement and are usually in a contracted condition. Two sporonts (gamonts) come together by their anterior ends with their protomerites lying opposed to each other (Text-fig. 5*a*). The deutomerites of the two gamonts contract further and further until at last they become rounded and secrete a common cyst-wall which later on becomes surrounded by a gelatinous covering 60μ to 180μ thick. The gametocysts thus formed measure 208μ to 672μ in diameter and are spherical in shape. The two protomerites




Text-fig. 6.—A gametocyst of *G. pixellae*: m. c., mucous covering; c. w., cyst-wall, g. gamont: ×70. (Live specimen stained with mucihaematin.)

at the place of their junction inside the freshly extruded cysts appear like a hollow biconvex lens under the coverglass (Text-fig. 6). The highly hygroscopic gelatinous layer is composed of numerous concentric layers, each layer probably indicating the quantity of the exudate oozing out of the body of the rounded up gamonts at one time. Various mucous stains, as suggested by Pixell-Goodrich, were tried but the cyst-wall proper did not take up these stains and according to her possibly it is made up of keratin. The cyst-wall is comparatively tense and offers more resistance to various infections (bacteria, fungi, etc.) than does the gelatinous layer. In fact, I have not encountered the mycelial infection (Mucoridae ?) inside the cysts as described by Léger and Duboscq and also by Pixell-Goodrich, although such infections were of frequent occurrence in the gelatinous layer.

Encystment of single individuals has also been noted but very little mucus is secreted in such cases and such individuals ultimately degenerate.

Healthy cysts were frequently found outside the peritrophic membrane, but they were also found within it, hence, it does not seem to be " a rule ", as mentioned by Pixell-Goodrich, that they are always external to this membrane. As regards the condition of freshly extruded cysts I agree with Léger and Duboscq's statement that such cysts are normally in an advanced stage of development, as the deutomerite nuclei of hundreds of fresh cysts of *Grebneckiella pixellae* were found to have already started dividing. Pixell-Goodrich has contradicted these authors and holds that freshly extruded cysts of her gregarine had " unchanged deutomerite nucleus"

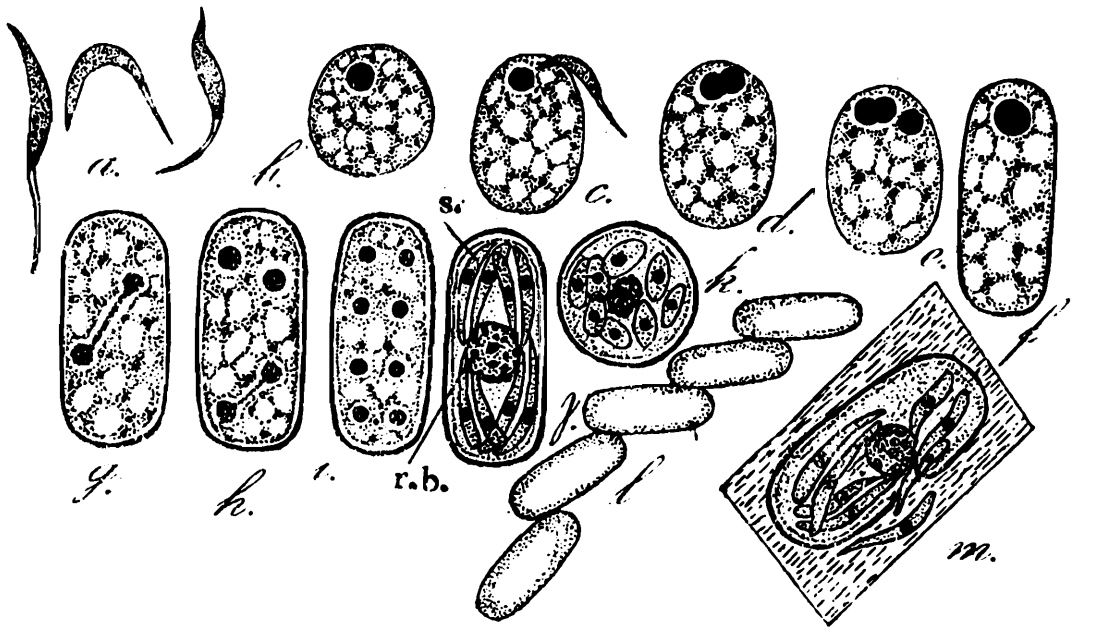
Autopsy of several specimens of *Scolopendra morsitans* revealed that cysts in the intestine had their nuclei unchanged but such cysts when " cultured " in hanging drops had their nuclei dissolved (divided) within one to three days. The fact that in the faecal matter the nuclei were generally found dissolved shows that it probably takes one to three days for the cysts to pass from the intestine to the exterior.

### (*d*) Gamete-formation and structure of the gametes.

The stages of nuclear division and gamete-formation studied from the sections of the gametocysts of *Grebneckiella pixellae* resemble those of *Grebneckiella gracilis*, as described and sketched by Léger and Duboscq (1909), and it is therefore unnecessary to describe them again. The haploid number of chromosomes is undoubtedly five, four of which are equal, being in two pairs, while one is extraordinarily long and unpaired and which ultimately forms the karyosome. Chakravarty (1938), has mentioned that the haploid number of chromosomes of *Grebneckiella navillae* is only two and that there is no axial (unpaired) chromosome.

The microgametes when examined alive under an oil-immersion lens showed marked agility, and fixed and stained preparations revealed that they are minute filamentous bodies measuring 5$\mu$ to 6$\mu$ in length. Each microgamete (Text-fig. 7*a*) consists of an elongated head or rostrum composed almost entirely of chromatin material and a drawn-out tail or flagellum which helps in its movement. At the apex of the head there is a small refringent granule. The undulating membrane, which,

according to Minchin (1903), " runs in a loose spiral from the rostrum to the base of the flagellum " could not be detected in my preparations.




Text-fig. 7.—*a*. A microgametes. ×4,184 ; *b*. a macrogamete ; *c*, showing entrance of a microgamete into a macrogamete ; *d*, pro-zygote stage ; *e*, a macrogamete showing three nuclei, presumably by the entrance of two microgametes ; *f*, a zygote ; *ca*. ; spores showing nuclear division and formation of sporozoites : note the residual body (r. b.) in *j* ; *k*, an abnormal spore : *b-k* × 2,272 ; *l*, a chain of spores attached obliquely, ×1,270 ; *m*, a mature spore showing liberation of spores in Ringer's solution. ×2,272. (l and *m*, Live specimens.)

The macrogametes are non-motile, spherical bodies measuring 7μ to 9μ in diameter (Text-fig. 7*b*), but after attaining maturity they tend to become oval, so much so that they assume a, more or less, cylindrical shape either after fertilization or even before it (Text-fig. 7 *c-e*). Each macrogamete has an excentrically located nucleus, 1·6μ in diameter, and its cytoplasm contains prominent reserve granules. In appearance the macrogametes resemble the telolecithal ova of Metazoa as mentioned by Minchin. It would appear, therefore, that the gametes of *Grebneckiella* present a striking instance of anisogamy among this group of Protozoa.

### (*e*) Fertilization and spore-formation.

It is held that minute apertures (never detected by me) are present in the partition membrane separating the two gamonts in the cyst and it is through these apertures that the microgametes escape from the male chamber into the female chamber where they fertilize the macrogametes. Microscopical examination of live gametes obtained by puncturing the cysts in Ringer's solution revealed that the microgamete is attracted towards that end of the macrogamete which contains the nucleus (Text-fig. 7*c*). After penetration the nucleus of the microgamete reaches that of the macrogamete, abuts against it, rests for some time (pro-zygote stage Text-fig. 7*d*) and then fuses with it to form the zygote nucleus (Text-fig. 7*f*). On a few occasions I have noted two nuclei besides the definitive female nucleus within the macrogamete (Text-fig. 7*e*). This is probably due to the entrance of two microgametes inside a macrogamete. The whole process after the entrance of the male nucleus

till the formation of the zygote nucleus takes about twenty minutes to one hour or at times even longer. The zygote elongates, becomes cylindrical, secretes a wall around it and thus forms a spore. The spores when liberated during the dehiscence of the cysts remain attached together in oblique chains (Text-fig. 7*l*); this adherence is brought about by the presence of an oily film around each spore.

(*f*) Structure of the spores and formation of the sporozoites.

The spores are cylindrical bodies measuring 10μ to 13μ in length and 4μ to 5μ in width, the most frequent measurements being 11μ × 4μ. Each spore has an excentrically located nucleus and its cytoplasm contains refringent granules. The sporocystic wall consists of two layers : an inner layer, the endospore, which is thin and delicate, and an outer layer, the epispore, which is thick and resistant. There is no operculum in the spores of *Grebneckiella pixellae* and the liberation of sporozoites takes place by the dissolution or rupture of the wall of the spores. In this respect the spores of *G. pixellae* differ from those of *Grebneckiella gracilis* described by Pixell-Goodrich. Moreover, she has given a period of over one year as the duration of viability of the spores of her gregarine, but in *G. pixellae* I have found that the spores are viable only for three to four months. I have found a few spores which were rounded and which had oval sporozoites (Text-fig. 7*k*), but such rounded spores were very rare and may be regarded as abnormal.

The nucleus of each spore divides into eight daughter nuclei by three successive divisions and its cytoplasm segments around each nucleus thus giving rise to eight sporozoites which are arranged in two tiers (Text-fig. 7 *g-j*). A definite residual cytoplasm consisting of refringent granules is left in the centre of the spore after the formation of the sporozoites. The whole process takes about 24 to 48 hours. Léger and Duboscq (1909), as mentioned by Pixell-Goodrich, have given 10μ to 11μ as the length of the sporozoites, whereas she has given 5μ to 6μ, maximum being 8μ, as the length of the sporozoites of *Grebneckiella gracilis*, and has mentioned that the sporozoites are about half the length of the spores. The measurements of the sporozoites of *Grebneckiella pixellae* approximate those given by Pixell-Goodrich. To my mind it appears that Léger and Duboscq measured the lengths of two sporozoites lying tandem. Usually the sporozoites of *G. pixellae* remain slightly curved inside the spore, and hence appear approximately to be half the length of the spore, but in an extended condition they are somewhat longer, as can be noticed by examining the live mature spores in Ringer's solution in which they often rupture.

(*g*) Remarkable stages in the developing cysts.

I have verified Pixell-Goodrich's observations on the developing cysts, stage by stage, and have found that my observations on the developing cysts of *Grebneckiella pixellae* agree with those described by her for the cysts of *Grebnekiella gracilis*. In hanging drops the whole process from the time of freshly extruded cysts till the liberation of spores takes 4 to 7 days. It may be remarked that at times the cysts

did not rise to the surface of the water and the spores were liberated within the water; presumably all the stages were gone through under water. Cysts kept in moist chamber but not actually within water also dehisced and liberated their spores. It seems probable that in the natural habitat of *Scolopendra morsitans* where only the moisture of the earth under stones, etc., is available, excepting during the rains, dehiscence of spores takes place in the usual way, *i.e.* by pseudocyst-formation, but the stages A, B and C as described by Pixell-Goodrich are not so well marked owing to insufficiency of water for the cysts to float upon.

(*h*) Mode of infection.

Infection is carried on from host to host through food and drink contaminated with infective spores and is more common in the adults than in young specimens. The maximum site of infection is just behind the proventriculus and at times the gregarines seem to block the lumen of the gut.

## DIAGNOSIS OF *GREBNECKIELLA PIXELLAE*, SP. NOV.

Sporonts solitary, measuring 1050μ to 4050μ in length; epimerite caducous, digitiform, with filaments; protomerite a mobile sucker, with two asymmetrical arms, one longer and nucleated, the other shorter, anucleated and bifid; bilaterally symmetrical; deutomerite elongated, widest behind the septum, terminates in blunt end; L. P.: L. T.:: 1: 15-23; W P.: W D.:: 1·2-2·5 : 1; cysts spherical, measuring 208μ to 672μ; dehinsce by pseudocyst; spores cylindrical or long ovoidal, with two envelopes, united in oblique chains, measuring 10μ to 13μ×4μ to 5μ; operculum absent in spores.

*Habitat.*—Mid-gut of *Scolopendra morsitans* Linn.

*Locality.*—Lucknow, U. P., India.

## NOTE ON THE FAMILY DACTYLOPHORIDAE.

The family Dactylophoridae Léger, 1892, of septate gregarines seems to have been loosely handled by protozoologists and "requires revision", as has been pertinently remarked by Pixell-Goodrich (1938). A perusal of the relevant literature shows that the definition of this family as given by Pixell-Goodrich is most plausible, but it would be complete were it added that sporonts are solitary and that dehiscence of cysts take place by simple rupture as well (*vide* Bhatia, 1938, p. 108).

Majority of the authors have included in this family the gregarines occurring in the gut of Chilopoda alone, but Kudo (1939) has included in it the gregarines occurring in the gut of other animals as well (*vide infra*), although while defining the family he mentions that its representatives occur "in guts of *chilopods*".[1] He has placed the following genera under the family Dactylophoridae: (1) *Dactylophorus* Balbiani, (2) *Echinomera* Labbé, (3) *Rhopalonia* Léger, (4) *Dendrorhynchus* Keilin, (5) *Trichorhynchus* Schneider, (6) *Nina* (=*Grebneckiella*) Grebnecki, (7) *Seticephalus* Kamm, (8) *Acutispora* Crawley, (9) *Metamera* Duke,

[1] Italics are mine.

(10) *Hentschelia* Mackinnon and Ray, (11) *Lecythion* Mackinnon and Ray. Firstly, it may be pointed out that out of these eleven genera, the following four genera occur in such hosts as do not belong to the order Chilopoda : (i) *Dendrorhynchus systeni* Keilin, occurs in the mid-gut of the larvae of *Systenus* sp., which is an insect (Dolichopodidae, Diptera), (ii) *Metamera schubergi* Duke, occurs in the gut of *Glossiphonia camplanata* and *Placobdella*[1] *marginata*, which are leeches (Glossiphonidae, Rhynchobdellidae, Hirudinea), while (iii) *Hentschelia thalassemae* and (iv) *Lecythion thalassemae* Mackinnon and Ray, occur in the gut of *Thalassema neptuni*, which is an Echiurid worm. Secondly, it may be noted that while classifying the *Cephalina* (Engregarinida, Gregarinida, Telosporidia) Kudo has defined the family Dactylophoridae along with others, as having characteristic extracellular development to distinguish them from the two families, Cephaloidophoridae and Stenophoridae, which are characterized by intracellular development. I have already emphasized that *Grebneckiella pixellae* passes through an intracellular phase of development during its early stages. Further, there are two other genera, namely, *Hentschelia* and *Lecythion*, which Kudo characterizes as those with extracellular development. For example, Mackinnon and Ray (1931, p. 451) write about *Hentschelia thalassemae*, " We have found a few young stages. These lie *within* the epithelial cells (fig. 14, Pl. 20) " Moreover, they have also mentioned (pp. 460-461) that " *Doliocystis* (*Lecudina* ?) and *Hentschelia* are *intracellular*[2] in the early stages of their life within the gut, and their epimeritic segment always remains intracellular " As regards *Lecythion thalassemae*, although Mackinnon and Ray have sketched its intracellular stage (*vide* their fig. 20, Pl. 20), they doubt the intracellular development of this parasite, as is evident from the question mark in connection with the explanation of that figure (p. 465), and from their statement (p. 454) that " in the adult condition, anyhow, it is never intracellular" In fact, there are several gregarines which show an intracellular growth during the early developmental stages but are entirely extracellular in the adult condition. It is possible that this is the case with *Lecythion*, although Mackinnon and Ray have not met with the intracellular stage. From these facts it would appear that at least two genera, namely, *Hentschelia* and *Grebneckiella*, and possibly also *Lecythion*, as exemplified by *H. thalassemae*, *G. pixellae* and *L. thalassemae* respectively, should be included in a family of the *Cephalina* whose members exhibit intracellular development, or, if they are to be included within the family Dactylophoridae, this family should not be characterized by having its " Development extracellular " as given by Kudo. For the present it would be better if the family Dactylophoridae were to be placed between those families of the *Cephalina* whose members exhibit intracellular development and those whose members develop entirely extracellularly. Finally, it may be mentioned that the whole family needs revision and its exact position amongst the septate gregarines needs to be accurately determined.

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[1] Duke in her original paper (*Quart. Journ. Micros. Sci.*, 1910, Vol. LV, pp. 261-286) mentions *Hemiclepsis* instead of *Placobdella*.

[2] Italics are mine.

Summary.

(1) A new gregarine, *Grebneckiella pixellae*, sp. nov., is recorded from *Scolopendra morsitans* Linn.

(2) This gregarine passes through an intracellular phase of development before attaining maturity.

(3) Various points dealing with the developing cysts, as described by H. Pixell-Goodrich (1938) for *Nina gracilis* Grebnecki, 1873, have been verified.

(4) The present position of the family Dactylophoridae Léger, 1892, has been discussed and it is concluded that its exact position amongst the septate gregarines requires to be accurately determined.

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