The life history of *Carcinoecetes hesperus* n. gen., n. sp., a gregarine parasite of the striped shore crab, *Pachygrapsus crassipes*, with observations on related forms [1].

By

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With 23 figures in the text and plate 14.

Introduction.

The striped shore crab, *Pachygrapsus crassipes* of the Pacific Coast of North America is commonly infected with a cephaline gregarine, which we designate here as *Carcinoecetes hesperus* n. gen., n. sp. There is little doubt but that this form belongs in the same genus as the gregarine found in *Pachygrapsus marmoratus* of the Mediterranean and known up to the present as *Cephaloidophora conformis* (Diesing) Léger and Duboscq. Study of species of *Cephaloidophora* from Cirripedia, however, indicates that the forms from the above Decapods can not be placed in the same genus as those from the barnacles, mainly because of differences in the method of discharge of the sporocysts from the oocyst.

The genus *Cephaloidophora* was created in 1908 by Mavrodiadi for a gregarine parasitic in three species of barnacles found in the Black Sea: *Balanus eburneus*, *B. amphitrite*, and *B. improvisus*. This was probably the same form as that described in the last of the above-named species by Solger from the Baltic in 1891. Mavrodiadi called his type species *Cephaloidophora communis*. He was unable

[1]) Aided by a grant from the Board of Research, University of California.

to follow the reproductive cycle but described and figured (Fig. 13 of MAVRODIADI) spores that he found in the alimentary tract of the host. These were solitary, about 5 μ in length, and resembled those of *Stenophora*. Because of this and other similarities, the new genus was placed by MAVRODIADI in the family Stenophoridae, to be transferred by KAMM in 1922 to a new family, the Cephaloidophoridae. In both families, one of the distinguishing characters is the presence of solitary sporocysts; i. e., sporocysts not united in chains. However, since at least some of the species of *Cephaloidophora* from barnacles discharge chains of sporocysts (Text-Fig. 21), while all of those I have examined from Decapods have solitary sporocysts, for this reason alone the genus would have to be broken up. In addition, the general morphology, behavior, and life history are so different in the two groups that it is strange that so artificial a grouping has been retained this long.

The entire group of Crustacea-inhabiting gregarines must undoubtedly be revised — a task which the present writer has neither the inclination nor the equipment to undertake. The proposed taxonomic changes are merely the minimum alterations that he feels are necessary in any attempt to treat the species which is the principal topic of this discussion, *Carcinoecetes hesperus* from *Pachygrapsus crassipes*.

Acknowledgements.

The writer wishes to express his deep appreciation to Professor ÉDOUARD CHATTON, Director of the Station Biologique de Sète, France, at whose laboratory he worked in the spring of 1935. In particular, the author is indebted to Professor CHATTON for pointing out certain developmental stages of *Cephaloidophora communis* MAVRODIADI, and for generously turning over to him all of his observations on this form. To Miss LIBBIE KRICHESKY, the writer desires to express his thanks for the translation from the Russian of MAVRODIADI's paper (1908).

Historical.

The species, here re-named *Carcinoecetes conformis* is of some historical interest for it is the first gregarine ever to be described. In 1787, CAVOLINI described and figured a gregarine parasite from the caeca of the Mediterranean "flat crab", *Cancer depressus*, at present known as *Pachygrapsus marmoratus*. He called this parasite a tapeworm, but there is no doubt from either his text or his drawing

that he was dealing with the form that up to now has been called *Cephaloidophora conformis*. RUDOLPHI (1810) placed the same organism among the worms of doubtful status and suggested that it might be a trematode. Some forty years later, DIESING (1851) recognized CAVOLINI'S parasite as a gregarine and named it *Gregarina conformis*. Unfortunately, however, FRENZEL (1885) in creating the genus *Aggregata* from *Portunus arcuatus*, described a life history which contained not only stages belonging to the parasite itself but also parts of the developmental stages of still another gregarine, *Porospora*, and included perhaps cysts of a species of *Carcinoecetes*, inasmuch as his figures 30 and 31 resemble so closely those found in this genus. But even if FRENZEL did not introduce *Carcinoecetes* into the life cycle of *Aggregata* directly, the confusion he created in mixing up *Aggregata* and *Porospora* had indirectly the same result. For although FRENZEL also recognized the validity of *Gregarina conformis* as a separate parasite of *Pachygrapsus marmoratus*, his combination of the coelom-dwelling *Aggregata* with the intestinal form *Porospora* resulted in the transfer by LABBÉ (1899) of several intestinal gregarines of Crustacea, including *Gregarina conformis* to the genus *Aggregata*. This classification was accepted by MINCHIN (1903).

LÉGER and DUBOSCQ (1906—1909) finally rectified FRENZEL'S error of twenty years before, and pointed out that the coelomic cysts found in Crustacea were the schizogonous stages of *Aggregata*, which undergoes its sporogony in various mollusks, and that the intestinal gregarines, sometimes present in the same host, included the parasites originally described by CAVOLINI. For these, they erected (1907) a new genus, *Frenzelina* to which they transferred various intestinal forms from other Crustacea. They described for the first time the complete life history, including the sporocysts, of *F. conformis*. However, traces of the confusion produced by FRENZEL remained; and in 1911, LÉGER and DUBOSCQ were still suggesting that *Frenzelina*, now renamed *Cephaloidophora*, was perhaps the sporogonic phase of *Porospora*, at that time known only from the Crustacea. Two years later, (1913), the same authors showed that *Porospora* was really a digenetic gregarine which continued its development in certain lamellibranchs and gastropods.

LÉGER and DUBOSCQ (1911) transferred these forms to the genus *Cephaloidophora*, which had been created, as stated above, by MAVRODIADI in 1908 for a gregarine from Black Sea barnacles. The resemblance between the two forms was based entirely on the trophozoites and sporonts, since MAVRODIADI did not figure any cysts

and merely conjectured that certain spores he found in the digestive tracts of the barnacles belonged to this parasite.

The present writer believes that the forms from the barnacle and from the crab are not members of the same genus for not only do they differ greatly in morphology and development, but the method of hatching of the cysts is radically different; in those from the barnacle, the sporocysts are discharged in chains, while those from the crab are set free singly.

Material and methods.

Pachygrapsus crassipes was obtained from various localities in Southern California and as far north as Monterey Bay. Most of the specimens were taken from the piling underneath the Santa Monica Pier on Santa Monica Bay, although infected specimens were obtained from other types of environment, comprising, in addition to Monterey Bay, stations from Point Vincente to Point Mugu, Ventura County, and including mud flats, rocky coasts, breakwaters, and piers. Crabs were kept in the laboratory in aerated aquaria, and after the first two weeks, fed at least once a week with fresh beef liver. If kept isolated and not fed for two weeks, *Pachygrapsus crassipes* loses its infection of unencysted gregarines; this is also about as long as captive crabs can be kept without food. Even if they are not provided with circulating sea water, crabs of this species can be kept alive in the laboratory for at least six months or more and will go through at least two molts. Cysts were maintained and their development followed in running sea water, which was circulated by means of an ingenious compressed-air pump, perfected by Dr. E. L. LAZIER of this Department.

The Mediterranean "flat crab", *Pachygrapsus marmoratus* was obtained from the vicinity of the Biological Station at Sète, France, either from the "Étang" or from the open ocean. Crabs were maintained in the tanks of running sea water at the aquarium. Specimens of *Balanus amphitrite* were taken from the same regions.

Smears of gut contents were fixed in various media, SCHAUDINN'S Fluid proving most satisfactory, followed by staining in HEIDENHAIN'S iron haematoxylin. Material for sectioning was fixed in BOUIN'S, BOUIN-DUBOSCQ, ZENKER'S, or CHAMPY'S fixatives, of which the first two solutions proved most suitable. Sections were stained either in DELAFIELD'S or in HEIDENHAIN'S haematoxylin with appropriate counter-stains.

Carcinoecetes hesperus in *Pachygrapsus crassipes*

Extent of infection.

The common striped shore crab of Southern California, *Pachygrapsus crassipes* is infected with *Carcinoecetes* to the extent of about 39 %. Six hundred and eighty-five specimens examined at different times of the year and from different localities are included in Table 1. The degree of infection is distributed more or less irregularly over the entire year. In some cases, as many as three-fourths of all the crabs in a particular lot proved to be infected. There was no correlation between the extent of the infection and the locality or the nature of the habitat in which the crabs lived. The parasites are acquired very early in the life of the host, since tiny crabs having carapaces only 5 mm. wide have been found infected. A comparison of crabs that had recently molted with those having an old carapace showed that the gregarines were capable of persisting through a molt. This is easily explained since the lining of the mid-gut is not shed at the time of molting, and parasites are frequently found in this portion of the digestive tract.

Table 1.

Record of examinations of *Pachygrapsus crassipes* for infection with *Carcinoecetes hesperus*. February 1930 to September 1937.

Month	Number Examined	Number Positive	Percent Positive
January	19	10	53 %
February	68	14	21 „
March	68	18	26 „
April	69	36	52 „
May	45	17	38 „
June	54	22	41 „
July	102	38	37 „
August	69	47	68 „
September	43	17	40 „
October	81	20	25 „
November	20	4	20 „
December	47	21	44 „
Total	685	264	39 „

Morphology and life history.

The intra-cellular trophozoite stage is a relatively brief one in this species, very few examples being observed in a large number of sections. One of these is represented in Fig. 1 of Pl. 14, embedded in an epithelial cell of the mid-gut. Most of the life history of *Carcinoecetes hesperus* is passed in the lumen of either the

mid- or the hind-gut. In this species, unlike *C. conformis* in *P. marmoratus*, the caeca are rarely infected. The sporonts, which follow the intra-cellular trophozoite stage, are found in the lumen of the gut. The epimerite, even at this period, is rudimentary. The sporonts are in various stages of growth, and consequently vary considerably

Fig. 1.

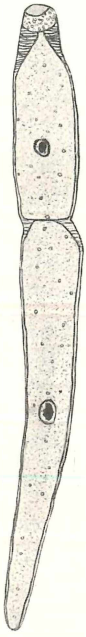


Fig. 2.

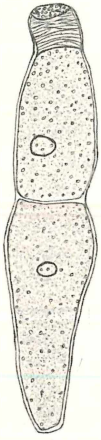


Fig. 3.

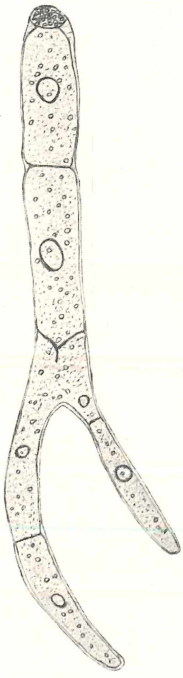


Fig. 4.

Carcinoecetes hesperus in *Pachygrapsus crassipes.*

Fig. 1. Sporont. Iron haematoxylin smear. Rudimentary epimerite present. × 340. — Fig. 2. Syzygy. Iron haematoxylin smear. Rudimentary epimerite present. Protomerite of satellite absent. × 210. — Fig. 3. Syzygy. From living specimen. Shows loss of epimerite. Satellite without protomerite. × 210. — Fig. 4. Multiple syzygy with forked tail. From living specimen. Penultimate satellites have partly fused and each has another satellite posteriorly. × 305.

in size, the smallest one seen was 18 μ × 8 μ, the largest 320 μ × 90 μ. One of them is shown in Text-Fig. 1.

In this species, the sporonts unite fairly early in development, so that most of the lumen-dwelling forms are in the nature of syzygies (Text-Fig. 2 and 3). The average length of a full-grown pair is 510 μ; the primite measuring 170 μ × 45 μ; the satellite

340 μ × 48 μ. The average of 50 pairs selected at random, and including both young and old syzygies, gave for total length 330 μ; the primite 110 μ × 27 μ; the satellite 220 μ × 26 μ. The smallest syzygy seen was only 30 μ in length, the longest 640 μ.

The great variability in relative sizes of the members of a syzygy make it inadvisable to employ few if any of the syzygy measurements as a species character, Occasionally, the primite may even exceed the satellite in length. The satellite is a little less wide than the primite during the growth period, but equals or slightly exceeds it in width in the full-grown syzygy. The most constant feature is the ratio of length of the protomerite to the total length of the primite, 1 : 7; the same ratio that holds for the solitary sporont.

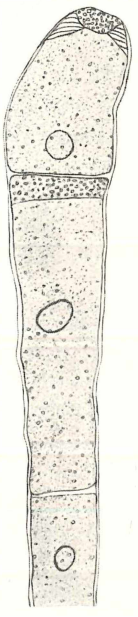


Fig. 5. Multiple syzygy. From living specimen. Protomerite present only in most anterior satellite. Syzygy consisted of 4 individuals × 450.

Multiple syzygies are very commen in this species; in different crab hosts, the relative numbers of members of a chain vary considerably. In some cases, pairs are the most common type; in others, groups of more than two are in the majority. The greatest number of individuals seen in a single chain was six. Usually, but not always, the posterior member of a multiple syzygy is the longest. Occasionally, an abnormal syzygy is formed by two individuals each attaching themselves to the posterior end of a chain, thus producing what appears like a bifurcated tail (Text-Fig. 4). In the great majority of cases of multiple syzygies, the more posterior satellites have no protomerites (Text-Fig. 4 and 5). I have been unable to determine whether these multiple individuals ever form mature cysts, but many of them, at least, begin the process of cyst formation.

The lumen-dwelling forms, either solitary or in syzygy, may occur free in the cavity of the gut, or else the protomerite may be deeply imbedded in the gut wall (Pl. 14 Fig. 5), without, however, actually rupturing the cell membranes. The parasite may produce a considerable thinning out or a sloughing of the gut tissues in heavy infections (Pl. 14 Fig. 5), due apparently to mechanical action, for the host shows no cellular reaction to any assumed lytic secretion. It is doubtful whether this destructive action, even in heavy

infections, is very harmful to the crab, for much of the gut wall remains undisturbed; and there is no evidence that infected crabs are less viable than non-infected ones.

The considerable variation in the size of the cysts is due to the fact that syzygies of *Carcinoecetes hesperus* encyst quite irreguarly regardless of the sizes of the members of a pair. The sizes of forty-four cysts from one host are given in Table 2. The behavior of two or more syzygyzing sporonts in the process of forming a cyst is rather striking. The members of a pair wrap themselves about one another to form a sphere, and begin to rotate in what may be called a „motile cyst" (Text.-Fig. 6). In the early stages of cyst formation, before there is any trace of a cyst wall, the two

Table 2.

Variation in size of oocysts of *Carcinoecetes hesperus* from hind-gut of a single *Pachygrapsus crassipes*.

95 μ × 93 μ	125 μ × 90 μ	130 μ × 110 μ	152 μ × 148 μ
102 „ × 90 „	125 „ × 100 „	130 „ × 112 „	155 „ × 120 „
105 „ × 100 „	125 „ × 101 „	133 „ × 118 „	158 „ × 155 „
115 „ × 108 „	125 „ × 112 „	134 „ × 110 „	160 „ × 110 „
116 „ × 100 „	125 „ × 118 „	135 „ × 108 „	162 „ × 145 „
117 „ × 107 „	128 „ × 110 „	140 „ × 120 „	165 „ × 150 „
119 „ × 110 „	128 „ × 110 „	140 „ × 122 „	170 „ × 123 „
120 „ × 100 „	128 „ × 115 „	140 „ × 138 „	170 „ × 145 „
120 „ × 100 „	130 „ × 100 „	143 „ × 148 „	170 „ × 152 „
122 „ × 120 „	130 „ × 110 „	145 „ × 120 „	171 „ × 150 „
125 „ × 80 „	130 „ × 110 „	145 „ × 130 „	220 „ × 200 „

or more members keep turning constantly within the same circumscribed space, aided apparently by their own mucous secretions. If properly stimulated, they will abandon their attempts at cyst formation and resume, temporarily at least, the life of active sporonts.

If cyst formation continues to completion, a process which can be followed on a slide, the two gametocytes, as they may now be called, keep up their ceaseless rotation, and in the course of about two hours, have secreted a thin gelatinous wall about themselves (Text-Fig. 7). Five to six hours later, a thick, gelatinous, and transparent wall has developed (Text-Fig. 8); a cyst 100 μ may have perhaps a wall of 20 μ. The gametocytes inside cease rotating as the cyst wall thickens, and with the maturing of the cyst, they gradually lose their identity. Fully formed cysts are found occasionally in the lumen of the hind gut (Text-Fig. 9), or else, more commonly, attached firmly to its wall or to that of the rectum (Text-Fig. 10 and Pl. 14 Fig. 4). Very rarely have cysts been seen attached to the hairs of the abdominal appendages or

to those fringing the abdomen, although they are found there in *P. marmoratus* somewhat more frequently. Neither have any cysts been recovered after centrifuging water from dishes in which heavily infested crabs containing mature cysts have been kept for several days. It seems probable, therefore, that in the Pacific species, the cysts continue their development under ordinary circum-

Carcinoecetes hesperus in *Pachygrapsus crassipes.*

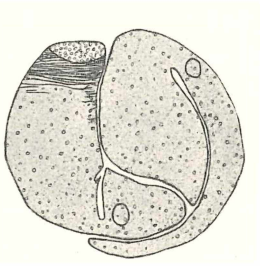


Fig. 6. "Motile cyst" in hind gut. From living specimen. × 155.

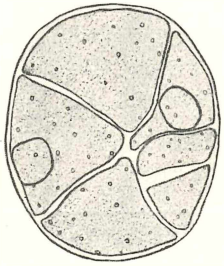


Fig. 7. Young cyst in hind gut. From living specimen. Syzygy has secreted gelatinous wall; slow rotation persists inside it. Pseudosegmentation due to compression of syzygy. × 340.

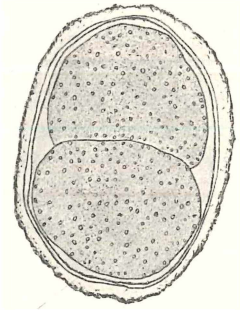


Fig. 8. Older cyst attached to abdominal hairs. From living specimen. The 2 gametocytes have rounded up but not fused. × 210.

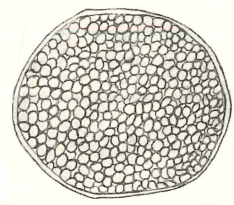


Fig. 9. Nearly mature oocyst free in lumen of hind gut. From living specimen. Developing sporocysts can be seen through oocyst wall. × 210.

stances while they are attached to the gut wall. For example, Pl. 14 Fig. 4 shows mature cysts developing in situ.

Contact with sea water seems to stimulate the mature gametocytes to encyst, and stages in the development of cysts up to those described above are fairly easy to obtain on a slide. Unfortunately, however, older cysts are attacked by bacteria and do not develop well in the laboratory. Various bactericidal and bacteriostatic substances have so far proved as lethal to the cysts as to the accompanying bacteria. The same difficulty in keeping mature cysts alive

in the laboratory was noted by LÉGER and DUBOSCQ (1909) in the case of *Carcinoecetes conformis*. The most successful method for the artificial cultivation of occysts proved to be to immerse them in a large volume of circulating sea water. Under these conditions, cysts become mature with the development of sporocysts in three to four days, about the same length of time as LÉGER and DUBOSCQ found necessary for *C. conformis*.

A nearly mature cyst is shown in Text-Fig. 10. Young cysts are frequently brown from the color of the contained gametocytes; as they become older the color changes to black or grey. In mature cysts, sporocysts can be seen through the cyst wall; such cysts are fragile and can be broken easily with a dissecting needle, freeing the sporocysts. The sporocysts (Text-Fig. 11) average 7.7 $\mu \times 8.6\ \mu$ and look rather like a life-preserver, with the sporozoites radiating from the center.

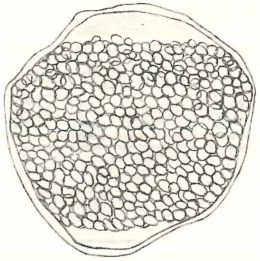


Fig. 10. Nearly mature oocyst attached to wall of hind gut. From living specimen. Oocysts do not mature beyond this stage in hind gut. × 210.

Fig. 11. Sporocysts with developing sporozoites and residual bodies (?). From living specimen. From oocyst which became mature after 72 hours in running sea water. × 1170.

Mature sporocysts contain apparently eight sporozoites. It is possible that the center is not actually hollow, but that the clear spot represents the so-called residual body of the spore. Except for the absence of a spiral arrangement of the sporozoites in the sporocyst, the picture resembles fig. 30 d of LÉGER and DUBOSCQ (1909) for *C. conformis*. The sporocysts are not discharged in chains. The sporozoites have not been seen set free from the sporocysts; hatching occurs probably in the lumen of the gut of the new host.

Method of infection.

The relative scarcity of cysts in sea water which had contained infected crabs, and particularly the rarity of intracellular trophozoites led the author, early in his investigation, to seek some possible supplementary method of transfer from one host to another. Examinations of probable intermediate hosts were all negative; these

included forms ordinarily associated with *Pachygrapsus crassipes* in its normal habitat, specifically, barnacles (*Mitella polymerus, Balanus glandula, Chthamalus fissus, Balanus tintinnabulum californicus*); mussels (*Mytilus californianus*); sand crabs (*Emerita analoga*); sea anemones (*Cribrina xanthogrammica*); limpets (*Acmaea* sp.); *Cancer antennarius* (frequently found buried in the sand at the bottom of the piling inhabited by *P. crassipes*); and fish feeding on *P. crassipes*, especially the California Sheepshead (*Pimelometopon pulcher*). This work was done before the author had had the opportunity to examine European material and provided further evidence that this was a monogenetic form allied to that found in *P. marmoratus* and in other Crustacea.

It was found that *P. crassipes*, if kept in isolation aquaria for two weeks without food, were rid completely of their infections of active gregarines by this time; only rarely did an infection persist as long as seven days under these conditions. The mortality of *P. crassipes* starved for a longer period was so high that it was not practicable to keep crabs without food beyond this time. They were fed subsequently once a week on fresh beef liver. As an additional precaution, no crab was used for infection experiments as parasite-free stock that had not been isolated in the laboratory for at least a month. The digestive tracts of heavily parasitized crabs, containing only sporonts or forms in syzygy (perhaps also intracellular stages, though this is doubtful because of their scarcity), were fed to these animals, which were then examined twenty-four to forty-eight hours later. Table 3 is a record of these feeding experiments and shows that these various stages can be transmitted to crabs free of lumen-dwelling parasites. For this reason, the author believes that infection is commonly acquired by one host feeding on another and thereby picking up the lumen-dwelling-forms. Since the pugnacious and gregarious habits of *P. crassipes* result in frequent cannibalism, it is probable that this is the principal method of keeping up the infection in nature.

Carcinoecetes conformis in *Pachygrapsus marmoratus*.

Although this gregarine, which is the one originally seen by Cavolini in 1787, has been fairly well described by Léger and Duboscq (1907, 1909), neither the published figures nor descriptions are sufficient to give one a complete picture of it.

Morphologically, *Carcinoecetes conformis* differs from *C. hesperus* in being longer and narrower, a full-grown sporont may reach the size of $650\,\mu \times 65\,\mu$ as against $320\,\mu \times 90\,\mu$ for the latter, and in

Table 3.
Results of feeding infected crab intestine to parasite-free crabs.
Carcinoecetes hesperus in *Pachygrapsus crassipes*.

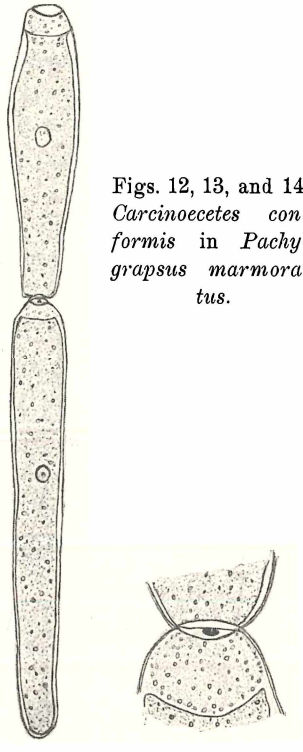
Date crab taken	Date crab fed infected gut	Date crab examined	Results
3/9/33	4/7/33	4/9/33	Negative
3/11/33	4/7/33	4/9/33	Syzygies in gut lumen
3/9/33	4/20/33	4/23/33	Syzygies and motile cysts in gut lumen
3/23/33	4/21/33	4/23/33	Negative (Found dead)
3/23/33	4/21/33	4/24/33	Negative
3/23/33	4/21/33	4/24/33	Negative
3/23/33	4/22/33	4/26/33	Negative
3/24/33	4/22/33	4/26/33	Negative
5/13/33	8/10/33	8/11/33	Negative
9/20/33	10/23/33	10/25/33	Negative
11/15/33	12/19/33	12/20/33	Negative
9/20/33	12/20/33	12/21/33	Negative
1/15/34	3/13/34	3/14/34	Syzygies and motile cysts in gut lumen
1/15/34	3/17/34	3/18/34	Negative
12/14/33	2/12/34	2/14/34	Negative
4/8/34	5/18/34	5/19/34	Negative
4/8/34	5/18/34	5/19/34	Negative
2/12/34	5/19/34	5/20/34	Negative
5/18/34	7/30/34	7/31/34	Negative

having the remnant of the epimerite well marked off anteriorly, even in old syzygies (Text-Fig. 12 a). In a large number of syzygies, a very distinct red granule can be seen at the junction of primite and satellite (Text-Fig. 12 b). Mature syzygies measure from 800 μ to 1050 μ in length. The ratio of length of protomerite to total length of the sporont is 1 to 10. Although the anterior member of a pair is frequently more swollen than the posterior one, the difference in shape between the two is by no means as constant a feature as LÉGER and DUBOSCQ (1909) were led to believe (cf. Text-Fig. 12 a with LÉGER and DUBOSCQ's Fig. 31). In this species, also, the protomerite of the second individual is rarely suppressed, and multiple syzygies are very scarce.

Whereas *Carcinoecetes hesperus* in *P. crassipes* lives mainly in the mid-gut and hind-gut, and rarely inhabits the intestinal caeca, *C. conformis* is found for the most part in the caeca and less commonly in the main portion of the gut. In the caeca, the parasites may be present by the hundreds. Here they form opaque masses, visible to the naked eye, and they may practically occlude the lumen (Pl. 14 Fig. 6), causing a sloughing or a thinning out of the epithelium. In heavy infections, they must interfere seriously with the func-

tioning of these organs. Furthermore, this location results in a much more persistent infection. While the Pacific Shore Crab, if not fed, loses its active infection usually in seven days and always in ten, the Mediterranean form still retains its lumen-dwelling forms after being starved for nearly three weeks.

Cyst formation is similar to the process described in *Carcinoecetes hesperus*, but, in the author's material, it was found to occur much less frequently. Although no cysts were seen attached to the wall of the posterior portion of the hind-gut, they were discovered firmly fixed to the hairs fringing the telson and the edge of the abdomen or to the hairs of the abdominal appendages. Thus, there is provided a very efficient mechanism for insuring reinfection and spreading the parasite to other individuals of the species, since the sporozoites are released where they can by ingested immediately. Cysts are probably formed in situ, as unencysted syzygies are found attached to these appendages and to hairs on the outside of the crab, and sea water has been shown to be a stimulus, at least in *Carcinoecetes hesperus*, for bringing about encystment in the laboratory.



Figs. 12, 13, and 14. *Carcinoecetes conformis* in *Pachygrapsus marmoratus*.

a b

Fig. 12 a—b. a, Syzygy in caecum. Formalin, unstained. Rudimentary epimerite present. × 75. b, Enlarged view of same specimen in region of junction of primite and satellite to show method of attachment and red granule at junction. × 287.

The writer did not succeed in causing cysts to develop under artificial conditions, and must draw upon the observations of Léger and Duboscq (1907) for the completion of the life cycle. The few cysts which they were able to save from destruction by bacteria orby a microsporidian, which in turn parasitizes this parasite, produced sporocysts in three to four days. These

Fig. 13. Very young sporont; extra-cellular. From gut section: Bouin-Duboscq; Delaf.-eosin; cut 5 μ. Posterior segment probably remnant of host cell. × 1020.

spores, each containing eight sporozoites, were not in chains. The youngest extracellular stage observed by the present writer was a young sporont 13 $\mu \times 5\ \mu$ (Text-Fig. 13). Text-Fig. 14 and Pl. 14 Fig. 2 show the intracellular trophozoite, a stage not previously figured for this species.

Cephaloidophora communis MAVRODIADI.

The morphological resemblance, the similarity in life history, and the close relationship of the hosts leaves little doubt but that the parasite from *Pachygrapsus crassipes* and that from *P. marmoratus* belong in the same genus. But for similar reasons, these two forms should not be placed in the genus *Cephaloidophora*, which was created for the gregarine described in 1908 by MAVRODIADI from three species of barnacles. LÉGER and DUBOSCQ (1911) transferred the forms they had described previously under the genus *Frenzelina* to MAVRODIADI'S *Cephaloidophora* upon finding that the former name was pre-empted. The validity of this transfer seems very doubtful to the present writer as it was made before the cysts of the parasite of the barnacle were known. It was based partly on a superficial resemblance of certain species of *Frenzelina* to MAVRODIADI'S parasite, and partly on an understandable failure at that time to recognize the great variety of gregarine parasites of Crustacea. In those days, it was believed that all Crustacean gregarines could be placed in very few genera. In addition to *Porospora*, say LÉGER and DUBOSCQ (1911, p. LIX) "La plupart des autres Grégarines de Crustacés semblant devoir rentrer dans les *Cephaloidophora*".

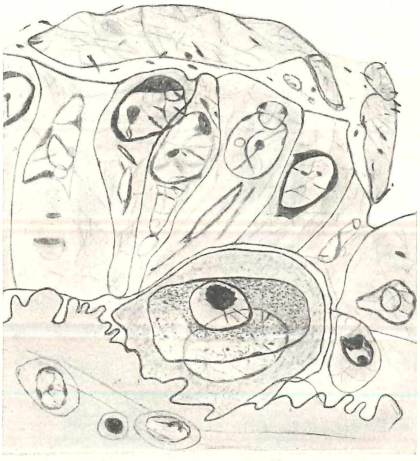


Fig. 14. Intracellular trophozoite; mid gut. From section: BOUIN'S; Delaf.-eosin; cut 5 μ. Parasite in cell at base of intestinal epithelium; host-cell nucleus visible. Cf. photograph: Pl. 14 Fig. 2. $\times$ 1250.

In the absence of complete life histories for most of the species in this genus, one cannot allocate all the members with certainty. From what evidence we have, however, the two species from *Pachygrapsus* and probably the majority of other forms listed under the name

of *Frenzelina* by LÉGER and DUBOSCQ (1909) and by SOKOLOW (1911) must be withdrawn from the genus *Cephaloidophora*. A new genus, *Carcinoecetes*, is proposed, containing two species definitely, *C. conformis* from *Pachygrapsus marmoratus* of the Mediterranean, and *C. hesperus* from *P. crassipes* of the Pacific Coast of North America. The author proposes to redefine the genus, *Cephaloidophora* MAVRODIADI by the addition of the character, "sporocysts discharged in chains" — a fact that apparently escaped TRÉGOUBOFF (1912), the last worker on *Cephaloidophora communis*. Nevertheless, he described the isolated sporocysts correctly.

The discovery of the chains of sporocysts among the molt cases of *Balanus amphitrite* infected by *Cephaloidophora communis* was made by Professor ÉDOUARD CHATTON while the author was working at the latter's laboratory in the spring of 1935. Professor CHATTON suggested that there might be a possible relationship between the two forms. The writer wishes to express his appreciation to Professor CHATTON for bringing the observation to his attention and for very generously turning over his sketches and notes of these developmental stages. The present author was able to demonstrate that the chains of sporocysts belonged actually in the life history of *C. communis*.

Figs. 15, 16, and 17. *Cephaloidophora communis* in *Balanus amphitrite*.

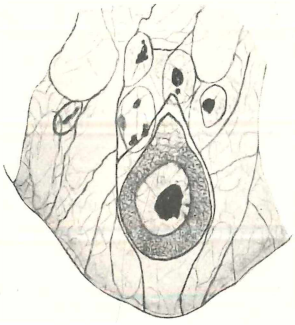


Fig. 15. Young intracellular trophozoite in epithelial cell of gut wall. From section: BOUIN's; iron haem.-eosin; cut 5 μ. $\times$ 1600.

Since the life history of this parasite is fairly well known as far as its intra-intestinal life is concerned (MAVRODIADI, 1908; TRÉGOUBOFF, 1912), we may pass through these stages very briefly. Unlike the forms in *Pachygrapsus*, *Cephaloidophora communis* spends a considerable portion of its life as an intracellular parasite. All stages from young non-septate forms to fully developed trophozoites are seen commonly in the epithelial cells of the gut wall, Text-Fig. 15 and Pl. 14 Fig. 7 and 8. Here they often excavate cavities in the bodies of the host cells (Pl. 14 Fig. 7). TRÉGOUBOFF undoubtedly interpreted a part of such a cavity as a hyaline, lens-shaped rudimentary epimerite of the parasite.

The lumen-dwelling stages bear little resemblance to those of *Carcinoecetes*, being only one-fifth to one-tenth as large, but rela-

tively twenty-five to fifty per cent wider than *C. hesperus*, and up to one hundred per cent wider than *C. conformis* (Text-Fig. 16 and 17). The protomerite in a fully grown sporont is about one-third of the total length, while in *C. conformis*, it is only about one-tenth, and in *C. hesperus* about one-seventh as long as the whole parasite. The primite and the satellite are much less intimately fused with one another, forming a rather loose junction between the two members. Multiple syzygies are very rare. One rather striking characteristic of these lumen-dwelling forms is their rapidity of movement, for they move with a speed comparing favorably with that of freeliving ciliates or flagellates in the same microscopic field.

Fig. 16. Syzygy. Formalin, unstained. × 575.

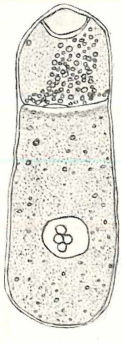


Fig. 17. Sporont. Formalin, unstained. × 575.

It is in the development of the cysts, however, that *Cephaloidophora communis* differs most markedly from *Carcinoecetes*. Cysts were never found in the intestine of the barnacle but were attached to the cuticle of the appendages either in the living animal or in the molt cases. Within the cyst, although the septum between protomerite and deutomerite soon disappeared, the two members of a pair retained their individuality for some time Usually, the two gametocytes differed morphologically, particularly in the size of their contained granules (Text-Fig. 18). Since, in many gregarines, the primite and the satellite give rise to gametes of different sexes, cysts having morphologically similar gametocytes (Text-Fig. 19) may have been sterile, although the writer has seen such cysts develop at least as far as the formation of the gametes.

In a developing cyst, the gametocytes can be seen to fuse suddenly, the resulting mass coming to fill the entire cavity (Text-Fig. 20). Within a quarter of an hour, the granules are thrown abruptly into a violent movement which continues unabated for about five minutes and then gradually subsides, — the so-called "dance of the sporoblasts". It is commonly believed that this behavior indicates the coming together of the gametes from the two individuals, brought about perhaps as the result of the activity of the residual body of the cyst.

The resulting zygotes develop into sporocysts which, contrary to the conditions in *Carcinoecetes*, are attached together in chains. These chains of a hundred or more sporocysts can be seen to emerge

Cephaloidophora communis in *Balanus amphitrite*.

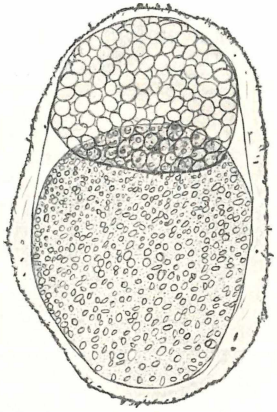


Fig. 18. Young oocyst attached to molt case. From living specimen. Gametocytes dissimilar. × 575.

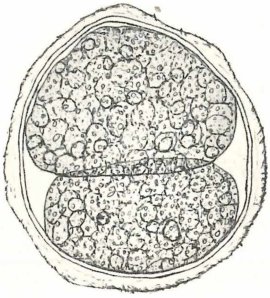


Fig. 19. Young oocyst attached to molt case. From living specimen. Gametocytes similar; beginning of gamete formation. × 575.

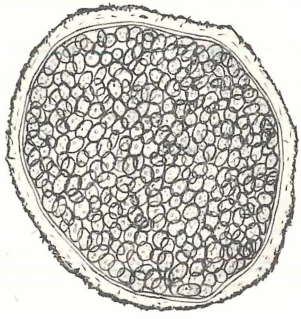


Fig. 20. Same oocyst one hour later. From living specimen. Gametocytes have fused; gametes formed. One-half hour before "dance of sporoblasts". Cysts wollen about 10% over previous stage. × 575.

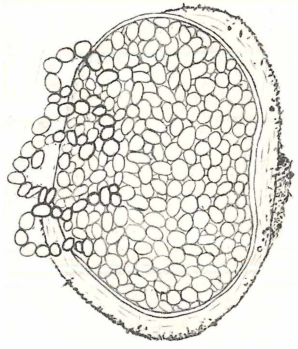


Fig. 21. Mature oocyst discharging chain of sporocysts; attached to molt case. From living specimen. × 575.

from the parent cyst (Text-Fig. 21 and 22) and to become entangled among the appendages and hairs of the body, particuiarly in the molt. The development of these cysts and spores on the outside of the body or in the molt cases is thus well suited for continuing the infection in forms as highly gregarious as are the barnacles, for

the molt frequently remains attached to the shell long after the time necessary for the development of the cyst and the release of the spores. The mature sporocysts each contain eight spirally arranged sporozoites and a refringent residual body (Text-Fig. 23).

The marked differences between the morphology and particularly the development of *Cephaloidophora communis* from *Balanus*

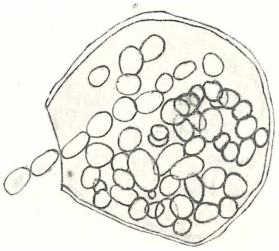


Fig. 22. Mature oocyst nearly empty of sporocysts; attached to molt case. From living specimen. Sporocysts being discharged in a chain. × 575.

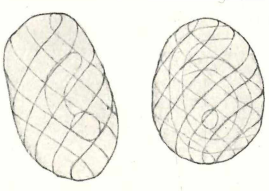


Fig. 23. Sporocysts found in chain entangled in molt case. From living specimen. Sporozoites and residual body visible. × 2900.

amphitrite and the above-described forms from *Pachygrapsus* are sufficient to warrant the removal of the latter from the genus *Cephaloidophora.* The genus *Carcinoecetes* is hereby created for them. Parenthetically, we may express the hope that *C. conformis*, the oldest of the gregarines, after so many vicissitudes has arrived at a permanent home — a wish, however, that may be looked upon with some scepticism in view of the past history of the parasite in question.

Diagnosis.

The genus *Cephaloidophora* is re-defined, following KAMM's description (1922).

Genus *Cephaloidophora* MAVRODIADI 1908. Sporonts associated usually in twos; syzygy occurring early. Development mainly intracellular. Rudimentary epimerite. Cyst without sporoducts, hatching by simple rupture. Sporocysts discharged in chains, sporocysts ovoidal. Monogenetic. Parasitic in digestive tracts of Crustacea.

Type species: *Cephaloidophora communis* MAVRODIADI.

Sporonts associated in twos. Size of solitary sporont up to 85 $\mu \times 27\ \mu$. Protomerite averages $^1/_3$ total length of sporont. Primite and satellite loosely fused. Sporonts capable of rapid movement. Cysts average 65 $\mu \times 54\ \mu$, found attached to cuticle of appendages or to molt cases. Sporocysts oval, 6.1 $\mu \times 4.8\ \mu$; each with eight

spirally arranged sporozoites and residual body. Intracellular stages common, up to 20 μ × 10 μ in size.

Parasitic in digestive tract of *Balanus amphitrite* (Sète, France) and probably also in *B. improvisus*, *B. improvisus gryphica*, *B. tintinnabulum communis*, *B. perforans*, and *B. eburneus*.

Genus *Carcinoecetes* n. gen. Sporonts associated in twos or more; syzygy occurring early. Intracellular development short. Rudimentary epimerite. Cyst without sporoducts, hatching by simple rupture. Sporocysts not in chains, sporocysts round to ovoidal. Monogenetic. Parasitic in digestive tracts of Crustacea.

Type species: *Carcinoecetes conformis* (Diesing) Ball.

Sporonts usually associated in twos. Size of solitary sporont up to 650 μ × 65 μ. Protomerite averages $^1/_{10}$ total length of sporont. Rudiment of epimerite well marked off. Primite and satellite intimately fused, with distinct red granule frequently at junction. Protomerite of satellite rarely suppressed. Cysts average 240 μ × 220 μ [1]; may be attached to appendages or to abdominal hairs of host. Sporocysts 6.4 μ × 5 μ; 5 μ × 4,7 μ, ellipsoidal with 8 sporozoites (Léger and Duboscq, 1907). Intracellular stages rare, up to 16 μ × 8 μ in size.

Parasitic in digestive tract, especially caeca, of *Pachygrapsus marmoratus*. Mediterranean.

Carcinoecetes hesperus n. sp.

Sporonts frequently associated in groups of more than two, up to 6. Size of solitary sporont up to 320 μ × 90 μ. Protomerite averages $^1/_7$ total length of sporont. Rudiment of epimerite indistinctly marked off. No red granule between primite and satellite. Protomerites of satellites frequently suppressed. Cysts average 140 × 123 μ in size. Attached usually to the wall of the hind gut. Sporocysts 7.7 μ × 8.6 μ, round to oval, with eight sporozoites radiating from center. Intracellular stages rare, up to 30 μ × 15 μ in size.

Parasitic in mid gut and hind gut, rarely in caeca, of *Pachygrapsus crassipes*. California: Pacific Grove to Point Vincente. Probably co-extensive with host.

Summary.

1. The life history is described of *Carcinoecetes hesperus* n. gen., n. sp., a gregarine from the striped shore crab, *Pachygrapsus crassipes* of the Pacific Coast of North America.

[1] Léger and Duboscq (1909) give 150 μ; this is too small for the large syzygies of 800—1000 μ; I have found them of the above size. Cf. size of *C. hesperus* cysts, which are formed by syzygies not exceeding 650 μ.

2. A comparison is made between this species and the form called up to the present, *Cephaloidophora conformis* (DIESING) LÉGER and DUBOSCQ, found in *Pachygrapsus marmoratus* of the Mediterranean.

3. New stages in the life history of *Cephaloidophora communis* MAVRODIADI are described from *Balanus amphitrite*.

4. On the basis of differences in the morphology and life history of *Cephaloidophora communis* and of *C. conformis*, the latter species is transferred to the new genus *Carcinoecetes*.

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Explanation of Plates.

Plate 14.

Carcinoecetes hesperus in *Pachygrapsus crassipes*, Figs. 1, 3, 4. *C. conformis* in *P. marmoratus*, Fig. 2. Bouin's; Delaf.-eosin.

Fig. 1. Intracellular trophozoite in epithelial cell of mid gut. Cut 10 μ. $\times$ 208.

Fig. 2. Intracellular trophozoite of *Carcinoecetes conformis* in cell at base of intestinal epithelium in mid gut of *Pachygrapsus marmoratus*. Cut 5 μ. $\times$ 650.

Fig. 3. "Motile cysts" in lumen of mid gut. Cut 12 μ. $\times$ 30.

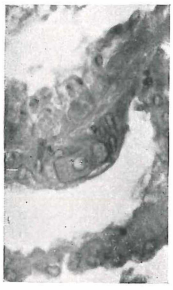
Fig. 4. Oocysts attached to lining of hind gut. Cut 12.5 μ. $\times$ 81.

Fig. 5. Section of mid gut of *Pachygrapsus crassipes*, heavily infected with *Carcinoecetes hesperus*. Bouin's; Delaf.-eosin; cut 10 μ. Note compression and sloughing of epithelium. $\times$ 84.

Fig. 6. *Carcinoecetes conformis* in caeca of *Pachygrapsus marmoratus*. Bouin's; Delaf.-eosin; cut 5 μ. Lumen practically occluded by parasites. Note protomerites deeply embedded in epithelium. $\times$ 47.

Fig. 7. Intracellular trophozoite of *Cephaloidophora communis* in epithelial cell of gut of *Balanus amphitrite*. Bouin's; iron haem.-eosin; cut 5 μ. Note hollow space in host cell in front of protomerite; this is the lens-shaped "cap" of Trégouboff. $\times$ 375.

Fig. 8. Mature intracellular trophozoite of *Cephaloidophora communis* dropping out of epithelial cell of gut of *Balanus amphitrite*. Bouin's; iron haem.-eosin; cut 5 μ. $\times$ 375.



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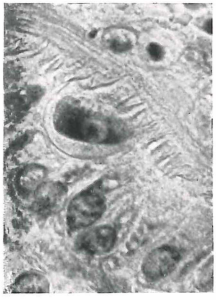

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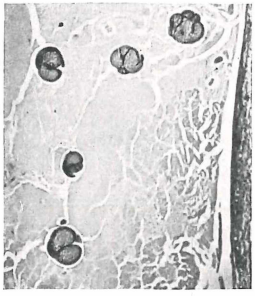

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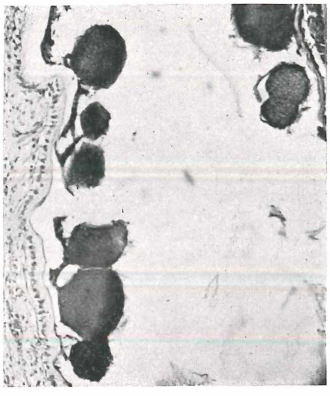

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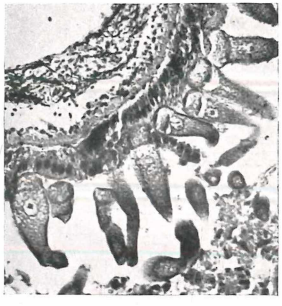

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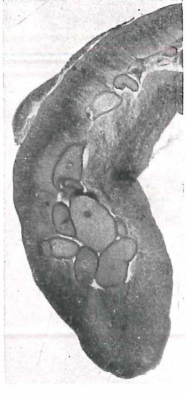

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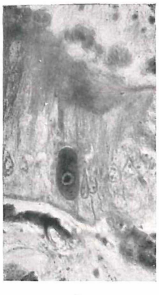

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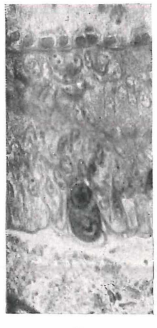

8

VERLAG VON GUSTAV FISCHER IN JENA