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## Hirmocystis termitis (LEIDY) and Kofoidina ovata gen. nov., sp. nov. from Termites.

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(With Plate 4.)

Although a voluminous literature has been developed concerning the Protozoa of termites, that dealing with the order Gregarinida is relatively scant. The first description of these forms from termites was given by LEIDY (1881) who described a gregarine from *Termes flavipes* and named it *Gregarina termitis*. This description, based on a single individual, gave the length as 60  $\mu$  and the width as 36  $\mu$ . The protomerite was 18  $\mu$  in length and 30  $\mu$  wide.

No further work on this species was done until PORTER (1897) reported the frequent occurrence of *G. termitis* in some termites. The parasites were found to be confined to the anterior part of the small intestine of the host. PORTER also reported the occurrence of cysts in sections of the anterior part of the intestine but his figures show that he undoubtedly mistook sporonts for cysts. Measurements taken from PORTER's illustrations show size variations from  $65.3 \mu$  to  $76.7 \mu$  for the length and from 43.3 to  $49.0 \mu$  for the width.

CRAWLEY (1903) failed to find gregarines in approximately a dozen termites which he examined.

Because of typographical errors in connection with measurements it is uncertain whether or not ELLIS (1913) observed G. termitis from Termes lucifugus. His figure of a sporont is similar to those of LEIDY and PORTER except in minor details. ELLIS gives 170  $\mu$  as the average length and 25  $\mu$  as the average width of the protomerite. The deutomerite averages 400  $\mu$  in length and 30  $\mu$  in width. These measurements are obviously in error as shown by his figure and probably represent  $17 \times 25 \mu$  for the protomerite and  $40 \times 30 \mu$  for the deutomerite. The total length would then be 57  $\mu$  rather than 570  $\mu$ , which is in accord with the measurements for *G. termitis* given by LEIDY and PORTER.

KAMM (1922) in a monograph on gregarines reviewed the literature on G. termitis and states that she has been unable to find gregarines by examinations of termites made at various times of the year.

BEQUAERT (1925) reports the finding of a gregarine apparently of the genus *Stylocephalus* (*Stylorhynchus*) lining the wall of the gut of *Neotermes castaneus*. From the description of the epimerite KIRBY (1927) considers that BEQUAERT was dealing with *Oxymonas*. The above mentioned authors have in all probability been

The above mentioned authors have in all probability been concerned with the single species, *G. termitis.* KIRBY (1927), on the other hand, has described a new species, *G. mirotermitis* from the tropical termite, *Mirotermes panamensis.* The two bi-associations upon which this description was based were examined in a stained preparation; for this reason the measurements which are given are not strictly comparable to those given for other species. The primite of *G. mirotermitis* had a total length of  $94 \mu$ ; the protomerite was  $15 \mu$  long and  $16 \mu$  wide, while the deutomerite was  $79 \mu$  long and  $39 \mu$  wide. The satellite of this bi-association had a total length of  $65 \mu$ ; the protomerite was  $6 \mu$  long and  $14 \mu$  wide, while the deutomerite was  $59 \mu$  long and  $27 \mu$  wide. The chief differentiating characteristic between this species and *G. termitis* is the greater relative length of *G. mirotermitis* as compared with its width and the small size of its protomerite.

KIRBY, in the same paper, also reports G. termitis in Reticulitermes hesperus and in Zootermopsis sp.

## Hirmocystis termitis (Leidy).

In the study of a large number of gregarines from the termites of the genus *Zootermopsis* many sporonts which were described as *G. termitis* by LEIDY have been encountered. The most outstanding characteristic of this species and one which has been found in most infections is the occurrence of the sporonts in associations of more

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than two. As bi-association is a generic characteristic of the genus *Gregarina* this species belongs in reality in the genus *Hirmocystis*, which has as one of its characteristics the occurrence of multiple associations. For this reason, the name *H. termitis* (LEIDY) is proposed for this species.

Hirmocystis termitis has been found very abundant in Zootermopsis angusticollis and Z. nevadensis from the western section of the State of Washington and in one colony of Z. angusticollis from central California. In all, thirty-five colonies of termites of the genus Zootermopsis have been examined and H. termitis was found in fourteen of these. Eleven of the colonies were found to be infected with this species of gregarine only, whereas, the other three contained H. termitis in addition to another species which is described below. Although the incidence of infection with gregarines in a single colony varies over a wide range due to the time of molt of the termites, the age and class of individuals and the life cycle of the parasite, the heavy infestations which do occur may be illustrated by citing a single example. One hundred and ninety-seven termites from a single colony were examined during a period of approximately three months. Of this group, 119 individuals contained sporonts of H. termitis, while only 78 were negative. The number of sporonts encountered in a single individual varied from one to two to several hundred; 200 or 300 gregarines in a single termite were not uncommonly found and in one individual 625 parasites were counted.

Cephalonts and sporonts of H. termitis occur almost exclusively in the anterior part of the mid-gut of Zootermopsis. In heavily parasitized individuals, sporonts may be found in the lower portion of the mid-gut. In lighter infections an occasional sporont is encountered in the lower area of the mid-gut or even in the hind-gut. These latter have in all probability been swept out of the intestine with the food column or may be dead or dying forms which are being cast out.

The normal sporonts in the mid-gut are found between the intestinal wall and the food column, lying parallel to the long axis of the gut. In heavily infected termites the sporonts may be seen through the intestinal wall as a relatively large opaque mass. The sporonts of H. termitis (Pl. 4 Fig. 1, 5) occur in asso-

The sporonts of *H. termitis* (Pl. 4 Fig. 1, 5) occur in associations of from two to eleven individuals, arranged in a linear formation. The majority of these associations consist of 2-5 sporonts, although it is probable that a number of the larger chains are

broken when the parasites are dissected from the gut. In addition to this linear arrangement it is not uncommon to find a single primite to which are attached two satellites, the attachment being somewhat more lateral than in the case of a single satellite. These satellites may in turn have other individuals attached posteriorly. The largest association of this type which has been encountered was composed of eleven sporonts. This deviation from the normal mode of attachment has been reported for other species of the genus *Hirmocystis*. In most cases the longer chains of sporonts are made up of immature individuals. The more mature sporonts almost invariably occur in pairs, although a third, usually small individual may be attached posteriorly. A variation of this tri-association is sometimes found consisting of a single mature, relatively large primite, with two smaller satellites each of which is directly attached to it. Although it is quite probable that the bi-associations consisting of two mature sporonts are in a stage just previous to encystment, it has not been determined whether or not the triassociations are able to form a single cyst.

Because of the noticeable difference in size between the mature bi-associations and the immature multiple associations, with the relatively infrequent transition forms, the possible presence of two species is suspected by the casual observer. However, closer study reveals the connecting forms. This scarcity of intermediate forms has been noted by WATSON (1915) in the species *H. harpali*.

Although some multiple associations were found which consisted of a greater number of individuals, the maximum length of a single association was  $1385 \mu$ . This chain consisted of ten sporonts. The longest bi-association was  $847 \mu$  and the largest single sporont,  $452 \mu$ . The maximum width of a sporont was  $146 \mu$ . From the measurements of 40 mature individuals the following ratios have been compiled. The ratio of the length of the protomerite to the total length of the sporont is as 1:4.2; the ratio of the width of the protomerite to the width of the deutomerite is as 1:1.2. Actual measurements in microns of typical associations and sporonts are given in the following table.

Total length of associations	614	606	803
Primite:			
Length protomerite	73	88	102
Length deutomerite	234	234	307
Width protomerite	73	102	95

Width deutomerite		102	131	131	
Total length sporont		307	322	409	
Ratio:					
Length protomerite: total length		1:4.2	1:3.6	1:4.0	
Width protomerite: width deutomerite		1:1.3	1:1.2	1:1.3	
Satellite :					
Length protomerite		49	58	58	
Length deutomerite		263	226	336	
Width protomerite		88	117	124	
Width deutomerite		102	146	161	
Total length sporont		307	<b>284</b>	394	
Ratio:					
Length protomerite: total length		1:6.2	1:4.8	1:6.7	
Width protomerite: width deutomerite		1:1.1	1:1.2	1:1.2	
Length of associations of m	nore than	ı two spo	ronts:		
Primite	248	161	175	175	
First satellite	219	161	131	146	
Second satellite	219	161	117	146	
Third satellite		131	131	131	
Fourth satellite		131	131	124	
Fifth satellite			131	146	
Sixth satellite			146	131	
Seventh satellite				131	
Eighth satellite				124	
Ninth satellite				131	
Total length of association	686	745	962	1 <b>3</b> 85	

Hirmocystis termitis (LEIDY) and Kofoidina ovata.

As workers who have previously studied *H. termitis* had in all cases, with the possible exception of ELLIS, observed only immature forms, it was thought advisable to compile the ratios based on the measurements of 20 immature sporonts, none of which exceeded 100  $\mu$  in total length. The ratios thus obtained together with those obtained by measurements of drawings included in the work of the above authors are given below.

LEIDY	L. P. : T. L. : 1 : 3.3
	W. P. : W. D. : 1 : 1.2
Porter	L. P. : T. L. : 1 : 3.5
	W. P. : W. D. : 1 : 1.3
Ellis	L. P. : T. L. : 1 : 3.3
	W. P. : W. D. : 1 : 1.2
HENRY (Less than $100 \mu$ in length)	L. P.: T. L.:1:3
	W. P. : W. D. : 1 : 1.3
HENRY (More than $270 \mu$ in length)	L. P.: T. L.: 1: 4.1
	W. P. : W. D. : 1 : 1.2

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I the error, due to the fact that the ratios were obtained from figures of one or two sporonts in all cases except those of the present author, is discounted, the ratios compited from the figures of LEIDY, PORTER, and ELLIS are seen to agree quite closely with those obtained from measurements of immature forms.

The sporont of *H. termitis* consists of a well defined protomerite, separated from the deutomerite by a definite septum. In the primite the protomerite is dome-shaped, slightly wider than long and flattened at the point of contact with the deutomerite, although there is a slight tendency toward rounding at this place due to a constriction at the septum. The deutomerite is slightly obese, being a little less than twice as long as wide. It is widest in the anterior third and tapers posteriorly to a blunt point. Situated in the enlarged anterior portion is the spherical or sub-spherical nucleus, which measures about  $25 \mu$  in diameter and which contains a single large karyosome. In the more opaque sporonts the nucleus may be completely hidden by the surrounding material.

The epicyte is uniform in thickness around the entire sporont except for a slight thickening on each side of the anterior end of the protomerite and at the septum. At the anterior end of the protomerite a cluster of relatively large granules is usually present beneath the epicyte.

The protomerite of the satellite differs from that of the primite in that it is compressed to such an extent at the point of attachment that it forms a cup-like depression into which the posterior end of the primite fits. Because of this compression the protomerite of the satellite is usually somewhat wider than is that of the primite. The epicyte surrounding the anterior end of the protomerite of the satellite is thickened to such an extent that ear-like projections extend out laterally.

The mature sporonts, although white by reflected light, appear dark by transmitted light. There is little difference in color between the protomerite and the deutomerite, and the granules of the endoplasm are of approximately the same size in both divisions.

In order to obtain the stages other than the sporonts in the life cycle of *H. termitis*, sections  $4-7 \mu$  in thickness, were made of the gut of infected termites. The digestive system of the termite (see IMMS, 1924) consists of an elongated oesophagus, a small crop, which is followed by the gizzard. Posterior to the gizzard the foregut protrudes into the cavity of the mid-gut, forming a large oesophageal valve. At the anterior end of the mid-gut, which is tubular

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and of uniform size throughout, are four short enteric caecae, which occur as outgrowths. At the junction of the mid- and hind-guts are found the eight Malpighian tubules. The hind-gut is enlarged to form a large chamber in which the enormous numbers of flagellates are found.

Sections of infected intestine show occasional cephalonts and immature sporonts in the enteric caecae and in the lumen of the mid-gut in the region of the oesophageal valve. The greatest number of both cephalonts and sporonts are found just posterior to this valve.

The most immature form of *H. termitis* which was encountered was the small cephalont in which the septum between the protomerite and deutomerite had already formed. In these younger stages as well as in the larger cephalonts, the epimerite occurs as a simple sphere, containing clear protoplasm. It may vary in diameter from  $7 \mu$  to  $14 \mu$ . The epimerite usually contains endoplasm only at the anterior end. In the young cephalonts (Pl. 4 Figs. 3, 4) the endoplasm of the protomerite and deutomerite is less concentrated than that of the older forms, and appears colorless or slightly gray by transmitted light. The epimerite apparently is pinched off when the cephalont breaks free from the intestinal cells as occasionally an indentation is seen at the anterior end of the protomerite when the epimerite has been lost.

The epimerite is extremely fragile and when the cephalont is freed from the intestinal wall soon bursts if placed in physiological salt solution, distilled water or glucose solutions of  $1 \, {}^0/_0$  or  $2 \, {}^0/_0$  concentration.

The epimerite, by which the gregarine is attached to the intestinal wall does not pierce the host cells but is attached in the crevices between two groups of cells (Pl. 4 Fig. 2). The protomerite and the anterior end of the deutomerite may also be enclosed by these cells in the early stages. As the cephalont increases in size it emerges more and more into the cavity of the gut. During this process, the deutomerite grows more rapidly than does the protomerite. This parasitism has no apparent effect upon the cells of the intestine, nor is the termite visibly affected by the presence of large numbers of gregarines.

The epimerite is lost while the cephalont is comparatively small and the freed sporonts form associations almost immediately. Groups of 6, 7, or 8 of these immature forms are frequently encountered. In associations the young sporonts differ from the more mature individuals in that there is no pronounced cup-like depression in the protomerite of the satellites. Those recently freed sporonts which fail to attach themselves to sporonts of similar size are frequently found attached to the posterior end of an association of larger individuals. As the sporonts mature, there is a tendency for the longer associations to subdivide until most of the adult sporonts occur in pairs. These pairs, as well as the longer associations, exhibit the motility typical of gregarines.

motility typical of gregarines. The rotary motion which is preliminary to cyst formation and which occurs commonly in some species has been noted only twice in *H. termitis*. In neither case did cyst formation occur. The failure to observe this preliminary step toward cyst formation frequently is consistent with the failure to observe more than a very few formed cysts, even though large numbers of mature sporonts have been encountered. In all, 6 cysts of *H. termitis* have been found, 2 in the mid-gut and 4 in the hind-gut. The cysts were spherical or sub-spherical in shape and varied in length from  $248 \,\mu$ — $321 \,\mu$ and from  $204 \,\mu$ — $292 \,\mu$  in width. The cyst is surrounded by a layer of varying thickness of granular debris. In 5 of the 6 cysts found both gametocytes and their nuclei could still be seen. After being examined the cysts were transferred to distilled

After being examined the cysts were transferred to distilled water in depression slides and cover-glasses were sealed over them with melted paraffin. In from 2 to 3 days the material within the cyst had become homogeneous and neither the gametocytes nor the nuclei could be distinguished. No further development occurred and the cysts eventually disintegrated.

nuclei could be distinguished. No further development occurred and the cysts eventually disintegrated. In practically all animals in which gregarines have been found in large numbers many cysts have been seen. That the life cycle of *H. termitis* differs in some respects from the majority of the gregarines is indicated by the fact that although 350 termites infected with *H. termitis*, some of them with extremely heavy infections, were examined, only the 6 cysts were found. This might be explained by assuming that the cyst stage is extremely short and that the oocysts are liberated within the original host. Failure to obtain development of the cysts in an environment which has proven satisfactory for the cysts of many gregarines also strengthens the belief that this is normally an *in vivo* process.

The study of a colony of termites in which an infection of *H. termitis* is present reveals conditions similar to those found where any infectious disease occurs in a community. In the case of the termites, the young nymphs which receive stomadeal feeding from older nymphs are free from gregarines. Soldiers, which receive

proctadeal feeding, are only rarely infected. The few queens and kings which were examined showed infection equal in extent to that found in the third form group. In addition to this caste difference, there is a definite difference in incidence of infection based on the molting of the infected groups. As the time of molting approaches, the parasites are found lower in the mid-gut than is normal and some degenerate sporonts are found in the hind-gut. Immediately after the molt, these termites are apparently free of infection with sporonts. During the period between molts the infection gradually increases until the next molt. This increase in sporonts in the newly molted individual may result from sporozoites still present in the walls of the intestine or from oocysts ingested, possibly at the time the cast is eaten.

Some evidence that immunity to H. termitis may be developed has been obtained from the study of an infected colony over a period of several months. While the infective agent was still present as shown by the fact that young individuals regularly become infected, the more mature individuals were found in most cases to be free of infection.

## Kofoidina ovata.

In addition to *Hirmocystis termitis*, a second gregarine was found infecting *Zootermopsis*. This parasite presented such striking differences from *H. termitis* and from other previously described gregarines that a new genus and species were found necessary for this form. The name *Kofoidina ovata* gen. nov., sp. nov. is proposed for this form, in honor of Professor C. A. KOFOID, who is responsible for most of our knowledge concerning the Protozoa of *Zootermopsis*.

of our knowledge concerning the Protozoa of Zootermopsis. Although K. ovata has been found in many colonies of Zootermopsis, the distribution of this parasite has not been determined as the hosts examined have been from a relatively restricted area in the western portion of the State of Washington. Likewise, the distribution of this parasite in genera and species of termites other than Z. angusticollis and Z. nevadensis has not been determined as yet.

Of the 35 colonies of Zootermopsis from Western Washington which were examined, 13 were infected with Kofoidina ovata; in 3 of these colonies H. termitis was also found. Some of the individual termites which were infected contained extremely large numbers of sporonts of K. ovata. The heaviest infection noted was one in which the number of sporonts was estimated to be 1500. In heavy infections the mass of gregarines can be seen as an opaque mass through the gut wall. The number of termites in a colony which may be infected with K. ovata is subject to the same variations as in H. termitis infections. In a colony of 243 termites examined over a period of 3 months, 162 were found to contain K. ovata, while 81 were not parasitized by this gregarine.

The sporonts of K. ovata (Pl. 4 Figs. 6, 8) occur in multiple and bi-associations. When mature the sporont is ovate in shape and is not differentiated into protomerite and deutomerite. The posterior end of the mature sporont is more rounded than is that of the sporont of *H. termitis*. The endoplasm is very dense but the nucleus is clearly visible. The mature sporont varies in length from  $58 \,\mu$ -117  $\mu$  and in width, from  $29 \,\mu$ -58  $\mu$ . The sporont is one and a half times as long as it is wide.

The immature sporonts (Pl. 4 Fig. 6) are colorless or nearly so and they differ considerably in shape from the mature forms. This difference in shape is chiefly due to a constriction at approximately the middle of the sporont, giving it a shape similar to that of a dumb-bell. However, the posterior enlargement is somewhat smaller than that of the anterior end.

The sporonts occur in associations similar to those described for H. termitis, although the associations tend to be somewhat longer than is the case with this latter species. The longest linear association seen consisted of 14 sporonts, the total length of which was  $635 \mu$ . Atypical associations in which 2 satellites are attached directly to a single primite are encountered. These satellites may have several other sproonts attached posteriorly. A striking characteristic of the associations in K. ovata is that the sporonts frequently decrease in size from the large primite down to the last satellite. This gradual dimunition results in a tapering of the entire chain which is more noticeable than actual measurements of the individuals would seem to warrant. Although the posterior end of the primite sets into a slightly developed cup in the anterior end of the satellite, the attachment is not so secure as is the case in *H. termitis*. Examples of the measurements of associations and associates which have been obtained of K. ovata are given, in microns, below.

Total length of association	153	168	184	194
Primite				
Length	73	80	92	92
Width	44	44	<b>58</b>	54
Satellite				
${f Length}$	80	88	92	102
Width	37	37	44	58

Length of associ	ations of	шоге спан	two spore	Juis:	
Total length of association	278	439	430	672	635
Primite	102	95	48	61	51
First satellite	105	88	51	82	61
Second satellite	37	88	48	61	54
Third satellite	<b>34</b>	80	54	68	51
Fourth satellite		44	48	68	48
Fifth satellite		44	48	61	44
Sixth satellite			44	61	44
Seventh satellite			48	44	37
Eighth satellite			41	44	41
Ninth satellite				44	41
Tenth satellite				37	37
Eleventh satellite				41	44
Twelfth satellite					41
Thirteenth satellite					41

Length of associations of more than two sporonts:

In addition to the immature and mature sporonts which are found in the anterior part of the mid-gut, large numbers of nonmotile bodies (Pl. 4 Fig. 7) are often seen in the posterior end of the mid-gut. These bodies are oval; the endoplasm is somewhat greenish in color and the nucleus, containing a single karyosome, is usually visible. These are the cephalonts. A vestigial epimerite is seen in some and in a few cases a slightly indented region at the anterior end shows where the epimerite was. The cephalont is very fragile in this region and after a few moments in normal salt solution or distilled water bursts open at this point.

Microscopic sections of the termite gut are necessary in order to observe the earlier stages. In the posterior part of the gut in a heavy infection great numbers of cephalonts (Pl. 4 Fig. 10) are found in the intestinal cells. In many the epimerite, a small knob, can be seen. As an epimerite is defined as the structure by which the cephalont is attached to the host cell, it is clear that this protuberance must be considered as a non-functional or vestigial epimerite. An epimerite is quite unnecessary when the parasite takes up an intra-cellular habitat.

Apparently in this species sporozoites do not break out of the oocyst until it reaches the posterior end of the mid-gut and in addition, probably, cyst dehiscence in a large number of cases occurs in the posterior part of the mid-gut and auto-infection occurs. The youngest stage seen in the intestinal cells was oval with a relatively large nucleus and measured  $8 \mu$  in length and  $7 \mu$  in width. An epimerite, which takes a slightly heavier stain than the rest of the cephalont in iron haemotoxylin, has been seen in slightly larger forms,

but there is no indication of the differentiation of a protomerite and deutomerite. The cephalont always lies parallel to the long axis of the intestinal cells and in every case the epimerite faces the muscular layer.

As the cephalont increases in size the nucleus of the host cell becomes pycnotic and in pushed toward the muscular layer. Eventually the invaded cell is destroyed and the parasite liberated into the lumen of the gut. Although large numbers of individual cells are destroyed, the parasite does not seem to cause any noticeable damage to the host. The death rate in a heavily infected colony is no greater than in normal colonies.

The discharged parasite takes up its place between the food mass and the epithelial cells. In the living condition the cytoplasm is greenish in color and fairly dense. Often epimerites in various stages of degeneration are seen. No motility occurs. By some means, this is transformed into a motile sporont, which is slightly dumbbell shaped and in which there is colorless or nearly colorless endoplasm. The immature sporont migrates to the anterior part of the mid-gut, associations are formed and the sporonts increase in size. In addition to this increase in size the protoplasm becomes more dense and the mature sporont appears black by transmitted light.

dense and the mature sporont appears black by transmitted light. The mature sporonts are usually found in pairs and eventually cysts (Pl. 4 Fig. 9) are formed. In 229 termites infected with K. ovata only 8 cysts have been seen. These varied in size from 73-88  $\mu$  in length and from 73-80  $\mu$  in width. They are subspherical in shape. In some both gametocytes could be seen. The cysts were recovered, for the most part, from the posterior end of the mid-gut. Each cyst was transferred from the contents to distilled water in a depression slide but all failed to develop further. For this reason, the time and manner of development of the cysts and the structure of the oocysts is not known.

## Systematic Position.

In older classifications gregarines are divided into two tribes, the Acephalina and the Cephalina. The Cephalina include those gregarines which are divided by septa into three parts, i. e. the epimerite, protomerite and deutomerite. In the Acephalina, on the other hand, no such division of the body occurs. Inevitably, however, some gregarines do not fit into either of these two categories. In some cases an epimerite is present but the body is not divided into protomerite and deutomerite or the protomerite is not present in the sporont. In other cases, no epimerite or a rudimentary one is found and yet a protomerite and deutomerite are present. For this reason, new classifications have been proposed to avoid these discrepancies. However, it is not necessary to discuss the merits of different classifications in this paper.

The family, Lecudinidae, occuring solely in polychaetes is generally considered as intermediate between the two groups mentioned above. A well defined epimerite is present and in the type species there is a differentiation in the endoplasm which suggests a protomerite and deutomerite although a septum is absent. The family, Cepha-loidophoridae, occurring in Crustacea and the Stenophoridae, in the Diplopoda, are considered somewhat less primitive than the Lecudinidae. Both families are intracellular in development and the epimerite is either rudimentary or lacking.

Several genera in several different families do not have a protomerite and deutomerite in the sporont. This is clearly the result of degeneration.

As the proposed genus *Kofoidina* can not be placed in any of the existing families, it has been found necessary to establish a new family, Kofoidinidae. It is of course difficult to fix unerringly the systematic position of the new family, Kofoidinidae, particularly as a knowledge of the oocysts is lacking. At first glance it would seem to be more primitive than the Lecudinidae because of the intracellular development of the cephalont. The rudimentary epimerite is the only thing which links it with the Cephalina. That this family has resulted from the degeneration of a cephaline gregarine rather than by evolution from the more primitive acephalines seems a logical conclusion. Although the intracellular habitat is a more primitive characteristic, it is difficult to conceive of the development of an attachment structure during the intracellular existence of the parasite. On the other hand, a return to an intracellular habitat and the loss of the septum separating the protomerite and deutomerite are both easily conceivable phenomena. The epimerite, a more primitive structure, might logically be expected to be retained after the disappearance of the septum between the protomerite and deutomerite.

## Family Kofoidinidae fam. nov.

Diagnosis. Gregarines with rudimentary epimerite. Deve-lopment intracellular. Body non-septate. Sporonts associative. Type genus. *Kofoidina* gen. nov.

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#### Kofoidina gen. nov.

Diagnosis. Epimerite rudimentary. Development intracellular. Body non-septate. Sporonts in associations of two to fourteen. Oocysts unknown.

Type species. K. ovata sp. nov. from the mid-gut of the genus, Zootermopsis, from the State of Washington.

#### Summary.

1. A study of the gregarines occurring in Zootermopsis augusticollis and Z. nevadensis has been made. These termites have been found to be heavily infected with two species of gregarines.

2. As a result of this study the systematic position of *Gregarina* termitis LEIDY has been changed and this species placed in the genus *Hirmocystis*.

3. A new gregarine from Zootermopsis has been described, which could not be placed in any of the existing genera or families. In order to classify this species a new family, Kofoidinidae, and a new genus, Kofoidina, are proposed and the form described as K. ovata.

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#### Explanation of Plate.

#### Plate 4.

(All drawings from living gregarines except figs. 2 and 10. Magnification  $\times$  545 unless otherwise stated.)

Figs. 1—5 Hirmocystis termitis (LEIDY).

Fig. 1. Association of four sporonts. Two small posterior satellites.  $\times 129$ . Fig. 2. Semi-diagram of portion of section through the mid-gut of the termite showing attached cephalont.

Figs. 3 and 4. Cephalonts.

Fig. 5. Association of five sporonts.  $\times$  129.

Figs. 6-10 Kofoidina ovata gen. nov., sp. nov.

Fig. 6. Immature sporont.

Fig. 7. Cephalont.

Fig. 8. Mature biassociation.

Fig. 9. Cyst in which both gametocytes and their nuclei can be seen.

Fig. 10. Semi-diagram of part of cross-section through the mid-gut of the termite showing four cephalonts.



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